SPRINGER BRIEFS IN APPLIED SCIENCES AND TECHNOLOGY · NANOTHERANOSTICS

Tomasz Jadczyk · Ewa Bryndza Tfaily
Sachin Mishra · Marek Jędrzejek
Marta Bołoz · Parasuraman Padmanabhan
Wojciech Wojakowski · Zdeněk Stárek
Sylvain Martel · Balázs Gulyás

Innovative Diagnostics and Treatment: Nanorobotics and Stem Cells

Springer

SpringerBriefs in Applied Sciences and Technology

Nanotheranostics

More information about this series at http://www.springer.com/series/13040

Tomasz Jadczyk · Ewa Bryndza Tfaily
Sachin Mishra · Marek Jędrzejek
Marta Bołoz · Parasuraman Padmanabhan
Wojciech Wojakowski · Zdeněk Stárek
Sylvain Martel · Balázs Gulyás

Innovative Diagnostics and Treatment: Nanorobotics and Stem Cells

Springer

Tomasz Jadczyk
Third Division of Cardiology
Medical University of Silesia
Katowice
Poland

Ewa Bryndza Tfaily
Department of Orthopaedic Surgery
University of Illinois at Chicago
Chicago, IL
USA

Sachin Mishra
Lee Kong Chian School of Medicine
Nanyang Technological University
Singapore
Singapore

Marek Jędrzejek
Third Division of Cardiology
Medical University of Silesia
Katowice
Poland

Marta Bołoz
Third Division of Cardiology
Medical University of Silesia
Katowice
Poland

Parasuraman Padmanabhan
Lee Kong Chian School of Medicine
Nanyang Technological University
Singapore
Singapore

Wojciech Wojakowski
Third Division of Cardiology
Medical University of Silesia
Katowice
Poland

Zdeněk Stárek
1st Department of Internal Medicine/Cardioangiology, International Clinical Research Center
St. Anne's University Hospital
Brno
Czech Republic

Sylvain Martel
NanoRobotics Laboratory, Department of Computer and Software Engineering, Institute of Biomedical Engineering
Polytechnique Montréal
Montreal, QC
Canada

Balázs Gulyás
Lee Kong Chian School of Medicine
Nanyang Technological University
Singapore
Singapore

ISSN 2191-530X ISSN 2191-5318 (electronic)
SpringerBriefs in Applied Sciences and Technology
ISSN 2197-6740 ISSN 2197-6759 (electronic)
Nanotheranostics
ISBN 978-981-10-4526-4 ISBN 978-981-10-4527-1 (eBook)
DOI 10.1007/978-981-10-4527-1

Library of Congress Control Number: 2017936892

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

Summary

Cancer and cardiovascular diseases are the two most common cause of death worldwide. Despite significant advancement in medical treatment, prognosis of patients with cardiovascular disease and cancer remains unsatisfactory. Clinical effectiveness of stem cell therapy to regenerate myocardium is still under evaluation, with no conclusive decision on a preferable cell type. Bone marrow as well as adipose tissue-derived mesenchymal stem cells (MSCs), referred as a second-generation stem cells, has been proposed to serve as a universal cell source due to beneficial immunomodulatory characteristics allowing allogenic application. Further improvement of MSC biological function is strongly investigated with genetic modification, pharmacological optimization, or preconditioned media. Moreover, novel strategies including injectable hydrogels are developed.

Apart from stem cells, innovative nanorobotic agents have a great clinical potential being able to perform advanced tasks at the molecular level. This unique feature opens a new chapter in medicine, where precise and personalized treatment is a cornerstone. This series highlights the current concepts and future directions in the field of stem cells and nanorobotic agents medical application.

This series highlights the current concepts and future directions in cancer and cardiovascular disease therapy with stem cells and nanorobotic agents.

Chapter 1
Advances Is Mesenchymal Stem Cell Application for Cardiovascular Disease Treatment

Abstract Novel strategies are developed to optimize MSC function. Among them genetic modification is a promising solution to improve cell survival/engraftment after transplantation as well as to enhance cardioprotective function. Following genetic modification are described: Bcl-2, CREG, Hsp20, Akt, PI3K-2α, ILK, periostin, CXCR4, TNFR, Ang1, VEGF, Wnt11. HO-1, GSK-3β, IGF-1, SDF-1, GATA-4. Pharmacological optimization or preconditioned media are also investigated to overcome current limitation in stem cell therapy. Pharmacological agent pretreatment strategy covered in this chapter includes application of diazoxide, estradiol, lysophosphatidic acid, lovastatin, oxytocin, phorbol myristate acetate, tadalafil, trimetazidine. Cytokine and growth factor pretreatment discussed below includes stromal-derived factor 1 alpha, angiopoietin-1, insulin-like growth factor-1, transforming growth factor-α, bone morphogenetic protein-2, fibroblast growth factor-2 and insulin-like growth factor-1 cocktail, interleukin-1β and transforming growth factor-β combination. Moreover, application of including injectable hydrogels are presented including cell containing injectable biomaterials, acellular scaffolds with incorporated bio-agents, and co-application of cells and bio-agents.

