SPRINGER BRIEFS IN MOLECULAR SCIENCE

Mariela A. Agotegaray
Verónica L. Lassalle

Silica-coated Magnetic Nanoparticles

An Insight into Targeted Drug Delivery and Toxicology

Springer

SpringerBriefs in Molecular Science

More information about this series at http://www.springer.com/series/8898

Mariela A. Agotegaray • Verónica L. Lassalle

Silica-coated Magnetic Nanoparticles

An Insight into Targeted Drug Delivery and Toxicology

Springer

Mariela A. Agotegaray
INQUISUR – CONICET
Universidad Nacional del Sur
Bahía Blanca, Argentina

Verónica L. Lassalle
INQUISUR – CONICET
Universidad Nacional del Sur
Bahía Blanca, Argentina

ISSN 2191-5407 ISSN 2191-5415 (electronic)
SpringerBriefs in Molecular Science
ISBN 978-3-319-50157-4 ISBN 978-3-319-50158-1 (eBook)
DOI 10.1007/978-3-319-50158-1

Library of Congress Control Number: 2016958970

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Nanotechnology applied to biomedicine represents one of the most important challenges currently facing science. The new properties that arise from a system reduced to the nano-scale make this discipline a novel tool to promote a revolution in terms of therapeutics in medicine. There are diverse fields where nanotechnology may contribute novel strategies to improve conventional therapeutics, with Nanomedicine emerging as a growing field of scientific research. Among the different strategies devoted to medical treatments, drug targeting is one where the convergence of different disciplines intends to give another approach to the current treatment of diverse diseases. Among different types of drug targeting, magnetic targeting presents the advantage related to nanosystems that may be easily guided by the aid of an external magnetic field. This property improves the targeting capability and increases their potential applications as target drug delivery systems or magnetic resonance image agents for diagnostic. However, in terms of medicine and in the application of new technologies for therapeutics, biocompatibility arises as one of the most important issues. So when a nanosystem intended for targeted drug delivery is designed and developed, the first topic a researcher should consider is not only the proper features of the biomaterial, but also the safety in terms of compatibility with the organism.

There are diverse types of biocompatible materials suitable for magnetic drug targeting at nanoscale, magnetite being the one of election. Coating of magnetic nanoparticles is another important topic when the nanosystems are intended for biomedical applications. The election of silica as coating material is a very proper choice in terms of its inert properties and in relation to the improvement of the stability and the physicochemical properties of the magnetic nanosystems. Anyway, solid silica-coated magnetic nanoparticles have not been explored extensively although the associated proper features make these systems suitable as drug targeting agents.

This book has been conceived as a means of disclosure of the remarkable properties related to solid-silica magnetic nanoparticles in biomedicine aiming to encompass from the synthesis to the biomedical applications of these nanosystems. In addition, the main aim of this book it to bring researchers detailed information about biomedical topics such as biocompatibility, bioavailability, biodistribution,

and toxicity. The goal is to join physicochemical properties with biological insights to better understand the relation between these approaches and the impact that they have in biomedicine.

We hope that readers may find here a specific site designed to cover all aspects related to the synthesis, physico-chemical, and biological properties of solid silica-coated magnetic nanoparticles. And that the reading of this book may open a new path in terms of developing more research devoted to these versatile and suitable systems for the treatment and diagnosis of various specific localized diseases. On the other hand, we hope that this work will serve as a stimulus not only for the development of new magnetic nanosystems based on solid silica but also to continue the study of their properties of biocompatibility. This is necessary for their effective application and implementation as commercially available medical treatments in the not too distant future.

Bahía Blanca, Argentina

Mariela A. Agotegaray
Verónica L. Lassalle

Contents

Chapter 1
Introduction

Abstract Nanotechnology is a scientific discipline involving multiple hard sciences such as chemistry, physic, biology, engineering, among others. The occurrence of novel properties when materials are reduced to nanosizes is the main reason for the scientific and technological interest in such discipline. In particular nanomedicine, that is nanotechnology applied to medicine, has suffered an exponential grow in the last decades. The possibility to target the drug to the diseased site, by avoiding side effects and lowering the required doses, strongly impulses the development of this kind of technology. Magnetic nanotechnology presents the additional advantage related to nanosystems that may be easily guided by the aid of an external magnetic field. This property improves the targeting capability and increases their potential in biomedical applications such as target drug delivery or MRI diagnostic. Iron oxides based nanosystems are currently the favorites to achieve these kinds of issues due to multiple reasons, but mainly to their low toxicity and biocompatibility. However, surface modification is often required to gain in stability, improve their physicochemical properties or even to raise the reactivity by means of functional groups incorporation. Silica appears as a highly attractive material to assess this objective.

In the Introductory section the general aspects of nanotechnology and nanomedicine are highlighted. Principles of iron oxides nanoparticles and their silica coat are described.

Keywords Nanotechnology • Nanomedicine • Iron oxide • Silica • Magnetic nanotechnology

The beginning of Nanotechnology dates back to last century and was conceived in the idea of Richard Feynman who, from his words referred to at the "Annual Meeting of the American Physical Society" 29th December 1959 at California Institute of Technology (CALTECH), opened the way to the wonderful and infinite world of nanotechnology. While nanotechnology is a very broad concept and can be applied to a wide variety of disciplines as he described, its conception and scope are inextricably linked to this man. In the conference entitled "There's Plenty of Room at the Bottom" he entered the wide concept related to the world of small dimensions, applied to "an enormous number of technical applications" [1]. Considering that Nanoscience is defined as the study of extremely small things that can be used and

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_1

employed in a wide variety of fields, as predicted by Feynman, manipulation of atoms and molecules was mandatory to develop this discipline. So it emerged once scientists were afforded the right and necessary tools such as scanning tunneling microscope (STM) and the atomic force microscope (AFM): by this, the Nanoscience and Nanotechnology age was born.

Nanometric scale or simply (Fig. 1.1) refers to structures with nanometric length considering 1 nm as the millionth part of a meter. In this dimension the properties of a material drastically change and quantum effect becomes relevant and evident rather than mass effects or volume, which are responsible for the properties of larger materials. Nanomaterials are, in general, defined as particles of size ranging between 1 and 100 nm [2]. However, this definition is limited because in terms of nanomaterials not only the size defines the special and specific properties. Nano-size benefits come from the properties and interactions that are unique to the nanoscale structure. Thus, particles larger than 100 nm can exhibit unique properties and can also be considered as nanomaterials.

Nanotechnology is described now as dedicated technology for design and handling of matter at molecular or atomic levels with applications in diverse fields. Feynman in his first speech mentioned the various areas of application of his brilliant idea related to the nanoscale: physics, chemistry, informatics, engineering, materials science, and biology. Especially, related to biology he mentioned in a section of the speech entitled "The marvelous biological system": "…A biological system can be exceedingly small. Many of the cells are very tiny, but they are very active; they manufacture various substances; they walk around; they wiggle; and they do all kinds of marvelous things—all on a very small scale. Also, they store information"… [1]. In this context, the application of nanotechnology in biomedicine becomes an entire field of science devoted to improve the point of view related

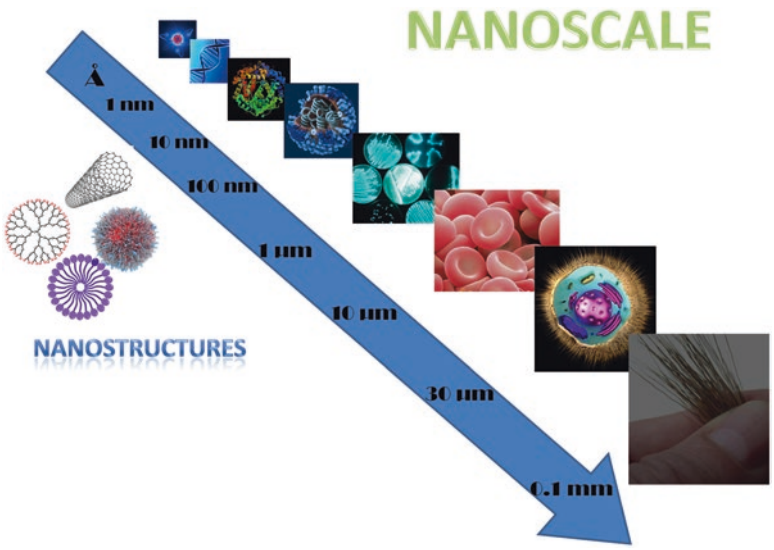

Fig. 1.1 Nanoscale

to the whole of medicine. It means nanoscale applied to diagnosis, therapeutics, and research on biomedicine.

Nanomedicine is one of the most promising areas in science and technology, leading to the convergence of nanotechnology and medicine toward improved diagnostic and therapeutic strategies that take advantage of the unique properties of materials at the nanoscale. Rapid developments in this field are taking place in terms of both our scientific understanding of applied nanoscience as well as the engineering design of programmable nanotechnology platforms with tunable and desirable features. While the earliest accomplishments in nanomedicine principally focused on cancer medicine, there has been a recent explosion on new frontiers of clinical application, including neuroscience and infectious diseases.

Shifting beyond classical examples of nanomedicines, we are also witnessing a greater diversity in relevant topics and applications as the lines between nanotechnology and medicine blur and lead to new innovations: platform technologies which redefine our vision of what nanomedicine is and what it can become.

Nanomedicine results as one of the most promising areas in science and technology by the convergence of various disciplines with the aim of taking advantage of the original and unique properties of nanomaterials toward improved diagnostic and therapeutic strategies in medicine.

The main area in which nanomedicine is currently applied is cancer therapy where major research has been developed. Diverse nanostructures have been designed and studied for detection and treatment of tumors: metal nanospheres, nanorods, nanoshells, nanotubes, liposomes, and polymer micelles [3]. However, recently there has been a boom in the use of nanotechnology applied to various fields of medicine, including neuroscience and infectious diseases. And considering the great versatility in the design of nanostructures, these materials are extremely interesting in the treatment of any ailment. Most applications of nanostructures in nanomedicine are now oriented to drug targeting, controlled drug delivery, and tissue engineering. Progress in this field involves the engineering design of nanostructures with tunable and desirable features. In this sense, the convergence of various disciplines (Fig. 1.2) such as chemistry, physics, engineering, biology, biochemistry, physiology, and other related is extremely necessary to achieve nanocomposites with specific characteristics for certain purposes.

Among the various applications intended for nanodevices in medicine, pharmacology, special drug targeting is a key where nanotechnology is developing new insights for therapeutics.

A major problem associated with conventional drug therapy is that, when drugs enter the body by any route of administration, they spread through the circulatory system to all organs and tissues. The aim for drug targeting by entrapment into nanoparticle systems is the reduction in the toxicity of the free drug to non-target organs and concomitantly this converges in an increase of therapeutic index as well as in the margin between the doses resulting in a therapeutic strategy to improve efficacy in the treatment.

The main aspect to consider when designing nanomaterials for medicine is biocompatibility. This feature is achieved by the selection of biomaterials or synthetic non toxic materials which provide a platform for in vivo applications.

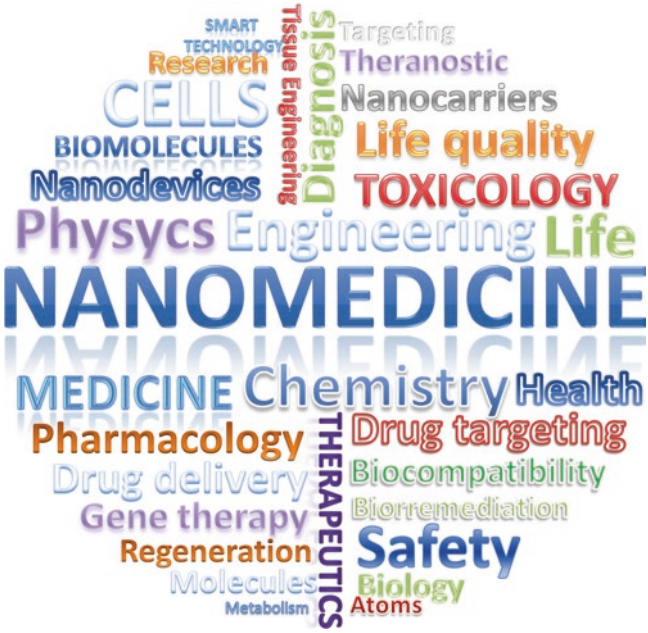

Fig. 1.2 Convergence of diverse scientific fields to the development and applications of nanomedicine

Nanocomposites are generally classified as organic and inorganic. The bulk of the research being developed in biomedical nanocomposites focuses primarily on the methods of synthesis and characterization of their properties.

Polymer nanoparticles, solid lipid nanoparticles, liquid crystal nanoparticles, liposomes, micelles, and dendrimers are the most common types among nanoparticles designed for drug delivery. Nanocapsules and nanospheres are both different arrangements in terms of drug incorporation. In addition, nanotubes and quantum dots are nanosystems greatly studied in nano-biomedicine. The strategy for therapeutics lies in the correct selection of the type of nanoparticles employed to a given delivery application based on the physicochemical properties of the drug of interest (Fig. 1.3).

Polymeric nanoparticles are highly studied and tested because of several benefits associated with biomedical applications. Polymers such as poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), poly-L-lactic acid (PLA), polycaprolactone (PCL), and chitosan are the most commonly used due to biocompatibility, biodegradability, and ease to functionalize. Among polymeric nanoparticles there are nanocapsules and nanospheres. Nanocapsules contain a drug-filled core surrounded by a polymer coating. The nanospheres contain the drug uniformly distributed among porous structure.

With regard to liposomes, their composition consists of phospholipid bilayers similar to cell membranes. The inner compartment formed by the hydrophilic head groups of the phospholipids can contain one or more hydrophilic drugs. In addition,

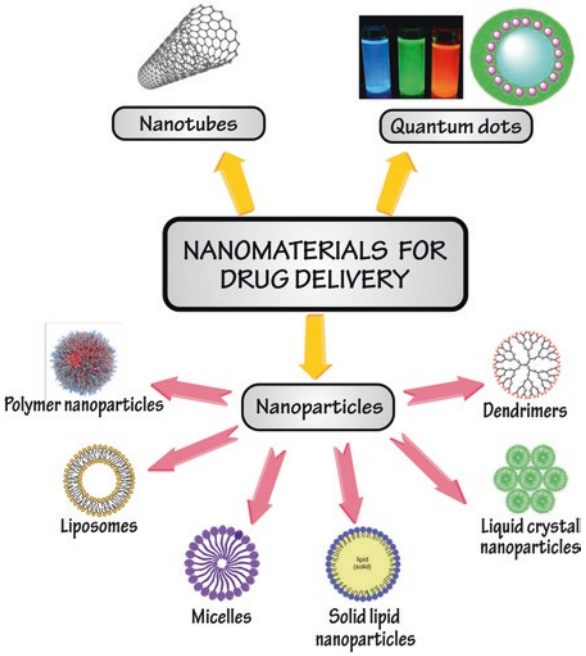

Fig. 1.3 Nanostructures for drug delivery

liposomes can carry lipophilic drugs which dissolve into the liposomal bilayer [4]. Liposomes show no adverse or toxic effects on healthy cells [5]. Current research on liposomes is devoted to optimization of size, surface charge, and number of lamellae [6]. Micelles present a monolayer of phospholipids in which the head group faces the outside and the hydrophobic tails the inner side. These nanodevices are intended for the delivery of lipophilic drugs.

SLNs contain a solid hydrophobic core surrounded by phospholipids. These nanoparticles are also intended for hydrophobic drug delivery. They are in general more stable than liposomes and new insights on their formulation consist of incorporation of liquid lipid into the solid structure, resulting in nanostructured lipid carriers [7].

Liquid crystal nanoparticles provide a useful and promising platform for drug delivery by the combination of both liquid and solid states associated with the ability to flow (liquid) and maintenance of an ordered crystalline structure (solids). They are intended for oral delivery because they can protect active ingredients from harsh gastrointestinal conditions. However, these nanostructures are not widely applied owing to the high costs associated with the manufacturing process [8].

Dendrimers represent rather monodisperse macromolecules containing symmetric branching units built around a small molecule or a linear polymer core. The term "Dendrimer" denotes an architectural motif but not a type of compound. These artificial macromolecules present a high number of functional groups in a compact structure. Dendrimers have become a delivery vehicle candidate because of their well defined structure and versatility, being extensively studied for applications in anticancer therapy and diagnostic imaging [9].

Nanotubes are described as a nanometer-scale tube-like structure. The research of these structures is in full swing. Different compositions of nanotubes have been studied for drug delivery and biomedical imaging: carbon nanotubes [10], magnetic-carbon nanotubes [11], titania nanotubes for local drug delivery on implant surfaces [12], and multifunctional hybrid-carbon nanotubes [13].

Quantum dots are semiconductor nanocrystals which present unique and fascinating optical properties that are not generally available for individual molecules or bulk semiconductor solids. The core is made up of semiconductor material, the shell surrounds the semiconductor core to improve its optical properties and the cap encapsulates the double layer quantum dots by different materials [14]. They have distinctive characteristics such as size-tunable light emission, improved signal brightness, resistance against photobleaching, and simultaneous excitation of multiple fluorescence colors. These particles, linked with bioaffinity molecules, lead to obtaining ultrasensitive and multicolor images of molecular targets in living cells and animal models. Current research is oriented to further develop this technology for clinical and translational research. One emerging application of quantum dots appears to be traceable drug delivery with applications to elucidate the pharmacokinetics and pharmacodynamics of drug candidates and to provide the design principles for drug carrier engineering [15].

There are many aspects to consider when designing a nanodevice with applications in medicine: size, surface chemistry and reactivity, shape, and biocompatibility are the most important regarding biological aspects among others. On the other hand, other aspects unrelated to the nanoparticles also influence biodistribution, uptake and toxicity such as physiology, histology of the organs, and route of administration. A precise knowledge about the effect of nanoparticles on normal cells and tissue distribution in the body is critical as a pre-clinical setting before any potential use. In general, after administration, it is expected that the major quantity of nanoparticles would be taken up by the liver due to the first-pass effects, and would be later redistributed to other organs [16]. Macrophages from the reticuloentothelial system play an important role in the capture of nanomaterials [17]. Other characteristics associated with the morphology of NPs are implicated in the biodistribution besides the reticuloendothelial system. Regarding the size, the observed trend is that larger nanoparticles are quickly taken up by the liver, diminishing circulation time in the blood. On the contrary, smaller MNPs present easier access to other organs and longer circulation time in the bloodstream [18]. Particle size and surface charge affect the efficiency and pathway of cellular uptake for liposomes [19], quantum dots [20], polymeric [21, 22], gold [23], and silica nanoparticles [24]. The nature of the material influences adhesion and interaction of nanoparticles with cells.

The use of nanoparticles as targeting agents to specific tissues or organs presents major benefits in drug delivery. Targeting improves drug bioavailability by increasing the amount of drug which reaches the targeted tissue. In addition, targeting strategies reduce toxic systemic effects associated with the drugs by the specific release on a specific and localized organ. Two different strategies on drug targeting exist: active and passive targeting. Active targeting consists of the attachment of a targeting ligand on the surface of the nanoparticles. For instance, nanoparticles with

folic acid are designed for the potential treatment of cancer. Many types of cancers cells overexpress the folic acid receptor of their surface. In this way, the attachment of folic acid to nanoparticles provides a targeting strategy to the delivery of chemotherapeutics to tumor. Passive targeting employs different approaches related to chemical or physical features of nanoparticles due to size, shape, and surface charge. It is often an effective and less-expensive option. For instance, targeting can also be achieved using external forces. The use of magnetic fields to direct a delivery system has gained some attention.

In this book, our purpose is to demonstrate to the reader a deepening of the development of magnetic nanoparticles as drug targeting strategy and, in particular, the role of silica as a functionalization agent and coating. The biomedical approach brings the author to the promising applications of these systems as well as to the beginning of a concrete vision about the clinical implementation of this nanotechnology.

References

1. Feynman, R. P. (1960). There's plenty of room at the bottom. *Engineering and Science, 23*(5), 22–36.
2. Auffan, M., Rose, J., Bottero, J. Y., Lowry, G. V., Jolivet, J. P., & Wiesner, M. R. (2009). Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nature Nanotechnology, 4*(10), 634–641.
3. Zare-Zardini, H., Ferdowsian, F., Soltaninejad, H., Ghorani Azam, A., Soleymani, S., Zare-Shehneh, M., et al. (2016). Application of nanotechnology in biomedicine: A major focus on cancer therapy. *Journal of Nano Research, 35*, 55–66.
4. Fahr, A., van Hoogevest, P., May, S., Bergstrand, N., & Leigh, M. L. (2005). Transfer of lipophilic drugs between liposomal membranes and biological interfaces: Consequences for drug delivery. *European Journal of Pharmaceutical Sciences, 26*(3–4), 251–265.
5. Yallapu, M. M., Jaggi, M., & Chauhan, S. C. (2012). Curcumin nanoformulations: A future nanomedicine for cancer. *Drug Discovery Today, 17*(1–2), 71–80.
6. Bonifácio, B. V., da Silva, P. B., dos Santos Ramos, M. A., Negri, K. M. S., Bauab, T. M., & Chorilli, M. (2014). Nanotechnology-based drug delivery systems and herbal medicines: A review. *International Journal of Nanomedicine, 9*, 1–15.
7. Üner, M., & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. *International Journal of Nanomedicine, 2*(3), 289–300.
8. Müller-Goymann, C. C. (2004). Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration. *European Journal of Pharmaceutics and Biopharmaceutics, 58*(2), 343–356.
9. Abbasi, E., Aval, S. F., Akbarzadeh, A., Milani, M., Nasrabadi, H. T., Joo, S. W., et al. (2014). Dendrimers: Synthesis, applications, and properties. *Nanoscale Research Letters, 9*(1), 247.
10. Karimi, M., Solati, N., Amiri, M., Mirshekari, H., Mohamed, E., Taheri, M., et al. (2015). Carbon nanotubes part I: Preparation of a novel and versatile drug-delivery vehicle. *Expert Opinion on Drug Delivery, 12*(7), 1071–1087.
11. Al Faraj, A., Shaik, A. P., & Shaik, A. S. (2015). Magnetic single-walled carbon nanotubes as efficient drug delivery nanocarriers in breast cancer murine model: Noninvasive monitoring using diffusion-weighted magnetic resonance imaging as sensitive imaging biomarker. *International Journal of Nanomedicine, 10*, 157.

12. Gulati, K., Kogawa, M., Maher, S., Atkins, G., Findlay, D., & Losic, D. (2015). Titania nanotubes for local drug delivery from implant surfaces. In D. Losic & A. Santos (Eds.), *Engineered nanoporous materials* (pp. 307–355). New York: Springer International Publishing.
13. Mehra, N. K., & Jain, N. K. (2016). Multifunctional hybrid-carbon nanotubes: New horizon in drug delivery and targeting. *Journal of Drug Targeting, 24*(4), 294–308.
14. Modani, S., Kharwade, M., & Nijhawan, M. (2013). Quantum dots: A Novelty of medical fields with multiple applications. *International Journal of Current Pharmaceutical Research, 5*(4), 55–59.
15. Qi, L., & Gao, X. (2008). Emerging application of quantum dots for drug delivery and therapy. *Expert Opinion on Drug Delivery, 5*(3), 263–267.
16. Kim, I. Y., Seo, S. J., Moon, H. S., Yoo, M. K., Park, I. Y., Kim, B. C., Cho, Ch. S. (2008). Chitosan and its derivatives for tissue engineering applications. *Biotechnology Advances, 26*, 1–21.
17. Varna, M., Ratajczak, P., Ferreira, I., et al. (2012). *In vivo* distribution of inorganic nanoparticles in preclinical models. *Journal of Biomaterials and Nanobiotechnology, 03*, 269.
18. Li, M., Al-Jamal, K. T., Kostarelos, K., et al. (2010). Physiologically based pharmacokinetic modeling of nanoparticles. *ACS Nano, 4*, 6303–6317.
19. Chono, S., Tanino, T., Seki, T., & Morimoto, K. (2007). Uptake characteristics of liposomes by rat alveolar macrophages: Influence of particle size and surface mannose modification. *Journal of Pharmacy and Pharmacology, 59*(1), 75–80.
20. Osaki, F., Kanamori, T., Sando, S., Sera, T., & Aoyama, T. (2004). A quantum dot conjugated sugar ball and its cellular uptake on the size effects of endocytosis in the subviral region. *Journal of the American Chemical Society, 126*(21), 6520–6521.
21. Wina, K. Y., & Feng, S. S. (2005). Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. *Biomaterials, 26*(15), 2713–2722.
22. Foged, C., Brodin, B., Frokjaer, S., & Sundblad, A. (2005). Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. *International Journal of Pharmaceutics, 298*(2), 315–322.
23. Chithrani, B. D., Ghazani, A. A., & Chan, W. C. W. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters, 6*(4), 662–668.
24. Lu, F., Wu, S. H., Hung, Y., & Mou, C. Y. (2009). Size effect on cell uptake in well-suspended, uniform mesoporous silica nanoparticles. *Small, 5*(12), 1408–1413.

Chapter 2
Magnetic Nanoparticles as Drug Delivery Devices

Abstract Nano-size in combination with magnetic properties gave rise to novel nanomaterials with improved properties, especially with regard to biomedical applications. This chapter is devoted to show the strong relationship between the design of nanoparticles and the final properties able to define its efficiency to the desired applications.

According to the literature, several inorganic materials may be chosen to assess magnetic nanodevices, however the iron oxides, such as magnetite and maghemite, are the preferred for several reasons. The property of superparamagnetism becomes crucial when the practical implementation of these nanosystems is intended in the biomedical field. This, and other properties strongly linked to the efficiency in biomedical applications are defined during the synthetic pathways. The most common preparative methods are here described highlighting the advantages and disadvantages as well as the properties of the obtained magnetic nanoparticles.

Coating of magnetic cores is strictly necessary to assess the interest and specific properties required for biomedical uses. In this regard, a classification of the most useful coatings is included highlighting the properties conferred by the selected coating material.

Characterization techniques able to evaluate the size, surface charge, functionality, and magnetism were also reported as a guide.

Keywords Superparamagnetism • Co-precipitation • Microemulsion • Size • Shape

A drug delivery system is described by two main characteristics: the first is the capacity to target which will enhance efficiency of the drug and decrease side effects. The second one is the ability to control drug release. Both features prevent drug systemic distribution and concomitantly diminish the undesired side effects associated with the interactions of the drug with healthy tissues [1, 2].

Nanosystems intended for drug delivery present several benefits imparted by nano-size related to the diminishing of irritant reactions at local injection and also enable better diffusion inside the body.

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_2

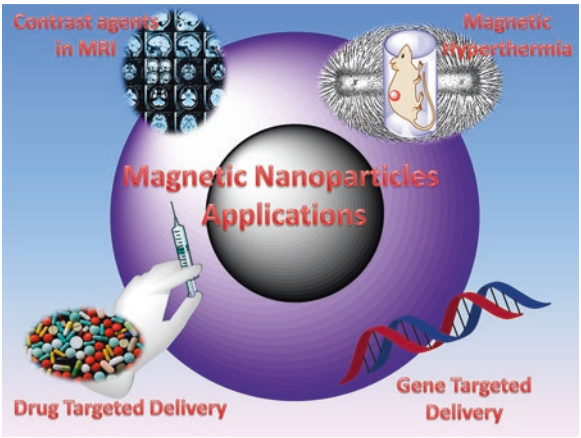

Fig. 2.1 Biomedical applications of magnetic nanoparticles

The idea of the use of magnetic particles for drug delivery dates back to the 1970s when Widder, Senyi, and colleagues [3] developed the first preclinical assays in rats devoted to study magnetic albumin microspheres for Doxorrubicin (a chemotherapeutic agent in the treatment of cancer) targeting and delivery.

