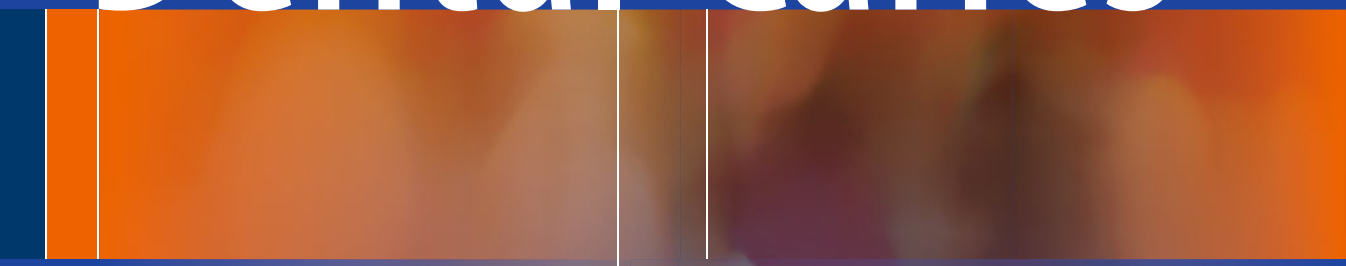
Zhou Xuedong
Editor

Dental Caries

Principles and Management



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State Key Laboratory of Oral Diseases
West China Hospital of Stomatology
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Contents

1 Tooth Development: Embryology of the Craniofacial Tissues 1
Zheng Liwei, Wang Chenglin, and Ye Ling

2 Biofilm and Dental Caries 27
Xu Xin, Zhou Yuan, Shi Wenyuan, Liu Yaling, and Zhou Xuedong

3 Saliva and Dental Caries 59
Wang Renke

4 Demineralization and Remineralization 71
Cheng Lei, Li Jiyao, Xu Hockin H.K., and Zhou Xuedong

5 The Diagnosis for Caries 85
Yang Liu, Li Boer, Wang Shuang, Zhang Yaru, and Peng Li

6 Dental Caries: Disease Burden Versus Its Prevention 91
Hong Xiao

7 Clinical Management of Dental Caries 107
Li Jiyao

8 Dental Caries and Systemic Diseases 129
Zou Ling and Hu Tao

9 Models in Caries Research 157
Huang Xuelian, Guo Qiang, Ren Biao, Li Yuqing, and Zhou Xuedong

Index 175

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Tooth Development: Embryology of the Craniofacial Tissues

1

Zheng Liwei, Wang Chenglin, and Ye Ling

1.1 Embryology of the Craniofacial Tissues

The development of craniofacial tissues is part of human prenatal development. Generally, human prenatal development goes through three stages: the proliferative 2-week period, when cell division is prevalent; the embryonic period, which extends from the second to the eighth weeks; and the fetal period, from eighth week to birth [1]. With normal accomplishment of the development, human body forms stepwise.

1.1.1 Origin of Human Tissue

The origin of tissue begins with fertilization, which is the fusion of spermatozoa and ova to form a zygote. Then the zygote moves to the uterine cavity where it will implant into the wall of the uterus and, meanwhile, undergoes a series of rapid divisions that lead to the formation of a fluid filled hollow ball, termed blastocyst, and small inner cell mass. When this blastocyst attaches to the sticky wall of the body of the uterus, uterine endometrium is digested, allowing blastocyst embedded in its surface and then deeper penetration. Implantation takes place.

On either side of the inner cell mass, two small cavities are formed. A small disk (the embryonic disk) develops in the center, where they reach each other. The embryonic disk becomes the embryo, composed of two layers of cells. One layer is lined with ectodermal cells, which will form the outer body covering (epithelium), called ectodermal layer. The cells on the ventral aspect are endodermal cells, forming the endodermal layer. This configuration is completed in the first 2 weeks, which is termed "proliferative period" [1].

During the third week, two-layered embryonic disk is converted to a three-layered disk. Cells that develop between the ectodermal and endodermal layers become the mesodermal layer. Next, major tissues and organs, including oral maxillofacial tissue such as tooth and facial bones, differentiate from these three layers [2]. Key events are the development of the nervous system, differentiation of neural crest tissue from the ectoderm, and folding of the embryo.

1.1.2 The Neural Crest

The nervous system begins with a specification of the neural plate, which develops as a thickening within the anterior ectodermal layer. Meanwhile, the neural plate develops raised folds at its margins. These folds in turn encompass and fuse so that neural tube forms and separates from the ectoderm.

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Upon closure of the neural tube, a unique population of cells known as neural crest cells separate from the lateral aspect of the neural plate. These cells have the capability of migration and differentiation. This is especially obvious in the head and neck region, and neural crest cells have an important role in the head development. They contribute to most of the embryonic connective tissue of facial region, which includes dental tissues such as the pulp, dentin, and cementum. Consequently, embryonic connective tissue in the head is termed as ectomesenchyme, reflecting its origin from the ectoderm, whereas connective tissue elsewhere is derived from the mesoderm and is known as mesenchyme. Although the neural crest tissues arise from neural ectoderm, they exhibit properties of mesenchyme [2, 3].

1.1.3 Head Formation

The head fold of the three-layered embryo is crucial and produces the primitive stomatodeum or oral cavity. When the stomatodeum first forms, it is surrounded by frontal prominence rostrally and by the cardiac bulge caudally. And it is separated from the foregut by buccopharyngeal membrane, a bilaminar structure consisting of ectoderm and endoderm, which breaks down soon so that the stomatodeum communicates with the foregut. Laterally the somatodeum becomes delimited by the first pair of pharyngeal arches [1, 2].

1.2 Enamel Development

Fully mature enamel comprises 80–90 % (v/v) carbonated calcium hydroxyapatite crystals, which is in contrast to bone and dentin, both with 10 % and 13 % (v/v) carbonated calcium hydroxyapatite crystals, respectively. The mechanisms of crystal initiation, crystal growth, as well as morphology are related to amelogenesis. In developing teeth, sequential and reciprocal interactions occur between the epithelium and the underlying mesenchyme, which derive from the cranial neural crest. Enamel formation associates with the differentiation of the tooth-specific cell types, the epithelial ameloblasts. This chapter will provide a brief overview in different aspects of tooth enamel development with a particular emphasis on the current knowledge of enamel morphogenesis, histogenesis, and cytodifferentiation.

1.2.1 Histogenesis and Morphogenesis

The consecutive phases during tooth morphologic changes, including lamina, bud, cap, and bell stages, are characterized by epithelial histogenesis. The segmentation of the dental epithelium occurs in the early tooth initiation, which indicates that a local epithelial thickening corresponds to the dental lamina. Experiment approaches suggested that Wnt/Shh interactions may regulate the delimitation between the dental epithelium and the oral ectoderm. Nevertheless, the molecules intervene in regulating epithelial cell apoptosis, and survival or compartmentalization of different elements is still poorly understood (Fig. 1.1).

1.2.1.1 Bud Stage

From the bud stage, the thickened presumptive dental epithelium, which forms the basal epithelium, can be distinguished from the round internal cells. Depending on the position, epithelial cells are evident in changes of the expression of different molecules, including γ-catenin, desmoglein, and P- and E-cadherins.

1.2.1.2 Cap Stage

During the cap stage, the dental epithelium becomes the enamel organ which consists of four different cell types: inner and outer dental epithelia, the stellate reticulum, as well as transiently the primary enamel knot. At this time, the inner enamel epithelium becomes discernible from the outer enamel epithelium. The histogenesis of the inner dental epithelium is coordinated by a change in the composition of the basement membrane.

The enamel knot is a dynamic transient structure and appears at the onset of mammalian tooth shape development, which is in contact with several cells, including peripheral cells, internal round cells, and basement membrane cells.

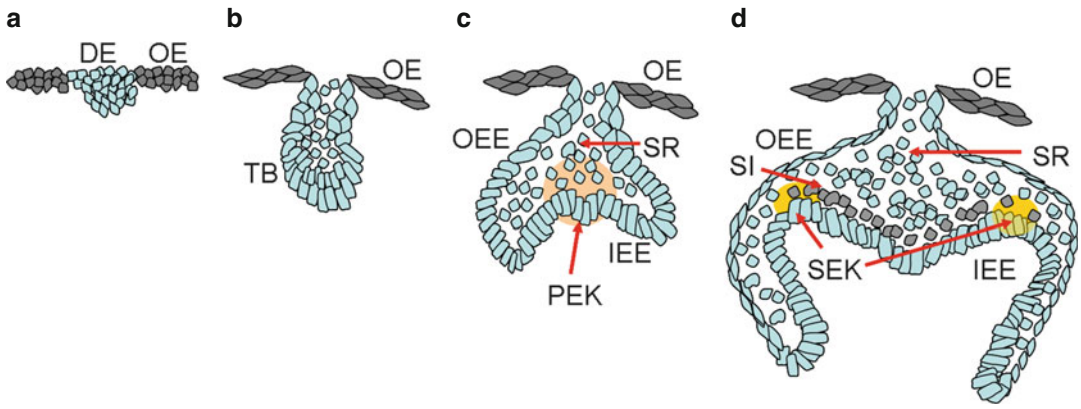

Fig. 1.1 (**a**–**d**) Morphogenesis of the tooth development (from bud to bell stage)

Studies indicated that the primary enamel knot represents a signaling center in formatting cusps, which may lead to unequal growth of the enamel epithelium and induce the formation of secondary enamel knots.

It has been suggested that the structure and organization of primary enamel knot rapidly change during the time. At the beginning of the cap stage, it appears as a long cylindrical shape and the shape will extend along the mesial–distal axis of the first lower molar. Soon after, some of internal cells begin apoptosis. While the first lower molar grows during cap formation, the primary enamel knot starts to extend in anterior and posterior directions. It is suggested that in the primary enamel knot, most cells do not divide and its proliferation needs the recruitment of cells within the enamel organ. However, the underlying mechanism is still not known due to differences in mouse strains or measuring stages of embryos.

1.2.1.3 Bell Stage

At the bell stage, the enamel organ delimitates the dental papilla and starts to form cusps. At this time, the secondary enamel knots form, which only precede cusp formation by a few hours. The secondary enamel knots are taken place at the tips of the forming cusps at the bell stage. The relationship between primary and secondary enamel knots has been suggested in several models. The gene expression patterns elucidated the primary enamel knot induces the secondary enamel knots by a reaction–diffusion-related mechanism.

During the bell stage of tooth development, the shape of the crown is determined. The growth of crown results from cell division and reorganization of inner dental epithelium. Furthermore, the programmed cell death is also accompanied by the regulation of cell number in the inner dental epithelium, suggesting its role in determining the final number of functional ameloblast cells.

1.2.2 Cytodifferentiation

From the lamina stage to the bell stage, changes in reorganization of the epithelium compartment can be distinguished. They not only regulate histogenesis but also determine the final number and specific positioning of functional ameloblasts.

Amelogenesis, or enamel formation, consists of two main steps. The first step creates partially mineralized enamel (about 30 %). The second step involves extreme influx of additional mineral while removing organic material and water to form more than 96 % mineral contents. The differentiation of epithelial cells into functional ameloblast cells includes several morphologic changes that occur in time and involve growth, elongation of the cytoplasm, polarization, and secretion of matrix protein. These epithelial cells exhibit a unique character of progressively changed phenotype. Amelogenesis has been described in as many as six stages but generally is divided into three functional phases, known as presecretory, secretory, and maturation stages (Fig. 1.2).

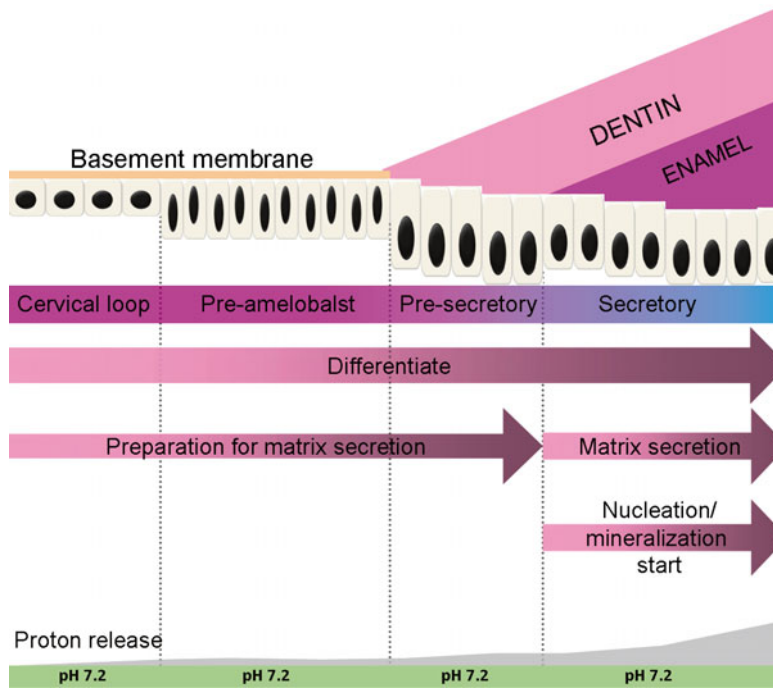

Fig. 1.2 Ameloblast differentiation

1.2.2.1 Presecretory Stage

During the presecretory phase, the cells of the inner enamel epithelium start to differentiate into ameloblast cells. At the morphogenetic phase, inner enamel epithelium cells are cuboidal or low columnar, with large centrally located nuclei and poorly formed organelles in the proximal portion of the cells. During differentiation phase, once stimulated, these cells elongate, their nuclei shift distally toward the stratum intermedium, and the Golgi elements increase and migrate distally. Moreover, the cytoplasm becomes filled with organelles which are needed for the synthesis and secretion of enamel proteins. At this time, a second junctional complex forms at the distal extremity of the cell, compartmentalizing the ameloblast cells into a body and a distal extension called Tomes' process, against which enamel develops.

Although the pre-ameloblasts have been regarded as nonsecreting cells, more and more researches demonstrate that the enamel protein secretion starts much earlier, even before the separation between pre-ameloblasts and pre-odontoblasts. Ameloblast cells are aligned closely with each other due to the tight junctional complex or attachment specializations [2]. These junctional complexes greatly involved in amelogenesis determine what may pass between cells to enter or leave the enamel at different times.

1.2.2.2 Secretory Stage

The newly formed ameloblasts near the dental papilla are flat and the matrix secreted is called rodless enamel matrix. During the secretory stage, the ameloblasts exhibit a tall columnar and polarized morphology and secrete an extracellular protein-rich matrix. The fine structure of secretory stage ameloblasts indicated their strong synthetic and secretory activity. The Golgi complex is intense and forms a cylindrical organelle surrounded by many cisternae of rough endoplasmic reticulum. Ameloblast secretion is constitutive, which means the secretion is successively, and the secretory granules are not stored for prolonged periods of time.

When enamel formation begins, Tomes' process comprises only a proximal portion. The secretory granules are released along the surface of the process against the newly formed mantle

dentin to create an initial layer of the enamel without enamel rods. The very first hydroxyapatite crystals formed interdigitate with the dentin crystals. After forming the initial enamel layer, ameloblast cells migrate from the dentin surface and form the distal portion of Tomes' process as an outgrowth of the proximal portion. The distal portion extends into and interdigitates beyond the initial layer of enamel, while the proximal portion penetrates from the distal junctional complex to the enamel layer surface [2].

It is believed that the distal portion of Tomes' process progressively lengthens as the enamel layer thickens and gradually turns to be thinner as the rod developing in diameter presses it against the wall of the interrod cavity. Eventually, the process is squeezed out of existent, leaving a narrow area which is filled with organic materials between the enamel rod and interrod enamel. When the outer layer of enamel is being formed, the distal portion of Tomes' process is altered and orientation also changed, leading to slightly difference of enamel rods in the outer third of layer with a more rectilinear trajectory. Finally, the ameloblasts become the same overall appearance as when initial enamel was forming. Without the distal portion of Tomes' process, the final enamel has no rods. Notably, the initial, interrod, and final enamels are developed by the same secretory surface and, indeed, form a continuum [2].

1.2.2.3 Maturation Stage

During the maturation stage, the ameloblasts aim at resorbing much of the water and organic matrix from the enamel in order to allow enough space for the growing enamel crystals [2]. This change results from the thickness and width growth of preexisting crystals seeded during amelogenesis formative stage, not due to additional crystal accumulation.

It is believed that the stratum intermedium cells are also related to secretory and absorptive functions of amelogenesis and desmosomes facilitate their close contact with ameloblast cells. The stratum intermedium cells appear less active as enamel maturation is near completion [4].

After immature enamel has fully formed, ameloblast cells undergo several morphologic changes in preparing maturing the enamel. At this time, a short transitional stage appears, during which ameloblasts become shorter and their volume and organelle content decrease. At the maturation stage, some ameloblast cells undergo programmed apoptosis; roughly about half of the ameloblasts is reduced during amelogenesis.

In summary, ameloblasts arise from the inner enamel epithelial cells and experience multiple morphologic and functional changes. Following the deposition of a layer of enamel, ameloblasts deposit enamel in the form of rods or prisms that become highly mineralized. The arrangement of ameloblasts with their Tomes' process plays a critical role in the formation of enamel rods. The process of amelogenesis is a series of successive phases of proliferation, differentiation, secretion, and maturation, eventually forming the enamel.

1.2.3 Microstructure of the Enamel

The enamel is a composite structure consisting of mineral and organic phases. At the nanometer scale, like most other naturally mineralized tissues, dental enamel has hierarchical structures and surface features [5, 6]. On the microscale, the enamel consists of highly organized architectural units known as enamel prisms. On the nanoscale, the enamel consists of highly crystalline nanorod-like calcium hydroxyl apatite crystallites that are arranged roughly parallel to each other [7] (Fig. 1.3).

1.2.3.1 Enamel Rod

Using the scanning electron microscope and following a short etching part, enamel rods can be observed in ground or fractured teeth. The enamel rod represents the mineralized progress of ameloblasts and Tomes' process. Enamel rods cross one another and follow an undulating course as they progress from the DEJ toward the enamel surface. When the arcades connect to each other, enamel rods have the features of keyholes or paddles, with the convex surface of the arcades oriented in an incisal or cuspal order. The enamel rods run nearly perpendicular to all parts of tooth surface, stopping at the final layer of aprismatic enamel [4].

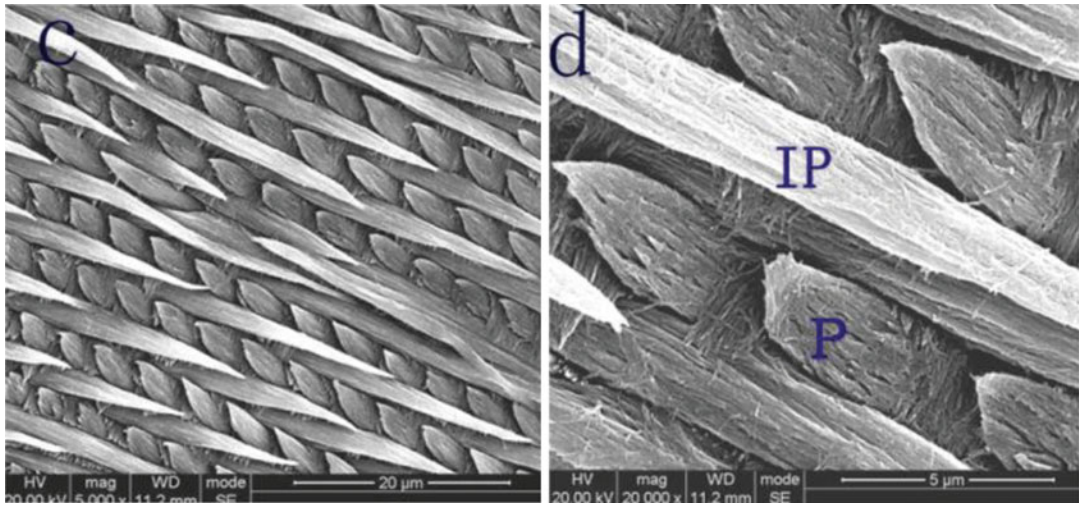

Fig. 1.3 Enamel structures

1.2.3.2 Enamel Spindle

Enamel spindles are generated during the differentiation stage of amelogenesis. When the initial enamel is formed, the enamel spindles become terminal extensions of the primary dentinal tubule into the enamel matrix. Spindles exhibit bulbous structures at DEJ region in mature tooth enamel.

1.2.3.3 Enamel Lamellae and Cracks

It is believed that enamel lamellae are the result of local failure of the maturation process. Enamel lamellae include thin sheets of organic materials that extend throughout the enamel mineralization and exhibit vertical orientation from incisal or cuspal regions to cervical area. Cracks share some similar characters with lamellae in ground section and usually appear as artifacts during teeth processing.

1.2.3.4 Enamel Tufts

Enamel tufts originate from the DEJ and extend 1/3 to 1/2 of the thickness of the enamel matrix. They are formed during Tomes' process development and also during the elaboration of the initial enamel of the enamel rod. They represent protein-rich regions that failed to mature in the enamel matrix.

1.2.3.5 Interpit Continuum

The secretory product is released from the ameloblast cells at two preferred sites. The comparatively superficial site forms the majority parts of all developing enamel surfaces. This site is interameloblastic and the product determines pits, naming interpit for this stage. In many circumstances, the interpit phase is continuous throughout vast parts of the tissue [8]. The second location at which the enamel matrix is released is from the secretory pole of Tomes' process proper, which aims at filling in the pit. At these sites, crystals may have orientations that merge with those from the interpit stage, building open-sided prism boundaries.

1.2.3.6 Functional Aspects of Enamel Structure

The particular organization of the enamel serves as enhancing hardness and wear resistance. The parallel formation of crystals perpendicular to the surface of the teeth brings about the best way for dense packing of the crystals as well as obviates the need to nucleate new crystals during enamel maturation. It has a microporous structure, which allows extra mineral flow in the crystals for further growth while degrading matrix components to be removed. Although single crystal is too

flexible, the perpendicular positions enable the growth of long whisker-like crystals, allowing the crystals form into larger domains to be stronger and stiffer.

Enamel crystals are the largest crystals found in the body. The primary structural unit of enamel is the enamel rod, which is formed by the secretory activity of ameloblasts. The orientation of crystals and the distribution of organic matrix are involved in maintaining structural properties of enamel.

1.2.4 Enamel Matrix Proteins

Enamel formation requires a remarkable orchestration of diverse and essential enamel-secreted proteins, including amelogenin, ameloblastin, enamelin, amelotin, tuftelin, dentin sialoprotein, and apin. Studies provide functional data showing that the disruption of synthesizing, secreting, and processing these genes can cause different subtypes of amelogenesis imperfecta (AI), indicating the indispensable role for enamel composition and maturation [9].

1.2.4.1 Enamelin

A number of studies have suggested that the first protein to be secreted by ameloblasts at the dentin–enamel junction (DEJ) region is enamelin [10, 11]. Enamelin is a novel acidic enamel protein that has been postulated to play an essential role in enamel mineralization. By high-resolution protein-A gold immunocytochemistry, the acidic feature of enamelin proteins has been reported to be in line with its capability to bind to enamel mineral crystallite surfaces [12]. Enamelin is rich in aspartic acid and could be arranged in β-sheet conformation that results in nucleation of the mineral component.

The enamelin proteins initially secreted at the very early phase of enamel formation are strictly expressed by ameloblasts and persist throughout enamel developing and maturing stages [10]. The mutations of enamelin such as enamelin-null phenotype are associated with aberrations of enamel, causing AIH2. Several studies have described the mutations of enamelin gene causing an autosomal-dominant AI phenotype [13]. In $Enam^{-/-}$ mice, the enamel layer is completely absent. The crust over the dentin is thin, irregular, and easily abraded [10]. These analyses indicate that enamelin is essential for enamel matrix organization and mineralization.

1.2.4.2 Amelogenin

The amelogenin proteins of developing dental enamel are tissue-specific components, rich in leucine, histidine, proline, and glutamyl residues. Among all the ameloblast-specific proteins, amelogenin is the most abundant extracellular protein. The initial enamel layer is dominated by amelogenin protein secretion. In human, the amelogenin gene has been shown to be located on both X and Y chromosomes [14]. Human amelogenin genes have 7 exons, with the principal variation of sequence homology occurring within exon 6, which codes for most amelogenin core and the C-terminus [15].

It has been shown that the amelogenin nanospheres, the supramolecular assembly of amelogenin, such as elastin, appear as a functional structural protein during enamel formation [16]. Two human pedigrees with an X-linked AI (AIH1) phenotype both share the same mutation in the amino-terminal, tyrosine-rich amelogenin peptide (TRAP) segment [17, 18]. The recombinant proteins of those two AIH1 point mutations have been compared with wild-type amelogenin, exhibiting altered nanosphere dimensions and amelogenin assembly kinetics [19, 20]. During in vivo enamel formation, the amelogenin nanosphere also can be observed adjacent to HAP crystallites [21].

It has been found that human-inherited enamel defect AI often associates with alterations in amelogenin X chromosome gene [22]. The mutations in amelogenin are known to hypoplastic or hypomineralized enamel [22, 23]. Amelogenin knockout mice also display abnormal teeth with chalky-white discoloration, broken tips of incisors and molars, as well as disorganized hypoplastic enamel, indicating amelogenin proteins play a major role in the regulation of enamel thickness and organization of crystal pattern [24] (Fig. 1.4).

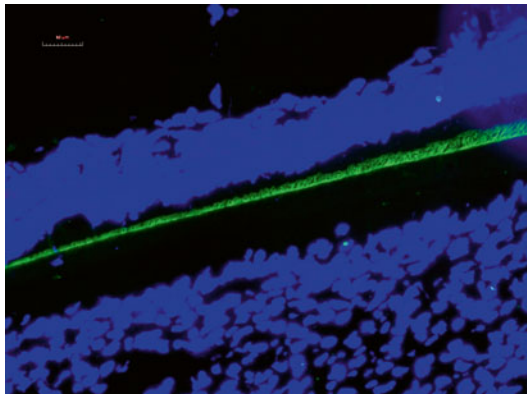

Fig. 1.4 Immunostaining of amelogenin

1.2.4.3 Ameloblastin

Ameloblastin, a cell adhesion molecule, is one of the unique tooth-specific proteins, expressed by secretory ameloblasts, yet the expression decreases during enamel maturation [25]. Shortly after dental epithelium initial differentiation, the cells are detached from the underlying matrix, resume proliferation, and lose polarity, reversing to undifferentiated one, indicating that ameloblastin maintains the differentiation state of ameloblasts at the secretory stage, by binding to ameloblasts and by inhibiting their proliferation [26].

At secretory amelogenesis, ameloblastin distribution following the ameloblast cell outline appears to be a 'fishnet'-like partitioning [27]. The ameloblastin null mice reveal severe enamel hypoplasia, and overexpression of ameloblastin in the enamel organ influences enamel crystallite habit and enamel rod morphology, resulting in a phenotype characteristic of AI. Undoubtedly, these data all suggest that in the enamel matrix, either gain of function or loss of function of ameloblastin can cause enamel alterations. It has also demonstrated that ameloblastin acts as a nucleator of crystallization because it is expressed at mineralization initiation sites within the enamel (Fig. 1.5).

1.2.4.4 Amelotin

Murine amelotin has been identified recently, which is the newest described enamel-specific protein. In developing murine incisors and molars, expression of amelotin mRNA was restricted to maturation-stage ameloblasts [28].

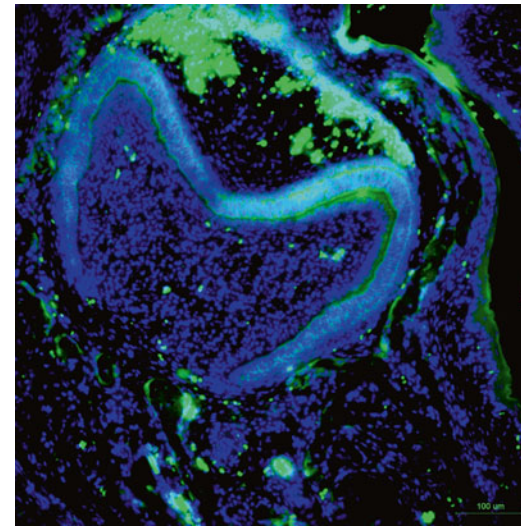

Fig. 1.5 Immunostaining of ameloblastin

Both murine and human amelotin genes contain 9 exons and 8 introns and are located on chromosomes 5 and 4q13.3, respectively, which are close to the enamelin and ameloblastin genes. The expression of amelotin mRNA is essentially limited in postsecretory ameloblasts, experiencing a dramatic increase from secretory to maturation phase ameloblasts and subsequently lessening toward the zone of reduced ameloblast cells. Less information is available describing whether or not amelotin is a candidate gene for AI.

1.2.4.5 Tuftelin

Shortly after differentiation, tuftelin, an acidic protein, is synthesized and secreted. Tuftelin gene localizes to chromosome 1q21 in human. In secretory stage, the secretory pathway of amelogenin to the extracellular space is from the Golgi complex and then to Tomes' processes [29]. However, in vivo tuftelin accumulates in cytoplasmic area other than the Golgi complex and secretes granules in both mineralizing and nonmineralizing tissues.

1.2.4.6 Proteolytic Enzymes

There are two major proteinases secreted into the enamel matrix, including matrix metalloproteinase-20 (MMP20, enamelysin) and kallikrein-4 (KLK4, enamel matrix serine proteinase-1, or serine proteinase 17).

Matrix Metalloproteinase-20 Human MMP20 gene consists of 10 exons and is part of the MMP gene clusters. The human MMP20 is located on chromosome 11q22.3, and an autosomal-recessive form of AI was recently discovered in a family that had a mutation in the intron 6 splice acceptor [13]. In porcine teeth, both ameloblast and odontoblast cells express MMP20. During the early stage of enamel formation, MMP20 activity accounts for virtually all of the known cleavage sites in amelogenin. The mutation of MMP20 exhibits hypoplastic enamel with improperly processed amelogenin and rod pattern [30]. In addition, the homozygous MMP20 mutation family reveals severely pigmented, brittle, and soft enamel, which is characterized by less radiodense.

Kallikrein-4 Human KLK4 gene is located on chromosome 19q13.41. KLK4 was first discovered in the teeth, but it also expressed in other tissues such as the prostate. In the teeth, KLK4 is secreted by different cell types, including odontoblasts and late-secretory and maturation phase ameloblasts [31]. KLK4 expression during enamel maturation correlates with the degradation of enamel proteins, thus indicating it is necessary for the enamel to achieve the high level of mineralization. KLK4 mutation was found in a family with autosomal recessive hypomaturation AI, showing yellow-brown discolored teeth. The enamel fractured from the teeth has normal thickness but with a decreased mineral content. Notably, the affected members are all females, so it is not sure whether KLK4 has an effect on the prostate. However, only the teeth were apparently altered by the homozygous KLK4 mutation [23].

1.3 Pulpodentin Complex

In a mature tooth, dentin is a unique, avascular mineralized connective tissue that forms the bulk of the tooth, and dentin encloses a richly innervated and highly vascularized soft connective tissue, the dental pulp. Dentin and pulp are derived from the dental papilla, whose cells migrate from the cranial neural crest. The tissues remain closely associated during development and throughout the life of an adult tooth and are hence most commonly referred to as the "pulpodentin complex."

During the process of tooth development, most attentions are focused on the common themes about odontoblast differentiation that have emerged and what is known about the influence of tooth-signaling molecules and transcription factors on the development and homeostasis of the pulpodentin complex. In addition, the focus is the theories about the general principles of dentin matrix formation, particularly the synthesis and secretion of extracellular matrix molecules and their postulated roles in the biomineralization of dentin, and the theories about the development and homeostasis of differentiated and undifferentiated or stem cell populations can be translated to regenerative approaches targeted at restoring the integrity of the adult pulpodentin complex.

1.3.1 Dentin

Fully mature dentin is composed of approximately 70 % inorganic material and 10 % water by weight. The principal inorganic component consists of $Ca_{10}(PO_4)_6(OH)_2$ (hydroxyapatite). Organic matrix accounts for 20 % of dentin. 91 % of organic matrix is collagen, and most of the collagen is type I, with a minor component of type V. Noncollagenous matrix components include phosphoproteins, proteoglycans, gamma-carboxyglutamate-containing proteins (Gla-proteins), acidic glycoproteins, growth factors, and lipids. By volume, inorganic matter makes up 45 % of dentin, while organic molecules and water 33 % and 22 %, respectively. A characteristic of human dentin is the presence of tubules that occupy from 20 to 30 % of the volume of intact dentin. These tubules house the major cell processes of odontoblasts. The elasticity of dentin provides flexibility for the overlying brittle enamel.

1.3.1.1 Structure of Dentin

Dentinal Tubules The characteristic of dentin is the presence of tubules, which host the major cell processes of odontoblasts. Tubules form

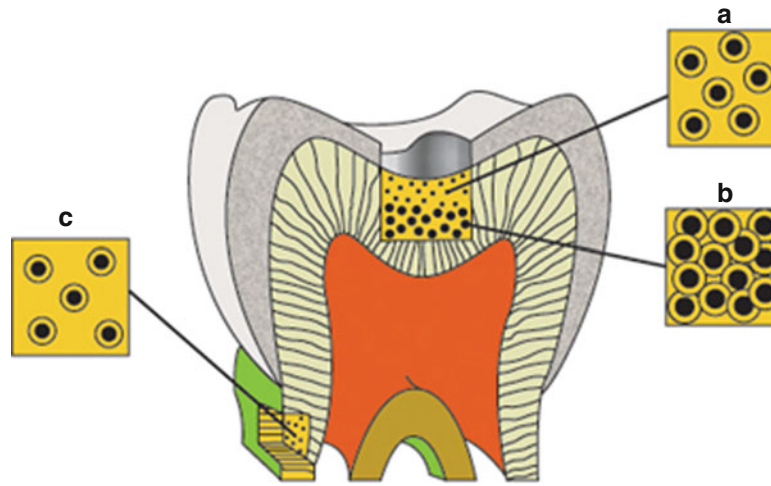

Fig. 1.6 Diagram illustrating the difference in size and number of tubules on the occlusal surface of coronal dentin (*A* and *B*) and at the cervical region of the root surface (*C*). This combination is responsible for the exponential increase in dentin permeability with depth (From Pashley [32], p. 106, figure 2)

around the odontoblast processes and thus transverse the entire width of the dentin from the DEJ or DCJ to the pulp. They are slightly tapered in the wider portion situated toward the pulp. This tapering is the result of the progressive formation of peritubular dentin, which leads to a continuous decrease in the diameter of the tubules toward the enamel.

In coronal dentin, the tubules have a gentle S shape as they extend from the DEJ to the pulp. The S-shaped curvature is presumably the result of the crowding of odontoblasts as they migrate toward the center of the pulp. As they approach the pulp, the tubules converge because the surface of the pulp chamber has a much smaller area than the surface of dentin along the DEJ.

The number and diameter of the tubules are different at various distances from the pulp, and the mean number and diameter of tubules decrease following the increased distance (Fig. 1.6) [32]. Investigators found the number and diameter of dentinal tubules to be similar in rats, cats, dogs, monkeys, and humans, indicating that mammalian orthodentin has evolved amazingly constantly [33].

Near the DEJ, the dentinal tubules ramify into one or more terminal branches; this is due to the fact that during the initial stage of dentinogenesis, the differentiating odontoblasts extended several cytoplasmic processes toward the DEJ, but, as the odontoblasts withdrew, their processes converged into one major process (Fig. 1.7).

Peritubular Dentin Dentin lining the tubules is termed *peritubular dentin*, whereas that between the tubules is known as *intertubular dentin* (Fig. 1.8). Presumably precursors of the dentin matrix that is deposited around each odontoblast process are synthesized by the odontoblast, transported in secretory vesicles out into the process, and released by reverse pinocytosis. With the formation of peritubular dentin, there is a corresponding reduction in the diameter of the process.

Peritubular dentin represents a specialized form of orthodentin not common to all mammals. The matrix of peritubular dentin differs from that of intertubular dentin in having relatively fewer collagen fibrils and a higher proportion of sulfated proteoglycans. Because of its lower content of collagen, peritubular dentin is more quickly dissolved in acid than is intertubular dentin.

Peritubular dentin is more highly mineralized and therefore harder than intertubular dentin. The hardness of peritubular dentin may provide added structural support for the intertubular dentin, thus strengthening the tooth. By preferentially removing peritubular dentin, acid etching agents used during dental restorative procedures enlarge the openings of the dentinal tubules, thus making the dentin more permeable.

Intertubular Dentin *Intertubular dentin* is located between the rings of peritubular dentin and constitutes the bulk of circumpulpal dentin. Its organic matrix consists mainly of collagen

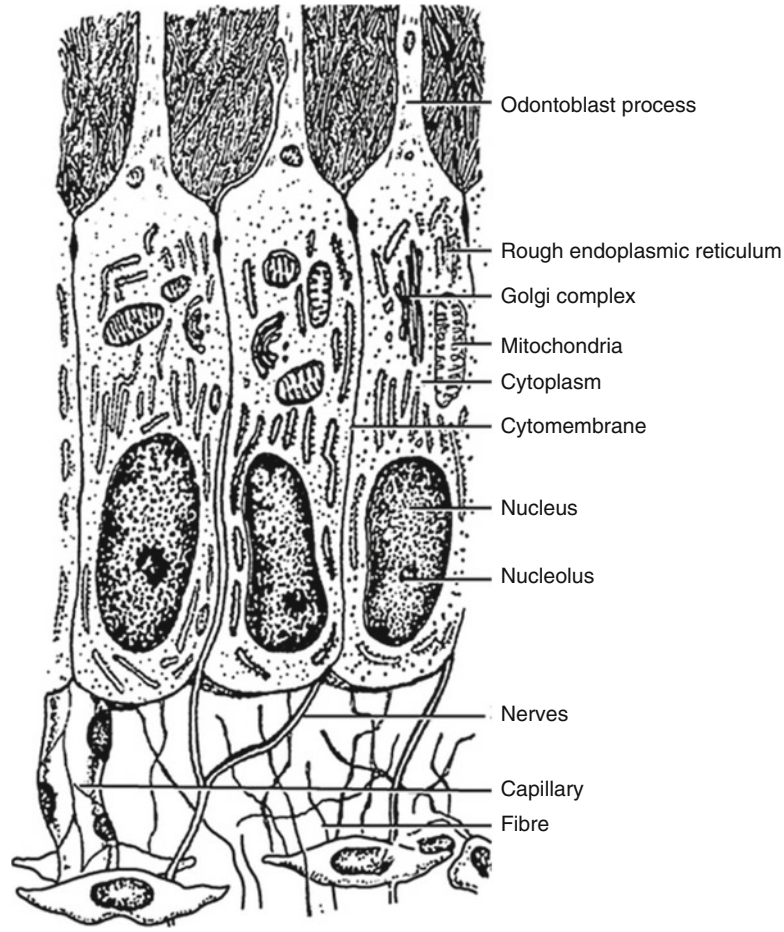

Fig. 1.7 Diagrammatic representation of the differentiated odontoblast

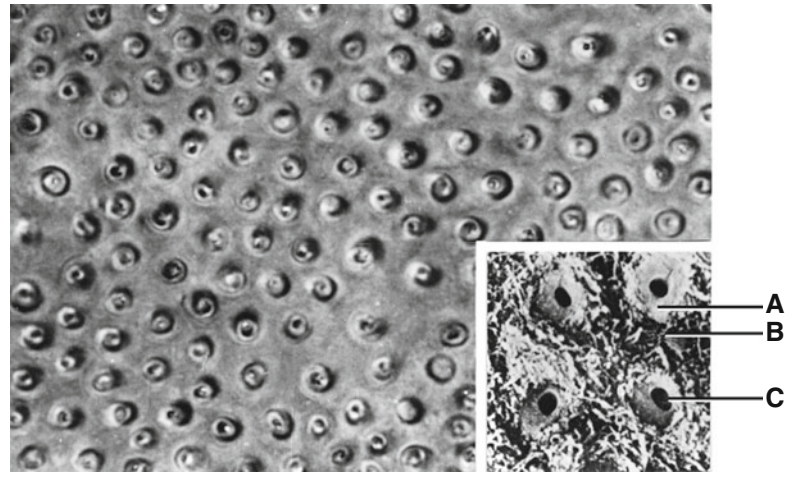

Fig. 1.8 The cross section of dentinal tubules. (*A*) Peritubular dentin; (*B*) intertubular dentin; (*C*) dentinal tubule

fibrils having diameters of 500–1000 Å. These fibrils are oriented approximately at right angles to the dentinal tubules.

Interglobular Dentin The term *interglobular dentin* refers to organic matrix that remains unmineralized because the mineralizing globules

fail to coalesce. This occurs most often in the circumpulpal dentin just below the mantle dentin where the pattern of mineralization is more likely to be globular than appositional. In certain dental anomalies (e.g., vitamin D-resistant rickets and hypophosphatasia), large areas of interglobular dentin are a characteristic feature.

1.3.1.2 Types of Dentin

Developmental dentin is that which forms during tooth development. That formed physiologically after the root is fully developed is referred to as the *secondary dentin*. Developmental dentin is classified as *orthodentin*, the tubular form of dentin found in the teeth of all dentate mammals. *Mantle dentin* is the first formed dentin and is situated immediately subjacent to the enamel or cementum. It is typified by its content of the thick fan-shaped collagen fibers deposited immediately subjacent to the basal lamina during the initial stages of dentinogenesis. Spaces between the fibers are occupied by smaller collagen fibrils lying more or less parallel with DEJ or DCJ. The width of mantle dentin in human teeth has been estimated at 80–100 μm [34].

Circumpulpal dentin is formed after the layer of mantle dentin has been deposited, and it constitutes the major part of developmental dentin. The organic matrix is composed mainly of collagen fibrils, approximately 500 Å in diameter that is oriented at right angles to the long axis of the dentinal tubules. These fibrils are closely packed together and form an interwoven network.

Predentin is the unmineralized organic matrix of dentin situated between the layer of odontoblasts and the mineralized dentin. Its macromolecular constituents include type I and type II trimer collagens, and noncollagen elements consist of several proteoglycans (dermatan sulfate, heparan sulfate, hyaluronate, keratan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate), glycoproteins, glycosaminoglycans (GAGs), Gla-proteins, dentin phosphoproteins (DPP), and a tissue-specific molecule which is unique to the odontoblast cell lineage. DPP is produced by the odontoblast and transported to the mineralization front, and it is thought to bind to calcium and play a role in mineralization.

1.3.1.3 Mineralization of Dentin

Mineralization of dentin matrix commences with the initial increment of mantle dentin. Calcium phosphate crystals begin to accumulate in matrix vesicles within the predentin [35]. Presumably these vesicles bud off from the cytoplasmic processes of odontoblasts. Although matrix vesicles are distributed throughout the predentin, they are most numerous near the basal lamina. The apatite crystals grow rapidly within the vesicles, and in time, the vesicles rupture. The crystals thus released mix with crystals from adjoining vesicles to form advancing crystal fronts that merge to form small globules. As the globules expand, they fuse with adjacent globules until the matrix is completely mineralized.

Apparently matrix vesicles are involved only in mineralization of initial layer of dentin. As the process of mineralization progresses, the advancing front projects along the collagen fibrils of the predentin matrix. Hydroxyapatite crystals appear on the surface and within the fibrils and continue to grow as mineralization progresses, resulting in an increased mineral content of the dentin.

1.3.1.4 Dentinal Sclerosis

Partial or complete obturation of dentinal tubules may occur as a result of aging or develop in response to stimuli such as attrition of the tooth surface or dental caries [36]. When tubules become filled with mineral deposits, the dentin becomes sclerotic. Dentinal sclerosis is easily recognized in histologic ground sections because of its translucency, which is due to the homogeneity of the dentin since both matrix and tubules are mineralized. Studies using dyes, solvents, and radioactive have shown that sclerosis results in decreased permeability of dentin. By limiting the diffusion of noxious substances through the dentin, dentinal sclerosis helps to shield the pulp from irritation.

One form of dentinal sclerosis is thought to represent an acceleration of peritubular dentin formation. This form appears to be a physiologic process, and in the apical third of the root, it develops as a function of age [36]. Dentinal tubules can also become blocked by the precipitation of hydroxyapatite and whitlockite crystals

within the tubules. This type occurs in the translucent zone of carious dentin and in attrited dentin and has been termed "pathological sclerosis."

1.3.1.5 Dentin Repair

Dentin that is produced in response to the injury of primary odontoblasts has been known by several different names: irregular secondary dentin, irritation dentin, tertiary dentin, and reparative dentin. The term most commonly applied to irregularly formed dentin is reparative dentin, presumably because it so frequently forms in response to injury and appears to be a component of the reparative process. It must be recognized, however, that this type of dentin has also been observed in the pulps of normal unerupted teeth without any obvious injury [37]. The reasons of this phenomenon and the difference between the development and repair of irregular dentin are still unclear.

It will be recalled that secondary dentin is deposited circumpulpally at a very slow rate throughout the life of the vital tooth. In contrast, the formation of reparative dentin occurs at the pulpal surface of primary of secondary dentin at sites corresponding to areas of irritation. For example, when a carious lesion has invaded dentin, the pulp usually responds by depositing a layer of reparative dentin over the dentinal tubules of the primary or secondary dentin which communicate with the carious lesion. Similarly, when occlusal wear removes the overlying enamel and exposes the dentin to the oral environment, reparative dentin is deposited over the exposed tubules. In general, the amount of reparative dentin formed in response to caries or attrition of the tooth surface is proportional to the amount of primary dentin that is destroyed. Thus, the formation of reparative dentin allows the pulp to retreat behind a barrier of mineralized tissue.

Compared to primary dentin, reparative dentin is less tubular and the tubules tend to be more irregular with larger lumina. In some cases, no tubules are formed. The cells that form reparative dentin are not as columnar as the primary odontoblasts of the coronal pulp and are often cuboidal. The quality of reparative dentin (i.e., the extent to which it resembles primary dentin) is quite variable. If irritation to the pulp is relatively mild, as in the case of a superficial carious lesion, the reparative dentin formed may resemble primary dentin in terms of tubularity and degree of mineralization. On the other hand, reparative dentin deposited in response to a deep carious lesion may be relatively tubular and poorly mineralized with many areas of interglobular dentin. The degree of irregularity of reparative dentin is probably determined by factors such as the amount of inflammation present, the extent of cellular injury, and the state of differentiation of the replacement odontoblasts.

The poorest quality of reparative dentin is usually observed in association with marked pulpal inflammation. In fact, the dentin may be so poorly organized that areas of soft tissue are entrapped within the dentinal matrix. In histologic sections, these areas of soft tissue entrapment impart a Swiss cheese appearance to the dentin. As the entrapped soft tissue degenerates, products of tissue degeneration further contribute to the inflammatory stimuli assailing the pulp.

1.3.2 Pulp

The pulp is a soft tissue of mesenchymal origin residing within the pulp chamber and root canals of the teeth. The primary role of the pulp is to produce dentin, by specialized cells, the odontoblasts, arranged peripherally in direct contact with dentin matrix. The close relationship between odontoblasts and dentin is one of several reasons why dentin and pulp should be considered as a functional entity, sometimes referred to as the pulpodentin complex. Following tooth development, the pulp retains its ability to form dentin throughout life. This enables the vital pulp to partially compensate for the loss of enamel or dentin caused by mechanical trauma or disease. How well it serves this function depends on many factors, but the potential for regeneration and repair is as much a reality in the pulp as in other connective tissues of the body.

The dental pulp is in many ways similar to other connective tissues of the body, but its special characteristic deserves serious consideration. Even the mature pulp bears a strong resemblance to

embryonic connective tissue. Certain peculiarities are imposed on the pulp by the rigid mineralized dentin in which it is enclosed. Thus it is situated within a low-compliance environment that limits its ability to increase in volume during episodes of vasodilatation and increased vascular permeability. The pulp houses a number of tissue elements, including vascular tissues, nerves, connective tissues, fibers, ground substance, interstitial fluid, odontoblasts, fibroblasts, antigen-presenting cells, and other minor cellular components.

1.3.2.1 Vascular Tissues

The pulp is actually a microcirculatory system whose largest vascular components are arterioles and venules. No true arteries or veins enter or leave the pulp. Unlike most tissues, the pulp lacks a true collateral system and is dependent upon the relatively few arterioles entering through the root foramina and occasional arteriole through a lateral canal. Since with age there is a gradual reduction in the luminal diameters of these foramina, the vascular system of pulp decreases progressively. Since the pulp is relative incompressible, the total volume of blood within the pulp chamber cannot be greatly increased, although reciprocal volume changes can occur between arterioles, venules, lymphatics, and extravascular tissue. In the pulp, therefore careful regulation of blood flow is of critical importance.

Blood from the dental artery enters the tooth via the arterioles, and these vessels pass through the apical foramen or foramina in company with nerve bundles. Smaller vessels may enter the pulp via lateral or accessory canals. As the arterioles pass into the coronal pulp, they fan out toward the dentin, diminish in size, and give rise to a capillary network in the subodontoblastic region. This network provides the odontoblasts with a rich source of metabolites.

Capillary blood flow in the coronal portion of the pulp is nearly twice that in the root portion [13]. Moreover, blood flow in the region of the pulp horns is greater than in other areas of the pulp. In young teeth, capillaries commonly extend into the odontoblast layer, thus assuring an adequate supply of nutrients for the metabolically active odontoblasts. The subodontoblastic capillaries are surrounded by a basement membrane, and occasionally, fenestrations (pores) are observed in capillary walls. These fenestrations are thought to provide rapid transport of fluid and metabolites from the capillaries to the adjacent odontoblasts.

1.3.2.2 Nerve Fibers

The pulp is a rather unique sensory organ capable of transmitting information from its sensory receptor to the central nervous system. Being encased in a protective layer of dentin, which in turn is covered with enamel, it might be expected to be quite unresponsive to stimulation; yet, despite the low thermal conductivity of dentin, the pulp is undeniably sensitive to thermal stimuli such an ice cream and hot drinks. The innervation of the pulp includes both afferent neurons, which conduct sensory impulses, and autonomic fibers, which provide neurogenic modulation of the microcirculation and perhaps regulate dentinogenesis.

Nerve fibers are usually classified according to their diameter, conduction velocity, and function. In the pulp, there are two main types of sensory nerve fibers, myelinated A fibers and unmyelinated C fibers. The A fibers include both A-8 and A-5 fibers. A-8 fibers may be slightly more sensitive to stimulation than the A-5 fibers, but functionally these fibers are grouped together. Approximately 90 % of the A fibers are A-8 fibers, and A-8 fiber terminals principally local in region of dentin–pulp junction, and the stimulation threshold of A fibers is relatively low contrast with C fibers which is probably distribute throughout the pulp and feel the pain of burning, aching.

In the human premolar, the number of unmyelinated axons entering the tooth at the apex reached a maximum number shortly after tooth eruption [38]. At this stage, an average of 1800 unmyelinated axons and more than 400 myelinated axons were observed, although in some teeth, fewer than 100 myelinated axons were present. The number of A fibers gradually increased to more than 700 five years after eruption. The relatively late appearance of A fibers in the pulp may help to explain why the electric pulp test tends to be unreliable in young teeth.

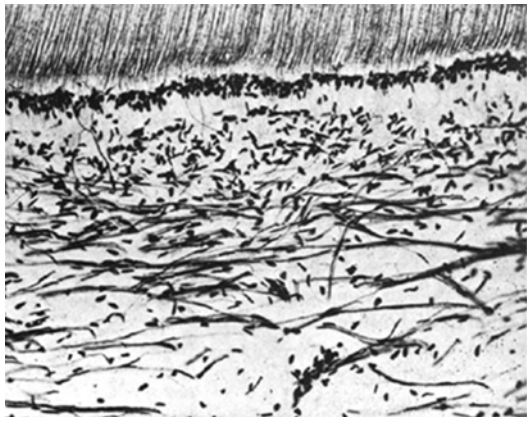

Fig. 1.9 Light microscopy showing the relationship of parietal layer of nerves (plexus of Raschkow) below to the cell-rich, cell-free zones, odontoblasts, and dentin at top of the picture (From Avery [39], p. 208, figure 8)

The nerve bundles pass upward through the radicular pulp together with blood vessels. Once they reach the coronal pulp, they fan out beneath the cell-rich zone, branch into smaller bundles, and finally ramify into a plexus of single-nerve axons known as the plexus of Raschkow (Fig. 1.9) [39]. Full development of this plexus does not occur until the final stages of root formation.

One study showed that a reduction in pulpal blood flow induced by stimulation of sympathetic fibers leading to the pulp results in depressed excitability of pulpal A fibers. The excitability of C fibers is less affected than that of A fibers by a reduction in blood flow. Pulpal nerve fibers contain neuropeptides such as neuropeptide Y, calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), tyrosine hydroxylase, and substance P. The release of these peptides can be trigged by such things as tissue injury, complement activation, antigen-antibody reactions, or antidromic stimulation of the inferior alveolar nerve.

Interestingly, pulpal nerve fibers are relatively resistant to pulp necrosis. This is apparently due to the fact that nerve bundles in general are more resistant to autolysis than other tissue elements. Even in degenerating pulps, C fibers might still be able to respond to stimulation. Furthermore, it may be that C fibers remain excitable even after blood flow has been compromised in the diseased pulp, for C fibers are better able to function in the presence of hypoxia. This may offer an explanation as to why instrumentation of the root canals of apparently nonvital teeth sometimes elicits a moderate level of pain.

1.3.2.3 Connective Tissue Fibers

Two types of structural proteins are found in the pulp: collagen and elastin. Elastin fibers are confined to the walls of the arterioles and, unlike collagen, are not a part of the ECM.

A single collagen molecule, referred to as tropocollagen, consists of three polypeptide chains, designated as either a-1 or a-2 depending on their amino acid composition and sequence. The different combinations and linkages of chains making up the tropocollagen molecule have allowed collagen fibers and fibrils to be classified into a number of types. Type I is found in the skin, tendon, bone, dentin, and pulp. Type II occurs in cartilage. Type III is found in most unmineralized connective tissues, and it is a fetal form found in the dental papilla and the mature pulp. Type IV and VII collagens are components of basement membranes. Type V collagen is a constituent of interstitial tissues. Type I collagen is synthesized by odontoblasts and osteoblasts, and fibroblasts synthesize types I, III, V, and VII.

In collagen synthesis, the protein portion of the molecule is formed by the polyribosomes of the RER of connective tissue cells. The proline and lysine residues of the polypeptide chains are hydroxylated in the cisternae of the RER, and the chains are assembled into a triple-helix configuration in the smooth endoplasmic reticulum. The product of this assembly is termed procollagen, and it has a terminal unit of amino acids known as the telopeptide of the procollagen molecule. When these molecules reach the Golgi complex, they are glycosylated and packaged in secretory vesicles. The vesicles are transported to the plasma membrane and secreted via exocytosis into the extracellular matrix, thus releasing the procollagen. Here the terminal telopeptide is cleaved by a hydrolytic enzyme, and the tropocollagen molecules begin aggregating to form collagen fibrils. It is believed that aggregation of tropocollagen is somehow mediated by the

GAGs. The conversion of soluble collagen into insoluble fibers occurs as a result of cross-linking of tropocollagen molecules.

In the young pulp, small collagen fibers stain black with silver impregnation stains and are thus referred to as argyrophilic fiber. They are very similar, if not identical, to reticular fibers in other loose connective tissues in that they are not arranged in bundles and tend to form delicate networks. The presence of collagen fibers passing from the dentin matrix between odontoblasts into the dental pulp has been reported in fully erupted teeth [40]; these fibers are often referred to as *von Korff fibers*. Large collagen fiber bundles are not argyrophilic but can be demonstrated with special histochemical methods such as the Masson trichrome stain or Mallory's triple connective tissue stain. These fibers are much more numerous in the radicular pulp than in the coronal pulp. The highest concentration of these larger fiber bundles is usually found in the radicular pulp near the apex.

1.3.2.4 Ground Substance

Connective tissue is a system consisting of cells and fibers, both embedded in the pervading ground substance. Cells that produce connective tissue fibers also synthesize the major constituents of ground substance. The term extracellular matrix ECM is used to describe ground substance, regarding it as the material into which fibers are deposited. Because of its content of polyelectric polysaccharides, the ECM is responsible for the water-holding properties of connective tissues.

Nearly all proteins of the ECM are glycoproteins. Proteoglycans are an important subclass of glycoproteins. These molecules support cells, provide tissue turgor, and mediate a variety of cell interactions. They have in common the presence of GAG chains and a protein core to which the chains are linked. Except for heparan sulfate and heparin, the chains are composed of disaccharides. The primary function of GAG chains is to act as adhesive molecules that can bond to cell surfaces and other matrix molecules.

Fibronectin is a major surface glycoprotein that, together with collagen, forms an integrated fibrillary network that influences adhesion, motility, growth, and differentiation of cells. Laminin, an important component of basement membranes, binds to type IV collagen and cell surface receptors. Tenascin is another substrate adhesion glycoprotein.

Degradation of ground substance can occur in certain inflammatory lesions in which there is a high concentration of lysosomal enzymes. Proteolytic enzymes, hyaluronidases, and chondroitin sulfatases of lysosomal and bacterial origin are examples of acid hydrolytic enzymes that can attract components of the ground substance. The pathways of inflammation and infection are influenced by the state of polymerization of the ground substance components.

1.3.2.5 Lymphatics

The existence of lymphatics in the pulp has been a matter of debate, since it is not easy to distinguish between venules and lymphatics by ordinary light microscopic techniques, although some studies utilizing light and electron microscopy have described lymphatic capillaries in human and in cat dental pulps.

1.3.2.6 Accessory Canals

Occasionally during formation of the root sheath, a break develops in the continuity of the sheath, producing a small gap. When this occurs, dentinogenesis does not take place opposite to the defect. The result is a small "accessory" canal between the dental sac and the pulp. An accessory canal can become established anywhere along the root, thus creating a periodontal–endodontic pathway of communication and a possible portal of entry into the pulp if the periodontal tissues lose their integrity. In periodontal disease, the development of a periodontal pocket may expose an accessory canal and thus allow microorganisms or their metabolic products to gain access to the pulp.

1.3.2.7 Morphologic Zones of Pulp

Odontoblast Layer The outermost stratum of cells of the healthy pulp is the odontoblast layer. This layer is located immediately subjacent to the predentin, with the odontoblast processes passing on through the predentin into the dentin. Consequently, the odontoblast layer is actually

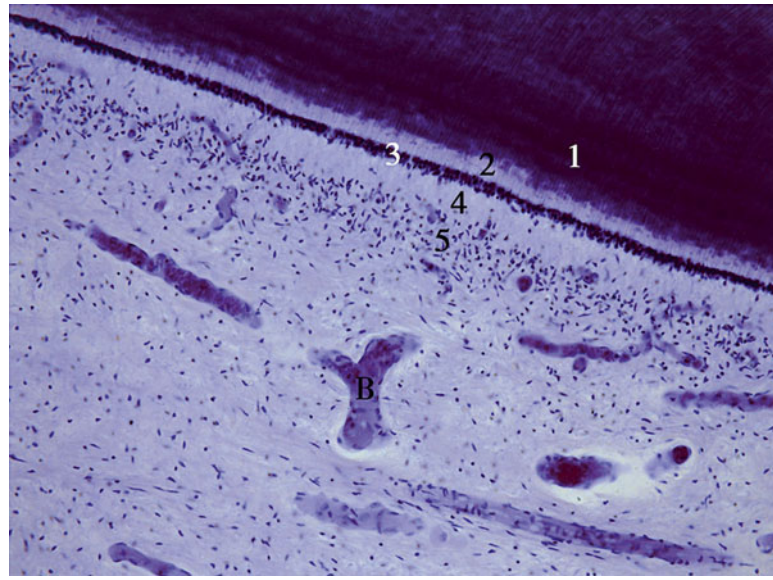

Fig. 1.10 Beside dentin (*1*) and predentin (*2*), there are tall columnar odontoblasts (*3*) of the coronal pulp. Note the presence of the cell-rich zone (*5*) and cell-poor zone (*4*)

composed of the cell bodies of the odontoblasts. Additionally, capillaries, nerve fibers, and dendritic cells may be found among the odontoblasts.

The odontoblast layer in the coronal pulp contains more cells per unit area than in the radicular pulp. Whereas the odontoblasts of the mature coronal pulp are usually columnar (Fig. 1.10), those in the midportion of the radicular pulp are more cuboidal. Near the apical foramen, the odontoblasts appear as a flattened cell layer. Since there are fewer dentinal tubules per unit area in the root than in the crown of the tooth, the odontoblast cell bodies are less crowded and are able to spread out laterally.

Cell-Poor Zone Immediately subjacent to the odontoblast layer in the coronal pulp, there is often a narrow zone approximately 40 μm in width that is relatively free of cells. It is traversed by blood capillaries, unmyelinated nerve fibers, and the slender cytoplasmic processes of fibroblasts. The presence or absence of the cell-poor zone depends on the functional status of the pulp. It may not be apparent in young pulps where dentin forms rapidly or in older pulps where reparative dentin is being produced.

Cell-Rich Zone Usually conspicuous in the subodontoblastic area is a stratum containing a relatively high proportion of fibroblasts compared with the more central region in the pulp. It is much more prominent in the coronal pulp than in the radicular pulp. Besides fibroblasts, the cell-rich zone may include a variable number of macrophages and lymphocytes.

On this evidence obtained in rat molar teeth, it has been suggested that the cell-rich zone forms as a result of peripheral migration of cells populating the central regions of the pulp, commencing at about the time of tooth eruption. Although cell division within the cell-rich zone is a rare occurrence in normal pulps, death of odontoblasts causes a great increase in the rate of mitosis. Since irreversibly injured odontoblasts are replaced by cells that migrate from the cell-rich zone onto the inner surface of the dentin, this motility ability is probably the first step in the formation of a new odontoblast.