Keywords Mesenchymal stem cells · Genetically modified stem cells · Pharmacologically optimized stem cells · Injectable hydrogels

1.1 Genetically Modified Mesenchymal Stem Cells

Combined stem cell and gene therapy represents a novel and promising approach to treat heart diseases. It has been shown that genetic modifications of mesenchymal stem cells (MSCs) can improve survival and engraftment of transplanted MSCs and their cardioprotective effect against post-ischemic myocardial dysfunction [1]. The latter one can be achieved e.g. by the use of MSCs overexpressing genes encoding proteins that promote angiogenesis.

Genetically modified MSCs represent a wide group of cells. Here we categorized them based on the primary effect of genetic modification on MSC transplantation

T. Jadczyk et al., *Innovative Diagnostics and Treatment: Nanorobotics and Stem Cells*, Nanotheranostics, DOI 10.1007/978-981-10-4527-1_1

and MSC function in cardiac repair. Specifically, MSCs overexpressing proteins that improve MSC survival and engraftment as well as factors that enhance MSC differentiation and their angiogenic potential are discussed (Fig. 1.1).

1.1.1 Genetically Modified MSCs with Improved Survival and Engraftment After Transplantation

Many different overexpressed genes have been employed in MSCs to improve their survival and engraftment after transplantation. Here we briefly describe MSCs overexpressing Bcl-2, CREG, Hsp20, Akt, PI3K-2α, ILK, periostin, CXCR4, TNFR. We discuss the effect of these genetic modifications on MSC transplantation and their use to increase efficacy of MSC-based therapy in heart disease treatment.

1.1.1.1 MSCs Overexpressing Bcl-2

Bcl-2 is an anti-apoptotic protein originally identified in B-cell lymphoma. Li and colleagues demonstrated that MSCs overexpressing Bcl-2 have better survival and engraftment after transplantation into infarcted myocardium. This subsequently can ameliorate LV remodeling and improve left ventricle (LV) function. Bcl-2 overexpression in MSCs also enhances vascular endothelial growth factor (VEGF) secretion under hypoxic conditions. This might provide cardioprotective effects on ischemia-damaged myocardiumby increasing capillary density and decreasing infarct area [1]. Taken together, transplantation of MSCs modified with Bcl-2 gene represents an effective approach in the treatment of myocardial infarction.

1.1.1.2 MSCs Overexpressing CREG

Cellular repressor of E1-stimulated genes (CREG) is a glycoprotein highly expressed in blood vessels. Deng and colleagues demonstrated that overexpression of CREG inhibits apoptosis of MSCs subjected to metabolic stress conditions, such as hypoxia and serum deprivation. This cytoprotective effect is probably based on activation of PI3K/Akt pathway and increased VEGF secretion from MSCs [2]. Taken together, modification of MSCs with CREG gene might become a potent strategy to increase survival of transplanted MSCs.

1.1.1.3 MSCs Overexpressing Hsp20

Hsp20, also known as HspB6, belongs to the small heat-shock proteins. It is an anti-apoptotic protein expressed at high levels in cardiac, skeletal and vascular