Further clinical and biomedical uses are being studied for magnetic nanoparticles (Fig. 2.1). Magnetic resonance image (MRI), magnetic hyperthermia treatment, tissue repair, contrast enhancement, and gene delivery are other applications in which magnetic nano-sized systems find promising issues.

2.1 Generalities of Magnetism in Biomedical Applications

Magnetic materials show some response in the presence of a magnetic field. These materials can be classified in different types depending on such response: ferromagnetic, paramagnetic, diamagnetic, antiferromagnetic, and ferromagnetic (Table 2.1).

Magnetic nanoparticles respond to the presence of a magnetic field. The key factor in this behavior comes from the large surface-to-volume ratio of the nanoparticles. This imparts novel physical, chemical, and mechanical properties compared to the corresponding bulk material. This feature also applies to magnetic properties regarding magnetic anisotropy [4].

A bulk ferromagnetic material is composed of small regions known as magnetic domains. The net magnetization of a magnetic material results from the addition of the magnetizations of all domains. Magnetic domains in ferromagnetic crystals have a minimum, around 100 nm that is the so called critical size. Below the critical size the ferromagnetic material cannot split up further into domains [5–7] and are

Table 2.1 Different types of magnetic materials. Composition and response when placed under the influence of a magnetic field

Type of magnetic material	Structure of the material	Response under an applied magnetic field	Response after removing the magnetic field	Example
Ferromagnetic	Atoms with parallel magnetic moments from unpaired electrons. The net magnetic moment induced points in some direction. The result is a zero net magnetic moment.	A large net magnetic moment is formed from the alignment of the domains' magnetic moments along the direction of the applied magnetic field.	Remains a residual magnetic moment.	Fe, Ni, Co
Paramagnetic	Unpaired electrons give rise atoms with a net magnetic moment. There are no magnetic domains.	A weak net magnetic moment is induced by the alignment of the magnetic moments of the atoms along the direction of the applied magnetic field.	No retention of magnetic moment.	Ga, Mg, Li, Ta, Cu
Diamagnetic	Zero net magnetic moment due to the absence of unpaired electrons in atoms.	Very weak response against an applied magnetic field.	Do not retention of magnetic moment.	Ag, Au, most of known elements
Antiferromagnetic	Two different atoms present equal magnetic moments in magnitude and opposite in direction. This gives rise to zero net magnetic moment.	A large net magnetic moment is induced by the alignment of the magnetic moments of the domains along the direction of an applied magnetic field.	A residual magnetic moment remains.	MnO, CoO, NiO, $CuCl_2$
Ferrimagnetic	Different atoms reside on different lattice sites with antiparallel magnetic moments. Magnetic moments do not cancel, resulting in a net spontaneous magnetic moment.	The magnetic moments of the domains align along the direction of the applied magnetic field forming a large net magnetic moment.	A residual magnetic moment exists after the removal of the magnetic field.	Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3)

called single domain particles. The MNP might be composed of a single magnetic domain if its size decreases below a critical limit. It might also display superparamagnetic [8, 9] behavior as long as the temperature is above the so called blocking temperature (*TB*). In the superparamagnetic state, the magnetic moments of the nanoparticles fluctuate around the easy axes of magnetization. Thus, each one of the MNPs will possess a large magnetic moment which orientation is continuously changing. When a magnetic field is applied, MNPs in the superparamagnetic state display a fast response to the changes of the magnetic field without remnant or residual magnetization and without coercivity. This term is applied to the magnetic field required to bring the magnetization back to zero. In the superparamagnetic state, a single MNP behaves as a paramagnetic atom with a very big spin. At temperatures below the blocking one, the thermal agitation becomes smaller and does not cause fluctuations in the orientation of the magnetic moments of the nanoparticles. Then, they freeze in random orientations.

2.2 Magnetic Nanoparticles: Synthesis of Magnetic Cores

There are many features to consider when designing magnetic nanoparticles for drug delivery. The typical structure for magnetic nanoparticles intended for drug targeting consists of a functionalized magnetic core. The feasibility of targeting a specific site of the body by the influence of a magnetic field turns these nanodevices into promising agents for localized treatment of diverse pathologies.

Size is determinant to pass through the endothelium which is the barrier they have to cross in blood vessels to arrive at the organs and tissues as well as to penetrate cell membranes to achieve targeted cells.

The biodistribution of superparamagnetic iron oxide nanoparticles has been studied regarding the in vivo effect of size. A study developed on magnetic nanoparticles with sizes in the range between 33 and 90 nm in mice revealed that larger particles were preferably distributed to the liver [10]. Others studies focused on nanoparticles with size around 200 nm also demonstrated the liver as the responsible organ for first uptake after administration [11, 12]. However, a recent research on magnetic nanoparticles intended for drug delivery showed that nanoparticles between 250 and 370 nm were feasible to avoid capture by the reticuloendothelial system of the liver [13]. Thus, reticuloendothelial system capture dependant on particle size would not be the only one responsible for tissue distribution. In this sense, other features of nanoparticles would determine distribution such as coating and surface charge. Surface charge is an important property which influences the biodistribution of magnetic nanoparticles. Neutral nanoparticles would be most adequate by their trend to avoid reticuloendothelial system capture. Zeta potentials near 0 mV on the surface are feasible to decrease opsonization [14]. On the other hand, positively charged magnetic nanoparticles have shown a rapid clearance by liver, diminishing the time of blood circulation while negatively charged magnetic

nanodevices presented an incremented blood circulating time with biodistribution profile similar to nearly neutrally charged nanoparticles [15].

The first stage in the obtaining of magnetic nanoparticles is to choose, design, and synthesize the magnetic core.

When designing a magnetic core for biomedical purposes it is mandatory to consider biocompatibility of the material employed.

Iron oxide based materials are of choice in accordance with relative safety and biocompatibility.

Among the eight species of iron oxides known [16], hematite (α-Fe_2O_3), magnetite (Fe_3O_4), and maghemite (γ-Fe_2O_3) are the most popular in biomedical applications.

Magnetite presents the general formula AB_2O_4 belonging to spinel group. The structure from the alternation of Fe^{2+} and Fe^{3+} gives rise to strong magnetization by ferromagnetic properties. As a topotactic oxidation product of magnetite, maghemite presents the same lattice structure as magnetite with all iron atoms as $Fe^{3+.}$

Iron (III) ions are present in the human body, by this, maghemite results as one of the most suitable magnetic materials for biomedicine considered to not cause significant side effects. Anyway, the cellular accumulation of this metal is a topic of hard rearch to ensure safety and biocompatibility.

Other materials based on iron oxides are issues of research in order to improve the quality of magnetic cores. Mixed oxides such as $CoFe_2O_4$, $NiFe_2O_4$, and $MnFe_2O_4$ consist of materials with similar spinel structure to magnetite (Fe_3O_4) [17]. In addition, alkaline earth metals mixed oxides were developed: $BaFe_{12}O_{19}$ and $SrFe_{12}O_{19}$.

Besides the development of much research on these materials, their application in biomedicine is limited due to the toxicity associated with the rare earth metals and transition metal clusters.

Iron alloys are being widely studied as materials for targeting and image contrast for diagnosis [18]. FePt, FeAu, and FeAg are promising compositions intended for biological applications.

Methodologies for the synthesis of core magnetic nanoparticles are generally divided regarding the medium and the number of steps involved in the procedure. Aqueous routes derive in low cost and sustainable methods and render water dispersible nanoparticles, which are in general desired for biomedical applications considering blood and plasma features in terms of hydrophilicity. Non-aqueous routes render nanoparticles soluble in non-polar solvents. These systems are of interest for encapsulation of lipophilic drugs although they involve functionalization strategies to disperse them in physiological media. The other classification of synthesis procedures for nano-magnetic cores lies in one-step or multi-step procedures. This classification is important to consider for the preparation of magnetic nanoparticles depending on environment and available instruments and facilities at disposal in the laboratory. It is remarkable that all methods present advantages and disadvantages. The election of a specific methodology depends on the final properties of the desired product. In this sense, a correct previous design upon the applications is mandatory.

2.2.1 Co-precipitation Method

Co-precipitation is one of the most conventional procedures to obtain magnetic nanoparticles. Iron oxide nanoparticles as well as mixed oxide particles can be obtained. The key in this method lies in the control of pH during the synthesis procedure. Mixed oxide magnetic nanoparticles can be obtained by co-precipitation of stoichiometric quantities of the ions involved.

Iron oxide magnetic nanoparticles synthesis consists of the mixture of ferric and ferrous salts in 1:2 iron molar ratios. The reaction can occur either at room temperature or at high temperature and the key is the increase of pH by the addition of an alkali. When pH is lower than 11 nucleation of Fe_3O_4 is facilitated meanwhile at an increase of pH over 11 it undergoes growth of the nucleus. The mechanism through which the reaction occurs is:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- + Fe(OH)_2 + 2Fe(OH)_3 \rightarrow Fe_3O_4 \downarrow + 4H_2O.$$

In general, the reaction needs gas protection to prevent oxidation of Fe(II) to obtain a better performance in the synthesis of magnetite as the final product. The co-precipitation method was first introduced by Massart [19], and after this pioneering study, new research was developed to improve the method with variants. Recent research involves the influence of temperature on morphology, size, and magnetic properties [20]. In addition, the implementation of different technologies to assist the synthesis has been analyzed. For instance, ultrasonic assistance to co-precipitation rendered magnetite nanoparticles 15 nm sized [21]. New variants are studied to provide easier conditions during the synthesis. The evaluation of the base is important to obtain the desired nanoparticles in terms of shape and size. For example, the use of alkanolamines as alkali rendered small magnetic nanoparticles of around 5 nm with acceptable magnetic properties, considering the trend based on the smaller the size, the lower the magnetic response to a magnetic field [17, 22]. Recent research has revealed that it is possible to obtain controlled size and shape nano-magnetite at room temperature and without control on inert atmosphere [23]. In order to improve the performance of co-precipitation, in addition to control some variants inherent in the method, new approaches have been studied related to the incorporation of stabilizers during synthesis. Their role is based on slowing down the nucleation process directly affecting the growth of nanocrystals. This may induce the formation of smaller and more dispersed nanoparticles. In this way different stabilizers were studied, such as oleic acid [24], poly(*N*-vinyl pirrolidone) (PVP) [25], and sodium dodecyl sulfate [23].

2.2.2 High Temperature Thermal Decomposition Method

In this method the precursors are mixed at room temperature and then heated in a closed or open reaction mixture. Precursors consist of organometallic or coordinated iron such as $Fe(CO)_5$, $Fe(acetylacetonate)_3$, iron oleate, Fe(*N*-nitrosophenylhydroxylamine)$_3$, Prussian blue ($Fe_4[Fe(CN)_6 \cdot 14H_2O]$, Fe–urea

complex ($[Fe(CON_2H_4)_6](NO_3)_3$), ferrocene ($Fe(C_5H_5)_2$), and $Fe_3(CO)_{12}$. The reactions occur in organic solvents. The resultant higher monodisperse nanoparticles and the narrow size distribution are due to nucleation separated from growth of nanocrystals [26, 27]. During the synthesis procedure, stabilizers can also be introduced in the reaction mixture.

2.2.3 Hydrothermal and Solvothermal Synthesis

This method consists of various wet-chemical techniques of crystallizing the substance in a sealed container from the high temperature aqueous or non-aqueous solution in the range between 130 and 250 °C under high vapor pressure (generally in the range 0.3–4 MPa) [28]. This is the method of election for the synthesis of highly crystalline iron oxide nanoparticles such as α-Fe_2O_3, γ-Fe_2O_3, and Fe_3O_4 because it maintains a good control on composition. Moreover, materials which present high vapor pressure near melting point are plausible to grow by the hydrothermal method. The "solvothermal" term is applied when an organic solvent is employed instead of water as reaction medium [29]. With this methodology it is also possible to obtain hollow iron oxide nanoparticles.

2.2.4 Sol–Gel Reactions and Polyol Method

The sol–gel process consists of a sol which is a stable dispersion of colloidal particles or polymers in a solvent and a gel composed of a three dimensional continuous network enclosing a liquid phase. The gel may be colloidal, where the network is built from the agglomeration of colloidal particles or polymer.

In general, precursors for the synthesis of magnetic iron oxdide nanoparticles consist of iron alkoxides and iron salts which are exposed to hydrolysis and polycondensation reactions [30]. These reactions are developed at room temperature, and further heat treatments are applied to achieve the final crystalline state. This method serves to obtain various sizes and shapes of magnetic nanoparticles as desired.

The polyol method consists of a reverse sol-gel process in which a reduction reaction occurs. Polyols serve as the solvent and also as reducing agents, playing an important role in stabilization to prevent aggregation and control of particle size as well as in magnetization [31]. The reaction process consists of the suspension of an iron precursor on a liquid polyol and heating to the boiling point of the liquid. It is not necessary to work at high pressures, as occurs in sol-gel approach.

2.2.5 Microemulsion

Microemulsions are described as stable and clear liquids mixtures composed of oil, water, and surfactant and in some cases, a co-surfactant. In this method, the aqueous phase usually contains the metal salts and pH regulators such as alkali and also

other compounds which can serve as coating agents. The oil phase is generally constituted by organic solvents such as hexane, for example, and surfactants are commonly bis(2-ethylhexyl) sulfosuccinate (AOT), sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), and PVP which are the most employed to obtain magnetic nanoparticles. Micelles formation enables the control on size, leading homogeneous size and shape of nanoparticles. The reversed micelle method where water is dispersed in oil is also employed for the synthesis of magnetic nanoparticles [32].

2.2.6 Sonolysis or Sonochemical Method

The sonolysis method employs sonochemical or ultrasound irradiation which enables work at lower temperatures and pressures as well as permits a diminution in reaction times [33].

The technique to obtain iron oxide magnetic nanoparticles consists of the sonication of iron salts solutions under ambient conditions. The ultrasound irradiation induces the formation of bubbles from expansive and compressive acoustic waves. The oscillating bubbles accumulate energy which is released after growth and collapse. Thus, new chemical structures arise from ultrasonic energy [33].

2.2.7 Microwave-Assisted Synthesis

The microwave-assisted synthesis method is very useful to synthesize iron oxide magnetic nanoparticles because it enables control of size and shape [34]. The basis of this method lies in the alignment of the dipoles of the molecules within an external field by the excitation caused by microwave radiation. The reorientation of the molecules with the electrical field induces an intense internal heating. This fact reduces time of processing as well as energy required because of the homogeneity and selectivity in heating. Times of reaction also diminish in comparison with other methods. In addition, the method permits control of the magnetic nanoparticles by changing experimental conditions [35].

2.2.8 Biosynthesis

This process occurs by reduction-oxidation reaction in vivo, from microbial enzymes and plant phytochemicals with redox properties. Magnetic nanoparticles were obtained from magnetotactic and iron reducing bacteria, *Geobacter metallireducens* and *Magnetospirillium gryphiswaldense* being the most commonly employed. The process consists of the reduction of iron salts to nanoparticles under

aerobic or anaerobic conditions depending on the bacteria involved [36]. Production of magnetic nanoparticles employing biotechnology is novel, so further research is necessary to control the size and shape of nanoparticles.

2.2.9 Other Methods

The above described methods are the most commonly employed for the synthesis of magnetic nanoparticles. Anyway, other chemical or physical methods can be practiced.

The electrochemical method is based on the oxidation-reduction reaction of iron salts. The advantage associated with this method is the high purity of the final product as well as the strict control of size by the adjustment of potential applied to the reaction [37].

The flow injection method introduces technology to improve the co-precipitation method by the addition of the different precursors with a pump at controlled flow rate. Several advantages are purchased from this method such as reproducibility, homogeneity in mixing precursors, and moreover a precise external control [38].

Spray aerosol technologies are also employed to obtain iron oxide magnetic nanoparticles. Spray pyrolisis consists of evaporation of ferric salts at high temperature atmosphere, drying, and pyrolisis of liquid drops in a controlled atmosphere. Laser pyrolisis improves the pyrolisis process by employing laser energy to heat the gaseous mixture of iron precursors and a flowing gas. This technology renders small and non-aggregated magnetic nanoparticles [39].

Continuous research is always developed to improve the properties of magnetic nanoparticles obtained. The main aims are based on reduction of costs and time production; the employment of sustainable methods pointing to green-chemistry protocols and supplies; the obtaining of controlled nano-sized and shaped nanoparticles for the desired applications with minimum aggregation. Currently, classical co-precipitation is the preferred route. Thermal decomposition is the other method most commonly employed to obtain nanoparticles smaller than 20 nm meanwhile the solvothermal method is performed when nanoparticles larger than 20 nm are desirable.

It is important to mention that the shape of the magnetic core may adopt different forms and this special feature is achievable by the control of the experimental variables. Spherical magnetic iron oxide nanoparticles are the most common found, but also cubic and rodlike cores have been achieved [23, 40].

Figure 2.2 provides the most remarkable topics of each method described above.

2.3 Description of Coating

The functionalization of the magnetic core of the nanoparticles is crucial for two main reasons: to avoid aggregation and to improve biocompatibility.

Fig. 2.2 Resumed description about the most common methods employed in the synthesos of magnetic nanoparticles

Because of bipolar anisotropic attraction, non-modified nanoparticles of iron oxide tend to aggregate in large groups losing the specific properties associated with single domains. Furthermore, the reactivity of the iron oxide particles significantly increases by reducing its size, undergoing rapid degradation when exposed directly to biological systems [41, 42]. To avoid these limitations, the coating is essential. To this end, numerous strategies have been used for functionalization, among which may be mentioned:

1. Employing monomeric stabilizers as carboxylates, phosphates, and sulfates;
2. Coating with a variety of polymers;
3. The use of inorganic materials. This type of coating provides not only good stability in aqueous medium but also helps to incorporate biological surface ligands considering that water dispersible nanoparticles NPs are necessary for biomedical applications.

Most biological media consist of nearly neutral aqueous solutions. The design of coating is important to achieve magnetic nanoparticles for specific applications, especially the maintenance of stability and magnetic response under an external magnetic field.

Two main ways exist to coat magnetic nanoparticles: in situ and post-synthesis coating.

Several materials are commonly employed for the coating of magnetic nanoparticles:

Organics: dextran, starch, poly(ethylene glycol), (PEG), poly (D,L-lactide) (PLA), polyethylenimine (PEI), especially for hydrophilic organic materials. These organic materials are elected because of their hydrophilic properties which are mandatory for a good dispersion in aqueous media.

Small molecules: Smalls molecules can provide spherical groups such as –OH, –COOH, $–NH_2$ or –SH which role is to provide anchorage for further attachment of biomolecules or drugs to specific biomedical applications. Protection of the magnetic component has been achieved by coating with oleic acid, for example [24, 28]. Oleic acid can be used even at high temperature to obtain stable iron oxide nanoparticles. This coating does not affect the magnetic behavior of resultant nanoparticles [43].

Amonoacids, citric acid, and vitamins are small molecules very useful for the synthesis of water-soluble magnetic nanoparticles.

In some cases in which the resultant formulation is stable but not aqueous soluble due to coating with hydrophobic small molecules, ligand exchange procedure is used to change polarity [44].

Polymers: A large number of either natural or synthetic polymers can be employed for the coating of magnetic nanoparticles. Polysaccharides in general, gelatin, alginate, polyethylene glycol (PEG), poly(D, L-lactide) (PLA), and chitosan are being extensively studied, among others, for biomedical applications—especially, as drug targeting and contrast agents. Biocompatibility is the main feature to achieve on these systems to ensure the expected applications.

Recently, we demonstrated the biosafety of a novel nano-system composed of oleic acid functionalized nano-magnetite coated with chitosan. In this study the

magnetic nanoparticles were evaluated for toxicity on endothelial cells revealing no cytotoxic effect. Moreover, the acute and sub-acute biodistribution profile was studied, concluding that chitosan and, in general, the coating, is responsible for the organs achieved by bloodstream [13].

Biomolecules: Coating with biomolecules has emerged especially for biological separation, detection, and sensors. Enzymes, antibodies, and proteins in general are biomolecules feasible to bind to nano-iron oxides [45].

Inorganic materials: Improving the stability in dispersion or solution is one of the most intended challenges to achieve by coating with inorganic materials. For biomedical applications, this kind of functionalization enables the binding of biological ligands to iron oxide cores. Metal oxides and silica are examples of inorganic coating agents. Silica is the protagonist in the development of this book, so it will be widely described in the next chapters as the inorganic coating and functionalization agent of magnetic nanoparticles for drug targeting.

Other coatings made of carbon are currently being studied due to chemical and thermal stability as well as conductivity. Metallic nanoparticles are also under investigation as coating materials. Gold (Au) and silver (Ag) are the most common metals employed, but further research is required to improve stability of the obtained nano-systems.

Figure 2.3 schematizes the diverse coating agents commonly used on magnetic nanoparticles.

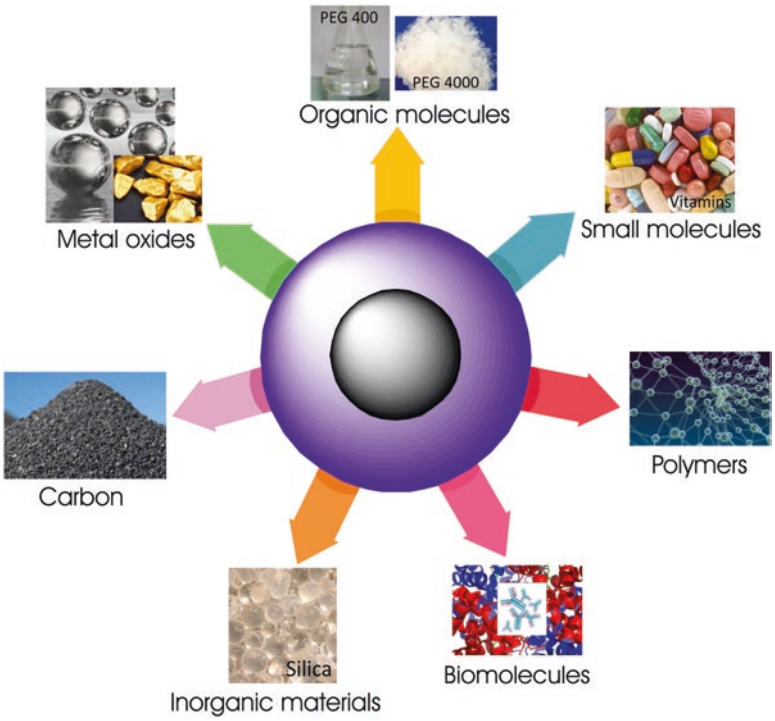

Fig. 2.3 Different materials commonly employed as coating agents for magnetic nanoparticles

2.4 Shape and Morphology

Many strategies on design and synthesis applied to magnetic nanoparticles led to four types of coated iron oxide nanosystems (Fig. 2.4).

Core shell structure consists of encapsulating a magnetic core in an inorganic or organic coating. This architecture may serve as a platform for biomolecules, becoming the most common design applied to drug targeting and gene delivery. When the magnetic core is not centered, the shape is called *"yolk structure"*. Among core shell structures, in the *inverse core shell* or also called *shell-core* the magnetic iron oxide particles act as the coating of a functional material.

In other cases, such as *matrix dispersed structure*, a matrix functions as the dispersant agent for magnetic nanoparticles to retain superparamagnetism and prevent aggregation into large clusters. *Janus structure nanoparticles* are made of two sides, a magnetic one and the other composed of a functional material. Finally, in shell-core-shell nanoparticles, the magnetic phase is located between two functional materials.

2.5 Strategies to Target by Magnetism

Magnetically responsive nanocarriers may be manipulated inside the body by external magnets. This is the principle to direct therapy to disease locations avoiding the general spread of therapeutics which cause side and undesirable effects on other tissue and organs.

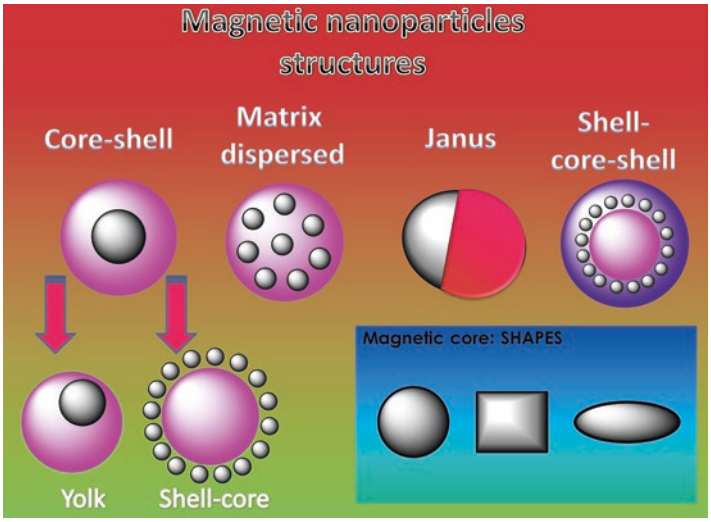

Fig. 2.4 Different structures adopted by iron oxide nanoparticles and coating and common shapes of magnetic core

Since this technology emerged as a potential therapeutic strategy nearly two decades ago, and significant advances in the field have been achieved, the application in human therapy is still a challenge.

The first trials performed in humans to target magnetic drugs to specific sites date from 1996, when Lube et al. published the clinical treatments developed in 14 cases of advanced cancer with biocompatible magnetic nanoparticles of 100 nm size loaded with epidoxorrubicin [46]. High-energy permanent magnets were used in patients, consisting of rare earths, especially neodymium as large ($8 \times 4 \times 2$ cm) and small ($3 \times 3 \times 1$ cm) blocks. The arrangements were performed according to each patient's situation of tumor. Magnetic field strengths of 0.5 and 0.8 T were reached. In general it was considered to assure a distance of 0.5 cm between tumor and magnet. Several parameters were evaluated in patients: hematological toxicity, concentration of magnetite in tumors, pharmacokinetics, and antitumor activity. The procedure resulted safe and effective for tumor located near the skin surface where the magnetic field was applied. Hematological studies resulted insufficient and there was a variable concentration of magnetite in tumors. So, the authors concluded the need to improve studies related to these topics and, moreover, to further evaluate the influence of size. Larger nanoparticules could be better attracted to the desired site under the influence of a constant magnetic field. On the other hand, other factors dependent on the patient, such as tumor blood flow and histology of tumor may influence nanoparticles distribution.

After almost 20 years, today the design of a therapy to magnetically guide nanoparticles to deep targets in human patients remains a challenge. During these years only a few clinical trials were performed. Between 2001 and 2002 a study was developed on four patients employing dual magnetic resonance imaging and a conventional angiography system [47]. Patients were sedated to alleviate discomfort from remaining in supine position during the procedure. The carrier consisted of magnetically active iron particles modified with activated carbon to reversibly bind Epidoxorubicin. The nanocarrier was administered intraarterially by the right femoral artery with the concomitant application of a small magnet (5 kg) on skin surface adjacent to the tumor over the patient's abdomen. Administration was performed by a catheter. The dose was 60 mg Epidoxorubicin-magnetic carrier (concentration of 0.7 mg/ml), administered in four steps of 15 mg at a rate of 2 mL/min. The magnet remained on the patient's skin for 15 min after administration of each dose. During the procedure, magnetic resonance images were obtained and angiography was constantly performed. In all cases both tumor size stabilization and reduction in tumor size were observed.