Pulp Proper The pulp proper is the central mass of the pulp. It contains the larger blood vessels and nerves. The connective tissue cells in this zone are fibroblasts or pulpal cells.

1.3.3 Cells in the Dental Pulp

1.3.3.1 Odontoblast

Because it is responsible for dentinogenesis both during tooth development and in the mature

tooth, the odontoblast is the most characteristic cell of the pulpodentin complex. During dentinogenesis, the odontoblasts form the dentinal tubules, and their presence within the tubules makes dentin a living tissue [41].

Differentiation of epithelial and mesenchymal cells into ameloblasts and odontoblasts, respectively, occurs during the bell stage of tooth development [42]. The preameloblasts differentiate at a faster rate than the corresponding odontoblasts so that at any given level, mature ameloblasts appear before the odontoblasts have fully matured. In spite of this difference in rate of maturation, dentin matrix is formed before enamel matrix. As the ameloblasts undergo differentiation, changes are happening across the basement membrane in the adjacent dental papilla. Before differentiation of odontoblasts, the dental papilla consists of sparsely distributed polymorphic mesenchymal cells with wide intercellular spaces. With the onset of differentiation a single layer of cells, the presumptive odontoblasts (preodontoblasts) align themselves along the basement membrane separating the inner enamel epithelium from the dental papilla. These cells stop dividing and elongate into short columnar cells with basally situated nuclei (Fig. 1.7). Several cytoplasmic projections from each of these cells extend toward the basal lamina. At this stage, the preodontoblasts are still relatively undifferentiated.

Dentinogenesis first occurs in the developing tooth at sites where the cusp tips or incisal edge will be formed. It is in this region that odontoblasts reach full maturity and become tall columnar cells. The production of the first dentin matrix involves the formation, organization, and maturation of collagen fibrils and proteoglycans. As more collagen fibrils accumulate subject to the basal lamina, the lamina becomes discontinuous and eventually disappears. This occurs as the collagen fibers become organized and extend into the spaces between the ameloblast processes. Concurrently the odontoblasts extend several small processes toward the ameloblasts. Some of these become interposed between the processes of ameloblasts, resulting in the formation of enamel spindles (dentinal tubules that extend into the enamel). Membrane-bound vesicles bud off from the odontoblast processes and become interspersed among the collagen fibers of the dentin matrix. These vesicles subsequently play an important role in the initiation of mineralization. With the onset of dentinogenesis, the dental papilla becomes the dental pulp.

As predentin matrix is formed, the odontoblasts commence to move away toward the central pulp, depositing matrix at a rate of approximately 4–8 μm per day in their wake. Within this matrix, a process from each odontoblast becomes accentuated and remains to form the primary odontoblast process. It is around these processes that the dentinal tubules are formed.

Dentinal tubule forms around each of the major odontoblastic processes which occupy space within the tubule and somehow mediates the formation of peritubular dentin. Microtubules and microfilaments are the principal ultrastructural components of the odontoblast process and its lateral branches. Microtubules extend from the cell body out into the process. These straight structures follow a course that is parallel with the long axis of the cell and impart the impression of rigidity. Although their precise role is unknown, theories as to their functional significance suggest that they may be involved in cytoplasmic extension, transport of materials, or simply the provision of a structural framework. The plasma membrane of the odontoblast process closely approximates the wall of the dentinal tubule. Localized constrictions in the process occasionally produce relatively large spaces between the tubule wall and the process. Such spaces may contain collagen fibrils and fine granular material, which presumably represents ground substance. The peritubular dentin matrix lining the tubule is circumscribed by an electron-dense limiting membrane.

The odontoblast is considered to be fixed postmitotic cell in that once it has fully differentiated, it apparently cannot undergo further cell division. If this is indeed the case, the life-span of the odontoblast coincides with the life-span of the viable pulp.

Apparently the odontoblast synthesizes mainly type I collagen, although small amounts of type V collagen have been found in the ECM. In addition

to proteoglycans and collagen, the odontoblast secretes phosphophoryn, a phosphoprotein involved in extracellular mineralization. Phosphophoryn is unique to dentin and is not found in any other mesenchymal cell lines. The odontoblast also secretes alkaline phosphatase, an enzyme that is closely linked to mineralization but whose precise role is yet to be illuminated.

In contrast to the active odontoblast, the resting or injured odontoblast has a decreased number of organelles and may become progressively shorter. These changes can begin with the completion of root development.

1.3.3.2 Pulp Fibroblast

The most numerous cells of the pulp tissues appear to be tissue-specific cells capable of giving rise to cells that are committed to differentiation as odontoblasts, given the proper signal. These cells synthesize type I and III collagen as well as proteoglycans and GAGs; since they degrade collagen, they are also responsible for collagen turnover in the pulp.

Although distributed throughout the pulp, fibroblasts are particularly abundant in the cell-rich zone. The early differentiating fibroblasts are polygonal and appear to be widely separated and evenly distributed within the ground substance. Cell-to-cell contacts are established between the multiple processes that extend out from each of the cells. Many of these contacts take the form of gap junctions, which provide for electronic coupling of one cell to another. As they mature, the cells become stellate in form and the Golgi complex enlarges, the RER proliferates, secretory vesicles appear, and the fibroblasts take on the characteristic appearance of protein-secreting cells. Along the outer surface of the cell body, collagen fibrils commence to appear. With an increase in the number of blood vessels, nerves, and fibers, there is a relative decrease in the number of fibroblasts in the pulp.

A colleague once remarked that the fibroblasts of the pulp are very much like Peter Pan in that they never grow up. There may be an element of truth in this statement, for these cells do seem to remain in a relatively undifferentiated modality as compared to fibroblasts of most other connective tissues. This perception has been fortified by the observation of large numbers of reticulin-like fibers in the pulp. However, it has been concluded that because of distinct histochemical differences, reticulin fibers, such as most of gingiva and lymphoid organ, are not present in the pulp. In the young pulp, the nonargyrophilic collagen fibers are sparse, but they progressively increase in number as the pulp ages.

Many experimental models have been developed to study wound healing in the pulp, particularly dentinal bridge formation following pulp exposure or pulpotomy. One study demonstrated that mitotic activity preceding the differentiation of replacement odontoblasts appears to occur primarily among fibroblasts. Thus, it appears that pulpal fibroblasts can be regarded as odontoprogenitor cells.

1.3.3.3 Macrophage

Tissue macrophage or histiocytes are monocytes that have left the bloodstream, entered the tissues, and differentiated into macrophages. They are usually found in close proximity to blood vessels. These cells are quite active in endocytosis and phagocytosis. Because of their mobility and phagocytic activity, they are able to act as scavengers, removing extravasated red blood cells, dead cells, and foreign bodies from the tissue ingested material destroyed by the action of lysosomal enzymes.

In addition, a proportion of macrophages, when primed by cytokines, participate in immune reactions by processing antigen and presenting it to lymphocytes. The processed antigen is bound to class II histocompatibility antigens on the macrophage, where it can interact with specific receptors present on immunocompetent T cells. Such interaction is obligatory for the induction of cell-mediated immunity. When activated by the appropriate inflammatory stimuli, macrophages are capable of producing a large variety of soluble factors including interleukin-1, tumor necrosis factor, growth factors, and other cytokines.

1.3.3.4 Dendritic Cell

Dendritic cells, like macrophages, are cells of the immune system. Similar cells are found in the

epidermis and mucous membranes, where they are called *Langerhans cells*. Dendritic cells are primarily found in lymphoid tissues, but they are also widely distributed in connective tissues, including the pulp. These cells are termed antigen-presenting cells and are characterized by dendritic cytoplasmic processes and the presence of cell surface class II antigens. Like macrophages, dendritic cells phagocytose and process antigens but are otherwise only weakly phagocytic. Together with macrophages and lymphocytes, dendritic cells are believed to participate in immunosurveillance in the pulp.

1.3.3.5 Lymphocyte

T lymphocytes and B lymphocytes were found in normal pulps from the human teeth. T_8 (suppressor) lymphocytes were the predominant T lymphocyte subset present in pulps. Lymphocytes have also been observed in the pulps of impacted teeth. The presence of macrophages, dendritic cells, and T lymphocytes indicates that the pulp is well equipped with cells required for the initiation of immune responses.

1.3.3.6 Mesenchymal Cell

Mesenchymal stem cells (MSCs) are found in many adult tissues and organs. In tissues that grow continuously or exhibit high rates of cell turnover, MSCs provide a renewable source of progenitor cells to differentiate and replace those lost. In the majority of tissues, MSCs are found in small numbers and remain quiescent until mobilized in response to tissue damage. In pulp tissue, some authors hold the opinion that primordial mesenchymal cells persist in adult pulp tissues as "undifferentiated" mesenchymal cells. However, during wound healing, well-differentiated fibroblasts undergo rapid serial division to give rise to new fibroblasts. Similarly, replacement odontoblasts are derived from mature fibroblasts. Consequently, there is no need to postulate that in the pulp new mesenchymal cells arise from cells other than pulpal fibroblasts. This controversy is still unclear and needs more researches.

1.3.3.7 Mast Cell

Mast cells are widely distributed in the connective tissues, where they occur in small groups in relation to blood vessels. This cell has been the subject of considerable attention because of its dramatic role in inflammatory reactive. But mast cells also present in the normal pulp tissue, although they are routinely found in chronically inflamed pulps.

1.4 Root Development

Root development is one of the important stages in tooth development process, following the bell stage. The interaction between dental epithelial and neural crest-derived mesenchymal cells is essential for tooth development.

1.4.1 The Initiation of Tooth Root Development

After the crown formation is nearly complete, the tooth root begins to develop. Then, the special bilayered epithelial sheath is formed from the outer and inner enamel epithelium at the neck ring of the crown and grows in the apical direction, termed Hertwig's epithelial root sheath (HERS). HERS, dental papilla cells, and dental follicle cells form the organ primordium of tooth development together [43]

HERS is the morphogenesis signal of the initiation of tooth root development [44]. Morphologically, the epithelial root sheath is located between the two regions of neural crest-derived mesenchyme: the dental papilla and the dental follicle. When the HERS grows apically, the dental papilla cells adjacent to the inner epithelial layer of the HERS and the epithelial basement membrane induced to become odontoblasts and later to form root dentin. After root dentin formation, the epithelial root sheath enveloping the root begins to be interrupted or perforated. The formation of a mesh-like structure in the HERS allows dental follicle cells to contact the newly formed root dentin surface through the epithelial root sheath. These dental follicle cells differentiate into cementoblasts to form cementum. In addition, some of the HERS cells undergo epithelial–mesenchymal transition to become

cementoblasts and form cementum [13]. At the same time, collagen fibers secreted by dental follicle cells are embedded into the new cementum matrix and fix the root in the jaw bone. Following tooth root development and elongation, the tooth erupts into the oral cavity to establish occlusal contacts with the opposing teeth and performs its physiological function.

Previous studies have shown that Hertwig's epithelial root sheath plays an important role in root development, but the fate of HERS has remained unknown. Until now, the fate of HERS and its function are not clear. At least 6 possible outcomes of HERS have been proposed: (1) epithelial cell rests of Malassez, (2) apoptosis, (3) incorporation into the advancing cementum front, (4) epithelial–mesenchymal transformation, (5) migration toward the periodontal ligament, and (6) differentiation into cementoblasts [10].

1.4.1.1 Epithelial Cell Rests of Malassez (ERM)

Epithelial cell rests of Malassez (ERM) are involved in the maintenance and homeostasis of the periodontal ligament. The ERM have a number of functions, such as to prevent root resorption, induce cementum formation, and maintain the homeostasis of the PDL [45]. On the other hand, ERM can be stimulated to proliferate in response to injury in rats. When stimulated by inflammatory cytokines, ERM can proliferate and differentiate into the lining epithelium of periapical cysts.

Primary HERS/ERM cells had typical epithelial cell morphology and characteristics, and they maintained for more than five passages. They expressed epithelial stem cell-related genes: ABCG2, ΔNp63, p75, EpCAM, and Bmi-1. Moreover, the expression of embryonic stem cell markers such as Oct-4, Nanog, and SSEA-4 was detected [46]

1.4.1.2 Induction of Differentiation of Mesenchymal Stem Cells

HERS, morphologically, is a structural boundary of two dental ectomesenchymal tissues: dental papilla and dental follicle [47]. It breaks up into epithelial rests and cords, allowing other cells to come in contact with the outer dentin surface. This sandwich structure plays at least two important roles during root formation: biomineralization (cementogenesis and dentin formation) and induction of root organization.

HERS cells may be involved in regulating differentiation of periodontal ligament stem cells (PDLSCs) and forming cementum in vivo [48]. Dental follicle cell sheets induced by HERS cells are able to produce periodontal tissues through epithelial–mesenchymal interactions.

HERS cells are detectable on the surface of the root throughout root formation and do not disappear. Most of the HERS cells are attached to the surface of the cementum, and others separate to become the epithelial rest of Malassez. HERS cells secrete extracellular matrix components onto the surface of the dentin before dental follicle cells penetrate the HERS network to contact dentin. HERS cells also participate in the cementum development and may differentiate into cementocytes.

1.4.2 Related Signaling Pathway of Root Morphogenesis

Recently, several genes were found to play crucial role in the process of root development.

1.4.2.1 TGF-β/BMP Signaling

The TGF-β superfamily of cytokines is composed of TGF-β, BMPs, activins, and related proteins. TGF-β signaling plays an important role in developmental biology, disease, and regeneration [49].

Smad4 is a central mediator of the canonical TGF-β signaling pathway. Deletion of Smad4 results in the blockage of TGF-β/BMP signaling. Ablation of Smad4 in the dental mesenchyme (Osr2-IresCre:Smad4$^{fl/fl}$ mice) results in short root formation and defects in odontoblast differentiation and dentin formation. Moreover, ectopic bone-like structures replaced normal dentin in the teeth of Osr2-IresCre:Smad4$^{fl/fl}$ mutant mice. Loss of Smad4 results in the upregulation of canonical Wnt signaling and downregulation of Dkk1 and Sfrp1, which are Wnt pathway inhibitors.

Comparing different animal models provides more detail about TGF-β signaling during root dentin formation. In Osr2-IresCre:Smad4$^{fl/fl}$ mice, dental mesenchyme differentiation is arrested at the late bell stage and secretory stage, with no detectable expression of Dspp. Odontoblast differentiation is delayed and Dspp expression is eventually detectable in mice lacking Tgfbr2 [50].

Bone morphogenetic protein 4 (BMP4) is secreted by mesenchymal cells, acting on the dental epithelium as a regulator of cell differentiation during crown formation. Studies assessed the localizations of BMP4, BMP4 receptor (BMPR-1B), and BMPR-2 during molar root formation in mouse [51]. In K14-Noggin transgenic mice, molar epithelial root sheath cell proliferation is abated, and root development is retarded [52].

In the early development of mouse molar root, mesenchymal progenitor cell markers such as STRO-1 with BMP receptors are expressed in the dental follicle, hence the hypothesis that mesenchymal STRO-1-positive cells are epithelial root sheath BMP signaling pathways of target cells.

1.4.2.2 SHH Signaling

Shh, a member of the hedgehog signaling family, is expressed in the dental epithelium and plays an essential role during tooth development. During root development, Shh is strongly expressed in the HERS, which suggests a function in root formation. Gli1, a transcript activated by Shh, is also detectable in the root epithelium (HERS) and mesenchyme [53].

1.4.2.3 Wnt Signaling

The Wnt family of proteins plays an important role in morphogenesis and cellular differentiation in many tissues. The canonical Wnt signaling pathway involves the stabilization and nuclear accumulation of β-catenin, which activates the expression of Wnt target genes. It is well known that Wnt/β-catenin signaling plays multiple roles in various stages of tooth morphogenesis [54]. During dentin formation, Wnt10a is expressed in odontoblast-lineage cells, and Axin2 is also expressed in developing odontoblasts and dental pulp cells. In addition, Dkk1, an inhibitor of Wnt signaling, is strongly expressed in preodontoblasts but is decreased in secretory odontoblasts. Moreover, it was recently reported that Lef-1 overexpression accelerates odontoblast differentiation of dental pulp cells and constitutive β-catenin stabilization in the dental mesenchyme can lead to excessive dentin formation. β-catenin has been shown to be strongly expressed in odontoblast-lineage cells and is required for root formation. Tissue-specific inactivation of β-catenin in developing odontoblasts produced molars lacking roots and aberrantly thin incisors. These reports strongly suggest that modulation of Wnt/β-catenin signaling may play an important role in odontoblast differentiation.

1.4.2.4 Notch Signaling

The Notch pathway regulates the renewal and fate decisions of stem cells in multiple tissues. Notch1 and Notch2 as well as the Notch target gene Hes1 are expressed in the putative stem cells in the continuously growing mouse incisors. Notch signaling is required for epithelial stem cell survival and enamel formation in the continuously growing mouse incisor [55].

At embryonic day 19, the molar and the incisor of rat began differentially developing: the molar formed double-layered cells of the root sheath while the incisor formed a cervical loop. By using the subtractive hybridization method, a subtraction cDNA library of the rat molar and incisor tissues was successfully constructed. Differentially expressed gene clones were evaluated by dot blot and sequencing. Sel1l, Nfic, Edar, GATA6, and some novel genes were found differentially expressed, which were strongly related to the tooth root patterning. The Notch signaling contributes to the maintenance of cervical loop stem cell niches. SEL1L, the negative regulating factor of notch signaling, was strongly expressed in the molars but weakly expressed in the incisors.

1.4.3 Tooth Eruption

Tooth eruption is the movement of the tooth from the original developmental site to the functional

position through the alveolar bone and gingiva in the mouth. It is a continuous process, which can be divided into 5 stages, respectively for pre-eruptive movement, intraosseous eruption, mucosal penetration, preocclusal eruption, and post-occlusal eruption. Each step involves intense reciprocal interactions between the tooth and its surrounding tissues and is temporally and spatially controlled to coordinate the growth of the jaw and the position of other teeth. However, the specific cellular, molecular, and genetic mechanisms governing tooth eruption remain unclear.

Until now, there are several theories explaining how the tooth erupts [56]. One of the most acceptable theories is that asymmetric bone remodeling around the tooth is responsible for the teeth moving into the oral cavity. When bone resorbed on the coronal side, bone formed on the basal side of the tooth. It is thought that the dental follicle, which is a loose connective tissue sac surrounding the enamel organ of each tooth, plays a critical role in regulating osteoclasts and osteoblasts during this process. In 1980s, it was firstly shown that the removal of the dental follicle from developing premolars of dogs prevented tooth eruption. Meanwhile, if the tooth germ was removed and replace with another artificial tooth, which the dental follicle was kept intact in situ, the artificial tooth still erupted on schedule. Furthermore, removal of either the coronal or basal halves of the dental follicle also prevented tooth eruption, with the coronal part controlling bone resorption and the eruption pathway, as well as the basal part causing bone formation. Recently, some important signaling molecules were identified at both these processes for tooth eruption, such as RANKL, BMP2, and so on. These studies provided convincing evidence that the dental follicle is essential for tooth eruption and also challenged the previously supposed requirement of dental pulp, periodontal ligaments, and roots in the process of tooth eruption.

For a long time, root development has been considered as the main force responsible for the tooth eruption. However, more and more studies found that rootless tooth can still erupt into the oral cavity in many species, including human, dogs, monkeys, and rodents. In some patients with special diseases, which may affect root development, the rootless tooth crown did erupt into the mouth. Developing premolar teeth with the removal of roots in dogs can erupt into oral cavity at normal speed, and the void created by the absence of roots during eruption was filled with the alveolar bone. All of these indicated that root formation is not required for tooth eruption.

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Biofilm and Dental Caries

2

Xu Xin, Zhou Yuan, Shi Wenyuan, Liu Yaling, and Zhou Xuedong

2.1 Dental Plaque and Microbial Biofilm

2.1.1 Bacterial Biofilm: An Advanced Mode of Life

Bacteria grow in two different ways: planktonic and biofilm forms. Decades ago, most microbiologists characterized and studied planktonic bacteria in liquid culture in which bacteria were floating free cells. In this period, plenty of fundamental work in microbiology was accomplished [7]. However, it is estimated that only less than 0.1 % of the microbial consortia in natural environment grow in planktonic state [40]. Then how do the most microbes live and grow? From the colorful microbial mats in Yellowstone geothermal area to the sophisticatedly structured microbial community on tooth surfaces, we have the answer.

2.1.1.1 The Concept and Discovery of Biofilm

Biofilm is defined as aggregates of bacterial cells attached to a surface and embedded in a polymeric matrix that is self-produced and helps the community to gain tolerance against antimicrobials and host defenses. Usually biofilm cells are adhered to a surface, for example, catheter or tooth surfaces. With the extensive biofilm studies, the surface attachment feature is not indispensable for biofilm recognition now [15].

The first observation of bacteria on surfaces was made by Antonie van Leeuwenhoek in 1684 [15]. He observed various types of the numerous "animalcules" in the tartar taken from his own teeth directly by microscope. He also found the preliminary evidence of antimicrobial tolerance of biofilm from the fact that the surface-attached cells on his teeth could survive after rinsing with vinegar, while cells removed from teeth could be killed by vinegar [146]. At that time, the knowledge and techniques in microbiology were too limited to understand the little communities on surfaces. Recently with molecular and bioinformatics techniques, the characterization of microbial growth and development in complex communities on surfaces have been highly promoted [82].

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2.1.1.2 Extracellular Polymeric Substances of Biofilm

The structure of biofilm is highly varied depending on the growth environment and component cells. In addition to the bacterial aggregates, another major component, the structural backbone of biofilm, is the extracellular matrix composed of self-secreted extracellular polymer substances (EPS) [55]. EPS is a highly diverse biopolymers mixture, including mainly polysaccharides, proteins, lipids, nucleic acids, and other substances from the environment or host (Fig. 2.1) [62]. As the immediate microenvironment surrounding cells, EPS serves various functions through the entire life cycle of biofilm. The parameters of EPS include molecular concentration, charge condition, quantity, hydrated level, components, and so on. These factors, varying because of the embedded cells, stages of biofilm, nutrient sources, and local environment/host, are essential for EPS functions.

Mechanically, EPS provides architectural stability for the biofilm entity, helps bacterial cells adhering to surfaces, immobilizes cells onto local substratum, and finally forms a three-dimensional network that supports the biofilm cells. Metabolically, at the extracellular retention space, EPS helps the external substances such as oxygen and carbon source to diffuse into the biofilm, as well as the internal metabolic waste moving out. From the multicellular and polymicrobial features of biofilm, EPS is also an external metabolism system containing free enzymes, metabolites, extracellular DNA (eDNA), and other substances from cell lysis. Therefore, within the matrix, gene horizontal transfer through eDNA, mutualism or antagonism interactions, and nutrient accumulation are active [62, 212]. By supporting vertical extension of biofilm, EPS also reduces the biomass density to buffer impact from external substances. Moreover, EPS can protect the microbial consortia from multiple external challenges, such as antibiotics, and allow the long-term existence of biofilm community. EPS not only provides a structural framework for biofilm but also keeps the homeostasis of microenvironment. This relative stable and interactive space offers chances for bacteria to survive and even persist in cruel conditions.

2.1.1.3 Biofilm Formation

The life cycle of surface-associated biofilm is well studied in in vitro models. The process is promoted by all different members in the community, regulated by associated genetic networks, and also influenced by the environment. Different standpoints are taken in recent study models of biofilm development, such as developmental genetic regulation network, environmental adaptation, and multispecies interactions.

The most accepted model describes the biofilm formation as a highly regulated spatiotemporal process. The formation of biofilm is generally summarized into five dynamic stages as (1) loose attachment of planktonic cells, (2) irreversible adherence, (3) microcolony formation, (4) mature

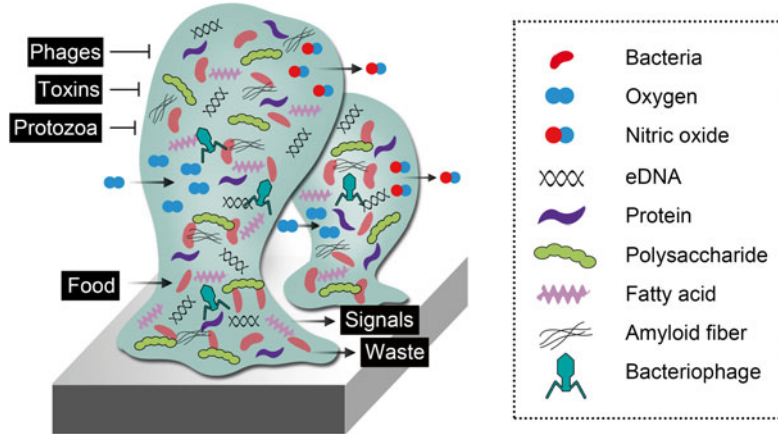

Fig. 2.1 The composition of extracellular polymeric substances of bacterial biofilms. Bacteria in the mature biofilm are embedded in an EPS matrix, which consists of polysaccharides, proteins, extracellular DNA (eDNA), amyloid fibers and bacteriophages, etc.

macrocolony formation, and (5) dispersal. The developmental model of biofilm focuses on the regulated transition through the cascade and emphasizes genetic pathway involvement in the group level development. Recent studies on the proteomics and genetic network of gram-negative bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilm have indicated that the developmental process of biofilm is sequentially regulated [181, 196]. *P. aeruginosa* biofilm is one of the earliest and most studied in medical microbiology field [114]. Stages 3 and 4 of its biofilm formation involve type 4 pili-mediated mechanism [109]. The general dynamic processes can be identified in most species, whereas distinct mechanisms reside in different bacteria, even for strains from the same species. For example, LapA from the biofilm-associated-protein (Bap) family is important in adhesion and transition from reversible to irreversible attachment in *P. fluorescens* and *P. putida*; conversely, no evidence indicates the involvement of Bap in the *P. aeruginosa* biofilm formation [57, 159].

Environmental impact is crucial to biofilm formation. Therefore, environmental adaptation model is employed to describe the biofilm formation. The environmental adaptation model suggests that the phenotypic or species heterogeneity within the biofilm is the response to the spatiotemporal change in the ecological niche. For instance, oxygen and carbon gradient is one of the driving forces for biofilm formation. Here the developmental stages can be sketched as (1) planktonic cells attachment; (2) initial seed cells growth with sufficient nutrients; (3) formation of microcolonies where outer cell layers restrain the nutrient availability of inner layers, thereafter gradient and heterogeneity showing within each colonies; (4) formation of macrocolonies, where some cells express high level of EPS and facilitate the vertical growth of community, resulting in the improvement of nutrient and oxygen distribution; (5) biofilm formation from mature macrocolonies, where structures like pores and channels form to further assist inner nutrient diffusion [159]. This model describes the changes in population and structure from the perspective of metabolism. The genes that regulate the environmental adaptation should also be included in the biofilm-specific gene network as described above.

In nature, a great portion of biofilm is composed of diverse species. This polymicrobial feature makes interspecies interaction an important factor and variable in biofilm formation. At the early stages of biofilm formation, distribution and roles of different bacteria and/or fungi depend on various factors, such as metabolic requirement, surface structures, mutualism, and quorum sensing regulatory system. For example, metabolic cooperation exists between oral commensal *Streptococcus gordonii* and pathogen *Aggregatibacter actinomycetemcomitans*. Peroxide produced by *S. gordonii* can be utilized by *A. actinomycetemcomitans* for further secretion of complement-resistant protein ApiA that can limit host complement-mediated killing [229].

So far, we still have a lot to ask about the mechanisms of biofilm formation, especially for that of in vivo biofilm. There is a long way to go to find out whether the discoveries from in vitro models are consistent with in vivo phenotypes.

2.1.1.4 Survival Advantages of Biofilm

The biofilm form of growth with its surface association offers bacteria remarkable advantages to survive and thrive in harsh environment compared to the planktonic form. Several hypotheses on how biofilm gains beneficial phenotypes and succeeds have been proposed. First, the underlying surface offers a relatively stable space within the environment and also a substratum for microbes to grow on, facilitating further communities building, nutrient accumulation, and interaction of cells in proximity. Second, microbial communities in biofilm mode gain protection and higher buffer ability against various challenges from the environment, such as ultraviolet exposure [56], metal toxicity [201], fluctuating pH [230], desiccation [231], phagocytosis [125], and antibiotics and antimicrobial agents [69, 137, 195].

The biofilm mode of survival has profound importance for prokaryotes in both the natural environment and human body, even being recognized as the most successful forms of life on the

earth. In environmental microbiology, biofilm fossil records from as early as 3.2 billion years ago were identified [82]. From the standpoint of evolution, we can speculate that biofilm as an advantageous form of growth offered space with homeostasis for the primitive prokaryotes in the rigid and ever-changing environment. Thus, nowadays, biofilms can be found almost everywhere even in extreme conditions, such as hot spring, deep-sea vents, and so on [15].

This survival advantage exists not only in the nature but also on and in our body. With the development of human microbiome project (HMP), we have recognized that we are living in close relationship with microbes, which are termed "the microbial part of ourselves" [228]. Human microbiome has several major components including the oral, skin, vaginal, gut, and nasal/lung microbiome, affecting the homeostasis of health and disease. Like microbes in nature, human microflora usually exists as sessile aggregates of cells with or without a surface. From thousands of recent in vitro and in vivo biofilm studies, we can summarize that biofilm is closely associated with chronic and persistent infections. Because bacterial biofilm is almost 1000-fold more tolerant to certain antibiotics compared to the planktonic state, the persistence of biofilm infection is most likely resulted from this important feature [15, 58].

2.1.2 Dental Plaque as a Typical Bacterial Biofilm

In 1886, Black first described the accumulated bacteria on early carious lesions as plaque [18]. In oral cavity, tooth as mineralized hard tissue provides non-shedding surfaces for oral microbes to colonize. Attached bacteria, embedded in polymeric matrix, form microcommunities called dental plaque. In recent years, dental plaque as a typical biofilm has been studied extensively. As a naturally formed biofilm, dental plaque acts actively as part of the host defense system against colonization of exogenous pathogenic microflora [145]. On the other hand, when the homeostasis of dental plaque is disrupted, biofilm-associated diseases like caries and periodontal diseases develop.

Dental plaque was the first biofilm being investigated at both composition and antimicrobial sensitivity aspects. With the help of molecular and bioinformatics techniques, more than 800 phylotypes of bacteria in the oral microbiome, including more than 300 species, have been identified [193]. The diverse and numerous residents make dental plaque a highly complex and varied community. Furthermore, a limited number of fungi and viruses are also found in oral cavity. Notably, more than half of oral bacteria cannot grow in pure culture in vitro, largely limiting our investigation on their physiological characteristics [111].

The tooth surfaces above gingival margin are mainly enamel or dentin, where they directly face the dynamic oral environment, whereas the surfaces below the gingival margin are mainly dentin or cementum, which are in close contact with gingival tissue and relatively less fluctuant. According to the location of the attached surfaces, dental plaque can be roughly divided into two categories: supragingival plaque and subgingival plaque. Each type has its own special physiological characteristics and relation to disparate diseases.

Oral cavity is a unique ecological niche, which is warm, moist and relatively opens to the outer environment. Tooth surfaces as well as dental plaque constantly encounter different challenges from food intake, speech, oral hygiene procedures, and so on. The oxygen and nutrient levels are changing constantly at different sites. For example, the oxygen level varies, from being anaerobic in subgingival plaque and basal layers of supragingival plaque to being almost aerobic in outer layers of supragingival plaque [142]. The local microbial diversity and metabolism also change enormously from health to disease. Apart from some specific pathogen-induced diseases, most oral diseases are caused by or associated with long-term multispecies microbial activity within dental plaque [111]. Here, we will take supragingival biofilm, for example, to demonstrate the association between dental plaque and caries.

2.1.3 Composition of Dental Plaque

Like other biofilms, dental plaque contains two major components: microbes and the biopolymer matrix. The bacterial species varies significantly at distinct surfaces, such as pits/fissures, smooth surfaces, approximal surfaces, and gingival crevice. The habitats are selective for the preferable bacteria that are able to grow, based on the different anatomy and biological features in the local environment [1, 145, 169]. The studies of microbial composition of biofilm have been shifting from culture-based to molecular approaches. Through 16 s rRNA gene-based sequencing, a plethora of new taxa have been discovered, although their physiological characteristics are largely unknown. In normal microflora on fissures and smooth surfaces, the predominant species are mostly gram-positive aerobic or facultative anaerobic bacteria, mainly streptococci. Other common species include *Gemella*, *Granulicatella*, *Actinomyces*, *Rothia*, *Veillonella*, *Neisseria*, *Prevotella*, *Abiotrophia*, *Capnocytophaga*, *Fusobacterium*, and so on [1]. In contrast, the subgingival species/phylotypes are more diverse, from facultative anaerobic to obligate anaerobic. In a locus with features of both supra- and subgingival surfaces, the approximal plaque had an intermediate microbial constitution [146]. The dental microflora is highly varied from person to person, depending on the complex genetic and environmental variations in population. Once established, the personal microbial community stays stable and in a dynamic balance (or homeostasis). This fluctuant balance is achieved by heterogeneous microflora from both metabolic mutualism and antagonism within the community. Staying in a relatively open niche, the resident community is under constant challenges or invasions. When the habitats change greatly and affect local microbial activities, the homeostasis will shift, and a new microbial composition may be established to fit new environmental conditions.

The biochemical constitution of dental plaque varies for different hosts and bacterial composition. Dental plaque is highly hydrated and contains about 80 % water. Changing with time before and after food intake, the glucan takes 10–20 % of plaque dry weight, while fructan takes 1–2 %. Protein comprises approximately 40 % of fry weight, which is usually from bacterial secretion and saliva. In addition, lipid, nucleic acid, and minerals like calcium, magnesium, phosphate, and fluoride are in varying amounts in plaque [39, 94, 167, 171].

The part of plaque without bacteria is the extracellular polymeric matrix, which is an essential component of biofilm [18, 221]. The extracellular matrix, as that of other biofilm, is a biopolymeric mixture of various components from multiple sources. The attached bacteria on tooth surfaces secrete macromolecules by active secretion or cell lysis. These molecules along with salivary proteins, host cells, and intake substances develop into a complicated three-dimensional network that surrounds cells and further forms dental plaque. The structure and elements of matrix are influenced by coresidents and the local habitat. It can be considered as a variable that reflects plaque status.

This matrix aids bacteria to adhere to tooth surface, promotes coaggregation, reserves nutrients and enzymes, and protects the community from external harms, such as diffusion of charged or bioreactive molecules. Besides keeping the local homeostasis, EPS contains high ratio of polysaccharides and also acts as a virulence factor in the pathogenesis of dental caries. The polysaccharides in matrix are the resources that acidogenic bacteria metabolize and generate acids to initiate further caries progress. For example, *S. mutans* and lactobacilli are able to metabolize sucrose in matrix into glucan or fructan polymers which are either water soluble or water insoluble (branching). The insoluble carbohydrate polymers produced by *S. mutans* offer adhesion sites for *S. mutans* and thus are important in the development and progression of dental caries [10, 18, 221]

2.1.4 Spatiotemporal Development of Oral Biofilms

When exposed to the oral environment, for example, as soon as a few seconds after the tooth eruption or after brushing, the tooth surfaces are

coated with a conditioning film consisting of proteins and glycoproteins. This film is called acquired pellicle, the sources of which are mainly saliva and gingival crevicular fluid (GCF) [100]. Selective salivary components absorb to the tooth surface, including amylases, mucins, proline-rich proteins, histatins, statherins, lysozymes, and so on [150, 190]. These molecules attach on tooth surfaces and offer bioactive sites that facilitate future colonizers to bind. Hence, acquired pellicle is recognized as the sign of the initiation of dental plaque formation [18, 68, 88].

Following acquired pellicle formation, a few bacterial species, mostly *Streptococcus* spp., start to absorb and attach loosely to the film by a wide range of nonspecific and relatively weak forces. These initial colonizers (e.g., *S. mitis* and *S. oralis*) bear specific surface-anchored proteins (adhesins) to form irreversible binding with host receptors in acquired pellicle. Electron microscopy has shown that cocci can be detected on enamel surfaces at as early as four hours after cleaning in vivo [83, 155]. As the initial attachment and proliferation go on, the bacterial metabolism changes the microenvironment in favor of future residents. As the biofilm develops, more early colonizers with surface adhesins or receptors interact and attach to the initial colonized bacteria, starting the cascade of biofilm proliferation and biodiversity flourish. In this dynamic process, coaggregation has been extensively studied as one of the major mechanisms in early biofilm succession. This typical bacterial adhesin and bacteria/host receptor interaction is often based on protein-carbohydrate (lectin) reaction and eventually leads to the construction of a more stable and diverse network. *S. oralis* and *Actinomyces oris* are one of the most studied pair of coaggregation partners. The receptor polysaccharides on the cell surface of *S. oralis* can interact with type 2 fimbriae on *A. oris* surface, forming potent cell aggregates in vitro [164]. With the involvement of more species, the complexity of biofilm composition and structure is ever growing. In some cases, in a single site, more than 50 different species can be found [1]. With community multiplication, inter- and inner-species communications become active. The quorum sensing regulatory pathway plays a critical role during the development of dental plaque community.

In supragingival plaque, oral bacteria are mostly saccharolytic and able to utilize metabolic substances from endogenous nutrients. Saliva, as the major local source of amino acids, proteins, and glycoproteins, is continuously available, whereas diet intake has a minor role in nutrient contribution due to its limited surface contact time and unavailability of some highly complex ingredients for bacteria. Salivary proteins and glycoproteins are essential for initial biofilm formation and bacterial growth, whereas host food intake and other activities enormously affect the maturation and dynamic transition of biofilm. As previously mentioned, bacteria produce biopolymers to build the matrix during biofilm formation. In health biofilm, the metabolism with salivary substrates induces minor change to the local pH. In contrast, when diet change induces local sucrose concentration increase, the acid production will rise and further cause the homeostasis shift, leading to the numerical predominance of *S. mutans* and finally dental caries [100, 168].

Taken together, dental plaque as a typical biofilm possesses the social traits and benefits from this community-based lifestyle. Microbial cells colonize following their own nutrient and interactive needs, and a gradient of nutrient, air, pH, and partners gradually forms in the community [20, 31]. The dental plaque as a microecosystem can offer a wide range of habitats for diverse microbes and integrate different residents into a highly organized and tolerant community. Within an immediate contact, oral bacteria have better chances to conduct more efficient metabolism through mutualistic interactions [170]. With the support from extracellular matrix, bacteria in plaque are less sensitive to inhibitory agents and host defenses [93, 210, 213]. Driven by environmental cues, dental plaque would shift to pathogenic biofilm with distinct microbial composition and metabolic activity and thus initiates oral infectious diseases such as dental caries.

2.2 Microbial Etiology of Dental Caries

2.2.1 Oral Microbiology at Early Stage

There is a long history of understanding the etiology of dental caries. A Sumerian text from 5000 BC describes a "tooth worm" as the cause of caries. The "tooth worm" theory has also been described in the literature of ancient China, India, Egypt, and Japan. During the European Age of Enlightenment, the "tooth worm" theory was gradually rejected by the European medical community. Pierre Fauchard, known as the father of modern dentistry, was one of the first to oppose the "tooth worm" theory and noted that sugar was detrimental to the teeth and gingiva.

Around 1680, van Leeuwenhoek (1632–1723) [67], a Dutch dry goods merchant, observed and described first microorganisms in the tartar from his teeth with his primitive microscopes. In his notebook, he recorded "I didn't clean my teeth for three days and then took the material that has lodged in small amounts on the gums above my front teeth…. I found a few living animalcules." The microbes sketched in his notebook are now known as some of the most abundant bacteria resided within oral cavity, including cocci, spirochetes, and fusiform bacteria. These fascinating observations at the birth of microbiology had already signaled the complexity of the oral microbial community [91].

2.2.2 Dental Caries as an Infectious Disease

W. D. Miller was arguably one of the most important scientists who substantially advanced the knowledge of dental caries and associated studies in oral microbiology. As a practicing dentist with a comprehensive training in natural sciences, he identified the "germs" responsible for tooth decay. In his 1890 book titled *Microorganisms of the Human Mouth*, he proposed a "chemoparasitic" theory of dental caries, which suggested that oral microorganisms can convert carbohydrate into acid and eventually result in the demineralization of teeth and development of dental caries in susceptible hosts. Miller's chemoparasitic theory, together with the description of "gelatinous microbic plaques", which are now commonly known as "dental plaque," by Black and Williams [16, 219], has laid the foundation for our modern knowledge of dental caries etiology.

Due to the limited bacterial isolation and cultivation technique available in the nineteenth century, Miller was unable to identify the causative agent(s) of dental caries. In 1924, Clarke first isolated a bacterial species from human dental caries site and named it *S. mutans*, which was capable of fermenting several sugars and producing a pH of 4.2 in glucose broth [38]. Unfortunately, Clarke was unable to demonstrate that this organism actually caused caries.

Since 1950, various experimental animal models have been employed to better elucidate the nature and etiology of oral diseases, including dental caries [105]. In 1960, two important articles were published which were considered the cornerstone of dental caries research. In the first paper, using hamster as animal model, Keyes revealed the infectious and transmissible nature of dental caries [108]. Then in another elegant paper, Fitzgerald and Keyes successfully demonstrated caries induction in an animal harboring a "conventional" microflora, by a single type of streptococcus that had been isolated by Clarke more than 30 years ago [61].

2.2.3 Dental Plaque as the Cause of Dental Caries

One of the early achievements in oral microbiology is to link dental plaques to dental and periodontal diseases. Dental plaque was one of the first substances van Leeuwenhoek examined under his microscope [67]. The living microorganisms recorded by him revealed for the first time the complex nature of dental plaque in terms of its microbial composition. Later on, both Erdi and Ficinus described the presence of

microorganisms within the "membrane" on the teeth [197]. However, the full implications of dental plaque were not realized until the publication of [16] paper, in which he referred dental plaque as "gelatinous microbic plaques," a gelatin-like substance that carried microorganisms [16]. Black believed that bacteria within dental plaque generate acids that dissolve the dental hard tissue. Black's work, together with Miller's chemoparasitic theory, underscored the important role of dental plaque in the etiology of dental caries and has become one of the essential paradigms of oral biology.

2.2.4 Association of *Streptococcus mutans* with Dental Caries

Considering the involvement of *S. mutans* in the pathogenesis of dental caries as demonstrated by early in vitro and in vivo animal models, epidemiologist tried to predict the development of dental caries by the detected number of *S. mutans* present on the teeth. Numerous studies suggested a positive correlation, and *S. mutans* was recognized as the chief culprit of dental caries. Hence, efforts were gradually shifted to the exploration of the cariogenic abilities of *S. mutans*. Researchers in this field proposed that the virulence of *S. mutans* resides in three core attributes, including the abilities (i) to metabolize dietary sucrose to form insoluble polysaccharides that mediate the persistent colonization of tooth surfaces, (ii) to produce large quantities of organic acids (primarily lactic acid) from a wide range of carbohydrates (acidogenicity), and (iii) to tolerate various environmental stresses, particularly low pH which are toxic to most of the other bacterial species present in the mouth (aciduricity).

The complete genome sequence of *S. mutans* published in 2002 further stimulated numerous studies of this bacterium at genetic level. However, the correlations between dental caries and *S. mutans* were not definitive at the level of individuals. Today, it is clear that there are individuals and population groups of high caries susceptibility with low levels of *S. mutans* and vice versa. While there is little doubt about the ability of *S. mutans* to cause dental caries in humans or animal models, its contribution to the pathogenesis and progression of dental caries is still unclear.

2.2.5 Nonspecific or Specific Plaque Hypotheses?

Miller's "chemicoparasitic theory" proposed a "nonspecific plaque" hypothesis, in which dental caries is the result of the overall interaction of all the groups of bacteria within plaque. However, the rat caries model and the positive correlation of *S. mutans* with dental caries in most cases suggested that this proposal might not be absolutely right. Hence, the "nonspecific plaque" hypothesis has been challenged for decades. An alternative view is that among the 200–300 indigenous species identified in the oral cavity, only a finite number of them can be recognized as dental pathogens. Thus, dental caries can be considered as specific, treatable infections. This proposal had the benefit of focusing studies on the control of specific microbial targets. However, although *Mutans streptococci* (including *S. mutans*, *S. rattus*, *S. sobrinus*, and *S. cricetus*) are strongly implicated with caries, the association is not unique; caries can occur in the apparent absence of these species, while *S. mutans* can persist without evidence of detectable demineralization. Indeed, in such circumstances, some acidogenic, non-*S. mutans* are implicated with disease. Due to the imperfection of both theories, the debate is still ongoing up until today.

2.2.6 Ecological Plaque Hypothesis

Unlike many known medical pathogens that are "foreign invaders with specific virulence factors," the oral "pathogens" such as *S. mutans* are part of the normal flora. From the initial isolation of *S. mutans* by J. K. Clarke in 1924 to the latest metagenomic studies, over 700 bacterial species have been identified from the human oral cavity. The oral microflora has been recognized as one of the most complex microbial communities in

the human body [122]. As proposed by Phil Marsh in his "ecological plaque hypothesis," it is not merely the presence of a single organism in a complex community that determines the properties of a biofilm, but it is the interactions between the biofilm residents that are crucial. In dental caries, there is a shift toward community dominance by acidogenic/aciduric gram-positive bacteria (e.g., *S. mutans* and lactobacilli) at the expense of the acid-sensitive species associated with sound enamel [110, 143].

The introduction of high-throughput metagenomic pyrosequencing has profoundly advanced our understanding of the overall oral microbial diversity and function. Data obtained from metagenomic level favorably support the ecological plaque hypothesis. A recent metagenomic study on microbiome in caries cavity showed that the caries cavities are not dominated by *S. mutans*, but are a complex community formed by tens of bacterial species [77]. The data support the polymicrobial etiology nature of caries. Another investigation on the oral microbiota of children with dental caries revealed that genera *Streptococcus*, *Veillonella*, *Actinomyces*, *Granulicatella*, *Leptotrichia*, and *Thiomonas* in plaques were significantly associated with dental caries [42], further supporting the idea that no one specific pathogen but rather pathogenic populations in plaque correlate with dental caries. In addition, a recent study on salivary microbiome of caries-active population further supports the ecological hypothesis that the shifts in community structure, instead of the presence or absence of specific groups of microbes, contribute to the occurrence of dental caries [232].

2.2.6.1 Microbial Ecology in the Oral Cavity

It has been estimated that the human body is made up of over 1×10^{14} cells, of which 90 % are the microorganisms that comprise the resident microflora of the host. Thus, this resident microflora has been currently proposed as a novel organ in human body. The resident microflora dynamically interacts with the human body, contributing directly and indirectly to the normal development of the physiology, nutrition, and defense systems of the host [143].

Despite the continual shift of these microorganisms, the composition of the resident microflora is distinct in different habitats/niches such as the oral cavity, gut, and vagina. The different key ecological factors present in these biohabitats have a great impact on the community structure and metabolic function of the resident microflora. Those ecological factors include appropriate receptors for attachment, essential nutrients and cofactors for growth, as well as an appropriate pH, redox potential, and gaseous environment. Take the oral cavity, for example, the tooth surfaces provide distinct binding factors for microorganisms. Moreover, the mouth is continuously bathed with saliva at a temperature of 35–36 °C and a pH of 6.75–7.25. The nutritional condition of the oral cavity is often described as "feast or famine", further exerting far-reaching influence on the composition of microflora. Consistently, our recent study by sampling microflora from various oral sites of different age groups has demonstrated that the oral cavity is a highly heterogeneous ecological system containing distinct niches with significantly different microbial communities. More importantly, the phylogenetic microbial structure varies with aging, and only a few taxa were present across the whole populations [231].

The first stages of dental plaque formation involve the attachment of bacteria to salivary proteins and glycoproteins that are deposited as pellicle on the surfaces of teeth/dentures and other oral tissues. Bacteria that first attach to the salivary pellicle are designated primary colonizers, including *S. oralis*, *S. mitis*, *S. sanguinis*, *S. parasanguinis*, and *S. gordonii*. In addition, *Actinomyces*, *Veillonella*, *Gemella*, *Abiotrophia*, and *Granulicatella* species are often detected. These early colonizers proliferate and change local environmental conditions, making the site suitable for colonization by more fastidious species (e.g., obligate anaerobes). These early colonizers also form the base layers of complex dental plaque biofilms. Subsequently, new microbial cells adhere via similar adhesin-receptor mechanisms (a process termed coaggregation or coadhesion). Subsequent colonizers such as

Fusobacterium and *P. gingivalis* are especially effective in attaching to earlier plaque colonizers and eventually form complex, structured, multispecies biofilms. Once established, the microbial composition of dental plaque remains relatively stable over time, and this microbial homeostasis is intricately maintained by the dynamic intermicrobial and host-microbial interactions [143].

2.2.6.2 Genetic and Environmental Factors and Oral Microbial Ecology

In order to fully understand the change of oral microbial community during the occurrence and development of oral diseases (e.g., dental caries), it is necessary to completely characterize the community structure of the oral microbiome and its influencing factors. Previous studies in healthy individuals have revealed significant inter- and intraindividual structure and function diversity in the human oral microbiome. Nurture (environment) and nature factors (host genotype), such as diet, hygiene, geography, cultural traditions, age, gender, and human genotype, work together to shape the oral microbiome [164].

Diet is one of the most important environment factors that impose profound influence on the structure and function of the human oral microbiome. Exogenous nutrients provided via the diet exert strong selection on the composition of the oral microbiota. Fermentable carbohydrates are the class of nutrients that most affect the microbial ecology of the mouth. They are catabolized to acids, inhibiting most of the species while promoting the growth of aciduric organisms. Frequent exposure to low pH can disrupt microbial homeostasis and lead to the enrichment of acidogenic/aciduric species within the dental plaque. Other environment factors, such as oral hygiene, medication, and geography, will also influence the composition of oral microbiota [143].

Host immunity plays an essential role in shaping oral microbiome. Saliva contains components of innate (e.g., lysozyme, lactoferrin, sialoperoxidase, antimicrobial peptides) and adaptive immunity (e.g., sIgA), can directly inhibit some exogenous microorganisms. Host tissues surrounding the oral cavity may secrete antimicrobial agents and immune modulators, which have substantial influence on the properties of dental plaque. In addition to host immunity, the tooth morphology is heavily determined to be influenced by genetic factors also contributes to the community structure of human oral microbiome [143].

In fact, most factors involved in the diversity in oral microbiome cannot be simply classified into nurtural or natural. Accumulating evidence from twin-pair model favors the "environment dominates" theory. By comparing the composition variation of salivary microbiome in a cohort of 27 monozygotic and 18 dizygotic twin pairs, Stahringer et al. proposed the philosophy of shared environment serving as the main determinant of microbial populations [193]. Nevertheless, a recent report by the Human Microbiome Project Consortium has shown that the ethnic or racial background association is one of the most robust associations with microbiome, indicating that the host genotype such as race and ethnicity may also be one of the major determinants for the human microbiome diversity [205]. Moreover, several recent studies have suggested that ethnicity tunes the oral microbiome at more specific levels [147, 239].

2.2.6.3 Interspecies Interactions and Dental Caries

The viability of oral microbial community is dependent not only on the host genetic and environmental factors but also on interactions between the microbial residents. The residents in the complex oral microbial community interact extensively, forming biofilm structures, carrying out physiological functions, and inducing microbial pathogenesis. Therefore, "war and peace" is usually used to describe the dynamic microbial interactions within a biofilm. In general, microbial interactions include (i) competition between bacteria for nutrients, (ii) synergistic interactions for the growth or survival, (iii) antagonistic interactions by secondary metabolites production, (iv) neutralization of a virulence factor produced by another resident, and (v) interference in the growth-dependent signaling mechanisms of each other [122].

S. mutans and other caries-associated organisms such as lactobacilli and *Actinomyces* species are capable of expressing certain pathogenic factors. A dynamic balance of both synergistic and antagonistic interactions with the coresidents plays an essential role in determining whether these pathogenic factors cause damage or not. Hence, in the case of dental caries, it is now generally recognized that this disease is not solely the result of the presence of *S. mutans* or any single organism in dental plaque. Rather, it is the net result of the interaction of multiple acidogenic/aciduric organisms such as *S. mutans* with other commensals within the dental plaque.

Factors Involved in Interspecies Interactions Metabolic interrelationship is one of the most common interspecies interactions within dental plaque. Nutrients could be available from the periodic intake of food, saliva, and nutrients provided by other organisms as well as polysaccharides present in dental plaque. *S. mutans* metabolizes sucrose to generate acid more efficiently compared to other common oral bacteria [83]. When sucrose is frequently consumed, *S. mutans* may take advantage of the substrate competition and acid selection to emerge as a predominant resident in caries-associated biofilms. In addition, mutualism between two organisms dependent on nutrients is also common. For example, lactate produced by streptococci is utilized directly by *Veillonella* for growth. As lactate is removed from the immediate environment by *Veillonella*, so the flux of glucose to lactate increases, thus in turn enhancing growth of streptococci [155]. Another example is the combined efforts of *A. naeslundii* and *S. oralis* in metabolizing salivary components to form extensive biofilms on saliva-coated surfaces [168].

The secondary metabolites of one organism also have effects on other coresidents within the same biofilms. One of the best examples is between *S. mutans* and *S. sanguinis*. The ecological antagonism between these two bacteria in the oral cavity has been noted for decades. Early colonization and high levels of *S. sanguinis* in an infant's oral cavity correlate with significantly delayed colonization by *S. mutans*. Similarly, high levels of *S. mutans* in the oral cavity correlate with low levels of *S. sanguinis* [132]. Animal study has also demonstrated a so-called competitive exclusion between *S. mutans* and *S. sanguinis* depending on the sequence of inoculation [156]. The lactic acid produced by *S. mutans* favors the growth of itself relative to that of other less aciduric oral streptococci including *S. sanguinis*. On the other hand, *S. sanguinis* can produce antimicrobial hydrogen peroxide to antagonize the growth of *S. mutans*, which lacks effective systems to detoxify hydrogen peroxide [117, 119]. In addition to antagonism, metabolic products of one organism may promote the growth of other organisms. For example, organisms which are able to metabolize oxygen would favor the growth of nearby anaerobic organisms [46].

Bacteriocin is another key factor involved in the interspecies competition. Bacteriocins are proteinaceous toxins produced by all major lineages of bacteria. Unlike traditional antibiotics, bacteriocins often have a narrow killing spectrum and inhibit the growth of related organisms [178]. In oral cavity, streptococci and a variety of oral bacteria including *A. actinomycetemcomitans* produce bacteriocins that are lethal to other bacteria. *S. mutans* produces a number of distinct bacteriocins (also known as mutacins) as an "arsenal" against its competitors. At least five different bacteriocins (mutacins I to V) produced by *S. mutans* have been identified [36, 174, 175]. In addition, at least nine other putative mutacin-encoding genes have been annotated in the genome of *S. mutans* UA159, suggesting a large repertoire that can be used against its competitors. Some of these bacteriocins are able to inhibit the growth of *S. sanguinis* and thus may be responsible, in part, for the negative correlation of the presence of *S. sanguinis* and *S. mutans* in the dental plaque [81]. Similarly, the expression of bacteriocins by some other biofilm residents may determine which organisms are coresidents in these structures.

Interactions mediated by signaling molecules are also prevalent in the dental biofilms. The quorum-sensing regulatory molecule autoinducer-2 (AI-2) is a potential signaling molecule

between heterogeneous bacteria within biofilms. One typical example is that the *luxS* mutants of *P. gingivalis* and *S. gordonii* cannot form mixed biofilms, but a mutation in either strain alone allows for such a biofilm formation [153]. In addition, AI-2 also mediates mutualistic biofilm formation by *S. oralis* and *A. naeslundii* strains [177]. Hence, AI-2 functions as one of the primary mediators of interspecies interactions within oral biofilms. In addition to AI-2, many of the oral streptococci, including *S. mutans*, *S. gordonii*, and *S. sanguinis*, signal by the competence stimulating peptide (CSP). Unlike the AI-2, CSP is highly species specific and is not likely to interfere with the activity of another distinct CSP molecule. Of note, the CSP still can be indirectly involved in interspecies interactions. In the case of *S. gordonii* vs *S. mutans* interactions, a protease (i.e., challisin) expressed by *S. gordonii* inhibits CSP-dependent properties of *S. mutans* [215]. In addition, *S. mutans* may acquire transforming DNA from the coresidents through the CSP-induced bacteriocin production [118].

Taken together, microbial homeostasis can only be achieved when an equilibrium is established among different species within the same biological niche. The aforementioned factors play an essential role in modulating interspecies interactions within biofilms, thus shaping the structure of the oral microbial community. Changes of these factors can perturb the established equilibrium, leading to the emergence of newly predominant bacteria more adaptive to the microenvironment (niche). In dental caries, this reestablished microbial community structure is characterized by a numeric predominance of acidogenic/aciduric bacteria (e.g., *S. mutans*) at the expense of the acid-sensitive commensal species (e.g., *S. sanguinis*) associated with sound enamel [110, 143].

The Role of Interspecies Interactions in Dental Caries The interspecies interactions within dental biofilms tune microbial composition, structure, and virulence factors of the oral microbial community and thus are involved in the onset and progression of dental caries [122].

Bacterial interactions can affect the growth of individual organisms or groups of related organisms, thus modulating the composition of the oral microbial community. For example, *S. sanguinis* gained competitive edge over mutacin-generating *S. mutans* by producing cytotoxic hydrogen peroxide under certain conditions. On the other hand, *gshAB* is essential for the competitiveness and prevalence of *S. mutans* by detoxifying hydrogen peroxide produced by *S. sanguinis* [238]. The *S. sanguinis*/*S. mutans* ratio resulting from the dynamic competition between these two bacteria may have ecological significance on the occurrence and incidence of dental caries.

Bacterial interactions could also affect the overall expression of bacterial virulence of the biofilm. Take *S. mutans*, for example, one of the core cariogenic virulences of *S. mutans* is the ability to produce large quantities of organic acids by metabolizing carbohydrates. In this regard, coresidents which are capable of neutralizing/depleting the acidic end products of *S. mutans* tend to reduce the cariogenicity of the biofilm. In dental biofilms, some commensal bacteria such as *Veillonella* are able to consume the lactic acid. In addition, some coresidents such as *S. salivarius*, *S. gordonii*, and *S. sanguinis* can generate alkali to neutralize the acid, through the metabolism of either urease by urease or arginine by arginine deiminase system [26]. The quorum-sensing systems also modulate the virulence properties of oral residents. *S. mutans* utilizes CSP-induced bacteriocin production to acquire transforming DNA from other coresidents [118], thus achieving a great genomic diversity for better environmental adaptation. The CSP-induced bacteriocin production also modulates the growth of related noncariogenic streptococci. In addition, the CSP-mediated quorum-sensing systems are involved in the antimicrobial resistance of *S. mutans* [149]. Therefore, biofilm residents which could affect the levels of the regulatory molecules present in the biofilm could indirectly exert profound effects on the biofilm virulence. Since noncariogenic *S. gordonii* can inactivate the CSP produced by *S. mutans*, its presence could thus modulate the virulence of these biofilms.

In conclusion, the interaction with the dental microbial community can not only affect the

growth of certain species but also modulate the virulence properties of oral residents, thus influencing the overall pathogenicity of the dental plaque. The interspecies interactions have ecological significance in the occurrence and progression of dental caries.

2.3 Dental Caries-Associated Bacteria

2.3.1 Carbohydrate Metabolism and Acidogenic Bacteria

Dental caries is a multifactorial and chronic infectious disease resulting in the localized destruction of dental hard tissue. Dental caries is one of the major disease burdens inflicting humans throughout history. The pandemic of dental caries has been linked to the two largest dietary shifts in human evolution in terms of the consumption of fermentable carbohydrates. With the advent of Neolithic farming, the increased consumption of domesticated grains positively correlates with a marked prevalence of caries. However, across the Neolithic and medieval period, the degree of caries was mild and prevalence remained relatively stable until 1850. After 1850, a sudden expansion of caries lesion occurred coinciding with the introduction of refined flour and sugar due to industrial revolution. Although the epidemiological history indicates a correlation of sugar with dental caries, the "culprit" of the dental caries remained enigmatic until the late nineteenth century, when Miller evidenced that the acidic microbial metabolites from dietary substrates contribute to the development of dental caries [157, 158]. These observations were further confirmed in 1940s by Stephan, who reported that microbial metabolism of carbohydrate in dental plaque could drive the local pH values below 3.0 after continuous sugar exposure [194]. Among those acidic microbial metabolites, lactic acid has been identified as the major contributor to the pH decline in dental plaque. Hence, the acidogenic bacteria are recognized as the culprit for caries initiation and progression. The accumulation of organic acids leads to continuous pH decline to the critical pH, below which tooth hard tissue demineralization begins and dental caries gradually occurs [143].

2.3.2 Major Acidogenic Bacteria

2.3.2.1 *Streptococcus mutans*

S. mutans was first described by J Kilian Clarke in 1924 [38]. It is gram-positive facultative coccus commonly arranged in chains. Oral streptococci are commensal bacterial but can opportunistically initiate caries. *Mutans streptococci* are a group of most important bacteria highly associated with caries, consisting of *S. mutans*, *S. sobrinus*, *S. rattus*, *S. cricetus*, *S. ferus*, *S. downeii*, and *S. macaca*. Their cariogenic virulence mainly involves several attributes, including (1) processing adhesins for initial attachment to the saliva-coated tooth surface; (2) the production of extracellular polysaccharide, i.e., glucan, fructan, to facilitate retention on tooth surface and plaque accumulation; and (3) the production of organic acid to generate acidic microenvironment and promote enrichment of aciduric microflora.

2.3.2.2 *Lactobacilli*

Lactobacillus is a genus of gram-positive facultative anaerobe or microaerophilic rod-shaped bacteria. *Lactobacillus* is able to convert lactose and other sugars to lactic acid, mostly through homofermentative metabolism. *Lactobacillus* count has been used to assess caries activity for years. *Lactobacillus* is acid tolerant and can carry out glycolysis at pH values as low as 3. However, lactobacilli are poor colonizer of smooth tooth surface. Therefore, lactobacilli are generally believed to exacerbate the initial enamel lesion to deep dentine lesion. After colonizing into the established dental plaque, the lactobacilli can further acidify the plaque and suppress the acid susceptible microorganism, further enriching acidogenic and aciduric bacteria.