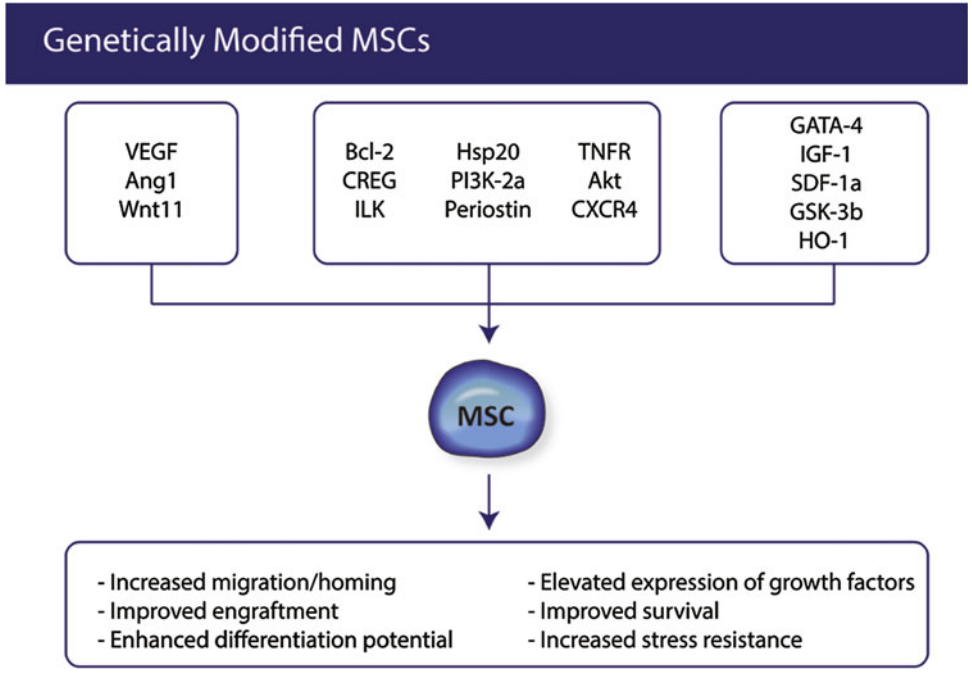


Fig. 1.1 Genetically modified mesenchymal stem cells (MSCs). *VEGF* vascular endothelial growth factor, *Ang1* angiopoietin-1, *CREG* cellular repressor of E1-stimulated genes, *Hsp20* heat shock protein 20, *PI3K-2α* phosphoinositide 3-kinase class II alpha, *ILK* Integrin-linked kinase, *TNFR* tumor necrosis factor (TNF)-α receptor, *CXCR4* CXC chemokine receptor 4, *HO-1* heme oxygenase 1, *GSK-3β* glycogen synthase kinase-3β, *IGF-1* insulin-like growth factor 1, *SDF-1α* stromal cell-derived factor 1α

smooth muscle [3]. In vitro studies have shown that overexpression of Hsp20 can reduce death rate in MSCs subjected to hypoxia. Moreover, transplanted MSCs overexpressing Hsp20 had better survival in the LAD ligation model. Hsp20-modified MSC improved subsequent cardiac function of infarcted myocardium, which was accompanied by reduced fibrosis and increased vascular density. The cytoprotective effect of Hsp20 overexpression on MSC survival is associated with enhanced Akt activation in MSCs and increased MSC secretion of growth factors, such as VEGF, FGF-2 and IGF-1 [4]. Taken together, transplantation of MSCs modified with Hsp20 might become an effective strategy for ischemic heart disease treatment.

1.1.1.4 MSCs Overexpressing Akt

Akt, also known as protein kinase B (PKB), is a serine/threonine kinase playing an important role in various cellular processes including cell survival and proliferation. It has been shown that Akt overexpression in MSCs significantly improved survival of MSCs transplanted into infarcted myocardium. This resulted in reduction of infarct size and improvement of cardiac function. Mangi and colleagues observed that transplantation of MSCs overexpressing Akt into ischemic myocardium inhibited the process of cardiac remodeling as it reduced intramyocardial inflammation, collagen deposition and cardiac myocyte hypertrophy. It also led to significant regeneration of lost myocardial volume and completely normalized systolic and diastolic cardiac function [5]. The mechanism by which MSCs modified with Akt repair ischemic myocardium involves secretion of various cytokines and growth factors from MSC which produce a protective milieu in damaged heart [5, 6]. Taken together, MSCs overexpressing Akt might be a potent source of cells for heart disease treatment.

1.1.1.5 MSCs Overexpressing PI3K-2α

Phosphoinositide 3-kinase class II alpha (PI3K-2α) is one of the isoforms of PI3K involved in regulation of cell survival via intrinsic death pathway. Eun and colleagues demonstrated that PI3K-2α overexpression in MSCs can be an effective way to enhance viability of MSCs transplanted into infarcted heart. Under in vitro hypoxic conditions, PI3K-2α stimulates production of pro-survival factors in MSCs leading to their better survival. Animal studies in rats have shown that transplantation of PI3K-2α-modified MSCs into infarcted hearts results in a significant reduction in infarct size and fibrosis area as well as increased density of microvessels and improved heart function [7].

1.1.1.6 MSCs Overexpressing ILK

Integrin-linked kinase (ILK) is a serine/threonine kinase binding to the cytoplasmic domain of β-integrin and participating in the regulation of many cellular functions including cell adhesion, growth and cell shape change. Song and colleagues demonstrated that overexpression of ILK in MSCs might increase efficiency of MSC transplantation as it enhances MSC survival in infarcted myocardium. These effects are mediated via activation of Act and ERK proteins, two key regulators of adhesion-mediated cell survival signals. The cytoprotective mechanism of ILK overexpression on MSC may be based on increased Bcl-2/Bax ratio and inhibition of caspase-3 activation in MSCs. Transplantation of ILK-modified MSCs into infarcted heart subsequently led to decrease in infarct size and fibrotic area as well as improvement in LV function [8]. Taken together, genetic modification of MSCs with ILK represents a promising strategy for infarcted myocardium treatment.

1.1.1.7 MSCs Overexpressing Periostin

Periostin is a secreted extracellular matrix molecule. It belongs to fascilin family and is involved in the regulation of cell adhesion, migration and growth. Studies by Cho et al. demonstrated that overexpression of periostin in MSCs improves survival of MSCs and increases their number in the infarcted heart. This effect is probably mediated via activation of Akt and associated with increased Bcl-2/Bax ratio and inhibition of caspase-3 activation. Transplantation of periostin-modified MSCs led further to the functional recovery of infracted myocardium. This was not only due to enhanced survival of MSCs but also anti-apoptotic effect of overexpressed periostin on cardiomyocytes [9].