In 2002, another study developed by Koda et al. [48] was performed devoted to the treatment of hepatocellular carcinoma with Doxorrubicin hydrochloride adsorbed to magnetic nanocarriers on 32 patients. Single or multiple treatment cycles did not cause significant toxicity.

The last clinical study was developed in 2005 to evaluate magnetic hyperthermia as a therapeutic strategy to treat a recurrent prostate cancer [49]. This study was developed after exhaustive studies on animal models [50–52]. A suspension of magnetic nanoparticles was administered transperineally into the prostate. A magnetic field applicator for humans was used and thermometry of the prostate was performed. The treatment developed for 6 weeks and because magnetic nanoparticles remained at the site only one administration was necessary enabling sequential hyperthermia treatment without repeated application. This treatment has been long studied by the authors [53] and successfully improved [54].

Current research is focused on the design of specific magnetic carriers for specific clinical needs. Several safe and effective magnetic nanocarriers have been developed [55]. Composition, size, shape, and drugs are variables to define for the design and, especially, the disease to treat in order to improve the efficiency of the nanocarrier.

The magnet is a special feature to consider for magnetic guidance. The main reason deep targeting is still a challenge is that the magnetic field falls off quickly with distance. Thus, the design of suitable external magnets is also mandatory to achieve successful targeting in association with real time imaging. Figure 2.5 gives the confluent variables to take into account for the design of magnetic targeting therapies.

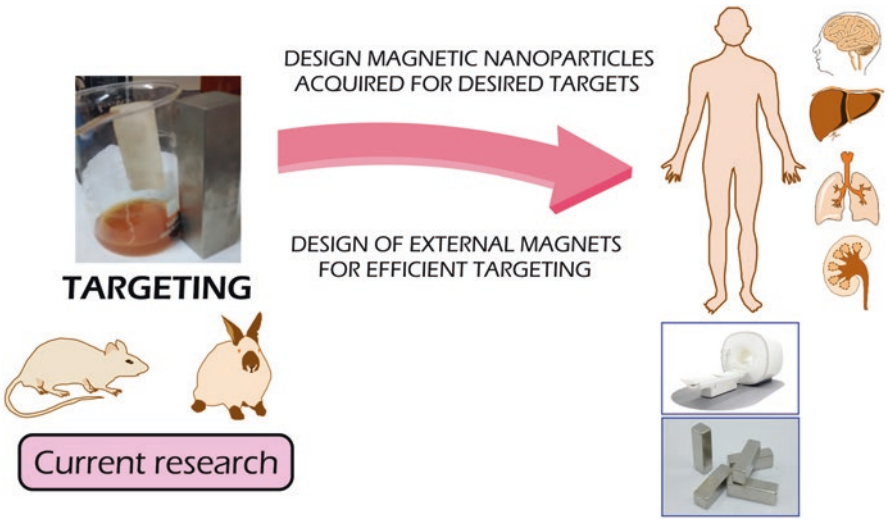

Fig. 2.5 Some features to consider in the design of magnetically guided therapy: characteristics of magnetic nanoparticles; the importance of magnet; extrapolation of results in animals to humans. The main challenge is to go on with clinical studies to implement therapies in human

References

1. Dobson, J. (2006). Magnetic nanoparticles for drug delivery. *Drug Development Research, 67*(1), 55–60.
2. Sun, C., Lee, J. S. H., & Zhang, M. (2008). Magnetic nanoparticles in MR imaging and drug delivery. *Advanced Drug Delivery Reviews, 60*(11), 1252–1265.
3. Senyei, A., Widder, K., & Czerlinski, C. (1978). Magnetic guidance of drug carrying microspheres. *Journal of Applied Physics, 49*, 3578–3583.
4. Gubin, S. P., Koksharov, Y. A., Khomutov, G. B., & Yurkov, G. Y. (2005). Magnetic nanoparticles: Preparation, structure and properties. *Russian Chemical Reviews, 74*, 489–520.
5. Frenkel, J., & Doefman, J. (1930). Spontaneous and induced magnetisation in ferromagnetic bodies. *Nature, 126*, 274–275.
6. Kittel, C. (1946). Theory of the structure of ferromagnetic domains in films and small particles. *Physics Review, 70*, 965–971.
7. Mørup, S., Hansen, M. F., & Frandsen, C. (2011). Magnetic nanoparticles. In G. Scholes, G. Wiederrecht, & D. Andrews (Eds.), *Comprehensive Nanoscience and Technology* (pp. 437–491). Amsterdam, The Netherlands: Elsevier.
8. Bean, C. P., & Livingston, J. D. (1959). Superparamagnetism. *Journal of Applied Physics, 30*, 120S–129S.
9. Bedanta, S., & Kleemann, W. (2009). Supermagnetism. *Journal of Physics D, 42*, 013001.
10. Chouly, C., Pouliquen, D., Lucet, I., Jeune, J., & Jallet, P. (1996). Development of superparamagnetic nanoparticles for MRI: Effect of particle size, charge and surface nature on biodistribution. *Journal of Microencapsulation, 13*, 245–255.
11. Jain, T. K., Reddy, M. K., Morales, M. A., et al. (2008). Biodistribution, clearance, and biocompatibility of iron oxide magnetic nanoparticles in rats. *Molecular Pharmaceutics, 5*(2), 316–327.
12. Mojica Pisciotti, M. L., Lima, E., Jr., Vasquez Mansilla, M. M., et al. (2014). In vitro and *in vivo* experiments with iron oxide nanoparticles functionalized with DEXTRAN or polyethylene glycol for medical applications: Magnetic targeting. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 102*, 860–868.
13. Agotegaray, M., Campelo, A., Zysler, R., Gumilar, F., Bras, C., Minetti, A., et al. (2016). Influence of chitosan coating on magnetic nanoparticles in endothelial cells and acute tissue biodistribution. *Journal of Biomaterials Science*. doi:10.1080/09205063.2016.1170417.
14. Aggarwal, P., Hall, J. B., McLeland, C. B., et al. (2009). Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeuic efficacy. *Advanced Drug Delivery Reviews, 61*, 428–437.
15. Papisov, M. I., Bogdanov, A., Jr., Schaffer, B., et al. (1993). Colloidal magnetic resonance contrast agents: Effect of particle surface on biodistribution. *Journal of Magnetism and Magnetic Materials, 122*, 383–386.
16. Cornell, R. M., & Schwertmann, U. (2003). *The iron oxides: Structures, properties, reactions, occurences and used*. Weinheim: Wiley.
17. Pereira, C., et al. (2012). Superparamagnetic MFe_2O_4 (M = Fe, Co, Mn) nanoparticles: Tuning the particle size and magnetic properties through a novel one-step coprecipitation route. *Chemistry of Materials, 24*, 1496.
18. Niendorf, T., Brenne, F., Hoyer, P., Schwarze, D., Schaper, M., Grothe, R., et al. (2015). Processing of new materials by additive manufacturing: Iron-based alloys containing silver for biomedical applications. *Metallurgical and Materials Transactions A, 46*(7), 2829–2833.
19. Massart, R. (1981). Preparation of aqueous magnetic liquids in alkaline and acidic media. *IEEE Transactions on Magnetics, 17*, 1247.
20. Wu, W., He, Q. G., Hu, R., Huang, J. K., & Chen, H. (2007). Preparation and characterization of magnetite Fe_3O_4 nanopowders. *Rare Metals Materials And Engineering, 36*, 238.
21. Wu, S., Sun, A. Z., Zhai, F. Q., Wang, J., Xu, W. H., Zhang, Q., et al. (2011). Fe_3O_4 magnetic nanoparticles synthesis from tailings by ultrasonic chemical co-precipitation. *Materials Letters, 65*, 1882.

22. Mascolo, M. C., Pei, Y., & Ring, T. A. (2013). Room temperature co-precipitation synthesis of magnetite nanoparticles in a large pH window with different bases. *Materials, 6*(12), 5549–5567.
23. Azcona, P., Zysler, R., & Lassalle, V. (2016). Simple and novel strategies to achieve shape and size control of magnetite nanoparticles intended for biomedical applications. *Colloid Surface A, 504*, 320–330.
24. Nicolás, P., Saleta, M., Troiani, H., Zysler, R., Lassalle, V., & Ferreira, M. L. (2013). Preparation of iron oxide nanoparticles stabilized with biomolecules: Experimental and mechanistic issues. *Acta Biomaterialia, 9*(1), 4754–4762.
25. Arsalani, N., Fattahi, H., & Nazarpoor, M. (2010). Synthesis and characterization of PVP-functionalized superparamagnetic Fe_3O_4 nanoparticles as an MRI contrast agent. *Express Polymer Letters, 4*(6), 329–338.
26. Sun, S., & Zeng, H. (2002). Size-controlled synthesis of magnetite nanoparticles. *Journal of the American Chemical Society, 124*, 8204.
27. Li, Z., Tan, B., Allix, M., Cooper, A. I., & Rosseinsky, M. J. (2008). Direct coprecipitation route to monodisperse dualfunctionalized magnetic iron oxide nanocrystals without size selection. *Small, 4*, 231.
28. Wu, W., He, Q. G., & Jiang, C. Z. (2008). Magnetic iron oxide nanoparticles: Synthesis and surface functionalization strategies. *Nanoscale Research Letters, 3*, 397.
29. Walton, R. I. (2002). Subcritical solvothermal synthesis of condensed inorganic materials. *Chemical Society Reviews, 31*, 230.
30. Pandey, S., & Mishra, S. (2011). Sol–gel derived organic–inorganic hybrid materials: Synthesis, characterizations and applications. *Journal of Sol-Gel Science and Technology, 59*, 73.
31. Caruntu, D., Caruntu, G., & O'Connor, C. J. (2007). Magnetic properties of variable-sized Fe_3O_4 nanoparticles synthesized from non-aqueous homogeneous solutions of polyols. *Journal of Physics D: Applied Physics, 40*, 5801.
32. Okoli, C., Sanchez-Dominguez, M., Boutonnet, M., Järås, S., Civera, C., Solans, C., et al. (2012). Comparison and functionalization study of microemulsion-prepared magnetic iron oxide nanoparticles. *Langmuir, 28*, 8479.
33. Xu, H., Zeiger, B. W., & Suslick, K. S. (2013). Sonochemical synthesis of nanomaterials. *Chemical Society Reviews, 42*, 2555.
34. Hu, L., Percheron, A., Chaumont, D., & Brachais, C. H. (2011). Microwave-assisted one-step hydrothermal synthesis of pure iron oxide nanoparticles: Magnetite, maghemite and hematite. *Journal of Sol-Gel Science and Technology, 60*, 198.
35. Jiang, F. Y., Wang, C. M., Fu, Y., & Liu, R. C. (2010). Synthesis of iron oxide nanocubes via microwave-assisted solvolthermal method. *Journal of Alloys and Compounds, 503*, L31.
36. Prathna, T. C., Mathew, L., Chandrasekaran, N., Raichur, A. M., & Mukherjee, A. (2010). Biomimetic synthesis of nanoparticles: Science, technology & applicability. In A. Mukherjee (Ed.), *Biomimetics learning from nature*. Rijeka: InTech.
37. Starowicz, M., Starowicz, P., Żukrowski, J., Przewoźnik, J., Lemański, A., Kapusta, C., et al. (2011). Electrochemical synthesis of magnetic iron oxide nanoparticles with controlled size. *Journal of Nanoparticle Research, 13*, 7167.
38. Salazar-Alvarez, G., Muhammed, M., & Zagorodni, A. A. (2006). Novel flow injection synthesis of iron oxide nanoparticles with narrow size distribution. *Chemical Engineering Science, 61*, 4625.
39. Costo, R., Bello, V., Robic, C., Port, M., Marco, J. F., Puerto Morales, M., et al. (2011). Ultrasmall iron oxide nanoparticles for biomedical applications: Improving the colloidal and magnetic properties. *Langmuir, 28*, 178.
40. Sayed, F. N., & Polshettiwar, V. (2015). Facile and sustainable synthesis of shaped iron oxide nanoparticles: Effect of iron precursor salts on the shapes of iron oxides. *Scientific Reports, 5*, 9733.
41. Santra, S., Tapec, R., Theodoropoulou, N., Dobson, J., Hebard, A., & Tan, W. H. (2001). Synthesis and characterization of silica-coated iron oxide nanoparticles in microemulsion: The effect of nonionic surfactants. *Langmuir, 17*, 2900.

42. Yang, H. H., Zhang, S. Q., Chen, X. L., Zhuang, Z. X., Xu, J. G., & Wang, X. R. (2004). Magnetite-containing spherical silica nanoparticles for biocatalysis and bioseparations. *Analytical Chemistry, 76*, 1316.
43. Barbeta, V. B., Jardim, R. F., Kiyohara, P. K., Effenberger, F. B., & Rossi, L. M. (2010). Magnetic properties of Fe_3O_4 nanoparticles coated with oleic and dodecanoic acids. *Journal of Applied Physics, 107*, 073913.
44. Song, M., Zhang, Y., Hu, S., Song, L., Dong, J., Chen, Z., et al. (2012). Influence of morphology and surface exchange reaction on magnetic properties of monodisperse magnetite nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 408*, 114.
45. Okuda, M., Eloi, J.-C., Jones, S. E. W., Sarua, A., Richardson, R. M., & Schwarzacher, W. (2012). Fe_3O_4 nanoparticles: Protein-mediated crystalline magnetic superstructures. *Nanotechnology, 23*, 415601.
46. Lübbe, A. S., Bergemann, C., Riess, H., Schriever, F., Reichardt, P., Possinger, K., et al. (1996). Clinical experiences with magnetic drug targeting: A phase I study with 4′-epidoxorubicin in 14 patients with advanced solid tumors. *Cancer Research, 56*(20), 4686–4693.
47. Wilson, M. W., Kerlan, R. K., Jr., Fidelman, N. A., Venook, A. P., LaBerge, J. M., Koda, J., et al. (2004). Hepatocellular carcinoma: Regional therapy with a magnetic targeted carrier bound to doxorubicin in a dual MR imaging/conventional angiography suite—Initial experience with four patients. *Radiology, 230*(1), 287–293.
48. Koda, J., Venook, A., Walser, E., & Goodwin, S. (2002). A multicenter, phase I/II trial of hepatic intra-arterial delivery of doxorubicin hydrochloride adsorbed to magnetic targeted carriers in patients with hepatocellular carcinoma. *European Journal of Cancer, 38*, S18.
49. Johannsen, M., Gneveckow, U., Eckelt, L., Feussner, A., Waldöfner, N., Scholz, R., et al. (2005). Clinical hyperthermia of prostate cancer using magnetic nanoparticles: Presentation of a new interstitial technique. *International Journal of Hyperthermia, 21*, 637–647.
50. Johannsen, M., Jordan, A., Scholz, R., Lein, M., Koch, M., Deger, S., et al. (2004). Evaluation of magnetic fluid hyperthermia in a standard rat model of prostate cancer. *Journal of Endourology, 18*, 495–500.
51. Johannsen, M., Thiesen, B., Jordan, A., Taymoorian, K., Gneveckow, U., Waldöfner, N., et al. (2005). Magnetic fluid hyperthermia (MFH) reduces prostate cancer growth in the orthotopic Dunning R3327 rat model. *Prostate, 64*, 283–292.
52. Johannsen, M., Thiesen, B., Gneveckow, U., Taymoorian, K., Waldöfner, N., Scholz, R., et al. (2006). Thermotherapy using magnetic nanoparticles combined with external radiation in an orthotopic rat model of prostate cancer. *Prostate, 66*, 97–104.
53. Johannsen, M., Gneveckow, U., Thiesen, B., Taymoorian, K., Cho, C. H., Waldöfner, N., et al. (2007). Thermotherapy of prostate cancer using magnetic nanoparticles: Feasibility, imaging and three-dimensional temperature distribution. *European Urology, 52*, 1653–1661.
54. Johannsen, M., Thiesen, B., Wust, P., & Jordan, A. (2010). Magnetic nanoparticle hyperthermia for prostate cancer. *International Journal of Hyperthermia, 26*(8), 790–795.
55. Hola, K., Markova, Z., Zoppellaro, G., Tucek, J., & Zboril, R. (2015). Tailored functionalization of iron oxide nanoparticles for MRI, drug delivery, magnetic separation and immobilization of biosubstances. *Biotechnology Advances, 33*(6), 1162–1176.

Chapter 3
Silica: Chemical Properties and Biological Features

Abstract Silicon dioxide, SiO_2, is commonly known as silica. It may be found polymerized alone or in combination with other metals known as silicates.

This Chapter focuses on the biological, physiological, and biomedical issues related to silica. Although it was, in principle, considered as a highly toxic compound, this belief was reverted when several benign natural properties were discovered. In fact, silicon is actually considered as an essential trace element, being the most abundant in the body after iron and zinc. Therefore, several types of silica based materials are actually recognized as highly efficient in several biomedical applications. Among them bioglasses, star gels, mesoporous materials, and solid silica nanoparticles are found. The main applications range from drug delivery systems, target drug delivery, tissue regeneration, and diagnosis. All these applications already require a strict control over the properties of the designed silica materials.

Keywords Silica • Bioavailability • Biomedical applications • Drug delivery

3.1 Generalities of Silica

Silicon (Si) is a chemical element characterized as a tetravalent metalloid. It represents the eighth most common element in the Universe in terms of mass. However, it rarely occurs as pure form in the Earth's crust because silicon does not exists as pure mineral due to facile reaction with oxygen and water in ambient. In our planet the oxide of silicon is widely distributed—26 % of the crust and the second most abundant compound after oxygen. Silicon dioxide, SiO_2, is known as silica. It can be found polymerized alone or in combination with other metals known as silicates. In nature it commonly occurs as quartz, being the largest constituent of sand. Other crystalline forms of SiO_2 are also found such as tridimite and cristobalite and it also exists in amorphous forms. It is also found in the skeleton of some living organisms. For example, siliceous sponges (the Demospongiae and the Hexactinellida) are metazoans which inorganic skeleton is formed by amorphous silica. The enzyme responsible for the formation of this structure is known as silicatein [1] (Fig. 3.1).

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_3

Fig. 3.1 Different forms of naturally occurring silica

Table 3.1 Water soluble forms of silica

Si acid form	Formula
Ortho	H_4SiO_4
Meta	H_2SiO_3
Di	$H_2Si_2O_5$
Pyro	$H_6Si_2O_7$

Silicic acid, $Si(OH)_4$ is the biologically relevant form because of its water solubility which enables absorption. There are different water soluble forms of silica (Table 3.1).

The association of silicic acid with anions occurs to balance the charge and to stabilize the acidic molecule. Depending on pH chemical interactions may be affected. By this, at pH lower than 9, silicic acid is predominant (H_4SiO_4). At low pH the acid tends to polymerize and form colloidal silica. When pH is beyond 8 bisilicate anions are formed such as $H_3SiO_4^-$ and $H_2SiO_4^{2-}$.

Synthetic compounds from silica have also been obtained which find applications in several fields such as computer chips, paints, rubber ceramics, metallurgical industry, silicon photovoltaic systems, and even in medicine and tissue engineering as silicone implants. It is important to mention that it is mandatory to not confuse silicon with silicone. Silicone corresponds to organosiloxane synthetic polymers based on alternating oxygen and silicon.

3.2 Biological Features of Silica

For a long time the research on silica and derivatives was focused on their toxic effects. These were due to inhalation of dust minerals containing silica, responsible for silicosis which is an inflammatory disease of the lungs causing fibrosis and chronic dyspnoea. Oral toxicity is also possible by ingestion of crystalline or amorphous silica. Toxicology of silica is currently associated with its crystalline form, but there are multiple non toxic forms in nature.

Currently, silicon is considered as a trace element essential for life in humans, being the most abundant in the body after iron and zinc. Biological studies related to the importance of silicon in biology started in the 1970s, revealing that dietary deficiency of silicon would cause alterations in connective tissue and skeletal system [2–4]. Bone and skin health depend directly on silicon presence [5].

Mucopolysaccharides are carbohydrate based compounds which form the base of bone and all collagenous tissues in the body. Silicon is an essential trace element in the composition of these systems and its presence is mandatory in the formation of all structural connections or architecture in these tissues as well as in the strength and stability. Tissues where silicon is found in high concentrations are skeleton, blood vessels, heart, muscles, skin, hair, ligaments, and cartilage. It is also found in liver, lungs, and brain. Nails are tissues where the highest concentration of silicon if found. Other functions developed by silicon are related to tissue healing, transfer of nutrients, and water in biochemical regulatory reactions in connective tissues and it also takes part in embryonic development and growth. Table 3.2 lists functions of silicon in the human body as well as effects of deficiency.

3.3 Diet and Silicon

Numerous sources of silica contribute to human contact or intake such as dust, pharmaceuticals, cosmetics, and biomedical devices with diet being the main source.

Cereals contribute 30 % of silicon in the diet, followed by fruits, drinks (cold or hot due to water presents high content), and vegetables. Together these foods provide more than 75 % of the daily intake of silicon (Fig. 3.2).

Table 3.2 Functions of silicon in human body and signs of deficiency

Functions	Effects of deficiency
• Formation and maintenance of connective tissue and skeletal architecture • Regulation of biochemical reactions in connective tissues • Regulation in the pathway of vitamin B1 • Bone formation • Regulation of tissue healing (prevention from atherosclerosis and heart disease; regulation of ulcers and joint and cartilage harm)	• Osteoporosis • Premature aging • Abnormal skeletal formation • Atherosclerosis • Heart disease

Fig. 3.2 Nutritional food pyramid indicating, according to content-rich silicon

3.4 Bioavailability and Absorption of Silicon

Chemical structure plays an important role in bioavailability and absorption of silicon based compounds. The grade of polymerization determines the grade of absorption in the intestine, monomeric silica as orthosilicic acid [$Si(OH)_4$] is the absorbable form in the human intestine [6].

Absorption also is related to solubility in the intestinal lumen. At intestinal pH, the solubility of silica is about 2–3 mmol/L [7]. In the common sources such as water and beverages in general silicon concentrations are lower than 2 mM. In these concentrations the monomeric species are predominant, which are easily absorbable [8].

Supplements present a concentration of silicon higher than absorbable ones. In general, at these quantities the forms are less absorbable polymers and colloids. So, it is important to consider when taking supplements the content of additives such as choline to improve solubility or to maintain the monomeric form and thus, the intestinal absorption [9]. The function of choline in silicon supplements is to prevent silica from polymerization and aggregation. Colloidal silica as supplement is not well absorbed because of the presence of particulate silica [10]. So, it is important to consider the form in which silicon is present in food and supplements to better understand absorption and bioavailability.

The uptake of silicon decreases with aging [11]. Both silicon uptake and metabolism are hormonally controlled in humans [12]. It is well established that silicon excretion occurs throw kidneys [13].

3.5 Different Types of Silica with Importance in Biomedicine

3.5.1 Bioglasses

Silica-based materials are of great interest in biomedicine. Silica glasses are bioactive materials widely studied for tissue healing and bone repair. Bioactive materials are those which are able to be incorporated into living tissue [14]. In the case of silica, silanol groups may be responsible for this property acting as the nucleation site [15, 16].

A glass consists of a three dimensional network structured substance which lacks in periodicity with the energy comparable to the corresponding crystal network. In other words, it may be define as a solid with liquid structure [17, 18]. Silicate glasses, composed of SiO_4 tetrahedra may present amorphicity depending on the way in which the oxygen ion links two tetrahedral structures. In the case of amorphous silica, it can form an open structure where other cations can be included giving rise to a wide range of silica glasses with specific applications in biomedicine. The modifiers impart reactivity in physiological medium. Therefore, silica glasses are plausible to use in bone regeneration and periodontal repair since hydroxyapatite formation may be induced. Even the mechanism of apatite formation has not yet been elucidated; the presence and exposure of silanol groups from ceramic glasses seems to be necessary as well as the porosity of the material.

3.5.2 Star Gels

Star gels are described as organic-inorganic hybrid materials formed by an organic core which is surrounded by inorganic structures with final alkoxysilane groups [19]. The intermediate behavior between glasses and rubbers makes these materials ideal for bioactive applications in tissue repair. They can be produced with homogeneous structures and better mechanical properties in comparison to common silicate glasses or ceramics.

The above mentioned materials find applications in periodontal repair and bone regeneration due to the solid-like properties. Drug delivery and targeting are biomedical applications greatly studied and with promising features, and they are the focus of this book. So, specific silica materials are described as biomaterials for pharmacological purposes as follows.

3.5.3 Mesoporous Silica

Ordered mesoporous silica materials consist of structures where SiO_4 tetrahedra arrangements form porous structures feasible to be loaded with drugs. The size of the pores can be controlled, as well as the homogeneity of the structure, leading to

a wide range of possibilities to obtain biocompatible materials for hosting molecules to diverse applications in drug delivery. Different synthetic strategies can be performed to obtain desired mesoporous silica-based materials, especially in the nanoscale. In general, mesoporous silica nanoparticles synthesis consists of a template-directed method employing a surfactant which acts as a structure-directing template [20, 21].

Three well known mesoporous silica frameworks exist.

- MCM-41 (Mobile Crystalline Material) consisting of a two-dimensional hexagonal planar structure, which belongs to the symmetry group *p6mm* with pores around 2 nm [22].
- SBA-15 (Santa Barbara Amorphous), presenting a similar structure as MCM-41 with pores around 10 nm [23].
- MCM-48 is a three-dimensional cubic framework with symmetry group Ia3*d*, presenting pores of near 3 nm [22].

Pore size of these materials is in the range of the molecular size of drugs. This fact turns ordered mesoporous silica into drug carriers able to absorb molecules on silanol groups exposed in the mesopore wall. Functionalization of silanol groups enables one to tune the pores and impart specific characteristics depending on the desired drug or molecule to transport.

The first reports regarding biomedical applications of MCM-14 date from 2001, when it was first evaluated as a drug delivery system [24]. From these studies, several research studies involving synthesis, characterization, in vitro, and in vivo evaluation were developed [25].

Figure 3.3 presents the major benefits of mesoporous silica as a material for drug delivery.

Regarding drug delivery, mesoporous silica finds specific applications leading to the development of diverse types of devices.