2.3.2.3 *Actinomyces*

Actinomyces is a genus of gram-positive facultative or strict anaerobic pleomorphic rod-shaped

bacteria. As a saccharolytic and acidogenic bacterium, *Actinomyces* spp., especially *A. naeslundii*, has been frequently isolated from both root caries lesions and sound root surface, suggesting their association with root caries. However, an in-depth knowledge about the involvement of individual *Actinomyces* spp. in root caries is still sketchy.

2.3.3 Acid Tolerance of Acidogenic Bacteria

The central pathogenesis of dental caries is the production of organic acid and the resultant decalcification of dental hard tissue. The dental plaque undergoes rapid, dynamic pH fluctuations ranging from pH 7.0 to 3.0 in less than 20 min upon carbohydrate intake [102]. The cariogenic bacteria emerge as numeric predominant species during this acidification process. Acid tolerance or acidurity is the most important attribute for those acidogenic bacteria to prevail in a cariogenic biofilm. Acidogenic bacteria such as *S. mutans* can function better at pH 6 and can even carry out glycolysis at pH below 4. Moderate acidophile, especially *Lactobacillus* spp., can also function better and carry out glycolysis at low pH values of 3–4. To survive and proliferate in acid conditions, the cariogenic bacteria have developed a large repertoire of strategies to maintain intracellular homeostasis, which involves both constitutive acid tolerance and acid-induced/adaptive tolerance [25]. Constitutive acid tolerance mainly relies on the F-ATPase proton pumps in the cell membrane. Taking *S. mutans*, for example, although the glycolytic pathway can catabolize sugars at pH values as low as 4.0, the *S. mutans* cell cannot grow at pH values lower than 5.0. In this case, the F-ATPase will use the ATP generated by glycolysis to pump out intracellular protons and thus maintain neutral cytosolic pH favorable for normal enzymatic function and cellular viability. In addition, the end metabolite, lactate, may also be extruded from cytoplasm by a membrane carrier for lactic acid, thus maintaining a relative neutral cytosolic pH. The adaptive acid tolerance response indicates the induction of enhanced survival ability of bacteria at a pH as low as pH 3.0 after exposure to a sublethal pH of approximately 5.5. The adaptive acid tolerance involves global cellular response, including alteration of metabolism, regulation of quorum sensing system, synthesis of chaperonin protein for damaged protein, increased activity of DNA protection/repair system, changes in membrane fatty acid composition, etc. [148]. Overall, the acid tolerance mechanisms allow the cariogenic bacteria to outcompete other acid-sensitive coresidents under acid selection, which would eventually lead to the enrichment of acidogenic/aciduric bacteria and continual acidification of the dental plaque favorable for caries formation.

2.3.4 Base Generation and Caries Protection

In addition to the aforementioned acid tolerance mechanisms, alkali generation is widespread among oral species. Alkali generation is particularly important for the survival of acid-sensitive commensal bacteria and thus plays an important role in modulating the microbial ecology within oral biofilm. The primary source of alkali production is through microbial metabolism of arginine, agmatine, and urea (Fig. 2.2). Alkali generated by these metabolism pathways might neutralize the acid metabolites produced by cariogenic microflora and thus provides a promising strategy for the development of ecological therapy against caries (Fig. 2.3).

2.3.4.1 Urease

The physiological concentration of urea present in saliva and crevicular fluids ranges from 3 to 10 mmol/L, roughly equivalent to those in serum. Some oral species, including *S. salivarius*, *A. naeslundii*, and oral haemophili, can convert urea by urease to ammonia and CO_2, thus increasing plaque pH [33, 129]. Urease is a nickel ion-dependent multisubunit metalloenzyme encoded by at least seven genes arranged as operon in most bacteria. *ureC*, *ureA*, and *ureB* encode the urease apoenzyme, consisting of α, β, and γ subunits. These subunits assemble into $(\alpha\beta\gamma)_3$ oligo-

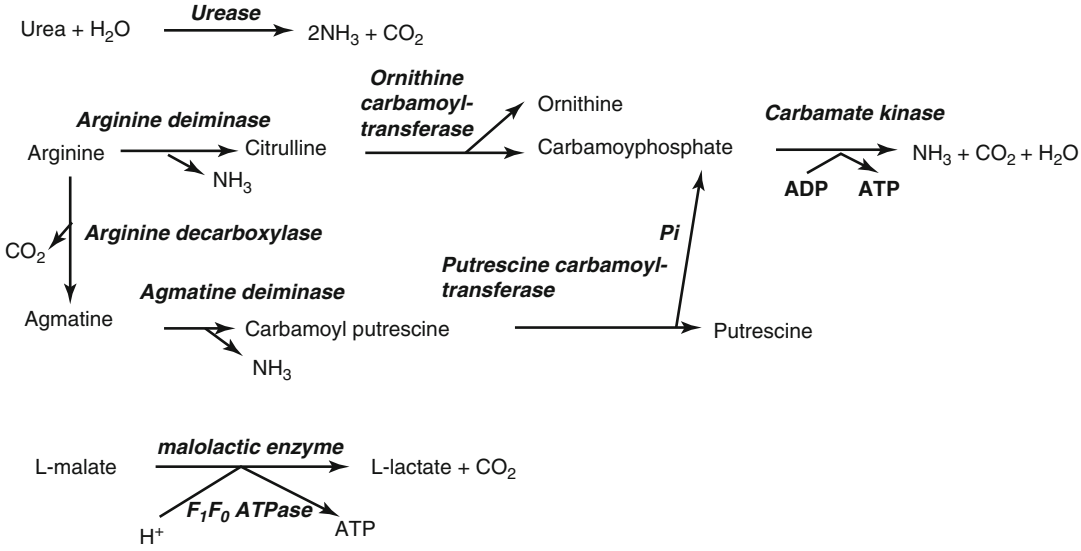

Fig. 2.2 Summary of alkali-generating pathways in the oral cavity (Figure is reproduced from Liu et al. [131])

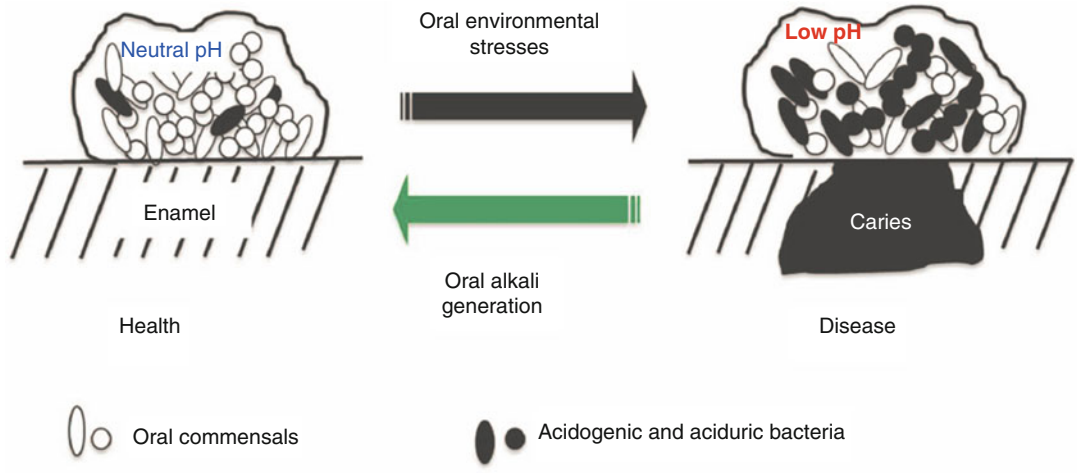

Fig. 2.3 The role of alkali generation in caries prevention. Dental biofilms in a healthy host maintain a microbial and pH homeostasis with a balanced demineralization/remineralization of dental hard tissue. The environmental cues, such as frequent sugar exposure, may enrich the acidogenic/aciduric bacteria via acid selection and further lead to plaque acidification in favor of demineralization of dental hard tissue. The continuous plaque acidification eventually results in the initiation and/or progression of caries lesion. Alkali generation by biofilm commensals can directly neutralize plaque pH, ease the competitive edge of acidogenic/aciduric bacteria, and restore a healthy microbial equilibrium (Figure is reproduced under the permission of Dr. Liu et al. [131])

meric complex with six nickel ions incorporated into the active site. *ureE*, *ureF*, *ureG*, and *ureD* encode chaperone complex essential for the incorporation of Ni^{2+} and CO_2 into the metallocenter. Other genes, such as *ureM*, *ureQ*, and *ureO*, encode a Ni^{2+}-specific ATP-binding cassette transporter, and *ureI* encodes urea transporter [32]. The expression of bacterial urease is regulated by multiple environmental cues, including low pH, the presence of urea, limited nitrogen source, carbohydrate availability, and rate of growth [32].

2.3.4.2 Arginine Deiminase System (ADS)

Arginine is abundant in salivary secretions as polypeptides with average concentrations in ductal saliva around 50 mmol/L [211]. Arginine is primarily metabolized by the microbial ADS to release ornithine, ammonia, and CO_2. ADS has been identified in many commensal bacteria, including *S. sanguinis*, *S. gordonii*, and *S. parasanguis*. Certain *Lactobacillus* and *Actinomyces* species, other oral streptococci, and some oral spirochetes have been also identified as arginolytic [26, 141]. Unlike urea hydrolysis by urease, arginine catabolism by ADS generates ATP, which could be further utilized to counter acid stress through both constitutive and adaptive acid tolerance pathways. Similar to urease, ADS-encoding genes are commonly arranged in an operon [47, 136]. *arcA* gene encodes arginine deiminase, which hydrolyzes arginine to generate citrulline and ammonia. *arcB* gene encodes ornithine carbamoyltransferase, which converts citrulline to ornithine and carbamoylphosphate. *arcC* gene encodes carbamate kinase that transfers a phosphate group from carbamoylphosphate to ADP to generate ATP, CO_2, and ammonia. Many organisms also possess an arginine/ornithine antiporter (*ArcD*) that is encoded in the same operon, and arginine aminopeptidases and transcriptional regulators are often encoded in ADS gene clusters [235]. ADS expression is induced by arginine and low pH. The operon is also sensitive to carbon catabolite repression (CCR) and can be downregulated in response to elevated oxygen levels [48]. In addition, ADS activity in *S. gordonii* has been reported to be upregulated when it coaggregated with *A. naeslundii* [99], indicating the ecological involvement of ADS expression in multispecies biofilm.

2.3.4.3 Agmatine Deiminase System (AgDS)

Agmatine present in the oral cavity can be obtained from foods, such as rice, milk, and beer, or be produced from arginine by bacterial arginine decarboxylase enzymes. The physiological concentration of agmatine is 0.75 mmol in dental plaque and 0.2 mmol in saliva [76]. Agmatine is primarily metabolized by AgDS to putrescine, ammonia, CO_2, and ATP. AgDS is present in many oral bacteria, including *S. mutans*, *S. sobrinus*, *S. downeii*, *S. rattus*, *S. uberis*, *S. mitis*, and *S. cricetus*, as well *Lactobacillus salivarius* and *L. brevis* [76]. AgDS is also encoded by genes arranged in operon (*aguBDAC*) [74]. *AguD* encodes agmatine-putrescine antiporter to allow the entry of free agmatine into the cell. Agmatine is then hydrolyzed to N-carbamoylputrescine and ammonia by the agmatine deiminase enzyme encoded by *aguA*. The putrescine carbamoyltransferase, encoded by *aguB*, further metabolizes N-carbamoylputrescine to yield putrescine and carbamoylphosphate. Finally, carbamate kinase encoded by *aguC* gene transfers a phosphate group from carbamoylphosphate to ADP to generate ATP, CO_2, and NH_3. The putrescine generated can also be used in exchange of agmatine via the antiporter [74]. *aguR* gene, which is located upstream of, and in the opposite orientation to, the *agu* operon in *S. mutans*, encodes a transcriptional activator of *agu* genes [75]. In oral streptococci, AgD activity is generally lower relative to that of arginine deiminase or urease [74]. Therefore, AgDS may not be sufficient to counter the plaque acidification. On the contrary, accumulating evidence has indicated that AgDS may actually favor the acid tolerance of *S. mutans* by elevating the cytoplasmic pH and generating extra ATP. In *S. mutans*, AgDS activity is growth phase dependent, and it can also be induced by the presence of agmatine and other environmental stresses, including low pH and heat shock [75]. Multiple two-component systems, including *CiaRH*, *ComDE*, and *VicRK*, also involve in the induction of AgDS genes under low pH or thermal stress [130].

2.3.4.4 Alkali Production and Biofilm Ecology

Dental biofilms are complex ecosystems with hundreds of metabolically and physiologically diverse species competing for nutrients. The ability of oral species to metabolize urea, arginine, or agmatine at low pH favors the growth of these

bacteria by cytoplasm alkalization. The generation of ATP could further enhance acid tolerance by providing energy for proton extrusion, growth, or maintenance [75]. The contributions of ADS, urease, and AgDS to oral biofilm pH homeostasis and microbial ecology can be quite different. In some oral commensals, such as *S. salivarius* and *S. gordonii*, urease and ADS can protect the bacteria from excessive environmental acidification [33]. In the meantime, other less-aciduric coresidents benefit from the local alkalization by ammonia generated from arginine or urea breakdown [48]. On the other hand, the caries pathogens *S. mutans* and *S. sobrinus* have no urease or ADS but possess AgDS that is expressed at a relatively lower level [76]. Consequently, AgDS of *S. mutans* or *S. sobrinus* probably cannot significantly alkalize the dental plaque, but instead, it enhances the acid tolerance of these acidogenic bacteria by neutralizing cytoplasm acid and generating extra ATP, thus promoting the prevalence of these bacteria during the development of caries.

2.3.4.5 Clinical Relevance of Alkali Production

Alkali production holds the promise to be a promising strategy for the management of dental caries. Firstly, it directly increases the pH of dental plaque in favor of dental hard tissue remineralization. Secondly, alkali generation favors the persistence of healthy commensals while preventing the overgrowth of cariogenic bacteria (e.g., *S. mutans*) dependent on acid selection. The activity of alkali generation of oral biofilm has shown negative correlation with the development and progression of caries. A genetically modified strain of *S. mutans* expressing the urease genes from *S. salivarius* showed potent anticaries effects in a rat caries model [37]. Chronic renal failure patients with salivary urea levels significantly higher than healthy population are more resistant to caries, despite their high dietary carbohydrate intake [172]. Consistently, caries-resistant subjects have higher ammonia concentrations and resting pH in their plaque compared with those of caries-free individuals [211]. Significantly higher levels of salivary ADS activity and plaque urease activity have been reported in caries-free population compared with subjects with dental caries. Salivary arginine levels are also positively correlated with caries resistance [163, 188]. A longitudinal study on children has further validated that ammonia generation by plaque urease is correlated with dental caries resistance [160]. The addition of arginine-bicarbonate to mouth rinse effectively can raise the plaque pH above the critical pH after a sucrose challenge [216]. More importantly, an increasing number of clinical trials have shown that arginine-containing oral hygiene products significantly reduced the incidence of dental caries [2, 3, 55]. Toothpaste containing 1.5 % arginine and 1450 ppm fluoride in a calcium base has also been proven more effective in arresting and reversing early carious lesions compared with dentifrice containing 1450 ppm fluoride alone [116, 192, 234].

2.3.5 Other Caries-Associated Bacteria

The oral cavity is inhabited by hundreds of bacterial species, forming complex ecology system [45]. The interplay between oral microflora and the host orchestrates the status of oral health and disease. The "specific pathogen hypothesis" has led to the identification of several other species including *Scardovia wiggsiae* and *Slackia exigua*, and *Bifidobacterium dentium* as the potential cariogenic pathogens [199]. In addition, *Atopobium*, *Olsenella*, *Propionibacterium*, and *Pseudoramibacter* genera are also indicated as bacteria associated with caries progress [173]. In contrast, the "nonspecific plaque hypothesis" supports the concept that caries is the consequence of the overall acid production activity of the total plaque microflora rather than a few specific bacteria. The "ecological plaque hypothesis" further proposes that the bacterial consortium in the dental plaque can interact in complex synergistic and antagonistic fashion, and it is the structure and function shift of the microbial community driven by environmental cues (e.g., frequent exposure to carbohydrate) that eventually lead to the net pathogenesis of dental caries [144].

2.4 Antimicrobial Approaches to the Management of Dental Caries

Dental caries is a biofilm-mediated disease. The composition of the biofilm associated with caries initiation and progression is diverse. The management of dental caries is to target the dental plaque, in particular to restore the microbial disequilibrium within the oral biofilm. This section is to provide an overview of various antimicrobial strategies applied in dentistry and related studies.

2.4.1 Chlorhexidine

Chlorhexidine is one of the most tested antiplaque agents and represents a gold standard against which the potency of other antiplaque agents is compared [8, 59]. Chlorhexidine molecules are positively charged (cations). Chlorhexidine binds strongly to most bacteria and surface structures in the oral cavity, including tooth surfaces and mucous membranes which are negatively charged (anions). When chlorhexidine binds to microbial cell walls, it destroys the surface structure, leading to an osmotic imbalance with consequent precipitation of cytoplasm causing cell death [59]. Chlorhexidine also possesses very good substantive properties [139]. The antimicrobial effect of chlorhexidine can be retained for up to 12 h or longer depending on the delivery dosage and form [4].

Chlorhexidine is a strong base, and it acts bacteriostatically when administered at low concentrations. It disrupts normal membrane functions or causes leakage of cell constituents [95]. At higher concentrations, chlorhexidine is bactericidal, inducing leakage of low molecular weight cell constituents and precipitation of cell contents. Chlorhexidine exhibits broad antibacterial spectrum with gram-positive microorganisms particularly sensitive than gram-negative microorganisms.

The efficacy of chlorhexidine mainly depends on the concentration and the frequency of application. Mouth rinses, sprays, gels and varnishes are some of the most often used delivery forms of chlorhexidine [191]. The usually prescribed dosage for chlorhexidine mouthrinses has been 10 ml of a 0.2 % solution, with twice-daily mouthrinses. By using 15 ml of a 0.12 % chlorhexidine mouthrinse, a comparable efficacy can also achieved [59].

2.4.2 Fluoride

Fluoride has been applied in dentistry for more than 70 years, and it is now recognized as the major contributor to the dramatic decline in caries prevalence worldwide [28]. Fluoride is a dual functional anticaries agent, acting on both tooth hard tissue and oral microbes [58, 114, 202]. Fluoride can disrupt enzyme activity and reduce acid production by oral bacteria [84], thus suppressing the enrichment of cariogenic bacteria within dental plaque [20, 21]. Amine fluoride [107] and stannous fluoride [151] can be bactericidal at higher concentrations against oral bacteria. Amine fluoride in a gel formulation can inhibit the growth of mixed bacterial populations in subgingival plaque [11, 24]. Amine fluoride and tin fluorides can also inhibit the adhesion of *S. sanguinis* to glass conditioned with either saliva or bovine serum albumin in vitro [54]. Preincubation of hydroxyapatite with amine fluoride can significantly decrease the growth of *S. sobrinus* in biofilm in vitro [183]. Of note, in vivo data reported by Weiger et al. [218] have shown that the amine/stannous fluoride mouthrinse possesses a transient antibacterial effect but no clear antiadhesive activity against oral bacteria.

The methods of fluoride delivery are either systemic (water, supplements, milk and salt) or topical (toothpaste, gels, varnishes, paint-on applications and mouthrinses) [59]. Although the role of fluorides in caries prevention represents one of the most successful stories in general public health, excessive fluoride intake during the period of tooth development can cause dental fluorosis. In addition, fluoride could nonselectively suppress the growth of commensal bacteria and thus disrupt the microbial equilibrium within

the dental plaque. Therefore, cautions should be taken in the application of fluorides in order to maximize the anticaries benefits while minimizing the risk.

2.4.3 Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) are surface-active agents with a wide antimicrobial spectrum against both bacteria and fungi [64, 98, 123]. QACs deliver their antimicrobial activity by binding to the cell membrane and causing cytoplasmic leakage, similar to those polycationic agents.

Cetylpyridinium chloride (CPC) is a commonly used QAC in a variety of mouthwashes over the past decades due to its antimicrobial property. The typically used concentration (W/V) of CPC is 0.05 %, although slightly higher concentrations (≥0.07) have also been used [44, 80, 214]. The antiplaque effect of CPC was first reported by Schroeder and Hirzel [79, 182]. Research has demonstrated that CPC mouthrinses have antiplaque activity when used alone or in conjunction with toothbrushing [79, 140, 222, 223]. Recently, a systematic review has further validated the plaque- and gingivitis-inhibiting effect of CPC-containing mouthrinses [87].

The CPC molecule allows ionic and hydrophobic interactions since it belongs to both hydrophilic and hydrophobic groups. It interacts with microorganisms via cationic binding, similar to chlorhexidine. Although CPC shows equal or better antimicrobial activity against planktonic culture compared to chlorhexidine, it exhibits an inferior inhibitory effect against biofilm [59]. This divergence may be attributed to the monocationic nature of CPC [53]. Initial retention of CPC is higher than that of chlorhexidine, but CPC is cleared from the oral cavity more rapidly [17]. CPC could be incorporated into dental materials, such as orthodontic adhesives, to control caries lesion formation around orthodontic brackets. Although CPC retains its antimicrobial properties, the clinical effect remains to be assessed [59].

Polymers containing QACs have also been incorporated into dental materials [34, 35, 236]. QACs are immobilized in the composite and not released or lost over time by copolymerizing with the resin through the formation of a covalent bond with the polymer network [209]. Therefore, the dental material has a durable and long-lasting antibacterial capability without significantly disturbing the biologic balance in the oral cavity and material's mechanic properties [96]. This is also due to the nonleaching properties of the material, since leaching leads to increased water absorption and solubility and decreased mechanical properties with time, decreasing the clinical longevity of these materials [227]. Previous study has presented that adhesive system containing QACs has similar antibiofilm properties and lasting at least 6 months of water aging [237]. Quaternary ammonium polyethylenimine (QAS-PEI) nanoparticles were incorporated in restorative materials to increase antibacterial action without further reduction on mechanical properties [12]. It has been demonstrated that the incorporation of 1 % QAC-PEI nanoparticles in dental composite resin has a strong antibacterial effect against *S. mutans* and can sustain over 1 month without alteration of the original mechanical properties [13]. QAC-PEI nanoparticles were also reported to have antibacterial effect against *S. mutans* and *L. casei* when incorporated in conventional glass ionomer [14].

2.4.4 Triclosan

Triclosan is a nonionic antimicrobial agent being used for more than 30 years as a preservative in products such as deodorants, soaps, and body powders. More recently, triclosan has been applied to dentifrices and mouthrinses as a prophylactic agent with the purpose of reducing dental biofilm formation and the development of gingivitis [59].

Triclosan is active against both gram-positive and gram-negative microorganisms and fungi. Oral bacteria such as *S. mutans*, *S. sanguinis*, and *S. salivarius* are susceptible to low concentrations of triclosan in vitro. At low concentrations,

triclosan is bacteriostatic [59]. Triclosan functions by specifically inhibiting bacterial lipid synthesis which further impairs cell membrane synthesis [152]. The antimicrobial/antiplaque properties of triclosan have been demonstrated in both in vitro and in vivo studies [78, 101, 176, 186, 198]. Triclosan and amine fluoride also show combinatory antibacterial and plaque-reducing effect in vivo [6].

The major concern of the widespread use of triclosan-containing products is the possibility of inducing antimicrobial resistance [124, 233]. Although triclosan is bactericidal at high concentrations, triclosan-containing products such as dentifrices leave residues that will dilute to sublethal concentration. Bacteria could develop reduced susceptibility to triclosan after repeated exposure to sublethal concentrations of this agent. A number of studies have verified the occurrence of triclosan resistance among dermal, intestinal, and environmental microorganisms [233]. More importantly, the widespread use of triclosan may also lead to the development of concomitant resistance to other clinically important antimicrobials through cross-resistance or coresistance mechanisms. Although studies regarding the concomitant resistance to other antimicrobials are still limited, cautions should be taken on the widespread use of triclosan [59].

2.4.5 Xylitol

Xylitol is an effective tooth decay-preventive pentitol accredited to its non-acidogenic property. Xylitol inhibits the growth of several bacterial species, among which *S. mutans* appear to be the specific target [19, 133, 208]. The *S. mutans* uptakes xylitol via a constitutive transport system specific for fructose and enriches xylitol intracellularly as a non-metabolizable metabolite. In this way, xylitol inhibits the growth of *S. mutans* and reduces the amount of plaque and the number of *S. mutans* in both the plaque and saliva of xylitol consumers [203]. Xylitol is able to disrupt the bacterial metabolism and consequently halt the pH drop in the dental plaque. In addition, xylitol can suppress the critical cariogenic factor of *S. mutans* by impairing their polysaccharide formation. Long-term consumption of xylitol also promotes the selection of xylitol-resistant *S. mutans*, which are believed to be less virulent than xylitol-sensitive strains [207]. All the aforementioned mechanisms suggest that xylitol is a promising cariostatic agent in controlling dental caries.

Xylitol has been incorporated as an active ingredient in dental hygiene products, such as xylitol-containing dentifrices currently available on the market. Anticaries effect of xylitol has been reported in longitudinal xylitol dentifrice studies [189]. Xylitol is an alternative sweetening agent to substitute sucrose in chewing gums. Besides the antimicrobial effect of xylitol, chewing itself promotes secretion of saliva and thus caries prevention. However, further well-designed randomized clinical studies with proper controls are needed to validate the caries-prophylactic effects of xylitol or superiority claims of xylitol over other polyols [59].

2.4.6 Phenolic Antiseptics

Phenols, either alone or in combination, have been known to be bactericidal since Lister used it as a surgical antiseptic in the 1860s [220]. Phenols are able to reduce plaque accumulation when used at high concentrations [63, 135]. Listerine, which is an essential oil/phenolic mouthwash, has been demonstrated in a number of clinical studies to possess moderate antimicrobial effect against dental plaque [43, 71]. However, Listerine has poor oral retention compared to chlorhexidine and thus lacks profound plaque inhibitory effect [53].

2.4.7 Natural Products

Natural products offer a wide variety of structurally different substances with a broad range of biological activities, which could be applied into the development of alternative or adjunctive anticaries therapies [104].

The antibacterial effect of *Camellia sinensis* (i.e., tea) against caries-related bacteria has been widely studied, using both in vitro and in vivo models [165, 166, 184, 185, 228–230]. Accumulating evidence has indicated that the bioactive components of green tea, polyphenolic catechins, in particular (−)-epigallocatechin gallate (EGCg) and (−)-epicatechin gallate (ECg), are able to inhibit the growth of the streptococci [200]. Catechins inhibit the growth of *S. mutans* and *S. sobrinus* with MICs ranging between 50 and 1000 μg/ml, which are within the concentrations determined in brewed tea [106, 180]. Catechins are also bactericidal at even higher concentrations. In addition, many of the "flavor compounds" (e.g., nerolidol) in green tea, although they are not antibacterial by themselves, might act synergistically with the abundant catechins to suppress bacterial growth [120]. Recent studies by our group have also demonstrated that tea catechins, particular EGCg, could not only inhibit the growth of cariogenic *S. mutans* at high concentrations but also suppress cariogenic factors involved in the acidogenicity, acidurity, and extracellular polysaccharide synthesis at sub-MIC levels [228–230]. The bioactivities of tea catechins at sub-MIC levels further support the translational application of tea extracts in vivo, considering the substantivity of these active components in the oral cavity after tea consumption.

Essential oils have also been extensively investigated for their antimicrobial activity against caries-related bacteria. Essential oils, odorous and volatile products of plant secondary metabolism, are typically a complex mixture of approximately 20–60 compounds that are in solution at various concentrations [104]. The main active component of essential oils is terpenoids, followed by aromatic and aliphatic constituents [9]. Thymol and eugenol inhibit the growth of a wide range of oral microorganisms including *S. mutans* [184, 185]. Essential oils appear to act against bacterial viability by disrupting the integrity of the bacterial membrane and cause the rapid efflux of intracellular bacterial components [185].

Propolis is a natural composite balsam collected by bees from tree buds and mixed with secreted beeswax [66, 89]. Propolis not only inhibits the growth of *S. mutans* and *S. sobrinus* but also suppresses the production of bacterial polysaccharides [65]. The application of propolis extract on rat molars also reduces the severity of carious lesions [179]. Specific flavanones (pinocembrin), dihydroflavonols (pinobanksin-3-acetate), and terpenoids (tt-farnesol) are believed to be the bioactive components of propolis [112]. tt-Farnesol is particularly effective against the proliferation of both planktonic and biofilm-associated *S. mutans* cells [113]. The antibacterial activity of tt-farnesol is likely associated with its lipophilic property and membranotropic effect, which might destroy the integrity of bacterial cell membrane [41, 97] and increase proton permeability [103].

Sanguinarine is a benzophenanthridine alkaloid originated from the alcoholic extraction of powdered rhizomes of the blood-root plant, *Sanguinaria canadensis* [73]. Sanguinarine at a concentration of 16 μg/ml could completely inhibit 98 % of microbial isolates from human dental plaque [49]. A sanguinarine mouthrinse and toothpaste regime has been shown to significantly reduce plaque by 57 % when given for 6 months during orthodontic treatment [86]. Another study of sanguinarine mouthrinse and toothpaste carried out in 120 subjects showed 13–17 % lower plaque scores compared with a placebo group after a 6-month treatment period [115].

There are many other reports available concerning the anticaries properties of various other plant extracts. Wu-Yuan et al. [225] and Li et al. [126] have identified that gallotannins from *Melaphis chinensis* and triterpenes (ceanothic acid and ceanothetric acid) from *Ceanothus americanus* possess antimicrobial activity against *S. mutans*. Furthermore, a chemically characterized extract of *Galla chinensis* (containing gallic acid and methyl gallate) can inhibit the growth of *S. mutans* and other caries-related organisms, including *L. rhamnosus* and *A. naeslundii*, within biofilms [226]. Ginkgoneolic acid from *Ginkgo biloba* has also been shown to be a potential natural anticariogenic agent which exhibits antimicrobial activity against *S. mutans* and suppresses specific virulence factors associated with its cariogenicity

[90]. 7-Epiclusianone, a prenylated benzophenone isolated from *Rheedia gardneriana*, and some cranberry flavonoids (e.g., myricetin, procyanidin A2, and A-type oligomers) have been shown to inhibit the acid-sensitive intracellular glycolytic enzymes by increasing the proton permeability of *S. mutans* cells [72, 161]. A crude extract of *Psidium cattleianum* can also inhibit the expression of proteins involved in general metabolism, glycolysis, and lactic acid production of *S. mutans* [23].

Despite the aforementioned efforts to identify the natural substances potentially active against cariogenic organisms, it is still challenging to determine the precise mechanisms of action and efficacy due to the complexity of chemistry and isolation procedures. Moreover, few studies have actually been conducted in vivo (e.g., using rodent models of dental caries) and even fewer have been evaluated in clinical trials [104]. The implementation of standardized randomly controlled trials is needed to further validate the application of natural products in the field of caries management.

2.5 Ongoing Direction of Oral Dental Plaque Study

2.5.1 Metagenomics and Oral Microbiome

The term "metagenomics" was first invented by Handelsman [85], and it is defined as "the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species." The advances in the refinements of DNA amplification, bioinformatics, and enhanced computational power for analyzing DNA sequences have enabled the adaptation of shotgun sequencing, such as chip-based pyrosequencing, to metagenomic samples [22, 52]. The approach randomly shears DNA, sequences many short sequences, and reconstructs them into a consensus sequence [22].

By performing metabolic function analyses on genes identified via metagenomic approach, researchers are able to retrieve information both on which organisms are present and, more importantly, what functions or metabolic processes are possible in that particular community [70]. Using comparative genetic studies coupled with expression experiments such as microarray and proteomics, microbiologist will be able to piece together a metabolic network that goes beyond species boundaries and gain valuable insight into the metabolism within the community. Recently, a comparative metagenomic study has been initiated, aiming to compare the microbial community within dental plaque associated with healthy and diseased sites. It is anticipated that such comparison will assist in identifying potential pathogenic organisms which may not have been detected using currently available technologies [121].

Our group has been working on the comparative study of oral microbiome using metagenomics-related techniques. By pyrosequencing the "hypervariable regions" of bacterial 16S rRNA of biological samples (i.e., saliva, supragingival plaque, and mucosal plaque) collected from healthy population at different age groups, we have demonstrated that the oral cavity is a highly heterogeneous ecological system containing distinct niches with significantly different microbial communities. More importantly, the phylogenetic microbial structure varies with aging, and only a few taxa were present across the whole populations [231]. By comparing the composition of supragingival plaque microbiota of acute lymphoblastic leukemia (ALL) pediatric patients with healthy controls, we have demonstrated that ALL patients have a structural imbalance of the oral microbiota, characterized by reduced diversity and abundance alterations, possibly involved in systemic infections, indicating the importance of immune status in shaping the structure of oral microbiota [217]. By characterizing the phylogenetic and functional gene differences between periodontal and healthy individuals, we have shown that the phylogenetic and functional gene structures of the oral microbiomes are distinctly different between periodontal and

healthy groups. Specifically, a variety of genes involved in virulence factors, amino acid metabolism, and glycosaminoglycan and pyrimidine degradation are enriched in periodontitis, while the genes involved in amino acid synthesis and pyrimidine synthesis are suppressed in people with periodontitis compared with healthy individuals [128]. Overall, metagenomics-related work from our group and those from others have provided new insights into our understanding of phylogenetic and functional gene structures of oral microbial communities interacting with the host. More cohort studies are needed to further elucidate the contribution of the comparative microbial difference to the pathogenesis and prognosis of oral infectious diseases and hence provide comprehensive knowledge for the development of novel approaches to a better control of dental caries.

2.5.2 Evidence-Based Dental Caries Diagnosis

Although the infectious nature of dental caries has been proved for more than 100 years, instead of treating it as infectious disease, traditional dentistry still focuses on treating the symptom (repair the damaged tooth) via surgical approaches. The recent advancement in caries pathogenesis allows us to understand that a comprehensive analysis of dental caries should be more than detecting tooth demineralization sites and repairing damaged teeth with surgical approaches. Instead, it should include the detection of cariogenic bacteria and plaque acidogenicity, followed by a comprehensive treatment of dental caries that includes the elimination of cariogenic bacteria, the reduction of plaque acidogenicity, and the enhancement of tooth remineralization [204]. The combination of accurate detection of oral bacteria and in situ monitoring of plaque pH, such as the polyaniline-based planer pH sensor developed by scientists at Jet Propulsion Laboratory and combined NMR confocal microscopy from Pacific Northwest National Laboratory which can monitor pH gradient within dental plaque with high sensitivity and in real time [138], is opening a new chapter for cariology. Early detection and quantification of cariogenic bacteria in plaque or saliva samples can help clinicians take preventive measures to stop caries development, much like early detection of cancer markers before overt/detectable cancerous lesions develop. New antibody- or nucleotide-based bacterial detection techniques have also been developed for the detection of cariogenic bacteria in chair-side or laboratory settings [187]. In conjunction with nanotechnology development, these tests can be further developed into different forms of nanochips for the detection of multiple pathogens in the clinical settings [127].

2.5.3 Novel Antimicrobial Therapies

In addition to "traditional" antimicrobial agents mentioned above, new approaches have been developed to maintain oral biofilm homeostasis. Such strategies are aimed to inhibit plaque biofilm formation without disturbing the biological equilibrium within the oral cavity.

2.5.3.1 Probiotics

Probiotics is defined by the World Health Organization as live microorganisms which, when applied in adequate amounts, will benefit the health of the host [5]. The use of probiotics, which has been successful established in the treatment of intestinal diseases, is now also considered for the treatment of oral diseases [162, 206]. Experimental studies and clinical trials have recently demonstrated that certain gastrointestinal bacteria, including *Lactobacillus* and *Bifidobacterium* spp., may help in controlling the proliferation of oral microorganisms, including cariogenic streptococci. *L. rhamnosus* CG [162], *L. casei* [27], *L. reuteri* [30], and Bifidobacterium DN-173 010 [29] have all been shown to have the potential to alter colonization of cariogenic bacteria and thus contributed in the prevention of dental caries. However, cautions should be taken when using the lactobacillus species as they can also produce acids [202]. Mechanisms of probiotic effect within the oral cavity are likely similar to those proposed in gastrointestinal studies [154]. The introduction of microorganisms as a

therapeutic tool for the management of dental caries could possibly act through colonization resistance and/or immune modulation within the oral environment [5].

2.5.3.2 Salivary Antimicrobial Substances

Antimicrobial substances in saliva and their synthetic analogues are also promising in caries control [92]. Whole saliva contains two peroxidase that oxidize thiocyanate (SCN^-) to hypothiocyanite ($OSCN^-$) in the presence of hydrogen peroxide. Hypothiocyanite is antimicrobial and inhibits some streptococci and lactobacilli in vitro [134]. The effect of the salivary peroxidase system is related to the availability of hydrogen peroxide, which is produced by various microorganisms as a metabolic end product [134]. Glucose oxidase produces hydrogen peroxide from glucose provided by the enzyme amyloglucosidase. The addition of these enzymes to oral products is suggested to generate adequate hydrogen peroxide so as to control the growth of microorganisms via enhanced peroxidase effect [59].

2.5.3.3 Specifically Targeted Antimicrobial Peptides (STAMPs)

The benefits of probiotics are mainly achieved by modulating existing microbial flora associated with the host, thus maintaining a balanced and healthy microbe-host relationship. In addition to the use of live organisms, microbiologists are now developing novel techniques and products that do not involve live bacteria, yet generate targeted effects against pathogenic factors or organisms and achieve similar probiotic effects [91]. One good example is the targeted antimicrobial therapy via a novel specifically targeted antimicrobial peptides technology [50, 51]. A "STAMP" is a fusion peptide with two moieties: a killing moiety made of a nonspecific antimicrobial peptide and a targeting moiety containing a species-specific binding peptide. The targeting moiety provides specific recognition of a selected pathogen and targeted delivery of an attached antimicrobial peptide. A pheromone produced by *S. mutans*, namely, competence-stimulating peptide (CSP), has been used as a STAMP targeting domain to mediate *S. mutans*-specific delivery of killing domain. Such STAMPs are potent against *S. mutans* grown in liquid as well as in biofilm cultures [50]. Importantly, STAMPs are able to eliminate *S. mutans* from multispecies biofilms without affecting other noncariogenic commensal residents [50, 51]. Hence, these molecules act as "probiotic" antimicrobials that may selectively eliminate pathogens while preserving the protective benefits of the normal flora. The success in the development of *S. mutans*-specific STAMPs may expand further to the design of other STAMPs specifically targeting various pathogens including periodontal pathogens within oral cavity and provides an ecological approach to the management of dental caries.

2.5.3.4 Light Active Killing

Light is more effective on bacteria in biofilm where chemical agent might encounter diffusion limitations. Light therapy could be used to reduce or modify the oral biofilm and may offer substantial health benefits. *S. mutans* biofilm cells can be killed by up to 3 $\log_{10}$ folds when treated with erythrosine and white light (500–650 nm) [224]. Exposure of *S. mutans* biofilm to blue light had an impeded effect on bacterial viability throughout the biofilm and a sustained antibacterial effect on biofilm newly formed by previously irradiated bacteria [60]. CO_2 laser (wavelength of 10.6 μm) under certain conditions is suggested as a potential novel preventive light therapy against biofilms. The activity of CO_2 laser irradiation on the viability of *S. mutans* in biofilm can reach in the deep layers. However, CO_2 may potentially damage the tooth surface, which limits its application in plaque control [60].

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Saliva and Dental Caries

3

Wang Renke

3.1 Salivary Flow and Composition

3.1.1 Formation of Saliva-Salivary Glands and Secretion

Whole saliva is mainly the mixture of secretion of salivary glands. It also contains gingival crevicular fluid, nonadherent microorganisms, food debris, and human cells including leucocytes and epithelial cells. In normal physiological conditions, the average daily flow of whole saliva is between 1 and 1.5 L [1]. About 90 % of the total volume of saliva is secreted by the three paired major salivary glands – the parotid glands, submandibular glands, and sublingual glands. There are also a number of minor salivary glands situated on the tongue, palate, and buccal and labial mucosa which produce only a small percentage of saliva. However, the function of the minor salivary glands is also important since about 70 % of the total volume of salivary proteins is secreted by them.

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The parotids are serous glands; upon stimulation, they produce watery saliva with high content of enzymes like amylase and lipase, while the secretions of sublingual glands are predominantly mucous, mucin-rich fluids, as same as of those minor salivary glands. And the secretions of submandibular glands are a mixture of mucous and serous fluids. Upon stimulation, the parotid glands produce watery saliva with high content of enzymes like amylase and lipase. The different properties of the secretions of these glands are determined by their different composition of the secretory endpieces, also called acini. Salivary gland fluid is produced by acini [2].

3.1.2 Salivary Composition

The normal salivary pH is slightly acidic, about 6–7, but pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow). Salivary fluid consists of approximately 99 % water and 1 % solutes, including electrolytes, glucose, nitrogenous products like urea and ammonia, and macromolecules. Salivary electrolytes include sodium, potassium, calcium, magnesium, bicarbonate, and phosphate. A variety of proteins are also found in saliva such as enzymes, immunoglobulins, mucins, other antimicrobial factors, mucosal glycoproteins, traces of albumin, some polypeptides, and oligopeptides [3]. The average concentration and function of saliva composition is shown in Table 3.1.

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Table 3.1 The average concentration and function of saliva composition

	Composition	Concentration	Function
Inorganic	Ca^{2+}	1–2 mM	Modulate demineralization and remineralization
	Mg^{2+}	0.2–0.5 mM	
	Na^{+}	6–26 mM	
	K^{2+}	14–32 mM	
	NH_4^{+}	1–7 mM	
	$H_2PO_4^{-}$ & HPO_4^{2-}	2–23 mM	Modulate pH, buffering, modulate demineralization and remineralization
	Cl^{-}	17–29 mM	
	HCO_3^{-}	2–30 mM	Modulate pH, buffering
	F^{-}	0.5–5 μM	Modulate demineralization and remineralization, antibacterial action
	SN^{-}	0.1–2 mM	Antibacterial action
Organic	Urea	2–6 mM	Modulate pH, buffering
	Uric acid	0.2 mM	Indication for kidney function
	Amino acids (free)	1–2 mM	
	Glucose (free)	50 μM	
	Lactate	0.1 mM	
	Fatty acids	10 mg/L	
Macromolecules	Proteins	1.4–2 g/L	Lubricant, cleanse, aggregate, attach to microorganism, dental plaque metabolism, modulate demineralization and remineralization, antibacterial action
	Glycoprotein sugars	0.11–0.3 g/L	Cleanse, aggregate, attach to microorganism, dental plaque metabolism
	Amylase	0.38 g/L	Digestion, antibacterial action
	Lysozyme	0.11 g/L	Antibacterial action
	Peroxidase	3 mg/L	Antibacterial action
	IgA	0.2 g/L	Antibacterial action
	IgG	14 mg/L	Antibacterial action
	IgM	2 mg/L	Antibacterial action
	Lipid	20–30 mg/L	

3.1.3 Salivary Flow Rate and Influence Factors

There is a broad normal range in individual salivary flow rate. The acceptable flow rate is no less than 0.1 mL/min for unstimulated saliva and 0.2 mL/min for stimulated saliva. In normal condition, the unstimulated whole saliva flow rate is 0.3–0.5 ml/min, while stimulated whole saliva flow rate is 1.5–2.0 ml/min and both of them have wide ranges.

The secretion of salivary glands is mainly controlled by parasympathetic impulses from the salivary nuclei. Several factors influence the salivary flow rate, including the nature and duration of stimulus, the emotional state, water balance of the body, and medication (which will be discussed later). The salivary flow and composition of saliva display large variations during the day [4]. The salivary flow rate usually increases during the day and reaches the acrophase in the late afternoon, around 15.00–17.00 h [2]. It's almost

zero during sleep. This rhythm could be related to change of hormone level during the day and indicates the importance of taking oral hygiene procedures before going to sleep. With the absence of protective effects of saliva, if there is much remaining food and dental plaque in the oral cavity, there would be a great chance for dental caries to happen.

Sympathetic impulses are more likely to influence salivary composition by increasing exocytosis from certain cells. Salivary composition may also be influenced by hormones such as androgens, estrogens, glucocorticoids, and peptide hormones.

3.2 Salivary Influences on Plaque PH and Oral Microflora

3.2.1 Salivary Influences on Plaque PH

Acid-producing bacteria, like *Streptococcus mutans* and *Lactobacillus* sp., existed in normal oral flora. These bacteria metabolize fermentable carbohydrates in food and produce variety of organic acids, leading to the reduction of pH value in dental plaque and causing demineralization of hard tissue of teeth.

However, because of the powerful salivary buffering capacity, dental caries is not likely to happen all the time. There are three major buffer systems in saliva: the carbonic acid/bicarbonate, the phosphate, and the protein buffer. The protein buffer mainly takes place in buffering below pH 5, while the optimal buffering range for the carbonic acid/bicarbonate and the phosphate buffer occurs at pH 6.3 and pH 7.2, respectively [5]. The phosphate buffer plays an important role in unstimulated saliva. The secondary phosphate ion, HPO_4^{2-}, could bind a hydrogen ion and form a primary phosphate ion $H_2PO_4^-$. During food intake, its effectiveness is limited due to insufficient concentrations of phosphate in saliva.

The carbonic acid/bicarbonate buffer is the most important buffering system in saliva during food intake and mastication. Bicarbonate is secreted within the ducts and. In resting saliva,

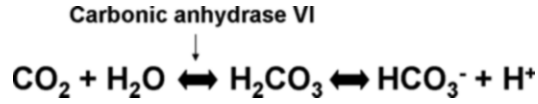

Fig. 3.1 The carbonic acid/bicarbonate equilibrium in saliva

HCO_3^- concentration is as low as 1–2 mM, but it increases with flow rate and reaches 60 mM in stimulated saliva. So during intake, two important events happen: (1) bacteria ferment carbohydrates and produce organic acrid, causing a drop in pH, and (2) the increased salivary flow rate leads to an increased HCO_3^- concentration. The carbonic acid/bicarbonate equilibrium (Fig. 3.1) shifts to the left to produce more carbonic acid. With the presence of carbonic anhydrase VI secreted by the serous acinar cells in parotid and submandibular glands, carbonic acid is further driven to be converted into carbon dioxide and water.

Concerning about caries, the pH of saliva may not be as important as the pH of dental plaque. Both fermentable carbohydrate remainders and salivary buffering capacity affected plaque pH. The resting plaque pH refers to the ph of plaque 2–2.5 h after the last intake of dietary carbohydrates and is usually between 6 and 7. Following exposure to fermentable carbohydrates, the plaque pH decreases rapidly during the first 5 min and reaches a minimum after approximately 5–20 min. Unless there is additional ingestion of fermentable carbohydrates, the plaque pH returns slowly to the starting level over 30–60 min. This plaque pH change over time is known as the Stephan curve.

The buffering capacity of saliva affects not only the rate at which the plaque pH decreases but also the minimum value of plaque pH and how long the pH stays at that minimum. The pH value of 5.6 is called a "critical value" which is the pH at which the tissues start to dissolve and cause demineralization. Low buffering capacity of saliva leads to increased rate of plaque pH fall after carbohydrate intake and prolonged time below critical pH, finally leading to an increased risk of dental caries.

Salivary stimulation after food intake also affects plaque pH. When gum is chewed, the flow of saliva increases from a resting value of 0.4–0.5 ml/min to approximately 5–6 ml/min. A series of studies confirmed an obvious and sustained rise in plaque pH when gum was chewed after sugar consumption. Both sugar-containing and sugar-free gum have such effect, but sugar-free gum displays a more powerful effect [6]. Chewing other material that could stimulate salivary secretion such as parafilm also works.

3.2.2 Salivary Influences on Oral Microflora

Besides affecting plaque pH, saliva has profound influence on oral microflora. Saliva provides a nutritious source for oral microflora. Salivary proteins, glycoproteins, peptides, and minerals can stimulate the growth of oral microflora and enhance biofilm formation. On the other hand, saliva displays antimicrobial activities with the presence of a series of immunologic (secretory IgA, IgG, IgM) and non-immunologic factors (enzymes, mucins, proteins, peptides). Lysozyme, lactoferrin, and histatins exhibit bacteriostatic, bacteriocidic activity, while immunoglobulins, mucins, and salivary agglutinin play a role in the oral clearance of bacteria by interfering with receptors on the microbial cell wall.

The immunoglobulins including IgG, IgM, IgA, and secretory IgA (SIgA) form the specific defense system in saliva against bacteria. SIgA is the most abundant immunologic component in saliva, mainly produced by plasma cells located in minor mucous glands. SIgA is non-detectable in neonates but become readily detectable 1 week after birth. It can neutralize pathogenic viruses, toxins, and enzymes produced by bacteria. SIgA can prevent bacteria forming colonies or attaching or penetrating host tissues, kill them directly, or activate complements or provide synergism with innate defense mechanisms. It's also able to aggregate or clump bacteria, promoting oral clearance. SIgA against streptococcus mutans can be detected in children at the age of 3 years old, and the quantity increases with the length of exposure. Other immunologic components occur in less quantity in saliva. IgG is the only detectable Ig in saliva of neonates and is mainly maternal origin. The concentration of IgG decreases to non-detectable after a few months after birth and appears again after tooth eruption. At this time, IgG mainly comes from gingival crevicular fluid, originating from sera.

Lysozyme is a cationic protein with low molecular weight from the basal cells of striated ducts in parotid glands as well as monocytes and macrophages. It is one of the most important nonspecific defense substances in saliva. Lysozyme catalyzes cleavage of beta-1,4 glycosidic bonds between muramic acid and *N*-acetylglucosamine residues in peptidoglycan; hence it hydrolyzes bacterial cell wall. Due to the external lipopolysaccharide layer, Gram-negative bacteria are more resistant to lysozyme. Furthermore, nonenzymatic bactericidic effect of lysozyme related to activation of bacterial autolysins is reported as well. It is also documented that this enzyme functions to aggregate bacteria and inhibit bacterial adherence.

There are two categories of salivary peroxidases: human salivary lactoperoxidase (HS-LPO), also termed sialoperoxidase, synthesized in salivary glands, and myeloperoxidase (MPO) formed in polymorphonuclear leucocytes. Salivary peroxidases have enzymatic activity and are able to catalyze the oxidation of salivary thiocyanate and hypothiocyanous acid, inhibiting bacterial metabolism [7].

Salivary chitinase secreted by salivary glands catalyzes the hydrolytic cleavage of chitin. Chitin is a cellular wall compound of yeast cells; hence, chitin may play a role in the protection against yeast.

Mucins are glycoproteins secreted from submandibular and sublingual glands that selectively modulate the microorganism adhesion. There are two genetically distinct mucin groups: highly glycosylated, high-molecular-weight MG1 (>1000 kDa) and single-glycosylated peptide chain, low-molecular-weight MG2 (200–300 kDa). The two groups of mucins display similar carbohydrate chain makeup but have different bacterial adherence ability. MG1 binds tightly to tooth surface and takes part in enamel pellicle formation. MG2 promotes the aggregation

and clearance of oral bacteria, including *Streptococcus mutans*. It's reported that MG2 predominates in saliva of caries-resistant individuals, while the level of MG1 is higher in caries-susceptible individuals [8].

Salivary agglutinin (SAG) is a high-molecular-weight (approximately 340 kDa), mucin-like glycoprotein aggregate cells in suspension that can be found in parotid and submandibular saliva. SAG is highly glycosylated and extremely sticky. It could bind to hydroxyapatite and is a component of the enamel pellicle and may be involved in the initial adherence of bacteria to the enamel surface.

SAG binds to streptococci surface receptor antigen I/II in a calcium-dependent manner. Furthermore, SAG also binds to SIgA, resulting in synergistic effect of bacterial aggregation which promotes clearance of microorganisms from the oral cavity [9, 10].

Lactoferrin is a glycoprotein with a molecular weight of about 80 kDa. It is a member of a transferring family and able to link to ferric iron in saliva and lead to bactericidal or bacteriostatic effects on bacteria requiring iron for metabolism including streptococcus mutans by iron-depriving effect. This process of starving bacteria of vital nutrients is termed nutritional immunity. It's reported that lactoferrin is a multifunctional protein having bacteriostatic, bactericidic, fungicidal, antiviral, anti-inflammatory, and immunomodulatory properties [11, 12].

Histatins are a family of peptides rich in histidine, arginine, and lysine residues. At least 12 histatin-like peptides are identified in human saliva. Histatins have antimicrobial activity against some strains of *Streptococcus mutans* and yeasts. They are implicated in pellicle formation, neutralization of lipopolysaccharides of the external membranes of Gram-negative bacteria, chelation of metal ions, and inhibition of proteinases including metalloproteins and cysteine proteinases.

Saliva provides nutrients to oral microorganisms and supports their growth. On the other hand, saliva has a protective function in maintaining oral health and microbial ecological balance by inhibiting pathogens and regulating pH in oral environment.

3.3 Xerostomia and Its Management

3.3.1 Etiology of Xerostomia

Xerostomia, also termed dry mouth or dry mouth syndrome, is referred to the subjective symptom of oral dryness, which is frequently, but not always, associated with salivary gland hypofunction and a significant reduction of unstimulated salivary secretion. As mentioned above, the normal flow rate of unstimulated saliva is about 0.3–0.5 ml/min, while that of stimulated saliva is about 1.5–2.0 ml/min. It would be considered as hypofunction of salivary glands if unstimulated flow rate is below 0.1 ml/min or stimulated flow rate is below 0.5 ml/min. As there is a wide range of salivary flow rate, a 50 % reduction of individual salivary flow rate would also be considered hypofunction [13, 14].

Saliva is secreted by three pairs of major salivary glands and the minor salivary glands. Any factors that could influence salivary gland function, including diseases of salivary glands, Sjögren's syndrome, radiotherapy in the head and neck area, the use of certain drugs, etc., might lead to the reduced output of salivary secretion, causing xerostomia. Besides iatrogenic factors, aging could be another important cause for xerostomia. As life expectancy in developed countries keeps increasing, xerostomia is becoming increasingly common in the result of an increased incidence of systemic diseases and a more extensive intake of medication and/or the degeneration and reduced volume of acini. Table 3.2 shows the common causes of xerostomia.

A lot of drugs have influence on salivary flow rate and composition, including medication for hypertension, depression, and allergies. Over 500 medications produce xerostomia as a side effect. A medication which is known to cause xerostomia may be termed xerogenic. Table 3.3 lists some drugs that may cause xerostomia.

Xerostomia is a common side effect of radiation therapy of tumors in the region of the head and neck. The parotid gland is the most radiosensitive gland and then the submandibular gland, then the sublingual gland, and the minor salivary

Table 3.2 Common causes of xerostomia

Class	Cause
Iatrogenic	Drugs
	Local radiation
	Chemotherapy
	Chronic graft-versus-host disease
Diseases of salivary glands	Sjögren's syndrome
	Sarcoidosis
	HIV disease
	HCV infection
	Primary biliary cirrhosis
	Cystic fibrosis
	Diabetes mellitus
Rare causes	Amyloidosis
	Hemochromatosis
	Wegner's disease
	Salivary gland agenesis (with or without ectodermal dysplasia)
	Triple A syndrome
Others	

Cited from Porter et al. [15]

Table 3.3 Medications associated with xerostomia

Class	Generic name
Anticonvulsants	Atropine, hyoscine
Antipsychotics	Phenothiazine
Antidepressants	Amitriptyline, fluoxetine, lithium, bupropion, dothiepin
Antihistamines	Diphenhydramine, cimetidine
Antihypertensives	Terazosin, prazosin, clonidine, atenolol, propranolol
Antireflux drugs	Omeprazole
Opioids	Meperidine
Antineoplastics	Cytotoxic drugs, retinoids, interleukin-2
Anti-HIV drugs	Protease inhibitors, didanosine
Diuretics	Chlorothiazide, furosemide, antisterone
Antiasthmatic	Ephedrine
Anxiolytics	Benzodiazepines

gland. As reported, a single dose above 52 Gy could lead to severe salivary dysfunction, so conventional radiation treatment of oral carcinoma at a dose of 60–70 Gy would cause a rapid reduction of salivary flow rate during the first week of treatment. By 5 weeks of the treatment, the salivary flow almost reduces to zero and hardly recovers completely after the treatment.

Besides the factors mentioned above, there are a number of additional disorders that may contribute to the presence of xerostomia, including thyroid dysfunction, Parkinson's disease, systemic lupus erythematosus, post-traumatic stress disorders, depression, anxiety, etc. These disorders influence the salivary flow either through pathophysiological process or the medical treatment of the disease.

Xerostomia is a common condition in population. It's reported that 25 % of general population complain of xerostomia or symptoms associated with it. For elderly, the incidence is as high as 40 %. With such a high incidence, the influence of xerostomia in life quality brings to great attention. As the quantity of saliva secretion reduced reversibly or irreversibly, patients could experience different extent of dysarthria, dysphagia, mucosal trauma and ulceration, candida infection, and dental caries. The clinical consequences and management had been comprehensively discussed in a review [15]; here we will mainly focus on dental caries related to xerostomia and its management [15].

One of the most important functions of saliva is to develop dental pellicle on enamel surface, protecting against demineralization and replenishing tooth surface minerals including calcium and phosphate. It also provides buffering activity, antibacterial activity, and effective carbohydrate clearance. As salivary output decreases, a series of respondents take place with the outcome of increased demineralization and decreased remineralization and virtually lead to dental caries (Fig. 3.2).

3.3.2 Management of Xerostomia

Xerostomia patients should be instructed to observe a variety of caries preventive procedures, including oral hygiene instruction, plaque control, low sugar dietary advice, daily use of topical fluoride (0.05 %), antimicrobial mouse rinses (e.g., chlorhexidine), and placement of sealants. The most important one is to reduce the intake of

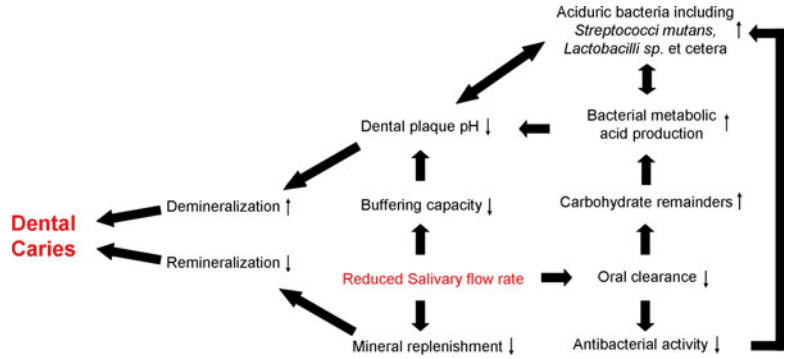

Fig. 3.2 The chain reaction of reduced salivary flow rate

sugars. Sugar intake should be confined to meals, and there should be no sugar consumption between meals. Non-cariogenic sugar substitutes including xylitol, sorbitol, aspartame, Lycasin, saccharin, and sucralose, which could not be fermented by acidogenic bacteria into organic acids, should be used to reduce sugar consumption [16].

Xerostomia patients should be recommended to brush teeth twice a day with a soft bristle tooth brush along with low abrasive, high fluorinated toothpaste. Fluoride is also available in other forms like foam, varnish, rinse, and gel. It is recommended by ADA that the daily use of fluoride rinses and toothpaste accompanied with fluoride varnish applied every 3 months is helpful in dental caries inhibition in xerostomia population [17].

The use of remineralization products is a relative new strategy against dental caries in xerostomia patients. It provides calcium and phosphate ions that are lacking because of reduced salivary output. These products usually contain different types of calcium and phosphate compound with or without additional fluoride in the form of paste or medication carrier (products without fluoride). Applying to the tooth surface, they can provide and attract calcium and phosphate ions to the tooth surface, aiding in remineralization.

3.4 Saliva and Caries Risk Assessment

Dental caries is a multifactorial infectious disease that is associated with complex interactions among acid-producing bacteria, fermentable carbohydrates, and host factors including saliva status. To estimate the caries risk before disease has been given more attention in recent years since dental caries is generally considered as a kind of progressive disease unless intervened surgically. Several caries risk assessment methods have been developed as indicators of caries susceptibility at the individual level. They can be used to estimate the probability of caries incidence, determine the need for therapeutic intervention, and are a part of treatment planning.

Generally, these tests are derived from the severity of past and current caries experience, diet, protective factors including fluoride, behavioral and physical factors, medical factors, socioeconomic status, measurement of saliva flow rate and buffering capacity, and estimation of caries-related microorganisms in saliva including *Streptococcus mutans* and *Lactobacilli* (Tables 3.4, 3.5 and 3.6) [19].

3.4.1 Caries-Associated Bacteria

There are more than 700 species of microorganisms inhabit the oral cavity. In healthy individuals, caries-associated bacteria are usually present in relatively small amount in saliva. But in conditions of biological or environmental change such as increased frequency of carbohydrate intake or poor oral hygiene, the etiological balance of oral microflora will shift to favor the aciduric bacteria and acidogenic bacteria, increasing the risk of dental caries.