1.1.1.8 MSCs Overexpressing TNFR

Tumor necrosis factor (TNF)-α is an inflammatory cytokine produced e.g. in ischemic heart. It can negatively affect the survival of transplanted MSCs thus some studies focus on developing strategies to increase its binding or removal from the heart environment at the site of MSC implantation. Bao and colleagues demonstrated that overexpression of TNF receptor (TNFR) in MSCs improved engraftment of these cells in the infarcted myocardium. Moreover, transplantation of TNFR-modified MSCs improved left ventricular function as it reduced the level of TNF-α in serum and cardiac tissue [10].

1.1.1.9 MSCs Overexpressing CXCR4

CXC chemokine receptor 4 (CXCR4) is a specific receptor for SDF-1, a chemokine that plays an important role in homing of progenitor cells to ischemic tissues. It has

been shown that CXCR4 overexpression increases MSC homing into infarcted myocardium. This, in turn, resulted in reduced remodeling and improved post-infarction recovery of LV function [11]. This was partly due to enhanced vascularization in the damaged myocardium induced by CXCR4-modified MSC [12]. Taken together, overexpression of CXCR4 in MSCs might become an important strategy to improve efficacy of MSC-based therapy for heart diseases.

1.1.2 Genetically Modified MSCs with Enhanced Cardioprotective Function

The role of MSCs in cardioprotection is based on their ability to secrete proangiogenic factors and differentiate into myocardial phenotypes. To improve the function of MSCs in damaged heart, these cells were modified with the genes encoding the following proteins: Ang1, VEGF and Wnt11. Here we briefly describe the benefits of MSC modification with these genes in improvement of the treatment of heart diseases.

1.1.2.1 MSCs Overexpressing Ang1

Angiopoietin-1 (Ang1) is an endothelial-specific growth factor and one of the key regulators of angiogenesis. Studies conducted by Lijie and colleagues confirmed that overexpression of Ang1 in MSCs enhances angiogenesis and arteriogenesis in infarcted heart by 11–35%. This led to the increased capillary density, reduction in the infarct area by 30% and improves LV function [13]. Taken together, MSC modified with Ang1 are a potent source of cells to improve cardiac function in myocardial infarction.

1.1.2.2 MSCs Overexpressing VEGF

VEGF is a heparin binding angiogenic growth factor highly specific for endothelial cells [14]. It is secreted from various types of cells, including MSCs. It has been shown that overexpression of VEGF of MSCs can increase the expression level of VEGF in MSCs that led subsequently to the enhanced myocardial neovascularization in infarcted heart. This subsequently led to the reduction of LV remodeling, accompanied by increased capillary density, reduction in infarct area and fibrotic tissue formation [15]. Despite of all the benefits of MSC modification with VEGF, this approach has one very important limitation. This is because continuous, high expression of VEGF in heart can lead to tumor vessel formation. Thus, strategies based on inducing overexpression of VEGF in MSCs should be carefully revised before it will be applied for heart disease treatment.

1.1.2.3 MSCs Overexpressing Wnt11

Studies have shown that Wnt11 plays a vital role in myocardial development as it promotes differentiation of cardiac progenitor cells into cardiac phenotypes. He and colleagues demonstrated that overexpression of Wnt11 in MSC can provide a strategy to also increase differentiation of MSCs into myocardial cells. The effect of this genetic modification may be partly due to upregulation of GATA-4 by Wnt11 in MSCs [16].

1.1.3 Genetically Modified MSCs Exhibiting Improved Transplantation Potential and Cardioprotective Function

Improvement of MSC transplantation as well as MSC function in cardiac repair might be achieved by a genetic modification with one or two genes. Here we briefly describe MSCs modified with HO-1, GSK-3β, IGF-1, SDF-1, GATA-4.

1.1.3.1 MSCs Overexpressing HO-1

Heme oxygenase 1 (HO-1) is an anti-apoptotic and anti-oxidant enzyme. It catabolizes heme into biliverdin/bilirubin, carbon monoxide and free iron and as such protects cells from death. Many groups demonstrated that overexpression of HO-1 in MSCs improves their viability after transplantation into infarcted myocardium. This is because HO-1-modified MSCs exhibit enhanced anti-apoptotic and anti-oxidative capabilities [17]. In consequence, higher number of MSCs survived in ischemic heart led to improvement in cardiac function after ischemia/reperfusion. This is mainly because of the increased angiogenic growth factor secretion (e.g. FGF-2, VEGF) by these cells as well as their ability to normalize the balance between metalloproteinases and tissue inhibitors of metalloproteinases in ischemic heart [18–20]. Higher secretion of angiogenic factors subsequently results in increased capillary density and reduction of infarct area whereas restored balance between metalloproteinases and tissue inhibitors of metalloproteinases contributes to the reversion of myocardial extracellular remodeling. This leads to further LV function improvement, accompanied by reduced fibrosis and LV dilatation, smaller chamber and thicker LV anterior walls [18, 20]. Taken together, MSCs overexpressing HO-1 can be a potent source of cells for heart disease treatment.