- *Mesoporous silica immediate drug delivery systems* (*IDDSs*)
- Mesoporous silica enables bioavailability of hydrophobic drugs leading to improved dissolution and absorption of this type of drug after oral dosage [26, 27].
- *Sustained drug delivery systems* (*SDDSs*)
- Sometimes depending on the drug implied and in the necessity of specific treatments, a long-term drug release is required. Mesoporous silica based materials may be employed as sustained drug delivery systems. There exist non-modified and modified silica based materials for these purposes. The sustained delivery by unmodified mesoporous silica may occur by controlling pore size and structure as well as the particle size of the material. Modified silica can be achieved by employing linkers with specific functional groups to conjugate the drug to the surface pore. This strategy enables sustained release in time [28].
- *Stimuli-responsive controlled drug delivery systems*
- Drug release from mesoporous silica nanoparticles has been overviewed in order to provide different strategies to control delivery. The main pathway proposed

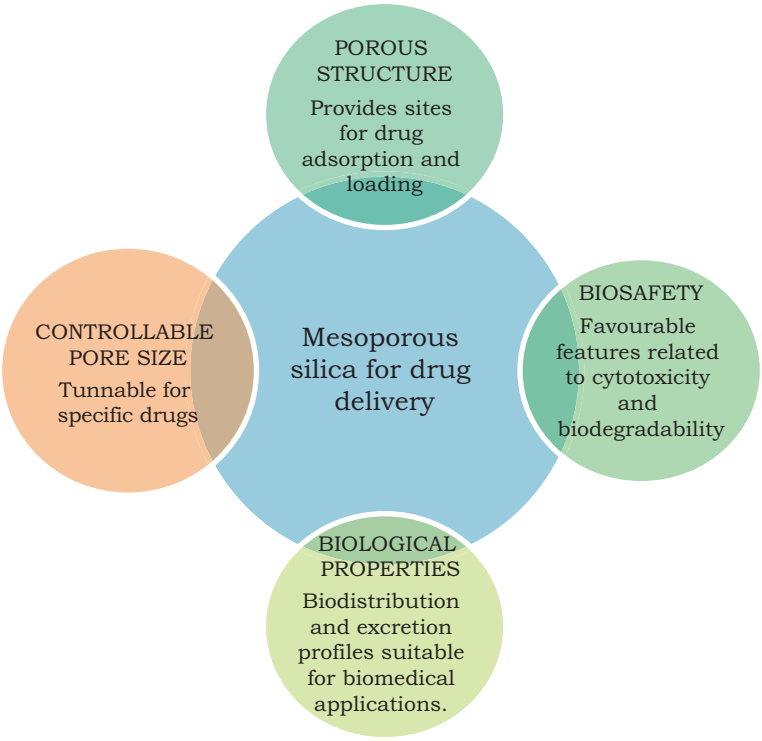

Fig. 3.3 Advantages of mesoporous silica as drug delivery systems and for active biomaterials

for this kind of system consists of keeping the drug within the pore until an external stimulus triggers its output. Figure 3.4 shows the different types of gatekeepers and stimuli to drug release.

- *Targeted drug delivery systems*
- Various strategies have resulted in the targeting of mesoporous silica nanoparticles. Enhanced permeation and retention strategy comes from the control of size and shape, leading to accumulation of the nanoparticles in desired tissues, such as tumors [29].
- Targeted delivery can also be achieved by the attachment to the silica surface of specific targeting ligands such as folate, endothelial growth factor (EGF), peptides or antibodies [30]. Conjugation of magnetic materials is another strategy to target mesoporous silica nanoparticles to a desired site in the body by the influence of an external magnetic field [31, 32].

The combination of different strategies leads to multifunctional drug delivery systems. These devices possess both targeting and stimuli-responsive abilities, so the drug is not released until the arrival at the specific site where the system finds proper conditions to open gatekeepers [33, 34].

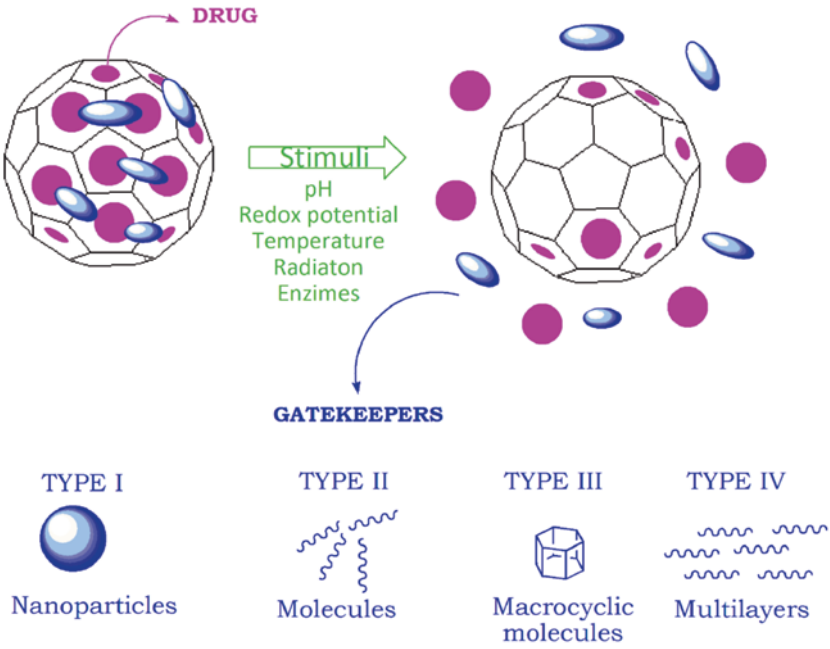

Fig. 3.4 Schematic representation of stimuli-responsive controlled drug delivery systems. Different types of stimuli and gatekeepers

Even with all existing studies on the potential applications of mesoporous silica in the biomedical field, practical consideration is still necessary regarding the impact in vivo of these devices. Beyond the in vitro and in vivo studies, it is necessary to establish specific research on mesoporous silica systems that correlate size, porous structure, biodegradability, and toxicology in living organisms to achieve concrete clinical implementation [35].

3.6 Solid Silica Nanoparticles

Tunable surface chemistry, ease of preparation, biocompatibility, and good transparency are some features regarding the use of solid silica as a biomaterial [36]. The research on solid/dense silica has emerged in the last decade because of diverse applications of these materials: catalysis, pigments, humidity sensors, electronics, photonics, Raman scattering, pharmacy, and biomedicine.

The pioneering studies of Stöber and co-workers established the base for the world of silica nanoparticles by employing sol-gel chemistry to obtain spherical silica nanoparticles [37]. The synthesis implies the dissolution of silicon alkoxides (tetraethylothosilicate, TEOS) in aqueous alcohol solution in the presence of basic medium. To this purpose ammonia is the alkali employed. From this research

diverse studies were developed to introduce modifications in order to improve the quality of the obtained silica nanoparticles and also to functionalize the surface for the desired purposes. In this sense, the above mentioned conditions were carefully explored finding that the size of the particles depends on the type of alkoxide used as well as on the alcohol and the alkali. For example, Rao et al. developed new methodology to obtain monodisperse silica nanoparticles from the implementation of ultrasound during the synthesis procedure. Several experimental conditions were evaluated, confirming that the type of reagents and the concentrations influence the size of the nanoparticles [38].

Solid silica coating around metal nanoparticles has become a very interesting issue regarding the potential uses of the new materials. The first coating with solid silica was developed by Liz-Marzan and co-workers [39]. It consisted of the silica coating on citrate reduced gold nanoparticles with (3-aminopropyl) trimethoxysilane and later hydrolysis/condensation on amino (NH_2) groups of silica, employing TEOS as described by Stöber.

Surface functionalization of silica nanoparticles with diverse groups such as amino-, mercapto-, carboxy- among others makes these nanodevices special for biomedical applications considering the wide range of possibilities in terms of the diversity of molecules that can be incorporated. Figure 3.5 illustrates many applications of solid silica in medicine.

The targeting of silica nano-materials to a specific site in the body is a topic of current research. Magnetic nanoparticles functionalized with silica and silica nanoparticles decorated with magnetic nanoparticles are the focus of exhaustive studies considering the possibility of external control by a magnetic field. The convergence of biocompatibility and rich chemistry to incorporate a wide range of molecules make these devices ideal for biomedical purposes—especially, drug targeting.

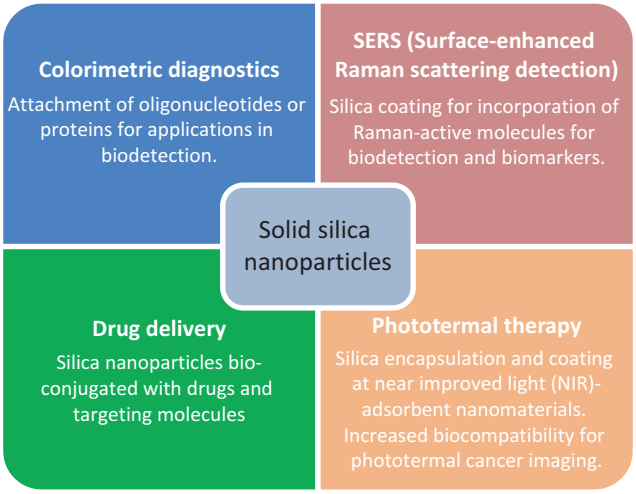

Fig. 3.5 Applications of solid silica nanoparticles in medicine

In the next chapters, attention is centered on solid silica magnetic nanoparticles for drug delivery: from synthesis to biomedical features.

References

1. Müller, W. E., Wang, X., Chen, A., Hu, S., Gan, L., Schröder, H. C., et al. (2011). The unique invention of the siliceous sponges: Their enzymatically made bio-silica skeleton. In *Molecular biomineralization* (pp. 251–281). Berlin: Springer.
2. Schwarz, K., & Milne, D. B. (1972). Growth promoting effects of silicon in rats. *Nature, 239*, 333–334.
3. Carlisle, E. M. (1986). Silicon as an essential trace element in animal nutrition. In D. Evered & M. O'Connor (Eds.), *Silicon biochemistry. Ciba Foundation Symposium* (Vol. 121, pp. 123–139). Chichester: John Wiley and Sons Ltd.
4. Carlisle, E. M. (1972). Silicon: An essential element for the chick. *Science, 178*, 619.
5. Jugdaohsingh, R. (2007). Silicon and bone health. *The Journal of Nutrition, Health & Aging, 11*, 99–110.
6. Reffitt, D. M., Jugdaohsingh, R., Thompson, R. P. H., et al. (1999). Silicic acid: Its gastrointestinal uptake and urinary excretion in man and effects on aluminium excretion. *Journal of Inorganic Biochemistry, 76*, 141–147.
7. Iler, R. K. (1979). *The chemistry of silica: Solubility, polymerisation, colloid and surface properties, and biochemistry*. New York: John Wiley & Sons.
8. Sripanyakorn, S., Jugdaohsingh, R., Elliott, H., Walker, C., Mehta, P., Shoukru, S., et al. (2004). The silicon content of beer and its bioavailability in healthy volunteers. *British Journal of Nutrition, 91*, 403–409.
9. Allain, P., Cailleux, A., Mauras, Y., et al. (1983). Digestive absorption of silicon after a single administration in man in the form of methylsilanetriol salicylate (in French). *Thérapie, 38*, 171–174.
10. Sripanyakorn, S., Jugdaohsingh, R., Dissayabutr, W., Anderson, S. H., Thompson, R. P., & Powell, J. J. (2009). The comparative absorption of silicon from different foods and food supplements. *British Journal of Nutrition, 102*(06), 825–834.
11. Anonymous. (1974). *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents anticaking agents. Silicon dioxide and certain silicates* (pp. 21–30). Geneva: World Health Organisation.
12. Charnot, Y., & Pérès, G. (1971). Modification de l'absorption et du métabolisme tissulaire du silicium en relation avec l'age, le sehe et diverses glandes endocrines. *Lyon Médical, 13*, 85.
13. Dobbie, J. W., & Smith, M. J. B. (1986). Urinary and serum silicon in normal and uraemic individuals. In D. Evered & M. O'Connor (Eds.), *Silicon biochemistry. Ciba Foundation Symposium* (Vol. 121, pp. 194–208). Chichester: John Wiley and Sons Ltd.
14. Vallet-Regí, M., & Balas, F. (2008). Silica materials for medical applications. *Open Biomedical Engineering Journal, 2*(1), 1–9.
15. Vallet-Regí, M. (2006). Revisiting ceramics for medical applications. *Dalton Transactions,* 5211–5220.
16. Vallet-Regí, M., Ragel, C. V., & Salinas, A. J. (2003). Glasses with medical applications. *European Journal of Inorganic Chemistry, 2003*, 1029–1042.
17. Zachariasen, W. H. (1932). The atomic arrangements in glass. *Journal of the American Chemical Society, 54*, 3841–3851.
18. Harper, C. A. (2001). *Handbook of ceramics, glasses and diamonds*. Blacklick: McGraw-Hill Professional Pub.
19. Vallet-Regí, M., Salinas, A. J., & Arcos, D. (2006). From the bioactive glasses to the star gels. *Journal of Materials Science: Materials in Medicine, 17*(11), 1011–1017.

20. Cai, Q., Lin, W.-Y., Xiao, F.-S., Pang, W.-Q., Chen, X.-H., & Zou, B.-S. (1999). The preparation of highly ordered MCM-41 with extremely low surfactant concentration. *Microporous and Mesoporous Materials, 32*, 1–15.
21. Wu, S. H., Mou, C. Y., & Lin, H. P. (2013). Synthesis of mesoporous silica nanoparticles. *Chemical Society Reviews, 42*(9), 3862–3875.
22. Beck, J. S., Vartuli, J. C., Roth, W. J., Leonowicz, M. E., Kresge, C. T., Schmitt, K. D., et al. (1992). A new family of mesoporous molecular sieves prepared with liquid crystal templates. *Journal of the American Chemical Society, 114*, 10834–10843.
23. Zhao, D. Y., Feng, J. L., Huo, Q. S., Melosh, N., Fredricksson, G. H., Chmelka, B. F., et al. (1998). Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 angstrom pores. *Science, 279*, 548–552.
24. Vallet-Regi, M., Rámila, A., del Real, R. P., & Pérez-Pariente, J. (2001). A new property of MCM-41: Drug delivery system. *Chemistry of Materials, 13*, 308–311.
25. Wang, Y., Zhao, Q., Han, N., Bai, L., Li, J., Liu, J., et al. (2015). Mesoporous silica nanoparticles in drug delivery and biomedical applications. *Nanomedicine: Nanotechnology, Biology and Medicine, 11*(2), 313–327.
26. Hu, Y., Zhi, Z., Wang, T., Jiang, T., & Wang, S. (2011). Incorporation of indomethacin nanoparticles into 3-D ordered macroporous silica for enhanced dissolution and reduced gastric irritancy. *European Journal of Pharmaceutics and Biopharmaceutics, 79*, 544–551.
27. Wang, Y., Sun, L., Jiang, T., Zhang, J., Zhang, C., Sun, C., et al. (2014). The investigation of MCM-48-type and MCM-41-type mesoporous silica as oral solid dispersion carriers for water insoluble cilostazol. *Drug Development and Industrial Pharmacy, 40*, 819–828.
28. Bossaert, W. D., De Vos, D. E., Van Rhijn, W. M., Bullen, J., Grobet, P. J., & Jacobs, P. A. (1999). Mesoporous sulfonic acids as selective heterogeneous catalysts for the synthesis of monoglycerides. *Journal of Catalysis, 182*, 156–164.
29. Tang, L., Gabrielson, N. P., Uckun, F. M., Fan, T. M., & Cheng, J. (2013). Size-dependent tumor penetration and in vivo efficacy of monodisperse drug–silica nanoconjugates. *Molecular Pharmaceutics, 10*, 883–892.
30. Pan, L., He, Q., Liu, J., Chen, Y., Ma, M., Zhang, L., et al. (2012). Nuclear-targeted drug delivery of TAT peptide-conjugated monodisperse mesoporous silica nanoparticles. *Journal of the American Chemical Society, 134*, 5722–5725.
31. Zhu, Y., Ikoma, T., Hanagata, N., & Kaskel, S. (2010). Rattle-type Fe_3O_4@ SiO_2 hollow mesoporous spheres as carriers for drug delivery. *Small, 6*, 471–478.
32. Huang, S., Yang, P., Cheng, Z., Li, C., Fan, Y., Kong, D., et al. (2008). Synthesis and characterization of magnetic Fe×O y@ SBA-15 composites with different morphologies for controlled drug release and targeting. *Journal of Physical Chemistry C, 112*, 7130–7137.
33. Chen, Z., Li, Z., Lin, Y., Yin, M., Ren, J., & Qu, X. (2013). Biomineralization inspired surface engineering of nanocarriers for pH-responsive, targeted drug delivery. *Biomaterials, 34*, 1364–1371.
34. Chan, M. H., & Lin, H. M. (2015). Preparation and identification of multifunctional mesoporous silica nanoparticles for in vitro and in vivo dual-mode imaging, theranostics, and targeted tracking. *Biomaterials, 46*, 149–158.
35. Lin, Y. S., Hurley, K. R., & Haynes, C. L. (2012). Critical considerations in the biomedical use of mesoporous silica nanoparticles. *Journal of Physical Chemistry Letters, 3*(3), 364–374.
36. Halas, N. J. (2008). Nanoscience under glass: The versatile chemistry of silica nanostructures. *ACS Nano, 2*(2), 179–183.
37. Stöber, W., Fink, A., & Bohn, E. (1968). Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science, 26*, 62–69.
38. Rao, K. S., El-Hami, K., Kodaki, T., Matsushige, K., & Makino, K. (2005). A novel method for synthesis of silica nanoparticles. *Journal of Colloid and Interface Science, 289*(1), 125–131.
39. Liz-Marzán, L. M., Giersig, M., & Mulvaney, P. (1996). Synthesis of nanosized gold-silica core-shell particles. *Langmuir, 12*(18), 4329–4335.

Chapter 4
Synthesis of Solid Silica-Coated Magnetic Nanoparticles for Drug Targeting

Abstract Coating of magnetic nanoparticles is strongly required in order to obtain nanocarriers with suitable properties in terms of stability (low aggregation in aqueous media), surface functionality, and magnetism. Silica appears as an attractive compound to assess these goals. Among preventing aggregation, it is able to provide biocompatibility and the easy linkage of multiple ligands to specific applications.

Methodologies adopted to incorporate a silica layer on a magnetic core are varied; among them the Stöber method is the most widely employed. To a lesser extent, microemulsion, sodium silicate hydrolysis methods, sonochemical method among others are usually used for the synthesis.

From the above mentioned procedures it is feasible to prepare magnetic silica coated nanoparticles or even other kinds of magnetic silica materials. These features are achieved by simply modifying the experimental variables inherent to each method.

A comparison between these methodologies leads to the most adequate preparation technique as a function of the intended applications.

Keywords Silica coating • Stöber method • Microemulsion • Hydrolysis

The benefits of employing silica as a magnetic iron oxides coating were highlighted in the previous section. Here we intend to review the most common methods to achieve the silica layer generating magnetic nanoparticles with suitable properties to be applied in the biomedical field. The focus is on the properties defined by the different preparation techniques regarding the end applications of the nanodevices. The aim of this chapter is to orientate the researcher in terms of the synthesis conditions and the expected characteristics of the nanoparticles in this regard. Hence, comparative analysis is included in this section.

In general silica coated MNPs are widely obtained via wet chemical synthesis routes. In this sense it is possible to find three groups of methods: seed growth mediated, non-seeded methods, and magnetic mesoporous silica techniques [1–3].

Here, we focus on seed mediated methodology that means achieving the modification from magnetite nanoparticles previously prepared. This appears as the most suitable strategy to assess a control over the silica shell width and hence over the size, shape, and magnetism of derived nanosystems. It is worth noting that the mentioned represents two critical properties in terms of the potential and efficiency of MNPs in the biomedical field [4].

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_4

4.1 Stöber Process

The Stöber process is by far the traditional method to assess the silica coating on the surface of inorganic and metallic oxides cores, including iron oxides nanoparticles.

This process emerged in the earlier twentieth century aiming to obtain monodisperse suspensions of spherical silica in the colloidal size range. The intention was to apply these materials in the study of hydro and aerosols. Besides, the potential use of them in the biomedical area greatly encouraged the research regarding the synthetic pathways [5].

The base of this Stöber process, regarding the magnetic nanoparticles modification, is the formation of a silica layer of variable width from the chemical reaction of tetraesters of silicic acid (tetraalkyl silicates) with certain solutions. In general it involves the hydrolysis and condensation of alkoxysilanes, such as tetraethyl orthosilicate (TEOS), in aqueous ethanolic solution, and the ammonia incorporation. Generally, continuous mechanical stirring is implemented and TEOS is slowly added to the dispersion, an average of 12 h is the time reported as suitable to achieve the formation of silica on the magnetite nanoparticles surfaces through hydrolysis and condensation of TEOS [6, 7]. Among the simplicity of the Stöber process, it was studied that altering some of the experimental variables associated with this method may give rise to different properties of Si@MAG NPs. The most interesting property susceptible to change as a function of the experimental conditions is the width of the silica layer. Therefore, by appropriately adjusting some factors it is possible to obtain from Si-monolayer solid Si@MAG to mesoporous materials containing magnetite nanoparticles [8].

The variables commonly analyzed applying the Stöber method are mainly the TEOS concentration (and also the TEOS/MAG ratio), the contact time, the ammonium concentration, and the sequence of reactive addition, among others. Altering the initial concentration of TEOS leads to a different layer width of silica around the magnetic core [9, 10]. The impact of experimental variables is appreciated not only by the amount of silica incorporated on MNPs core, but also in the morphology and stability of the achieved formulation (Fig. 4.2). To illustrate, TEM micrographs of MNPs@Si obtained from different Stöber based protocols are shown in Fig. 4.1 and compared with the bare Magnetite@citric acid nanoparticles (MAG@CA). It is worth noting that these formulations were developed in our labs.

As a consequence it is highly feasible to find different articles reporting modified Stöber method to prepare silica coated magnetite nanoparticles [11, 12]. The use of other catalysts was reported, for instance employing lysine or arginine instead of ammonia [13–15]. Other common modifications include the use of surfactants in the reaction media aiming to restrict the growth of the silica layer [16–19].

The incorporation of PEG, a polymeric stabilizer, was found to affect the morphology of the Si coated MNPs [20].

Although the Stöber process is commonly performed under magnetic stirring, the use of ultrasound was recently reported as a modification to improve the coating properties. The reasons for this behavior are related to the better dispersion of

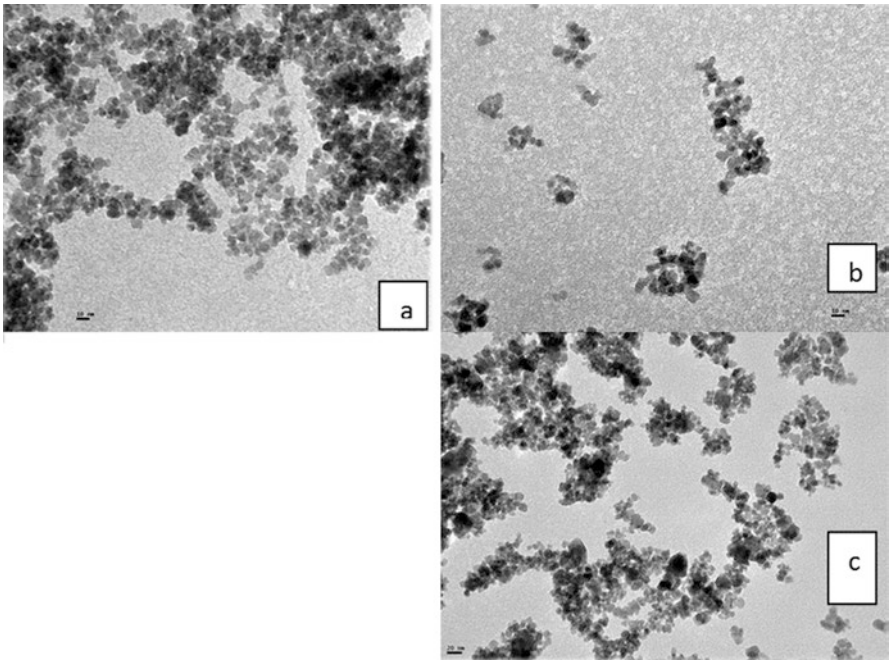

Fig. 4.1 TEM micrographs of: (**a**) MAG@CA., (**b**) MNPs@Si (1): Addition of NH_4OH, then addition dropwise of TEOS; (**c**) MNPs@Si (2): Addition of NH_4OH and TEOS simultaneously. The scale is: 3 mm = 20 nm

MNPs, the acceleration of the hydrolysis and condensation reactions with the consequent limitation in the MNPs aggregation [21].

Another alternative to traditional Stöber takes place when magnetic nanoparticles are incorporated in a dispersion of silica NPs precursors just during the aggregation of the primary silica particles. In fact Fe_3O_4 NPs are added to the TEOS solution. In this case a near monolayer of silica could form surrounding various magnetic cores; such number is adjusted by modifying the MNPs concentrations. The formation of NPs is regulated by measuring the conductivity. The conductivity increased slowly during the formation and aggregation of the primary particles. Once the colloidal stable particles were formed, the conductivity became constant since the condensation rate is much faster than that of the hydrolysis at this time [22].

The use of a functionalizing agent on the magnetite nanoparticle surface is a common practice to increase affinity to silica. To this end it is feasible to employ citric acid, polymers (such as polyvinyl pirrolidone, PEG), and other chemical agents containing thiol groups [23, 24].

In Table 4.1 advantages and disadvantages of the Stöber method are listed and compared with other common methods.

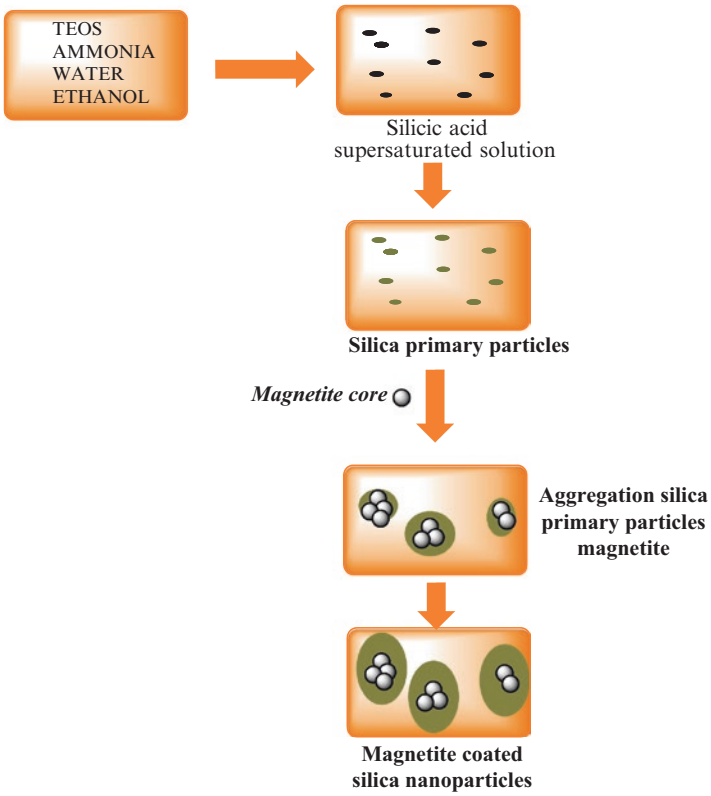

Fig. 4.2 Schematic representation of the Stöber process to achieve Si@MAG

4.2 Microemulsion Method

In general, microemulsion methods are based on a system composed of water, oil, a surfactant and, eventually, a co-surfactant. The use of this strategy to incorporate a silica coating on an iron oxide core was explored. Commonly, magnetic nanoparticles previously coated with hydrophobic ligands are required [25, 26]. Those are dispersed in an organic media. The dispersion is then contacted with aqueous alkaline solution containing the surfactant under ultrasonic treatment, determining the microemulsion formation. In the final stage, a silica precursor, generally TEOS is added and allowed to react during a determinant period of time [27] (Fig. 4.3).