Plaque bacterial composition is most related to dental caries since acid causing demineralization is mainly produced by plaque bacteria. Since

Table 3.4 ADA caries risk assessment form for age 0–6

		Low risk	Moderate risk	High risk
Contributing conditions				
I	Fluoride exposure (through drinking water, supplements, professional applications, toothpaste)	Yes	No	
II	Sugary foods of drinks (including juice, carbonated or non-carbonated soft drinks, energy drinks, medical syrups)	Primarily at mealtimes	Frequent or prolonged between meal exposures/day	Bottle or sippy cup with anytime other than water at bed time
III	Eligible for government programs (WIC, Head Start, Medicaid, or SCHIP)	No		Yes
IV	Caries experience of mother, caregiver, and/or other siblings	No carious lesions in last 24 months	Carious lesions in last 7–23 months	Carious lesions in last 6 months
V	Dental home: established patient of record in a dental office	Yes	No	
General health conditions				
I	Special health-care needs (developmental, physical, medical, or mental disabilities that prevent or limit performance of adequate oral health care by themselves or caregivers)	No		Yes
Clinical conditions				
I	Visual or radiographically evident restorations/cavitated carious lesions	No new carious lesions or restorations in last 24 months		Carious lesions or restorations in last 24 months
II	Non-cavitated (incipient) carious lesions	No new lesions in last 24 months		New lesions in last 24 months
III	Teeth missing due to caries	No		Yes
IV	Visible plaque	No	Yes	
V	Dental/orthodontic appliances present (fixed or removable)	No	Yes	
VI	Salivary flow	Visually adequate		Visually Inadequate

plaque bacteria can be released into saliva, it is documented that the level of certain species of bacteria in saliva can reflect their level in dental plaque, making salivary microflora an effective biomarker of the health and disease status of oral cavity [20, 21].

Streptococcus mutans and *Lactobacilli* have been implicated as important contributory species in dental caries. It is reported that high level of salivary *Streptococcus mutans* (>10^5 CFU/ml) is associated with an increased risk of dental caries [22–24]. Although the amount of lactobacilli is less sensitive in predicting caries incidence than the amount of mutans streptococci [25], the high level of *Lactobacilli* (>10^6 CFU/ml) [26] could be an indicator for increased frequency of carbohydrate consumption [27] and caries progression [28].

Most of tests regarding caries-associated bacteria require incubation which makes them less convenient for dental practitioners. Recently, culture-independent technologies including checkerboard DNA-DNA hybridization, 16S rRNA sequence analysis, and T-RFLP have been utilized for dental caries microbial analysis. These molecular methods have not applied clinically in large scale but definitely suggest new possibilities for fast, convenient, and culture-independent methods of caries-associated bacteria test.

Table 3.5 ADA caries risk assessment form for age>6

		Low risk	Moderate risk	High risk
Contributing conditions				
I	Fluoride exposure (through drinking water, supplements, professional applications, toothpaste)	Yes	No	
II	Sugary foods of drinks (including juice, carbonated or non-carbonated soft drinks, energy drinks, medical syrups)	Primarily at mealtimes		Frequent or prolonged between meal exposures/day
III	Caries experience of mother, caregiver, and/or other siblings (for patients ages 6–14)	No carious lesions in last 24 months	Carious lesions in last 7–23 months	Carious lesions in last 6 months
IV	Dental home: established patient of record in a dental office	Yes	No	
General health conditions				
I	Special health-care needs (developmental, physical, medical, or mental disabilities that prevent or limit performance of adequate oral health care by themselves or caregivers)	No	Yes (over age 14)	Yes (ages 6–14)
II	Chemo-/radiation therapy	No		Yes
III	Eating disorders	No	Yes	
IV	Medications that reduce salivary flow	No	Yes	
V	Drug/alcohol abuse	No	Yes	
Clinical conditions				
I	Cavitated or non-cavitated (incipient) carious lesions or restorations (visually or radiographically evident)	No new carious lesions or restorations in last 36 months	1 or 2 new carious lesions or restorations in last 36 months	3 or more carious lesions or restorations in last 36 months
II	Teeth missing due to caries in past 36 months	No		Yes
III	Visible plaque	No	Yes	
IV	Unusual tooth morphology that compromises oral hygiene	No	Yes	
V	Interproximal restorations, 1 or more	No	Yes	
VI	Exposed root surfaces present	No	Yes	
VII	Restorations with overhangs and/or open margins; open contacts with food impaction	No	Yes	
VII	Dental/orthodontic appliances present (fixed or removable)	No	Yes	
IX	Severe dry mouth (xerostomia)	No		Yes

Table 3.6 The strength of association between salivary characteristics and caries risk

Strong association	Weak-to-moderate association	No association
Flow rate	Buffering capacity; calcium/phosphate; specific sIgA immunoglobulin	pH, glucose clearance rate/concentration; other electrolytes and small organic molecules; total sIgA; IgG; innate immunity factors

Cited from Leone et al. [18]

3.4.2 Chemical and Physical Aspects of Saliva

Leone et al. had reviewed 96 references and divided the chemical and physical characteristics of saliva into three groups according to their strength of association with dental caries (Table 3.6) [18].

It has been discussed earlier in this chapter that low salivary flow rate is a risk factor of dental caries. Statistical data suggests that unstimulated salivary flow less than 0.3 ml/min and the stimulated salivary flow lower than 0.8–1.0 ml/min indicate increased caries risk strongly. But these values should not be treated as an absolute standard for caries risk screening since there is a wide range of salivary flow rate among individuals. An obvious reduction of salivary output in one individual should be paid attention to as well.

A number of studies showed the correlation between low salivary buffering capacity and dental caries, while high buffering capacity indicates better neutralizing capacity and more resistance to demineralization.

The level of specific secretory IgA showed a relationship with caries risk, and the literature is almost equally divided for and against an anticaries role of specific secretory IgA. No evidence has indicated sufficient association between caries risk and salivary innate non-immunoglobulin factors including lysozyme, lactoferrin, peroxidase/myeloperoxidase, praline-rich proteins, statherins, and histatins. On the other hand, Mungia et al. found significant associations between caries and specific individual submandibular/sublingual salivary proteins including lactoferrin, albumin, lysozyme, mucin, and cystatin recently [29]. But if it is an indicator of caries risk still needs more data and further investigation.

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4 Demineralization and Remineralization

Cheng Lei, Li Jiyao, Xu Hockin H.K., and Zhou Xuedong

Dental caries is caused by acids produced from bacterial metabolism diffusing into dental hard tissue and dissolving the mineral. The process of dental caries is a continuum which results from many de-/remineralization cycles [1, 2]. Dental hard tissue consists of enamel, dentin, and cementum. Dental enamel is highly mineralized, and it comprises 80–90 % by volume of a calcium-deficient carbonate hydroxyl apatite. Other calcified tissues (bone or dentine) contain considerably lower amounts of inorganic minerals. Mature human enamel crystallites are 26.3 ± 2.2 nm thick, 68.3 ± 13.4 nm wide, and between 100 and 1,000 nm long [3]. Dentine and cementum contain a much greater proportion of organic matrix. Dentine is made up of approximately 50 vol.% mineral, 30 vol.% collagenous and non-collagenous proteins, and 20 vol.% fluids. The dentinal matrix is mainly composed of type I collagen fibrils forming a three-dimensional scaffold matrix, reinforced by hydroxyl apatite crystallites, measuring approximately 20 nm in size [3].

Sound enamel and dentine crystals are comprised of a hydroxyapatite-like mineral containing many impurities and inclusions of other ions. The mineral phase of the dental hard tissues is not pure hydroxyapatite (HAP = Ca_{10} $(PO_4)_6$ OH_2). Hydrogen phosphate, carbonate, and magnesium ions are incorporated into the HAP lattice to form a less stable, more soluble apatite. For example, approximately 1 out of 10 of the phosphate ions in enamel is replaced by carbonate ions and 1 out of 5 in dentine [4, 5]. So the mineral of enamel and dentine is much more soluble than pure hydroxyapatite or fluorapatite. But the partial substitution of fluoride ions for OH groups

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in the crystal lattice can stabilize the apatite structure less susceptible to acid attack.

4.1 Dynamics Process of De-/Remineralization

Dental caries is a disease that is manifested as a dynamic process of de-/remineralization in the mouth (Fig. 4.1). Demineralization is a continual imbalance between pathological and protective factors that results in the dissolution of apatite crystals and the net loss of calcium, phosphate, and other ions from the tooth [6]. The first stage of demineralization is occurring at the atomic level far before it can be seen visually as gross demineralization. During this step, fermentable carbohydrates are metabolized by bacteria in dental plaque to produce organic acids. The acids diffuse into the dental hard tissue through the water among the crystals and could reach a susceptible site on a crystal surface. Calcium and phosphate are dissolved into the surrounding aqueous phase between the crystals [2]. This is considered as the first step in the continuum of the dental caries process which can eventually lead to cavitation.

The oral fluids (saliva, biofilm fluid) have calcium (Ca) and phosphate (Pi) in supersaturated concentrations with respect to the mineral composition of enamel. At physiological conditions (a neutral pH of 7), low ion concentrations are sufficient to keep dental hard tissues in equilibrium. If the pH drops because of acid produced by the dental plaque, higher ion concentrations are needed to prevent dissolution of dental hard tissue. Calcium (Ca) and phosphate (Pi) ions are continually deposited on the enamel surface or are redeposited in enamel areas where they were lost. At a pH of ca. 5.5, undersaturation begins, that is, the calcium- and phosphate-ion concentrations in the plaque fluid are not sufficient to maintain the enamel in stable equilibrium; thus, the enamel starts to dissolve.

The term "remineralization" is used to described mineral gain. Remineralization is the body's natural repair process for subsurface non-cavitated carious lesions [7]. In the process of remineralization, calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain.

De-/remineralization cycles continue in the mouth as long as there are factors including cariogenic bacteria, fermentable carbohydrates, and saliva present. The balance between pathological factors and protective factors determines whether demineralization or remineralization is proceeding at any one time [2].

4.2 Investigations of De-/Remineralization

4.2.1 Models

The de-/remineralization process can be studied using different kinds of models, such as in vitro model, in situ model, animal models, or

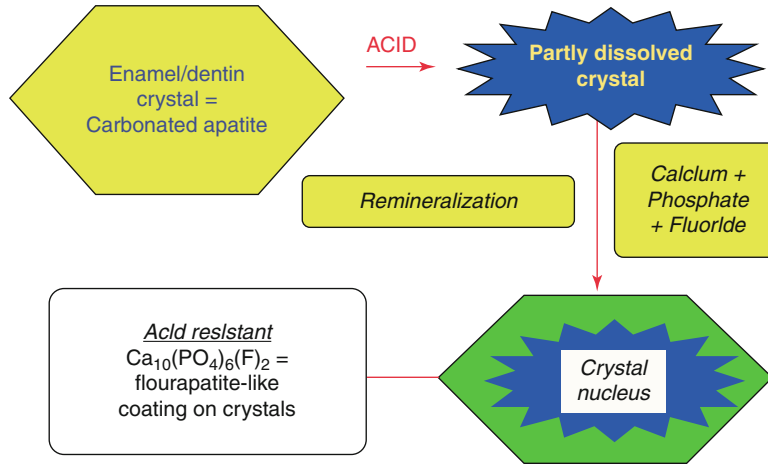

Fig. 4.1 The caries process including demineralization and subsequent remineralization to form a low solubility surface on the crystals [2]

in randomized controlled clinical trials [8–10]. The induction of artificial carious lesions in bovine and human teeth is an important tool to study strategies for the prevention or treatment of carious lesions. Various models can be selected according to different purposes. Each model has its advantages along with disadvantages. For example, in vitro experiments are the most commonly applied methods. They can be performed over a short period of time, require fewer staff than in situ studies, avoid participant compliance issues, and are relatively inexpensive. But they cannot replicate the oral environment with all of the biological variations known to influence de-/remineralization process [11, 12].

4.2.1.1 In Vitro Chemical Model

The modern pH-cycling models were first produced by ten Cate and Duijsters [13]. In vitro pH-cycling models are widely used, especially for the in vitro evaluation of the efficacy of fluoridated dentifrices for caries control [14]. They are also broadly used in profile studies for rapid and inexpensive testing of developing and recently marketed products [9]. There are several advantages of in vitro pH cycling: (i) the model can mimic the dynamics of mineral loss and gain involved in caries formation; (ii) the high level of scientific control and the resulting lower variability intrinsic to in vitro models; and (iii) it requires smaller sample size [14].

4.2.1.2 In Vitro Biofilm Model

Dental plaque biofilms play a pivotal role in the progression of dental diseases, so in vitro biofilm models are developed to produce artificial caries lesions [15]. Two main aspects should be considered when the most suitable biofilm model for a de-/remineralization investigation was chosen: (1) to select pure cultures or defined communities or microcosms and (2) to select closed system biofilm models or open system biofilm models [15].

Streptococcus mutans are considered the most cariogenic microorganisms in dental biofilm due to their capacity to use dietary carbohydrates to synthesize extracellular polysaccharides (EPS) and because of their acidogenic and aciduric properties [16]. And *S. mutans* biofilm models as single-species biofilm model are widely used in de-/remineralization investigations [17–19]. Multiple species biofilm models using defined consortia could achieve a high degree of reproducibility between experimental runs which cannot be relied upon when using complex inocula [15, 20, 21]. Microcosms are able to maintain much of the complexity and heterogeneity of the original sample. Saliva was usually collected to form microcosm biofilms to replicate enabling in situ bacterial community dynamics within the laboratory environment [22, 23].

Biofilm models can be divided into two groups according to whether they are "closed" or "open" with respect to nutritional availability [15]. Closed system biofilm models are analogous to batch culture and usually based on multi-well plates [18, 24]. Silva TC et al. applied an active attachment biofilm model as a high-throughput demineralization biofilm model for the evaluation of caries-preventive agents (Fig. 4.2a) [18]. Zürich biofilm model is another typical closed system biofilm models applied in de-/remineralization experiments [21, 25]. Open system biofilm models are analogous to continuous culture. McBain AJ. et al. reviewed different kinds of open system biofilm models [15]. The constant depth film fermentor (CDFF) was widely used for de-/remineralization investigations [23, 26, 27]. The CDFF allows the generation of large numbers of replicate biofilms which can be maintained at a constant depth by static scraper blades (Fig. 4.2b).

4.2.1.3 In Situ Model

In situ models are also widely used for de-/remineralization experiments now. In situ models involve the use of devices creating conditions that simulate the process of dental caries. Enamel and dentin samples are the hard tissue substrates used in in situ models to assess de- and remineralization [8]. The in situ models are designed to simulate the natural process of de-/remineralization and also to provide information in a short period of time without causing damage to the natural teeth of volunteers [28]. These models serve as a link between the clinical uncontrolled situation and the highly controlled laboratory experiments. The in situ caries model designs are highly

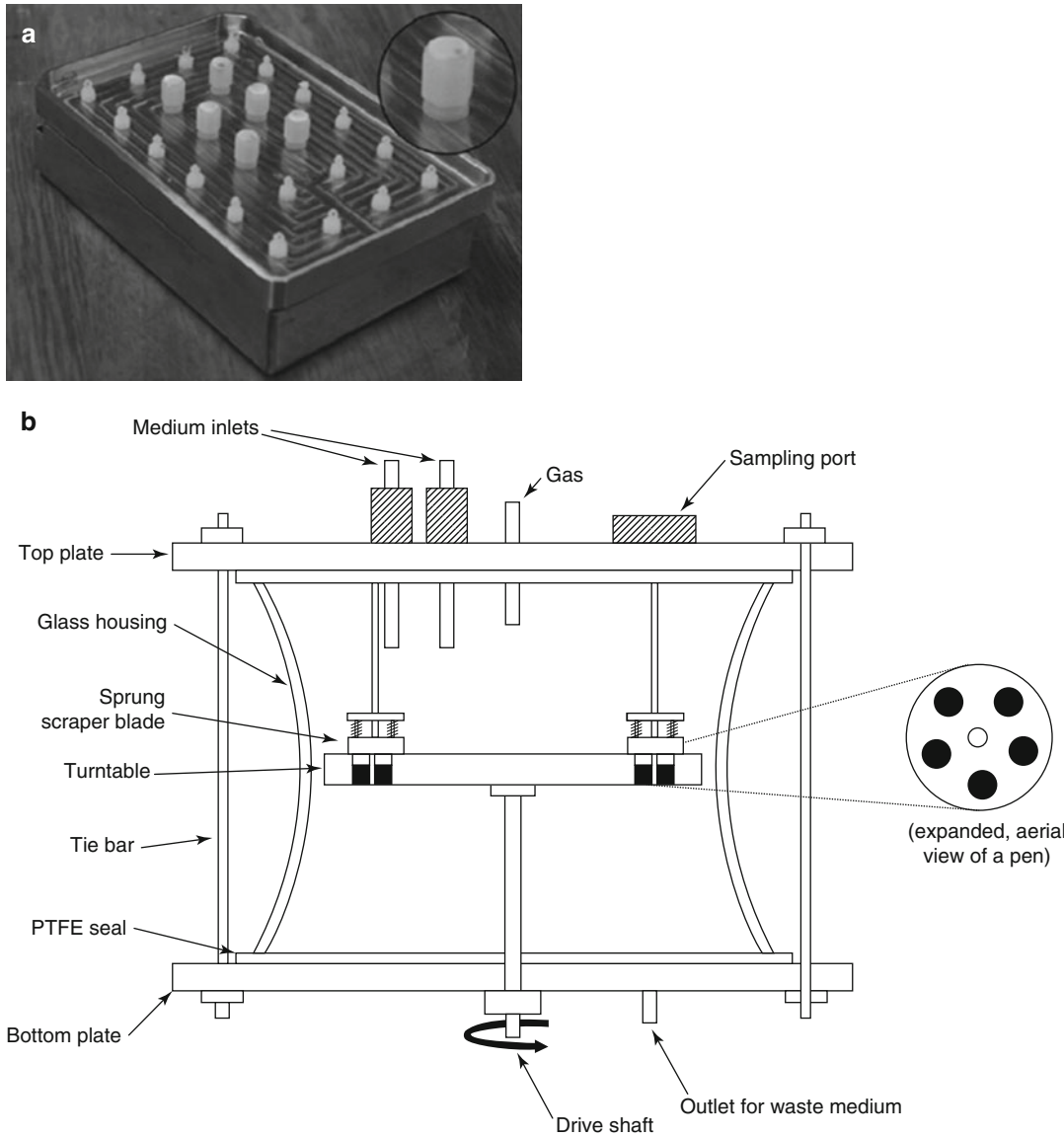

Fig. 4.2 In vitro biofilm models, (**a**) an active attachment biofilm model [18]; (**b**) CDFF [15]

variable because of the variations of the in situ study designs. All in situ studies must have appropriate controls including a positive and negative control where possible [8].

4.2.1.4 Animal Model

For many reasons—particularly time considerations, animal availability, and attendant costs—rodents have been the most commonly used species for experimental caries studies [21]. According to Stookey et al.'s investigations, the following rat caries models could be used for evaluating fluoride dentifrices: Francis' hypomineralized area model, Gaffar's CARA rat model, the Connecticut model, and the Indiana model [29].

4.2.2 Detection and Measurement Methods

Various techniques have been used to investigate the mineral loss and gain during de-/remineralization process, including destructive and nondestructive methods.

4.2.2.1 Transversal Microradiography (TMR)

TMR or contact-microradiography is a highly sensitive method used to measure the morphology of and the change in mineral content of dental hard tissue [30]. But the method is usually destructive to dental hard tissue, so it cannot be used to study any longitudinal mineral changes in exactly the same lesions [31]. To prepare the samples for TMR investigation, thin slices (about 80 μm for enamel samples and 200 μm for dentine samples) are cut perpendicularly to the tooth surface. A microradiographic image is made on high-resolution film by X-ray exposure of the sections together with a calibration aluminum step wedge. The mineral can be automatically calculated from the gray levels of the images of section and step wedge. Parameters of interest are mineral loss (Delta Z in Vol%.μm), lesion depth (Lesd in μm), ratio or average loss of mineral content in the lesion area (Delta Z/Lesd in Vol%), and the mineral vol% and position of the subsurface layer and lesion body [32, 33].

4.2.2.2 Indentation Techniques

Indentation techniques have been used to measure the hardness of the dental hard tissue surface. There are two kinds of indentation techniques for de-/remineralization investigations: microindentation (surface hardness) [32] and nanoindentation (ultra-microindentation) [34]. During the process of both microindentation and nanoindentation, a diamond tip of known dimensions is pressed onto a surface with a given load and duration. The microindentation technique yields data in arbitrary units, usually Knoop hardness number (KHN) or Vickers hardness number (VHN), and nanoindentation yields hardness and reduced elastic modulus in the SI unit of Pascals (Nm^{-2}) [35]. Moron BM et al. revealed that surface hardness analysis should not be interpreted with respect to dentine mineral loss [11]. This was expected as the high organic content, and thus, the elastic properties of the dentine influence the hardness measurement [36].

4.2.2.3 Micro-CT

Micro-CT investigation is a nondestructive method to measure the mineral changes of dental hard tissue. So it is possible to measure and visualize longitudinal mineral changes during de- and remineralization in the same lesion [37]. Published papers proved that micro-CT could offer the quantitative analysis of the de- and remineralization based on CT intensity data [31]. The key point of using micro-CT is to find out how to converse the CT intensity into mineral content. Neves et al. reported a linear correlation between CT intensity (or gray scale value) and the mineral density using three apatite phantoms, the linearity covering a range of 0.25–3.14 g/cm^3 [38]. Schwass et al. found a good linearity using six phantoms covering a range from 0.07 to 2.95 g/cm^3 [39].

4.2.2.4 Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) is able to identify tissue-emitting fluorescent signal and can be used to detect the mineral loss of dental hard tissue [40–42]. Demineralizing dentin has a strongly increased autofluorescence compared to sound dentine [43]. Some previous studies stained thick enamel samples with a fluorescent dye (0.1 m*M* rhodamine B) and analyzed using CLSM for quantitating demineralization and remineralization of enamel specimens [44].

4.2.2.5 Quantitative Light-Induced Fluorescence

Quantitative light-induced fluorescence (QLF) is a quantification system for assessing early demineralization or remineralization of human enamel. When high-intensity blue light illuminates the teeth, the resultant autofluorescence of enamel is detected by an intraoral camera. The emitted fluorescence has a direct relationship with the mineral content of the enamel. The intensity of the tooth image at a demineralized area is darker than the sound area. The software of QLF systems can process the image to provide user quantitative parameters such as lesion area, lesion depth, and lesion volume [45–47].

4.2.2.6 Optical Coherence Tomography

Optical coherence tomography (OCT) is a three-dimensional optical imaging technique which works in a similar way to ultrasound, but uses high-frequency light (around 820 nm) instead of high-frequency sound [48]. It is a noninvasive, cross-sectional imaging system that can visualize

the internal structures nondestructively [49]. Amaechi et al. developed a quantitative method to detect the demineralizing lesions of dental enamel using an OCT system [50]. This system was able to collect A-scans (depth versus reflectivity curve), B-scans (longitudinal images), and C-scans (transverse images at constant depth). The area (R) under the A-scan could be quantified to indicate the degree of reflectivity of the tissue.

4.3 Methods to Influence the De-/Remineralization Process

4.3.1 Traditional Methods

4.3.1.1 Fluoride

Fluoride was introduced into dentistry over 70 years ago, and it is now recognized as the main factor responsible for the dramatic decline in caries prevalence that has been observed worldwide [51]. Fluoride can be obtained in two forms: systemic and topical. Systemic methods include water fluoridation, salt fluoridation, milk fluoridation, and supplements. Later in the 1940s, the well-conducted water fluoridation program was established in the United States. Though some dentists and researchers have conflicting opinions about their safety and benefits, these systemic methods are still recommended in many countries and receive support from recognized international committees and associations. Due to the widespread application of fluoride and the updated knowledge about its mechanisms of action, topical applications of fluoride (e.g., fluoride toothpastes, gels, varnishes, and mouthwashes) are considered to be more effective methods for caries prevention than systemic use of fluorides [52].

It is believed that fluoride could inhibit demineralization and enhance remineralization [7, 53–55]. Numerous studies were designed to investigate the mechanism of fluoride in inhibiting demineralization of dental hard tissue. According to previous studies, fluoride could incorporate into the enamel apatite structure, enhance the resistance of the dental hard tissue to acidic challenges, and thus inhibit lesion development [7, 56]. In addition, calcium-fluoride-like deposits could form on dental hard tissues and act as a protective barrier on the surface and serve as a reservoir for fluoride [53]. Other researchers have some different opinions. They demonstrate that the incorporation of fluorides into the mineral components of enamel only slightly reduced its solubility [54, 55, 57]. Small amounts of free fluoride ions in solution around the dental hard tissue play a much more important role in inhibiting demineralization. These fluorides have a much greater caries-protective potential than a large proportion of FAP incorporated in enamel mineral [58]. Free fluoride ions are in part adsorbed onto the crystalline surface and are in dynamic equilibrium with the fluoride ions in solution around dental hard tissue. So it forms an equilibrium or supersaturation relative to fluor(hydroxy)apatite, and the adsorption of fluoride on the crystals can offer direct protection from demineralization. Therefore, according to this theory, fluoride should be present in the right place (biofilm fluid or saliva) and at the right time (when biofilm is exposed to sugar or right after biofilm removal), and even small amount of fluoride (below ppm values) available is effective.

In addition to its ability of inhibiting demineralization, fluoride is thought to be effective to promote remineralization. However, fluoride's ability to enhance net remineralization is limited by the availability of calcium and phosphate ions [59]. If adequate salivary or plaque calcium and phosphate ions are available, fluoride ions can drive the remineralization of extant non-cavitated caries lesions. Fluorhydroxyapatite forms more rapidly even in slightly acidic condition than do the other calcium phosphate phases, so fluoride can accelerate and promote remineralization of dental hard tissue.

It has been demonstrated that fluoride used in enamel remineralization also work in dentine remineralization, and even their remineralization mechanisms are similar [60, 61]. However, dentin demands a considerably higher fluoride concentration in its surrounding solution than enamel does to reach an equivalent degree of demineralization inhibition [62].

4.3.1.2 Calcium Phosphate

Calcium and phosphate ions play important roles in enhancing remineralization of dental caries. Investigators have tried various solutions containing calcium and phosphate ions in their experiments, in which solutions contained between 1 and 3 mM calcium ions with phosphate ions in the ratio of 1:1 [63, 64] or 1.66:1 [63],often with the addition of 1 ppm fluoride ions. Higher concentrations are difficulty to be used because of the instability of the solutions. So the low solubility of calcium phosphates, particularly in the presence of fluoride ions, limited the clinical application of calcium and phosphate remineralization systems. Therefore, novel calcium-phosphate-based delivery systems are developed to combat the demineralization of dental hard tissue. New commercial products are available based on three types of novel systems—crystalline, unstabilized amorphous, or stabilized amorphous formulations.

4.3.2 Novel Methods

4.3.2.1 CPP–ACP and CPP–ACFP

CPP–ACP has been shown to promote remineralization of initial enamel lesions and to prevent demineralization in laboratory, animal, and human experiments [59, 65]. Casein phosphopeptides (CPP) can stabilize calcium and phosphate as nanoclusters of ions through the formation of amorphous nanocomplexes (diameter of 2.12 nm) in metastable solution [66]. But it can also prevent the growth of the nanoclusters to the critical size required for nucleation and phase transformation [65]. CPP contains the active sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu. Phosphorylated seryl residues are regarded as responsible for the interactions between casein and the calcium and phosphate ions in the nanocomplexes [67].

CPPs can bind to the more thermodynamically favored surface of an apatite crystal face in the caries lesion due to its high binding affinity for apatite. And CPPs prefer binding to the (100) and (010) faces of hydroxyapatite crystals (Fig. 4.3). So crystal growth would be allowed to continue only at the hydroxyapatite (001) plane or along the c-axis, which is the pattern of crystal growth during amelogenesis. Therefore, CPPs are able to regulate anisotropic crystal growth and also inhibit crystal demineralization in the enamel subsurface lesion [68].

The amorphous calcium phosphate (ACP) is an important compound because it is a precursor that can convert to apatite, similar to the minerals in tooth enamel and dentin. ACP is an unstabilized calcium and phosphate system. When a calcium salt (e.g., calcium sulfate) and a phosphate salt (e.g., potassium phosphate) are delivered separately, the calcium ions and phosphate ions are mixed and result in the immediate precipitation of ACP or, in the presence of fluoride ions, amorphous calcium fluoride phosphate (ACFP). In the intraoral environment, these phases (ACP and ACFP) are potentially very unstable and may rapidly transform into a more thermodynamically stable, crystalline phase (e.g., hydroxyapatite and fluorhydroxyapatite). However, before phase transformation, calcium and phosphate ions should be transiently bioavailable to promote enamel subsurface lesion remineralization.

4.3.2.2 Natural Medicine

Previous studies indicated some nature medicines were able to influence the de-/remineralization balance of dental hard tissue. The extracts of Galla chinensis (GCE) could inhibit the demineralization and enhance the remineralization of enamel and dentin [69–72]. In addition, this polyphenol compounds had combined effects with traditional remineralizing agents, like fluoride [69], nano-hydroxyapatite [73]. However, the mechanism of GCE is still unclear, and more investigations are still needed.

4.3.2.3 Laser

Several types of lasers, such as erbium-doped yttrium aluminum garnet (Er:YAG) [74, 75], neodymium-doped yttrium aluminum garnet (Nd:YAG) [74, 76], and carbon dioxide (CO_2) [76–79], with different parameter settings, have been used for caries inhibition. It is believed that the use of the high-intensity laser on the dental structure can lead to a more acid-resistant sur-

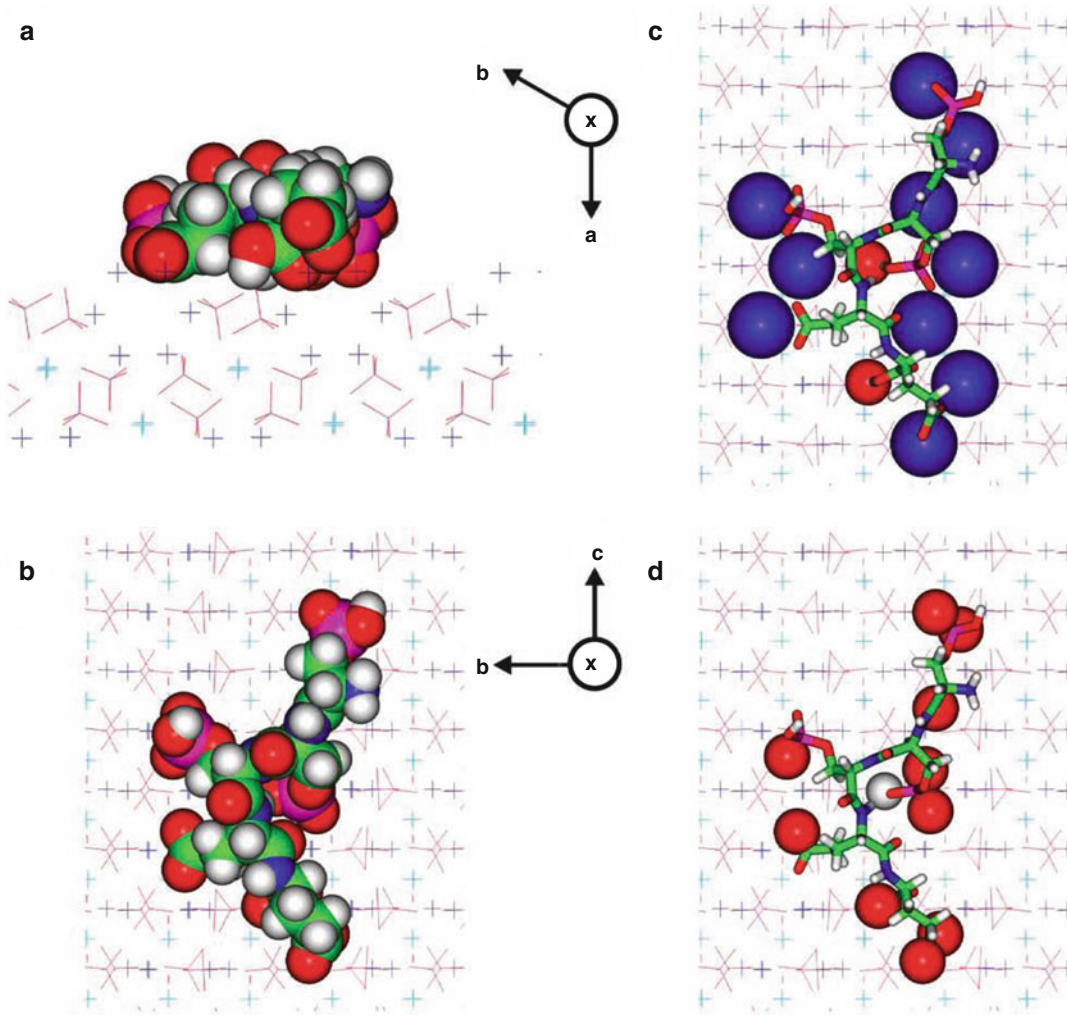

Fig. 4.3 A molecular model of the Ser(P)-Ser(P)-Ser(P)-Glu-Glu motif bound onto the face of hydroxyapatite (HA). The atoms are colorcoded as follows: calcium (1) atoms are *light-blue* crosses, calcium (2) atoms are *dark-blue* crosses, oxygen atoms are *red*, phosphorus atoms are *magenta*, carbon atoms are *green*, nitrogen atoms are *blue*, and hydrogen atoms are *grey*. The symbol X indicates a crystallographic axis projecting into the paper. Four views are presented: (**a**) a side view along the c-axis, with the peptide rendered in CPK and the crystal atoms in 'line' form; (**b**) as in (**a**), but viewed from above, looking down on the HA (100) face; (**c**) as in (**b**), with the peptide displayed in stick form and the atoms in the HA surface within 0.25 nm of the peptide rendered in CPK; and (**d**) as in (**b**), with the peptide displayed in stick form and the atoms of the peptide within 0.25 nm of the HA surface rendered in CPK. [68]

face, and previous investigations showed that lasers could inhibit enamel demineralization and reduce enamel permeability [80].

4.3.2.4 Nanoparticles

Currently, nanotechnology is experiencing rapid growth, with many potential applications in caries prevention and treatment. It is defined as the creation of functional materials, devices, or systems through control of matter on the nanometer scale (1–100 nm). Nanotechnology has motivated mimicking of the nanostructural features of natural human enamel and development of bioinspired strategies for remineralization and caries therapy, respectively [81]. Previous researches have tried to apply nanoparticles in dental caries

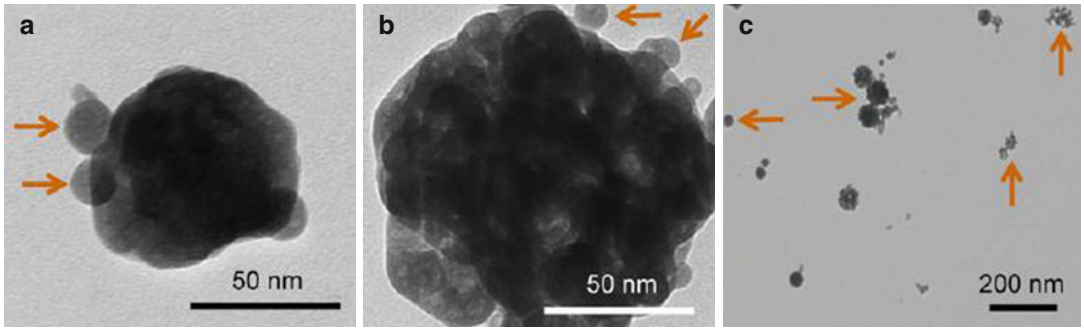

Fig. 4.4 TEM micrographs of the spray-dried nanoparticles: (**a**) small ACP nanoparticles, (**b**) ACP cluster, (**c**) CaF_2 nanoparticles [89]

prevention and treatment, including spherical, cubic, and needlelike nanoscaled particles (approximately 5–100 nm) and near-nanoscaled devices (up to micrometers) [82]. When the sizes of particles are reduced from micrometer to nanometer, the resultant properties can change dramatically. For example, hardness, active surface area, chemical reactivity, and biological activity are all altered [83]. The application of nanoparticles in dentistry can be categorized into two directions: preventive dentistry and restoration dentistry [81].

In recent years, various types of nano-sized hydroxyapatite or calcium carbonate are applied to combat early caries lesions [73, 84, 85]. Some in vitro studies indicated that 10 % suspension of nano-hydroxyapatite particles (10–20 nm diameter, 60–80 nm length) promotes remineralization of the superficial layer of initial caries lesions measuring 20–40 μm, but little remineralization could be obtained by nano-hydroxyapatite in the body of the lesion [73, 84]. Carbonate hydroxyl apatite nanoparticles have also been reported to be effective in repairing micrometer-sized tooth-surface defects in vitro [86]. And some nanocrystals have been incorporated into toothpastes or mouth-rinsing solutions as commercial products.

Clinical investigations indicate that secondary caries and restoration fracture are still the main reasons for dental restoration failure, which limits the longevity of dental restorations, especially the resin composites [87]. Approximately half of all dental restorations fail within 10 years, and replacing them consumes nearly 60 % of the average dentist's practice time [88]. Investigators try to improve the resin compositions, filler particles, and cure conditions. Calcium phosphate (CaP) particles and calcium fluoride particles have been used as fillers in resin composites (Fig. 4.4). These resin-based CaP or CaF composites can release calcium (Ca), phosphate (PO4), or fluoride ions. These additives enable the resin composite to release calcium and phosphate when the pH is dropped down under in vitro conditions, providing caries-inhibiting properties [90]. These calcium and phosphate or fluoride ion-releasing nanofillers include nanoparticles of dicalcium phosphate anhydrous (112 nm in size), amorphous calcium phosphate (ACP) (116 nm in size), and so on [91–95].

Nanoparticles of ACP can be synthesized via a spray-drying technique. Briefly, a spraying solution was prepared by dissolving calcium carbonate and dicalcium phosphate anhydrous ($CaHPO_4$) into an acetic acid solution. Then this solution was sprayed through a nozzle into a heated chamber. The water and volatile acid were evaporated and expelled into an exhaust hood. The dried particles were collected by an electrostatic precipitator.

CaF_2 nanoparticles can be synthesized using the same spray-drying apparatus. A two-liquid nozzle was employed during the procedures. Two solutions are mixed during atomization: $Ca(OH)_2$ and NH_4F. And the two solutions are atomized leading to the formation of CaF_2 nanoparticles: $Ca(OH)_2 + NH4F \rightarrow CaF_2 + NH_4OH$. The NH_4OH is removed as NH_3 and H_2O vapors [96].

4.3.3 Biomineralization

Biomineralization is the process by which living organisms produce minerals, often to mineralized tissues. The formation of teeth is a process of biomineralization. Though traditional methods have been proven to be effective to combat dental caries, dental hard tissue is unable to heal and repair itself after demineralization of the surface and subsequent cavitation due to its non-regenerative nature. Understanding the mechanism of dental hard tissue inspired the researchers who studied the remineralization of dental hard tissue. Numerous studies have tried to find special templates to achieve biomineralization of dental hard tissue in vitro [89]. There are numerous evidences indicating that organic templates or scaffolds would be a prerequisite for bioinspired formation of enamel-like structures [97]. However, there are some problems about the clinical application of biomimetic approaches in vitro. Firstly, the suspected biocompatibility of nonbiological surfactants strongly limits their clinical application. Secondly, most of biomimetic approaches need various hydrothermal conditions, including nonphysiological temperature or pressure [98], which cannot be applied in clinical practice. So some investigators have focused on the biomimetic approaches in physiological-like condition. For example, single crystalline hydroxyapatite micro-ribbons were used as substitutes for amelogenin templates to control HAP crystallization at biophysical conditions at 37 °C [99].

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The Diagnosis for Caries

5

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According to the glossary of endodontic terms, caries is defined as a localized and progressive bacterial infection that results in disintegration of the tooth, usually beginning with the demineralization of enamel and followed by bacterial invasion. It usually takes 6–12 months for caries to appear. Generally, we can make a correct diagnosis with routine inspection. For some difficult cases, dental radiographs or other special inspections are supplementary methods for caries diagnosis (Table 5.1).

5.1 Conventional Diagnosis Methods

5.1.1 Inspection

When making an examination for dental caries on suspected sites, we can find black or chalky area or a formed cavity. The interproximal marginal ridge area has ink stain discoloration under the enamel or a visible cavity. For observation on tooth cervical areas, the cheek and tongue should be pulled away to fully expose buccal and lingual surface of posterior teeth. Depending on the inspection, we can get a general scope of carious damage.

5.1.2 Probing

The sharp probe is used to inspect the suspicious areas. With the help of the probe, the depth and extension of cavity can be examined. If a proximal cavity is suspected and cannot be located through an inspection, the probe is useful to locate affected area when it is hooked by the edge of the cavity. The probe can also be used on the tooth surface to locate the area of dentinal hypersensitivity. Pulp exposure can also be located while examining the deep carious lesion.

5.1.3 Percussion

Caries does not cause periodontal and periapical inflammation, so the reaction to percussion is always negative.

5.2 Special Diagnostic Methods

5.2.1 Radiographic Examination

Radiographic examination can be helpful in locating proximal caries and undermining caries and secondary caries [1]. It can also be used to assess the proximity of caries to pulp chamber. Periapical

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Table 5.1 The diagnosis for caries

Conventional diagnosis methods	Inspection	Black or chalky area or a formed cavity can be seen
	Probing	Probe can be used to locate affected area, the area of dentinal hypersensitivity, and pulp exposure
	Percussion	The reaction to percussion is always negative
Special diagnostic methods	Radiographic examination	Radiolucency on hard tissue due to demineralization is identified as carious lesion
	Cold and hot irritation test	The response of dental pulp toward cold and hot irritation can determine the severity of caries
	Dental floss examination	Dental floss can diagnose caries on proximal contact area
	Diagnostic cavity preparation	After removing supportless enamel, the practitioner can obtain well a vision on hidden carious lesion
The new technology of caries diagnosis	Fiber-optic transillumination	This system uses fiber transillumination for potential caries diagnosis
	Electrical impedance technology	This technology is an alternative way for occlusal pit and fissure caries diagnosis
	Ultrasonic technique	It is a new method for caries detection by measuring the wave that reflects back from tooth structure
	Elastomeric separating modulus technique	Elastomeric separating modules are used to separate apart adjacent tooth temporarily for examination of proximal surfaces
	Staining technique	This technique can show the presence of caries and estimate the depth of carious lesion
	Quantitative laser fluorescence technique	Autofluorescence is light emission phenomenon of biological structure
The differential diagnosis of the superficial caries	Enamel hypocalcification	The key points of the differential diagnosis for caries are glossiness and smoothness, predilection site, symmetry of the lesion, and progress of the lesion
	Enamel hypoplasia	
	Dental fluorosis	

and bite-wing radiographs are commonly used for clinical assessment of caries. Radiolucency on hard tissue due to demineralization is identified as carious lesion. A series of researches have revealed that more than half of proximal caries are seen on the radiograph. Since the radiograph is a two-dimensional image, the diagnosis result should be analyzed and combined with clinical examination. Proximal caries should be distinguished from normal triangular low-density areas in the cervical region of the tooth. Secondary caries should be differentiated from low-density basing materials on the floor of the cavity.

5.2.2 Cold and Hot Irritation Test

The response of dental pulp toward cold and hot irritation is examined during cold and hot irritation test. Examination is done by putting chloroethane-soaked cotton ball or hot gutta-percha stick on the surface of the tooth, and the patient's response is evaluated. These external stimuli can elicit acute pain. The pulp is supposed to be healthy if pain disappears right after removal of stimuli. But if the pain is lingering, the pulp is likely to be in irreversible inflammatory. When cold water is used as stimulus, it is

important to note that the flow of water may affect the accurate location of carious cavity. At last, caries should be differentiated from dentin hypersensitivity.

5.2.3 Dental Floss Examination

Caries on proximal contact area is difficult to be examined by inspection and probing. Dental floss can be used as a convenient method. By putting a dental floss across the embrasure of the suspicious tooth surface and moving the floss horizontally in a seesaw motion, the examiner can experience the roughness of the surface. The floss will be torn if caries is present. Examination with floss can be misled by dental calculus.

5.2.4 Diagnostic Cavity Preparation

After removing supportless enamel, the practitioner can obtain well a vision on hidden carious lesion. To identify the scope and depth of cavity, the infectious dentin on the floor and wall of the cavity should be removed completely. Dying the decalcified dentin with 0.5 % basic fuchsin can help dentists to identify and remove the infectious dentin. After that, the tooth decay area and pulp condition can be easily defined.

5.3 The New Technology of Caries Diagnosis

Tooth caries is chronic, progressive, and bacterial diseases. The main characters of tooth caries are the changes in color, shape, and quality of tooth hard tissue. The typical pathological changes have important reference value for caries diagnosis. At present, the methods for dental caries diagnosis are mainly based on clinical inspection and X-ray examination. However, it is difficult to identify early caries which is in the hidden area of the tooth. With the latest development in science and technology, some techniques and methods are being used for caries diagnosis. These methods have greatly improved the accuracy and sensitivity of caries diagnosis.

5.3.1 Fiber-Optic Transillumination, FOTI

A new diagnostic technique for caries was called fiber-optic transillumination. This system uses fiber transillumination for potential caries diagnosis [2, 3]. The principle is based on the fact that the light transillumination index in decayed tissue is lower than that in normal tissue. Generally, the decayed area shows dark shadow.

5.3.2 Electrical Impedance Technology

The electrical impedance technology is an alternative way for caries diagnosis by examining tooth potential difference. The carious cavity is filled with dead and decayed tissue, saliva, and electrolytes. Therefore, this area becomes more electric conductive than the normal tissue. Following this principle, the resistance offered by the tooth surface is measured under controlled drying. The conductivity is measured by probe in occlusal fissure, and current passes through the pulp to the ground through handheld lead forming a circuit.

The electric caries detector device measures the bulk electric resistance and potential difference. This method is simple, sensitive, and stable for occlusal caries detection.

5.3.3 Ultrasonic Technique

The ultrasonic technique is a new method for caries detection by measuring the wave that reflects back from the tooth structure. This ultrasonic wave is received by a sensor when reflecting back from the tooth surface. The normal tooth surface and the decay one are supposed to have different reflecting waves. Currently, 18 MHz frequency wave was used for caries diagnosis.

5.3.4 Elastomeric Separating Modulus Technique

Elastomeric separating modules are used to separate apart adjacent tooth temporarily for examination of proximal surfaces. This method can be helpful especially when proximal surface caries was examined.

5.3.5 Staining Technique

The staining technique usually stains the degraded collagen which is present in the carious cavity but never stains the intact collagen. For this reason, the dye is used in carious cavity to stain dead and decayed dental tissue. With this technique, the dentist can determine the presence of caries and estimate the depth of carious lesion. The commonly used dye is 1 % basic fuchsin.

5.3.6 Quantitative Laser Fluorescence Technique

Autofluorescence is light emission phenomenon of biological structure. The autofluorescence of dental tissue decreases in demineralization of the tissue. Quantitative laser fluorescence devices use high-intensity halogen lamp to stimulate the tooth to emit the fluorescence in green spectrum [4, 5]. This reflected light is detected by spectrum and recorded in computer and demineralization is quantified. The related other new technologies are dye-enhanced laser fluorescence (DELF), quantitative light-induced fluorescence, light scattering, and confocal laser scanning microscope [6, 7].

5.4 The Differential Diagnosis of the Superficial Caries

The diagnosis of the pit and fissure caries can be done based upon its location. However, the diagnosis of the smooth surface caries is more challenging due to its appearances. Common clinical presentation of caries is described below.

5.4.1 Enamel Hypocalcification

This is a condition where the enamel is formed without adequate mineralization. The lesion is characterized by irregular, opaque, and chalky spots or plaque on tooth surface [8] (Fig. 5.1a).

5.4.2 Enamel Hypoplasia

Enamel hypoplasia is a deficit in enamel formation. The enamel is thin and deficient in amount. It is seen clinically as dot or banded sunken defects with chalky, yellowish, or brownish discoloration [9] (Fig. 5.1b).

5.4.3 Dental Fluorosis

Dental fluorosis is a developmental disturbance, due to exposure of tooth bud to high concentration of fluoride during its development. In mild type of fluorosis, tiny white streaks or specks are seen in the enamel, but discolored and pitted in severe type [10]. The spots and stains left by fluorosis are permanent and may darken over time [11] (Fig. 5.1c).

5.4.4 The Key Points of the Differential Diagnosis for Caries

Glossiness and Smoothness The enamel lesions that are caused by developmental disturbance may have color changes. However, the glossiness, smoothness, and hardness are not affected. The caries teeth have chalky or snuff colored spots without gloss. The enamel surface can also be rough in appearance.

Predilection Site Pit and fissure, proximal surface, and cervical part are caries' predilection sites. It could hardly be found on the self-cleaning areas like cusps or any other smooth surface. The developmental disturbance is caused by abnormal development or irregular mineralization on

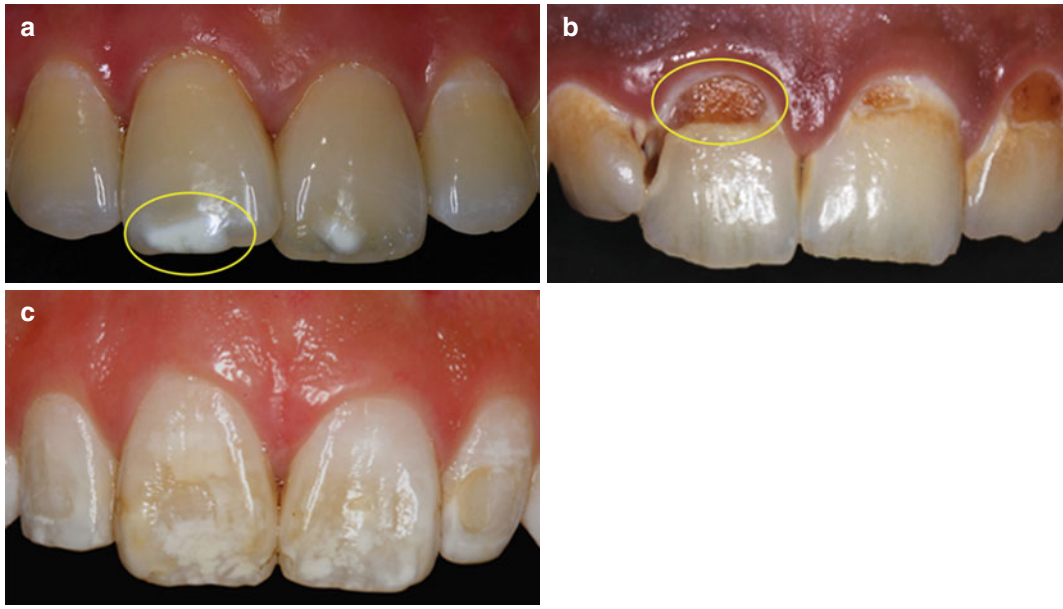

Fig. 5.1 Different clinical images of tooth diseases. (**a**) Chalky plaques can be seen on the labial surface of the central incisors, diagnosed as dental hypocalcification. (**b**) The *yellow circle* shows sunken defect with yellow discoloration. It is known as enamel hypoplasia. (**c**) White specks are seen on the labial surface of maxillary anterior teeth

the tooth surface. Based on the stage of tooth development, the lesion can be seen on different areas.

Symmetry of the Lesion The developmental disturbances affect the tooth during the period of development. Therefore, developmental lesion can be found bilaterally and in a similar manner.

Progress of the Lesion The lesions of developmental origin will become quiescent as soon as the tooth erupts in the oral cavity. It will neither develop nor disappear. The caries is progressive by its nature. The color of carious lesion changes from chalky white to dark brown with its progress toward the pulp chamber. The initial enamel lesion which is chalky white in appearance can be arrested by the tooth remineralization.

The Differential Diagnosis of the Medium Caries The teeth with medium size caries are sensitive to sweet, acid, heat, and cold stimulations. Patients usually complain that they feel pain with these stimuli. This is different from dentinal hypersensitivity. The dentin hypersensitivity is stimulation of nerve endings in dentinal tubule due to change of temperature or pH in oral cavity. The sensation can range all the way from irritation to intense, shooting pain. This sensitivity can also be found on wear-away tooth, decayed tooth, or tooth with exposed root surface.

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Dental Caries: Disease Burden Versus Its Prevention

6

Hong Xiao

6.1 Global Trends of Caries Burden

6.1.1 Oral Diseases: One of the Most Costly Diseases to Treat

Oral diseases, despite their non-life-threatening nature, have been ranked the fourth most expensive disease to treat in most industrialized countries. In the high-income industrialized countries, 5–10 % of public health spending is used for oral health care [1]. Back in the year 2000, the European Union spent a total of €54 billion on oral health care, and in the United States, the 2004 expenditures for oral health care were US$ 81.5 billion [2]. However, such figures did not include the additional costs of dental care performed under the medical umbrella. Among the variety of oral diseases, dental caries has long been the main factor attributable as the most important source of oral disease burden.

Not surprisingly, these costs will require unaffordable financial resources from low- and middle-income countries. For example, the costs of treatment to control periodontal diseases in children on basis of the Community Periodontal Index (CPI) of Treatment Needs (CPITN) data greatly exceeded the total national health-care budget of Kenya [3, 4]. Take Nepal as another example: the total costs of restoring dental caries cavities of the child population would exceed the total health-care budget for children of the entire country [5].

This fact should be interpreted on the background that, for low-income countries, a comprehensive national essential health-care package at a cost of US$ 4 will reduce the burden of mortality and disability among the 0- to 14-year-old children by 30 %!

However, planning traditional curative intervention strategies entirely on the basis of epidemiological data without considering the social, economic, and political conditions is doomed to fail. In particular the national economic conditions appear to be an important determinant of restorative care. If we take the Care Index (F/DMFT*100 %) for 34- to 44-year-olds as an indicator of the country's restorative care level, it appears that no country with a gross domestic product (GDP) below US$ 5,000 has a Care Index higher than 30 %, which further underpins that restorative treatment is unaffordable in low-income countries [5, 6].

6.1.2 Uneven Distribution of Oral Disease Burden Around the World

In the recent several decades, we have witnessed a time of dramatic decline of caries status worldwide. This trend can be attributed to

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widespread use of topical fluoride and increasing awareness of the importance of maintaining good oral hygiene. However, in some parts of the world, dental caries still remains a common disease affecting children and adults, posing tremendous economic burden.

If the burden of disease is described using years lived with disability (YLD) per million people, the highest disease burden of the world can be found in sub-Saharan Africa closely followed by India. Both the countries carry heavy disease burdens mainly because of communicable diseases. However, in the high-income countries, noncommunicable diseases are the main source of disease burden [7].

When it comes to oral diseases, including caries, periodontal diseases, and edentulism, they make very little contribution to the total YLD/million population of each country with the worldwide average of 1.6 %. While the Middle Eastern Crescent is found to have the highest oral disease contribution to the total YLD/million population, China is found to be among those countries with lowest oral disease contributions.

With respect to relative contribution of caries to oral disease burden of different regions of the world, contribution of caries was found to be over a half of most countries or regions, ranging from as low as 46.7 % in established economy market countries to as high as 89.8 % in Latin America and Caribbean.

Usually, caries status can be monitored through national epidemiological surveys by recording the dmf/DMF indices (decayed, missing, and filled tooth/surface indices). Although the recording of such indices disregards the impact of perceived pain and discomfort as a result of dental caries, it is able to reveal some valuable objective information especially from a professional perspective. The proportion of each component varies greatly among high-, middle-, and low-income countries. Data collected between 1990 and 2004 indicate that, in high-income countries, only about one-fifth of the caries lesions in the primary dentition were treated. Such proportion of treatment dramatically decreased to about 5 % in the middle-income countries. Worst findings were found in the low-income countries, where the proportion of treated caries lesions could be totally neglected.

Situations in the permanent dentition told a better story. The high-income countries had a Care Index (F/DMF score) of over 50 %, while the middle-income countries had about 20 %. However unfortunately, the Care Index was found to be within 5 % for the low-income countries.

The temporal trends in dental caries experience of 12-year-old children in developing and developed countries can be illustrated in Figs. 6.1 and 6.2. In most developing countries, the levels of dental caries were low. However, the prevalence rates of dental caries seem to increase in recent years. This might be attributed to increased consumption of sugar and inadequate use of fluoride.

However, the industrialized countries tell a different story. A steady decline of caries experience is found. This result from a number of public health measures including effective use of fluorides, together with changing living conditions, lifestyles, and improved self-care practices.

Worldwide, the prevalence of dental caries among adults is high as the disease affects nearly 100 % of the population in the majority of countries. Figure 6.3 illustrates the levels of dental caries among 35–44-year-olds, as measured by the mean DMFT index. Most industrialized countries and some countries of Latin America show high DMFT values.

6.1.3 Developing Global Policies Highlighting the Importance of Oral Health

The policy of the WHO Global Oral Health Programme emphasizes that oral health is integral and essential to general health and that oral health is a determinant factor for quality of life. The WHO Global Oral Health Programme has implied that greater emphasis is put on developing global policies based on common risk factors and approaches should be coordinated more effectively with other programs in public health [8–10]:

- To adopt measures to ensure that oral health is incorporated as appropriate into policies for the integrated prevention and treatment of chronic noncommunicable and communicable

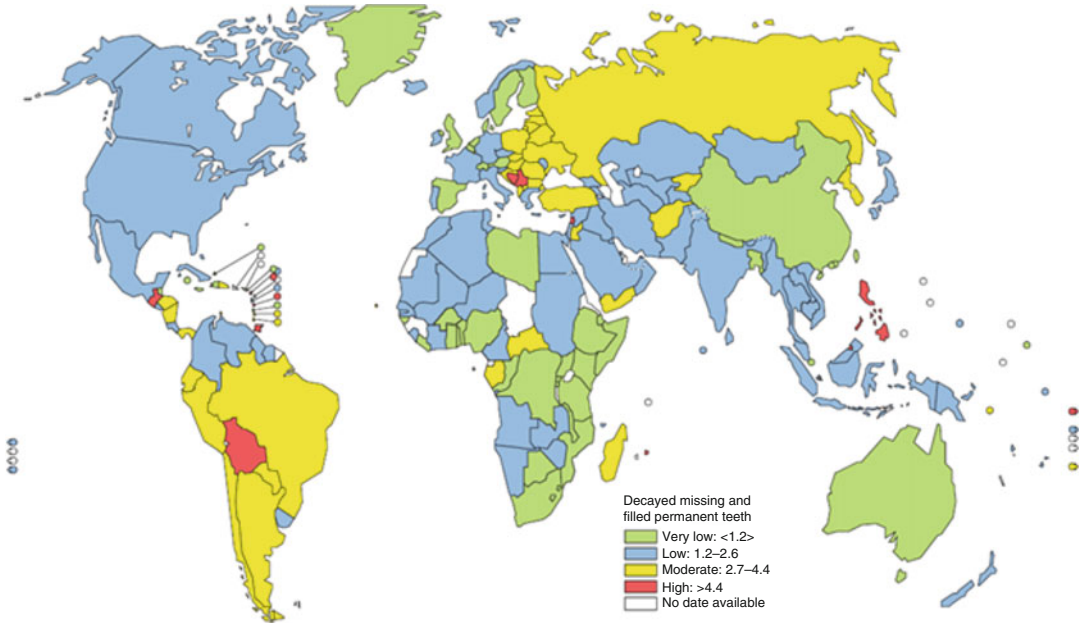

Fig. 6.1 Dental caries levels (decayed, missing, and filled teeth (DMFT) index) among 12-year-olds worldwide (Reproduced, with the permission of the publisher, from *Bulletin of the World Health Organization*, Petersen et al. [7]. http://www.who.int/bulletin/volumes/83/9/petersen-0905abstract/en/)

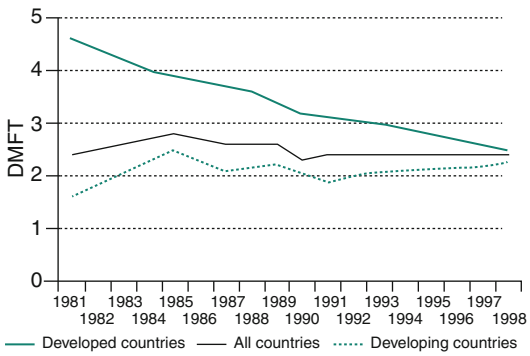

Fig. 6.2 Changing levels of dental caries experience (decayed, missing, and filled teeth (DMFT) index) among 12-year-olds in developed and developing countries (Reproduced, with the permission of the publisher, from *Bulletin of the World Health Organization*, Petersen et al. [7]. http://www.who.int/bulletin/volumes/83/9/petersen-0905abstract/en/)

diseases and into maternal and child health policies

- To take measures to ensure that evidence-based approaches are used to incorporate oral health into national policies as appropriate for integrated prevention and control of noncommunicable diseases
- To consider mechanisms to provide coverage of the population with essential oral health care, to incorporate oral health in the framework of enhanced primary health care for chronic noncommunicable diseases, and to promote the availability of oral health services that should be directed toward disease prevention and health promotion for poor and disadvantaged populations, in collaboration with integrated programs for the prevention of chronic noncommunicable diseases
- For those countries without access to optimal levels of fluoride, and which have not yet established systematic fluoridation programs, to consider the development and implementation of fluoridation programs, giving priority to equitable strategies such as the automatic administration of fluoride, for example, in drinking water, salt, or milk, and to provide affordable fluoride toothpaste
- To develop and implement the promotion of oral health and prevention of oral disease for preschool and school children as part of activities in health-promoting schools
- To scale up capacity to produce oral health personnel, including dental hygienists, nurses,

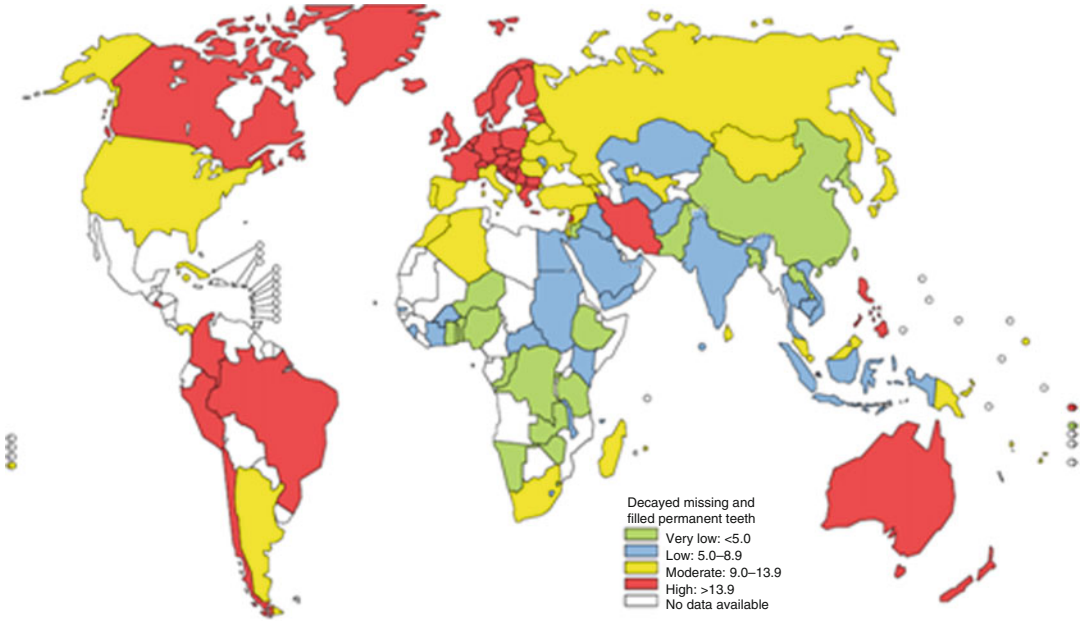

Fig. 6.3 Dental caries levels (decayed, missing, and filled teeth (DMFT) index) among 35–44-year-olds worldwide (Reproduced, with the permission of the publisher, from *Bulletin of the World Health Organization*, Petersen et al. [7]. http://www.who.int/bulletin/volumes/83/9/petersen-0905abstract/en/)

and auxiliaries, providing for equitable distribution of these auxiliaries to the primary-care level and ensuring proper service back-up by dentists through appropriate referral systems

- To incorporate an oral health information system into health surveillance plans so that oral health objectives are in keeping with international standards and to evaluate progress in promoting oral health
- To strengthen oral health research and use evidence-based oral health promotion and disease prevention in order to consolidate and adapt oral health programs and to encourage the intercountry exchange of reliable knowledge and experience of community oral health programs
- To address human resources and workforce planning for oral health as part of every national plan for health
- To increase, as appropriate, the budgetary provisions dedicated to the prevention and control of oral and craniofacial diseases and conditions
- To strengthen partnerships and shared responsibility among stakeholders in order to maximize resources in support of national oral health programs

6.2 Caries Burden in China

6.2.1 The First and Second National Epidemiological Investigation of Oral Health in China

China has organized two nationwide epidemiology investigations. The first was conducted between 1982 and 1984, which had examined the status of caries and periodontal diseases in primary and middle school students of 29 provinces, autonomous administration regions, and municipalities directly under the central government. There were altogether 131,340 students examined. The first national epidemiological investigation of oral health in China is the first large-scale epidemiological investigation organized since the establishment of P.R. China.