1.1.3.2 MSCs Overexpressing GSK-3β

Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine kinase that regulates various intracellular functions, including cell growth and differentiation. It has been

shown that overexpression of GSK-3β in MSCs increases survival and cardiomyocyte differentiation of MSCs after injection into infarcted myocardium, thus becoming a potent strategy to treat heart diseases. MSCs modified with GSK-3β may also produce factors that induce e.g. proliferation of residential cardiac progenitor cells and promote recruitment of these cells to the ischemic heart. This, in turn, leads to repair of damaged heart. The beneficial effects of GSK-3β-modified MSC transplantation into damaged myocardium included suppression of cardiac remodeling and improvement of LV function, largely through VEGF-dependent/independent mechanisms [21].

1.1.3.3 MSCs Overexpressing IGF-1

Insulin-like growth factor 1 (IGF-1) stimulates cardiac growth and contractility and as such plays an important role in the heart. It has been shown that overexpression of IGF-1 in MSCs can increase survival of both MSCs and myocytes in infarcted myocardium. This effect is mediated via PI3K/Akt signaling pathway activation. Genetic modification of MSCs with IGF-1 enhances also release of various growth factors, including VEGF and SDF-1α. This subsequently leads to increased angiogenesis in heart and results in massive stem cell mobilization into damaged myocardium. The MSC-based delivery of IGF-1 into infarcted heart contributes to further improvement of indices of LV contractile function, including LV wall thinning and dilatation [22]. Taken together, IGF-1 overexpression can represent a potent strategy to improve MSC transplantation and MSC function in damaged heart repair.

1.1.3.4 MSCs Overexpressing SDF-1α

Stromal cell-derived factor 1α (SDF-1α) is a chemokine that modulates several biological functions via its receptor, CXCR4. It is involved in regulation of cell growth, proliferation, cell migration and apoptosis. Tang and colleagues demonstrated that MSCs overexpressing SDF-1α exhibits enhanced survival and paracrine function after injection into infarcted heart. They can also differentiate into cardiomyocytes and endothelial cells in the ischemic myocardium as well as promote angiogenesis and decrease the expression of collagens (type I and II) and metalloproteinase (type 2 and 9). These effects are mediated via activation of Akt as well as increased level of VEGF secretion by SDF-1α-modified MSCs. Transplantation of MSCs overexpressing SDF-1α into infarcted myocardium increased vascular density and thickness of LV wall and reduced infarct size and fibrosis. The last effect is probably mediated via enhanced HGF secretion from SDF-1α-modified MSCs [23]. Taken together, transplantation of MSCs overexpressing SDF-1α is a promising approach to improve LV function and reduce LV remodeling in infarcted heart.

1.1.3.5 MSCs Overexpressing GATA-4

GATA-4 is a member of GATA transcription factors family that regulates differentiation, growth and survival of various cell types. Li and colleagues demonstrated that overexpression of GATA-4 in MSCs can have a beneficial effect not only on their transplantation but also function in damaged heart repair. This is because GATA-4 overexpression results in increased survival of MSCs in ischemic heart and enhances VEGF release from MSCs, which promotes endothelial cell angiogenic response. Transplantation of GATA-4-modified MSCs reduced infarct size and increased capillary density as well as local blood flow. This, in turn, ameliorated LV remodeling and improved LV function [24].

1.1.4 Future Directions

Despite of all the beneficial effects of above described genetic modifications, researchers are still searching for a "golden" gene or gene combination that being overexpressed in MSCs could enhance the efficacy of MSC-based therapy for heart disease treatment. Tang and colleagues demonstrated that overexpression of SDF-1 and VEGF in MSCs can result in better survival of MSCs in infarcted heart as well as their better angiogenic potential and differentiation into cardiomyocytes compared to MSCs overexpressing only SDF-1 or VEGF. Combined MSC, chemokine and angiogenic growth factor therapy subsequently significantly reduced infarct size, prevented remodeling, increased capillary density, thickness of the LV wall and improved cardiac function in acute myocardial infarction rat model [25]. The therapeutic effect of MSCs in myocardial infarction treatment can be also enhanced by MSC modification with Csx/Nkx2.5 and GATA-4. It has been shown that overexpression of these cardiac genes in MSCs markedly increased the survival and differentiation of MSCs as well as angiogenesis in infarcted myocardium. Transplantation of MSCs modified with Csx/Nkx2.5 and GATA-4 resulted in ameliorated LV remodeling and LV function [26]. Taken together, genetic modification of MSCs with two or more genes might significantly advance the efficacy of MSC-based therapy for cardiac disease treatment. Thus, effect of different gene combination on MSC transplantation and function in damaged heart should be intensively investigated.