The growth of the silica coating on the magnetic surface is regulated by experimental parameters such as TEOS concentration, amount of water, MNPs-TEOS contact time, among others [28, 29].

Whereas, the incorporation of a single magnetic core or clusters of iron oxide NPs is mainly controlled by the presence and concentration of the surfactant [30, 31].

Table 4.1 Advantages and disadvantages of the most useful methods to assess MNPs coated with silica

Method	Advantages	Disadvantages	References
Stöber	Simple Low cost Relatively monodisperse and homogeneous silica coverage on magnetic surface	The formation of large aggregates and polydispersed products is always inevitable because of the high and uncontrollable hydrolysis rate of TEOS and the quite low critical concentration for homogeneous nucleation of silica	[6–10]
Microemulsion	–Good control of size and shape of composite particles in which micelles or inverse micelles are used to confine and control the coating of silica on core nanoparticles –Highly suitable for unstable in the classical Stöber reaction medium	Many factors affecting the reaction, such as the kinds of surfactants and the ratio of surfactant and water phase, reaction time, etc.	[23–25]
Sodium silicate	Relatively simple Providing a high silica coverage level on the magnetic core	Highly sensitive to the molar ratio of sodium silicate and MNPs	[36, 37]
Sonochemical	Simple, low cost, rapid Magnetic core almost retains its original Ms values	Well dispersed MNPs and silica particles are required to succeed with US method	[41, 42]

The role of the surfactant result is crucial in terms of the size and shape control of the nanodroplets, which lastly will determine the homogeneity of the coating.

Ingepal®, CO-520 (poly(5)oxyethylene-4-nonylphenyl-ether), is reported among the most widely used and efficient surfactants to assess the silica coating on the surface of iron oxide NPs though microemulsion [31, 32]. In addition, other non ionic surfactants such as oxyethylene derivatives and polymeric were reported to these ends. Triton X was employed to perform a silica shell on previously prepared magnetite nanoparticles by means of the microemulsion method [33–37].

In general, the hydrodynamic sizes and the magnetization saturation were not substantially modified after the silica coating. In fact using the microemulsion method the crystallite sizes increased between 1.8 and 20 % after silica deposition. In the case of magnetization saturation an enhancement of the magnetic properties was evidenced promoted by the silica layer [38].

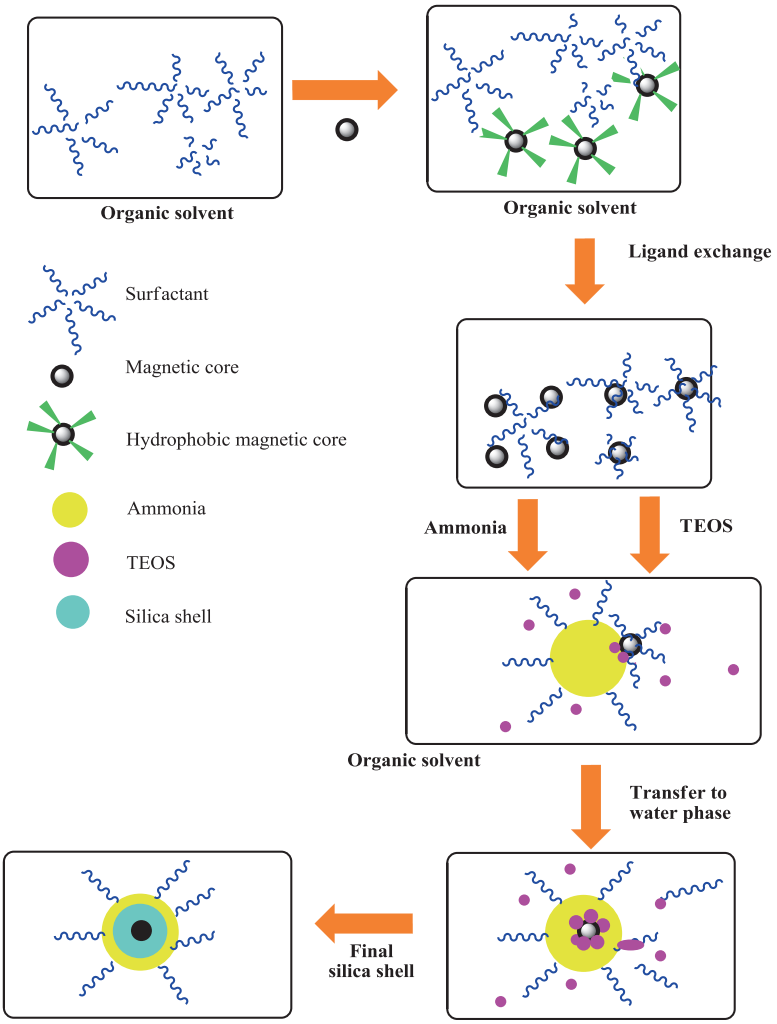

Fig. 4.3 Schematic representation of microemulsion procedure

4.3 Sodium Silicate Hydrolysis Methods

This method is based on the hydrolysis of sodium silicate in any acidic or alkaline media to induce the condensation of a silica layer on the nanoparticle surface. From this procedure a multi coating of silica is achieved by several cycles with sodium silicate. Hence, the thickness of the shell is regulated by varying the number of cycles (Fig. 4.4).

The sodium silicate hydrolysis method appears as a suitable one to assess a great coverage level of the magnetite nanoparticle surface. Research attributed this fact to the different types of silica produced in each step [27].

$$\equiv Si - OR + H_2O \leftrightarrow \equiv Si - OH + ROH$$

$$\equiv Si - OR + \equiv Si - OH \leftrightarrow \equiv Si - O - Si\equiv + ROH$$

$$\equiv Si - OH + \equiv Si - OH \leftrightarrow \equiv Si - O - Si\equiv + H_2O$$

Fig. 4.4 Mechanism involved in the preparation of silica by sodium silicate hydrolysis

Generally speaking, the procedure involves the dispersion of magnetite nanoparticles in aqueous media, where a solution of Na_2SiO_3 was added in a controlled way. The pH was maintained at 6 by regulating with HCl.

The mixture was stirred for 3 h at 80 °C under N_2 inert atmosphere. Different levels of surface coverage are achieved by conveniently modifying the magnetitie:SiO_2 molar ratio [39, 40].

4.4 Sonochemical Deposition

This, relatively novel methodology, involves the treatment of magnetite and silica nanoparticles previously prepared with external force such as ultrasound. The ultrasound acts in the collision acceleration, meaning intra and interparticles collision. In fact, the collapsed bubbles and shockwave generated from the ultrasonic irradiation produce a great force that enables the incorporation of magnetic nanoparticles on the silica [41].

Sodipo et al. prepared silica coated magnetic nanoparticles from silica particles using the US method. They employed silica particles previously obtained from sol-gel technique and magnetic nanoparticles prepared by co-precipitation. These authors postulated three possible hypotheses for the MNPs incorporation on the silica framework, i.e., (1) the total inelastic collision between MNPs and silica; (2) structure and physical properties of silica particles, and (3) the colloidal stability of the MNPs. The MNPs dispersability appears as a key factor in relation to withstand the influence of the turbulent flow from the ultrasonic irradiation and shock waves.

The stability of MNPs in dispersion is strongly dependent on the pH of the media. Therefore, the pH needed adjusting in order to avoid the pH of the PZC, where the MNPs suspension became instable [41].

The main advantage recognized along this method is related to the magnetic properties. As expected, the saturation magnetization (Ms) fell with respect to uncoated MNPs. However, the US method ensures a lower M reduction when compared with other preparation methods [42].

4.5 Miscelaneous Methods

Other, less common procedures recently emerged as efficient alternatives to the preparation of silica coated magnetic nanoparticles. For instance, the procedure reported by Abbas et al. based on one pot synthesis of magnetite and silica coating.

These authors propose the preparation of MNPs in PEG media, inducing the iron oxide formation by alkalinization up to pH 10–11. After precipitation, different concentrations of TEOS were added at high temperatures (almost 300 °C). Satisfactory poldispersion, in terms of the size distribution, was achieved whereas saturation magnetization was also acceptable and its reduction considered as indicative of the silica present around the magnetic core [43].

Aerosol pyrolisis allows the direct preparation of silica-coated magnetic nanoparticles [44, 45]. It involves the use of an aerosol reactor where aerosol is formed by heating and evaporation cycles. Spherical particles are obtained by heating treatments. For instance, silica coated maghemite nanoparticles were prepared by aerosol pyrolysis of methanol solutions containing iron ammonium citrate and TEOS. The authors reported satisfactory Ms values as well as suitable nanosizes and almost monodisperse samples [46].

Sol-gel approaches are also well known to promote the silica coating on MNPs surface. However, this methodology is more oriented to the obtention of mesoporous silica structures. Besides, some articles report the use of sol gel based method with modifications to assess a silica layer [47].

References

1. Qu, H., Tong, S., Song, K., Ma, H., Bao, G., Pincus, S., et al. (2013). Controllable in situ synthesis of magnetite coated silica-core water-dispersible hybrid nanomaterials. *Langmuir, 29*(33), 10573–10578.
2. Wang, T., Zhang, L., Su, Z., Wang, C., Liao, Y., & Fu, Q. (2011). Multifunctional hollow mesoporous silica nanocages for cancer cell detection and the combined chemotherapy and photodynamic therapy. *ACS Applied Materials & Interfaces, 3*(7), 2479–2486.
3. Deng, Y., Qi, D., Deng, C., Zhang, X., & Zhao, D. (2008). Superparamagnetic high-magnetization microspheres with an Fe_3O_4@ SiO_2 core and perpendicularly aligned mesoporous SiO_2 shell for removal of microcystins. *Journal of the American Chemical Society, 130*(1), 28–29.
4. Sodipo, B. K., & Aziz, A. A. (2016). Recent advances in synthesis and surface modification of superparamagnetic iron oxide nanoparticles with silica. *Journal of Magnetism and Magnetic Materials, 416*, 275–291.
5. Stöber, W., Fink, A., & Bohn, E. (1968). Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science, 26*(1), 62–69.
6. Deng, Y. H., Wang, C. C., Hu, J. H., Yang, W. L., & Fu, S. K. (2005). Investigation of formation of silica-coated magnetite nanoparticles via sol–gel approach. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 262*(1), 87–93.
7. Kobayashi, Y., Horie, M., Konno, M., Rodríguez-González, B., & Liz-Marzán, L. M. (2003). Preparation and properties of silica-coated cobalt nanoparticles. *The Journal of Physical Chemistry B, 107*(30), 7420–7425.
8. Hui, C., Shen, C., Tian, J., Bao, L., Ding, H., Li, C., et al. (2011). Core-shell Fe_3O_4@ SiO_2 nanoparticles synthesized with well-dispersed hydrophilic Fe_3O_4 seeds. *Nanoscale, 3*(2), 701–705.
9. Kuzminska, M., Carlier, N., Backov, R., & Gaigneaux, E. M. (2015). Magnetic nanoparticles: Improving chemical stability via silica coating and organic grafting with silanes for acidic media catalytic reactions. *Applied Catalysis A: General, 505*, 200–212.

10. Kassaee, M. Z., Masrouri, H., & Movahedi, F. (2011). Sulfamic acid-functionalized magnetic Fe_3O_4 nanoparticles as an efficient and reusable catalyst for one-pot synthesis of α-amino nitriles in water. *Applied Catalysis A: General, 395*(1), 28–33.
11. Lu, Y., Yin, Y., Mayers, B. T., & Xia, Y. (2002). Modifying the surface properties of superparamagnetic iron oxide nanoparticles through a sol-gel approach. *Nano Letters, 2*(3), 183–186.
12. Barrera, E. G., Livotto, P. R., & dos Santos, J. H. (2016). Hybrid silica bearing different organosilanes produced by the modified Stöber method. *Powder Technology, 301*, 486–492.
13. Greasley, S. L., Page, S. J., Sirovica, S., Chen, S., Martin, R. A., Riveiro, A., et al. (2016). Controlling particle size in the Stöber process and incorporation of calcium. *Journal of Colloid and Interface Science, 469*, 213–223.
14. Hartlen, K. D., Athanasopoulos, A. P., & Kitaev, V. (2008). Facile preparation of highly monodisperse small silica spheres (15 to >200 nm) suitable for colloidal templating and formation of ordered arrays. *Langmuir, 24*(5), 1714–1720.
15. Yokoi, T., Wakabayashi, J., Otsuka, Y., Fan, W., Iwama, M., Watanabe, R., et al. (2009). Mechanism of formation of uniform-sized silica nanospheres catalyzed by basic amino acids. *Chemistry of Materials, 21*(15), 3719–3729.
16. Yokoi, T., Sakamoto, Y., Terasaki, O., Kubota, Y., Okubo, T., & Tatsumi, T. (2006). Periodic arrangement of silica nanospheres assisted by amino acids. *Journal of the American Chemical Society, 128*(42), 13664–13665.
17. Lee, Y. G., Park, J. H., Oh, C., Oh, S. G., & Kim, Y. C. (2007). Preparation of highly monodispersed hybrid silica spheres using a one-step sol-gel reaction in aqueous solution. *Langmuir, 23*(22), 10875–10878.
18. Meng, Z., Xue, C., Zhang, Q., Yu, X., Xi, K., & Jia, X. (2009). Preparation of highly monodisperse hybrid silica nanospheres using a one-step emulsion reaction in aqueous solution. *Langmuir, 25*(14), 7879–7883.
19. Roy, I., Ohulchanskyy, T. Y., Pudavar, H. E., Bergey, E. J., Oseroff, A. R., Morgan, J., et al. (2003). Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: A novel drug-carrier system for photodynamic therapy. *Journal of the American Chemical Society, 125*(26), 7860–7865.
20. Bharali, D. J., Klejbor, I., Stachowiak, E. K., Dutta, P., Roy, I., Kaur, N., et al. (2005). Organically modified silica nanoparticles: A nonviral vector for in vivo gene delivery and expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America, 102*(32), 11539–11544.
21. Yu-Xiang, Y., Li-Ping, Z., Xiao-Cui, X., Ya-Ni, Z., Jian-Guo, S., & Xiang-Nong, L. (2011). Synthesis of chain-like and core–shell spherical Fe_3O_4@ SiO_2 complex. *Advanced Science Letters, 4*(1), 96–103.
22. Lu, Z., Dai, J., Song, X., Wang, G., & Yang, W. (2008). Facile synthesis of Fe_3O_4/SiO_2 composite nanoparticles from primary silica particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 317*(1), 450–456.
23. Rho, W. Y., Kim, H. M., Kyeong, S., Kang, Y. L., Kim, D. H., Kang, H., et al. (2014). Facile synthesis of monodispersed silica-coated magnetic nanoparticles. *Journal of Industrial and Engineering Chemistry, 20*(5), 2646–2649.
24. Mohmood, I., Lopes, C. B., Lopes, I., Tavares, D. S., Soares, A. M., Duarte, A. C., et al. (2016). Remediation of mercury contaminated saltwater with functionalized silica coated magnetite nanoparticles. *Science of the Total Environment, 557*, 712–721.
25. Dung, C. T., Quynh, L. M., Linh, N. P., Nam, N. H., & Luong, N. H. (2016). Synthesis of ZnS:Mn–Fe_3O_4 bifunctional nanoparticles by inverse microemulsion method. *Journal of Science: Advanced Materials and Devices, 1*(2), 200–203.
26. Guerrero-Martínez, A., Pérez-Juste, J., & Liz-Marzán, L. M. (2010). Recent progress on silica coating of nanoparticles and related nanomaterials. *Advanced Materials, 22*(11), 1182–1195.
27. Li, C., Ma, C., Wang, F., Xi, Z., Wang, Z., Deng, Y., et al. (2012). Preparation and biomedical applications of core–shell silica/magnetic nanoparticle composites. *Journal of Nanoscience and Nanotechnology, 12*(4), 2964–2972.

28. Digigow, R. G., Dechézelles, J. F., Dietsch, H., Geissbühler, I., Vanhecke, D., Geers, C., et al. (2014). Preparation and characterization of functional silica hybrid magnetic nanoparticles. *Journal of Magnetism and Magnetic Materials, 362*, 72–79.
29. Yuan, Y., Rende, D., Altan, C. L., Bucak, S., Ozisik, R., & Borca-Tasciuc, D. A. (2012). Effect of surface modification on magnetization of iron oxide nanoparticle colloids. *Langmuir, 28*(36), 13051–13059.
30. Sun, Q., Zhao, G., & Dou, W. (2016). An optical and rapid sandwich immunoassay method for detection of Salmonella pullorum and Salmonella gallinarum based on immune blue silica nanoparticles and magnetic nanoparticles. *Sensors and Actuators B: Chemical, 226*, 69–75.
31. Park, J. N., Forman, A. J., Tang, W., Cheng, J., Hu, Y. S., Lin, H., et al. (2008). Highly active and sinter-resistant Pd-nanoparticle catalysts encapsulated in silica. *Small, 4*(10), 1694–1697.
32. Kishida, M., Tago, T., Hatsuta, T., & Wakabayashi, K. (2000). Preparation of silica-coated rhodium nanoparticles using water-in-oil microemulsion. *Chemistry Letters, 9*, 1108–1109.
33. Stjerndahl, M., Andersson, M., Hall, H. E., Pajerowski, D. M., Meisel, M. W., & Duran, R. S. (2008). Superparamagnetic Fe_3O_4/SiO_2 nanocomposites: Enabling the tuning of both the iron oxide load and the size of the nanoparticles. *Langmuir, 24*(7), 3532–3536.
34. Lai, C. W., Wang, Y. H., Lai, C. H., Yang, M. J., Chen, C. Y., Chou, P. T., et al. (2008). Iridium-complex-functionalized Fe_3O_4/SiO_2 core/shell nanoparticles: A facile three-in-one system in magnetic resonance imaging, luminescence imaging, and photodynamic therapy. *Small, 4*(2), 218–224.
35. Lin, Y. S., Wu, S. H., Hung, Y., Chou, Y. H., Chang, C., Lin, M. L., et al. (2006). Multifunctional composite nanoparticles: Magnetic, luminescent, and mesoporous. *Chemistry of Materials, 18*(22), 5170–5172.
36. Lee, J., Lee, Y., Youn, J. K., Na, H. B., Yu, T., Kim, H., et al. (2008). Simple synthesis of functionalized superparamagnetic magnetite/silica core/shell nanoparticles and their application as magnetically separable high-performance biocatalysts. *Small, 4*(1), 143–152.
37. Xu, H., Cui, L., Tong, N., & Gu, H. (2006). Development of high magnetization Fe_3O_4/polystyrene/silica nanospheres via combined miniemulsion/emulsion polymerization. *Journal of the American Chemical Society, 128*(49), 15582–15583.
38. Jia, L., & Kitamoto, Y. (2015). Influence of silica coating process on fine structure and magnetic properties of iron oxide nanoparticles. *Electrochimica Acta, 183*, 148–152.
39. Zhao, X., Shi, Y., Wang, T., Cai, Y., & Jiang, G. (2008). Preparation of silica-magnetite nanoparticle mixed hemimicelle sorbents for extraction of several typical phenolic compounds from environmental water samples. *Journal of Chromatography A, 1188*(2), 140–147.
40. Roto, R., Yusran, Y., & Kuncaka, A. (2016). Magnetic adsorbent of Fe_3O_4@ SiO_2 core-shell nanoparticles modified with thiol group for chloroauric ion adsorption. *Applied Surface Science, 377*, 30–36.
41. Lewandowska-Łańcucka, J., Staszewska, M., Szuwarzyński, M., Kępczyński, M., Romek, M., Tokarz, W., et al. (2014). Synthesis and characterization of the superparamagnetic iron oxide nanoparticles modified with cationic chitosan and coated with silica shell. *Journal of Alloys and Compounds, 586*, 45–51.
42. Sodipo, B. K., & Aziz, A. A. (2015). Non-seeded synthesis and characterization of superparamagnetic iron oxide nanoparticles incorporated into silica nanoparticles via ultrasound. *Ultrasonics Sonochemistry, 23*, 354–359.
43. Abbas, M., Rao, B. P., Islam, M. N., Naga, S. M., Takahashi, M., & Kim, C. (2014). Highly stable-silica encapsulating magnetite nanoparticles (Fe_3O_4/SiO_2) synthesized using single surfactantless-polyol process. *Ceramics International, 40*(1), 1379–1385.
44. Tartaj, P., del Puerto Morales, M., Veintemillas-Verdaguer, S., González-Carreño, T., & Serna, C. J. (2003). The preparation of magnetic nanoparticles for applications in biomedicine. *Journal of Physics D: Applied Physics, 36*(13), R182.

45. Ruiz-Hernández, E., Lopez-Noriega, A., Arcos, D., Izquierdo-Barba, I., Terasaki, O., & Vallet-Regí, M. (2007). Aerosol-assisted synthesis of magnetic mesoporous silica spheres for drug targeting. *Chemistry of Materials, 19*(14), 3455–3463.
46. Das, H., Arai, T., Debnath, N., Sakamoto, N., Shinozaki, K., Suzuki, H., et al. (2016). Impact of acidic catalyst to coat superparamagnetic magnesium ferrite nanoparticles with silica shell via sol–gel approach. *Advanced Powder Technology, 27*(2), 541–549.
47. Takeda, Y., Komori, Y., & Yoshitake, H. (2013). Direct Stöber synthesis of monodisperse silica particles functionalized with mercapto-, vinyl-and aminopropylsilanes in alcohol–water mixed solvents. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 422*, 68–74.

Chapter 5
Drug Loading and Release for Specific Diseases

Abstract Biomedical applications of solid silica-coated magnetite nanoparticles are not highly reported in literature. Conventional biomedical uses are almost restricted to diagnostic issues. Other magnetic-silica materials such as mesoporous were last explored as target drug delivery.

Among the scarce literature reports, the existent are devoted to the use of solid silica coated magnetic nanoparticles as target drug delivery in the treatment of oncological diseases and in gene delivery and transfection. The use of these kinds of nanosystems as theranostics was also reported in the last few years. In this sense, the possibility is being explored to design a nanocarrier to be useful in more than one diagnostic technique, leading to multiple theranostics tools. In most cases, hard work on the design is required in order to assess selectivity and specificity in their function. To these ends silica coated magnetic nanoparticles are commonly modified with suitable ligands able to interact with biomarkers associated with the disease to treat.

Keywords Theranostics • Targeted drug delivery • Specific ligands • Drug release

In general, targeted drug delivery systems encompass three targeting strategies, which are passive, active targeting, and magnetic field directed targeting. Passive refers to the preferential accumulation of nanoparticles at the disease site in the absence of targeting ligands. The accumulation efficiency is purely determined by the physiochemical properties of nanoparticle, such as size, shape, surface charge, etc. Active is an approach that can enhance the preferential accumulation of nanoparticles in the disease site through surface modification with ligands that have selectivity and affinity toward tissue, organ or cell. Magnetic targeting is an improved strategy combining the active target with the ability of the nanocarrier to respond to external stimuli (i.e., magnetic field) [1]. In Fig. 5.1 three kinds of targeting are schematized.

Nanosized formulations involving silica in their composition were investigated over the past decade. In spite of this, unfortunately, none of them reached clinical application. This is, mainly, because of the lack of in depth and reliable information regarding the behavior of these kinds of nanosystems in the physiological environment [2].

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_5

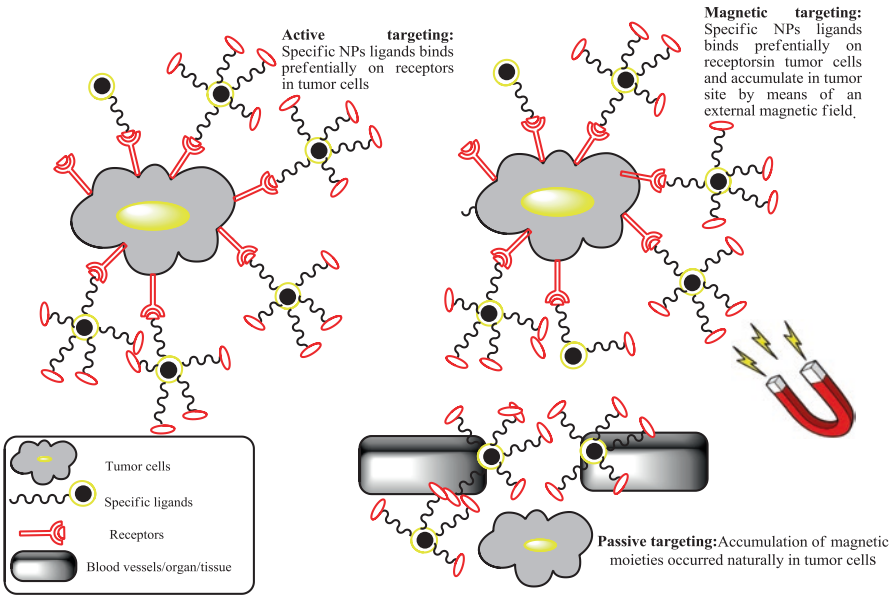

Fig. 5.1 Scheme of the three types of targeting found wih MAG@Si NPs

Silica based nanomaterials themselves exhibit therapeutic potential based on their intrinsic properties. For example, in Saos-2 cells that are similar to osteoblasts, bio-silica matrices stimulate increased hydroxyapatite deposition. This suggests that synthesized bio-silica is a promising route for tooth reconstruction in vivo [3].

In general terms, two types of Si based nanomaterials are actually found in open literature regarding biomedical interests: on one side mesoporous silica nanoparticles [4–8]; and, on the other side, solid core/shell silica nanoparticles. Besides, the information regarding the last nanosystems is considerably more limited. Even when hybrid solid magnetite/silica nanoparticles are considered the reports in open literature are scarce. Between them the majority refer to applications in imaging diagnostic [6, 7, 9, 10]. In Fig. 5.2a the differences between drug loading in mesoporous magnetic nanocomposites and solid silica coated magnetic nanoparticles are shown. Whereas, in Fig. 5.2b the most common strategies to load therapeutic agents in MAG@Si solid NPs are schematized.

The selection of silica as the coating of magnetic nanoparticles intended as target and drug delivery systems is motivated not only by its biocompatibility but mainly by the surface reactivity that allows the incorporation of specific and well determined ligands. Moreover, it helps to convert hydrophobic NPs into hydrophilic water-soluble particles, rendering highly selective nanosystems, potentially useful for treatment of specific diseases [11].

Table 5.1 summarizes the reported MAG@Si based formulations intended as biomedical applications, in particular diagnostic and target drug delivery.