The second national epidemiological investigation of oral health in China took place from 1995 to 1998. This investigation had included 6 age groups covering 5–74-year-olds from 11 provinces, autonomous administration regions, and municipalities directly under the central government. A total of 140,712 people at 396 sample sites were selected.

Results of these two epidemiology investigations have provided valuable first-hand data for the central government to make oral health-related policies, set up targets for oral health service, and planned future human resource allocation in oral health service.

According to the second national survey which was conducted in 1995, caries prevalence rates for 5-year-olds, 12-year-olds, 35–44-year-olds, and 65–74-year-olds were 76.6 %, 45.8 %, 63.0 %, and 64.8 %, respectively.

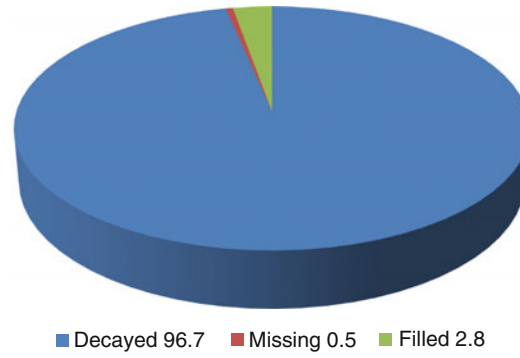

Fig. 6.4 Constitution of decayed, missing, and filled teeth in 5-year-olds in China (%)

6.2.2 The Third National Epidemiological Investigation of Oral Health in China

The third national epidemiological investigation of oral health in China was implemented in 2005. At the planning period, the sampling methods have been carefully designed as stratified multilevel randomized sampling [11]. A total of 93,826 people were selected covering 5–74-year-olds in 6 age groups. The sample well represents the overall status of oral health in China. In addition to dental examinations, a comprehensive questionnaire investigation was simultaneously conducted to investigate the oral health-related concepts and behaviors of the selected sample.

Caries status obtained from stratified populations is detailed as follows.

6.2.2.1 Caries Status of 5-Year-Olds

The 5-year-olds have a caries prevalence rate of 66.0 % in the primary dentition. Children who live in the cities have a slightly lower caries prevalence rate (62.0 %), while in the rural areas, children have a higher caries prevalence rate (70.2 %).

Average DMFT of the 5-year-olds is 3.5, while most of these children have a DMFT of 2, accounting for 11.1 % of the total subsample. The constitution of DMFT indices can be viewed in Fig. 6.4, which has shown that an astonishing proportion of carious teeth have never been treated (96.7 %). Analysis of the frequency distribution of DMFT in children has aroused our attention when it is found that 79.3 % of all carious teeth concentrate in about one-third of the examined children, who present a significant caries index of 8.33.

Approximately half of the subsample have their guardians (mostly parents and grandparents) completed the questionnaire. It has been found that 13 % children began to brush their teeth before the age of 3, and the remaining vast majority has just begun to brush their teeth. Around one-fifth of children don't brush their teeth.

There were 49 % parents aware of the fluoride toothpaste, and 39 % of 5-year-olds use the fluoridated toothpaste when brushing their teeth. It should be paid attention to that only 22 % children visit dentists periodically. The most common reasons for dental visits are acute or chronic toothache and other reasons such as dental emergency.

Multiple regression analysis was performed to examine the main reasons for caries in children. It has been found that urban/rural distribution, sugar consumption before sleeping, toothbrushing starting age, and oral hygiene by parents all correlate with caries status of children.

6.2.2.2 Caries Status of 12-Year-Olds

Caries prevalence rate of the 12-year-olds is 28.9 %. No difference is observed between urban and rural areas, and boys have a statistically significant lower caries prevalence rate than girls (25.4 % vs. 32.6 %).

The most often affected teeth are mandibular first molars, maxillary first molars, and mandibular second molars. Unfortunately, 88.8 % of all carious teeth have not been treated (Fig. 6.5). In China, 1.45 % of 12-year-olds have pit and fissure

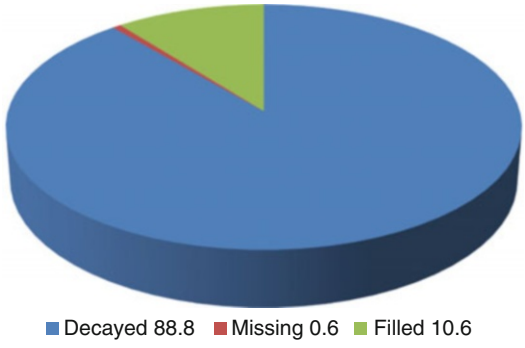

Fig. 6.5 Constitution of decayed, missing, and filled teeth in 12-year-olds in China (%)

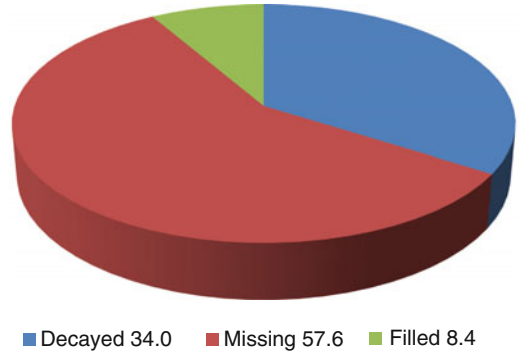

Fig. 6.6 Constitution of decayed, missing, and filled teeth in 35–44-year-olds in China (%)

sealants, and there is a huge difference between urban area and rural area (2.75 % vs. 0.19 %).

The investigated 12-year-olds have completed the questionnaire by themselves. As this age group is the most important age group, it has been receiving continuous attention from the central government. In addition to questions on oral health-related knowledge and behavior, the questionnaire has also included questions on dental visits and self-perceived impact of oral health.

It has been found that 82 % of the 12-year-olds investigated brush their teeth daily; however, only 28 % of them brush their teeth at least twice a day. Ninety-three percent of the children report that they have never used dental floss. The percentage of children who use fluoridated toothpaste is 46 %. Sixty-nine percent of the children have daily consumption of sugar-containing food, such as sweetened milk, dissert, candy, carbonated soft drink, and sweetened fruit juice. It is satisfactory that only a small fraction of the investigated children smoke (boys 5.7 % and girls 0.5 %) and that the percentage of daily consumption of cigarette and alcohol is further reduced to 1.0 % and 0.1 %, respectively, for boys and girls.

With regard to oral health-related knowledge, only one-tenth of 12-year-olds understand dental plaque as the cause for caries and periodontal diseases. However, over 60 % of them have realized that bleeding during toothbrushing is abnormal.

In the past 12 months, 21 % of 12-year-olds have visited a dentist. However, 47 % of the total subsample have never seen a dentist. The most common reasons for their dental visit are acute or chronic toothache. It is sad that only 28 % have formed the habit of frequent dental check-ups.

6.2.2.3 Caries Status of 35–44-Year-Olds

The 35–44-year-olds is the WHO recommended age group for adults. It has been found that the vast majority of this age group (88.1 %), quite unfortunately, has been affected by caries. Not much difference was found between the urban and rural populations (89.1 % vs. 87.1 %). However, women are found with statistically significant higher caries prevalence rate than men (91.3 % vs. 84.9 %). The constitution of the DMFT indices is shown in Fig. 6.6. However, the prevalence rate for root caries is near one-third (32.7 %), signifying bad periodontal status.

All of the investigated subgroup answered the questionnaire. Among all the 35–44-year-olds, 89 % have reported that they brush their teeth daily, and 35 % have reported to be brushing their teeth at least twice a day. Better teeth brushing habits are found in cities, women than in rural areas and men.

On a daily basis, 27 % use toothpicks, but over 99 % do not use dental floss. Fluoridated toothpaste is used in 46 % people. Daily consumption of sugar is found in 21 % of those investigated. There are 32 % questionnaire respondents reported to be smokers. There is a huge gender difference in daily alcohol consumption, while 23 % men, in contrast to 2 % women, drink alcohol daily.

Among the 35–44-year-olds, 69 % believe there is a need for them to receive oral treatment,

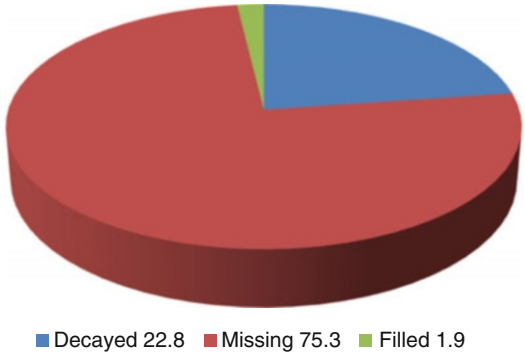

Fig. 6.7 Constitution of decayed, missing, and filled teeth in 65–74-year-olds in China (%)

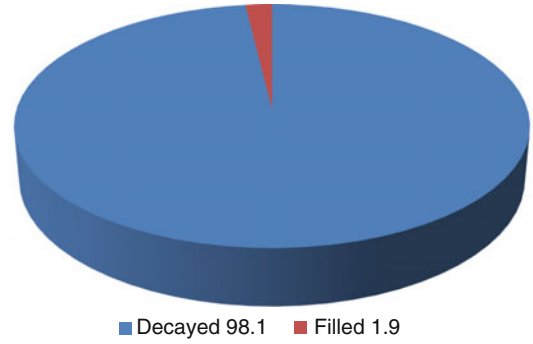

Fig. 6.8 Constitution of decayed and filled root caries in 65–74-year-olds in China (%)

and the percentage is found to be statistically significantly higher in urban populations than rural (73 % vs. 65 %). However, in the past 12 months, only 16 % of them have been to a dentist. Quite surprisingly, even at this age, there are as many as 46 % 35–44-year-olds who have never visited a dentist ever. For the remaining 54 %, the majority of them have attributed their dental attendance to be because of acute or chronic toothache or other dental situations, and few of them have formed the habit of regular dental check-ups.

Multiple regression analysis has revealed that the caries status of the 35–44-year-olds is closely related to the use of fluoride toothpaste, consumption of sugar-containing foods, toothbrushing frequencies, and other behavioral variables. Those with no use of fluoridated toothpaste, with daily consumption of sugar-containing foods, and who are not brushing their teeth on a daily basis manifest with high caries risk.

6.2.2.4 Caries Status of 65–74-Year-Olds

Caries prevalence in the age group of 65–74-year-olds is found to be very high. The prevalence rate is as high as 98.4 %, and not much difference is found between urban and rural populations. Also, not much difference is discovered between both genders. The mean DMFT score is 14.7, the majority of which is missing due to caries (75.3 %), and only a fraction of the iceberg is treated (1.9 %) (Fig. 6.7).

Root caries is another important issue that should not be neglected. The age group of 65–74-year-olds are found to be heavily affected by caries in the root. Rural population have a root caries prevalence rate of 67.2 %, higher than the urban population (60.0 %). It is found that 66.1 % females are affected by root caries, higher than males (61.1 %). It is further discovered that only as low as 1.9 % of all the carious lesions in the root have been treated (Fig. 6.8).

About half of the examined subjects have handed in the questionnaire. It has been found that three-quarters of the questionnaire respondents brush their teeth on a daily basis and that slightly over one-quarter brush their teeth twice a day. This self-oral hygiene behavior is found to be better in cities and females. In 27 % of the questionnaire respondents, fluoridated toothpaste is used; the percentage is found to be higher in cities than in rural areas. There are 26 % of the respondents who report they use toothpick daily, but almost none of the respondents have ever used dental floss.

Daily sugar-containing food consumption is found in 27 % questionnaire respondents. An average of 27 % of the respondent smoke, and there is a huge difference between the two genders. And it is found that among those who smoke, 90 % of them have been smoking for more than 20 years. Alcohol consumption is found in 13.6 % respondents; the percentage of male is almost six times that of the female.

Over half of the elder population believe they are in need of oral examination and treatment; however, only 19 % of the respondents report that

they have visited a dentist within the past 12 months. The most common reason for dental referral is acute and chronic toothache. However, it is unfortunate that about one-third of the all the respondents have never been to a dentist for any form of oral examination or treatment. For the expenditure on dental visits, 83 % of the respondents report that they are fully self-financed, not to be able to find a source to partly cover the bill.

When we analyze the oral health impact on daily life, it is sad to find that over half the respondents have experienced toothache, and the majority of the elders are not satisfied with their oral hygiene conditions.

6.3 Caries Preventive Strategies

Dental caries results from a variety of contributing factors. There are three most important factors, namely, cariogenic microflora (termed as dental plaque), carbohydrate-rich diet, and susceptible teeth. Given time, the interrelation and interaction between these three factors will produce the chronic oral disease which brings pain and discomfort to the majority of the population worldwide since an early age. Therefore, effective caries-preventive strategies almost always require removal of any or all of these contributing factors.

There is a hierarchy of caries-preventive strategies which include the following.

6.3.1 Primary Prevention

Primary prevention is the most important strategy as it stresses and deals with combating the etiological factors. Self-awareness with regard to caries prevention is often highlighted in this stage. People are motivated by a variety of means, either with propaganda via mass media or face-to-face educational lectures, so that they will be self-conscious about the importance of keeping good oral hygiene, restricting the consumption of sugars and frequent dental visits.

Many countries have set up their own launches or campaigns to deliver the concept of oral health education. China, for example, has launched its own national campaign for over a decade. Each year on the "love teeth day" on September 20th, there will be dental health educational and promotional activities held across the country. There is a unique theme every year. Usually manuals containing educational information are distributed free of charge. In many cities where there are dental schools or colleges, free dental check-ups are available.

Removal of etiological factors is very important in this stage. The main methods include dental plaque control, use of fluoride, and other preventive methods that are to be introduced in the following section.

6.3.2 Secondary Prevention

Secondary prevention mainly refers to the early diagnosis and treatment of lesions found to be still at their early stages. For a long time, caries diagnosis mainly relies heavily on the dentists themselves who can only resort to their naked eyes and self-practicing experiences. This has resulted in countless mistaken diagnosis and omission of countless lesions which had been left not dealt with. Later, the invention of X-ray has offered another possibility to visualize lesions that hide from usual visual examination.

6.3.2.1 Conventional Caries Detection Methods

Traditional diagnostic methods, such as visual inspection, appear to have very low sensitivity and high specificity in diagnosing caries [12–14]. Continuous attempts to improve the sensitivity have been made. Ekstrand et al have proposed a new caries scoring system as presented in Table 6.1. Sensitivity and specificity for detection of dentinal lesions were found to be ranging between 0.92 and 0.97 and 0.85 and 0.93, respectively [15]. A conclusion from one study was that although good results were obtained regarding sensitivity and specificity as well as operator agreement, it takes more time to learn the method. Although improvements in visual inspection with new scoring systems (take, ICDAS, for another

Table 6.1 Detailed criteria for visual inspection of occlusal surfaces introduced by Ekstrand et al

Classification	Visual inspection
0	No or slight change in enamel translucency after prolonged air drying (>5 s)
1	Opacity or discoloration hardly visible on wet surface but distinctly visible after air drying
2	Opacity or discoloration distinctly visible without air drying
3	Localized enamel breakdown in opaque or discolored enamel and/or grayish discoloration from the underlying dentin
4	Cavitation in opaque or discolored enamel exposing the dentin

example) seem promising, further clinical validation is still needed.

The use of explorers has received some dispute as some believe it cannot increase diagnosing accuracy while tends to damage tooth tissue and contaminate sound areas. Loesche et al, in a study on intraoral transmission of pathogenic microorganisms, showed that sterile fissures might be inoculated by probing after previous contact with an infected fissure [16]. For detection of occlusal dentinal lesions, the sensitivity of the explorer is reported to be only about 0.5–0.6. A number of reports have demonstrated that probing with a sharp explorer may cause damage to newly erupted teeth or even create a cavity at the site of a superficial carious lesion. Therefore, its use has been questioned by several authors.

The use of film radiograph for caries detection has a long history and is still the most widely used diagnostic technique. Bitewing radiography has been found to be useful for dentinal caries detection on both occlusal and approximal surfaces. However, it has no value for occlusal enamel caries detection and only a limited value for approximal enamel caries detection.

6.3.2.2 Fiber-Optic Transillumination (FOTI)

Fiber-optic transillumination (FOTI) is a widely used caries detection adjunctive to dental professionals [17–20]. Its use can be traced back to the 1970s. It brings special advantages in diagnosing caries in the proximal surfaces which are difficult to discover with the naked eye under normal means of illumination. In FOTI, white light from a cold-light source is passed through a fiber to an intraoral fiber-optic light probe that is placed on the buccal or lingual side of the tooth. The surface is examined using the transmitted light, seen from the occlusal view. Decayed tooth structure manifests itself as the dark shadow under illumination of ultraviolet. Demineralized areas appear darker compared with the surrounding sound tissue. The contrast between sound and carious tissue is then used for detection of lesions.

FOTI has been evaluated in a number of studies for detection of posterior approximal carious lesions and has shown low-to-good sensitivity and good specificity. Cortes et al showed, in an in vitro study, that a combination of FOTI and visual inspection was useful for determination of occlusal lesion depth.

Using the digital image of a tooth (seen from the occlusal view during transillumination) through computer image analysis, researchers have attempted to improve the performance of FOTI. This quantitative method, digitized fiber-optic transillumination (DI-FOTI), has been evaluated in a few studies, and the initial results indicate that both the sensitivity and specificity are very high. However, this method needs to be developed further before it can be applied in clinical situations.

6.3.2.3 New Caries Detection Methods

Till now, many new and high-tech diagnosing technologies have become available, providing new possibilities in caries diagnosis at its earliest stages. Moreover, caries can not only be detected but also can be quantified. This has very important implications as thanks to these high technologies, longitudinal quantitative monitoring of carious lesions is now a reality.

New Caries Detection Methods: Optically Based Light interacts with the dental hard tissues in different ways. It can be either reflected, scattered, transmitted, or absorbed. The different phenomena can occur alone or in combination.

A possible consequence of absorption is fluorescence, in which electrons of a lower-energy status are moved to a higher status. When they fall back to the original level, energy is emitted in the form of light, called fluorescence. In other words, fluorescence occurs as a result of the interaction of electromagnetic radiation with molecules in the tissue.

The cause of enamel fluorescence is still unclear. Most of the fluorescence is induced by organic components, proteinic chromophores, but some can be probably attributable to apatite. It has been proposed that fluorescence in dentin is caused by inorganic complexes, as well as some organic components. In sound enamel, the path lengths are long so that there is a high probability that the photons will hit a chromophore. Thus, fluorescence is relatively intense. Demineralization of dental hard tissue will result in the loss of autofluorescence (the natural fluorescence).

The amount of autofluorescence loss can be measured by devices to quantify the severity of tooth decay. Some of these high-tech caries detection devices are introduced below.

DIAGNOdent DIAGNOdent is developed based on the finding that natural tooth structure will absorb the 655 nm wavelength light, while decayed tooth structure produces fluorescence in the near-infrared spectrum [21–23]. Intensity of the near-infrared fluorescence reflects the extent of tooth decay. By comparing the numerical value of the test site with that of the comparison site (usually the same location at the contralateral tooth in the same dentition), one is able to tell whether the site of examination is diseased or not and at what severity is the disease. This device is very sensitive so that it can be used to analyze the initial stage of caries while causing no damage to the tooth structure or pain to the patient.

It has been found to be able to help identify the most caries-susceptible site of pits and fissures on fully erupted first permanent molars in both caries-active and caries-free children, thus providing helpful information to decide cost-effective, targeted prevention for pediatricians.

The version called DIAGNOdent (Kavo, Biberach, Germany) is designed to detect occlusal and smooth surface caries. A new device, named the DIAGNOdent pen (Kavo), has recently been developed for the use in approximal and occlusal surfaces, which has the same mode of functioning. Several in vitro and in vivo studies have been conducted to evaluate the devices. In a study by Lussi et al, good to excellent sensitivity and excellent reproducibility were reported.

In recent years, a new approach has been proposed to improve the performance of DIAGNOdent in detecting early caries lesions by using fluorescent dyes. This proposed approach has been tested by Alencar CJ et al, who had used a fluorescent dye (tetrakis N-methylpyridyl porphyrin, TMPyP) to enhance the performance of DIAGNOdent and DIAGNOdent pen. Mineral loss was determined with gold standard method. Correlation was observed between the amount of mineral loss and DIAGNOdent measurements. They found that DIAGNOdent and DIAGNOdent pen are capable of identifying demineralization around brackets bonded with resin-modified glass ionomer cements. They also found that the DIAGNOdent pen associated with TMPyP was more capable of identifying this difference in mineral loss as well as the gold standard method.

Quantitative Light-Induced Fluorescence (QLF) Quantitative light-induced fluorescence (QLF) also detects caries lesions based on fluorescence findings (laser-induced autofluorescence of natural tooth structure) [24–28]. The tooth is illuminated by a broad beam of blue-green light which is transported through a liquid-filled light guide. The source of light illumination can be either an argon + ion laser, producing diffuse monochromatic light at a wavelength of 488 nm, or blue light from a 50 W xenon microdischarge arc lamp, which has an optical band-pass filter with a peak intensity of 370 nm. Using a color CCD video camera and a frame-grabber, fluorescent-filtered images can be captured and stored in a computer.

Demineralized areas appear as dark spots viewed by QLF. Software (Inspektor Research Systems BV, Amsterdam, the Netherlands) can be customized to analyze the collected images. After calculating the loss of fluorescence which

indicates the severity of a lesion, three measures are given: lesion area (mm^2), ΔF (average change in fluorescence, in %), and ΔQ (area*ΔF). Therefore, compared with other caries detection methods, QLF has a huge advantage in direct visual demonstration for patients. In addition, countless studies have confirmed its accuracy and reproducibility. Intra-class correlation coefficients (ICCs) for each QLF metric were found to be high with intra-examiner ΔQ 0.91, ΔF 0.80, and area 0.92, according to a study of 91 in vivo samples. It can be used to reliably monitor the caries status of an individual over time. However, it seems to be limited to a lesion depth of about 400 μm.

To now, the QLF method has been found to be more suitable for smooth surface caries examinations. The possibility of adapting it for occlusal caries diagnosis as well as secondary caries is still under development. However, it has already been employed in a number of clinical studies which evaluated the anticaries efficacy of various kinds of oral hygiene maintaining products such as toothpaste, mouth rinse, etc.

It has been found that longitudinal measurements of QLF could detect differences in remineralization of early enamel caries on buccal surfaces of anterior teeth following supervised daily brushing with sodium fluoride (NaF; 1,450 ppm F), sodium monofluorophosphate (MFP; 1,450 ppm F) dentifrices, or a herbal, non-fluoride placebo dentifrice.

New Caries Detection Methods: Electrical Impedance Based Electrical caries monitor (ECM) is developed on the basis of analyzing the conductivity of tooth structures. Natural enamel is no good conductor for electricity. However, under diseased condition, its conductivity is greatly increased because of expanded intercrystal space and fluid content inside. The more demineralized the tissue, the lower the resistance becomes. Then, by measuring the electric conductivity of the test site and comparing it with findings of the contralateral or adjacent tooth in the same dentition, the extent of the carious lesion can be derived [29].

Measurements can be performed by closing a circuit of a very weak alternating current through the patient. A probe is placed on the site that is to be measured, an earth-unit is held in the patient's hand, and both are connected with the device by a cord. During examination, the measuring site should be isolated from saliva. When a tooth surface is of interest, the probe tip can be placed in an electrolyte which covers the surface.

This equipment is mainly devised to detect caries lesions at approximal sites of teeth. In a number of in vitro and in vivo studies, the reported sensitivity for ECM in diagnosing dentinal carious lesions of permanent premolar and molar teeth ranges from 0.67 to 0.96. And the specificity ranges from 0.71 to 0.98, which could be regarded as acceptable.

However, under some circumstances, false-positive or false-negative readings may be recorded. These circumstances include examined teeth which have only erupted into the oral cavity for less than 6 months, excessive dehydration of the adjacent or other reference sites, and occlusal surface with too complex morphology. Temperature variations may also influence the outcome of the measurements in various ways.

Other high-tech caries detection technologies include electrochemical impedance spectroscopy (EIS), optical coherence tomography (OCT), and near-infrared (NIR) technology. These technologies are less frequently used in recent times, and further studies are still ongoing.

At the same time, latest inventions and breakthroughs in new dental material have tremendously reshaped the conception of dental treatment for carious lesions. Tooth structures are no longer subjected to unnecessary damages in order to compromise for a more stable restoration. In this way, tooth is much better protected than ever before. All those new possibilities are unimaginable without the forthcoming of adhesive dental materials.

6.3.3 Tertiary Prevention

Tertiary prevention is a last-resort strategy which is not intended to be utilized but has to be turned to when caries at late stages are discovered. At this stage, when dental caries already come into

being, all that should be done is to prevent the undesirable complications it will induce if not dealt with. Among those possible and often clinically observed complications are acute or chronic pulpitis, alveolar abscess, periapical inflammation, alveolar osteomyelitis, and a number of other less-frequently encountered caries derivatives.

When things become worse, damage to the integrity of dentition occurs as a result. In this case, prosthetic dentistry is needed to restore a full dentition and maintain proper masticatory functions.

6.4 Methods for Caries Prevention

6.4.1 Dental Plaque Control

Dental plaque control refers to maintaining the amount of plaque accumulation on tooth surfaces at a reasonable level which does not allow the initiation and progression of carious lesions. Theoretically, caries can be prevented through perfect oral hygiene. Usually, dental plaque control is performed by individuals at home. Under some circumstances, it is performed by a hygienist or dentist as indicated necessary.

Chemical removal of dental plaque can be achieved by administration of mouth rinses at different frequencies. This often involves different kinds of key effective chemical components in the mouth rinse solutions, such as triclosan, chlorhexidine, essential oil, benzethonium chemicals, and many others. However, there are undesirable consequences as microbes can start to tolerate the effects of these chemicals given enough time. Therefore, removal of dental plaque with chemicals can only be used as an adjunctive to mechanical methods or used as a substitute for it when mechanical removal is not possible due to some reasons.

Many mechanical methods can be employed for this purpose. Among those, toothbrushing is the most frequently advocated and most widely exercised [30–32]. However, the efficiency of toothbrushing is not always satisfactory, when the level of which is measured by calculating the plaque removal rates. Therefore, what is to be stressed not only involves stressing the adequate frequency of daily toothbrushing and its duration of time but also includes the correct toothbrushing methods. However very unfortunately, people seem not to pay enough attention to this fact that only by performing correct toothbrushing methods can they effectively remove the maximum amount of dental plaque. Moreover, brushing the teeth in a wrong way can also bring about undesirable consequences, such as multiple wedge-shaped defects, posing potential danger in pulp exposure or even tooth fracture.

There are a number of toothbrushing methods to choose, and each method has its own focus. Horizontal vibrating method (also known as the Bass method) stresses the removal of plaque in the gingival sulcus and the interproximal spaces. However, this method is very much time-consuming and difficult to exercise for most people, because it demands dynamic equilibrium of direction and force. Another method to be suggested is reported by Fones. It mainly involves repeated rotary movements of the toothbrush between the maxillary and mandibular dentition. This method is so easy to conquer that it can even be mastered by children and adolescents to perform effective self-oral hygiene.

Despite the brushing technique, the toothbrush itself is to be carefully selected. Now there are different kinds of toothbrushes in the market. Each kind serves a targeted subpopulation of a certain age or of particular physical characteristics. Some toothbrushes are specially designed for different subgroups, such as children or the disabled who require a toothbrush to have a smaller head and less-likely-to-slip handle. For the majority however, a regular-design toothbrush with soft round-end bristles is well enough to maintain everyday oral hygiene.

In addition, electronic toothbrushes are invented to better serve the disabled and the elder. Toothbrushing movements of different groups of bristles have been preprogrammed, and these movements are often very complex which include different rhythms of vibration and rotation in all directions. Although numerous clinical studies have revealed no statistically significant

differences in the cleaning ability between an ordinary toothbrush that is powered by hand and one that is powered by electricity, the target group are greatly facilitated in maintaining good oral hygiene.

However, the toothbrush fails to reach some remote parts of the oral cavity. Therefore, additional accessories can be utilized to perform the cleaning. Among the many choices, there are dental floss, toothpicks, interdental brushes, and electrical dental syringe. Dental floss, either waxed or not, can effectively help remove dental plaque on interproximal surfaces.

Toothpicks can be used to remove food debris; however, care is needed so that it won't hurt the adjacent soft tissue. For some subgroup of people who have lost the protection from gingival papillae, e.g., in the case of periodontitis or attachment loss therefore leaving the individuals at greater danger of caries attack, interdental brushes can be used to clean those places.

Electrical dental syringe can produce high-speed projection of water which contains rich foam and can remove food debris for hard-to-reach places, such as the surface under the orthodontic wires or partial removable prosthetic denture.

6.4.2 Restriction on Sugar Consumption and Use of Sucrose Substitute

Oral bacteria, especially the species capable of acid production and acid toleration, metabolize sugar contained in food debris to get energy to survive. This process, which often takes place in deep part of dental plaque where oxygen concentration is much lower than the superficial part, produces acid. This metabolism end-product will accumulate inside dental microflora and demineralize enamel crystals if the dental plaque is not wiped away and remain on the tooth surface for a long time.

Among all kinds of sugar-containing food (food that contains glucose, fructose, maltose, lactose, and many others), sucrose is found to be the most dangerous in causing dental decay. A lot of studies, both in vivo and in vitro, have proved that consumption of sucrose-rich diet will result in the unbalance of oral microbial micro-ecology where cariogenic species will tremendously outnumber those non-cariogenic species. Therefore, the initiation of dental decay will continue to progress once started. Therefore, restricting the consumption of such cariogenic-potent sugars is an important caries-preventive strategy. Such restriction can be applied to either the amount of consumption or the frequency of consumption or both.

However, despite of its undesirable cariogenicity, sugar is very important for the general health of human as a vital source of energy and nutrition. Therefore, it is very difficult to completely replace it by other means. Then it comes the advocacy of the use of sugar, especially sucrose, substitutes. These sugar substitutes are sugars in nature but have been found to be hard for dental microflora to use or will produce very limited amount of acid even if some species have managed to metabolize them. This special group of sugars includes those with very high sweetness such as aspartame, benzoic imine, cyclamate, and stevia sugar and those with low sweetness such as xylitol, sorbitol, mannitol, maltose, maltulose, and many others. Recently, these sugar substitutes have also entered the market for the general public to choose, where xylitol-containing products (mainly in forms of chewing gum or soft drinks) dominate.

Also in recent years, studies on isomaltitol are very hot. Isomaltitol is a mixture of α-d-glucopyranosyl-1,6-D-sorbitol (GPS) and α-d-glucopyranosyl-1,1-D-sorbitol (GPM) which are mixed at a mol ratio of 1:1, which has been found to be able to hydrolyze into glucose, sorbitol, and mannitol and is hard to be metabolized by cariogenic bacteria. Hopefully in the future, it might bring us a new choice of sugar substitute.

6.4.3 Reinforce Tooth Resistance to Acid

One important aspect as well as an important prerequisite for tooth resistance to acid is that first of

all a tooth must have natural anatomy and proper amount of inorganic and organic components. Therefore, before any individual is born, the proper oral health of the mother is of immense significance. Clinical observations and clinical studies have revealed the causal relationship between gingivitis and periodontitis of the mother and premature delivery as well as low birth weight, both of which will have substantial impact on the tooth formation and maturation for both the primary and permanent dentitions. Also, malnutrition of the mother will influence such critical tooth formation process of the child adversely.

After birth, the first few years serves as the most pivotal stages for a natural dentition to erupt into the oral cavity gradually and develop various physical functions of the oral cavity as well as the masticatory system. However, in such developmental stage, the permanent dentition is still under formation, while the primary dentition remains very vulnerable to local contributing factors to dental decay. Therefore, maintenance of proper oral hygiene and good general nutrition is the only way leading to a healthy full permanent dentition that will manifest itself years later (usually at the age of 12) and serve the individual lifelong (under ideal conditions).

However, the other side of the story must not be neglected. Normal anatomy and composition are no guarantee of the lifelong service of the dentition. Local contributing factors, including cariogenic bacteria and sugar-containing food consumption, exist in the oral cavity continuously. Weapons we have against the war of dental decay can be a variety of choices other than simple personal oral hygiene maintenance. Alongside with regular dental check-ups, fluoride in its various forms can provide potent protection to our teeth.

From the time of its discovery many years ago, when it is found to be existing in the drinking water which led to mottled enamel, Dr. Dean had noticed the reverse relationship between its existence in drinking water and reduced rate of caries. Further laboratory studies have convinced such underlying mechanism. Fluoride is proven to be able to reduce enamel solubility in acid solutions because it is able to react with tooth crystals (mainly exist in the form of hydroxyapatite, HA) and form fluorohydroxyapatite (FHA) or fluorapatite (FA), which are very resistant to acid. At the same time, fluoride is able to suppress bacterial metabolism of carbohydrates; therefore, the production of lactic acid is greatly reduced.

Since the discovery of this potent anticaries chemical, its various forms have been tested and proven to be suitable for clinical use. Many different kinds of fluoride are used worldwide, among which most frequently used includes sodium fluoride (NaF), calcium fluoride (CaF_2), sodium monofluorophosphate (SMFP), stannous fluoride (SnF_2), and silicon fluoride (SiF_2).

Systemic and topical applications of fluoride have been widely accepted and promoted. Systemic application of fluoride refers to addition of fluorides into various kinds of vehicles so that the effective component fluoride ion gets into the body and continuously secreted into the oral cavity alongside with saliva. Such vehicles which have come into being in the western industrialized countries for many decades include water (fluoridation of water supply), milk, and salt and agents such as tablets or drips usually administered by professionals. However, developing countries, which do not have adequate resources or infrastructure (such as water supply equipment), fail to have this means of fluoride administration that will benefit the general public.

On the other hand, topical fluoride application serves as an equally effective means of fluoride supply. This category involves a variety of application methods that are more feasible and flexible than systemic fluoride administration. These methods include fluoridated toothpaste, mouth rinses, varnish, gel, and foams. The first two methods are easy for personal use at home and have been widely accepted as important means of daily oral hygiene maintenance. The rest are usually applied by professionals.

Many clinical studies have shown very encouraging results with regard to reductions on caries incidence rates. Two to three decades later, epidemiological surveys have shown a steady decrease in caries prevalence rates worldwide, indicating

the tremendous achievements by widespread use of fluoride in caries prevention dentistry has ever managed to achieve.

6.4.4 Pit and Fissure Sealing

Pit and fissure are created in the process of tooth development. They have distinct anatomical features that render them very much vulnerable to caries attack. These depressions of occlusal surface (together with the buccal and lingual surfaces of the molars) always have the bottom part at deep enamel, enamel-dentinal junction (EDJ), or even at the dentine. What's worse, these narrow places are hard to reach with routine examination and cleaning methods.

As a result, it is not surprising that there is almost always presence of accumulation of multispecies microbial biofilm, food debris, remains of enamelogenic epithelium, organic plug, etc. Therefore, pit and fissure are most often discovered as the first site of carious lesions of an individual. Data of a clinical study show that about 67 % of caries lesions of children at the age of 3 are pit and fissure caries. Another epidemiological survey of 25,000 school children revealed that 80 % of all diagnosed carious lesions are located at pit and fissure.

In the 1960s, on the basis of research findings on enamel etching and adhesion, pit and fissure sealant was invented. The underlying strategic thinking is that if a posterior tooth erupts fully into the oral cavity and gets all the vulnerable caries-susceptible sites concealed by dental material the pit and fissure are no longer open to the oral cavity and therefore will not be influenced by the microbes that inhabit the mouth and their dangerous acidic metabolic products. And this invention has been proven to be a huge success.

During the following years, the components of pit and fissure sealants have been refined greatly. Now, the most often used sealants are resin in nature, often belonging to the Bis-GMA system which has many advantages under clinical condition, such as they are easy to use and aggregate, and its volume shrinkage after aggregation is small. However, in recent years there is also some dispute about its safety.

A great number of clinical studies have verified that pit and fissure sealing can substantially reduce caries onset. Systematic reviews on randomized clinical trials have found similar encouraging results. In this sense, pit and fissure sealing is strongly recommended by FDI, ADA, IADR, WHO, and many other professional organizations. And in many countries, it has already been included as part of governmental budget for caries prevention programs.

6.4.5 Preventive Resin Restoration

Preventive resin restoration is actually a variation of pit and fissure sealing. It involves treatment of susceptible or early carious lesions in pit and fissure (preservative cavity preparation and restoration with fluid resin mainly) and combines it with subsequent pit and fissure sealing. This method has adopted concepts of minimal invasive dentistry (MID) into caries prevention. However, its application is restricted to early caries lesions only.

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Clinical Management of Dental Caries

7

Li Jiyao

Dental caries can be classified clinically as cavitated and noncavitated lesions according to substantial damage to tooth morphology. The non-operative approach is usually used for non-cavitated lesions or as a preventive measure for susceptible teeth. For caries with substantial loss of dental tissue, an operative measure such as restoration is taken. The development of material science provides a variety of choices for dental caries management.

Dental restoration is an operation on an organ with unique biological properties, which involves theoretical knowledge such as mechanics, biology, materials science, aesthetics, etc. It is possible that the pulp–dentin complex could be irritated or damaged in the process of treatment; hence it is vital and important to protect the dentin and pulp throughout the process.

7.1 The Development of Caries Treatment Theory

At the end of nineteenth century, G.V. Black created a dental restoration system according to the susceptible sites and dental anatomical relationship of dental caries, which involved the clinical demands of retention and resistance, in addition to the property of amalgam, and laid the foundation for modern restoration dentistry. In the 1950s, Buonocore introduced the acid-etching technique into restorative dentistry. Through the acid-etching technique, resin composite was able to bind with dental hard tissue with a mechanical lock. The binding mechanism was different from amalgam. From then on, restorative dentistry entered the new age of "adhesive dentistry." After the twenty-first century, restorative dentistry entered the era of microdentistry, by proposing the concept of early diagnosis, prevention, and micro treatment; the operative dentistry was directed toward a biological approach, to prevent the development of caries and to preserve healthy dental tissue as far as possible [1].

7.1.1 G.V. Black and Modern Restorative Dentistry

G.V. Black made a huge contribution in forming the concept and principles of modern dental restoration. At the end of the nineteenth century, with a rigorous scientific attitude, he carried out

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an in-depth investigation into the damage of caries in susceptible sites and its relationship with dental anatomy. Combining the demands of retention and resistance required by restoration, in addition to the property of amalgam, he created a complete restoration system. The system explained the corresponding principle and developed a series of basic principles and requirements of cavity preparation, material mixing, and filling. Restoration dentistry then embarked upon a scientific, standardized pathway and the foundations of modern restoration dentistry were laid.

The content of restoration dentistry proposed by G.V. Black was to prepare the cavity upon the already weakened dental tissue or after removing the damaged dental tissue base on the principle of retention, resistance, and protection of the pulp–dentin complex and then restore its original form and function with a specific material through a certain process.

The knowledge of cavities is a major part of G.V. Black's contribution to restoration dentistry. According to the caries location, and in combination with the tooth structure, in addition to the characteristics of the design and preparation, he divided cavities into five categories, which generally covered the basic types of caries. Two more types were added afterward to supplement them. Clinically, this category is still being widely used.

According to the properties of amalgam, he also proposed a principle based on the demands of auxiliary retention and resistance of cavity preparation, such as the depth, contour of the cavity, a cavity with a dovetail, a ladder-shape retention form, the undercut, and the removal of weakened enamel. He also proposed certain demands for each category of cavity. The description of these systems laid the foundations for the development of modern restoration.

7.1.2 Adhesive Bonding Technique and Dental Restoration

The development of dental restoration and that of dental material are inseparable. At the end of the nineteenth century, G.V. Black created a scientific, standardized restorative system, using mechanical retention. In 1955, for the first time, Buonocore used acid to process the enamel surface, to increase the bonding between composite resin and the tooth surface and started to revolutionize dental practice. As the resin material and bonding material developed, the concept of restoration had already been changed; the preservation of more dental tissue was widely accepted as a bonding technique.

In the past 20 years, there has been a great leap in the development of resin composite material and the acid-etching technique has matured. Bonding systems for dentin have been continuously updated, including total etching and self-etching techniques and the procedure was simplified as well. The clinical application of composite resin has become increasingly widespread and the effectiveness of bonding restoration has also been proven because of its long-term results.

The bonding technique revolutionized traditional dental restoration. In terms of cavity design and preparation, it preserved more healthy dental tissue by discarding the sacrifice certain depth of cavity and dove tail, ladder-shaped retention form. Combined with the theory of bonding resin composite, more careful preparation of the cavosurface angle and bevel was required.

7.1.3 The Foundation and Principle of Minimally Invasive Caries Treatment

Traditional dental restoration was based on the principle created by G.V. Black in 1908, removing a lesion by operation and then restoring the damaged part. This approach was launched based on the foundation of amalgam properties, which could certainly cause vast damage of dental tissue. In the twenty-first century, modern dentistry suggested a more reasonable theory, which was minimally invasive treatment. Minimally invasive treatment is a branch of preservation dentistry. In the literature, terms such as minimal intervention dentistry, minimally invasive

dentistry, and micro dentistry were used. In the Modern Oxford Dictionary, minimally invasive dentistry has the same meaning as microdentistry. MI is an abbreviation of minimal intervention dentistry, but not minimally invasive dentistry [2]. Tyas et al. believed that minimal intervention dentistry focuses on the knowledge of how caries develop, including early diagnosis, prevention, and treatment, and placed emphasis on the treatment switch from dental operation to biological method, to prevent the development of dental caries and preserve as much healthy dental tissue as possible. Peters et al. believed that minimally invasive dentistry focuses on the preservation of healthy dental tissue when removing caries lesions, instead of the "extend to prevent" principle of G.V. Black.

In terms of the biological study of dental tissue, in addition to the etiology of caries, especially the process of remineralization, the revolution of diagnosis measurement, and the novel view of prevention, the development of dental material has laid the foundation for minimally invasive dentistry.

Martin et al. proposed four basic principles for minimal invasive dentistry: lesion control, remineralization of early caries, minimal surgical trauma, and restoration, but not replacement, of dental caries lesions.

Modern material science proved that dental restoration material could not match the healthy dental tissue in terms of physical, mechanical, and biological properties. Removing healthy dental tissue and restoring the cavities with traditional filling material certainly could not meet the functional requirements. With the development of adhesive material, in addition to the traditional chemical dentin bonding, there was also micromechanical bonding in the hybrid layer. The dentin bonding system developed from no-etching bonding to total-etching bonding (1980s), total-etching wet bonding (1990s), self-etching primer adhesive systems (1990s–2000), and then one-step adhesive systems (2000s). The micromechanical bonding mechanism produced solid bonding with dental tissue, decreased the sacrifice of healthy dental tissue to obtain sufficient retention, and decreased the chance of microleakage.

In the past, the process from the demineralization of dental hard tissue to the degradation of dentin collagen and finally the formation of cavity was generally considered irreversible. As a matter of fact, the process of dental tissue gaining and losing calcium and phosphate ions was carried out alternately; i.e., a demineralization–remineralization cycle on the surface of the tooth. When the pH of the interface between dental tissue and plaque was below 5.5, it showed that the enamel and dentin demineralized. As the pH recovered, it showed remineralization. Fluorine played an important role in this cycle. It could strengthen the absorption of calcium and phosphate and form fluorapatite on the tooth surface. Fluorapatite is highly acid-resistant; demineralization starts at $pH < 4.5$, but when in fluorine-containing environments, remineralization of the tooth and anticarious ability are greatly enhanced.

For now, the method of diagnosis for detecting the different statuses of caries, such as early demineralization, small caries lesions, and small cavity were varied, including bacterial counts, saliva buffering capacity, fiber-optic transillumination technology, electrical impedance technology, laser fluorescence technique, nonvisible light imaging technology, etc. These techniques were particularly used for diagnosing hidden caries, early caries, proximal surface caries, and caries in pits and fissures, which greatly enhanced the sensitivity and accuracy of early diagnosis.

The new idea about prevention has highlighted the importance of the interaction between doctors and patients when a dental operation switches to nonsurgical treatment. It is widely believed and accepted that caries are initially reversible, mainly caused by bacterial infection, and constitute a multi-factorial chronic disease. Treatment from the doctor is merely a small part of the complete treatment, to fulfill caries prevention; long-term cooperation between doctors and patients must be built to achieve that.

New techniques of prevention include enamel angioplasty, pit and fissure sealant, and preventive resin restorations; the purpose is to retain as

much of the healthy tooth structure as possible and change the conditions for the development of dental caries, thereby reducing the incidence of dental caries.

Modern caries treatment pays more attention to the biological response of the pulp–dentin complex. At the same time, the relationship between the restored tooth and periodontal health, between occlusion and periodontal health, and the proximal contact between the prosthetic and the adjacent teeth also needs to be considered. Minimizing patients' anxiety and pain caused by the fear of dental treatment should also be considered.

Atraumatic restoration, sandblasting caries removal, chemical–mechanical caries removal, the laser treatment of dental caries, and other new technologies, have overcome the excessive loss of the healthy tooth structure caused by traditional dental drilling, which can easily cause complications when near pulp, especially in the children with an extreme fear of dental treatment, and the risk of cross-infection. New techniques such as amalgam bonding, tooth colored material, composite resin inlay, use new materials or technologies in cavity restorations, making it possible to get rid of the traditional preventive expansion during cavity preparation.[3]. The concept of a minimally invasive dental treatment plan is reflected in the following three aspects:

1. Early diagnosis and personal treatment. Through individual caries risk assessment such as plaque and saliva, and evaluation of *Streptococcus mutans*, in addition to early caries monitoring, the establishment of personalized dental files, the enhancement of patient management, an increase in patient communication, and improvement of patient loyalty.
2. Treatment and effective control. Based on the diagnosis to build up prevention treatment programs, to implement minimum intervention, and prevent secondary caries, using smart materials, to treat rather than simply replace the damaged tissue, to maximize the preservation of dental health.
3. Focused prevention and effective intervention. Control of cariogenic bacteria, plaque control, reduce the amount of sugar intake, and improve salivary function. Explain to patients the causes of dental caries, correcting the patient's poor eating habits and oral hygiene habits, guide patients to benefit from professional dental methods, and to control the demineralization and promote remineralization. Implement the concept of professional dental care and early caries treatment.

Minimally invasive dental operation process: patient visits; diagnosis through treatment planning system, assess caries risk, prevent caries activity through the history of dental caries, general oral condition, and to confirm treatment the plan by performing observation, prevention, or treatment.

Lesion control is a prerequisite for successful remineralization and filling. To control lesions, the presence of pathogens first needs to be controlled. At the early stage of the lesion, through the effective use of modern diagnostic techniques and prevention systems to control bacteria before irreversible loss of dental tissue, thus preventing the development of dental caries. For patients at a high risk of caries, the use of mouthwash, a change of bad oral habits, eating habits, etc. will be needed to control bacteria or adjust the pH of the saliva, flow, and viscosity to achieve the goal of caries prevention.

Minimal surgical intervention is the most effective method of restoration of tooth structure. When caries progress to dentin, the enamel layer forms cavities. Plaque accumulates in the cavities, which affects calcium, phosphorus, fluoride ion uptake, and is difficult to remove. Remineralization repair is then unlikely to succeed, and only by the use of dental surgical methods. The cavity can be roughly divided into two layers from outside to inside:

1. Infected layer: this layer of the tooth structure has been completely denatured and bacteria settled.
2. Demineralized layer: this layer has a certain level of demineralization, but the collagen scaffold still exists and can be re-mineralized. In the past it was thought that the demineralized

layer should be removed, but recent studies suggest that the demineralized layer is a precarious status instead of caries-active status. With the promotion of mineralized material to repair, this layer can be remineralized. Therefore, the modern view is that the removal of diseased tooth structure should be limited to the infected layer.

Based on the understanding regarding the early diagnosis of dental caries, focused prevention, and minimally invasive treatment, the twenty-first century has ushered in the era of the minimally invasive treatment of dental caries.

7.2 Current Management of Dental Caries and Its Development

Under the influence of minimally invasive dentistry, dental caries treatment has shifted from dental surgery to biological treatment. According to the progressive stages of decay, the development in the treatment of dental caries focuses on the prevention and management of people susceptible to dental caries, nonsurgical treatment at an early stage, and the surgical treatment of the cavity. Accordingly, dental caries treatment technology has also developed considerably. The clinical application of the minimally invasive treatment of dental caries and the systematic evaluation of the effect of composite resin restoration has provided the foundation for the application of new technologies and materials. Current dental caries treatment should be an optimized treatment regime, using the best technology to achieve the best treatment outcomes.

7.2.1 Minimally Invasive Treatment Technique

The minimally invasive treatment of dental caries emphasizes the preservation of as much of the tooth structure as possible, keeping surgical intervention to a minimum. Broadly speaking, all of the caries treatment techniques should emphasize a conservative approach. The following briefly summarizes the minimally invasive treatment techniques; the nonsurgical treatment technologies are also included. These techniques are mainly used in the early stages where dental caries have not yet developed, in people susceptible to dental caries, and in the prevention of caries in sensitive areas.

7.2.2 Minimally Invasive Cavity Preparation

7.2.2.1 Nonmachinery Preparation

Air Abrasion Air abrasion was first introduced in 1945 with the first air abrasion machinery available in 1951. The air abrasion system has evolved greatly since then. The principle of air abrasion is to apply highly pressurized, nontoxic particles, such as aluminum oxide ions, to accurately remove the enamel, dentin, carious tissue, and old fillings. Air abrasion can partially replace the high-velocity gas turbine cavity preparation. It is quieter, more time- and energy-efficient, and requires no anesthesia as it does not produce vibration and heat. It is well received by patients and maximizes the conservation of the tooth structure. The interior of the prepared cavity is smooth, making it easier to fill. It reduces the stress between the filling and the tooth structure, reduces the likelihood of microfracturing, hence prolonging the life of the fixture.

To aid early the detection of caries on the occlusal surface, the blackened part of the cavity should be examined. After visual examination, the air abrasion system aims strong abrasive particles toward the affected areas in the cavity. If the blackened area is a stain, it can be removed with ease. If it is accompanied by decay, a beam of strong abrasive particles can reveal and blast the stain away in addition to the decay. As dentin is moist, thick, and elastic, the particles bind on its surface rendering it unable to exert its effect. Hence, it should be removed manually or using a mechanical machine before using air abrasion.

The disadvantage of this method is that because it is easier to remove dentin than enamel, it causes the overhang of enamel, which requires trimming of the enamel with the drill. Contraindications to air abrasion include patients with:

1. A severe allergy to dust, asthma, and chronic obstructive pulmonary disease.
2. Open wound or recent tooth extraction.
3. Active periodontal disease.
4. Recent placement of an orthodontic appliance
5. Subgingival caries

Air Polishing Air polishing delivers high-pressure jet of sodium bicarbonate to the surface of the tooth, producing a cutting effect. Air polishing differs by using an aqueous friction solution, which does not cause a significant amount of sodium bicarbonate aerosol. This technique was originally designed for stain removal and it is now also used to remove crown fillings. Air polishing is not very selective when cutting the tooth structure and can damage healthy dentin and cementum. It is mainly used in the final preparation of the caries to remove any remaining decayed dentin.

Lasers Lasers can be used to perform surgeries on dental soft tissues as they do not cut through dental hard tissues. Hence, surgeries involving hard tissues need to employ other types of lasers. The ideal laser should be able to manage both dental hard and soft tissues. Clinically used lasers that can cut through dental hard tissues include erbium:yttrium-aluminum-garnet (Er:YAG), carbon dioxide (CO_2), neodymium:yttrium-aluminum-garnet (Nd:YAG), and Ar:F. They all have selective abrasive properties whilst conserving healthy tooth tissue. Er:YAG is the most selective of all lasers. Laser cavity preparation is precise, nonvibrating, has no smell, and does not require anesthetics. As lasers can seal dentinal tubules, they can also prevent hypersensitivity postoperatively. On the downside, the machinery is bulky and expensive, thus limiting its role in clinical practice.

Chemomechanical Caries Removal Chemomechanical caries removal (CMCR) uses chemical agents to soften the dental tissues before eliminating infected tissue using machinery. In 1985, the first CMCR system, Caridex, was introduced into dentistry. Caridex involves the intermittent application of pre-heated N-chlorinated-DL-2-amino butyric acid (GL-101E) into the cavities. This solution causes the partial disintegration of the collagen in the cavity, accelerating the removal of dental caries. The uptake of this technology was not great as it is expensive, time-consuming, and requires a lot of additional equipment, including memory cell, a heater, pump, and special hand piece.

Recently, CMCR has been re-introduced to the dental industry, providing a new alternative to caries removal and preparation. A new CMCR system, Carisolv was introduced – Carisolv gel and Carisolv hand tools. The Carisolv reagent consists of two component mixtures. One of the component mixtures is a red gel composed of leucine, lysine, glutamic, and sodium hypochlorite. After applying the mixed Carisolv reagent to the cavity, a hand tool can be used to remove the softened carious tissue. This method can selectively dissolve carious tissue quickly (around 30s), whilst not affecting any healthy dentin. Compared with other caries removal techniques, CMCR can effectively remove the smear layer of the cavity, reinforce the bond between the filling and the tooth, there is no noise, vibration or anesthetics, and patient acceptance is high. However, when compared with the high-velocity turbine, the operating time is longer and requires alternative methods to gain access to and repair some undermining caries.

The CMCR procedure can handle most dental caries. It can be used alone or combined with other traditional caries removal techniques. In those caries that cannot be directly accessed or have existing fillings, CMCR can be used in conjunction with a dental drill to gain access to the cavity area or remove the old filling, before using Carisolv to remove the caries.

The CMCR method should be first considered for the following patient group: root/cervical caries, coronal caries (especially deep coronal

caries), caries located on the edge of the crown or bridge abutment, completion of canal preparation, those in whom anesthetic is contraindicated, especially needle-phobic patients, those with a dental phobia, and pediatric patients.

7.2.2.2 Mechanical Rotary Technique

The mechanical rotary technique uses high-velocity turbine hand tools to prepare dental cavities. To maximize healthy tooth conservation, burs in proper size are chosen in practice in relation to the principles of minimally invasive dentistry.

Tunnel Preparation Tunnel preparation refers to the occurrence of caries located on the proximal surface of the teeth, if the lesion is more than 2.5 mm apart from the marginal ridge, cavity preparation can enter from the occlusal surface, to maintain the integrity of the marginal ridge but it is not prepared as the classical proximal–occlusal cavity. If the tooth surface that is adjacent to the lesion has demineralized but not actually damaged, and it is necessary to maintain the integrity of its surface, this is also called internal preparation. Advantages of this method are:

1. The marginal ridge of the tooth can be kept intact, thereby enhancing the resistance of the remaining tooth structure.
2. Injury of the proximal surface of the adjacent tooth is avoided during cavity preparation.
3. Normal contact relationship with the adjacent tooth is maintained.
4. Overhanging of repair materials is prevented.

Slot Preparation Slot preparation, also known as mini box preparation, is designed mainly for proximal caries. It can be divided into the following cases:

1. Caries is close to the marginal ridge and cannot preserve its integrity or the marginal ridge has been destroyed.
2. Caries is located underneath the interproximal contact. Designing of cavity preparation focuses on carious tissue; if the marginal ridge cannot be preserved, the cavity is only prepared into a box shape without a dovetail. When the caries is underneath the interproximal contact, carious tissue is approached from the buccal or lingual surface, and prepared into a box or disk shape with the auxiliary help of groove retention. Then glass ionomer or composite resin is used for restoration. In most cases, this method can provide normal interproximal contact between adjacent teeth.

Microscopic Preparation Techniques To minimize invasive trauma, the technique of using micro drills for microscopic preparation, under the microscope or loupe, to obtain precise tooth preparation is called "microscopic dentistry." Micro drills can be round, oval, and conical, and the operator can select different drills for fissure caries preparation and finishing. The shank of the burs is longer than that of traditional ones; thus, the operator's sight is not blocked by the head of the hand piece. Using this drill together with tooth-colored bonding materials for repairs can better reflect the purpose of minimally invasive treatment.

7.2.2.3 Minimal Invasive Prevention Technique

Pit and Fissure Sealing Pit and fissure sealing is an effective method of preventing fissure caries. The occlusal surface of the tooth formed a different shape and varying depths of the fissure during development, and oral bacteria, metabolites, and food residue often accumulate at caries predilection sites. Pit and fissure sealants may isolate the fissure and the oral environment, preventing bacteria and food debris from entering, to achieve the purpose of caries prevention. Fluoride sealants can continuously release fluoride, provide barriers, and promote remineralization.

Pit and fissure sealing is mainly used for suspicious fissure and pit caries as well as deep grooves adjacent to filled fissure caries on the occlusal surface. Sealant is mainly made of resin, a diluent, an initiator, and a number of auxiliary components, such as fillers, fluoride, dyes, and other components. The main body of sealant resin material, bisphenol-A-glycidyl methacrylate

(Bis-GMA), is a commonly used resin with good performance.

Enameloplasty Noncaries fissures were drilled out to form a shallow plate shape, making it easy to clean and keep free from caries, i.e., enameloplasty. This method removed minimal tooth structure, but produced lasting anti-caries effects, and no worries regarding fallen sealer. A simple operation, with few technical requirements. The suitable depth of the fissure is no more than half the enamel thickness. Using a high speed hand piece with a pear-shaped diamond, ball diamonds, or a sand stone point bur the site should be gently removed. For the grooves on the cusp surface, a diamond bur can be used to remove evenly parallel to the surface, until the groove is no longer brown. Enameloplasty can be used on fissures that are close to or across the molar buccal lingual ridge. The prepared cavity can be extended to 2 mm away from the buccal–occlusal junction or lingual–occlusal junction, and the rest can undergo enameloplasty, but not following the G.V .Black principle, which extended to the buccal and lingual surface.

Preventive Resin Restorations In 1977, Simonsen suggested performing "preventive resin restorations" to treat suspicious fissure caries and provides a new approach to the treatment of fissure caries. Preventive resin restorations only remove the infected enamel or dentin at the lesions, according to the size of the caries, using etching technology and the resin material filling up the early fissure caries, and the occlusal surface coated with sealant. It is a preventive measures combined with pits and fissure sealing and fissure caries filling. Because it does not use the traditional extension for prevention, only a amount of carious tissue is removed and restored with composite resin or glass ionomer, the pit and fissure caries without caries is protected by the sealant, thus preserving more healthy dental tissue, and is an effective method for preventing the further development of caries.

The advantage of preventive resin restorations is using glass ionomer composite resin as filling and binding with enamel mechanically or chemically, then bonding with sealant by chemical bonding reduces the possibility of generating micro-leakage.

Caries Pharmacotherapy Caries pharmacotherapy is the use of drug treatment to stop the development of caries, or get rid of superficial caries. Drug therapy is mainly applied to early enamel caries in teeth on a easy-to-clean smooth surface (such as buccal, lingual), on which cavities have not yet formed; superficial caries of anterior primary teeth on the proximal surface; secondary enamel hypoplasia, which causes extensive shallow caries, and difficulties with prepare cavities preparation.