1.2 Pharmacologically Optimized Mesenchymal Stem Cells

Recent studies have shown that efficiency of MSC-based therapy in treatment of heart diseases can be increased via MSC preconditioning with various pharmacological agents as well as cytokines and growth factors (Fig. 1.2). Importantly, this

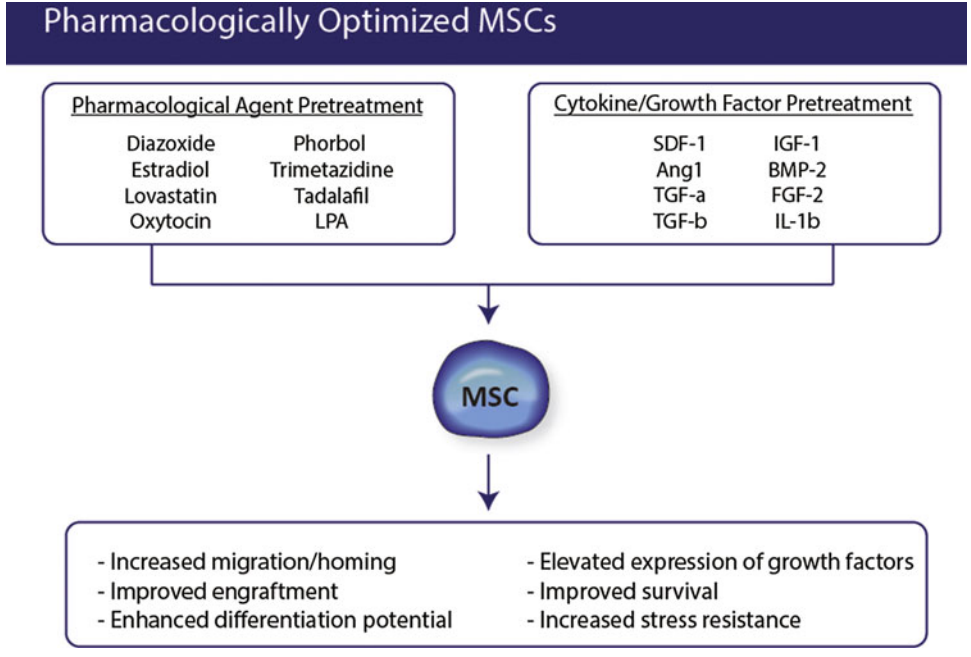


Fig. 1.2 Pharmacologically optimized mesenchymal stem cells (MSCs). *LPA* lysophosphatidic acid

strategy can be easily applied to a large number of cells. Moreover, pretreatment of MSCs has a transient effect on cells. This might be crucial to overcome the tumorigenecity problem related to some genetic modifications of MSCs. Thus, this strategy might be an alternative for genetic engineering of MSCs.

1.2.1 Mesenchymal Stem Cells Optimized via Pharmacological Agent Pretreatment

Pharmacological agents used to improve therapeutic potential of MSCs in heart disease treatment represent a wide group of compounds. Here we briefly characterize some of them and describe their effect on MSCs and their role in repair of cardiac tissue injury. Specifically, diazoxide, estradiol, lysophosphatidic acid, lovastatin, oxytocin, phorbol, tadalafil and trimetazidine are discussed.

1.2.1.1 Diazoxide

Diazoxide is a selective mitochondria ATP-sensitive potassium (mito-K_{ATP}) channel opener, which has been proven to have a protective effect on the heart after ischemia/reperfusion injury [2, 3]. Cui and colleagues demonstrated that preconditioning of SMSCs (selected MSCs expressing c-kit and Nxk2.5) with diazoxide improves survival of the transplanted SMSCs. This is due to the ability of diazoxide to protect mitochondrial membrane integrity and prevent translocation of cytochrome c into cytoplasm. The cytoprotective effect of diazoxide might also be associated with secretion of paracrine factors, such as bFGF and HGF that activate the PI3K/Akt survival pathway by binding to their receptor tyrosine kinases. It has also been shown that transplantation of SMSCs pretreated with diazoxide reduced the infarct size and increased LV function. This was mainly due to better survival of SMSCs undergoing myogenic differentiation and improved SMSC paracrine function in the infarcted heart [4]. Taken together, preconditioning SMSCs with diazoxide represent an effective approach to promote survival of SMSCs subjected to oxidative stress and attenuate cardiac injury after myocardial infarction.

1.2.1.2 Estradiol

Estradiol (E2 or 17β-estradiol) is a sex hormone synthesized in various steroidogenic cells and tissues including ovarian granulosa cells, placenta adipose tissue, skin, endometrium, vaginal mucosa, breast, liver, blood vessels, and heart [5]. Estradiol induces diverse biological effects in various tissues and organs. One of them is increased cardiovascular system protection. It has been shown that estradiol modulates vascular tone, as it induces vascular vasodilation, and prevents vascular

remodeling [6]. Erwin and colleagues demonstrated that estradiol can also enhance MSC paracrine function in injured cardiac tissue. It has been shown that MSCs pretreated with estradiol produce more VEGF than untreated MSCs. Moreover, preischemic infusion of estradiol-treated MSCs protects myocardial function (LVDP and EDP) and viability following ischemia/reperfusion injury [7]. These findings suggest that pretreatment of MSCs with estradiol may represent an important approach for ischemia/reperfusion injury treatment.