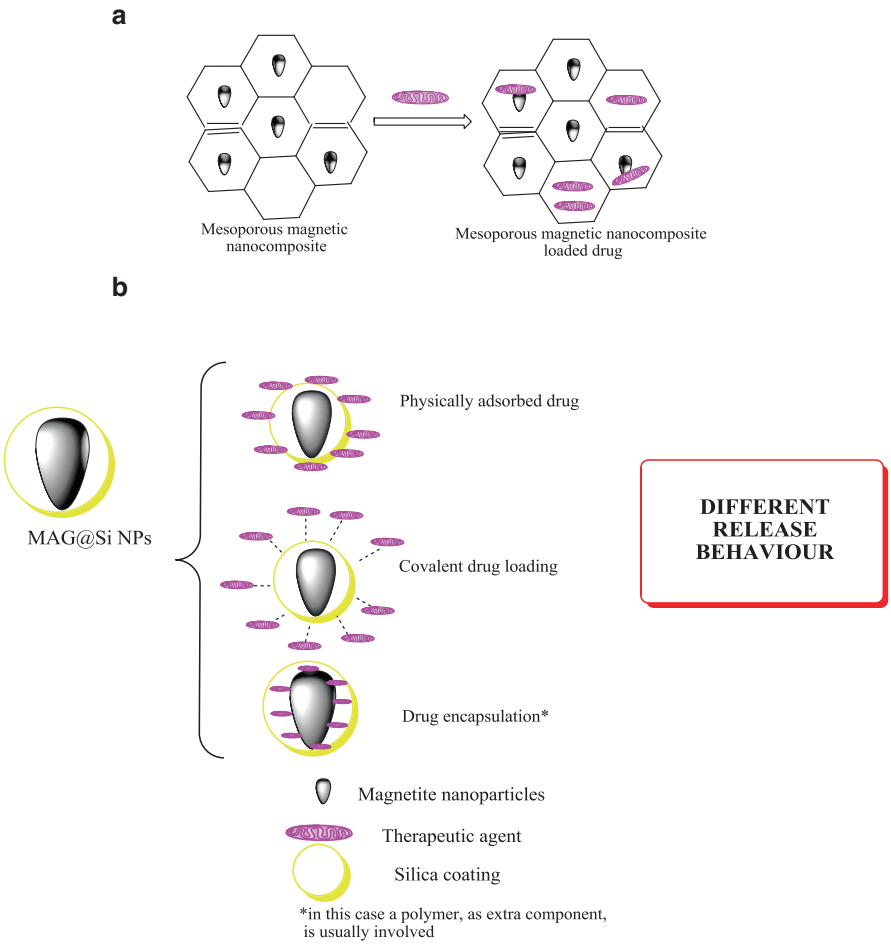

Fig. 5.2 (**a**) Mesoporous magnetic material loaded drug. (**b**) Different kinds of drug loading on MAG@Si NPs

Table 5.1 Summary of the nanosystems based on magnetite and silica intended as target and diagnostic applications

Kind of magnetic nanosystem	Ligand	Drug	Action: therapy, diagnostic or theranostic	References
MAG@Si	PEG	Doxorrubicin	Tumor therapy	[14]
MAG@Si	APTS	Folic acid	Diagnostic	[27]
MAG@Si	–	Mycophenolic acid (MPA)	Immunosuppressing MRI-therapy	[20]
MAG@Si	–	Mebrofenin	Liver targeting MRI	[6]
MAG@Si	–	CdTe	Diagnostic	[28]
MAG@Si	–	Methylene blue	Diagnostic and therapy	[29]

5.1 Oncological Diseases

After a survey of the open literature, it emerges that silica based magnetic formulations are not, in general, associated with the treatment of any specific pathology. However, it is possible to find articles devoted to the use of these kinds of nanosystems in diverse therapies.

Oncological diseases appear as one of the most explored because of the well known negative impact of these diseases in all societies around the world. In this regard Si coated MNPs appear as a valid tool to achieve two aspects: therapy and diagnostic leading to the novel term Theranostics [2, 12].

In a recent work, silica-coated superparamagnetic iron oxide NPs were conjugated with a specific monoclonal antibody CHO 31.1, which recognizes gpA33, a membrane glycoprotein overexpressed in 50 % of pancreatic cancers and 95 % of colorectal cancers. This represents a clear example of the role of silica as a linker to providing specificity to the nanosystems action. The coupling silica–antibody was achieved by means of PEG reaction [13].

In a similar approach the tumoral therapeutic agent, Doxorubicin was conjugated in the magnetic core surface. In a later step, the drug conjugated magnetic core was coated with silica and in a posterior step a PEG was incorporated as the biocompatibilizer. In this case, it is clear that the role of silica is not as the stabilizer of the magnetic core or linker of the drug. Here it is intended, in combination with PEG, to confer biocompatibility and potential to MRI diagnostics to the designed nanosystems [14]. The sequence followed to obtain the described MNPs@Si-Doxo is represented in Fig. 5.3.

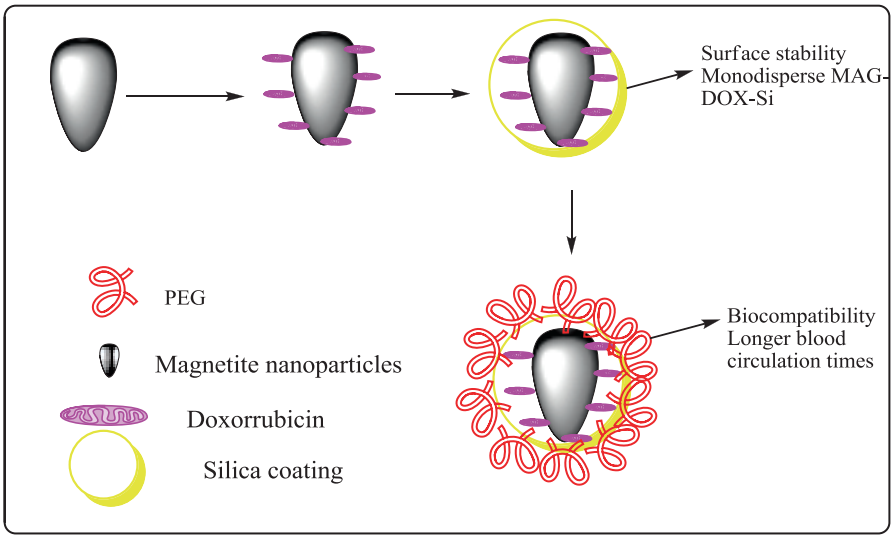

Fig. 5.3 Representation of the pathways to obtain a nanocarrier for Doxorubicin target

5.2 Gene and Antibodies Target and Delivery

The first proofs regarding the use of MNPs for target and delivery of DNA were recorded at the University of Florida by C. Mah, B. Byrne, and Col. in early 2000. They coated adeno associated virus (AAV) encoding green fluorescent protein (GFP) to the surface of MNPs using a cleavable heparin sulfate linker. They found that AAV conjugated MNPs gave increased transduction efficiency in both in vitro and in vivo assays [15].

Actual research studies opened the possibility of using nanotechnology tools as vehicles for nucleic acid based drugs. This feature offers new insights to treat genetic diseases.

A wide volume of recent articles reporting: silica nanoparticles transporting DNA to affect gene expression; to DNA delivery; transporting small interfering RNA (siRNA); to enhance transfection efficiency; etc. are available [11, 16].

However, only a few include magnetic moieties as part of their structure. Therefore, the possibilities of target, accumulation, and orientation conferred by the magnetic core have not been, until now, exploited enough.

The coupling of antibodies on Fe_3O_4-SiO_2NPs was reported between the scarce articles devoted to the use of MNPs coated silica as target drug delivery. One of the selected antibodies was (αT-IgG). To do this a sequential functionalization of NPs surfaces with 3-glycidoxypropyltrimethoxysilane (GPS) and 1,1′-carbonyldiimidazole (CDI) was attained. The derivatization of MAG-SI NPs rendered highly efficient not only to antibodies binding but also plasmatic protein affinity such as BSA. This has interesting consequences in terms of the interactions with the physiological media. The αT-IgG loaded Fe_3O_4-SiO_2-GPS-CDI- are seen as promising tools for targeting T cells which should be ex vivo eliminated from bone marrow prior to transplantation [17].

In another work core shell magnetite/silica nanoparticles of about 5–400 nm were prepared and intended for oligonucleotides target and delivery. To do so particles required an extra modification, so disulfide coupling chemistry was employed to induce the oligonucleotide immobilization on the silica coating on MNPs surface [18]. The formulated nanocarriers have the ability to electrostatically bind, condense, and project plasmid DNA from cleavage [19].

Mycophenolic acid (MPA) is the active ingredient of the immunosuppressant mycophenolate mofetil, which is widely used in organ transplants to prevent acute rejection or in autoimmune diseases to reduce autoreactive immune responses. To assess the successful target and delivery of this drug, a specific strategy is required, considering the hydrophobic nature of this drug.

The use of silica as coating of MNPs is a reliable tool to achieve this purpose. The layer of silica in the MNPs surface improves the solubility of MNPs with MPA by hydrophobic or electrostatic interactions.

The proposed nanosystems exhibited a dual role: on one side the target and delivery of MPA and, at the same time, these MNPs would provide MR contrasts, which would allow molecular imaging of the nanocarriers and MPA activities. From the

therapeutic point of view, the MNPs–Si-MCA administration was found to lower the amount of drug doses required to be effective by at least tenfold.

The release of the MCA was induced by the silica coated degradation. It is well reported that the degradation of silica NPs occurs after roughly 7 days. This evidence involved the incubation of Si-NPs carrying FITC dye in cell cultures. In fact, the US Food and Drug Administration (FDA) already approved an aqueous suspension of Si coated, MNPs as a clinically acceptable product (i.e., GastroMARK, AMAG Pharmaceuticals) in the 1990s [20]. However, this formulation was intended only to apply in diagnostic for MRI [21].

5.3 Other Drugs and Therapies

The efficient administration of insulin, in the treatment of diabetes is a challenge that persists among scientists all around the world. Nowadays, strategies to improve the oral insulin bioavailability are associated with the development of drug delivery systems in the nanosize. Nanodevices containing silica in its formulation are attractive candidates for this purpose due to its high porosity, specific surface area, biocompatibility, and ease of surface functionalization. In fact, these properties make Si containing nanoformulations highly suitable to the delivery of several proteins. In particular, the presence of residual silanol groups (Si-OH) on the silica coating activates the reactive sites for its surface modification by specific organic groups [22–25].

The incorporation of a transition metal such as Zn to the above mentioned formulation conferred selectivity to the MNPs to preferentially bind to specific proteins. In fact, Ma et al. showed that Fe_3O_4-SiO_2-GPS coated with imino diacetic acid (IDA) and Zn^{2+} to form Fe_3O_4-SiO_2-GPS-IDA-Zn^{2+} core-multishellNPs can only bind bovine haemoglobin (BHb protein) rather than bovine serum albumin (BSA) protein [26].

A number of strategies have been implemented in the field of in vitro and in vivo gene silencing. These include cationic polymeric nanoparticles and cationic liposomal nanoparticles used to deliver siRNA. Recently, silica nanoparticles, encapsulating QDs and surface animated, demonstrated suitable properties to efficiently bind and deliver DNA [26].

In a similar approach, plasmid vector containing a short hairpin RNA (shRNA) sequence targeting TurboGFP, an improved variant of the green fluorescent protein CopGFP, was adsorbed electrostatically on the surface of 25 nm SiO_2NPs modified with amine groups. This investigation proved that silica based NPs act as very satisfactory carriers of DNA with optimum transfection agent properties, determining a slow, but incisive silencing of tGFP expression. It is worth mentioning that the cell viability was not affected by the NPs presence, which encourages the development of these kinds of formulations in the gene delivery area.

References

1. Yang, Y., & Yu, C. (2016). Advances in silica based nanoparticles for targeted cancer therapy. *Nanomedicine: Nanotechnology, Biology and Medicine, 12*(2), 317–332.
2. Nagesetti, A., & McGoron, A. J. (2016). Multifunctional organically modified silica nanoparticles for chemotherapy, adjuvant hyperthermia and near infrared imaging. *Colloids and Surfaces B: Biointerfaces, 147*, 492–500.
3. Müller, W. E., Boreiko, A., Wang, X., Krasko, A., Geurtsen, W., Custódio, M. R., et al. (2007). Morphogenetic activity of silica and bio-silica on the expression of genes controlling biomineralization using SaOS-2 cells. *Calcified Tissue International, 81*(5), 382–393.
4. Xie, M., Xu, Y., Shen, H., Shen, S., Ge, Y., & Xie, J. (2014). Negative-charge-functionalized mesoporous silica nanoparticles as drug vehicles targeting hepatocellular carcinoma. *International Journal of Pharmaceutics, 474*(1), 223–231.
5. Wu, X., Wu, M., & Zhao, J. X. (2014). Recent development of silica nanoparticles as delivery vectors for cancer imaging and therapy. *Nanomedicine: Nanotechnology, Biology and Medicine, 10*(2), 297–312.
6. Yazdani, F., Fattahi, B., & Azizi, N. (2016). Synthesis of functionalized magnetite nanoparticles to use as liver targeting MRI contrast agent. *Journal of Magnetism and Magnetic Materials, 406*, 207–211.
7. Vivero Escoto, J. L., Slowing, I. I., Trewyn, B. G., & Lin, V. S. Y. (2010). Mesoporous silica nanoparticles for intracellular controlled drug delivery. *Small, 6*(18), 1952–1967.
8. Bae, S. W., Tan, W., & Hong, J. I. (2012). Fluorescent dye-doped silica nanoparticles: New tools for bioapplications. *Chemical Communications, 48*(17), 2270–2282.
9. Chitra, K., & Annadurai, G. (2014). Rapid capture and exemplary detection of clinical pathogen using surface modified fluorescent silica coated iron oxide nanoparticles. *Biocybernetics and Biomedical Engineering, 34*(4), 230–237.
10. Deng, Z., Zhen, Z., Hu, X., Wu, S., Xu, Z., & Chu, P. K. (2011). Hollow chitosan–silica nanospheres as pH-sensitive targeted delivery carriers in breast cancer therapy. *Biomaterials, 32*(21), 4976–4986.
11. Malvindi, M. A., Brunetti, V., Vecchio, G., Galeone, A., Cingolani, R., & Pompa, P. P. (2012). SiO_2 nanoparticles biocompatibility and their potential for gene delivery and silencing. *Nanoscale, 4*(2), 486–495.
12. Li, L., Tang, F., Liu, H., Liu, T., Hao, N., Chen, D., et al. (2010). In vivo delivery of silica nanorattle encapsulated docetaxel for liver cancer therapy with low toxicity and high efficacy. *ACS Nano, 4*(11), 6874–6882.
13. Nagalla, R., Liao, Z. W., Webb, A., Flynn, N., & Kolodny, N. (2014). Functionalizing silica-coated iron oxide nanoparticles for imaging and targeted cancer therapeutics (780.3). *The FASEB Journal, 28*(1 Suppl), 780–783.
14. Chen, F. H., Zhang, L. M., Chen, Q. T., Zhang, Y., & Zhang, Z. J. (2010). Synthesis of a novel magnetic drug delivery system composed of doxorubicin-conjugated Fe_3O_4 nanoparticle cores and a PEG-functionalized porous silica shell. *Chemical Communications, 46*(45), 8633–8635.
15. McBain, S. C., Yiu, H. H., & Dobson, J. (2008). Magnetic nanoparticles for gene and drug delivery. *International Journal of Nanomedicine, 3*(2), 169.
16. Ta, T. K. H., Trinh, M. T., Nguyen, L. V., Nguyen, T. T. M., Nguyen, T. L. T., Thuoc, T. L., et al. (2016). Synthesis and surface functionalization of Fe_3O_4-SiO_2 core-shell nanoparticles with 3-glycidoxypropyltrimethoxysilane and 1,1′-carbonyldiimidazole for bio-applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 505*, 376–383.
17. Tan, W., Wang, K., He, X., Zhao, X. J., Drake, T., Wang, L., et al. (2004). Bionanotechnology based on silica nanoparticles. *Medicinal Research Reviews, 24*(5), 621–638.

18. Kneuer, C., Sameti, M., Haltner, E. G., Schiestel, T., Schirra, H., Schmidt, H., et al. (2000). Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. *International Journal of Pharmaceutics, 196*(2), 257–261.
19. Frank, J. A., Zywicke, H., Jordan, E. K., Mitchell, J., Lewis, B. K., Miller, B., et al. (2002). Magnetic intracellular labeling of mammalian cells by combining (FDA-approved) superparamagnetic iron oxide MR contrast agents and commonly used transfection agents. *Academic Radiology, 9*(2), S484–S487.
20. Hwang, J., Lee, E., Kim, J., Seo, Y., Lee, K. H., Hong, J. W., et al. (2016). Effective delivery of immunosuppressive drug molecules by silica coated iron oxide nanoparticles. *Colloids and Surfaces B: Biointerfaces, 142*, 290–296.
21. Andreani, T., de Souza, A. L. R., Kiill, C. P., Lorenzón, E. N., Fangueiro, J. F., Calpena, A. C., et al. (2014). Preparation and characterization of PEG-coated silica nanoparticles for oral insulin delivery. *International Journal of Pharmaceutics, 473*(1), 627–635.
22. Andreani, T., Kiill, C. P., de Souza, A. L. R., Fangueiro, J. F., Fernandes, L., Doktorovová, S., et al. (2014). Surface engineering of silica nanoparticles for oral insulin delivery: Characterization and cell toxicity studies. *Colloids and Surfaces B: Biointerfaces, 123*, 916–923.
23. Liu, D., He, X., Wang, K., He, C., Shi, H., & Jian, L. (2010). Biocompatible silica nanoparticles – insulin conjugates for mesenchymal stem cell adipogenic differentiation. *Bioconjugate Chemistry, 21*(9), 1673–1684.
24. Jain, R. N., Huang, X., Das, S., Silva, R., Ivanova, V., Minko, T., et al. (2014). Functionalized mesoporous silica nanoparticles for glucose and pH stimulated release of insulin. *Zeitschrift für Anorganische und Allgemeine Chemie, 640*(3-4), 616–623.
25. Ma, Z. Y., Liu, X. Q., Guan, Y. P., & Liu, H. Z. (2006). Synthesis of magnetic silica nanospheres with metal ligands and application in affinity separation of proteins. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 275*(1), 87–91.
26. Bardi, G., Malvindi, M. A., Gherardini, L., Costa, M., Pompa, P. P., Cingolani, R., et al. (2010). The biocompatibility of amino functionalized CdSe/ZnS quantum-dot-Doped SiO_2 nanoparticles with primary neural cells and their gene carrying performance. *Biomaterials, 31*(25), 6555–6566.
27. Wortmann, L., Ilyas, S., Niznansky, D., Valldor, M., Arroub, K., Berger, N., et al. (2014). Bioconjugated iron oxide nanocubes: Synthesis, functionalization, and vectorization. *ACS Applied Materials & Interfaces, 6*(19), 16631–16642.
28. Zhang, L., Zhang, F., Wang, Y. S., Sun, Y. L., Dong, W. F., Song, J. F., et al. (2011). Magnetic colloidosomes fabricated by Fe_3O_4–SiO_2 hetero-nanorods. *Soft Matter, 7*(16), 7375–7381.
29. Andhariya, N., Chudasama, B., Upadhyay, R. V., & Mehta, R. V. (2011). Methylene blue loaded silica encapsulated magnetite nanoparticles: A potential drug delivery vector for photodynamic therapy. *International Journal of Nanoscience, 10*, 1061–1065.

Chapter 6
Biomedical Features

Abstract The study of biodistribution and pharmacokinetics of nanosystems devoted to biomedical applications is mandatory, especially for nanodevices with potential applications as agents for targeted drug delivery. The physicochemical properties of silica coated magnetic nanoparticles such as composition, size, and surface charge play important roles in the biological impact of the nanosystems. The biodistribution pattern of the nanoparticles gives information about those organs which are feasible for target and also provides information to develop new strategies to improve targeting. Pharmacokinetics and metabolism are two important issues in terms of the behavior of the magnetic nanoparticles in the organism to ensure a good combination between nanoparticles and specific drugs to treat the desired pathologies.

This chapter reviews the biodistribution, pharmacokinetics, and metabolism of silica, iron oxide nanoparticles, and silica-coated magnetic nanoparticles in order to understand the role of each component in the biological features proposed.

Keywords Biodistribution • Pharmacokinetics • Metabolism

This chapter is devoted to the description of evidence on pharmacokinetics, biodistribution, and metabolism of magnetic solid silica nanoparticles, focused on solid silica and magnetite as the main components of the nanosystems. This point of view is intended to clarify and to give tools for the understanding of the behavior of these devices in vivo, considering that at present little is known about magnetic nanosystems coated with solid silica for drug targeting and delivery. Thus, this chapter addresses the blood circulation parameters, the tissue distribution, the clearance, and the excretion of solid silica as coating and the same features related to magnetic cores of nano-iron oxides.

6.1 Pharmacokinetics, Biodistribution Metabolism, and Excretion of Solid Silica Nanoparticles

The knowledge about biodistribution and pharmacokinetics of nanosystems intended for drug delivery systems is extremely important for their biomedical applications. Pharmacokinetics of solid silica nanoparticles is a field where reports

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_6

are not extensively available due to the lack of suitable tracking methods. On the other hand, biodistribution about magnetic nanoparticles is being widely studied to better understand not only the biological impact, but also the tissues and organs where these iron oxide-nanoparticles are distributed after administration.

When a nanosystem is designed for drug delivery, it is essential to study the pharmacokinetics of both parent drug and the incorporated drug in the nanosystem considering that it may be different. In addition to the core composition, size, surface functionalization, and other physicochemical properties play crucial roles in the biodistribution pattern of composed nanosystems.

Figure 6.1 shows the main factors influencing biodistribution of silica nanoparticles.

When nanoparticles enter the bloodstream, they find blood components and serum proteins bind to their surface. The structure formed by these interactions is the "protein corona". A lot of work has been devoted to the formation and composition of the structure of the protein corona around a nanostructure. Possible interactions are so great because the number of proteins present in plasma is about 3700 [1] and they may depend on the equilibrium bonding constants of each protein. The protein corona is the biological identity of a nanoparticle—what a cell identifies and interacts with. Composition is dynamic considering the exchange processes which occur by nanoparticles redistribution among different compartments within the cell as well as to other tissues and organs by the bloodstream [2].

Many factors inherent to nanoparticles are responsible for the formation of protein corona, but surface charge seems to be the most relevant. In general, positively charged nanoparticles tend to increase protein adsorption and to adsorb proteins with isoelectric points (p*I*) lower than 5.5, as in the case of albumin [3]. On the other hand, negatively charged nanoparticles interact with proteins presenting p*I* higher than 5.5, such as IgG [4]. It is believed that the increasing interaction of the positively charged surface of nanoparticles with proteins is due to the negative charge of plasmatic proteins. Surface charge is also relevant in the effect

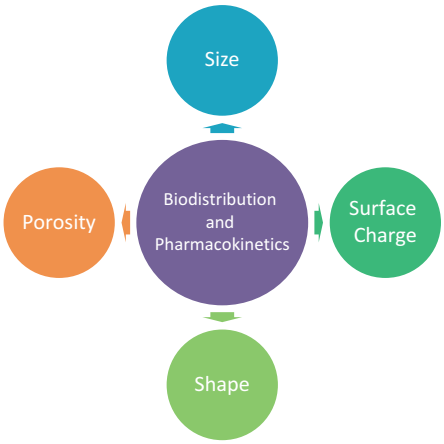

Fig. 6.1 Main factors related to nanoparticles physic-chemistry responsible for tissue distribution and pharmacokinetics

on the proteins upon interactions, considering that either positive or negative surface charge may induce denaturalization, meanwhile neutral charge tends to maintain the nature of the proteins [2]. Other factors which determine protein adsorption are:

- Hydrophobicity: hydrophobic nanoparticles seem to present more sites of interactions with proteins, favoring opsonization [3].
- Size and shape: protein-binding affinity and the composition of protein corona are affected by size and curvature of nanoaprticles [5]. The highly curved surfaces of nanomaterials decrease protein–protein interactions and may undergo conformational changes. The surface area available for protein binding increases with decreasing particle size, so smaller nanoparticles adsorb higher amounts of protein [6].

Silica nanoparticles in contact with plasma tend to bind albumin, fibrinogen (α, β, and γ chains), complement factor C8, heavy chain-immunoglobulin (γ and κ), and apolipoprotein A [7].

Lesniak et al. have reported an interesting research about the effects of protein corona formation by comparing the impact of silica nanoparticles with and without protein corona on the uptake and cytotoxicity in cells [8]. The authors showed that silica nanoparticles were differently internalized in terms of degree and process when they were exposed to A549 cells in complete medium or in serum-free conditions. Bare silica nanoparticles, in the absence of proteins from serum presented direct physical association with cell membrane and concomitant increased degree of adhesion, leading to accumulation in lysosomes and to the presence of free nanoparticles in cytosol. The observation of these free nanoparticles inside the cytoplasm was associated with increased cellular damage.

The particles exposed to cells in serum-free conditions presented a different adsorbed layer in comparison to those exposed with serum. Protein corona in the first case was composed by cell surface proteins, cytosolic proteins as well as membrane lipids. This composition may be associated with the observed cellular damage caused by free serum conditions. In this sense, it may be concluded that formation of protein corona is a determinant step in the consolidation of all the features associated with cell interactions. It is dependent not only on the nature of coating, but also is strictly associated with medium. So, it is mandatory to report not only the conditions of assays but also consider the criteria of the in vitro conditions because protein corona formation and composition determine the nanoparticles interaction with cells as well as cytotocity and metabolic features.

Once nanoparticles enter the bloodstream and after formation of protein corona, the endothelial wall is the first barrier they may pass to achieve tissues and organs. Anyway, certain tissues such as tumors, liver, spleen, and bone marrow present leaky structure in endothelium, so nanoparticles can be taken up by these structures. In general, it is believed that nanoparticles may pass through gaps between endothelial cells depending on their size, impacting this feature in the uptake by certain tissues [9].

In this sense, size and dispersion in physiological medium is very important when nanoparticles are intended for biomedical applications. Aggregation may form large clusters which cause transient embolism in capillaries, this is dangerous in lung and brain, in the case that nanoparticles pass through the blood brain barrier.

Besides the endothelial pass, the arrival of nanoparticles to specific tissues is also governed by the presence of macrophages primarily located in liver, spleen, lungs, and bone marrow. They take part in the so-called mononuclear phagocyte system (MPS), involved in the uptake and metabolism of foreign structures. Macrophages recognize protein corona around the nanoparticles and internalize them via the scavenger receptor. This leads to a decrease in the amount of nanoparticles in circulation [10].

Silica nanoparticles are taken up mainly by macropahges in liver and spleen via endocytosis and phagosomes formation [11]. Size is again the main property inherent in the nanoparticles governing the cellular uptake [12]. Smaller nanoparticles tend to adsorb a larger amount of proteins, increasing the protein corona effect. Thus, the recognition by macropage surface receptors increases, augmenting the cell uptake. Besides this, the smaller size of nanoparticles enables easy biding to the receptor and, thus internalization because it does not influence in extreme the receptor diffusion kinetic through membrane [13]. Metabolism within the cells depends on the chemical stability of the nanoparticles involved.

In general, the lack of studies related to biodistribution of silica nanoparticles lies, as mentioned above, in the necessity of specific methods to track and detect the nanoparticles in vivo. He et al. employed an in vivo optical system to study biodistribution and pharmacokinetics [14]. To this purpose, they doped 45 nm-sized silica nanoparticles with RuBPY dye to study the blood circulation, clearance half-life, and biodistribution in mice. Another parameter that they evaluated was surface modification, by introducing carboxyl groups and poly(ethylene glycol) (PEG) by water-in-oil microemulsion. The research consisted of administration of the nanoparticles intravenously in tail and the posterior evaluation by in vivo images of the fluorescence emitted from the nanoparticles. PEG modified silica nanoparticles exhibited longer circulation time, meanwhile both naked silica and carboxylated-silica nanoparticles were mainly captured by liver. Circulation lifetime obtained by in vivo imaging revealed that PEG coated silica nanoparticles present a blood circulation time estimated at 180 ± 40 min. This time resulted longer than the observed for the hydroxyl-modified counterparts which was about 80 ± 30 min and much longer than the carboxylated-silica nanoparticles, found at 35 ± 10 min.