Fluoride, such as 75 % sodium fluoride and glycerol paste, 8 % stannous fluoride solution, 2 % sodium fluoride, sodium monofluorophosphate solution, or a fluoride gel are commonly used drugs for the treatment. By local application, these fluorides can penetrate into the enamel to form insoluble acid fluorapatite, and promote remineralization of enamel, and also to prevent bacterial growth, inhibit bacterial metabolism, acid and polysaccharide synthesis. Fluoride has no corrosive stimulation to soft tissue, no tooth discoloration, and is considered safe and effective.

The main agents of silver nitrate are 10 % silver nitrate solution or ammonia silver nitrate solution. Silver nitrate is a strong corrosive agent, which can combine with proteins to form precipitates. When in low concentrations, it provides convergence and an antibacterial effect; in high concentrations it has a strong corrosive effect and can kill bacteria. When it is applied to zones of caries, with the addition of clove or 10 % formalin solution, it can turn into black reduced silver, and with the addition of 25 % iodine it results in off-white silver iodide. Both of these two formulations can penetrate into the enamel and dentin to coagulate proteins, kill bacteria, plugging gaps in enamel and dentin tubules, thus blocking and terminating the development of the caries lesion.

Remineralization Treatment For early enamel caries that have been demineralized and softened, the appropriate drug treatment to re-deposit

calcium and remineralize, thereby removing its hardness and eliminating caries, is called remineralization treatment.

Early enamel caries on the smooth surfaces (buccal, labial, lingual, palatal or proximal), such as white spots, and people susceptible to caries are suitable for remineralization therapy.

There are many types of mineralized fluid, which divided into single component and complex components. The single component is mainly fluorine-containing (e.g., NaF: 0.2 g; DH_2O: 1,000 ml), the complex component mainly containing different ratios of calcium, phosphate, and fluoride salts, while calcium or fluoride salt is the main ingredient (e.g., CaCl: 9 g; KH_2PO_4: 6 g; KCl: 1.1 g; KF0.2 g; DH_2O: 1,000 ml).

In recent years, a new remineralization agent, CPP-ACP, has been used clinically. Casein (casein phosphopeptide, CPPs) used casein as a raw material, by hydrolysis, separation, and purification to obtain a class of phosphoserine-rich bioactive peptides. Under neutral or alkaline conditions, CPPs can form soluble chelates with the amorphous calcium phosphate (ACP), i.e., casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). CPP-ACP has a wide range of applications in biology, including the promotion of remineralization of the tooth surface and bone calcification, promoting the absorption of minerals, and has an effect on cariogenic bacteria. Currently, CPP-ACP is used in the treatment of early caries remineralization of dentin hypersensitivity, dental erosion treatment, and as prevention in caries-susceptible patients. The remineralizing agent with CPP-ACP as the main ingredient shows broad application prospects in caries prevention.

Atraumatic Restorative Technique Atraumatic restorative technique (ART) refers to using only hand instruments such as a spoon excavator to remove caries tissue, then using glass ionomer cement or other cementitious filling materials for repair.

The ART meets the requirements of modern minimally invasive restoration, the use of a bonding of glass ionomer materials, with as little cavity preparation and tooth damage as possible, and the best preservation of tooth structure.

7.3 Current Silver Amalgam and Techniques for Direct Restorations [4]

7.3.1 The Controversy Over Silver Amalgam

Silver amalgam has a history of more than 160 years as a dental restorative material. Because it has the ideal wear resistance, inoxidizability, good mechanical strength, and it is inexpensive and simple to operate, silver amalgam also displays great longevity and a low rate of secondary caries in posterior teeth. Although silver amalgam is not satisfactory with regard to aesthetics and conducts heat and electricity, it is still widely applied in dental practice.

In China, it was recorded that silver paste filling has been in use since the Tang Dynasty. In 1826 in France silver amalgam was used for tooth repair; in the mid-1930s, it started being applied in American dental practice. With the development of material preparation and the improvement in property, the silver amalgam in restorative dental material has obtained the approval of many international health organizations, including the WHO.

However, there was doubt regarding silver amalgam and its application in tooth repair because of the mercury content. There have been reports that mercury can cause adverse effects, such as kidney function damage, reduced immunity, nerve toxicity, etc. However until now, there still has been no evidence reporting the direct causal relationship between silver amalgam use and adverse effects. Moreover, there are also cases of silver amalgam allergy, the main manifestations of which are a lichen planus-like response affecting the local mucosa and the occurrence of skin erythema, but this type of allergy is only limited to the region adjacent to the silver amalgam, and had no obvious effect on the body; moreover, the symptoms may be

relieved after the elimination of the silver amalgam restoration, with no need for special treatment.

From the perspective of public health, protection from silver amalgam pollution should be strengthened, and the presence of mercury vapor in the clinic should be closely monitored. Nowadays, the mixing of silver amalgam has been automated, closed off, and there is minimal mercury vapor pollution in the oral clinic.

It is noteworthy that with the continuous development of dental restorative materials and technology, the new idea of a minimally invasive technique has been put forward in the twenty-first century, and the status of silver amalgam in tooth repair has changed. Because of the shortage of the physical and chemical properties of composite resin and glass ionomer cement tooth-colored material, it still cannot completely replace the silver amalgam in posterior tooth repair at present, although some research has been carried out that has shown that the longevity of composite resin is greater than for the same sized silver amalgam restoration [5].

7.3.2 Indications and Contraindications

Silver amalgam can be used in almost all posterior teeth restoration, unless the preparation is not appropriate for the retention form, or if there is a mercury allergy. However, with composite resin-bonded fixed technology widely used in dental restoration, the developed countries in Europe have been gradually reducing the use of amalgam. It is believed in the following situations that amalgam is preferred: a large complicated cavity in a molar, the need to restore the tooth cusps, and direct restorations of teeth. If retention is expected to increase, the bonded amalgam should preferably be used and/or a box-shaped, grooved, circular retention groove retention, and then consider dentin pins should be considered. If there is large area of crown damage, the cementing material post and core restoration is superior to the silver amalgam. Afterward, the entire crown is used to cover it. Moreover, the use of cementing material is not suggested for repairing cracked spots in silver amalgam restoration. The silver amalgam may also be used as post and core material. Then, the amalgam-fractured parts repair, the repair parts are not comfortable using a bonding material.

However, it is not recommended to use silver amalgam in the following situations: early tooth decay; premolar and molar occlusal surface or adjacent surface cavity of small to medium size; premolar large occlusal surface or interproximal caries; all tooth neck cavity repair; root canal filling; pregnant women.

7.3.3 Silver Amalgam Restoration Technique

7.3.3.1 Cavity Shape Preparation

The principle of cavity shapes of silver amalgam restorations consists of removing the decayed tissue, outlining the cavity border, removing all unsubstantial enamel and sharp ridges, emphasizing the protection of the dental pulp–dentin complex and the functional reconstruction after filling, without damaging the periodontal tissues.

The cavity shape for silver amalgam restoration requires a high level of resistance and retention. It is the basic requirement that tooth tissues and silver amalgam restorations should not fracture or deform and restorations should not be taken off, shift, under normal chewing forces with proper chewing movements.

The amalgam restorations must have a certain thickness to ensure basic resistance. The depth of the occlusal cavity of posterior teeth should be at least 1.5–2 mm. The distance of healthy tooth tissue between two amalgam restorations on the same tooth should be at least 1 mm.

7.3.3.2 Silver Amalgam Filling

After the cavity preparation the rubber dam and suction are used to isolate the operation area from moisture and saliva.

To confine the silver amalgam to the cavity and complete the compression backfill, a box-shaped cavity with a restrictive wall and bottom

is needed. For a class I cavity on only one tooth surface, the structure itself has satisfied this request. However, for a cavity involving two or more surfaces, it is essential to use a matrix bond surrounding to turn it into a box shape. Before the filling, the matrix bond is used to develop the artificial wall. On one hand, it is conducive to the compressive backfill of silver amalgam; simultaneously, it may also effectively prevent the silver amalgam from overflowing and periodontal overhang, which stimulates the periodontal tissue.

For a class II cavity of proximal tooth damage, the use of a forming piece is extremely convenient if it has proximal tooth support, but for severe tooth body damage, the proximal tooth cannot play a supportive role; thus, other methods are needed to assist. The wedge is a common piece of auxiliary equipment used in the forming of the proximal surface. When the matrix band cannot fit the tooth neck well, the wedge may be inserted between the necks of the teeth. Because the matrix band with the teeth closed, in the case of the silver amalgam pressed out to form an overhang from the gingiva. The shape and size of the wedge should be appropriate. If it is too small it is not easy to set tight, while if it is too big it will affect the adjacent shape of the restoration.

After cleaning, disinfecting, and drying, the cavity can be filled. Before silver amalgam filling, coating and painting can reduce the occurrence of secondary caries; the commonly used varnish is made from resin materials. Using the silver amalgam autostirrer completes the attrition evenly. When backfilling, the principle of little by little filling, of layer upon layer compression, and of the dotted angle to the line angle should be followed. The backfill process makes silver amalgam go into the cavity and fit with the tooth body closely, and a silver amalgam plugger is used to carry out the process. When compression causing the unnecessary mercury to squeeze out and causing the air which mixes with it to discharge, it is more advantageous to the completion of the operation, thus strengthening the intensity of the restoration and the mechanical properties. In class II cavity filling of the side of the cavity shape, the surgical principle is lateral compression, and layer upon layer compaction. When the cavity shape is large, more attention should be paid to the principle of layer upon layer compression. It is generally believed that a thickness of 1 mm is able to provide full compression. When applying pressure, in addition to considering the vertical direction, attention should also be paid to both the horizontal and the lateral direction to ensure that the silver amalgam restoration has a three-dimensional adaptation.

When filling is completed, polishing should be carried out promptly, using an ovoid hoe light modulator to act in mesiodistally and buccolingually to remove excess silver amalgam and further compact the edge of the silver amalgam restoration. Then, the restoration should be carved according to the anatomical form and adjacent relations. Usually, an instrument with a sharp edge can be used. A special carving instrument is available, but a digging machine and hoe can also be used. Silver amalgam carving should be done in accordance with the anatomical shape, forming the ideal tooth shape and bulge, but not a deep pit and fissure. It should not appear sparse or feather-edged on the border of the restoration with the tooth tissue. The sculpting of the silver amalgam should be performed before removal of the forming sheet. This is advisable before the silver amalgam is fully solidified, generally using a short push and pull, but attention should be paid to the direction, which should be from the tooth body to the restoration. In trimming the edge of the crest of the adjacent and occlusal cavity, attention should be paid to the height of the edge of the crest of the adjacent tooth, and then to forming an outreach gap. Next, the occlusion should be checked and adjusted to prevent a high bite, and the early contact point of middle occlusion, lateral occlusion, and stretching occlusion should also be removed. General occlusal adjustment can be divided into two steps, namely immediately after the filling is completed and the second day after the filling. The subsequent polishing, called polishing after carving, is to ensure that the surface of the silver amalgam restoration is smooth, to improve the corrosion resistance of

the silver amalgam restoration, and to prolong the life of the restoration.

7.4 Resin Composites and Direct Bonding Restoration Technique

Resin composites are types of polymers that are used in dentistry as a restorative material developed based on acrylate. They are mainly composed of Bis-GMA monomers or some Bis-GMA analog, a filler material, and a photo initiator. The properties of resin composites have been greatly improved since Bowen invented them in 1962. In particular, the development of bonding technology has led to resin composites becoming more widely used and becoming the ideal tooth-colored restorative materials at currently available.

In 1955, Buonocore first treated the tooth surface with acid to promote the bonding between resin and the tooth surface, which improved the stability of restorations. This technique was then used in clinical practice and universally acknowledged at the annual meeting of the International Society of etching techniques in 1974, which brought about great changes in dental practice.

7.4.1 Resin Composites

There are many kinds of resin composites, which can be divided into anterior tooth type, posterior tooth type, and universal, according to the positions in which they are used in the clinic. Anterior resin composites emphasize color and polishing, while posterior tooth type enhances the mechanical strength and abrasion resistance. According to the inorganic filler particle size, resin composites can be divided into macrofill resin composite, microfill resin composite, microhybrid resin composite, hybrid resin composite, and nanocomposite. For macrofill resin composite, the filler particle size ranges from 10 to 100 μm and the filler volume fraction is 70–80 %. As early products, such materials possess good mechanical properties, but poorer surface polishing characteristics, so that they are less frequently used at present. For the microfill resin composite, the filler particle size ranges from 0.01 to 0.1 μm and the filler volume fraction is 35–50 %. This kind of material, which was produced in the late 1970s has great color and polishability, but poor mechanical properties; thus, they are suitable for class III, IV, and V restorations, tiny tooth reshaping, and partial discolored tooth restoration. Microhybrid resin composite refers to the compound filler of large particles and small particles. The filler particle size ranges from 0.04 to 4 μm and the filler volume fraction is 56–66 %. Because small particles fill the voids between the large particles, the microhybrid resin composite has better abrasion resistance and transparency; however, they are not as good as ultra-microfill resin composites. They are suitable for class III, IV and V restorations, resin veneers, dental morphology shaping, discolored teeth restorations, and also posterior teeth restorations. For hybrid resin composite (namely universal resin composite), the filler particle size is 1–3 μm, the filler volume fraction is 70–77 %, and they are suitable for class I, II, and V restorations, and dentin restoration for classes III and IV. However, their surface polishing performance is inferior to that of microfill and nanocomposite. The nanocomposite was first reported in 2002, and the filler particle size ranges from 25 to 75 nm with a precise arrangement. This composite has excellent mechanical properties, improved surface polishability, and decreased polymerization shrinkage. Thus, they have been considered to be the best universal composites, which are suitable for repairing all kinds of cavities, resin veneers, dental morphology shaping, and discolored teeth restorations.

7.4.2 Etching Adhesive Systems and Bonding Mechanisms

Modern etching systems can be divided into the total-etch systems and the self-etch systems based on processing for smear layer by etching systems [6]. The total-etch system is also called

"etch and rinse adhesive" because there is a separate etching step that completely removes the smear layer by an acidic gel and demineralizes hydroxyapatite under the smear layer of the dentin. The self-etch system only makes the smear layer permeable without completely removing it because of the lack of the separate etching step.

The development of dentine adhesives has changed a lot to simplify the procedure, for example, the combination of etching and pretreatment. However, the various simplified methods are all from the two systems. The classification of dentin adhesive systems is shown in Fig. 7.1.

7.4.3 Total-Etch Systems

The total-etch system completely removes the smear layer using an acidic gel (usually phosphate acid) and demineralizes hydroxyapatite under the smear layer of the dentin. The adhesive of the one bottle etching system is synthesized by dissolving the resin monomer to form an organic solvent. After etching and rinsing, the adhesive was applied to the treated tooth surface; then, the resin monomers penetrate into the demineralized dentin through the gap, which is filled with water between the dentin collagen (this gap was originally occupied by the hydroxyapatite) to form a "hybrid layer". The hybrid layer is between the adhesive and dentin and is composed of collagen, resin monomer, residual hydroxyapatite, and water. The hybrid layer helps to reduce postoperative sensitivity and form a better marginal seal; at the same time, buffering the shrinkage stress produced by the polymerization of the resin composites as an "elastic buffer".

7.4.4 Self-Etch Systems

A self-etch system combines the etching and pretreatment as one step when processing enamel and dentin without the separate etching step. The mixture combined with etch and primer penetrates into and dissolves a part of the smear layer to form a "hybrid zone" with hydroxyapatite so that the "hybrid zone" is composed of the hybrid layer and the residual smear layer.

For both the etch and the primer in the two-step system and the self-etch adhesive in the one-step system, the composition is substantially the same, i.e., a mixture of water and the acidic monomer. The acid monomer is typically phosphate ester or carboxylic acid ester, and its pH is above that of the phosphoric acid gel. Water is a key component of self-etch systems because it is involved in the ionization of acidic components.

According to its acidic strength, self-etching adhesive can be divided into three categories: mild ($pH > 1.5$), medium ($1.0 < pH < 1.5$), and strong ($pH < 1.0$).

7.4.5 Enamel Bonding

Etching is a key step in enamel bonding. There are large quantities of hydroxyapatite in enamel, in which the surface layer turns into water-soluble monocalcium phosphate during phosphoric acid treatment. Meanwhile, the dental plaque, material

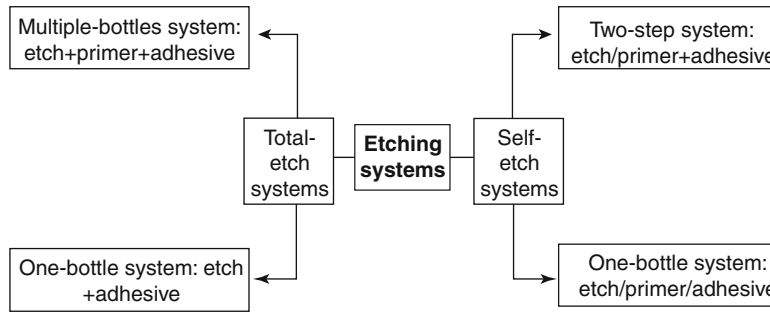

Fig. 7.1 Classification of dentin adhesive systems

alba, and food residues attached to the tooth surface are removed, thereby exposing a clean fresh surface layer. Owing to the orientation of hydroxyl and amino on the tooth surface after phosphoric acid treatment, a polarized surface is formed. The increased surface energy of enamel is beneficial for wetting and penetrating of the adhesive. It is generally accepted that 30–50 % of phosphoric acid enables even demineralization of the enamel surface; thus, total-etch systems are considered a better adhesive system for enamel bonding.

After the etching process, the wettability of the rough enamel surface is improved so that the adhesive more easily penetrating the microstructure of the tooth surface, thus strengthening the interaction of adhesive and fresh enamel. When the adhesive is cured, the bonding interface generates a considerable mechanical interlocking force. It can be observed by SEM that a large quantity of cured adhesive filled in the demineralized interprismatic area of the enamel, and countless micro-protuberances (usually called resin tags) are formed on the enamel side of the bonding interface. These mechanical anchored structures formed by resin tags and enamel provide the prime bonding force for the materials and enamel.

Etching technology is the general method for performing enamel bonding. In this method, mechanical interlocking is obtained by etching treatment, and the formation of resin tags is the main adhesive mechanism. The etched enamel prisms and interprismatic areas are demineralized, and the low-viscosity adhesive penetrates into the micropores of the enamel via the capillaries. Then, the polymerization of the adhesive occurs, which forms the resin tags that can generate the micromechanical interlocking system.

For the two-step self-etch system, the demineralized enamel layer is thinner than that treated by phosphoric acid gel because of the higher pH of adhesive. There are two methods of improving the bonding strength of self-etch systems:

1. Removing the rodless enamel to obtain a rough enamel surface.
2. Using phosphate acid to pre-etch the enamel before self-etching. The bonding strength is clinically acceptable for dentin and etched enamel treated using the self-etch method, but it is insufficient for untreated enamel and sclerotic cementum.

For the one-step self-etch system, the bonding strength is very low and there is no enamel pre-etching. However, the bonding strength is acceptable when the enamel is beveled and prepared.

In spite of the self-etch system gaining in popularity, phosphoric acid etching of the enamel is still the gold standard for testing new bonding material.

7.4.6 Dentin Bonding

The surface property and interior structure of the dentin is sophisticated. The dentin contains a lot of organic components, water, dentinal tubule connected with pulp, and liquid effused from the tubules. In addition, there is a smear layer caused by instrumental cutting of the dentin. Therefore, it is difficult to perform the treatment of dentin.

The smear layer is caused by the formation of metamorphic organics and inorganics during the bonding surface preparation, and its thickness is 0.5–15 μm. The degree of treatment of the smear layer directly influences the bonding effect of dentin. In the total-etch system, the smear layer can be thoroughly removed, but probably leads to excessive opening of the dentinal tubule, which will increase the postoperative sensitivity.

In the case of the self-etch system, the weak acid can partially remove the smear layer and lead to the appropriate opening of the dentinal tubules, thus bringing about the benefit for dentin bonding. Consequently, whether the one-step or the two-step self-etch system is used, the treatment of dentin includes three aspects: removal of the smear layer; improving the surface activity of dentin; promoting the penetration and bonding strength of the adhesive. It is widely believed that the prime mechanism of dentin bonding is the formation of a hybrid layer or hybrid zone.

The treatment of dentin is described as follows. At first, the dentin surface is treated by the etch so that the smear layer can be partially removed. After demineralization of the

intertubular dentin, the microporous stent of collagenous fiber is exposed to form a porous belt and the opening of dentinal tubules; then, the primer is used. It can become wet and penetrate into the micropores of collagen fibers and dentinal tubules to facilitate the subsequent penetration of the adhesive; at last, the adhesive is coated. After the primer and the adhesive are cured in situ, they form the hybrid layer (or hybrid zone) with dentin collagen fibers, which will obtain a solid bond with the dentin because this zone contains many resin micro-protrusions and large resin protrusions of the dentinal tubules. Meanwhile, the residual unsaturated ethylene of the adhesive copolymerizes with the resin monomer; thus, the resin composites can be bonded to dentin. Along with the development of the dentin bonding system, the processes mentioned above are simplified into a one-step or two-step procedure.

The durability of the self-etch bonding system is a significant issue. At the early stage, the bonding strength is acceptable. As time goes on, the bonding strength is continuously decreased, especially for the one-step self-etch system, owing to the hydrophilicity of the acidic monomer in addition to the high water content to maintain ionization of the acidic monomer, and this may ultimately even affect the bonding to enamel. Meanwhile, the inadequate penetration of the resin into the tooth structure may also accelerate the degradation of the bonding interface. Shrinkage stress caused by the polymerization of resin composites, which act on the bonding interface, results in reduced dentin bond strength if the dentin bond strength cannot resist it. This will bring about the formation of gaps or edges resulting in secondary caries and dentin sensitivity.

7.5 Resin Composite Bonding Restoration Technique

7.5.1 Indications and Contraindications

Currently, resin composite has been widely used indirect restorative dentistry. Almost all the dental defects can be repaired via a resin composite, which can also be used for an abnormal shape or the color of teeth in cosmetic restoration, in addition to the restoration of endodontic treatment teeth.

The following situations should be taken into account: in the anterior restoration of a class IV cavity, except for a crossbite and clenching, the teeth deficit, which does not exceed one half, can be considered a direct composite restoration. For posterior teeth restoration, a severe attrition and cusp defect need to be excluded. We do also not use resin composite if the cavity cannot be completely isolated from saliva, gingival crevicular fluid, and blood.

7.5.2 Requirements for Restoration Design

Acid etching followed by bonding provides retention, while increasing the resistance of the remaining tooth structure. The tooth types, the position of the teeth in the dental arch, the size and type of defect, whether the treatment is for the placement of the original prosthesis, the occlusal function, and the relationship between the edges of the tooth preparation need to be considered in bonding restoration. What is more, the quantity and quality of the remaining hard tissue also need to be considered, the mechanical force of remaining tooth structure is exposed to the defect, and the reserve area extends to the range of the sound dental tissue.

7.5.3 Cavity Preparation

The principles of cavity preparation for resin composite restoration are based on the principles of amalgam cavity preparation, combined with the characteristics of bonding restoration. The principles emphasize preserving as much of the tooth structure as possible in the premise of removal of infected tissue and caries staining. Cavity shape is determined by the area of the lesion, and retention of the restoration relies on the etching. The extension for prevention is not needed.

Class I The cavity shape only involves the carious parts and developmental defects. For enamel caries, the depth of the cavity should be limited to the enamel, without proceeding to the dentin, or increasing the supporting retention; to remove a large shallow dish caries, the cavity should be extended at the buccal and tongue groove, and then prepare the bevel at the edge of the cavity, adding the auxiliary retention ditch at the bottom and side walls. However, at the occlusal contact points in the occlusal cusp the edge bevel is not needed.

Class II The abrasion of composite resin material is not as good as that for the silver amalgam. Therefore, the occlusal factors should be considered, especially the functional occlusal tip occlusion. On the occlusal surface, the cavity preparation should embody the preservation principle, and the cavity edge and line angle should be more obtuse than with silver amalgam, to facilitate closing together. For the occlusal cavity, the beveled edge could increase the cavity width, which means the wear of the prosthesis is greater than for the conventional cavity; however, at these parts of the restoration, especially the edges, flakes often form and breakage is easy by force. Therefore, the preparation of the bevel at the non-occlusal contact at the occlusal surface remains controversial, and in contact with occlusal cusp, the bevel should be avoided.

For the proximal cavity, buccal and lingual walls should be introverted, and the enamel bevel edge can be prepared, without extending to the self-cleaning area. An additional retention groove could be prepared at the axiofacial and axiolingual line angles, in the same way as the silver amalgam. There are pulp walls at the occlusal surface and an axial wall in the proximal surface, and a large part is involved in dentin; thus, less enamel is available in the proximal cavity, which is not conducive to bonding restorations. Therefore, to preserve as much tooth tissue as possible, especially the enamel thickness, for the gingival wall parts, carious tissue should be removed without extension to the root side.

Class III Cavity preparation should be started from the lingual surface, trying to save the lip surface integrity; if the labial enamel has been stained, or the edge of the facial surface has been damaged, the preparation can be entered directly from the facial surface. Small to medium-sized cavities, should be designed as conservatively as possible, without making a special cavity shape, or as an aid to the retention form. For enamel caries, the retention depends mainly on the retention wall and resin bonding. Therefore, the bevel edge should be prepared, without going deep into the dentin. For a large area of caries, the retention groove should be made in the axiogingival line angle, and the undercut should be prepared in the axiofaciopulpal point angle, while the bevel is at the enamel wall. The gingival wall, stretching to the root surface, where there is no enamel, should not be beveled. Withstanding a greater bite force at the tongue surface, the tongue edge does not make a beveled edge.

Class IV The beveled edges and the size of the dental defects in class IV cavities should be considered for resin composite restoration. For the tooth in which defect is limited to one side and the incisal part is intact, the carious structure and weak enamel should be removed, and all the enamel edges beveled. In cases with defects exceed mesiodistally half the width of the incisal part, or the distance between the incisal and gingival surface exceeds 2/3 of the crown length, with intact pulp, only a short bevel of at least 1.0 mm around the cavity needs to be prepared. Increasing the width of the bevel could enhance enamel bond strength, and the spreading of the material along the plane can also attain a better aesthetic effect.

Class V For small to medium-sized class V cavities, preparation should be as conservative as possible, with no special shape, and the bevel edge only needs to be prepared for the enamel wall slope. Generally, the retention groove is not needed, and when there is no enamel on the gingival wall, the slide ditch should be prepared at the axiogingival line angle. Retention grooves

not only increase retention, but can also reduce polymerization shrinkage and micro-leakage in the bite force of the resin.

Class V cavities, in which the area is large and involves the root surface, need to be prepared routinely, similar to the box-like cavity in silver amalgam restorations. The retention groove should be prepared at the angle of the gingival and occlusal axis, while the bevel edge should be shaped on the occlusal, mesial and the distal edges of the wall.

7.5.4 The Importance of Postprocessing Decoration

After the restoration, contouring, blending, grinding, and polishing are needed. For a long time, this part was not given serious clinical attention. Very simply, by removing the flash side at the adjacent surface, trimming the shape carefully, and polishing the smooth surface, the occlusal surface and the adjacent surface from coarse to fine, can form a smooth surface, a suitable edge, and a good occlusal relationship, which can achieve minimal plaque adhesion, easy bacteria removal, and better aesthetics. The success of grinding and polishing of the resin composite is determined by the repair materials and devices. The surface quality of the resin is not only impacted by the quality of the polishing apparatus and polishing paste, but also by the relevant component and filling characteristics of the resin. A new composition with small particles and micro-mixed resin has changed the filler formulation. The size, shape, orientation, and the aggregation of the resin have been enhanced, and the physical and mechanical properties improved, while the character of the polishing is better. The hardness of the inorganic filler and the matrix is different, which is caused by the difference in the wear rate of these two components, and this results in a surface roughness. The surface gloss of restoration materials should be similar to that of the dental interface, because it affects the color-matching of the restoration. At present, high-quality restoration not only requires decoration for the anatomical shape, contour, marginal integrity, occlusal function, and improvement of the smoothness of the surface, but also pays attention to the concept of aesthetics. For the best bonding aesthetic, the surface should be smooth to prevent plaque accumulation and dyeing; the restoration should have a perfect contour and form, to improve the organizational flexibility. The appropriate dressing also makes the anatomical shape of the prosthesis adapt to occlusal requirements, and match the color of the surrounding teeth. The integrity and the adaptability of the edge is also improved. Therefore, the restoration is durable and aesthetically pleasing, and the life of the restoration is extended.

7.5.5 Problems of Direct Resin Composite Restoration

7.5.5.1 Polymerization Shrinkage

Polymerization shrinkage stress is one of the main problems that affects the longevity of direct composite resin restoration. The dental composite resin is mostly formed of dimethacrylate molecules, whose polymerization reaction produces a polymer network and volumetric contraction. Owing to the restrictions of the cavity, the volumetric contraction resin showed that the volume changes the effect on the tooth structure and bonding interface stress, causing tooth deformation and resulting in bond failure, postoperative sensitivity, microleakage, secondary caries, and other adverse consequences. The volumetric contraction in the bonded resin composite restorations may cause shrinkage stress at the resin composite/tooth structure interface and/or within the tooth or the resin composite. The resulting shrinkage stress may result in adhesive failure, tooth deformation, postoperative hypersensitivity, secondary caries or microleakage. Shrinkage stress is not only dependent on the characteristics of the composite resin, but also on the tooth structure, cavity shape, characteristics, and restoration techniques. Therefore, the correct understanding of resin composite polymerization significantly offers numerous clinical advantages in reducing the shrinkage stress generated in polymerizing dental composites.

Material related factors Resin composite consists of organic matrix and inorganic fillers. The polymerization shrinkage is mainly caused by the volumetric contraction of the organic matrix, with an average of 2.6 %~7.1 vol% [7]. In this process, the space occupied by inorganic fillers is not involved in the shrinkage. Therefore, the main strategy in reducing polymerization shrinkage in methacrylate-based composites focused on increasing the filler load. Compared with resin composites with a low filler load, the resin composites with a high filler load have some good physical properties, lower shrinkage, and greater hardness. With a constant amount of shrinkage, the resulting shrinkage stress grows with the increase in the elastic modulus of the composite resin.

Conventional composite resins have filler particles larger than 400 nm, but nanocomposites contain filler particles between 20 and 100 nm. Compared with hybrid and microfilled resins, nanocomposite resin particles have a high filler load and extensive filler distribution, resulting in low polymerization shrinkage. Compared with conventional composite resin, flowable composite resin contains less inorganic filler, which reduces the mechanical performance, increases the polymerization shrinkage, and produces more shrinkage stress at the interface. However, owing to the reduction of inorganic filler during the curing process, concave deformation at the surface of a flowable resin composite can compensate for the volume contraction within the resin; thus, the flowable resin composite can be placed between the tooth surface and the nonflowable composite as a stress buffer layer. Currently, there are differences of opinion about the flowable resin composite used as a base material to reduce polymerization shrinkage.

C-factor The configuration factor is the ratio of the bonded to unbonded surface of the restoration. As the C-factor decreases, the polymerization shrinkage is limited to only one direction, and at the early stage of polymerization, the resin flows freely to prevent polymerization shrinkage stress. As the C-factor increases, the resin is limited in direction and becomes less flowable at the early stage of polymerization; therefore, the shrinkage stress increases, the bond strength with dentin declines, resulting in greater marginal microleakage.

Incremental techniques are always recognized as a major factor in the reduction of C-factor and shrinkage stress. Incremental layering is considered to be the conventional technique for reducing polymerization shrinkage, compared with the bulk technique. For incremental layering, the shrinkage stress of each increment can be compensated for by the next increment. Because of the reduction of C-factor, the contact surface between the resin and the cavity walls is minimized and the resin is relatively flowable during polymerization, which can decrease the shrinkage further. Incremental layering techniques include horizontal occlusogingival layering, wedge-shaped oblique layering, and the successive cusp build-up technique. In recent years, the use of incremental layering techniques for the purpose of reducing polymerization shrinkage has been questioned by some authors, who argued that different restoration techniques had no significant difference on the shrinkage stress of composite resin. Nevertheless, incremental layering techniques are still widely used in clinics.

The polymerization rate is the rate at which the monomer of composite resin is converted to polymer. The higher the rate, the more polymerized monomers there are, the higher the polymerization stress. The use of a high-intensity curing light can increase the polymerization rate and mechanical properties of composite resin, but the polymerization shrinkage stress is high. Slowing down the curing process can release stress during polymerization. Therefore, a new "soft" curing technique has been reported. A relative low-intensity curing light was used during the first few seconds of the light curing (10 s), and then a high-intensity light is applied for the final curing. For this technique, the light intensity is low at the beginning, which makes the surface polymer sufficiently cross-linked, but prolongs the polymerization of the underlying resin to inhibit potential stress. In addition to the curing methods, the curing units also

have different effects on polymerization shrinkage stress. Compared with the traditional halogen unit, a light emitting diode unit using low-intensity light can effectively reduce the shrinkage stress.

7.5.5.2 Technique Sensitivity

Technique sensitivity, which is totally dependent on the practitioner, includes the failures caused by improper selection of indications, inadequate understanding of material and mechanism, neglect of the product manual, and incorrect operation by not following the manufacturer's instructions. Technique sensitivity is an important factor that affects the result of direct composite resin restorations. Any carelessness in procedure and difference in technical proficiency may lead to the failure of restoration.

First of all, selection of the appropriate resin composite and adhesive according to the operating instructions is essential. It requires consideration of the tooth position, location, and volume of the cavity, filler types of resin to meet the requirements of the mechanical characteristics of the posterior teeth, or the aesthetic properties of the anterior teeth, or both. Total-etch adhesive is recommended for bonding with the enamel surface, and for bonding with the dentine surface, use of a self-etching adhesive is recommended. Strictly following the product manual and operating instructions is the foundation of successful direct composite resin restorations.

The selection of the appropriate indications is also very important. Direct resin restoration will definitely fail if inappropriate cases are selected, such as cusp defect, severe attrition, or a subgingival cavity restored without gingival retraction. Cavity preparation is another important factor. Although cavity preparation of direct resin restoration does not need strict retention and resistance form, there are certain principles that need to be followed. If the position and angle of the cavity walls, and the bevel of the cavity edge, are not properly prepared, adhesive failure will result. In addition, the adhesive surface must be thoroughly cleaned. Debris, plaque, pigment or carious tissue have an influence on the etching and binding effect on the healthy tooth tissue.

7.5.5.3 Postoperative Sensitivity

Postoperative sensitivity is described as a moderate pain, of short duration, that is initiated by mastication, hot and cold stimulus, immediately after resin restoration or later. Typically, there are two types of postoperative sensitivity. First, when the cavity is deep and there is not enough base material, the cavity need re-filling with sufficient base material and resin. Second, when the cavity is shallow but reaches the enamel–dentin junction (EDJ), the sensitivity may be due to opened dentinal tubules caused by the etching process, or insufficient marginal seal if the adhesive is too thick, or shrinkage stress-induced microleakage, or exposed dentin surface due to excessive occlusional adjustment. The incidence of such sensitivity is high and it is difficult to solve.

7.6 The Prospect of the Treatment of Dental Caries

7.6.1 Individualized Ideas of Treatment

Scholars describe dental treatment in the twenty-first century as micro-invasive dentistry, cosmetic dentistry, and adhesive dentistry. These concepts reveal that modern dental treatment, especially treatment of dental caries, requires as little trauma as possible from the treatment, the best functional reconstruction, and the best aesthetic performance. Above are the foundations for the individualized treatment of dental caries.

7.6.2 The Importance of Individualized Treatment of Dental Caries

Dental caries is a multi-factorial disease based on the ecological plaque hypothesis. Factors including individual systemic health, social economic

status, sucrose intake, the buffer capacity of saliva, the implementation of oral health care, the past history of caries, and the colonization of caries-related microbes are closely related to dental caries. Individualized treatment plans for dental caries against specific risk factors are proposed, after integrated evaluation of the factors described above. For example, the risk of caries among children and young people lies in deep pits and fissures, while among middle-aged and elderly people gingival recession and root exposure contribute to dental caries. Some individuals have caries because of a preference for sweets and a tendency to ignore oral health, while others suffer from caries because of the local accumulation of plaque due to poor restoration. Specific measures and targeted intervention should be taken against different risk factors for dental caries to obtain the best effect.

7.6.3 The Risk Evaluation Is the Premise of Individualized Treatment of Dental Caries

The evaluation of the risk of dental caries is an assessment of the degree of risk of developing new caries or continuing the progress of existing caries under the circumstances of fairly constant etiological factors, including diet, time, a susceptible tooth surface, and plaque levels of an individual at the time. The functions of the risk evaluation of dental caries are listed as follows:

1. To control dental caries effectively. Recent epidemiological studies show that caries activity is not evenly distributed among the general population and a small proportion of people with high caries activity suffer from most of the dental caries among the general population. More than 60 % of dental caries occur in only 20 % of the overall population. Taking precautions in the general population can lower the overall prevalence of dental caries, but the efficacy of precautions cannot be noticeably increased. Filtering people at a high risk of dental caries and combining multiple prophylaxes before the occurrence of dental caries can greatly enhance the efficacy of the prevention of dental caries.
2. To make suitable precautionary treatment plan for individuals. As dental caries is a multifactorial disease, the risk evaluation can help us to define risk factors for an individual so that specific treatment and precautionary plans can be made.
3. To cure caries at an early stage. The handling of incipient caries has been paid increasing attention in the individualized treatment of dental caries. Remineralization is one of the effective measures. The risk evaluation of dental caries is the major tool for predicting the success rate during handling incipient caries using the remineralization method. The success rate is relatively low if the individual is at a high risk of caries; otherwise, the success rate is relatively high.

7.6.4 The Development of Technology and Material Provides a Guarantee for the Individualized Treatment of Dental Caries

The development of technologies, including early diagnosis, risk evaluation, micro-invasive treatment, colony prevention, and the progress of dental adhesive restorative materials, provide the premise and guarantee for the individualized treatment of dental caries. The risk evaluation of dental caries predicts the risk of caries of an individual by combining analysis of the results of multiple risk factors to achieve the goal of early detection, early prevention, and early treatment of individual caries, focusing on populations at a high risk of caries. Therefore, the appearance and development of a highly sensitive and specific screening technology to filter population at risk of caries, a quantitative technology of the early diagnosis of caries, a micro-invasive treatment technology of caries, and a preventive technology with low side effects, together with definite efficacy, provide a guarantee for the realization of the real sense of individualized treatment of caries.

7.7 Biological Treatment Methods

Nowadays, there are two directions of research into the regeneration of tooth tissue: first, achieving the goal of regeneration of tooth tissue by mimicking biomineralization through the design of organic matrix using bionic theories; second, realizing the regeneration of tooth tissue by cultivating stem cells in scaffold material using tissue engineering methods. These ideas and studies provide an exciting future for restorative dentistry [8].

7.7.1 Restorative Therapy Based on Tissue Engineering of Tooth Regeneration

The development of tissue engineering for tooth regeneration has made the self-repair of dental damage, including cavitated and noncavitated, or even missing teeth, possible. By directly implanting teeth cultivated in vitro instead of traditional implants or dentures to restore missing parts of teeth or by conditioning the microenvironment of tooth damage in situ can help us to realize the goal of the self-repair of tooth damage.

There is currently no direct evidence supporting the introduction of the specific technology of tissue engineering into the field of tooth-filling restoration, or related reports on the final effect. However, lots of related reviews have pointed out that tissue engineering may promote tooth filling treatment under specific circumstances.

Classifying tooth-filling restoration technologies by the degree of tooth damage may predict the indication for tissue engineering-related technology in the restoration of tooth damage. The chart below summarizes accessible traditional filling restoration and the possible tissue engineering technology against tooth damage of different degrees.

Degree of tooth damage	Minor	Severe	Parts of active tissue lost	Most of active tissue lost	Active tissue lost completely
Common cases	Incipient nonstatic caries	Arrested caries	Arrested or chronic deep caries involving pulp	Pulp exposed because of caries or trauma	Total cavitation or total dislocation accidentally
Common filling restoration at present	Pit and fissure sealant or filling after removal of lesion	Filling after removal of lesion	Removing softened dentin fractionally or pulp capping	Pulp capping, root canal treatment or tooth extraction	Implant or restoration after debridement
Possible tissue engineering technology	Replacement therapy by constructing lower-toxicity or inactivated strains using genetic engineering technology to change the composition of the oral microbe	Inducing the pulp–dentin complex to defend outer stimuli and produce reparative dentin using growth factor	Promoting regeneration of pulp tissue and repair of dentin structure using growth factor	Reconstructing missing pulp and hard tissue of the tooth by implanting stem cells	Implanted teeth induced in vitro to restore missing teeth

Application of tissue engineering in the field of tooth restoration includes terminating or avoiding the progress of caries by replacing the composition of oral microbes with lower-toxicity or inactivated strains constructed by gene engineering technology, inducing the regeneration of dentin through the slow release of a sustained release carrier into dental tissue by adding various growth factors to the filling materials, inducing the production of a new tooth structure

by implanting multifunctional stem cells for teeth severely cavitated or severely damaged by trauma, and implanting tooth tissue cultivated in vitro to restore the integrity of dentition for teeth that are missing or that cannot be restored through the method of filling.

Tissue engineering has provided an exciting future for tooth restoration, but related biosecurity problems, technological problems, and relatively high costs have limited its possibility of wide deployment. It seems that we can only rely on the optimization of traditional treatment methods to improve prognosis.

7.7.2 Restorative Therapy Based on Bionics

Nowadays, composite resin, metal, and ceramics are the main materials in clinical application. However, insolvable problems such as margin leakage, aging of the materials, and the lack of fracture toughness remain. Also, removal of a large amount of tooth tissue during surgery causes the patient a lot of pain. Therefore, regenerating new tooth tissue where the tooth tissue is damaged in situ has become the dream of dental scholars. At present, designing organic matrix to conduct biomineralization using bionic theories may enable us to regenerate teeth. Therefore, research into bionic materials to simulate tooth tissue using a bionic methods has been a hotspot of current studies. For example, in vitro studies to simulate the biomineralization of enamel and dentin have enabled simple nucleation and sedimentation of hydroxyapatite and are going deeper toward the highly densely arrayed and regular structures such as enamel. When it comes to tooth tissue mineralization in situ, we are trying to implement the mineralization procedure, in which crystal grows layer by layer in a well-organized way and closely controlled by organic matrix, while the exploration of forming enamel on the surface of dentin continues. As studies on the forming mechanism of enamel and dentin go deeper and frontier science such as the self-assembly of the supermolecule develops, we believe that we can obtain tooth tissue that is highly bionic in form and function and that we will finally implement the restoration of tooth damage in situ.

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Dental Caries and Systemic Diseases

8

Zou Ling and Hu Tao

As stated by World Health Organization, oral health is fundamental to overall health and well-being and a determinant of quality of life [1]. According to "Oral Health in America: A Report of the Surgeon General," the mouth and face are mirrors of health and disease. A physical examination of the mouth and face can reveal signs of general health status. Imaging of the oral and craniofacial structures (x-ray, MRI, SPECT) may provide early signs of skeletal changes such as those occurring with osteoporosis and musculoskeletal disorders and salivary, congenital, neoplastic, and developmental disorders. For example, the research group of Dr. David Wong from UCLA has initiated a series of concerted efforts to spearhead the scientific and translational frontiers of salivary diagnostics. The potential use of saliva, a totally noninvasive biofluid without the limitations and difficulties of obtaining blood and urine, for oral and systemic disease detection, disease progression, and therapeutic monitoring is a highly desirable goal [2, 3]. In other words, oral health refers to the health of our mouth and, ultimately, supports and reflects the health of the entire body [4]. In a sense, oral disease is not just a minor ailment of the soft and hard tissues of the mouth, and it may be a disease of the body that happens to begin in the mouth. If left unchecked, oral disease can contribute to other more harmful diseases that can seriously affect the quality of life [5].

As Hani T. Fadel from University of Gothenburg wrote in his doctoral thesis, "the link between oral and general health has been suggested since early times, almost as early as history itself. The concept of local or systemic diseases secondary to a localized chronic infection (e.g., in the oral cavity) is usually called focal infection. Its origin can probably be traced back to the time of Hippocrates" [6, 7]. Recently, a report titled "Links between oral health and general health – the case for action" from Dental Health Services Victoria summarized that oral health and general health are related in four major ways:

1. Poor oral health is significantly associated with major chronic diseases.
2. Poor oral health causes disability.
3. Oral health issues and major diseases share common risk factors.
4. General health problems may cause or worsen oral health conditions.

Dental caries and periodontal disease are the two biggest threats to oral health and are by far the most

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common oral infection diseases in the United States and Australia [8]. It has been well proven that the oral cavity contains some of the most varied and vast flora in the entire human body, not only including those linked to dental caries and periodontal disease but also including systemic diseases that affect general health. In addition to bacterial organisms, oral microorganisms can include fungal, protozoal, and viral species. It is well accepted that our body is negatively affected by infection of any kind, no matter where it is located. Moreover, the more serious the infection and the longer it is present, the greater its potential for affecting systemic health. Infection can also seriously stress the immune system and diminish its ability to deal with other infections and diseases. Its effect on the immune system is directly related to the extent, type, and duration of the infection [5]. Over 100 years ago the theory of focal sepsis, although lacking empirical scientific evidence, hypothesized that chronic infections in the mouth caused systemic diseases [9]. The concept has been neglected for several decades and still is a subject of controversy [10]. Since many teeth were extracted without evidence of infection, thereby providing no relief of symptoms, the theory was discredited and largely ignored for many years [11]. Interestingly, increasing evidence over the past 30 years suggests that, due to dental bacteremia, the oral cavity can indeed serve as a reservoir for systemic dissemination of pathogenic bacteria and their toxins, leading to infections and inflammation in distant body sites, especially in immunocompromised hosts such as patients suffering from malignancies, diabetes, or rheumatoid arthritis or having corticosteroid or other immunosuppressive treatment [12].

Most studies stated above concerning the relationship between oral infection and systemic diseases are related to periodontal disease [13]. And according to Thomas McGuire, the most important of oral diseases in regard to their impact on general health are:

(a) Periodontal disease
(b) Infected root canals
(c) Cavitations (infected extraction sites)
(d) Other diseases of the oral cavity, such as oral cancer [5]

"Dental caries" cannot be directly found in this list. Thomas McGuire explained the reasons as the following: certainly, dental caries can have an effect on a person's overall health. For example, it can interfere with the mastication process and thereby affect digestion. It can cause tooth loss, again affecting digestion. The main difference is that, unlike periodontal disease, dental caries is not an infection that has access to the systemic body. Clearly, it can contribute to systemic health problems, but its effects on overall health are significantly less than the effects of periodontal disease [5].

As mentioned before, dental caries is one of the most common causes of pulpitis and periapical diseases by penetrating through the enamel and dentin to reach the pulp. Untreated decay can become so advanced that the tooth must be removed (extraction). Most studies supported that dental caries was the main cause for tooth loss, but a few studies revealed that a greater proportion of tooth extractions were due to periodontal disease, especially in patients over 40 years old. Overall, 70 % of tooth loss is due to tooth decay, 20 % due to periodontal diseases, and 10 % due to other causes [14, 15]. It was reported that caries accounted for a higher proportion of extractions than periodontitis at all ages over 20 years in 1968 and only up to 45 years of age in 1988 [16]. According to another study, although there is an increase in orthodontic extractions or a decline in extractions for caries in under-21-year-olds, when extractions from the population as a whole are considered, caries and its sequelae remain the principal reason for loss of all tooth types apart from lower incisors which were extracted mainly for periodontal reasons [17]. In 2012, a quantitative study evaluated the prevalence and factors related to tooth loss due to dental caries among workers in industrial estates in central Thailand. There were 457 adult (283 males; 174 females) between 19 and 53 years participants. The results showed that 62.2 % participants had tooth loss due to caries [18]. The latest study also proved that dental caries and its complications were the leading reasons for extraction. Their study included a total of 2,620 teeth extracted from 1,382 patients. The highest rate (36.9 %) of extraction occurred for those of 41–60 years of

age. Tooth loss due to caries was 51 %; periodontal disease was 14.4 %; and supernumerary and tooth impaction were 13.9 %. Although 86 % of teeth extracted for periodontal disease were in patients over 40 years of age, caries was still the main reason for extraction even in elderly patients, but to a less degree than in younger ones [19].

Our goal of this chapter is to discuss the relationship between dental caries and general health; we will summarize the limited recent advances in this topic. Since the effect of dental caries on the overall quality of health and well-being has not been well studied, in order to enrich the content of this chapter, studies associating systemic diseases with periapical diseases, tooth loss, root canal treatment, and other conditions caused by dental caries directly are also included [20].

This chapter explores what the dental caries can reveal about general health, describes the role the mouth plays as a portal of entry for infection, and concludes with studies that are associating oral infections with serious systemic diseases and conditions. Following this introduction and overview, the remainder of the chapter is organized as follows: first defining dental caries and bacteremia, head and neck cancer, and children growth; then briefly describing dental caries and atherosclerosis, cardiovascular disease, and heart attack; next discussing dental caries and immune system disease and kidney diseases; and last describing dental caries and gastrointestinal diseases, diabetes mellitus, and respiratory infections.

8.1 Dental Caries and Bacteremia

Bacteremia is an invasion of the bloodstream by bacteria. The blood is normally a sterile environment [21]. So the detection of bacteria in the blood (most commonly accomplished by blood cultures) is always abnormal. This may occur through a wound or infection or through a surgical procedure or injection when other foreign bodies are entering the arteries or veins. Bacteremia may cause no symptoms and resolve without treatment, or it may produce several consequences like fever and other symptoms of infection. In some cases, the immune response to the bacteria can cause sepsis and septic shock, a potentially life-threatening condition which has a relatively high mortality rate. Bacteria can also use the blood to spread to other parts of the body (which is called hematogenous spread), causing infections away from the original site of infection.

The oral cavity is intensely colonized by bacteria. Recent advances in bacterial identification methods, particularly culture-independent approaches such as 16S rRNA gene sequencing, have shown that the oral cavity is inhabited by more than six billion bacteria representing in excess of 700 species belonging to at least nine different phyla [22]. Bacteremia occurs with various frequencies following dental procedures and has been well documented. As early as 1990, Heimdahl et al. detected the patients with bacteremia after dental extraction, third-molar surgery, dental scaling, endodontic treatment, and bilateral tonsillectomy by means of lysis filtration of blood samples with subsequent aerobic and anaerobic incubation. Their results showed that bacteremia was observed in 100 % of patients after dental extraction, 55 % of patients after third-molar surgery, 70 % of patients after dental scaling, 20 % of patients after endodontic treatment, and 55 % of patients after bilateral tonsillectomy. And anaerobic microorganisms were isolated more frequently than aerobic microorganisms [23]. Transient bacteremia is produced not only as a result of dental manipulation. Even daily life activities such as eating, chewing gum, brushing the teeth, or using toothpicks also induce bacteremia detectable by means of blood cultures in a variable percentage of subjects [24].

Three mechanisms or pathways linking oral infections to secondary systemic effects have been proposed for several years [6]. Li et al. summarized the mechanisms as the following: metastatic spread of infection from the oral cavity as a result of transient bacteremia, metastatic injury from the effects of circulating oral microbial toxins, and metastatic inflammation caused by immunological injury induced by oral microorganisms [11].

Till now, there is no direct evidence to prove the connection between the dental caries and

bacteremia, but we can find some clues from published papers. Debelian et al. used phenotypic and genetic methods to trace microorganisms released into the bloodstream during and after endodontic treatment back to the root canal. Microbiological samples were taken from the root canals of 26 patients with asymptomatic apical periodontitis of single-rooted teeth. The blood of the patients was drawn during and 10 min after endodontic therapy. The results found that microorganisms from the root canal and blood presented identical phenotype and genetic characteristics within the patients examined,which demonstrated that endodontic treatment can be the cause of anaerobic bacteremia and fungemia. Interestingly, some cariogenic bacteria were also isolated from the blood, such as *Streptococcus sanguinis* [10]. *Streptococcus mutans* and *Streptococcus sanguinis* are most consistently been associated with the initiation of dental caries. The results not only illustrated that dental caries is the most common cause of pulpitis and periapical diseases but also showed a clue that cariogenic bacteria may be related to bacteremia.

These bacteria are normally harmless as long as they are kept in check by the body's natural barriers and the immune system. In the oral cavity there are several barriers to bacterial penetration from dental plaque into the tissue: a physical barrier composed of the surface epithelium; defensins, which are host-derived peptide antibiotics, in the oral mucosal epithelium; an electrical barrier that reflects the Eh difference between the host cell and the microbial layer; an immunological barrier of antibody-forming cells; and the reticuloendothelial system (phagocyte barrier) [25]. However, once the equilibrium is disturbed by an overt breach in the physical system (e.g., trauma) or immunological barriers (e.g., through neutropenia, AIDS), organisms can propagate and cause both acute and chronic infections with increased frequency and severity [25, 26]. In addition, medical treatment (e.g., immunosuppressant therapy) may bring a person in contact with new types of bacteria that are more invasive than those already residing in that person's body, further increasing the likelihood of bacterial infection.

8.2 Dental Caries and Head and Neck Cancer

8.2.1 Dental Caries and Head and Neck Cancer Treatment

Head and neck cancer accounts for more than 550,000 cases annually worldwide. The incidence rate in males exceeds 20 per 100,000 in regions of France, Hong Kong, the Indian subcontinent, central and eastern Europe, Spain, Italy, and Brazil and among African Americans in the Unites States. Mouth and tongue cancers are more common in the Indian subcontinent [27]. Surgical resection, radiotherapy, and chemotherapy, either used singly or in combination, are the three most common modalities used in head and neck cancer treatment. Despite their effects in eradicating the tumor, they also negatively impact the normal head and neck structures surrounding the tumor. Surgical resection removes abnormal tissue, while radio- and chemotherapy frequently cause direct damage to the oral soft and hard tissue, and indirect damage may also arise from systemic toxicity.

Firstly, we will discuss the radiotherapy because radiation caries is a common disease in clinic. We all know that saliva in the oral cavity protects hard tissues against acid attacks and demineralization. Salivary glands are very susceptible to radiation, and any disturbances in their function are detrimental to the hard tissues in the oral cavity. Radiation caries is mainly an indirect effect of irradiation-induced changes in salivary gland tissue that result in hyposalivation [28]. Hyposalivation leads to accelerated dental caries through changes in salivary composition, a shift in oral flora toward cariogenic bacteria, and dietary changes [29]. It is reported that the initial caries usually occur around the third week of treatment [30]. Mohammadi et al. [31] reviewed 27 cases with head and neck cancers undergoing radiotherapy. Of these cases, class V dental caries of posterior teeth were evaluated in three intervals: before treatment, 3 weeks after the initiation of the treatment, and at the end of the treatment. The baseline is that there were no class V decays prior to radiotherapy. Their

results found that mean percentages of class V caries 3 weeks after radiotherapy and at the end of radiotherapy were 28.42 % ± 14.41 and 67.05 % ± 19.02, respectively. These findings are in accordance with the results of other studies [28, 32]. Since the severity of xerostomia is related to the radiation dose, dose rate, and amount of salivary tissue irradiated, the authors also pointed out that further studies should evaluate the effects of new techniques such as intensity-modulated radiotherapy on occurrence of dental caries, in which a higher dose is beamed at the tumor site without increased received dose of the surrounding tissues [31].

Secondly, we talk about which one of the three modalities, either used singly or in combination, is the most common cause of dental caries after therapy. In order to determine the prevalence of dental caries in cancer survivors, Catherine et al. conducted a systematic literature search with assistance from a research librarian in the databases MEDLINE/PubMed and Embase for articles [33]. Finally, 64 published papers between 1990 and 2008 were reviewed. Dental caries was assessed by the present (Y/N), DMFT/dmft, and DMFS/dmfs indexes if available. Their results showed that the weighted overall prevalence of dental caries was 28.1 % and was determined from 19 studies. The weighted prevalence of dental caries in patients who received only chemotherapy was 37.3 %. The weighted prevalences of dental caries in patients who were post-radiotherapy and those who were post-chemotherapy and post-radiotherapy were 24 and 21.4 %, respectively. The authors attributed the discrepancy to the distinct differences in the dental management of patients prior to radiotherapy versus those being prepared for chemotherapy. Another explanation for the unanticipated caries prevalence may be because 12 of the 19 studies included were carried out on children undergoing hematologic malignancies who were treated largely by curative chemotherapy. They could have higher caries activity because of the need to frequently consume highly cariogenic dietary supplements for weight maintenance or are taking sucrose-rich medications. In addition, their oral hygiene may be ignored. In contrast to the caries prevalence, the DMFT index is expectedly highest in patients who were post-radiation therapy compared to patients who were post-chemotherapy and healthy controls [33].

8.2.2 Dental Caries and Head and Neck Squamous Cell Carcinoma

Recently, an interesting paper published online in *JAMA Otolaryngology – Head and Neck Surgery* showed that the bacteria that caused tooth decay are linked to an immune response, which may be protective against cancer [34]. The researchers from the University at Buffalo, NY, set out to determine if there is a significant link between dental cavities and head and neck squamous cell carcinoma (HNSCC). The study involved 399 patients newly diagnosed with HNSCC and 221 participants without the cancer who were all selected from the Department of Dentistry and Maxillofacial Prosthetics at Roswell Park Cancer Industry between 1999 and 2007. The dental history of all patients, particularly their history of dental cavities, was analyzed by measuring the number of decayed, missing, and filled teeth. Of the 399 patients with HNSCC, 146 (36.6 %) had oral cavity squamous cell carcinoma (SCC). Oropharyngeal SCC occurred in 151 (37.8 %) patients, while 102 (25.6 %) had laryngeal SCC. The results of the study overall showed that those who had high cavity numbers were less likely to have HNSCC, compared with participants who had low cavity numbers. The authors explained that "Caries is a dental plaque-related disease. Lactic acid bacteria cause demineralization (caries) only when they are in dental plaque in immediate contact with the tooth surface. The presence of these otherwise beneficial bacteria in saliva or on mucosal surfaces may protect the host against chronic inflammatory diseases and HNSCC. We could think of dental caries as a form of 'collateral damage' and develop strategies to reduce its risk while preserving the beneficial effects of the lactic acid bacteria" [34].

8.2.3 Tooth Loss and Head and Neck Cancer Risk

As previously mentioned, caries is one of the most common reasons of tooth loss; we will include the relationship between tooth loss and tumors in this chapter. Actually, multiple epidemiologic studies regarding the potential association of tooth loss with head and neck cancer risk have been published nowadays [35–38]. But considering the modest sample size and different study designs, the evidence still remains controversial. Therefore, a quantitative and systematic summary of the evidence using rigorous methods is necessary. We know that meta-analysis is the use of statistical methods to combine results of individual studies. This allows us to make the best use of all the information we have gathered in our systematic review, and by statistically combining the results of similar studies, we can improve the precision of our estimates of treatment effect and assess whether treatment effects are similar in similar situations. Recently, some Chinese researchers from Guangxi Medical University conducted a meta-analysis involving 5,204 patients and 5,518 controls to assess the inconsistent results from published studies on the association of tooth loss with head and neck cancer risk [39]. Their overall estimates provided evidence that tooth loss was significantly associated with increased risk of head and neck cancer. In addition, the moderate [6–15] tooth loss and the severe (>15) tooth loss experienced a significantly increased risk of head and neck cancer by 18 and 54 %, respectively. Furthermore, the moderate [6–15] tooth loss was associated with a 45 % increase in the risk of larynx cancer. The authors also summarized that several plausible mechanisms may explain why a significant increased association of tooth loss with head and neck cancer was observed in their analysis.

Whether tooth loss is an independent risk factor of head and neck cancer is an interesting question. But the answers have not reached consistent conclusions yet. Guha et al. observed that missing 6–15 teeth increased the odds ratio of esophageal squamous cell carcinoma by more than twofold in both Latin America and central Europe. However, when missing teeth were more than 15 in number, no increase risk was observed [40]. On the other hand, Wang et al. found that moderate and severe tooth loss did not change such an association, suggesting that tooth loss is probably an independent risk factor of head and neck cancer [39].

8.2.4 Cariogenic Bacteria and Oral Cancer

Alcohol is one of the main risk factors for oral cancer. Alcohol itself is not carcinogenic, but it is oxidized to carcinogenic acetaldehyde in saliva by the ADH enzyme of some oral microbes of the normal oral microflora. Oral streptococci, especially *S. mutans*, are the primary pathogens causing dental caries, and *Neisseria* strains are related to the early stage of caries. About a decade before, some *Neisseria* strains are found to be able to produce significant amounts of acetaldehyde, probably via their high alcohol dehydrogenase (ADH) activity [41]. *Neisseria* strains are considered to be part of the normal oral flora, but they are found only in low numbers in the oral cavity. Later, in 2007, oral streptococci were proved to contribute significantly to the normal individual variation of salivary acetaldehyde levels after alcohol drinking and thereby also to the risk of oral cancer [42]. We believe that the effect of cariogenic bacteria on oral cancer provides some evidence between caries and cancer from another side.