1.2.1.3 Lysophosphatidic Acid

Lysophosphatidic acid (LPA) is an important signaling molecule that belongs to a family of bioactive lipid phosphoric acids. It is present in all mammalian cells, tissues and blood. The biological activity of LPA is mediated via activation of five specific G-protein-coupled receptors, termed LPA1-LPA5. Acting through these receptors LPA regulates diverse cell functions, such as proliferation, survival and apoptosis [8, 9]. Liu and colleagues demonstrated that treatment of MSCs with LPA inhibits apoptosis of MSCs subjected to hypoxia and serum deprivation. The antiapoptotic effect of LPA on MSC was also confirmed in vivo in an animal model of myocardial infarction. It has been shown that LPA treatment improved survival of MSCs transplanted into the ischemic heart. Moreover, LPA-treated MSCs promoted angiogenesis and thus improved capillary density in ischemic myocardium. In vitro studies have shown that LPA also elevated secretion of VEGF from MSCs subjected to hypoxia and serum deprivation. This may lead not only to increased angiogenesis but also better survival of cardiomyocytes and MSCs in vivo. Taken together, preconditioning of MSCs with LPA represents a promising approach to promote MSC survival and angiogenesis in ischemic hearts [10].

1.2.1.4 Lovastatin

Lovastatin is a cholesterol-lowering drug commonly used in prevention of cardiovascular disease. It is isolated from *Monascus* and a strain of *Aspergillus terreus*, and acts by inhibiting 3-hydroxy-3methylglutaryl-coenzyme A reductase (HMG-CoA reductase), an enzyme involved in conversion of HMG-CoA into mevalonate [11, 12]. Mevalonate is one of the building blocks required for cholesterol biosynthesis which production can be reduced by lovastatin via competitive inhibition of HMG-CoA. In vitro studies have shown that lovastatin protects MSCs from apoptosis under hypoxia and serum deprivation conditions. The antiapoptotic effect of lovastatin is based on its ability to activate a mitochondrial pathway, preventing release of cytochrome-c and activation of caspase-3/CPP32. Protective mechanisms of lovastatin on MSCs also involve activation of cell survival signaling pathways, such as PI3K/Akt and MEK/ERK2. In vivo studies are needed now to confirm the effects of lovastatin demonstrated in in vitro experiments. However, in vitro results

suggest that MSCs preconditioning with lovastatin may support transplantation of MSCs into damaged heart after myocardial infarction [13].

1.2.1.5 Oxytocin

Oxytocin is a neurohypophyseal neuropeptide, originally considered to be a pregnancy hormone produced primarily in paraventricular and supraoptic nuclei of the hypothalamus. Recent literature however shows that oxytocin is also synthesized in the heart. It acts via G protein-coupled receptors that are expressed mainly in endothelial cells and cardiomyocytes [14–16]. Activation of oxytocin receptor in the heart stimulates multiple cardioprotective reactions, e.g. negative inotropy and chronotropy, parasympathetic neuromodulation, and release of nitric oxide. Moreover, oxytocin receptor-specific signaling induces differentiation of embryonic stem cells that have been shown to convert to cardiac muscle cells [14]. It has been shown that pretreatment with oxytocin can enhance migration of umbilical cord blood-derived MSCs (UCB-MSCs) via induction of matrix metalloproteinase-2 and can increase MSC engraftment in the infarcted heart [17]. Moreover, oxytocin pretreated UCB-MSCs express cardiac proteins, such as connexin 43 (Cnx43), cardiac troponin I (cTnI) and α-sarcomeric actin (α-SA). Thus, oxytocin treatment can stimulate MSC differentiation into cardiomyocytes and enhance cardioprotective function of transplanted MSCs in the infarcted heart [18].

1.2.1.6 Phorbol Myristate Acetate

Phorbol myristate acetate (PMA) is a protein kinase C (PKC) activator derived from the oil of the seeds of the Croton plant, *Croton tiglium*. It is widely used in biomedical research, mainly in various models of carcinogenesis [19]. Song and colleagues demonstrated that PMA induces MSC differentiation into cardiogenic cells. MSCs pretreated with PMA expressed cardiac-specific markers, such as cardiac troponin T (cTnT), myosin light chain (MLC), myosin heavy chain (MHC) and connexin 43 (Cnx43) as well as cardiac-specific transcription factor NkX2.5. They also expressed Ca^{2+} homeostasis-related proteins and showed adrenergic receptor signaling by norepinephrine. It has been shown that MSC pretreatment with PMA prevented sudden death in rats after MSC transplantation into infarcted hearts. This is due to better electromechanical synchronization of cardiomyocytes derived from PMA-modified MSCs with surrounding cardiomyocytes in host myocardium. Infarct hearts implanted with MSCs treated with PMA exhibited restoration of conduction velocity as well as improved myocardial contractility. Taken together, PMA treatment of MSCs represents a promising therapeutic strategy, because it enhances electromechanical integration of transplanted MSCs with host myocardium [20].