Cho et al. conducted a research in mice about biodistribution of different sized-fluorescent silica nanoparticles. They compared tissue distribution of three different silica nanoparticles of 50, 100, and 200 nm after intravenous injection [15]. Nanoparticles were captured by macrophages in the spleen and liver and remained there until 4 weeks after administration. The 200 nm particles were taken up faster and more intensively, disappearing thereafter. Considerable amounts were found in the kidneys and no nanoparticles were observed in brain.

These study results were interesting to evaluate general qualitative biodistribution, but a more precise knowledge was necessary about quantitative data. To this purpose, Xie et al. [11] developed a complete and clarifying research about

biodistribution and internalization of silica nanoparticles by a long term quantitative tissue and sub cellular distribution study over 30 days. They employed radioactive iodine labeling and radioactive counting to track and quantify 20 and 80 nm sized-silica nanoparticles in mice. Nanoparticles were surface modified with amine groups, employing 3-aminopropyltriethoxysilane (APTS). This research revealed that liver, spleen, and lungs were the organs in which the nanoparticles were predominantly accumulated after administration. More precisely, 20 nm sized silica nanoparticles were found in higher quantity in liver and spleen in comparison to the bigger ones. This is reasonable, considering the previous discussion about the fact that smaller nanoparticles tend to be easier cell-internalized. After 1 day from injection, 80 nm sized nanoparticles remained in lungs, decreasing this accumulation after 7 days. This fact was associated with aggregation and post-disaggregation with re-distribution to liver and spleen. After 30 days, a considerable amount of both nanosystems in liver and in spleen was found. The study of sub-cellular distribution revealed that silica nanoparticles were taken up mainly by macrophages and no nanoparticles were observed in other cells of these tissues.

Yu et al. conducted an interesting study about biodistribution of radioactive silica nanoparticles with different surface properties mainly in terms of geometry and porosity [16]. They studied solid silica nanoparticles and their counterparts surface-modified with amino groups by APTS; mesoporous silica nanoparticles and silica nanorods. Besides the physic-chemical properties of the nanoparticles evaluated, they were found mainly accumulated in liver and spleen. Distribution to lung was dependent on their properties. Solid silica nanoparticles obtained by the Stöber process as well as their amine-modified counterparts presented a high tendency to accumulate in liver rather than in other organs. Accumulation in lung was due to transient association with capillary given that no internalization was observed. Naked solid silica nanoparticles did not exhibit high affinity for lung, in comparison to mesoporous silica nanoparticles and amine-modified solid silica. This is ascribed to the larger size of mesoporous silica nanoparticles as well as to high surface area of mesoporous silica which may improve inter-nanoparticle interaction and, thus, aggregation and accumulation in thin capillaries. Figure 6.2 presents the organs in which silica nanoparticles tend to accumulate in studies performed in mice. Pharmacokinetics analysis of these nanosystems demonstrated that clearance of solid silica nanoparticles and amino-modified solid silica nanoparticles from circulation was higher in comparison to mesoporous silica nanoparticles, revealing their improved association with the reticuloendothelial system.

As previously described, liver and spleen are the main tissues involved in silica nanoparticles accumulation. However, when the nanoparticles are intended to specific targeting, it is necessary to tune their surface in order to direct them to a desired blank, as well as to increase circulation time by avoiding rapid clearance by the reticuloenthelial system. These drawbacks make naked solid silica nanoparticles lowly biocompatible. In general, silica dispersions are stable in aqueous media. However, this stabilization comes from Coulomb repulsion between reduced hydroxyl groups on the surface which may lead to particle

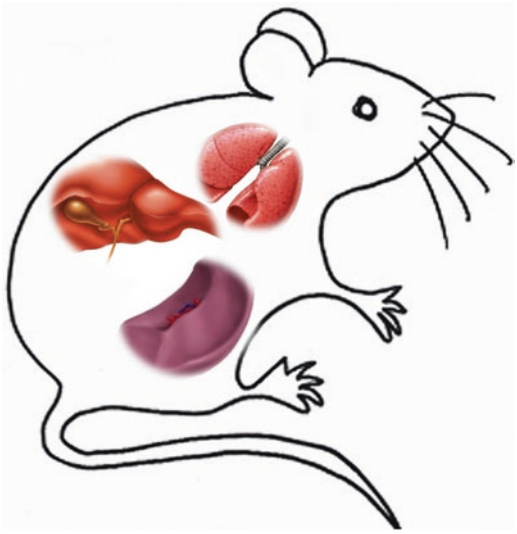

Fig. 6.2 Silica nanoparticles are mainly distributed to liver, spleen, and lung after intravenous administration in mice

aggregation when they are administered intravenously [17]. Aggregation plus increased opsonization enables rapid clearance by the reticuloendothelial system. These facts impart solid silica nanoparticles a poor pharmacokinetics and limit their biocompatibility by the improvement in accumulation in lung capillary vessels, by causing possible embolism [18]. In relation to magnetic silica nanoparticles applied as drug targeting systems, unmodified silica coating tends to rapidly release the drug [19].

In this sense, functionalization of silica surface improves pharmacokinetics and biodistribution as well as induces a sustained release in time. Diverse methods have been developed for surface modification of silica nanoparticles aiming to couple silica to biomolecular targets [20]. Different functional groups, including amino groups, can be introduced easily onto the SiNPs for conjugation with biomolecules.

Zhao et al. carried out a biodistribution research in mice employing multifunctional gelatin-silica nanovectors as gene delivery system. Functionalization consisted of modifying silica surface with a membrane-destabilizing peptide, a tumor target agent, and polyethylenglicol (PEG) for tumor targeting. Study of biodistribution revealed that functionalization increased the capture of nanoparticles by the tumor and extender blood circulation time, with the concomitant diminution of accumulation and toxicity in liver [21].

Evidence suggests that silica nanoparticles are eliminated by urine and also by feces.

The three types of modified-solid silica nanoparticles described above, consisting of bare silica, carboxylated, and PEG modified silica with sizes around 45 nm were mainly excreted by urine. The in vivo fluorescence imaging evidenced the presence of nanoparticles in some organs related to formation and excretion of urine such as

kidneys and bladder. The study of urine pools 4.5 h after intravenous injection obtained by bladder puncture demonstrated that the three types of silica nanoparticles were excreted intact in urine. The study of remanent fluorescence in mice after 24 h revealed relatively little excretion [14].

The silica radio labeled nanoparticles studied by Yu et al. consisting of solid silica nanoparticles, amine surface-modified silica nanosystems, mesoporous silica nanoparticles, and silica nanorods revealed interesting data in terms of excretion [16]. The study consisted of the intravenous administration of a 20 mg/kg dose of the different silica nanoparticles and the collection of urine and feces after 2, 24, and 48 h post injection.

Samples were immediately weighed and their radioactivity was measured. Radioactivity was found in urine and feces indicating the excretion of either the nanoparticles or their degraded product. Accumulation in the kidneys was low as demonstrated by the biodistribution assay. It is possible that nanoparticles were degraded into orthosilicic acid species and were cleared through the renal route. Evidence also exists of excretion by urine of large silica nanoparticles, around 100 nm [22]. So, both whole silica nanoparticles and degradation products may be excreted by urine. The examination of feces indicated that silica nanoparticles may be eliminated through the hepatobiliary route. Evidence about silica nanoparticles excretion from this research work suggests that these nanosystems or their biodegradation products may be eliminated by urine or feces.

6.2 Magnetic Core: Biodistribution and Elimination of Magnetite Nanoparticles

While the biodistribution of magnetic nanoparticles is governed by the coating, which was described in terms of silica in the previous section, in this section biodistribution and pharmacokinetic parameters of the nanoparticulate magnetite as magnetic core are involved.

Magnetite nanoparticles tend toward aggregation due to the bipolar anisotropic attraction. So, surface modification is mandatory to achieve stabilization and monodispersion as well as to improve biocompatibility. In this sense diverse studies have been performed to evaluate the influence of simple functionalization agents for magnetite either to stabilize or to provide a platform for subsequent coating. So in literature, research is found about the biodistribution profile of modified-nano magnetite with diverse agents but not research on naked magnetite because of aggregation.

The superparamagnetic character of iron oxide based nanoparticles enables in vivo detection by magnetic and nonmagnetic methods to perform biodistribution studies. Non-magnetic methods are based on the quantification of iron content using elemental analyses such as inductively coupled plasma optical emission spectrometry and Prussian blue staining. These methods are not specific to differentiate

endogenous iron and iron from nanoparticles. Moreover, they are not able to detect low doses, considering that the amount of iron administered in the form of nanoparticles is around 10 % of the natural content of iron in liver [23]. On the other hand, magnetic methods are based on the variation of magnetic properties of the nanoparticles. This imparts specificity by avoiding the contribution of physiologically endogenous iron from tissues and blood.

Alternating current susceptibility represents a sensitive method for quantification by employing a magnetometer. The disadvantages associated with this method are the requirement of strict drying of the samples and the performance of measurements in a vacuum medium at low temperatures [24]. Another method is based on the use of magnetization curves at room temperature to quantify magnetic moments. For example, Zysler et al. developed a technique to quantify superparamagnetic iron oxide nanoparticles in different tissues and organs of mice based on the analysis of magnetization versus magnetic field curves [25].

Ruiz et al. performed a research devoted to study biodistribution and elimination of iron oxide nanoparticles coated with meso-2,3-dimercaptosuccinic acid (DMSA) and conjugated to PEG-derived molecules by 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) chemistry [26]. The 12 nm-sized nanoparticles biodistribution pattern was evaluated in a rat model, revealing that PEG coating was responsible for the increased time of residence in blood and consequently, accumulation in liver and spleen was reduced. The specific study on liver revealed a uniform distribution into this organ.

The metabolism and elimination of iron oxide nanoparticles depends strictly on iron metabolism. Once opsonized, iron oxide nanoparticles are captured by cells via endocytosis and they accumulate in endosomes which are compartments with acidic medium to undergo degradation. In this process the iron metabolic process is activated and two proteins take part in iron metabolism: ferrireductase and divalent metal transporter (DMT1) are responsible for reducing ferric to ferrous iron and releasing it into cytoplasm. After this, iron enters the cell metabolism process and it is incorporated in the synthesis of the heme group in mitochondria as well as stored as ferritin [27]. The transport to the exterior of the cell is governed by ferroportin, which is a transmembrane transporter. In the extracellular medium it undergoes metabolism to be transported to other tissues and organs. Iron is then oxidized by ceruloplasmin and hephaestin. Apo-transferrin is the protein which recognizes the oxidized form of the complex transferrin-Fe^{3+} which is transported by the bloodstream, and it is recognized by cells expressing the transferring receptor, mainly those in which iron contributes to specific functions such as hepatocytes in liver, erythroblasts in bone marrow, and red blood cells [23]. Coating of iron oxide nanoparticles may introduce some altering mainly in the uptake by cells. Anyway, the metabolic pathway which nanoparticles undergo tends to respond to the iron.

Figure 6.3 schematizes all the topics discussed in this chapter related to silica and magnetic nanoparticles metabolism.

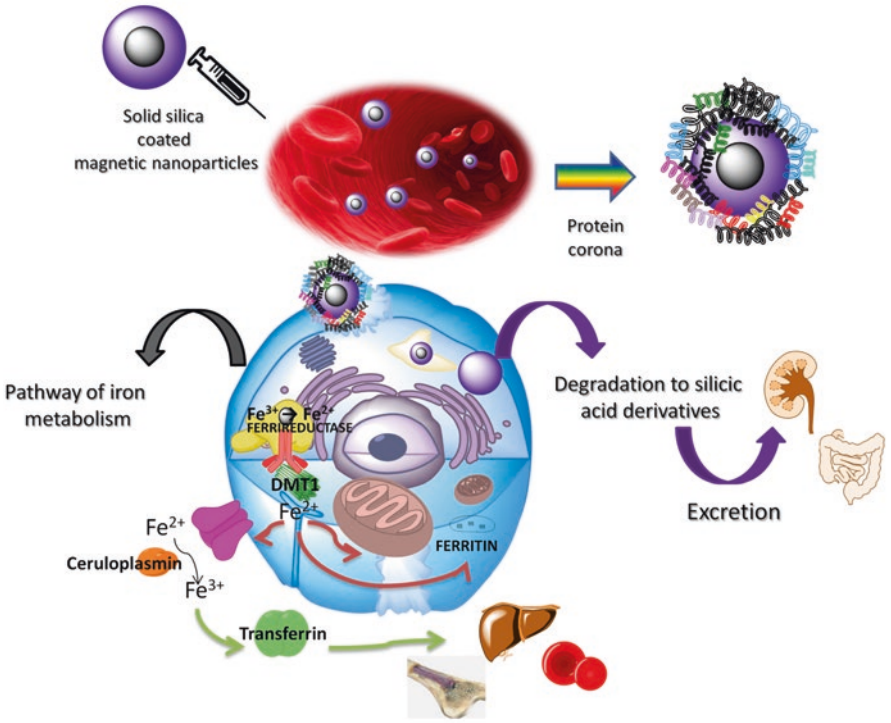

Fig. 6.3 Metabolic pathway followed by silica magnetic nanoparticles when they enter the bloodstream

6.3 Magnetic Silica Nanoparticles

The only magnetic nanosystem coated with solid silica has been developed and biologically studied by Kim et al. The authors synthesized biocompatible silica-overcoated magnetic nanoparticles labeled with rhodamine B isothiocyanate (RITC). The synthesis procedure enabled controlled thickness of the silica shell. The biological impact of 50 nm sized nanoparticles was researched in vivo by the intraperitoneal injection in mice of 100, 50, and 25 mg/kg doses during 4 weeks. Biodistribution patterns exhibited that these nanodevices were detected in diverse organs such as liver, lungs, kidneys, spleen, heart, testes, and uterus time-dependently. The most interesting finding was that the silica coated magnetic nanoparticles were able to penetrate the blood brain barrier as they were detected in brain. Unlike commonly observed in silica nanoparticles, in this study the significant presence of magnetic nanoparticles coated with silica in the lung was not found. Localization of the MNPs in the liver, lungs, and spleen was not consistent with the RES system. These results suggested that several factors determine the biodistribution of magnetic-silica nanoparticles [28].

In summary, the biodistribution on silica coated-iron oxide nanoparticles depends mainly on silica and formation of protein corona is determinant in cellular uptake, metabolism, and cytotoxicity. Anyway, factors other than coating govern magnetic-silica biodistribution considering the differences found between silica nanoparticles and magnetic-silica nanoparticles. Metabolism and excretion pathways are dependent on the composition of the nanoparticles: silica coating would undergo specific cell processes to be metabolized and nanosized iron oxide may follow iron metabolism for accumulation or re-distribution.

References

1. Muthusamy, B., Hanumanthu, G., Suresh, S., Rekha, B., Srinivas, D., Karthick, L., et al. (2005). Plasma Proteome Database as a resource for proteomics research. *Proteomics, 5*(13), 3531–3536.
2. Lynch, I., & Dawson, K. A. (2008). Protein-nanoparticle interactions. *Nano Today, 3*(1), 40–47.
3. Aggarwal, P., Hall, J. B., McLeland, C. B., Dobrovolskaia, M. A., & McNeil, S. E. (2009). Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Advanced Drug Delivery Reviews, 61*(6), 428–437.
4. Gessner, A., Lieske, A., Paulke, B. R., & Müller, R. H. (2002). Influence of surface charge density on protein adsorption on polymeric nanoparticles: Analysis by two-dimensional electrophoresis. *European Journal of Pharmaceutics and Biopharmaceutics, 54*(2), 165–170.
5. Monopoli, M. P., Walczyk, D., Campbell, A., Elia, G., Lynch, I., Baldelli Bombelli, F., et al. (2011). Physical–chemical aspects of protein corona: Relevance to in vitro and in vivo biological impacts of nanoparticles. *Journal of the American Chemical Society, 133*(8), 2525–2534.
6. Mahmoudi, M., Lynch, I., Ejtehadi, M. R., Monopoli, M. P., Bombelli, F. B., & Laurent, S. (2011). Protein–nanoparticle interactions: Opportunities and challenges. *Chemical Reviews, 111*(9), 5610–5637.
7. Deng, Z. J., Mortimer, G., Schiller, T., Musumeci, A., Martin, D., & Minchin, R. F. (2009). Differential plasma protein binding to metal oxide nanoparticles. *Nanotechnology, 20*(45), 455101.
8. Lesniak, A., Fenaroli, F., Monopoli, M. P., Åberg, C., Dawson, K. A., & Salvati, A. (2012). Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. *ACS Nano, 6*(7), 5845–5857.
9. Li, S. D., & Huang, L. (2008). Pharmacokinetics and biodistribution of nanoparticles. *Molecular Pharmaceutics, 5*(4), 496–504.
10. Opanasopit, P., Nishikawa, M., & Hashida, M. (2002). Factors affecting drug and gene delivery: Effects of interaction with blood components. *Critical Reviews in Therapeutic Drug Carrier Systems, 19*, 191–233.
11. Xie, G., Sun, J., Zhong, G., Shi, L., & Zhang, D. (2010). Biodistribution and toxicity of intravenously administered silica nanoparticles in mice. *Archives of Toxicology, 84*(3), 183–190.
12. Chithrani, B. D., Ghazani, A. A., & Chan, W. C. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters, 6*, 662–668.
13. Gao, H., Shi, W., & Freund, L. B. (2005). Mechanics of receptor-mediated endocytosis. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 9469–9474.
14. He, X., Nie, H., Wang, K., Tan, W., Wu, X., & Zhang, P. (2008). In vivo study of biodistribution and urinary excretion of surface-modified silica nanoparticles. *Analytical Chemistry, 80*(24), 9597–9603.

15. Cho, M., Cho, W. S., Choi, M., Kim, S. J., Han, B. S., Kim, S. H., et al. (2009). The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. *Toxicology Letters, 189*(3), 177–183.
16. Yu, T., Hubbard, D., Ray, A., & Ghandehari, H. (2012). In vivo biodistribution and pharmacokinetics of silica nanoparticles as a function of geometry, porosity and surface characteristics. *Journal of Controlled Release, 163*(1), 46–54.
17. Yoon, T. J., Yu, K. N., Kim, E., Kim, J. S., Kim, B. G., Yun, S. H., et al. (2006). Specific targeting, cell sorting, and bioimaging with smart magnetic silica core–shell nanomaterials. *Small, 2*(2), 209–215.
18. Barbe, C., Bartlett, J., Kong, L., Finnie, K., Lin, H. Q., Larkin, M., et al. (2004). Silica particles: A novel drug-delivery system. *Advanced Materials, 16*(21), 1959–1966.
19. Li, Z. Z., Wen, L. X., Shao, L., & Chen, J. F. (2004). Fabrication of porous hollow silica nanoparticles and their applications in drug release control. *Journal of Controlled Release, 98*(2), 245–254.
20. Mader, H., Li, X., Saleh, S., Link, M., Kele, P., & Wolfbeis, O. S. (2008). Fluorescent silica nanoparticles. *Annals of the New York Academy of Sciences, 1130*(1), 218–223.
21. Zhao, X., Wang, J., Tao, S., Ye, T., Kong, X., & Ren, L. (2016). In vivo bio-distribution and efficient tumor targeting of gelatin/silica nanoparticles for gene delivery. *Nanoscale Research Letters, 11*(1), 1–9.
22. Huang, X., Li, L., Liu, T., Hao, N., Liu, H., Chen, D., et al. (2011). The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility in vivo. *ACS Nano, 5*(7), 5390–5399.
23. Andrews, N. C. (1999). Disorders of iron metabolism. *New England Journal of Medicine, 341*(26), 1986–1995.
24. Gutiérrez, L., Mejías, R., Barber, D. F., Veintemillas-Verdaguer, S., Serna, C. J., Lázaro, F. J., et al. (2011). Ac magnetic susceptibility study of in vivo nanoparticle biodistribution. *Journal of Physics D: Applied Physics, 44*(25), 255002.
25. Zysler, R. D., Lima, E., Jr., Mansilla, M. V., Troiani, H. E., Pisciotti, M. L., Gurman, P., et al. (2013). A new quantitative method to determine the uptake of SPIONs in animal tissue and its application to determine the quantity of nanoparticles in the liver and lung of Balb-c mice exposed to the SPIONs. *Journal of Biomedical Nanotechnology, 9*(1), 142–145.
26. Ruiz, A., Hernandez, Y., Cabal, C., González, E., Veintemillas-Verdaguer, S., Martinez, E., et al. (2013). Biodistribution and pharmacokinetics of uniform magnetite nanoparticles chemically modified with polyethylene glycol. *Nanoscale, 5*(23), 11400–11408.
27. Levy, M., Luciani, N., Alloyeau, D., Elgrabli, D., Deveaux, V., Pechoux, C., et al. (2011). Long term in vivo biotransformation of iron oxide nanoparticles. *Biomaterials, 32*(16), 3988–3999.
28. Kim, J. S., Yoon, T. J., Yu, K. N., Kim, B. G., Park, S. J., Kim, H. W., et al. (2006). Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicological Sciences, 89*(1), 338–347.

Chapter 7
Toxicological Insights

Abstract Many factors govern the toxicological feature of silica-coated magnetic nanoparticles and they are associated with intrinsic features of the nanoparticles and also cell type. In vitro studies on cells and in vivo experiments trend to reveal the biocompatibility of silica and magnetite in the nano-scale.

The knowledge about toxicology of magnetic silica nanoparticles is intended not only to ensure safety of new devices intended for biomedical purposes such as drug targeting and delivery, but also as a tool for therapeutic insights if nanoparticles are present in selective toxicity. Thus, it is important to evaluate toxicological features associated with each novel nanodevice. This chapter describes the mechanisms associated with the toxicology of nano-silica, iron oxide magnetic nanoparticles, and the effects of the combination of the magnetic core with silica in different types of cells as well as the impact in vivo in different animals. All the aspects that govern toxicity are carefully considered and described to provide a global knowledge about the effect on the whole organism.

Keywords Iron oxide nanoparticles • Silica • Toxicity • Cells • Toxicological mechanisms

The safety and bioeffects of magnetic solid silica nanoparticles is a key factor in the development of new devices intended for biomedical purposes such as drug targeting and delivery. In this chapter we will discuss toxicology of magnetic silica nanoparticles approaching in vitro and in vivo studies focused on revealing the biocompatibility of silica and magnetite in the nano-scale.

7.1 Toxicological Studies in Cell Culture and In Vivo of Solid Silica Nanoparticles

The first studies devoted to reveal the toxicity associated with silica were performed at macro or microscale in order to understand the linkage between inhalation of micro-silica by humans and silicosis. Silicosis is a pulmonary disease product of the direct cytotoxicity of silica on lung cells which undergo inflammatory processes

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_7

related to the release of lipases and proteases, activation of oxidant production by pulmonary phagocytes activate the antioxidant defenses leading to lipid peroxidation, protein nitrosation, cell injury, and lung scarring [1]. Previous to these studies, inhalation of crystalline silica was associated with pulmonary fibrosis and cancer [2]. On the other hand, research performed on amorphous silica revealed that it does not pose risks to health.

Thus, the study about toxicity of nano-sized silica on biologic milieu is mandatory to ensure safety in biomedicine, considering the promising applications of solid silica in nanobiotechnology.

Pioneering research studies aimed at evaluating the toxicology of silica nanoparticles were done in cell cultures. One of them was performed by Chen et al. in 2005 and provided valuable information regarding effects of fluorochrome-labeled SiO_2 nanoparticles sized between 40 nm and 5 μm in epithelial cells [3]. They observed by confocal laser scanning microscopy and differential interference contrast that all sized nanoparticles penetrated the cell; meanwhile, only particles sized between 40 and 70 nm had access to the nucleus, achieving localization in distinct subnuclear domains of nucleoplasm, except nucleoli. Larger particles, between 200 nm and 5 μm located in cytoplasm. The authors observed that smaller SiO_2 nanoparticles induced nucleoplasmic clusters of topoisomerase I which is an enzyme that solves the topological problems which emerge while DNA replication, transcription, chromatin assembly, recombination, and chromosome segregation by introducing transient breaks into the DNA helix [4]. Aggregation of other proteins such as ubiquitin, proteasomes, cellular glutamine repeat proteins, and huntingtin was also observed. These effects are associated with inhibition in replication and transcription. On the other hand, alterations in cell viability or proteasomal activity were not observed. Results obtained in this study suggest that silica nanoparticles able to enter the nucleus may trigger a subnuclear pathology related to neurodegenerative disorders induced by alterations in polyglutamine. These facts also reveal a size-dependant toxicological effect.

Lin et al. conducted research about the effect of 15 and 46 nm sized silica nanoWWWparticles cultured human bronchoalveolar-carcinoma derived cells [5]. They assayed different doses between 10 and 100 μg/mL during 24, 48, and 72 h, revealing both a dose and time dependant decrease in cell viability. Cytotoxicity was not size dependant in this case. In order to provide more concise information about the mechanisms associated with cytotoxicity, the authors analyzed different indicators of oxidative stress such as reactive oxygen species, glutathione, malondialdehyde, and lactate dehydrogenase. The study revealed an increased production of reactive oxygen species concomitant with reduced glutathione levels in the cancer lung cells exposed to the silica nanoparticles. In addition, lipid peroxidation and membrane damage was evidenced from an increase in the production of malondialdehyde and lactate dehydrogenase release from cells.

Free radicals are a family of reactive oxygen species (ROS) composed of the anion-radical superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). They are commonly generated as subproducts from cellular metabolism: electrons emerging from the transport chain during aerobic respiration, enzyme activity, auto-oxidation of biological macromolecules or cyclic redox properties of

some compounds present in biological fluids. The deleterious effects of these species are counteracted by certain defense mechanisms in cells, mediated by enzymes with antioxidant activity such as superoxide dismutase, catalase and glutathione peroxidase, as well as by antioxidant molecules of low molecular weight. The excessive production of reactive oxygen species is very dangerous because under conditions of oxidative stress it can induce significant damage on the cellular and molecular level when they react with biomolecules such as deoxyribonucleic acid (DNA), lipids, and proteins. These events can lead to the development of diseases such as cancer, atherosclerosis, asthma, and neurodegenerative disorders [6].

Chang et al. conducted interesting research about the dependence of silica cytotoxicity with cell type [7] by investigating the effects of 80 nm sized silica nanoparticles exposure at different doses (up to 500 μg/mL milieu) on several types of normal fibroblasts of lung, stomach, intestine, and skin as well as on diverse types of cancer cells. The authors found that amorphous silica nanoparticles did not exert cytotoxicity at low doses. High doses induced cell membrane damage. Regarding cell type, fibroblast were more susceptible than cancer cells to the exposure to silica nanoparticles. These data reveal that the metabolic activity is determinant in the cytotoxic effect. In the same research, a nanocomposite synthesized with chitosan and silica was evaluated. This nanodevice presented a lower cytotoxic effect than silica nanoparticles. Thus, combination of silica with other biocompatible materials may be a suitable strategy for developing novel nanoparticles for biomedical applications.