8.3 Dental Caries and Children Growth

There's no doubt that dental caries constitutes the single most common chronic disease of childhood: as many as 60 % of school children have experienced dental caries, and the data can reach as high as 90 % in some countries according to the report of World Health Organization (WHO) [43]. Among 5- to 17-year-olds, dental decay is five times as common as asthma and seven times as common as hay fever [44]. Current evidences

show that dental caries is a multifactorial disease and complexly modulated by genetic, behavioral, social, and environmental factors [45]. A recent descriptive cross-sectional study assessed dental caries experience among 12-year-old school children from low socioeconomic status background attending public primary schools in Zimbabwe. The results showed that there was a high prevalence of dental caries in both urban (59.5 %) and rural (40.8 %) children [46]. While most people in rural areas in Zimbabwe cannot afford and perceive these sugary products as non-beneficial, affording them is often considered as a symbol of higher socioeconomic status. Another retrospective cohort study gave support to the idea that children who lived in urban areas showed 75 % greater probability of presenting caries when compared to those children residing in rural areas [47]. This disparity between urban and rural children has been partially attributed to increased access and consumption of high sugar-containing foods and beverages in urban areas [48]. Based on the recent studies, socioeconomic status has been shown to be a major risk for caries incidence. Children living in poverty represent a large population of high-risk individuals who have undiagnosed and untreated diseases coupled with limited access to care. Nearly twice the proportion of US children with family incomes less than the federal poverty level (FPL) show decay of the primary or permanent dentition (55 %), compared to those whose family incomes are greater than 200 % of the FPL (31 %). Low-educated and low-income families that pay less attention to the dental hygiene of their children may be one of the reasons [49].

Apart from structurally weakening teeth, dental caries can lead to infection, pain, abscesses, chewing problems, poor nutritional status, and gastrointestinal disorders. Moreover, serious caries can damage a child's sense of self-esteem, which in turn may affect his or her school performance, ability to learn, and potential to thrive [50]. Specifically, in young children, there is a relationship between dental caries and childhood obesity [51, 52]. Dental caries can also contribute to poor nutritional status and affect the growth of adult teeth [53]. In addition, children with extensive dental caries may need to undergo treatment under general anesthesia in hospital. This is a significant side effect of childhood caries that is widely acknowledged by the experts. It is essential to remember that dental caries is one of only very few common childhood diseases which cause large numbers of the child population to undergo general anesthesia.

The relationship between dental caries and child's body weight was firstly noticed by Miller 30 years ago [54]. Caries of the primary teeth or "early childhood caries" (ECC) is one of the most prevalent health problems in infants and toddlers [55]. A recent study found a positive correlation between severe early childhood caries (S-ECC) and body mass index (BMI) of 3- and 6-year-old children, which means the mean BMI of S-ECC children is significantly more than the caries-free children [56]. We know that if caries involve the pulp, the eating of some foods will cause pain; therefore, toothache and infection alter eating and sleeping habits, dietary intake, and metabolic processes [57]. For example, some of the patients may thereby avoid certain nutritious foods and select high-calorie, high-fat food, which is recognized as risk factors for obesity. On the other hand, some patients cannot pulverize the foods well and may have an adverse effect on the internal absorption of nutrients. But if such bad oral condition has been changed, the children's growth will be better. In 2009, Malek et al. conducted a longitudinal clinical trial study to examine whether the removal of carious teeth affected children's growth relative to that of a standard population. Five- and six-year-old children who attended for extraction of carious teeth under general anesthesia took apart in this study. The children's dental caries levels, weight, and height were measured prior to extraction using standard criteria and a single trained examiner, and they were then remeasured 6 months later. The participants had a mean dmft of 7.18 (SD 3.27) at baseline, and at follow-up children showed a statistically significant gain in BMI SDS and a small gain in height SDS [58]. In their another longitudinal birth cohort, Kay et al. found that children who had caries at 61 months had slower increases in weight and height than those without

decay at the same age [59]. These observations were consistent with a recent study which examined the association between untreated dental caries in primary and permanent teeth with age-adjusted height and weight among 6–12-year-old children in Bangladesh [60].

However, the relationship between dental caries and child's growth is inconclusive so far. A research from the department of cardiology, endodontology, and pedodontology in Academic Centre for Dentistry Amsterdam (ACTA) has been published in *Clin Oral Investig* in 2011. The study has two objectives: first, to assess the relation between dental caries and body proportions cross-sectionally in a Suriname caries child population and, second, to investigate whether dental treatment had a significant influence on body growth of these children in a randomized controlled trial using different treatment strategies. Three hundred eighty 6-year-old children with untreated dental decay participated in the study. Participants were evaluated after 6 months and 1, 2, and 3 years. However, negative correlations were observed between anthropometric measures and the number of untreated carious surfaces and caries experience of the children. Next, no significant differences in growth pattern between the treatment groups were observed. Thus, the authors suggested that caries activity is a negative predictor for body growth in children, and dental intervention does not show significant improvement within 3 years [61]. Later, Merrilyn et al. undertook an updated systematic review of the relationship between body mass index and dental caries in children and adolescents. The authors searched MEDLINE, ISI, Cochrane, Scopus, Global Health, and CINAHL databases and conducted lateral searches from reference lists for papers published from 2004 to 2011, inclusive. Finally, a total 48 studies were included. Three main patterns of relationships were found between dental caries and BMI: 23 of the 48 studies found no association between BMI and dental caries, 17 found a positive relationship between BMI and dental caries, and 9 found an inverse relationship. The reasons that authors analyzed may be method of dental examination, sample differences, dental caries prevalence, and BMI distribution. And they also recommend that future research investigate the nature of the association between body mass index and dental caries in samples that include a full range of body mass index scores and explore how factors such as socioeconomic status mediate the association between body mass index and dental caries [62].

8.4 Dental Caries and Atherosclerosis, Cardiovascular Disease, and Heart Attack

Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is a specific form of arteriosclerosis in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol and triglyceride. *Cardiovascular disease* (*CVD*) is the broad term used to categorize any abnormal condition characterized by dysfunction of the heart and blood vessel system, principally referring to cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease. Evidence suggests a number of traditional risk factors for atherosclerosis and CVD: age, gender, high blood pressure, high serum cholesterol levels, tobacco smoking, excessive alcohol consumption, sugar consumption [63], family history, obesity, lack of physical activity, psychosocial factors, diabetes mellitus, and air pollution [64]. However, these factors cannot explain all the deaths from CVD. For example, about 40 % of coronary heart disease (CHD) deaths occur in people with cholesterol levels that are lower than the population average [65]. Therefore, medical researchers' attention has focused in recent years on identifying additional risk factors that are non-traditional but may play major roles in explaining some of the variability in atherosclerosis and CVD risk.

During the last three decades, there has been an increasing interest in the impact of oral health on atherosclerosis and subsequent cardiovascular disease (CVD). Just as Meurman et al. wrote in their paper which was published in *Crit Rev Oral Biol Med*: "chronic infections caused by a variety of micro-organisms are thought to be involved in

the etiopathogenesis of CVD by releasing cytokines and other pro-inflammatory mediators that may initiate a cascade of biochemical reactions and cause endothelial damage and facilitate cholesterol plaque attachment. Yet, due to the multifactorial nature of dental infection and CVD, confirming a causal association is difficult, and the published results are conflicting. The main deficit in the majority of these studies has been the inadequate control of numerous confounding factors, leading to an overestimation and the imprecise measurement of the predictor or over adjustment of the confounding variables, resulting in underestimation of the risks" [66].

8.4.1 Dental Caries and Atherosclerosis and Cardiovascular Disease

Many studies have looked at poor dental care as a risk factor for cardiovascular disease (CVD). The results have been inconsistent, but most studies support a modest association between them [67]. Mattila et al. may be one of the first researchers to indicate a relationship between orofacial infections and cardiovascular disease. In 1989, they published an article in *British Medical Journal (BMJ)* and reported that there was an unexpected correlation between dental disease and systemic disease. After adjusting for age, exercise, diet, smoking, weight, blood cholesterol level, alcohol use, and health care, people who had caries and periodontal disease had a significantly higher incidence of acute myocardial infarction [68]. Another prospective cohort study, published in 1993, found that patients with periodontal disease had a 25 % increase in CVD, and men younger than 50 years had a significantly higher risk. However, no association between extent of active dental decay and risk of coronary heart disease was observed. Since tooth loss in people under 60 is usually caused by dental caries, the authors said they cannot rule out the possibility that the increased risk of coronary heart disease among young men with no teeth may have been related to previous dental decay [69]. These important discoveries resulted from the study is not the only reason that makes it noteworthy. Actually, the 9,760 subjects included in this work make it the largest sample size of its kind at that time. Since then, investigating the relationship between dental disease and CVD has become a priority.

Later, in 2001, a prospective cohort study in Stockholm, Sweden, followed 1,393 individuals for 27 years and concluded that oral health was a risk factor for death due to CVD, especially in combination with smoking, another risk factor. In this investigation, a significant correlation between caries and death due to CVD when adjusted for age and gender was demonstrated, indicating that this possible etiological pathway should be further investigated in the future. And the number of tooth surfaces with caries and presence of plaque were significantly increased for smokers compared to nonsmokers [69].

Maharaj and Vayej studied 44 black patients with severe rheumatic heart disease before they had cardiac surgery in 2012. Abnormalities were detected in the panoramic radiographs of 84.1 % of patients. The most frequent lesion was caries, present in 56.8 % of patients, followed by missing teeth in 54.5 %, and impacted teeth in 25 % of patients. Retained roots were present in 22.7 % and periapical pathology was detected in 18.1 % of patients [70].

It is clear that minimal carious lesions, caries with and without involvement of the pulpal cavity, and chronic apical periodontitis (CAP) represent different stages of the same inflammatory process. A recent study shows for the first time that dental caries, pulpal caries, and chronic apical periodontitis are associated positively, while restorations are associated inversely, with aortic atherosclerotic burden [71]. The authors' result showing that not only CAP but also caries with pulpal decay or no visible pulpal decay was associated with a greater atherosclerotic burden was somewhat surprising. We know that early stage of caries is an inflammatory process localized in the oral cavity that does not affect the pulpal cavity or the bone, indicating that a lesser extent of association of the early stage of caries with the atherosclerotic burden was expected than with the other two serious stages. One obvious explanation for

this finding may be the covariance of these factors, as pulpal caries and CAP occur primarily in patients with extensive tooth decay. The initial carious lesion and caries not yet affecting the pulpal cavity exist for a longer period compared with the pulpal decay, which can precede pulpal decay by a number of years. An explanation other than the disease lasting many years is that even forms of caries not yet involving the pulp are not merely local inflammatory lesions but rather disease affecting the entire body. The authors suggested that prospective studies are required to confirm these observations and answer the question of possible causality [71].

8.4.2 Root Caries and Cardiac Dysrhythmia and Gerodontology

Cardiac dysrhythmias, especially atrial fibrillation, are known to cause ischemic heart disease. Many studies suggested that inflammation plays a prominent role in the onset of atrial fibrillation [72]. With respect to the result of logistic regression analysis, cariogenic bacteria have a specific impact on the pathogenesis of cardiac diseases, especially dysrhythmias [73]. In 2005, researchers from University of Copenhagen designed a cross-sectional study to examine whether caries is associated with cardiac arrhythmias in community-dwelling people aged 80 and older. The primary finding of the multivariate logistic regression analysis was that persons with three or more active root caries lesions had more than twice the odds of cardiac arrhythmias than persons without active root caries. The findings indicated that there may be a link between active root caries and cardiac arrhythmias in the oldest old [74]. In order to explain the link, we should turn to the immune response because several studies have reported that an increase in dental caries is associated with a heightened immune response. In addition, dental caries affects the production of IgG and induces acute-phase proteins. The inflammatory-mediated cytokines and acute-phase proteins are practical markers of increased risk of cardiovascular disease, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) [75]. Bacterial species lying within the root surfaces supporting structures induce systemic inflammation and immune response, thereby increasing levels of serum CRP and serum IgG. In 2011, Kaneko et al. conducted a longitudinal study to elucidate the relationship between root caries and the onset of dysrhythmias on electrocardiographs in community-dwelling persons aged 75 and older. Serum CRP level was used as a variable to link root caries with dysrhythmias directly. They found that a high mean CRP serum level group had a significantly higher number of sites with root caries than a low CRP group. Moreover, number of sites with root caries events was significantly associated with cardiac dysrhythmia among nonsmokers. These results confirmed that root caries is related to the incidence of dysrhythmias in nonsmokers [73].

8.4.3 *Streptococcus mutans* and Atherosclerosis

We know that *Streptococcus mutans* (*S. mutans*) is a major cariogenic pathogen that is a normal inhabitant of the oral cavity in most individuals. *S. mutans* has also been isolated from the blood of patients with infective endocarditis (IE), which strongly suggests a close relationship of the pathogen with IE [76, 77]. Ullman et al. pointed out that their experience agrees with the literature and indicates that *S. mutans* is primarily a pathogen in elderly patients with heart disease and may be associated with IHSS [78]. In 2006, Nakano from Osaka University Graduate School of Dentistry and coworkers published the first study to analyze the presence of streptococcal species in diseased heart valve and atheromatous plaque specimens, as well as in dental plaque samples from the same subjects by a PCR method. Unexpectedly, *S. mutans* was detected at high frequencies and quantities in both heart valve tissues and atheromatous plaque samples in their study. Their conclusion indicated that *S. mutans* is a possible causative agent of cardiovascular disease [79]. In addition, when using DNA

fingerprinting to compare *S. mutans* isolated from dental plaque and an infected heart valve from a patient who underwent heart surgery, Nomura and colleagues demonstrated that the oral isolates differed from those found in the heart valve [80]. Three years later, Nakano et al. published another paper titled "Detection of oral bacteria in cardiovascular specimens" in *Oral Microbiology and Immunology*. This time, they found that *S. mutans* was the most frequently detected species in the cardiovascular specimens, followed by *Aggregatibacter actinomycetemcomitans*. Furthermore, the positive rate of *S. mutans* in cardiovascular specimens from patients whose dental plaque specimens were also positive for *S. mutans* was 78 %, which was significantly higher than any other tested species when the same analysis was performed [81]. Collectively, these findings lend credence to the idea that there are subpopulations of *S. mutans* carried in humans that, while not necessarily associated with caries, may have an enhanced capacity to interact, and possibly invade, the cells of the cardiovascular system [82]. *S. mutans* is classified into four serotypes (c/e/f/k) based on the chemical composition of its cell surface serotype-specific rhamnose–glucose polymers (RGPs), which form a backbone of rhamnose polymers with side chains of glucose polymers. Serotype c is reported to be the most prevalent in oral isolates at approximately 70–80 %, followed by e, f, and k. Serotypes e and f have been found to invade endothelial cells [82]. Serotype k, with a defect of the glucose side chain in RGPs, was found to show low cariogenicity but high virulence in blood as compared to the other serotypes, due to alterations of several cell surface structures [83]. When it comes to the possible reasons of the link between *S. mutans* and cardiovascular system diseases, it may be summarized as the following:

1. One crucial step for the development of atheromatous plaque lesions is formation of foam cells, which are macrophages that accumulate from excess cholesterol, and *S. mutans* strain GS-5 has been shown to enhance their formation [84].
2. In addition, *S. mutans* were shown to induce platelet aggregation, which presumably lead to thrombus formation. It was also found that *S. mutans* cells bind to extracellular matrix molecules and fibrinogen with contribution from the major surface protein antigen Pac [85, 86].
3. The infection with *S. mutans* expressing collagen-binding protein (CBP) is a potential risk factor for hemorrhagic stroke [87]. Lately, two types of cell surface collagen-binding proteins, Cnm and Cbm, have been studied to see if they play a role in *S. mutans* attachment and invasion of human umbilical vein endothelial cells (HUVEC). The results found that most of the Cbm-positive strains showed higher levels of binding to type I collagen as well as higher rates of adhesion and invasion with HUVEC as compared to the Cnm-positive strains. Furthermore, the gene encoding Cbm was detected significantly more frequently in heart valve specimens from IE patients than from non-IE patients [88].

8.4.4 Tooth Loss and Cardiovascular Disease and Stroke

While some studies have shown that decay is not a direct risk factor, it can and does cause tooth loss, which has been demonstrated to be a greater risk for cardiovascular disease [89, 90]. Recent evidence showed a direct link between oral health and CVD and that the number of teeth can be used to assess increased risk of CVD in adults. They drew the conclusion from a fairly large ($n = 7{,}647$), prospective study with a long follow-up period (1976–2002) that presents for the first time a dose-dependent relationship between number of teeth and both all-cause and CVD mortality. The authors found that a person with fewer than 10 of their own teeth remaining is seven times more likely to die of coronary disease than someone with more than 25 of their own teeth [91].

As pointed out by Watt et al., although there is increasing epidemiological evidence linking poor oral health with the development of chronic diseases

and mortality, these associations are still doubtful due to imprecise measurement of important risk factors of systemic disease. Indeed, most previous studies exploring the link between tooth loss and systemic disease have been conducted in selected samples and have failed to control adequately for socioeconomic, behavioral, and general health status [92]. Thus, their recent prospective cohort study of a national sample of Scottish adults published in *PLOS ONE* caught our attention. The sample consisted of 12,871 participants and they were followed for 8.0 (SD: 3.3) years. During 103,173 person-years, there were 1,480 cases of all-cause mortality, 498 of CVD, and 515 of cancer. After adjusting for demographic, socioeconomic, behavioral, and health status, edentate subjects had significantly higher risk of all-cause and CVD mortality compared to subjects with natural teeth only. Their findings confirm previous studies which have shown a small but significant association between tooth loss and all-cause and CVD deaths after controlling for a range of potential confounding factors [92].

However, there were different opinions from other studies, such as the study using the data from the Glasgow Alumni Cohort to investigate whether oral health in young adulthood is independently associated with cause-specific mortality after accounting for childhood socioeconomic background and other risk factors in young adulthood. Over 12,000 subjects (30 years or younger at baseline) were traced during up to 57 years of follow-up, and 1,432 deaths occurred among subjects with complete data, including 509 deaths from CVD and 549 from cancer. When the number of missing teeth was treated as a categorical variable, there was evidence that students with nine or more missing teeth at baseline had an increased risk of CVD compared with those with fewer than five missing teeth. When the number of missing teeth was transformed using fractional polynomials, there seemed to be a nonlinear relation between missing teeth and CVD mortality [93].

Stroke remains the third leading cause of death (after heart disease and cancer) in most developed countries. Cerebrovascular ischemic strokes are the commonest kind of stroke and occur as a result of an obstruction, usually a clot, within a blood vessel supplying blood to the brain. Heitmann and Gamborg examined if the number of remaining teeth was associated with the development of cardiovascular morbidity and mortality over 5–12 years. The prospective observational study among1474 men and 1458 women born 1922, 1932, 1942 or 1952 from The Danish MONICA follow up study (monitoring trends in and determinants of cardiovascular disease) in 1987–88 and 1993–94. Their results showed that tooth loss was strongly associated with incidence of stroke and, to a lesser extent, incidence of cardiovascular disease and coronary heart disease, during averagely 7.5 years of follow-up [94]. Choe et al. conducted a prospective cohort study of stroke in Korea on hypertension, diabetes, smoking, and tooth loss to characterize their independent effects and interactions. They confirm that tooth loss is independently associated with increased risk of stroke, and hypertension does interact antagonistically, particularly for hemorrhagic stroke [95]. A recent study found that stroke patients in their 50s and 60s had significantly fewer remaining teeth than did patients hospitalized for other conditions in the corresponding age groups. Moreover, the number of remaining teeth was significantly lower among stroke patients in their 50s than data reported for that age group in the Survey of Dental Diseases, suggesting the possibility that stroke patients may have lost teeth at a younger age. The authors also pointed out that the association between stroke and tooth loss can be explained by common risk factors associated with lifestyle such as hypertension, diabetes, smoking, and alcohol intake. It is quite difficult to rule out all common risk factors as confounding variables; therefore, the exact mechanisms of the relationship between stroke and tooth loss are difficult to identify [96]. Interestingly, periapical lesions, normally resulting from an infected root canal (caused by caries), are also a factor in stroke risk. This is another example of how dental caries can play a role, however indirectly, in heart disease [5].

8.4.5 Pulpal Periapical Diseases and Coronary Heart Diseases

Endodontic inflammation occurs after bacteria or their byproducts enter a tooth's pulp chamber.

Apical periodontitis is an acute or chronic inflammatory lesion around the apex of a tooth root which is caused by bacterial invasion of the pulp of the tooth. Despite numerous differences between chronic inflammatory disease of periodontal and endodontic origins, Caplan et al. summarized their similarities, primarily that: (1) both conditions share a common microbiota that often is associated with Gram-negative anaerobic bacteria, and (2) elevated systemic cytokine levels have been observed in conjunction with both disease processes [97]. Since several epidemiologic investigations have uncovered relationships between chronic periodontal disease and coronary heart disease, links between endodontic inflammation and cardiovascular outcomes are biologically plausible. Mechanisms linking endodontic disease to CHD might be similar to those hypothesized for associations between periodontal disease and CHD, in which a localized inflammatory response to bacterial infection leads to release of cytokines into the systemic circulation and to subsequent deleterious vascular effects [98]. Frisk and colleagues are one of the first groups who specifically studied endodontic variables as risk factors for the development of CHD. However, they did not reveal a significant association between endodontically treated teeth and CHD nor between teeth with periapical disease and CHD from their population-based study of Swedish women [99]. Joshipura et al. found a possible modest association between pulpal inflammation and CHD, although dental caries was not associated with CHD. They observed a greater CHD incidence among men with a positive self-reported history of ET in the Health Professionals Follow-Up Study [100].

8.4.6 Summary

As stated above, several epidemiologic investigations have uncovered relationships between dental caries (including its subsequent pulpal periapical diseases and tooth loss) and coronary heart disease. However, other studies have not found significant relationships, sparking questions about the proposed association. In the future, more direct evidence should be found to support this connection.

8.5 Dental Caries and Immune System Disease

It's no doubt that infection stresses the immune system. So dental infections, especially long-term disease (such as periapical abscesses), should have a deleterious effect on the immune system. Dental caries is an infectious disease that occurs because of imbalance in the homeostasis between the host and microbiota [101]. Salivary innate and adaptive immune defenses may influence in the bacterial colonization, and some disorders can affect these systems such as general immune deficiencies associated with malnutrition, inherited or medication disorders, or other factors that affect salivary flow and saliva composition [102].

8.5.1 Salivary Immunoglobulin A

Oral microorganisms and aerodigestive antigens are continuously influenced by the two major antibody classes in saliva: SIgA and IgG. The former is the dominant immunoglobulin in the healthy mouth which is produced by gland-associated immunocytes. It can agglutinate oral bacteria, modulate enzyme activity, and inhibit the adherence of bacteria to the buccal epithelium and to enamel. It does well at interfering with the initial colonization of caries-associated microflora on the tooth surface. Thus, a protective role for salivary sIgA was postulated [103–105]. However, about the relationship between Salivary IgA and dental caries, different studies got contrary results and conclusions. In 1978, Challacombe S. J. did not find that salivary antibodies in man play a role in protection against caries [106]. Several years later, Gregory et al. found that caries-free subjects or individuals with low caries susceptibility exhibited significantly higher levels of naturally occurring salivary immunoglobulin A (IgA) and serum IgG, IgA, and IgM antibodies to a *Streptococcus mutans* ribosomal preparation than subjects with high caries susceptibility [107]. Recently, some Indian researchers from MAR Dental College carried out a protocol-driven cross-sectional pilot study to check the purported association between salivary

sIgA and dental caries with special reference to RA. Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated. Forty-eight patients with RA and 102 non-RA, healthy case controls were taken part in the study. The decay, missing teeth, filled teeth (DMFT) index was used to classify caries. Whole unstimulated saliva was collected to assay sIgA using a commercial ELISA kit. The results showed that there were no statistically significant differences between RA and non-RA subjects with respect to salivary sIgA and the extent of caries [104]. It's hard to explain all the contrary results, since all the studies had their limitations, such as the study designs, if the sample size was insufficient or not, or technical variables also influence biomarkers and antibodies in oral secretions. Further studies should be carried out to find the truth.

8.5.2 HIV and Dental Caries

According to the World Health Organization, globally, 34.0 million [31.4–35.9 million] people were living with HIV at the end of 2011. In 2004, Phelan J. A. et al. published a paper in Journal of Dental Research titled "Dental caries in HIV-seropositive women." The authors conducted the study to determine if there was an association between HIV infection and dental caries among women enrolled in the Women's Interagency HIV Study. Subjects included 538 HIV+ and 141 HIV− women at baseline and 242 HIV+ and 66 HIV− women at year 5. Caries indices included DMFS and DFS (coronal caries) and DFSrc (root caries). Cross-sectional analysis of coronal caries data revealed a 1.2-fold-higher caries prevalence among HIV+ women compared with HIV− women. Longitudinally, DMFS increased with increasing age and lower average stimulated salivary volume. Root caries results were not significant except for an overall increased DFSrc associated with smoking [108]. At the same year, Mulligan R. et al. also reported that seropositive women who fit the Center for Disease Control (CDC) AIDS criteria were also more likely to have more DMF teeth ($P=0.004$), DMF surfaces ($P=0.003$), and decayed and/or filled (DF) root surfaces ($P=0.0002$) compared to seropositive women without AIDS [109].

It is estimated worldwide that there are 2.3 million human immunodeficiency virus (HIV)-positive children from 0 to 14 years infected by mothers [110]. However, prior to 1992, information about dental caries in HIV-infected children was very limited. Between 1992 and 1996, there were three published cross-sectional studies of dental caries in the primary teeth of HIV-infected children [111]. These studies showed that there was a higher prevalence of dental caries (including early childhood caries (ECC)) in the primary dentition of HIV-infected children as compared to healthy children [112–114]. However, it has not always been clear from past studies exactly what factors bring about these differences that exist in dental caries between HIV-infected children and a noninfected control group. In order to exclude the common environmental factor, Tofsky et al. compared the baseline prevalence and 2-year incidence of dental caries found in both the primary and secondary dentition among a group of HIV-infected children as compared to their HIV-negative household peers. They found that HIV-infected children have a high prevalence of dental caries in the primary teeth and a low prevalence in permanent teeth, while the incidence of permanent tooth dental caries is less than that of a group of noninfected household peers [115]. And reasons are still unknown.

It has been hypothesized that immunodeficiency and a progressive decrease in CD4+ T-lymphocytes resulting from HIV infection might alter salivary flow rate and impair the secretory immune system, thus contributing to increased bacterial colonization in the oral cavity implying that cariogenic bacteria may also increase in the oral cavity [116]. Those changes could be contributing factors for the development of HIV-associated oral diseases, including increased prevalence of dental caries. A recent study by Liu and coworkers examined the effect

of HIV infection on the level and genotypic characteristics of *S. mutans* colonization. They found that HIV+ individuals experienced significantly higher levels of *S. mutans*. Interestingly, the level of *S. mutans* significantly correlated with CD8+ count, but not with viral load or CD4+ counts, which is clearly suggesting that other HIV-associated factors mechanistically mediate *S. mutans* colonization in saliva. And they also revealed decreased salivary flow rate in the HIV+ group [117]. Since more dental caries was evidenced in HIV+ individuals from this study and others, additional studies are required to elucidate and understand the correlation between the colonization of other cariogenic microbes, including *S. mutans*, and the status of immunosuppression at the advanced stages of HIV infection.

8.6 Dental Caries and Kidney Diseases

Chronic kidney disease (CKD), also known as chronic renal disease, is a progressive loss in renal function over a period of months or years. The symptoms of worsening kidney function are nonspecific and might include feeling generally unwell and experiencing a reduced appetite. Often, chronic kidney disease is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with high blood pressure or diabetes and those with a blood relative with chronic kidney disease. Chronic kidney disease may also be identified when it leads to one of its recognized complications, such as cardiovascular disease, anemia, or pericarditis. In addition to the systemic manifestations and complications that arise from the disease and its treatment, changes in the mouth are common in patients with chronic kidney disease (CKD). Poor oral health in CKD patients may thus represent an important, but often overlooked, problem [118, 119].

Several studies have demonstrated higher rates of oral pathology in dialysis patients with one or more oral symptoms such as xerostomia, taste disturbances, uremic odor, tongue coating, mucosal inflammation, oral ulceration, or enamel hypoplasia. We know that xerostomia (or dryness of the mouth) may predispose to caries and gingival inflammation as well as contribute to difficulties with speech, denture retention, mastication, dysphagia, sore mouth, loss of taste, and infections. Studies in the general population suggest that edentulous subjects are prone to have an inappropriate dietary intake (such as ingesting too little protein and too much calorie-rich, high-fat food) as compared with dentated persons. Whereas the number of teeth is of importance for masticatory function, having premolar and molar teeth is especially important for nutritional status. The increased periodontitis and dental caries rates of CKD patients lead to tooth loss, which may result in chewing difficulties because of inadequate occlusive surfaces or the limitations of prostheses. On the other hand, approximately 30 % of patients with advanced CKD are reported to have a “bad” or a “metallic” taste in their mouths, which has been associated with metabolic changes, diverse drugs, a reduced number of taste buds, and changes in both salivary flow rate and composition. Increased dental calculus has been observed, perhaps as a consequence of a high salivary urea and phosphate levels.

When it comes to the relationship between dental caries and CKD, different researches got different results, and sometimes the conclusions were contradictory. Some studies showed that uremic patients have higher rates of decayed, missing, and filled teeth, loss of attachment, and periapical and mucosal lesions than the general population. The consequences of poor oral health may be more severe in CKD patients because of advanced age, common existing additional diseases such as diabetes, concurrent medications, and a state of reduced immune function that may increase the risk for consequences of periodontitis and other oral and dental conditions [120]. Some researchers reported that the prevalence of dental caries was low in children with renal disease [121, 122]. As early as 1985, Peterson and his colleagues got data to support the hypothesis that the relative paucity of caries in patients with chronic renal failure results from alteration of plaque by metabolic end products of urea

metabolism. The data further suggested that transplanted patients whose renal function is normal may be at increased risk of caries, especially if enamel hypoplasia is present and oral hygiene is poor [123]. Others have not found any evidence that the prevalence of dental caries in permanent teeth is significantly different in CKD children when compared with healthy children [124, 125].

A recent systematic review published in *Pediatric Nephrology* tried to determine whether there is any evidence in the literature referring to a lower prevalence of dental caries in children and adolescents with chronic kidney disease (CKD) compared to healthy individuals. After the evaluation of title, keywords, and abstracts of the articles selected, six articles met the inclusion criteria. Three of these six articles included studies which showed susceptibility to bias and possible confounding factors. A subsequent assessment of the six studies revealed that the mean caries indices in both primary (dmf) and permanent (DMF) teeth were lower in the children and adolescents with CKD compared with healthy individuals. In these patients, the low prevalence of dental caries may be associated with salivary characteristics, especially the neutralization of end products of bacterial plaque due to the increased pH resulting from urea hydrolysis in the saliva. So the authors concluded that data in the literature weakly support a lower prevalence of caries in children and adolescents with CKD than in their healthy counterparts [126]. There is still a lack of well-designed studies that provide better scientific evidence in support of this conclusion.

8.7 Dental Caries and Gastrointestinal Diseases

To date, the most significant relationship between dental caries and gastrointestinal diseases is from chewing pain and tooth loss. As we mentioned before, carious teeth become pulpitic; the eating of some foods will cause pain; therefore toothache and infection alter eating and sleeping habits, dietary intake, and metabolic processes. Moreover, the edentulous patient without dentures is the most vulnerable to gastrointestinal and other related problems. In the edentulous person with a deficient masticatory performance, reduced consumption of fiber-rich foods that are hard to chew could provoke gastrointestinal disturbances. One study provided a sound basis for why the denture wearer does not achieve the necessary breakdown of food substances. The research indicated that the chewing efficiency of those wearing dentures was about one-sixth that of a person with natural teeth. In addition, evidence suggests that nutritional deficiencies, regardless of their cause, are associated with impaired immune responses [5].

There are also some interesting researches that connected dental caries and gastrointestinal diseases, such as gastroesophageal reflux disease and the effect of *S. mutans* on the ulcerative colitis.

8.7.1 Dental Caries and Gastroesophageal Reflux Disease

Gastroesophageal reflux disease (GERD) is a chronic symptom of mucosal damage caused by stomach acid coming up from the stomach into the esophagus [127]. GERD is usually caused by changes in the barrier between the stomach and the esophagus, including abnormal relaxation of the lower esophageal sphincter, which normally holds the top of the stomach closed, impaired expulsion of gastric reflux from the esophagus, or a hiatal hernia. These changes may be permanent or temporary. The oral lesions resulting from GERD are not usually noticed until they cause significant damage. Despite the pruritus and burning on the oral mucosa, tooth sensitivity, aphthae, sour taste, and decrease in the vertical dimension of occlusion to irreversible damage, dental caries is still our focus in this chapter.

However, the results have been contradictory. Some studies on the oral health of patients with chronic reflux reported an inverse relation between caries and gastroesophageal reflux and

showed a small number of caries in individuals with GERD as compared to control groups [128]. They attributed this to the low prevalence of bacteria (lactobacilli and streptococci) observed in the saliva of patients with chronic reflux [129]. On the other hand, Linnett et al. conducted for 52 children (31 boys and 21 girls) with a definitive history of GERD. They found that caries experience was higher in GERD patients compared to controls. Although there were more subjects with *Streptococcus mutans* in the GERD group compared to the control group (42 % vs 25 %), the difference was not statistically significant [130]. Silva et al. [23] did not find relationship between GERD and changes in the oral cavity by saliva tests, oral clinical examination, or histopathologic examination of the palatal mucosa [131]. The differences among the different studies may be explained by the different research design, the patients from different areas, and sample size. Future studies are needed to explore the truth.

8.7.2 *S. mutans* and Ulcerative Colitis

Ulcerative colitis (Colitis ulcerosa, UC) is a chronic, or long-lasting, disease that causes inflammation and sores in the inner lining of the large intestine. The main symptom of active disease is usually constant diarrhea mixed with blood, of gradual onset. The cause of UC is unknown though theories exist. People with UC have abnormalities of the immune system, but whether these problems are a cause or a result of the disease is still unclear. Current theories suggest that indigenous gut microbiota play a key role in the pathogenesis of inflammatory bowel disease. Moreover, regulation of mucosal immune response to unidentified components of normal intestinal microbiota in a genetically susceptible host is at the core of these diseases [132, 133].

In 2011, Nakano et al. found the specific strains of *S. mutans* that express collagen-binding protein (CBP) caused hemorrhagic damages in the murine brain and other tissues because of the ability to bind the collagen and resistance to phagocytosis [87]. In normal situations, only a limited number of strains are possible risk factors for aggravation of UC caused by *S. mutans-induced* bacteremia. In contrast, the detection frequency of specific strains of *S. mutans*, such as CBP-positive and phagocytosis-resistant strains from UC patients, was extremely higher than in non-UC control subjects. Thus the authors speculated that such specific strains of *S. mutans* may be involved in the pathogenesis of UC. So the next year, in 2012, they published another paper to show that infection of specific strains of *S. mutans* is one of the risk factors in aggravating inflammation of UC. They stated that it's the first paper describing the involvement of oral bacteria in UC pathology [134].

8.8 Dental Caries and Diabetes Mellitus

It's no doubt that diabetes mellitus is a rapidly growing health concern in both developed and developing nations. According to the World Health Organization (WHO), in 2011, approximately 364 million people globally suffer from diabetes mellitus (DM), with projections that DM-related deaths will double from 2005 to 2030 [135]. The Center for Disease Control (CDC) estimates that in the United States alone, 25.8 million Americans, or 8.3 % of the population, suffer from DM, with 7 million currently undiagnosed [136]. Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes, and "other specific types." Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. Type 2 diabetes is the most common type. In China, type 2 diabetes mellitus affects almost 92.4 million (9.7 %) Chinese adults, and 148.2 million adults are in the prediabetes. Based on case numbers in 2007 and projected case numbers in 2030, Wang W. et al. estimated that the direct medical costs of T2DM and its complications were estimated to be 26.0 billion USD in

2007 and were projected to be 47.2 billion USD in 2030. The results indicated that T2DM consumes a large portion of healthcare expenditures and will continue to place a heavy burden on health budgets in the future [137]. In 2008, Patiño et al. carried out a cross-sectional study involving 175 subjects to determine the frequency of caries, periodontal disease, and tooth loss in patients affected by diabetes mellitus types 1 and 2. Their results showed a difference between the two types among the study variables [138]. Since we care about the overall association between diabetes mellitus and dental caries prevalence, we did not make a distinction between type 1 and type 2 diabetes mellitus in this chapter.

It is widely understood that diabetes patients are at an increased risk for oral complications such as candidiasis, erosion, xerostomia, and periodontal disease [139, 140]. Studies have reported that patients with diabetes are susceptible to oral sensory, periodontal, and salivary disorders, which could increase their risk of developing new and recurrent dental caries [141]. However, although the relationship between diabetes and dental caries has been investigated since the last century, no clear association has been clarified till now [142].

8.8.1 Epidemiological Studies of Diabetes and Dental Caries

In 2004, Taylor et al. reviewed the post-1960 English-language literature on the relationship between diabetes and oral health, specifically focusing on periodontal disease, dental caries, and tooth loss. The literature does not describe a consistent relationship between type 2 diabetes and dental caries. It reports increased, decreased, and similar caries experiences between those with and without diabetes. This review suggests that currently there is insufficient evidence to determine whether a relationship between diabetes and risk for coronal or root caries exists [143].

Here are two studies that reported a greater history of dental caries in people with diabetes. In 2002, a study evaluated the caries incidence in 64 children and adolescents (8–15 years of age) with type 1 diabetes mellitus over a 3-year period from the onset of the disease in relation to metabolic control and to caries-associated risk factors. Results showed that patients with less good metabolic control exhibited higher glucose levels in resting saliva and a significantly higher caries incidence compared to those with good metabolic control. The most influential determinants for high caries development during the 3-year follow-up period were metabolic control, poor oral hygiene, previous caries experience, and high levels of salivary lactobacilli [144]. Later, Miralles et al. conducted the other work which comprised 90 type 1 diabetics between 18 and 50 years of age, and a group of non-diabetic controls matched for age and sex. Their results showed that under similar conditions of oral hygiene and salivary flow, the diabetic group showed a higher incidence of caries than the control group. Likewise, on specifically analyzing the diabetic group, the metabolic control of the disease, the duration of diabetes, and the existence of complications of the disease exerted an influence upon the development of dental caries [145].

Other researchers, however, have detected no significant difference or even a decrease in caries susceptibility between diabetic and nondiabetic patients. A decade ago, Moore et al. published a paper to describe the prevalence of coronal and root caries in an adult type 1 diabetic population and evaluate demographic, dietary, behavioral, physiologic, salivary, and medical variables associated with decayed and filled surfaces in the crown (DFS) or root (RDFS). The authors found that adult type 1 diabetic subjects did not have significantly higher DFS rates as compared with their control subjects or published age-adjusted NHANES III findings. However, the prevalence of RDFS was higher in the diabetic subjects as compared to recruited control subjects [146]. In 2006, another study in Lithuania comprised 68 10–15-year-old diabetics and 68 age- and gender-matched nondiabetic controls. Diabetics were categorized into well-to-moderately controlled and poorly controlled groups. They found that diabetics had fewer caries and plaque, lower salivary flow rates and buffer effect, and more frequent growth of yeasts than their nondiabetic

controls. Well-to-moderately controlled diabetics had fewer decayed surfaces and lower counts of mutans streptococci and yeasts than poorly controlled diabetics, but the level of metabolic control of diabetes had no influence on salivary flow rates and buffer effect. High caries levels in diabetics were significantly associated with age, plaque score, and decreased unstimulated salivary flow rate but were not associated with the level of metabolic control of diabetes. High caries experience in this study population could be related to plaque accumulation and/or to changes in saliva induced by diabetes mellitus [147]. Two years later, the same authors published another paper to analyze possible associations between caries increments and selected caries determinants in children with type 1 diabetes mellitus and their age- and sex-matched nondiabetic controls, over 2 years. A total of 63 (10–15 years old) diabetic and nondiabetic pairs were examined for dental caries, oral hygiene, and salivary factors. Salivary flow rates, buffer effect, concentrations of mutans streptococci, lactobacilli, yeasts, total IgA and IgG, protein, albumin, amylase, and glucose were analyzed. Means of 2-year decayed/missing/filled surface (DMFS) increments were similar in diabetics and their controls. No differences were observed in the counts of lactobacilli, mutans streptococci, or yeast growth during follow-up, whereas salivary IgA, protein, and glucose concentrations were higher in diabetics than in controls throughout the 2-year period. Their results also suggested that, in addition to dental plaque as a common caries risk factor, diabetes-induced changes in salivary glucose and albumin concentrations are indicative of caries development among diabetics [148]. In 2009, in her thesis for Master degree, Abay conducted a study that aimed to answer the research question, "Is there an association between diabetes mellitus and prevalence of severe dental caries in adults?" Data of 701 subjects with diabetes and 3,636 subjects without diabetes from the National Health and Nutrition Examination Survey (NHANES) conducted from 2003 to 2004 were used. Findings of her study suggested that prevalence of severe caries, prevalence of severe untreated caries, and prevalence of at least one root surface with caries or filling did not differ between adults with and without diabetes [149].

In experimental diabetic rodent animals, there are also contradictory reports; some studies reported that diabetes enhanced the incidence of dental caries whereas another did not. As early as 1957, Nichols and Shaw reported that in terms of carious molars and carious legions, normal rats did not differ from caries-susceptible rats that received intravenous injections of alloxan monohydrate [150]. However, Hartles and Lawton reported that the mean number of carious teeth per rat and the mean caries score were significantly higher for the injected animals than for the controls. And the authors proposed that the most probable way in which the injection of alloxan can influence the incidence of dental caries is by causing an alteration in the salivary secretions, either directly or indirectly [151]. A recent study published by some Japanese authors also confirmed that diabetic conditions enhance dental caries in WBN/KobSlc rats [152].

As stated above, results of these studies have shown conflicting conclusions. In spite of the difference between diabetes mellitus types 1 and 2, the reasons may be methodological difficulties, such as small sample size, the absence of standard criteria for caries evaluation, and nonconventional cutoffs to classify good and poor diabetes control [149]. Thus, further studies of the potential association between diabetes mellitus and dental caries are suggested.

8.8.2 Root Caries and Diabetes

According to its location, dental caries can be divided into coronal caries and root caries. The latter poses a complex challenge for dental practitioners, which is different to those challenges presented by the former [153]. Beck et al. found that coronal and root caries do tend to appear together in the same individuals. They also found that people who experience both types of caries had more gingival recession at baseline [154]. As world's population ages and retention of teeth increases, there will be increasing numbers of older patients at risk of root-surface caries. Root caries has a

higher prevalence among older adults than any other age group. Many of these individuals may be more likely to have chronic systemic disease. So we discuss the relationship between root caries and diabetes separately. Currently, insufficient evidence exists to support or refute an association between diabetes and root caries [155].

As we mentioned before, Moore et al. described the prevalence of coronal and root caries in an adult type 1 diabetic population in 2001. Although no significantly higher DFS (decayed and filled surfaces in the crown) rates were noticed, the prevalence of RDFS (decayed and filled surfaces in the root) was higher in the diabetic subjects as compared to recruited control subjects [146]. In 2007, another stratified cross-sectional study was conducted in Thailand to determine the effect of type 2 diabetes mellitus on coronal and root-surface caries. Subjects of 105 type 2 diabetic patients and 103 nondiabetic at the same age and gender were included. Their results found that type 2 diabetic patients compared with nondiabetic subjects had a higher prevalence of root-surface caries and a higher number of decayed/filled root surfaces. The authors also found that the factors associated with root-surface caries included type 2 DM, a low saliva buffer capacity, more missing teeth, and existing coronal caries [156].

To analyze the possible factors connected the root caries and diabetes, we have to talk about periodontal diseases, firstly. Diabetes has been found to be bidirectionally linked with periodontal disease and subsequent loss of attachment. As a result, gingival recession will cause the exposing of tooth's root and contributing to the risk of root caries [157, 158]. Secondly, considering that saliva is responsible for establishing protective environment against dental caries, we will talk about the effect of salivary factors like salivary flow rate and adequate level of calcium, phosphate, and fluoride in diabetes mellitus. Recently, Jawed et al. evaluated the possible protective role of salivary factors in diabetes mellitus type 2 patients with dental caries. In their study, a total of 398 diabetes mellitus type 2 patients with dental caries and 395 age- and sex-matched nondiabetic subjects with dental caries were included as controls. The blood glucose, HbA1c, and DMFT indices were found to be significantly high, while the salivary flow rate, calcium, phosphate, and fluoride were found to be significantly low in diabetic patients as compared to controls [159]. Salivary flow is known to be reduced in long-standing diabetes. This is thought to be due to neuropathy affecting the salivary glands as a result of chronic hyperglycemia [159, 160]. Therefore, in diabetes, periodontal disease and associated attachment loss and gingival recession may mediate increased root caries, compounded by reductions in salivary flow and elevated gingival crevicular glucose levels in people with poorly controlled diabetes [155].

Since oral microbiota plays an important role in the root caries process, the change of microbiology in the oral cavity of diabetes patients may also have relationship with root caries. Root caries was thought to be associated with *Streptococcus mutans*, *Lactobacillus* (spp.), and *Actinomyces* (spp.) based on the culture method [161, 162]. So far, the use of culture-independent methods has played a key role in the discovery of previously unrecognized species in the oral cavity as well as in redefining the pathogenesis of the major oral infections. And the authors found that the microbial flora associated with root caries was far more complex than previously assumed. Except for these three bacterial species we just mentioned, additional species, such as *Atopobium* spp., *Olsenella* spp., *Pseudoramibacter alactolyticus*, and *Propionibacterium* sp. strain FMA5, were also commonly found [163]. The study showed that significantly more diabetic subjects had higher levels of *Treponema denticola*, *Prevotella nigrescens*, *Streptococcus sanguinis*, *Streptococcus oralis*, and *Streptococcus intermedius* in their supragingival plaque than nondiabetic subjects. Root-surface caries was associated with an increased count of mutans streptococci, lactobacilli, and yeasts in saliva and of *Streptococcus mutans* in supragingival plaque samples [164].

8.8.3 Tooth Loss and Diabetes

The results of Lin et al.'s study suggested that diabetes and poor glycemic control may not be

associated with an increased prevalence of past coronal and root-surface caries experience in older adults, but there is a tendency for more active caries lesions and missing teeth [142]. In 2011, a Korean study has identified a relationship between total tooth loss from any cause and diabetes [165]. In addition, a recent study found that having 19 or fewer teeth was associated with high HbA1c among men aged 40–64 years but not among those aged 65–79 years. Since diabetes is a major risk factor for periodontal disease, the significant associations of tooth loss with HbA1c among the middle-aged men may reflect associations of periodontal disease with HbA1c, although they had no information on the causes of tooth loss [166].

8.9 Dental Caries and Respiratory Infections

The anatomical continuity between the lungs and the oral cavity makes the latter a potential reservoir of respiratory pathogens. It is well known that the respiratory system includes the nasal and oral cavity: the sinuses and larynx as the upper airway and the trachea, bronchi, bronchioles, and alveoli as the lower airway. Thus it's not surprising that many of the diseases that occur in the oral cavity could be also found in the upper airway regions. However, it's not easy for an infective agent to reach the lower respiratory tract. It must defeat sophisticated immunological and mechanical defense mechanisms. The latter is so efficient that, in healthy patients, the distal airway and lung parenchyma are sterile, despite the heavy bacterial load found in the upper airway. An infection occurs when the host's defenses are compromised, the pathogen is particularly virulent, or the inoculum is overwhelming. The microorganisms may enter the lung by inhalation, but the most common route of infection is aspiration of what pneumologists have long referred to as oropharyngeal secretions. Therefore, it is plausible that oral microorganisms might infect the respiratory tract [166].

Dental plaque and poor oral health have been associated with nosocomial pneumonia and chronic obstructive pulmonary disease (COPD) [167]. Since community-acquired pneumonia and lung abscesses may be due to anaerobic bacteria, there are lots of anaerobes implicated in the destruction of periodontal tissues that have also been isolated from infected lungs, for example, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Pseudomonas aeruginosa*. Pascual-Ramos et al. found a strong association between third-grade caries and pneumonia in 30 consecutive women with SLE, hospitalized because of pneumonia, compared with two groups of patients with SLE, hospitalized and ambulatory, matched to cases by age, sex, and hospitalization date. Compared with ambulatory controls, the oral health of patients with pneumonia was worse as reflected by a higher frequency of periapical lesions and cervical and third-grade caries and a higher number of caries per patient [168]. There is also evidence that the occurrence of respiratory tract infections during the first year of life is associated with a significantly increased risk for developing early childhood caries during subsequent years [169]. A research conducted by Eldem et al. from Turkey showed the possible association between poor oral hygiene and upper respiratory tract infection (URTI) rates. Children without any systemic disease were enrolled in the study and divided into two groups: 100 children with dental caries as patient group and another 100 children without caries as control group. URTI rates and antibiotic usage in both groups since birth were identified according to the medical records. Dental caries was scored according to decayed, missing, and filled teeth index. And their results showed that the URTI rates were significantly higher among children with poor oral hygiene and dental caries [170]. Pascual-Ramos et al. made a summary of the different mechanisms that have been proposed to explain the potential role of oral bacteria in the pathogenesis of respiratory infections. Among them are the aspiration of oral pathogens into the lungs, the modification of mucosal surfaces by periodontal disease-associated enzymes that promote adhesion and colonization by respiratory pathogens, the destruction of salivary pellicles by periodontal disease-associated enzymes that modify clearance of pathogenic bacteria from the

mucosal surface, and alteration of the respiratory epithelium by cytokines originating from periodontal tissues to promote infection by respiratory pathogens [168, 171].

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Models in Caries Research

9

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1. In vitro models in caries research
2. In situ models in caries research
3. Animal models in caries research

Clinical trials are always large, time-consuming, and costly; furthermore, due to ethical problems, some studies are not available in human subjects [1]. Therefore, models have played a substantial role in caries research, which help to establish the multifactorial etiology of dental caries, define the impact of numerous factors contributing to the initiation and progression of dental caries, and identify agents or measures with the ability to prevent or reduce the incidence of dental caries [2]. Models in caries research can be divided into three categories: in vitro model, in situ model, and animal model [3].

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9.1 In Vitro Models in Caries Research

In vitro models or laboratory models are the most common models applied in dental research with several advantages [4]:

1. Less costly and comparatively rapid
2. Can carry out single-variable experiments under highly controlled conditions, which are more sensitive and precise
3. Best approach to screen large numbers of agents and to determine their modes of action [5]

However, in vitro models have significant limitations, mostly related to their inability to simulate the complex biological processes involved in caries.

As dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms, two basic methods are developed to study dental caries: de- and remineralization in the teeth (e.g., in vitro chemical models) and microbial systems on the teeth (e.g., in vitro microbial models) [6]. Recently, microbial-based de- and remineralization models are also developed, which are closer to the tooth decay process in oral cavity.

9.1.1 In Vitro Chemical Models

Chemical induction of caries by organic acids is one of the principal approaches to study the

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mechanisms in de- and remineralization of enamel and dentin. In vitro chemical models allow strict control of the experimental environment and are relatively simple and cost-effective; however, their applicability is limited to factors which directly influence the de- and remineralization process. Between de- and remineralization models, substrates are one of the major differences. Different from remineralization methods which typically use lesions as substrate, demineralization models utilize a wider variety of substrates, including those pretreated and remineralized with agents, on natural enamel and dentin [5]. (For details, please also see "demineralization and remineralization" part.)

9.1.1.1 Demineralization Models

Tooth decay is the result of progressive mineral loss from dental tissues. In vitro demineralization models enable researchers to examine those fundamental processes, and their applications include [5] mechanistic studies of solution/substrate factors affecting demineralization, studies of factors contributing to the intrinsic resistance of mineralized tissue to acid demineralization, and efficacy evaluation of caries-preventive agents (e.g., fluoride, natural products [7, 8]) or the application of laser irradiation [9] which may inhibit enamel and dentin dissolution in acid attack.

9.1.1.2 Remineralization Models

In vitro remineralization models can be applied to investigate the mechanism involved in caries lesion repair and evaluating the efficacy of treatments or agents which are favorable to enhance remineralization. Remineralization protocols can be grouped into three general categories:

In pH-Lattice Ion "Drift" Protocol

Substrates are exposed to constant-volume supersaturated remineralization solutions. They have the advantage of direct chemical measurement of remineralization within a given exposure period [10]; however, in remineralization solutions, calcium and phosphate ion concentration will decrease, and the pH will also decrease, if the systems are not well buffered. Those changes cannot only decrease the amount of mineral formation but also change both the type and location of mineral deposition [5, 11].

Constant Composition Protocols

Lattice ion concentrations and pH remain constant throughout remineralization. In details, two techniques were involved: flow-through techniques and titration-controlled techniques. In the former, supersaturation is kept constantly by means of a high volume of remineralization medium, and it permits multi-group studies to be carried out. The measurement of remineralization is confined to substrate changes [5]. In the latter, controlled addition of calcium phosphate lattice ions and buffer titrants monitored potentiometrically by pH and/or calcium ion-selective electrodes was involved. The measurement of remineralization is used titrant [5].

"pH Cycling" Protocols

The "pH cycling" protocols consist of numerous cycles of demineralization and remineralization, which are correlated to alternant acidification and alkalinization phases in oral cavity. The genesis of modern pH cycling protocols was produced by ten Cate and Duijsters [10], which have become favorable choice to study remineralization in vitro, because they provide better simulation of the caries process for both mechanistic studies and for evaluations of some caries-preventive agents. Recently, a particular in vitro remineralization model, called Featherstone pH cycling model, was recommended as an appropriate alternative to animal testing, particularly for ionic fluoride based dentifrices, for the reason that it demonstrated excellent correlation with the currently accepted animal caries models [12].

9.1.2 In Vitro Microbial Model

In vitro microbial models provide means for studying complex microbial ecosystems on the teeth and their roles on the development of dental caries [6]. Microbial models can be used to (1) investigate prevention of carious lesions through antimicrobial agents or measures, (2) compare the cariogenic potential of different microorganisms, and (3) assess the cariogenicity of carious diets.

Microbial models can be divided into two main classifications: closed (batch) system and open system (continuous culture). The closed system, in which there is no flow into or out of the reactor during the cultivation and microorganisms are provided with finite nutrients and growth rates are rapid, is comparatively rare in nature [13, 14]. It is the simplest and most frequently used in vitro microbial models. During the growth process, the environment in the enclosed system will change (e.g., nutrients become depleted, signaling molecules and metabolites accumulate), unless the fluid is regularly replaced. This system is far from physiological; however, better repeatability, less contamination, less cost, and high throughput are its marked advantages. This system also allows researchers to easily vary multiple parameters including the composition of growth media, incubation temperatures, humidity, presence or absence of shear stress, and O_2 and CO_2 concentrations. Those features make it valuable system for initial screening assays [14, 15].

In dynamic continuous system, fresh medium flows into the bioreactor continuously, and part of the medium in the bioreactor is withdrawn from the fermenter at the same flow rate of the inlet flow. Then some metabolites can be eliminated from the bioreactor, and it is more like oral cavity than batch cultivation [16]. This system enables better control of growth rates and other variables [13]; however, it is more likely contaminated and not easy to repeat the results.

9.1.2.1 Inoculum

An important factor in the design of in vitro microbial models is the choice of inoculum [3]. Pure culture, defined consortium, and microcosm are all used currently. Pure culture is broadly used in vitro, in which physiological studies are normally done. It is much easier to manipulate the variation of the single test organism. However, it is far from mimicking the oral cavity.

Microcosm is used to closely mimic the physicochemical, microbiological, and nutrient conditions present in oral cavity [13]. In in vitro microbial models for dental caries, dental plaque or saliva can be used. They have the advantage of maintaining the complexity and heterogeneity and enabling in situ bacterial community dynamics to be replicated in laboratory environment. However, there are still some disadvantages. Firstly, it is hard to find out a medium to support all organisms in the plaque or saliva to grow as they do in oral cavity. Secondly, they are often poorly characterized. As the system is so complex, it is hard and costly to analyze its metabolism, composition shift, and interaction within the ecosystems. Thirdly, it would have been difficult to standardize the plaque inoculum in replicate experiments and to manipulate its composition for experimental purposes.

In order to overcome these problems, inoculum with a defined consortium has been constructed by pooling pure cultures of plaque bacteria in various combinations. The communities that develop from them are stable with time and establish reproducibly in replicate experiments. It also allows detailed control and study of the properties of the individual bacterial species present. Defined inoculum comprised of two or more organisms in combination, with organisms chosen for their relevance in health and disease and for their ease of identification; subsequently, individual species can be added or deleted for experimental purposes. One of the good examples is often referred to as the "Marsh Consortium" which is composed of nine bacteria [17]. In another batch culture study, a mixture containing *Actinomyces naeslundii*, *Veillonella dispar*, *Fusobacterium nucleatum*, *Streptococcus sobrinus*, and *Streptococcus oralis* was cultivated to form supragingival plaque, which was used to evaluate antimicrobial agents. A yeast species, *Candida albicans*, was added later, for the reason that a valid prescreening test should rule out the possibility of fungal overgrowth due to selective interference with bacterial ecology [18, 19]. In a root caries model system developed in artificial mouth, four putative root-caries pathogens, *Streptococcus mutans*, *Streptococcus sobrinus*, *Actinomyces naeslundii*, and *Lactobacillus rhamnosus*, form multispecies consortia biofilms and furthermore form the caries lesion [20].

9.1.2.2 Closed (Batch) System Microbial Models

In closed system microbial models, some small reactor vessels and test tubes were frequently used for planktonic culture, and microtiter plate

(MTP)-based system is among the most frequently used biofilm model systems. In MTP-based system, biofilms are either grown on the bottom and the walls of the microtiter plate (most commonly a 96-well microtiter plate) or on the surface of a substrata (glass, hydroxyapatite, enamel, or dentin disks) placed in the wells of the microtiter plate (most commonly a 6-, 12-, or 24-well microtiter plate). The former is generally based on bacterial sedimentation, in which the metabolites accumulate, and the latter can form through attachment, which is more close to the reality than the former.

Some modifications were developed for the 96-well microtiter plate, during which transferable solid phase (TSP) biofilm model was used to screen antimicrobial activity [21]. TSP is commercially available and contains a 96-well microtiter plate and a lid with 96 pegs placed on the plate. Biofilm can form on the surface of pegs through attachment. Another MTP-based commercially available method is the Biofilm Ring Test, which provides a kit including microplates (12 polystyrene strips of 8 wells), toner (magnetic bead solution), contrast liquid (a nontoxic and inert opaque oil used for reading step), dedicated block test (magnet support), and plate reader (scanner) [22]. With this technology, the immobilization of inert paramagnetic beads included in the culture medium during the formation of the biofilm is measured. A magnet is used to collect the non-immobilized beads into a single spot which is then quantified through specialized image algorithms [14]. This model was once used to confirm that AI-2-based quorum sensing affects biofilm formation in *Streptococcus mutans* [23].

Zürich biofilm model, a multispecies model, based on 24-well plates, is a classical batch culture approach, in which the host of environmental variables can be rigorously controlled. At present, six microorganism representatives are used to generate biofilms for supragingival plaque, which are *Streptococcus oralis*, *Streptococcus sobrinus*, *Actinomyces naeslundii*, *Veillonella dispar*, *Fusobacterium nucleatum*, and *Candida albicans* [18, 19]. Cells are cultivated anaerobically in a saliva-based medium on substrata coated with a salivary pellicle. As shear forces are absent in a batch culture system, those disks are dipped in saline three times daily, and at each time point, the biofilms are dipped three times in saline, thereby being subjected to passages through an air-liquid interface [18]. The validation of the in vitro caries model was assessed, which confirms the repeatability of biofilm formation after 40.5 h and 64.5 h in repeated independent trials, and demonstrated the produced losses in viability from brief exposures of biofilms to chlorhexidine or triclosan were similar to those observed in vivo, inferring that this biofilm model was very useful for preclinical testing of prospective antiplaque agents at clinically relevant concentrations [24]. Actually, the main application of this model was to evaluate antimicrobial compounds. The model could also be used to achieve demineralization and remineralization of bovine enamel under biofilms [18]. More recently, a novel high-throughput active attachment model (Fig. 9.1) was also used to evaluate antimicrobial compounds and contributing factor on dental caries [7, 26]. The model consisted

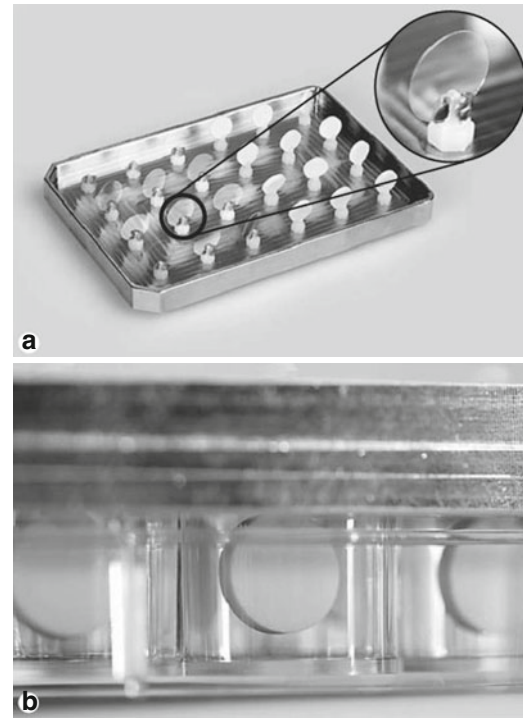

Fig. 9.1 Pictures of the biofilm model used in this study. (**a**) Custom-made stainless steel lid on which 24 clamps are fixed. Substrata glass cover slips or HA disks are shown. (**b**) Position of the substrata (HA disks) in the 24-well plate at the time of biofilm growth [25]

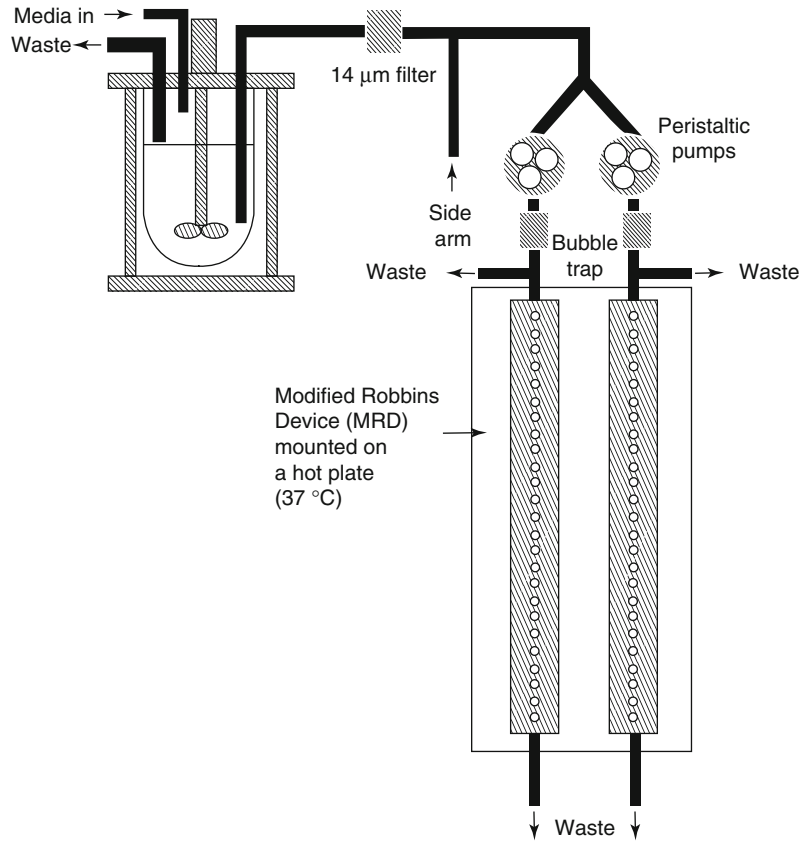

Fig. 9.2 Modified Robbins device [28]

of a custom-designed lid containing substrata that fit on top of standard 24-well plates. Single species biofilms and polymicrobial biofilm derived from saliva can be formed in the systems [25]. This model is also applicable for evaluating novel caries-preventive agents on both biofilm and demineralization inhibition at the same time when bovine dentin disks were used as substrata [27].

9.1.2.3 Open (Continuous Culture) System Microbial Models

Flow Cell Biofilm Model and Modified Robbins Device

Flow cell biofilm model and modified Robbins device [28] (MRD, Fig. 9.2) shared similar principles for the operation, whereby culture fluid is passed through a tube or cell and biofilms may be monitored microscopically (in flow cells) or formed on coupons (in some flat plate flow cells) or pegs (Robbins devices) [29]. Flow cells are commercially available devices with glass chambers that are particularly well suited for real-time nondestructive microscopic analyses of biofilms [14]. This model is not inexpensive, and the design can be versatile in the selection of material for the substratum [30]. They still have some disadvantages: Firstly, sterilization can be difficult because many of the common materials used for flow cell construction do not respond well to autoclaving, especially to repeated autoclaving. Secondly, peristaltic pumps can produce some pulsation in liquid delivery. Finally, in cases of high biomass within the flow cell [30], it is conceivable that a gradient in nutrients could be established over the length of the flow cell. As commonly used biofilm models, flow cells can be used for single-species biofilm or multispecies biofilm. The co-adhesion of *Streptococcus gordonii* with *Streptococcus oralis* was studied in a two-species in vitro oral biofilm flow cell system, in which green fluorescent protein was used as a species-specific marker [31]. In MRD, the tube can be plastic or metal, into which pegs can be

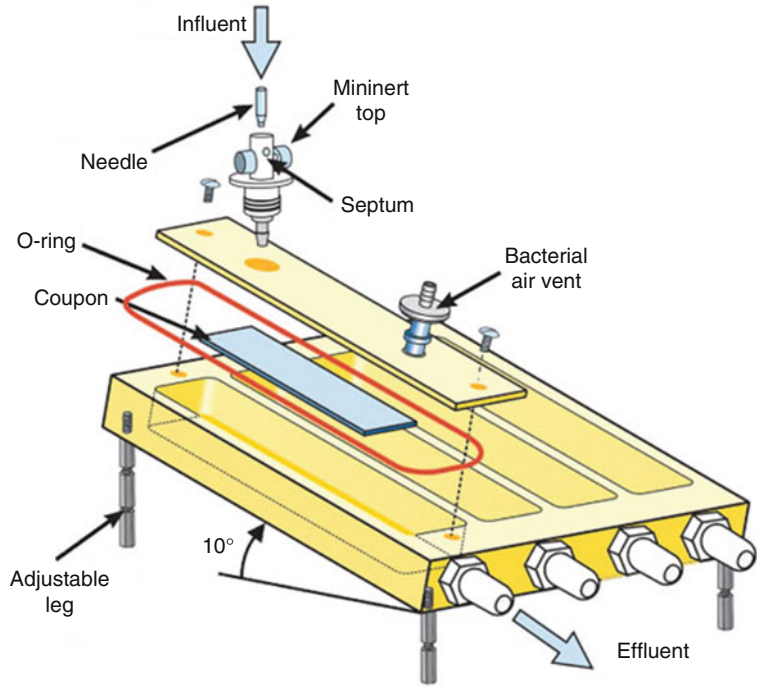

Fig. 9.3 Schematic diagram of a drip flow reactor [33, 34]

inserted so that when in place, the end of the peg forms part of the wall of the tube [13]. In a typical experiment, the MRD is filled with a suspension of microorganisms and is flipped over to improve the adhesion of the planktonic cells to the disks [14]. After the adhesion phase, the pump is started to allow a continuous flow of the growth medium and biofilm development on the disks. The advantage of this system is that the biofilms can be sampled by removing plugs at any time, once they are replaced to maintain a closed system. This model was established as an appropriate biofilm model for susceptibility testing of oral microorganisms [32].

Drip-Fed Biofilm Model

Among drip-fed biofilm models, drip flow biofilm reactors are a simple sort, in which biofilms are grown on angled slides continuously irrigated with small volumes of (inoculated) media, thereby providing a low-shear environment with dispersive mixing (Fig. 9.3) [14, 33, 34]. This system has been developed as an interproximal laboratory model to compare the potential effectiveness of powered brushing to remove biofilm plaque from interproximal spaces beyond the reach of bristles [35]. Different substrata can be used such as hydroxyapatite or teeth tissues. Care is required in experimental design and in sampling because medium flow over the surface of the slide may not be uniform and hence there may be significant aerial heterogeneity over the surface of the substratum [13]. In constant depth film fermenter (CDFF), biofilms are fed by the drip-wise addition of growth medium onto the turntable, and excess and/or spent medium flows downward through a waste outlet [13]. The biofilm developed on a surface is limited to a predetermined depth by mechanically removing excess biofilm, a situation mimicking the movement of the tongue over the teeth [14]. In this system, 200 or 300 μm is most commonly used for maintenance of dental plaques [13]. This system can develop single-species biofilm, defined consortium biofilm, or microcosm biofilm to evaluate antibacterial agent and explore the etiology of dental caries [36, 37].

Perfused Biofilm Fermenters

Perfused biofilm fermenters (PBF) are constructed such that nutrients are supplied by continuous perfusion of growth medium, which is pumped through the substratum (a permeable

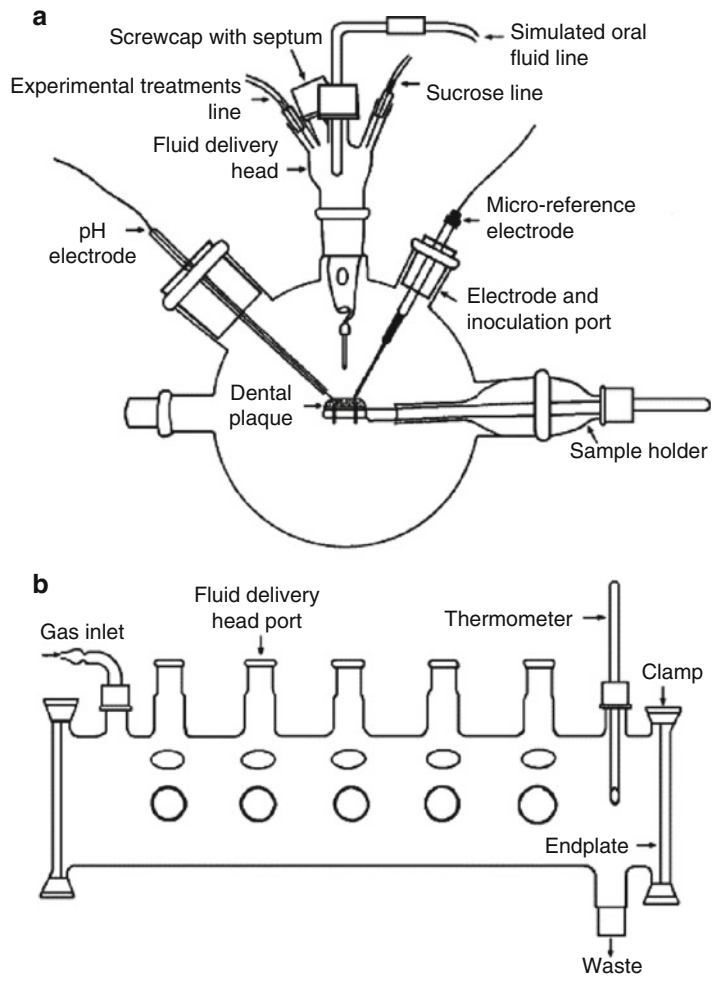

Fig. 9.4 Artificial mouth [39, 40]. (**a**) Cross-section of biofilm growth station, (**b**) longitudinal section of culture chamber

membrane) and hence through the biofilm [13]. The media flow can be accurately controlled, the growth rate of the biofilm can also be well modulated, and dynamic steady states can be achieved. The multiple Sorbarod device (MSD) is one kind of PBF which is proved to be an ideal system to grow oral biofilms by McBain and co-workers. It uses a simple two-piece stainless steel housing, yields relatively large amounts of biomass, and enables continuous monitoring of population dynamics through the analysis of perfusates (spent culture fluid) [38]. It has been validated for the maintenance of complex salivary microbial ecosystems and for the in vitro reproduction of interindividual variation within oral microbiotas. Disadvantages of MSD are related to the development of heterogeneous biofilms [13].

Artificial Mouth

Artificial mouth models mimic the in vivo oral niches and habitats to act as a laboratory "microcosm" [39]. It consists of a vessel in which a surface (or surfaces) is inoculated and supplied with a continuous or intermittent nutrient supply; during such experimental procedures, real-time growth and development of dental plaque/biofilm can be investigated (Fig. 9.4) [3, 39, 40]. This system has progressed from simple and basic apparatus to the currently available, highly sophisticated, computer-controlled, multi-station artificial mouth systems. The advanced multiple artificial mouth (MAM) system which was developed by Sissons and his co-workers could be employed for the long-term growth of multiple plaque samples within a standardized, simulated

oral environment generated by computer-controlled facilities [40]. The environment and the biofilm pH range can be controlled and manipulated, and oral fluids and periodic pulses of sucrose to model meals were simulated. A chemically defined saliva-like oral fluid analogue is named defined medium with mucin (DMM), which can work well as a "saliva substitute" [41]. Plaque samples grown over several weeks in this system exhibited metabolic behavior and pH profiles typical of natural plaque. It was possible to analyze aliquots during plaque development without contaminating the mature samples [40]. These current applications contain evaluating microbial interactions in simulated dental plaque and similar biofilms and monitor their physical, chemical, biological, and molecular features to a very high degree of accuracy, evaluating potential antimicrobial agent [40, 42]. Besides, several artificial mouth models have been established to study factors influencing the development of primary carious lesions and evaluate caries-preventive agents, which will be represented in "microbial-based mineralization model" part in detail.

Chemostat

Chemostat can provide a homogeneous liquid environment for microbial growth, under highly defined and controllable conditions [3]. Bacteria can be grown at fixed growth rates, enabling single parameters to be varied independently so that true cause-and-effect relationships can be established; the culture can also be sampled repeatedly, facilitating statistical analysis. However, if excessive bacteria grew in chemostat, operational problems may arise from blocked tubing [3]. Prevention of wall growth (change of cells from planktonic to biofilm forms adhering to the chemostat vessel) is also very important, which will cause deviations from "steady-state" growth [43]. Pure culture and mixed culture can both grow in chemostat systems. Marsh developed a consortium, composed of nine bacteria to investigate carbohydrate pulses and pH on population shifts within oral microbial communities [44] and later focus on pH-driven disruption [17]; both of the two studies are very important to his "ecological plaque hypothesis." The control of pH at neutral values during sugar pulsing was possible only with this type of laboratory model; this experiment could not have been performed in animal or human model studies because of the inevitable pH change when carbohydrates are metabolized [3]. The applications of chemostat for the usual microbiology laboratory are costly and have space considerations. A lot of medium are needed, especially when multiple parallel cultures are needed. Nowadays, some modifications are developed to alleviate the hurdles [43]. For classic chemostat, one of the shortcomings is that the organisms are not in a biofilm; however, recently, there are some modifications for chemostat to involve relevant surfaces to form biofilms. Sterile hydroxyapatite rods or disk can be inserted into chemostat head and immersed in steady-state planktonic cultures for certain periods [45]. Chemostat has also been linked to flow cells to form biofilm. S. Herles and co-worker developed a chemostat flow cell system (Fig. 9.5), in which the chemostat was provided with a continuous source of five species of oral bacteria grown in an artificial "saliva-like" medium. This mixture was pumped through six flow cells, each containing two types of surfaces in which plaque formed, and was subsequently used to compare the antiplaque agents [46]. Furthermore, chemostat can also be used to inoculate a novel biofilm generating model system, the thin film fermenter, in which biofilms of a predetermined depth can be generated, of which a key feature is that biofilms of a predetermined depth can be generated [3].

9.1.3 Microbial-Based De- and Remineralization Model

The use of microbial-based de- and remineralization model permits more clinical relevant in vitro investigations of dental caries etiology and the properties of caries-preventive agent. In this system, primary caries, secondary caries, pit and fissure caries, root caries, and so on can be developed. Batch culture techniques can be used, and continuous culture techniques were also applied in this system, during which "artificial mouth" was generally involved.

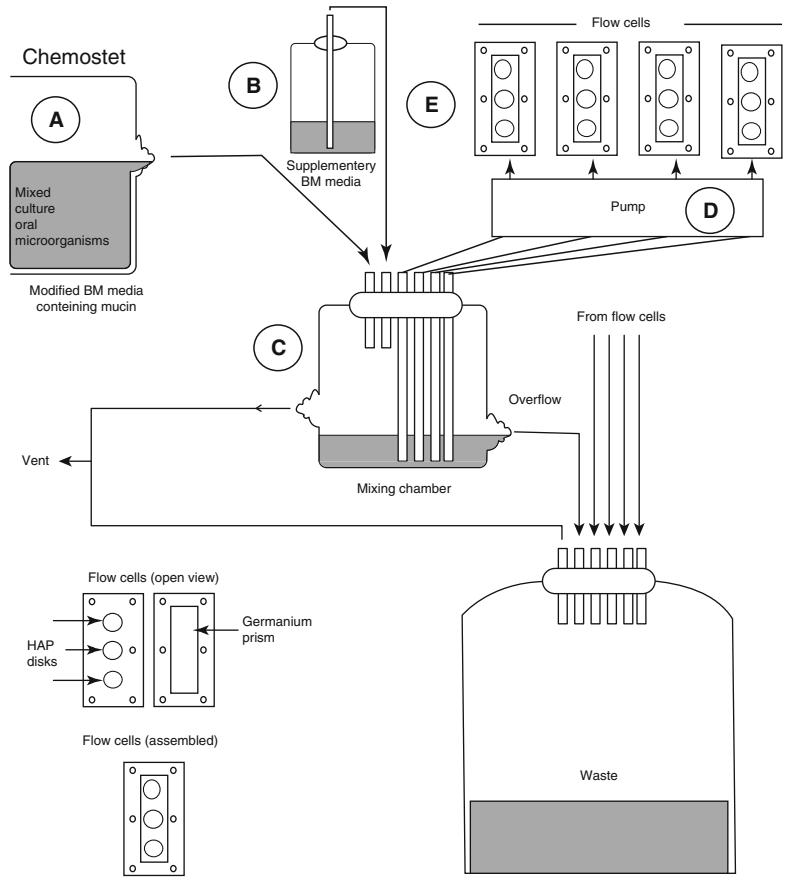

Fig. 9.5 Chemostat flow cell system [46]. *A* Chemostat containing a mixed culture of 5 oral bacteria, *B* Vessel containing a supply of supplementary BM medium (without glucose), *C* The mixing chamber containing the flow from A (1 ml/min) plus 5 ml/min from B, *D* The pump supplying flow (1 ml/min) from the chemostat to 6 low cells (1ml/min), *E* Flow cells containing the HAP disks and germanium ATR prisms

In an in vitro secondary caries model, the occlusal surface of the tooth was sealed with a conventional resin-based fissure sealant; during the process, certain part of the tooth was slightly moistened (contaminated) with saliva to produce marginal gaps, the teeth were infected with *Streptococcus mutans* in artificial mouth, and the secondary caries can be developed in contaminated regions [47]. This model can also be used to study the relationship of gap size and secondary caries, in which gaps were created by inserting shim stocks with different thickness at the tooth/resin interfaces and *Streptococcus mutans* was also used as inoculum [48].

Katz and his colleagues developed a pit and fissure caries in artificial mouth. They selected extracted human premolars and molars and inoculated pit and fissures with a concentrated *Streptococcus mutans* inoculum, which was overlaid with a nutrient layer of 15 % agar, 15 % glycerin, and 5 % sucrose. A filter paper and a thin layer of collodion were placed over the artificially created plaque. These specimens were incubated at 37 °C and continuously washed with artificial saliva (pH neutral) at a regular rate. The acrylic blocks with the tooth specimens were removed daily, and 0.24 % sodium fluoride dentifrice was applied for 3 min with a toothbrush. The latter procedure was repeated 5 days a week, and the whole experiment was conducted for 8 weeks. Subsequently, stereomicroscopic evaluations revealed that the artificial lesions were very similar to those of natural pit and fissure caries. This in vitro caries model contributed somewhat to the study of pit and fissure caries including the study of remineralization due to agents incorporated in toothpaste [40].

When root tissue (disks) was used and putative root-caries pathogens were used, root caries can be modeled in the bacterial system. M. Shu

and colleagues developed such root caries model using four putative root-caries pathogens, *Streptococcus mutans*, *Streptococcus sobrinus*, *Actinomyces naeslundii*, and *Lactobacillus rhamnosus* [20]. Also, saliva can also be used as inoculum to develop the root caries [49].

9.2 In Situ Model in Caries Research

In situ caries models involve the use of appliances or devices which create defined conditions in human mouth to simulate the natural process of dental caries. These models attempt to provide clinically relevant information in a relatively short period without causing irreversible tissue changes in the natural dentition [50]. The advantages of in situ caries model systems compared with clinical trials include [1, 51]:

1. They have fewer ethical and logistical problems.
2. They are less costly.
3. Experimental design can be more flexible, allowing hypotheses to be tested.
4. The results are acquired in much shorter time.
5. Better control with the study subjects and better compliance.

Compared with in vitro caries models, in situ caries model is complicated by dietary eating habits, the presence of physiologically secreted saliva, plaque of varying composition and thickness, and a pellicle-coated tooth surface [50], which make it more close to oral cavity than in vitro caries models.

Compared with animal caries model, in situ caries models are conducted in human beings, while animal caries models are conducted in animals. Due to so many differences between animals and human beings, some of the results from animal caries models may not extrapolate to human beings.

The disadvantages of in situ caries model are still obvious:

1. The number of subjects in in situ caries model is generally limited. Whether the small study population can represent the general population is raised as a question.
2. The validation of the studies is generally heavily dependent on compliance of the test subjects.

By using in situ caries models, we have the potential to study both fundamental aspects of the caries process, such as to form artificial carious lesions [52], to study de- and remineralization, and to obtain dental biofilm directly in the human mouth [53], as well as more applied research problems such as the effect of food on dental caries and the role of caries-preventive agent in caries prevention [54, 55] in human subjects without actually causing caries in the natural dentition [50]. In situ study designs are highly variable, with models using different hard tissue substrates, a variety of intraoral sites, different exposure periods, the inclusion or exclusion of participants' diet, the use of different depth recesses and gauze to encourage plaque retention, the use of mesh to protect surface from mechanical disturbance and allow plaque accumulation, and the use of various mineral quantification methods [52, 56].

Both enamel and dentin can be used as hard tissue substrates. For enamel, both the natural enamel surface, which is more suitable for formation of dental plaque biofilm [57], and enamel slab (pieces of extracted teeth), which is suitable for mineralization study [54] to make similar baseline, can be used.

When the in situ caries model was used to assess de- and remineralization, enamel specimens were more frequently used than dentin; however, dentin is useful to simulate root caries. In remineralization study, it is better to produce standardized demineralized lesions in laboratory firstly. These lesions can be sectioned to provide one half lesion for the intraoral appliance and the other half used as the baseline lesion control [56].

However, the outcome of in situ caries models may differ substantially depending on their design, and therefore, the choice of model may significantly influence the conclusions drawn from such studies [58]. For example, the microbial composition of plaque will vary among different teeth in the same mouth, on the same tooth

in different people, and even on different surfaces on the same tooth. So the selection of patients for in situ caries model should be carefully done. It makes sense to recruit subjects with similar salivary flow and buffer capacity, with desired microbiological pattern, and even with a preferred immunological profile [3]. Also, plaque that forms with the aid of gauze does not fully resemble "natural" plaque, in either structure or microbial composition [59].

9.2.1 Classification of In Situ Models

In 1990, Wefel grouped in situ model systems into three general types: removable appliances, single-section models, and banding models [60]. Nowadays, some new models were developed, and some models were less used; in situ caries models can be roughly divided into two categories: removable appliances and fixed appliances, in terms of the mobility characteristic.

9.2.1.1 Removable Appliances

Removable appliance is the widely used in situ caries model which can be constituted with acrylic appliances or denture and hard tissue substrates. The model can be exposed extraorally to the challenge, or to a therapeutic regime, to decrease variability in individual mouths. This kind of treatment method can permit the testing of agents or procedures which might be harmful to the natural dentition or ethically unacceptable [61]. If dietary challenges were not considered or oral hygiene is not the influence factor, the model can be taken out of the mouth when subjects are eating or carrying out oral hygiene, to give a high degree of control and to diminish compliance. In an in situ artificial dentin carious lesion study, acrylic palatal appliances containing two bovine dentin specimens, protected with a plastic mesh to allow biofilm development, were involved. The volunteers dripped a 20 % sucrose solution on each specimen four times a day for 14 days; finally, the in situ model produced a deep lesion with a high R value but with a thin surface layer [52]. A removable appliance with three 200-μm-wide grooves cut into bovine dentin disks was used to accumulate plaque, which was later treated with chlorhexidine. Then the in situ plaque with and without chlorhexidine treatment can be well investigated to extrapolate the similar conditions in oral cavity [53].

9.2.1.2 Fixed Appliances

Fixed appliances in in situ caries model are the appliances that can only be removed in the end of the study. They have various forms: banding model such as orthodontic band model which can develop orthodontic non-cavitated (white spot) lesion and can produce a plaque accumulation niche for demineralization. The crown single-section model relies mainly on placement of the sections in plaque-retentive areas below contact points [60]. The enamel can even be bonded to the teeth directly to collect natural plaques on the natural enamel surface [57]. Orthodontic non-cavitated (white spot) lesion models have been used regularly for testing the efficacy of novel remineralization agents; however, this approach cannot be extrapolated to model other types of non-cavitated lesions, for after removing the band, the lesion will not continue to develop [62]. A special in situ caries model is that non-cavitated lesions formed under plaque retention sites on the teeth that are destined for extraction, and the effects of remineralization agents can be tested [56].

9.3 Animal Model in Caries Research

In 1995, the US Food and Drug Administration (FDA) issued the Anticaries Drug Products for Over-the-Counter (OTC) Human Use final rule, establishing the requirement that "all OTC anticaries dentifrice drug product formulations be tested in the animal caries reduction test" [12].

Animal caries models have a long history of successful use in caries research. They are invaluable tools to simulate the natural progression of caries under true biological conditions. Unlike in situ and in vitro systems, which measure isolated components of caries process, animal caries models truly measure caries [4]. Animal caries

model has played a critical role in caries research due to its unique advantages. Compared with in situ caries model and in vitro caries model, it is the closest caries model. The whole saliva is present, and it can provide components of the host defenses and simulate more accurately the clearance of test compounds [3]. However, there are also limitations, including differences on the composition of the oral flora and dental plaque, eating habits, saliva, food retention, dentition, and the morphology of the teeth [2, 50]. It may be difficult to inoculate and establish some human bacterial strains in animals, while the pattern of caries in rodents is different from that observed in humans.

Various experimental animal species have been used in animal caries model, including non-human primates, rats, hamsters, and mice, during which the rodent models are the commonest. The development of genetically modified animals makes utility of the model more broadly. With respect to the general considerations in animal caries models, William H. Bowen has a very excellent review [29]. In short, several important considerations should be paid attention to: selection of animals, litter effect, age of animals, sex, caging of animals, and diet. When those considerations are deliberately under control, which makes the baseline the same, the animal caries model may show the effect brought by the experimental factors. Animal caries models are very valuable to study the etiology of dental caries and evaluate the anticaries products.

9.3.1 Study on Etiology of Dental Caries

Animal caries models can be used to evaluate the cariogenicity of diet. The first recorded use of rats in caries research was published in 1922 by McCollum et al., who was primarily interested in the role of diet in the etiology of dental caries. Most of the early studies were focused on the influence of diet [29]. Nowadays, some special food was still evaluated by animal model. For example, cariogenicity of milk and formula was compared in Wistar rat. Sucralose, a sugar substitute, was found to have low cariogenicity in Sprague-Dawley rat. In SPFOM rat, high-glucose diet was found to have higher cariogenic capacity than pure starch [63].

As the inherent difference in the dentition between animals and humans, the host factors investigated in animal caries models mainly focus on the saliva factor. Data from the animal models, especially desalivated animal models, have enhanced our understanding of the importance of saliva in maintaining oral health. The incidence of caries increased significantly in rats which had their salivary glands removed surgically [29]. Conventionally, rats can be desalivated partially or completely by surgery to test the role of saliva in the development of caries or to make caries formation faster. An alternative strategy is to genetically modify the test animal system to exclude the host factor of interest. In mice with targeted deletion of the gene encoding aquaporin-5 (*Aqp5*$^{-/-}$), a water channel involved in the production of saliva, there was a significant increase in caries, indicating that caries susceptibility increases with a reduced salivary flow that is associated with decreased water content of saliva [64]. The interaction of bacteria and host can also be evaluated in genetically manipulated rats. In NOD/SCID.*e2f1*$^{-/-}$ mice that show hyposalivation, lower salivary antibody, and an extended life span compared to the parent strain, the roles of several salivary components in *Streptococcus mutans* colonization in mice were evaluated, suggesting that there are multiple effects exerted by sIgA in *Streptococcus mutans* colonization, with synergistic effects evident under the condition of sIgA and limited nutrients on colonization in NOD/SCID.*e2f1*$^{-/-}$ [65].

Comparing the lesion formation in germfree and conventional rodents can make sure the role of microorganism in dental caries, and animal caries models are a good tool to study the microbial etiology of dental caries. In addition, animals can be inoculated with mutants of cariogenic bacteria that lack putative virulence traits or have putative anticaries traits, so that this type of model can be valuable in determining factors involved in the pathogenesis of caries [3]. A strain of *Streptococcus mutans*, which lacks

urease, was genetically engineered to express the urease genes of *Streptococcus salivarius*. Rats were infected with the parent strain or the alkali-producing *S. mutans* and fed a cariogenic diet supplemented with urea. The rats infected with the recombinant strain had a dramatically lower incidence and severity of all types of caries compared with controls, showing that alkali generation inhibits caries [66]. Furthermore, the development of transgenic animals together with genetic manipulation of microorganisms will facilitate the utility of the model greatly and may lead to development of novel approaches to the prevention of disease [29].

9.3.2 Evaluate Anticaries Agent

The benefits of animal caries models also lie in their role in evaluating fluoride and antimicrobial compounds and vaccination on plaque formation and caries development. It has been pointed out that no caries-protective agent currently in human use has failed in a rodent test. However, it has been argued that the experimental conditions in animal caries model may be too severe to mimic the condition in human being, for example, a 56 % sucrose diet with ad libitum feeding, and infection with *S. sobrinus*, which is particularly adapted to colonizing and causing caries on smooth surfaces. The outcome is that some promising agents may be incorrectly discarded [3].

Animal caries models have been demonstrated to be suitable to evaluate the caries-preventive effect of fluoride, for those reasons: These models develop incipient and more advanced coronal caries which resemble clinical caries structurally and etiologically, and the response of animal caries models to fluoride demonstrates dose responses [2].

There are several models that have been used to evaluate the efficacy of fluoride-containing dentifrices, such as Francis' hypomineralized area (HMA) model, Gaffar's CARA rat caries model, Connecticut rat caries model, and Indiana rat caries model. G.K. Stookey and co-workers have reviewed those models in detail [2]. Shortly speaking, HMA model is designed to assess the impact on incipient enamel lesions, while the other three models utilize more overt caries lesions. HMA model relies upon an indigenous flora; the other three models involve the infection of the animals with cariogenic microorganisms.

It is also possible to develop animal caries models to evaluate non-fluoride antimicrobial agents. As antimicrobial agents utilize different mechanism with fluoride, the former mainly work on microorganism, and the latter mainly work on mineralization. It is wise to modify the animal caries model.

9.4 The Role of Saliva in Caries Models

The periods of alkalinization in dental biofilm, which promote remineralization and restore the integrity of the enamel, are primarily attributable to diffusion of acids from the biofilms, buffering by salivary bicarbonate, salivary peptides, bacterial cells, and bacterial metabolism of urea and arginine [66]. In this process, the role of saliva is very important. This role is also demonstrated by a lot of clinical evidence.

In animal caries model and in situ caries models, in which the whole saliva is present, salivary factor is inevitably involved, while desalivated animals (salivary glands are partially or completely removed or drug-induced hyposalivation) can mimic the extreme cariogenic challenge commonly observed in patients suffering from salivary hypofunction [64]. Also, current techniques to alter gene expression in animals allow direct analysis of the saliva in caries development [64].

In in situ caries models, it is well realized that salivary composition and flow differ at different sites, which may lead to fluctuating results. So it is better to put the model in the same dentition site, even if there are still some differences among different individuals. To overcome this problem, it is better to recruit subjects with similar salivary flow and buffer capacity. Those are all important considerations in designing in situ caries study.

Furthermore, the role of fluoride in saliva is also well realized. Fluoride may reach saliva directly from the ingesta or from topical

treatments, or indirectly from the bloodstream via the salivary glands or gingival crevicular fluid, or from temporary intraoral reservoirs of fluoride, including surface deposits on the teeth of calcium fluoride-like material and dental plaque [67]. After local application of fluoride and initial rapid clearance phase, the saliva can have a low concentration of fluoride over long periods of time, which is as important as a brief exposure to relatively high fluoride concentrations for shorter periods of time [68]. So it is necessary to consider this factor related to saliva, when evaluating the caries-preventive role of fluoride in caries model. Then another question is also raised, whether other caries-preventive agents have their "reservoirs" in oral cavity.

However, in in vitro caries models, the role of saliva was not always emphasized. In some cases, the effect of saliva was objected, for the reason that caries lesions form only in "stagnant" sites where the benefit of saliva is not working and thus that models where salivary influences are excluded will best represent the conditions of caries lesion formation [67].

On the other hand, there are some models focusing on the saliva's role in the oral cavity and further on its role in dental caries, such as saliva-plaque interface, salivary clearance of bacterial substrates, fluoride, and acid [69, 70]. The studies of the Stephan curve showed that if salivary stimulation is mimicked, the rate of pH rise in a model plaque is highly dependent upon the bicarbonate concentration and the velocity of the film of saliva in both thick and thin plaques, indicating that salivary benefits can be exerted even with thick plaques [67].

Even in caries models considering the effect of salivary factor, saliva tends to be considered more as a nonspecific diluent or sink rather than as a fluid with a complex chemistry that may interact with the plaque and the teeth in significant ways [67]. Saliva possesses an array of activities that appear to have been seldom considered to in many caries models. However, those bioactivities should not be ignored as the following facts.

Firstly, saliva has some properties regarding de- and remineralization of hard tissue of teeth, such as the saturation of calcium and phosphate ions, calcium-ion binding by salivary macromolecules, and some precursors for the adsorbed protein films or pellicles, found on teeth surfaces, which have a significant effect on the interactions of dental mineral with overlying fluids, particularly with respect to diffusion rates of acids into and calcium and phosphate ions out of the enamel [71].

Secondly, it is well demonstrated that alkali generation from salivary substrates, especially arginine (or polypeptides and proteins) and urea, could play major roles in plaque pH homeostasis and in the inhibition of dental caries [66]. Arginine and urea can be secreted by salivary glands, and polypeptides and proteins containing arginine residue belong to saliva proteins. Interestingly, the presence of saliva itself will favor the base formation and then the pH rise [72]; the reason may be attributed to its buffer capacity [26].

Thirdly, salivary proteins adsorbed onto surfaces of apatitic minerals profoundly affect bacterial adhesion onto those surfaces; those effects may significantly influence the initial bacterial colonization of teeth and, therefore, the microbial nature of dental plaque.

Fourthly, there are some antibacterial systems in saliva which contain sialoperoxidase, lysozyme, lactoferrin, histatins, peroxidases, and other basic polypeptides, which have less specific antibacterial effect and also other bioactivities.

Collectively, in oral cavity, saliva plays a crucial role in the initiation and progression of dental caries, chemical and physiological process of de- and remineralization of the teeth, dental biofilm formation and metabolism, clearance, buffer and neutralization of acid, and so on. As a result, in dental caries model, saliva should not be ignored.

In summary, much of our present understanding of the etiology and initiation and progression of dental caries as well as the identification of caries-preventive agents or measures is attributed to the findings of studies on models. However, no single, ideal model is optimal for studying all aspects of caries, and different models have specific roles in studying specific aspects. They have their advantage and disadvantage from both

experimental design and experiment cost. Models must be modified or refined to achieve the desired goals.

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Index

A
Academic Centre for Dentistry Amsterdam (ACTA), 136
Accessory canals, 16
ACFP. *See* Amorphous calcium fluoride phosphate (ACFP)
Acid-etching technique, 107, 108
Acidogenic bacteria
  acid tolerance of, 40
  Actinomyces, 39–40
  carbohydrate metabolism and, 39
  Lactobacilli, 39
  Streptococcus mutans, 39
ACP. *See* Amorphous calcium phosphate (ACP)
Actinomyces
  A. actinomycetemcomitans, 37
  acidogenic bacteria, 39–40
  A. naeslundii, 37, 40, 47
  A. oris, 32
  arginine deiminase system, 42
Acute lymphoblastic leukemia (ALL), 48
ADA caries risk assessment, 65–67
Adhesive dentistry, 107
ADS. *See* Arginine deiminase system (ADS)
Aggregatibacter actinomycetemcomitans, 29, 139
Agmatine deiminase system (AgDS), 42
Air abrasion, 111–112
Air polishing, 112
Alkali production
  ADS, 42
  AgDS, 42
  and biofilm ecology, 42–43
  in caries prevention, 41
  clinical relevance of, 43
  urease, 40–41
ALL. *See* Acute lymphoblastic leukemia (ALL)
Ameloblastin, 8
Amelogenin, 7–8
Amelotin, 8
Amorphous calcium fluoride phosphate (ACFP), 77
Amorphous calcium phosphate (ACP), 77, 79, 115
Animal model
  de-/remineralization, 74
  in research, 167
    anticaries agent evaluation, 169
    etiology study, 168–169
    saliva role, 169
Anticaries agent, animal model, 169
Antimicrobial approaches
  chlorhexidine, 44
  fluoride, 44–45
  natural products, 46–48
  phenolic antiseptics, 46
  quaternary ammonium compounds, 45
  triclosan, 45–46
  xylitol, 46
Antimicrobial substances, in saliva, 50
Apical periodontitis, 141
Arginine deiminase system (ADS), 42
ART. *See* Atraumatic restorative technique (ART)
Arteriosclerotic vascular disease (ASVD). *See* Atherosclerosis
Artificial mouth
  open system microbial models, 163–164
  Streptococcus mutans in, 165
Atherosclerosis, 136–138
  Streptococcus mutans and, 138–139
Atopobium, 43
Atraumatic restorative technique (ART), 115

B
Bacteremia, 131–132
Bacterial biofilm
  concept and discovery, 27
  extracellular polymeric substances, 28
  formation, 28–29
  survival advantages of, 29–30
Bap family. *See* Biofilm-associated-protein (Bap) family
Bell stage, 3
Bicarbonate, 61
Bifidobacterium spp., 49
  B. dentium, 43
Biofilm-associated-protein (Bap) family, 29
Biofilm ecology, alkali production and, 42–43
Biofilm model
  drip-fed, 162
  flow cell, 161–162
  systems, 160–161
  TSP, 160
  Zürich biofilm model, 160

Z. Xuedong (ed.), *Dental Caries: Principles and Management*, DOI 10.1007/978-3-662-47450-1

Biological treatment methods, 127–128
Biomineralization, 80
Bionics, and restorative dentistry, 128
Bis-GMA system, 105
Bitewing radiography, for caries detection, 99
Black, G.V., and restorative dentistry, 107–108
B lymphocytes, 20
Body mass index (BMI), 135–136
British Medical Journal (BMJ), 137
Bud stage, 2

C
Calcium fluoride particles, 79
Calcium phosphate (CaP), 77
  particles, 79
  stage, 2–3
Camellia sinensis, 47
Candida albicans, 159
CaP. *See* Calcium phosphate (CaP)
Carbohydrate metabolism, 39
Carbonate hydroxyl apatite nanoparticles, 79
Cardiac dysrhythmias, 138
Cardiovascular disease (CVD), 136–140
Care Index, oral diseases, 91
Caries-associated bacteria, 65–66
Caries pharmacotherapy, 114
Caries-preventive strategies
  dental plaque control, 102–103
  pit and fissure sealing, 105
  preventive resin restoration, 105
  primary prevention, 98
  reinforce tooth resistance to acid, 103–105
  secondary prevention
    conventional caries detection methods, 98–99
    DIAGNOdent, 100
    electrical caries monitor, 101
    FOTI, 99
    QLF, 100–101
  sugar restriction and substitute for, 103
  tertiary prevention, 101–102
Cariogenic bacteria
  logistic regression analysis, 138
  oral cancer, 134
Casein phosphopeptide (CPP), 115
  CPP-ACFP, 77
  CPP-ACP, 77
Cavity preparation
  minimally invasive treatment
    air abrasion, 111–112
    air polishing, 112
    atraumatic restorative technique, 115
    caries pharmacotherapy, 114
    chemomechanical caries removal, 112–113
    enameloplasty, 114
    lasers, 112
    mechanical rotary technique, 113
    microscopic preparation techniques, 113
    nonmachinery preparation, 111–113
    pit and fissure sealing, 113–114
    preventive resin restorations, 114
    remineralization treatment, 114–115
    slot preparation, 113
    tunnel preparation, 113
  resin composite, 121–123
CBP. *See* Collagen-binding protein (CBP)
CDC. *See* Center for Disease Control (CDC)
CDFF. *See* Constant depth film fermenter (CDFF)
Ceanothus americanus, 47
Cell-poor zone of pulp, 17
Cell-rich zone of pulp, 17
Center for Disease Control (CDC), 142, 145
Cetylpyridinium chloride (CPC), 45
CHD. *See* Coronary heart disease (CHD)
Chemical models, in research, 157–158
Chemomechanical caries removal (CMCR), 112
Chemoparasitic theory, 33, 34
Chemostat
  flow cell system, 165
  open system microbial models, 164
Childhood, dental caries, 134–136
Chlorhexidine, 44
Chronic apical periodontitis (CAP), 137–138
Chronic kidney disease (CKD), 143–144
Chronic obstructive pulmonary disease (COPD), 149
Chronic renal disease, 143–144
Circumpulpal dentin, 12
CKD. *See* Chronic kidney disease (CKD)
Clin Oral Investig in 2011, 136
Closed system microbial models, in research, 159–161
CMCR. *See* Chemomechanical caries removal (CMCR)
Cold and hot irritation test, 86–87
Colitis ulcerosa. *See* Ulcerative colitis (UC)
Collagen-binding protein (CBP), 139, 145
Community Periodontal Index (CPI) of Treatment Needs (CPITN), 91
Competence-stimulating peptide (CSP), 38, 50
Confocal laser scanning microscopy (CLSM), 75
Connective tissue fibers, 15–16
Constant depth film fermenter (CDFF), 73, 162
Contact-microradiography. *See* Transsal microradiography (TMR)
Coronal caries, cross-sectional analysis of, 142
Coronary heart disease (CHD), 136, 140–141
CPC. *See* Cetylpyridinium chloride (CPC)
CPP. *See* Casein phosphopeptide (CPP)
Craniofacial tissues embryology
  head formation, 2
  neural crest, 1–2
  origin of tissue, 1
C-reactive protein (CRP), 138
CSP. *See* Competence-stimulating peptide (CSP)
CVD. *See* Cardiovascular disease (CVD)
Cytodifferentiation, enamel development, 3
  maturation stage, 5
  presecretory stage, 4
  secretory stage, 4–5

D
Decayed, missing, and filled teeth (DMFT) index, 92–97, 133, 142
  5-year-olds, 95
  12-year-olds, 93, 95–96
  35–44-year-olds, 94, 96–97
  65–74-year-olds, 97–98
  salivary immunoglobulin A, 142
Demineralization models, caries research, 158
Dendritic cell, in pulp, 19–20
Dental caries
  5-year-olds, 95
  12-year-olds, 95–96
  35–44-year-olds, 96–97
  65–74-year-olds, 97–98
  assessment, 133
  burden in China, 94–98
  detection methods, 98–101
  DMFT index, 92–97, 133, 142
  Nepal example, 91
  prevalence, 92, 133
Dental drilling, 110
Dental enamel, 71
Dental floss examination, 87
Dental fluorosis, 88
Dental microflora, 31
Dental plaque
  and bacterial biofilm, 30
  as cause of dental caries, 33–34
  composition, 31
  control, 102–103
  ecological plaque hypothesis, 34–35
  nonspecific/specific plaque, 34
Dentin
  bonding, 120–121
  mineralization, 12
  repair, 13
  sclerosis, 12–13
  structure, 9–12
  types, 12
Dentinal tubules, 9–10
Dentin-enamel junction (DEJ), 7
Dentin phosphoproteins (DPP), 12
De-/remineralization
  biomineralization, 80
  calcium phosphate, 77
  CPP-ACFP, 77
  CPP-ACP, 77
  detection and measurement methods, 74
    CLSM, 75
    indentation techniques, 75
    micro-CT, 75
    OCT, 75–76
    QLF, 75
    TMR, 75
  dynamics process of, 72
  fluoride, 76
  investigations models, 72–73
    animal model, 74
    in situ model, 73–74
    in vitro biofilm model, 73
    in vitro chemical model, 73
  laser, 77–78
  nanoparticles, 78–79
  natural medicine, 77
Detection methods, dental caries, 98
  DIAGNOdent, 100
  electrical caries monitor, 101
  fiber-optic transillumination, 99
  quantitative light-induced fluorescence, 100–101
Diabetes mellitus (DM), 145–146
  epidemiological studies of, 146–147
  root caries, 147–148
  tooth loss and, 148–149
DIAGNOdent, 100
Diagnosis for caries
  cold and hot irritation test, 86–87
  dental floss examination, 87
  dental fluorosis, 88
  diagnostic cavity preparation, 87
  elastomeric separating modulus technique, 88
  electrical impedance technology, 87
  enamel hypocalcification, 88, 89
  enamel hypoplasia, 88, 89
  fiber-optic transillumination, 87
  glossiness and smoothness, 88
  inspection, 85
  lesion
    progress of, 89
    symmetry of, 89
  medium caries, 89
  percussion, 85
  predilection site, 88–89
  probing, 85
  quantitative laser fluorescence technique, 88
  radiographic examination, 85–86
  staining technique, 88
  ultrasonic technique, 87
Diagnostic cavity preparation, 87
Diet, 36, 103, 166, 168, 169
Digitized fiber-optic transillumination (DI-FOTI), 99
Direct bonding restoration technique, 118
DMFT index. *See* Decayed, missing, and filled teeth (DMFT) index
DPP. *See* Dentin phosphoproteins (DPP)
Drip-fed biofilm model, 162

E
Early childhood caries (ECC), 135
ECM. *See* Electrical caries monitor (ECM)
Ecological plaque hypothesis, 34–35
EDJ. *See* Enamel–dentinal junction (EDJ)
eDNA. *See* Extracellular DNA (eDNA)
Elastomeric separating modulus technique, 88
Electrical caries monitor (ECM), 16, 101
Electrical impedance technology, 87
Electron microscopy, 32

Embryology of craniofacial tissues
head formation, 2
neural crest, 1–2
origin of tissue, 1
Enamel bonding, 119–120
Enamel–dentinal junction (EDJ), 105, 125
Enamel development
cytodifferentiation, 3
maturation stage, 5
presecretory stage, 4
secretory stage, 4–5
enamel matrix proteins
ameloblastin, 8
amelogenin, 7–8
amelotin, 8
enamelin, 7
proteolytic enzymes, 8–9
tuftelin, 8
histogenesis and morphogenesis
bell stage, 3
bud stage, 2
cap stage, 2–3
microstructure
enamel lamellae and cracks, 6
enamel rod, 5
enamel spindle, 6
enamel tufts, 6
functional aspects, 6–7
interpit continuum, 6
Enamel hypocalcification, 88, 89
Enamel hypoplasia, 88, 89
Enamelin, 7, 8
Enamel lamellae and cracks, 6
Enamel matrix proteins
ameloblastin, 8
amelogenin, 7–8
amelotin, 8
enamelin, 7
proteolytic enzymes, 8–9
tuftelin, 8
Enameloplasty, 114
Enamel rod, 5
Enamel spindle, 6
Enamel tufts, 6
Endodontic inflammation, 140
Environmental factors, oral microbial ecology, 36
Environment dominates theory, 36
Epithelial cell rests of Malassez (ERM), 21
Erbium:yttrium-aluminum-garnet (Er:YAG), 77, 112
Essential oils, 47
Etching adhesive systems, bonding mechanisms, 118–119
Evidence-based dental caries diagnosis, 49
Extracellular DNA (eDNA), 28
Extracellular polymer substances (EPS), 28
Extracellular polysaccharides (EPS), 73

F

Featherstone pH cycling model, 158
Fertilization, craniofacial tissues, 1
Fiber-optic transillumination (FOTI), 87, 99
Fibronectin, 16
Film radiograph, for caries detection, 99
Fixed appliances, in situ caries models, 167
Flow cell biofilm model, 161–162
Fluorapatite, 109
Fluorescence, in dental caries, 100
Fluoride, 44–45, 76, 114
saliva role in research, 169–170
Focal infection, 129
Focal sepsis theory, 130
FOTI. *See* Fiber-optic transillumination (FOTI)
Fusobacterium, 36

G

Galla chinensis (GCE), 77
Gastroesophageal reflux disease (GERD), 144–145
GCF. *See* Gingival crevicular fluid (GCF)
Gelatinous microbic plaques. *See* Dental plaque
Genetic factors, oral microbial ecology, 36
GERD. *See* Gastroesophageal reflux disease (GERD)
Gerodontology, 138
Gingival crevicular fluid (GCF), 32
Ginkgo biloba, 47
Glossiness, and smoothness, 88

H

Head and neck cancer
tooth loss with, 134
treatment, 132–133
Head and neck squamous cell carcinoma (HNSCC), 133
Head formation, craniofacial tissues, 2
Hertwig's epithelial root sheath (HERS), 20–21
Histatins, 63
Histogenesis, enamel development
bell stage, 3
bud stage, 2
cap stage, 2–3
HIV, 142–143
HNSCC. *See* Head and neck squamous cell carcinoma (HNSCC)
Human microbiome project (HMP), 30
Human salivary lactoperoxidase (HS-LPO), 62
Human umbilical vein endothelial cells (HUVEC), 139
Hypomineralized area (HMA) model, 169
Hyposalivation, 132

I

IE. *See* Infective endocarditis (IE)
Immune system disease
effect on, 130
HIV, 142–143
salivary immunoglobulin A, 141–142
Indentation techniques, 75
Individualized treatment
importance, 125–126
risk evaluation, 126
technology and material, 126

Infectious disease, dental caries as, 33
Infective endocarditis (IE), 138
Inoculum, microbial models, 159
In situ model
de-/remineralization, 73–74
in research
classification, 167
disadvantages, 166
outcome, 166
saliva role, 169
Inspection of caries, 85
Interglobular dentin, 11–12
Interspecies interactions
and dental caries, 36–39
factors involved in, 37–38
metabolic interrelationship, 37
Intertubular dentin, 10–11
Intra-class correlation coefficients (ICCs), QLF, 101
In vitro biofilm model, de-/remineralization, 73, 74
In vitro chemical model, de-/remineralization, 73
In vitro models, in research
chemical models, 73, 157–158
microbial-based de-and remineralization model, 164–166
microbial models, 158–164
saliva role, 170

J
JAMA Otolaryngology–Head and Neck Surgery, 133

K
Kallikrein-4, 9

L
Lactic acid bacteria, 133
Lactobacillus, 31, 61, 66
acidogenic bacteria, 39
arginine deiminase system, 42
L. casei, 49
L. reuteri, 49
L. rhamnosus, 47
probiotics, 49
Lactoferrin, 63
Laminin, 16
Langerhans cells, 20
Lasers
cavity preparation, 112
de-/remineralization, 77–78
Lesion
control, 110
progress of, 89
symmetry of, 89
Light active killing, 50
Lymphatics, 16
Lymphocyte, in pulp, 20
Lysozyme, 62

M
Macrophage, in pulp, 19
MAM system. *See* Multiple artificial mouth (MAM) system
Mantle dentin, 12
Marsh Consortium, 159
Mast cell, in pulp, 20
Matrix metalloproteinase-20 (MMP20), 9
Maturation stage, enamel development, 5
Mechanical rotary technique, 113
Melaphis chinensis, 47
Mesenchymal stem cells (MSCs)
induction of differentiation, 21
in pulp, 20
Mesenchyme, 2
Metagenomics, oral microbiome, 48–49
Microbial-based de-and remineralization model, 164–166
Microbial ecology, in oral cavity, 35–36
Microbial models, in research, 158
classifications, 159
closed system, 159–161
inoculum, 159
open system
artificial mouth, 163–164
chemostat, 164
drip-fed biofilm model, 162
flow cell biofilm model, 161–162
modified robbins device, 161–162
perfused biofilm fermenters, 162–163
Micro-CT investigation, 75
Microindentation technique, 75
Microorganisms of the Human Mouth (Miller), 33
Microscopic preparation techniques, 113
Microtiter plate (MTP)-based system, 159–160
Mineralization of dentin, 12
Mini box preparation. *See* Slot preparation
Minimally invasive treatment, 108–111
cavity preparation
air abrasion, 111–112
air polishing, 112
atraumatic restorative technique, 115
caries pharmacotherapy, 114
chemomechanical caries removal, 112–113
enameloplasty, 114
lasers, 112
mechanical rotary technique, 113
microscopic preparation techniques, 113
nonmachinery preparation, 111–113
pit and fissure sealing, 113–114
preventive resin restorations, 114
remineralization treatment, 114–115
slot preparation, 113
tunnel preparation, 113
demineralized layer, 110–111
early diagnosis and personal treatment, 110
infected layer, 110
prevention and effective intervention, 110
treatment and effective control, 110
MMP20. *See* Matrix metalloproteinase-20 (MMP20)
Modified robbins device (MRD), 161–162

Morphogenesis, enamel development
bell stage, 3
bud stage, 2
cap stage, 2–3
Morphogenetic protein 4 (BMP4), 22
MSCs. *See* Mesenchymal stem cells (MSCs)
MSD. *See* Multiple Sorbarod device (MSD)
MTP-based system. *See* Microtiter plate (MTP)-based system
Mucins, 62
Multiple artificial mouth (MAM) system, 163
Multiple regression analysis, 95, 97
Multiple Sorbarod device (MSD), 163
Mutacins, 37
Myeloperoxidase (MPO), 62

N
Nanoindentation technique, 75
Nanoparticles, 78–79
National Health and Nutrition Examination Survey (NHANES), 147
Natural medicine, de-/remineralization, 77
Neisseria strains, 134
Nerve fibers, pulp, 14–15
Neural crest, 1–2
Notch signaling, for root development, 22

O
OCT. *See* Optical coherence tomography (OCT)
Odontoblast, 16–19
Olsenella, 43
Open system microbial models, in research
artificial mouth model, 163–164
chemostat, 164
drip-fed biofilm model, 162
flow cell biofilm model, 161–162
modified robbins device, 161–162
perfused biofilm fermenters, 162–163
Optical coherence tomography (OCT), 75–76
Oral biofilms, spatiotemporal development of, 31–32
Oral cancer, cariogenic bacteria and, 134
Oral cavity, 30, 130
Care Index, 91, 92
CPITN, 91
high-income industrialized countries, 91
microbial ecology in, 35–36
uneven distribution, 91–92
YLD/million population, 92
Oral fluids, 72
Oral haemophili urease, 40
Oral health, 129
epidemiological investigation in China, 94–98
global policies, 92–94
Oral Health in America: A Report of the Surgeon General, 129
Oral microbial ecology, 36
Oral microbiology, at early stage, 33
Oral microbiome, 48–49
Oral microbiota, 148
Oral microflora, saliva on, 62–63
Orthodentin, 12

P
PBF. *See* Perfused biofilm fermenters (PBF)
PCR method, 138
Pediatric Nephrology, 144
Percussion for caries, 85
Perfused biofilm fermenters (PBF), 162–163
Periapical diseases, 130
Periodontal disease, 129–130, 149
Peritubular dentin, 10, 11
"pH cycling" protocols, 158
Phenolic antiseptics, 46
Pit and fissure sealant, 105, 113–114
Plaque pH, salivary influences on, 61–62
Polymerization shrinkage, 123–125
configuration factor, 124–125
material related factors, 124
Porphyromonas gingivalis, 36
Postoperative sensitivity, 125
Predentin, 12
Predilection site, 88–89
Presecretory stage, enamel development, 4
Preventive resin restorations, 105, 114
Probing for caries, 85
Probiotics, 49–50
Proliferative period, 1
Propionibacterium, 43
Propolis, 47
Proteolytic enzymes, 8–9
Pseudomonas
P. aeruginosa, 29
P. fluorescens, 29
P. putida, 29
Pseudoramibacter, 43
Pulp, 13–14
accessory canals, 16
cells in
dendritic cell, 19–20
lymphocyte, 20
macrophage, 19
mast cell, 20
mesenchymal cell, 20
odontoblast, 17–19
pulp fibroblast, 19
connective tissue fibers, 15
fibroblast, 19
ground substance, 15–16
lymphatics, 16
morphologic zones of pulp, 16–17
nerve fibers, 14–15
proper, 17
vascular tissues, 14
Pulpal periapical diseases, 140–141
Pulpitis, 130
Pulpodentin complex
cells in dental pulp
dendritic cell, 19–20
lymphocyte, 20

macrophage, 19
mast cell, 20
mesenchymal cell, 20
odontoblast, 17–19
pulp fibroblast, 19
dentin
mineralization, 12
repair, 13
sclerosis, 12–13
structure, 9–12
types, 12
pulp, 13–14
accessory canals, 16
connective tissue fibers, 15
ground substance, 15–16
lymphatics, 16
morphologic zones of pulp, 16–17
nerve fibers, 14–15
vascular tissues, 14

Q
Quantitative laser fluorescence technique, 88
Quantitative light-induced fluorescence (QLF), 75, 100–101
Quaternary ammonium compounds (QACs), 45

R
RA. *See* Rheumatoid arthritis (RA)
Radiation caries, 132
Radiographic examination for caries, 85–86
Remineralization
models in research, 158
treatment, 114–115
Removable appliances, in situ caries models, 167
Resin composite bonding restoration technique
cavity preparation, 121–123
dentin bonding, 120–121
enamel bonding, 119–120
etching adhesive systems, 118–119
indications and contraindications, 121
polymerization shrinkage, 123–125
postoperative sensitivity, 125
postprocessing decoration, 123
requirements for, 121
self-etch systems, 119
technique sensitivity, 125
total-etch system, 118–119
Resin composites, 118
Respiratory infections, 149–150
Restorative dentistry, 107
based on bionics, 128
biological treatment methods, 127–128
Black, G.V. and, 107–108
bonding technique and, 108
direct bonding restoration technique, 118
individualized treatment, 125–126
minimally invasive treatment (*see* Minimally invasive treatment)
resin composite
cavity preparation, 121–123
dentin bonding, 120–121
enamel bonding, 119–120
etching adhesive systems, 118–119
indications and contraindications, 121
polymerization shrinkage, 123–125
postoperative sensitivity, 125
postprocessing decoration, 123
requirements for, 121
self-etch systems, 119
technique sensitivity, 125
total-etch system, 118–119
resin composites, 118
silver amalgam
cavity shape preparation, 116
controversy, 115–116
filling, 116–118
indications and contraindications, 116
Rheedia gardneriana, 48
Rheumatoid arthritis (RA), 142
Root caries
cardiac dysrhythmias, 138
diabetes, 147–148
Root development
ERM, 21
initiation, 20–21
mesenchymal stem cells, 21
signaling pathway
notch signaling, 22
SHH signaling, 22
TGF-β/BMP Signaling, 21–22
Wnt signaling, 22
tooth eruption, 22–23
16S rRNA gene sequencing, 131

S
SAG. *See* Salivary agglutinin (SAG)
Saliva, 73
carbonic acid/bicarbonate equilibrium in, 61
caries-associated bacteria, 65–66
characteristics and caries risk, 67
chemical and physical aspects, 68
composition, 59–60
critical value, 61
flow rate, 60–61, 65
formation and secretion, 59
low buffering capacity, 61
on oral microflora, 62–63
on plaque pH, 61–62
potential use of, 129
role in research
animal model, 169
fluoride, 169–170
oral cavity, 170–171
in situ model, 169
in vitro models, 170
xerostomia
etiology of, 63–64
management of, 64–65

Salivary agglutinin (SAG), 63
Salivary antimicrobial substances, 50
Salivary glands, 132
Salivary immunoglobulin A (SIgA), 141–142
Sanguinaria canadensis, 47
Sanguinarine, 47
Scardovia wiggsiae, 43
S-ECC. *See* Severe early childhood caries (S-ECC)
Secondary dentin, 12
Secretory IgA (SIgA), 62, 68
Secretory stage, enamel development, 4–5
Self-awareness, caries prevention, 98
Self-etch systems, 119
Severe early childhood caries (S-ECC), 135
SHH signaling, for root development, 22
Signaling pathway, for root development
  notch signaling, 22
  SHH signaling, 22
  TGF-β/BMP Signaling, 21–22
  Wnt signaling, 22
Silver amalgam, 115–116
  cavity shape preparation, 116
  controversy, 115–116
  filling, 116–118
  indications and contraindications, 116
Slackia exigua, 43
Slot preparation, 113
Smoothness, glossiness and, 88
Specifically targeted antimicrobial peptides (STAMPs), 50
Staining technique, 88
Stephan curve, 61
Streptococcus
  S. gordonii, 29, 38, 42, 161
  S. mutans, 31, 45, 61, 63, 66, 132
    acidogenic bacteria, 39
    acid tolerance of, 40, 42
    AgDS activity, 42
    in artificial mouth, 165
    atherosclerosis, 138–139
    in biofilm, 50, 73, 160
    catechins inhibit growth of, 47
    colonization in mice, 168
    colonization in saliva, 143
    ecological plaque hypothesis, 34–35
    genetically modified strain, 43
    in GERD group, 145
    inoculum, 165
    interspecies interactions, 37–39
    involvement, 34
    risk assessment, 110
    salivary immunoglobulin A, 141
    salivary level, 66
    SIgA against, 62
    STAMPs targeting, 50
    strain of, 168–169
    ulcerative colitis, 145
    xylitol, 46
  S. oralis, 32, 37, 161
  S. parasanguis, 42
  S. salivarius, 38, 40, 169
  S. sanguinis, 38, 132
    arginine deiminase system, 42
    interspecies interactions, 37
Stroke, 139–140
Survey of Dental Diseases, 140

T
T2DM. *See* Type 2 diabetes mellitus (T2DM)
Technique sensitivity, 125
TGF-β/BMP signaling, for root development, 21–22
Tissue engineering, 127–128
T lymphocytes, 20
TMR. *See* Transsal microradiography (TMR)
Tomes' process, 4, 5
Tooth eruption, 22–23
Tooth loss, 130–131
  diabetes, 148–149
  head and neck cancer risk, 134
Tooth regeneration, 127–128
Tooth worm, 33
Total-etch system, 118–119
Transferable solid phase (TSP) biofilm model, 160
Transversal microradiography (TMR), 75
TRAP segment. *See* Tyrosine-rich amelogenin peptide (TRAP) segment
Triclosan, 45–46
Tuftelin, 8
Tunnel preparation, 113
Type 2 diabetes mellitus (T2DM), 145–146
Tyrosine-rich amelogenin peptide (TRAP) segment, 7

U
Ulcerative colitis (UC), 145
Ultrasonic technique, 87
Upper respiratory tract infection (URTI), 149

V
Vascular tissues, pulp, 14
Veillonella, interspecies interactions, 37, 38
Visual inspection
  classification, 99
  improvements in, 98
von Korff fibers, 16

W
WHO Global Oral Health Programme, 92
Wnt signaling, for root development, 22

X
Xerostomia
  causes of, 64
  etiology of, 63–64
  management of, 64–65
  medications, 64
  severity of, 133
  sugar intake, 65
Xylitol, 46

Z
Zürich biofilm model, 160