The first in vivo research about toxicity of silica nanoparticles was conducted by Park et al. aiming to evaluate stress and inflammatory response associated with the nanodevices [8]. After a single treatment by intraperitoneally injection in mice with different doses of 12 nm sized silica nanoparticles (50; 100, and 250 mg/kg) the viability of several cell types harvested from treated mice was evaluated. When peritoneal macrophages were cultured and studied, their activation as mRNA expressions of inflammation-related genes were elevated. An increased release of nitric oxide was also detected for these cells. The viability of splenocytes from treated mice was tested, finding a dose dependant decrease: cell proliferation was affected at doses of 100 and 250 mg/kg. The spleen leukocyte subtypes analysis revealed a change with respect to control: T and NK cells were elevated meanwhile type B lymphocytes were decreased. From these results and to elucidate the pro-inflammatory mechanism exerted by the in vivo exposure to silica nanoparticles, the authors conducted an in vitro study on a cell line derived from mouse peritoneal macrophages (RAW264.7 cells). The results revealed that the treatment with silica nanoparticles induced an increase of reactive oxygen species and a concomitant decrease of intracellular glutathione. Thus, this study also demonstrated that toxicity of silica nanoparticles is exerted by activation of oxidative stress in macrophages and the induction of an inflammatory response.

Meanwhile, Nishimori et al. investigated the relationship between silica nanoparticles size (70, 300, and 1000 nm), and toxicity. The authors demonstrated hepatotoxic effects exerted by 70 nm sized silica nanoparticles in mice. Doses of 50 and 100 mg/kg were intravenously injected. Administration of 50 and 100 mg/kg of the

70 nm sized particles resulted lethal and the examination of liver indicated degenerative necrosis of hepatocyes. On the other hand, the administration of the same doses corresponding to 300 and 1000 nm caused abnormalities neither in liver, spleen, lung nor kidney. A dose of 30 mg/kg for the 70 nm-sizes silica nanoparticles did not exert toxic effects on the mentioned organs; however, toxicity in liver was observed. Thus, in this research size and doses are two influencing factors in the induction of toxicity. The chronic administration of 70-nm particles caused liver fibrosis at doses that were non-toxic in a single injection [9].

The in vivo assay exerted by Xie et al. to evaluate biodistribution and toxicity of amine modified-silica nanoparticles with 3-aminopropyltriethoxysilane showed interesting results [10]. They intravenously injected mice with doses of 1 mg/kg of 125-iodine radio-labeled silica nanoparticles of 20 and 80 nm. Toxicologic assay demonstrated that lungs and spleen did not display significant changes in morphology in comparison to control. On the other hand, liver showed signs of pathological changes by the presence of mononuclear inflammatory infiltrate at the portal area as well as hepatocyte necrosis at the portal triads.

Yu et al. evaluated the impact of silica nanoparticles on cellular toxicity and hemolytic activity in terms of surface characteristics [11]. To this purpose, they evaluated the cytotoxic effects of non-porous silica nanospheres (115 nm diameter) obtained by the Stöber method, mesoporous silica nanospheres (120 nm), mesoporous silica nanorods with aspect ratio of 2, 4, and 8 (width by length 80×200 nm, 150×600 nm, 130×1000 nm), and their cationic counterparts on macrophages, lung carcinoma cells, and human erythrocytes.

Nonporous Stöber nanoparticles presented high cellular association. This was associated with the highest silanol density on the continuous external surface. The corresponding amine-modified counterparts presented lowest association ascribable to a decrease of silanol groups. Anyway, when the positive surface charge increases over 30 mV, the association also increases significantly because the interaction with the negative surface charge receptor of cells becomes predominant. Association and internalization evaluated in RAW 264.7 cells shows that Stöber nanoparticles led to a significant increase in cellular association after 24 h incubation at 37 °C. This research provided much knowledge about the interaction of amorphous silica nanoparticles with different cells, to better understand the clearance and also the cytotoxicity resulting from the internalization. Another observation plausible from this work is that toxicity of silica nanoparticles is cell-type dependent—mainly governed by surface charge. The hemolysis assay performed showed that the hemolytic activity was dose dependant; there was a rapid onset of hemolysis as the concentration of nanoparticles increased. An increase in surface charge might enhance interactions of nanoparticles with RBCs and the resultant elevated hemolysis by amine-modified silica nanoparticles.

Going on with their research, these authors proposed that one of the main adverse effects of silica nanoparticles, limiting bio-safety, is due to mechanical obstruction in the vasculature. This paper asserts that the effects on vasculature are responsible for organs congestion and subsequent inflammation and failure rather than the cytotoxic effects. In this sense, size seems to be the main feature of nanoparticles involved

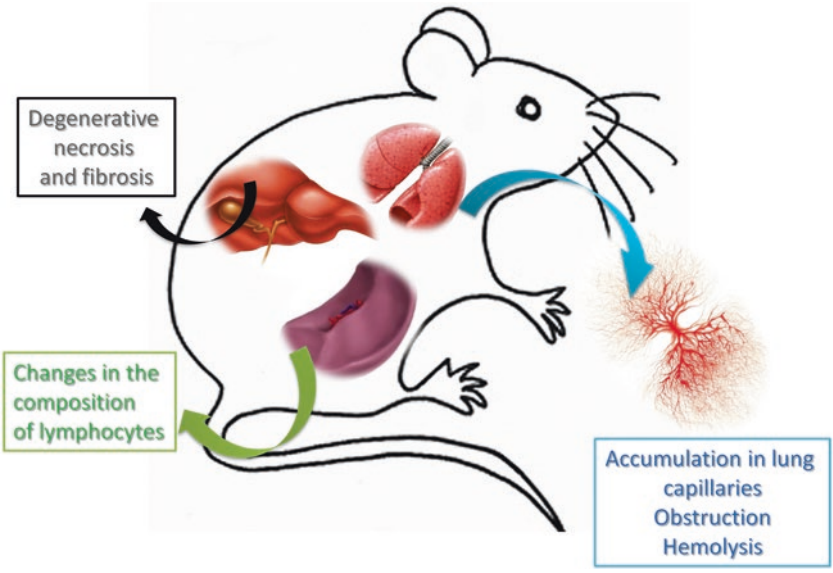

Fig. 7.1 Main toxic effects exerted in different organs and tissue induced by silica nanoparticles in mice. Please see text for more detailed information about dependence on size, surface charge, and doses

in vasculature impact. Lung and kidneys are the most susceptible organs to suffer from nanoparticles obstruction in vasculature due to their abundant blood supply and special anatomic structures. Anyway, solid silica nanoparticles, by suitable dispersion in physiological milieu as well as due to relative intermediate size do not cause vasculature obstruction [12].

A recent study on amorphous silica nanoparticles, both bare and modified with amine functional groups by 3-aminopropyltriethoxysilane, revealed no cytotoxicity in a human lung cancer epithelial cell line (A549) at doses below 200 μg/mL. An in vivo assay was also performed on mice to assess lung inflammation. To this end, mice were administered with doses of 0.1 and 0.5 mg NPs/mouse of bare or amine-functionalized silica nanoparticles via intra-tracheal instillation. At the highest dose of bare silica nanoparticles, an over expressed inflammatory response was observed, evidenced by an increase in neutrophils and protein level in bronchoalveolar fluid. Functionalization of silica nanoparticles with amine groups reduced inflammation in murine lung evidencing an improvement of the nanomaterial biocompatibility [13]. Figure 7.1 presents the tissues and organs affected by silica nanoparticles in studies performed in mice. All the observed toxic effects were dose and size dependant.

In summary, with the evidence collected from all research performed to evaluate toxicological aspects of solid silica nanoparticles, the cytotoxicity exerted by these nanodevices depends on factors inherent to the nanoparticles such as surface charge and size; and also is cell type dependent. Positively charged silica nanoparticles with values higher than 30 mV tend to associate intensively with cells, so all the mechanisms related to cytotoxicity are triggered in these cases. The mechanisms by

which silica nanoparticles result cytotoxic are related to the generation of oxidative stress in cytoplasm and to accumulation in nucleus, in the case of smaller nanoparticles, where they alter the normal functionality of gene transcription.

7.2 Toxicology of Iron Oxide Magnetic Nanoparticles

As previously described in Chap. 6, iron is a natural occurring ion in the body and several mechanisms involved in its metabolism are known and described [14]. Iron from MNPs is gradually cleared and degraded to Fe^{3+} by different endogenous metabolic pathways. Then it enters the pool of body iron to be used in the generation of red blood cells. The excess is excreted by the kidneys [15].

The cytotoxicity attributed to iron oxide magnetic nanoparticles is considered dose-dependent [16] and it is associated with diverse effects. Iron oxide nanoparticles may interfere with normal cell surface interactions when attached to the external membrane [17]. Other plausible cytotoxic effects may emerge from an imbalance of cytoplasmic iron ions which may cause oxidative stress leading to cellular toxicity, impaired cell metabolism, and concomitant increment in apoptosis [18]. The production of reactive oxygen species is associated with diverse mechanisms triggered by the iron oxide nanoparticles:

- The surface of the nano-magnetic iron oxide may induce reactive oxygen species generation.
- The leaching of the metal in case of coated nanoparticles.
- The oxidants released by metabolic enzymatic degradation of the magnetic nanoparticles.

Once iron oxide nanoparticles have entered the cell, they are accumulated or metabolized. These facts may lead to different possible sources of toxicity. Degradation products possibly react with various components of the body or cells.

Diverse studies were performed aiming to clarify the mechanisms associated with toxicity of iron oxide nanoparticles. It is always important to consider each nanosystem in terms of magnetic core and coating to evaluate specific toxicological insights Was composition, size, and surface charge. Coated and un-coated iron oxide nanoparticles present different reactivity ascribable to the different surface properties.

Magnetite nanoparticles have been proven to induce oxidative damage on DNA of a human lung ephitelial cell line while this effect was not observed for maghemite [19].

The research work conducted by Mahmoudi et al. revealed that uncoated magnetite nanoparticles exerted DNA damage on the mouse fibroblast cell line L929 in comparison to the polyvinyl alcohol (PVA)-coated counterparts. The treatment with iron oxide magnetic nanoparticles induced the formation of gas vesicles in the cytoplasm possibly due to interactions of nanoparticles with proteins causing their denaturalization [20].

Regarding the toxicology of nanosystems composed of magnetic silica, only Kim et al. have synthesized and evaluated the toxicity in mice of these devices.

The 50 nm sized silica coated-magnetite nanoparticles labeled with rhodamine B isothiocyanate (RITC) were administered sub-acutely in vivo to a murine model in order to evaluate serum biochemistry, hematological impact, and histopathology. In addition, in vitro mutagenesis assays were performed to evaluate genotoxicity. This research demonstrated that after 4 weeks of exposure the magnetic silica nanoparticles persisted in mice organisms without causing apparent signs of toxicity [21].

Figure 7.2 presents the cytotoxic mechanisms induced by magnetic-silica nanoparticles and Fig. 7.1 shows the main organs and systems affected in murine models.

All this data recovered from an extensive analysis of bibliographic data indicates that more research is needed in terms of toxicology of magnetic silica nanoparticles. There exist many factors and diverse mechanisms implied in the toxicological effects of the nanoparticles, related to both intrinsic features of nanoparticles and cell type. The knowledge and understanding of the mechanisms associated with toxicology of these nanodevices is extremely valuable not only for the side effects associated with potential therapy but also as a tool for therapeutic insights. For example, if these nanoparticles are associated with oxidative damage, reactive oxygen species can be used in cancer therapies to destroy abnormal cells. Magnetic guidance may be a very useful tool to guide the particles to a desired site, such as a tumor. Silica coating may

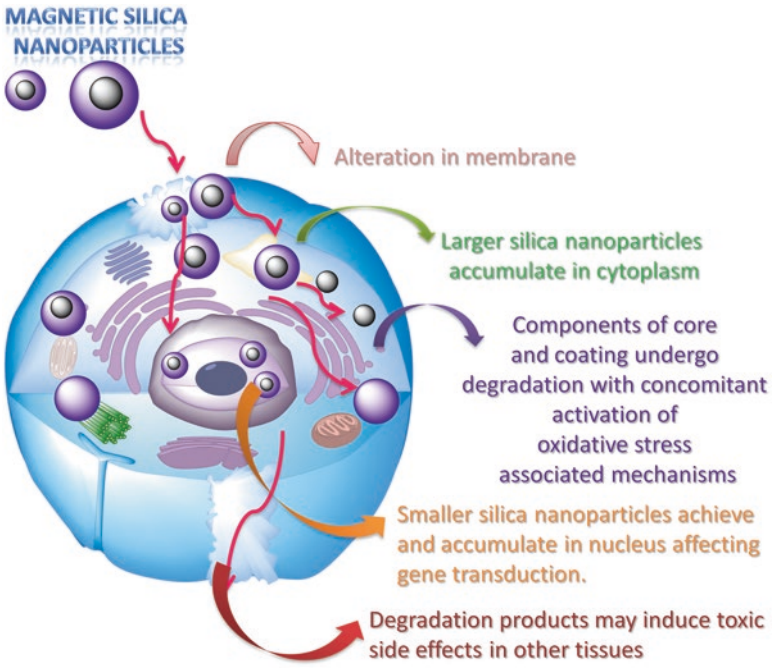

Fig. 7.2 Cytotoxic mechanism induced by magnetic-silica nanoparticles

be useful for the attachment of therapeutic drugs. In this sense, the carrier may also act as a therapeutic agent improving the effect of the drug.

Thus, it is also important to evaluate toxicological features associated with each particular nanodevice considering all the aspects that govern toxicity.

References

1. Castranova, V., & Vallyathan, V. (2000). Silicosis and coal workers' pneumoconiosis. *Environmental Health Perspectives, 108*(Suppl 4), 675.
2. Saffiotti, U. (1991). Lung cancer induction by crystalline silica. *Progress in Clinical and Biological Research, 374*, 51–69.
3. Chen, M., & von Mikecz, A. (2005). Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles. *Experimental Cell Research, 305*(1), 51–62.
4. Champoux, J. J. (2001). DNA topoisomerases: Structure, function, and mechanism. *Annual Review of Biochemistry, 70*(1), 369–413.
5. Lin, W., Huang, Y. W., Zhou, X. D., & Ma, Y. (2006). In vitro toxicity of silica nanoparticles in human lung cancer cells. *Toxicology and Applied Pharmacology, 217*(3), 252–259.
6. Abuhijleh, A. L., & Khalaf, J. (2010). Copper (II) complexes of the anti-inflammatory drug naproxen and 3-pyridylmethanol as auxiliary ligand. Characterization, superoxide dismutase and catecholase–mimetic activities. *European Journal of Medicinal Chemistry, 45*(9), 3811–3817.
7. Chang, J. S., Chang, K. L. B., Hwang, D. F., & Kong, Z. L. (2007). In vitro cytotoxicitiy of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. *Environmental Science & Technology, 41*(6), 2064–2068.
8. Park, E. J., & Park, K. (2009). Oxidative stress and pro-inflammatory responses induced by silica nanoparticles in vivo and in vitro. *Toxicology Letters, 184*(1), 18–25.
9. Nishimori, H., Kondoh, M., Isoda, K., Tsunoda, S. I., Tsutsumi, Y., & Yagi, K. (2009). Silica nanoparticles as hepatotoxicants. *European Journal of Pharmaceutics and Biopharmaceutics, 72*(3), 496–501.
10. Xie, G., Sun, J., Zhong, G., Shi, L., & Zhang, D. (2010). Biodistribution and toxicity of intravenously administered silica nanoparticles in mice. *Archives of Toxicology, 84*(3), 183–190.
11. Yu, T., Malugin, A., & Ghandehari, H. (2011). Impact of silica nanoparticle design on cellular toxicity and hemolytic activity. *ACS Nano, 5*(7), 5717–5728.
12. Yu, T., Greish, K., McGill, L. D., Ray, A., & Ghandehari, H. (2012). Influence of geometry, porosity, and surface characteristics of silica nanoparticles on acute toxicity: Their vasculature effect and tolerance threshold. *ACS Nano, 6*(3), 2289–2301.
13. Morris, A. S., Adamcakova-Dodd, A., Lehman, S. E., Wongrakpanich, A., Thorne, P. S., Larsen, S. C., et al. (2016). Amine modification of nonporous silica nanoparticles reduces inflammatory response following intratracheal instillation in murine lungs. *Toxicology Letters, 241*, 207–215.
14. Hall, J. E. (2015). *Guyton and Hall textbook of medical physiology*. Philadelphia, PA: Elsevier Health Sciences.
15. Anzai, Y., Piccoli, C. W., Outwater, E. K., Stanford, W., Bluemke, D. A., Nurenberg, P., et al. (2003). Evaluation of neck and body metastases to nodes with ferumoxtran 10-enhanced MR imaging: Phase III safety and efficacy study 1. *Radiology, 228*(3), 777–788.
16. Boyer, C., Whittaker, M. R., Bulmus, V., Liu, J., & Davis, T. P. (2010). The design and utility of polymer-stabilized iron-oxide nanoparticles for nanomedicine applications. *NPG Asia Materials, 2*(1), 23–30.

17. Solanki, A., Kim, J. D., & Lee, K. B. (2008). Nanotechnology for regenerative medicine: Nanomaterials for stem cell imaging. *Nanomedicine, 3*(4), 567–578.
18. Markides, H., Rotherham, M., & El Haj, A. J. (2012). Biocompatibility and toxicity of magnetic nanoparticles in regenerative medicine. *Journal of Nanomaterials, 2012*, 13.
19. Moore, A., Marecos, E., Bogdanov, A., Jr., & Weissleder, R. (2000). Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model 1. *Radiology, 214*(2), 568–574.
20. Mahmoudi, M., Hofmann, H., Rothen-Rutishauser, B., & Petri-Fink, A. (2011). Assessing the in vitro and in vivo toxicity of superparamagnetic iron oxide nanoparticles. *Chemical Reviews, 112*(4), 2323–2338.
21. Kim, J. S., Yoon, T. J., Yu, K. N., Kim, B. G., Park, S. J., Kim, H. W., et al. (2006). Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicological Sciences, 89*(1), 338–347.

Chapter 8
Future Perspectives on Silica-Coated Magnetic Nanoparticles in Biomedicine

Abstract The lack of enough knowledge about the biological impact of silica-coated magnetic nanoparticles makes these systems non well-explored devices. However, the potential they have in terms of biomedical applications is really huge. Therefore, several applications in medicine are waiting to be explored and developed for silica-coated magnetic nanoparticles.

Keeping in mind the advances of nanotechnology applied to medicine or, in fact, nanomedicine as an independent emerging field, it is clear that magnetic and silica nanosystems, individually, are located at the top of the most promising materials intended for these ends. However, as demonstrated throughout the chapters of this book, the combination of both materials in a unique nanocarrier has not been yet well exploited regarding their whole potential.

The concept of theranostics has rapidly arisen as a research area highly impulsed by the improved efficiency compared with traditional therapy and diagnostics tools. In spite of this it is true that clinically available theranostics are still not on the market. This reveals a lack of reliable information mainly associated with biosafety aspects.

In this context the future perspectives are envisaged in this sense. In fact, recent insights in open literature are devoted to the design of theranostics involving targeting of specific drug combined with other suitable moieties sensitive to different diagnostic techniques [1].

Near IR photoacoustic has appeared as a non-invasive technique to imaging diagnostic that may be taken as an alternative to the widely used MRI.

Hemoglobin, the iron containing and oxygen transport protein in the red blood cells, has the property to absorb in the near IR spectral region (NIR). As a consequence it may be inferred that superparamagnetic iron oxides nanoparticles could act as a contrast agent to this imaging technique. By combining this with other clinically available imaging techniques (e.g., MRI) new multimodality systems with an augmented diagnostic capability beyond the inherent limitations of individual components may be achieved [2, 3]. Besides, as bare magnetite nanoparticles are not strong light absorbers in the near-IR, the silica coating results are crucial to improve the contrast efficiency [4].

Current research published the preparation of silica nanoparticles intended as multifunctional tools for diagnostic and therapy of oncological diseases. The nanocarriers

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1_8

were loaded with Doxorubicin (DOX) and Indocyanine Green (ICG), an NIR sensitive dye. Therefore, Doxorubicin plays a role as a chemotherapeutic agent; and ICG may induce cancer cell killing by hyperthermia when laser exposure of NIR occurs [5, 6].

In another work, the NIR dye was entrapped inside the silica matrix whereas DOX was loaded via physical adsorption (called FDSIR820) or via a covalent linkage (called CDSIR820) on the surface of the organically modified silica nanoparticles.

In this case a trifunctional device would act in chemotherapy, adjuvant hyperthermia, and NIR imaging. A deep study of design was developed in terms of the suitable physicochemical properties such as size and aqueous stability. These features ensured optimal cell uptake and deep penetration into tumors. Covalent loading of DOX on the silica particle surface slowed the release of DOX compared to physical adsorption, which resulted in rapid release of DOX from the particles. Exposure to near infrared laser caused an increase in temperature and also favored the release of DOX [7].

It is worth mention that in the cited article magnetic NPs were not included in the formulation. The presence of minimal iron oxide nanoparticle's concentration may make these nanosystems MRI able and would allow an improved accumulation in the tumor site by simply applying an external magnetic field.

Phototherapy is another approach deserving increasing interest, with regard, mainly, to cancer therapy and diagnostic. It is a noninvasive therapy that can be applicable to both neoplastic and non-neoplastic disease [8, 9]. This therapy's fundament lies in the fact that certain therapeutic molecules, named photosensitizers (PS), may accumulate preferentially in malignant tissues, and when these PSs are activated with light of appropriate wavelength, they pass on their excess energy to surrounding molecular oxygen. This results in the generation of reactive oxygen species, such as free radicals, which are toxic; leading to cell death and the consequent activation of the immune system [10]. Due to the extent of the investigations, data on pre-clinical and clinical studies resulted in the approval of a PS based drug, Photofrin®, intended to attack selected tumors [11]. Combining the properties of PS with magnetic nanoparticles, nanosystems with great potential to accumulate in the disease site by simply applying an external magnetic field could be obtained, causing absolute damage to the tumor cells. This strategy would lead to an increment of the treatment efficiency, and a reduction of the PS doses required to reach the desired effects.

A recent article reports the fabrication of a magnetic target drug delivery, loaded with the PS drug methylene blue (MB). In this investigation magnetite nanoparticles were first coated with silica to improve the capability to bind to MB, and to offer better PS properties [12].

In summary, the advance of silica coated magnetic nanosystems in the biomedical field goes in the direction of multiple diagnostic tools combined with multiple therapy, especially when attention is centered on oncological or other high impact diseases. Figure 8.1 schematizes the future scientific and medical perspectives regarding these nanodevices.

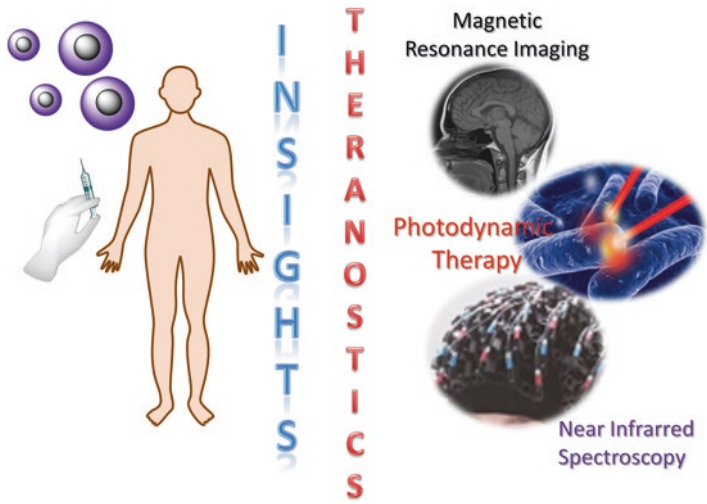

Fig. 8.1 Future insights on biomedical applications of magnetic silica nanoparticles

References

1. Friebel, M., Do, K., Hahn, A., & Mu, G. (1999). Optical properties of circulating human blood in the wavelength range 400–2500 nm. *Journal of Biomedical Optics, 4*(1), 36–46.
2. Jin, Y., Jia, C., Huang, S. W., O'Donnell, M., & Gao, X. (2010). Multifunctional nanoparticles as coupled contrast agents. *Nature Communications, 1*, 41.
3. Qu, M., Mallidi, S., Mehrmohammadi, M., Truby, R., Homan, K., Joshi, P., et al. (2011). Magneto-photo-acoustic imaging. *Biomedical Optics Express, 2*(2), 385–396.
4. Alwi, R., Telenkov, S., Mandelis, A., Leshuk, T., Gu, F., Oladepo, S., et al. (2012). Silica-coated super paramagnetic iron oxide nanoparticles (SPION) as biocompatible contrast agent in biomedical photoacoustics. *Biomedical Optics Express, 3*(10), 2500–2509.
5. Tang, Y., & McGoron, A. J. (2009). Combined effects of laser-ICG photothermotherapy and doxorubicin chemotherapy on ovarian cancer cells. *Journal of Photochemistry and Photobiology B: Biology, 97*(3), 138–144.
6. Fernandez-Fernandez, A., Manchanda, R., Lei, T., Carvajal, D. A., Tang, Y., Kazmi, S. Z. R., et al. (2012). Comparative study of the optical and heat generation properties of IR820 and indocyanine green. *Molecular Imaging, 11*(2), 99–113.
7. Nagesetti, A., & McGoron, A. J. (2016). Multifunctional organically modified silica nanoparticles for chemotherapy, adjuvant hyperthermia and near infrared imaging. *Colloids and Surfaces B: Biointerfaces, 147*, 492–500.
8. Sibata, C. H., Colussi, V. C., Oleinick, N. L., & Kinsella, T. J. (2000). Photodynamic therapy: A new concept in medical treatment. *Brazilian Journal of Medical and Biological Research, 33*(8), 869–880.
9. Chen, Z. L., Sun, Y., Huang, P., Yang, X. X., & Zhou, X. P. (2009). Studies on preparation of photosensitizer loaded magnetic silica nanoparticles and their anti-tumor effects for targeting photodynamic therapy. *Nanoscale Research Letters, 4*(5), 400.
10. Park, S. I., Lim, J. H., Kim, J. H., Yun, H. I., & Kim, C. O. (2005). In vivo and in vitro investigation of photosensitizer-adsorbed superparamagnetic nanoparticles for photodynamic therapy. *IEEE Transactions on Magnetics, 41*(10), 4111–4113.

11. Dougherty, T. J., Gomer, C. J., Henderson, B. W., Jori, G., Kessel, D., Korbelik, M., et al. (1998). Photodynamic therapy. *Journal of the National Cancer Institute, 90*(12), 889–905.
12. Andhariya, N., Chudasama, B., Mehta, R. V., & Upadhyay, R. V. (2011). Nanoengineering of methylene blue loaded silica encapsulated magnetite nanospheres and nanocapsules for photodynamic therapy. *Journal of Nanoparticle Research, 13*(9), 3619–3631.

Index

M.A. Agotegaray, V.L. Lassalle, *Silica-coated Magnetic Nanoparticles*,
SpringerBriefs in Molecular Science, DOI 10.1007/978-3-319-50158-1

N