1.2.1.7 Tadalafil

Tadalafil belongs to the group of long-lasting phosphodiesterase 5 (PDE5) inhibitors and it has been proven to protect heart against ischemia/reperfusion injury [21]. Haider and colleagues demonstrated that tadalafil might be also used to improve survival of MSCs in ischemic heart. Tadalafil pretreatment of MSCs protected MSCs against oxidant stress and promoted their proliferative potential via cGMP/protein kinase G (PKG) signaling. Moreover, treatment of MSCs with tadalafil enhanced survival of MSCs during the acute phase in myocardial infarction model in rats. Engraftment of tadalafil-pretreated MSCs subsequently supported angiomyogenic response and protection of the ischemic myocardium. This resulted in attenuated infarct size and preservation of LV contractile function indices [22]. Taken together, tadalafil may become an important component of stem-cell based therapy for heart.

1.2.1.8 Trimetazidine

Trimetazidine (1-[2,3,4-trimethobenzyl]piperazine, TMZ) is an anti-ischemic drug commonly used to reduce ischemia-induced metabolic damage [23]. TMZ inhibits fatty acid oxidation and increases glucose oxidation rates during low-flow ischemia in the heart. It acts by inhibiting long-chain 3-ketoacyl CoA thiolase, an enzyme involved in β-oxidation of fatty acids. Studies by Wisel et al. demonstrated that MSC preconditioning with TMZ significantly protected MSCs against H_2O_2-induced loss of cellular viability, membrane damage, and oxygen metabolism. Additionally, TMZ-treated MSCs exhibited higher levels of HIF-1α, survivin, phosphorylated Akt, Bcl-2 protein level and Bcl-2 gene expression. Moreover, transplantation of MSCs preconditioned with TMZ improved recovery of cardiac function, decreased tissue fibrosis and led to up-regulation of pAkt and Bcl-2 in infarcted heart [24]. Thus, TMZ preconditioning seems to be very effective for MSC-based therapy for the repair of myocardial ischemic damage.

1.2.2 Mesenchymal Stem Cells Optimized via Cytokine and/or Growth Factor Pretreatment

Cytokines and growth factors are known to affect MSC function and may modulate efficacy of MSC-based therapy for heart diseases. Here we present effects of SDF-1, Ang1, IGF-1, TGF-α alone as well as the cocktail of FGF-2, IGF-1, BMP-2, and interleukin-1β in combination with TGF-β on MSC survival and function in damaged cardiac tissue.

1.2.2.1 Stromal-Derived Factor 1 Alpha

Stromal-derived factor 1 alpha (SDF-1) is a member of the chemokine CXC subfamily. Acting through G protein-coupled receptor CXCR4, SDF-1 plays an important role in cell trafficking [25]. Pasha and colleagues demonstrated that preconditioning with SDF-1 enhanced homing and proliferation of MSCs in infarcted heart and promoted neovascularization and myogenesis. Moreover, preconditioned MSCs secreted VEGF, which improved survival of MSCs under ischemic conditions. The combined effect of SDF-1 treatment of MSCs resulted in reduced infarct size and LV remodeling [26]. Taken together, preconditioning of MSCs with SDF-1 represent an effective approach to minimize adverse effects of ischemia on cell death and cardiac remodeling.

1.2.2.2 Angiopoietin-1

Angiopoietin-1 (Ang1) is a proangiogenic growth factor that acts through the Tie2 receptor. Biological effects of Ang1/Tie2 signaling include inhibition of apoptosis, activation of endothelial cell migration, vessel growth and angiogenesis. It has been shown that MSC preconditioning with Ang1 improved MSC survival both in vitro and in vivo. This is due to activation of the PI3K/Akt signaling pathway, elevated expression level of Bcl-2 and increased ratio of Bcl-2/Bax. Transplantation of Ang1-pretreated MSCs into infarcted heart resulted in reduction of infarct size and better heart functional recovery, contributing later to improvement of heart function [27]. Taken together, MSC preconditioning with Ang1 provides a promising approach for enhancing MSC survival and can improve efficacy of MSC-based therapy for ischemic heart diseases.

1.2.2.3 Insulin-like Growth Factor-1

Insulin-like growth factor-1 (IGF-1) is a hormone, primarily prodyced by liver. It acts through two receptors, IGF-1R and IGF-2R, and helps to preserve tissues from hypoxia, ischemia and oxidative stress [28]. Enoki and colleagues demonstrated that IGF-1 increases adhesion and inhibits apoptosis in MSCs subjected to hypoxia via activation of PI3K/Act signaling pathway. Moreover, IGF-1 added to transplanted MSC suspension improved engraftment of MSCs in infarcted heart. This, in turn, resulted in increased neovascularization and inhibition of host cardiomyocyte death, and subsequent improvement of LV function and prolongation of survival [29].

1.2.2.4 Transforming Growth Factor-α

Transforming growth factor-α (TGF-α) belongs to the epidermal growth factor (EGF) family of cytokines. It acts via EGF receptor and is a potent mitogen for

epithelial cells and fibroblasts. It can also function as an angiogenic factor for capillary endothelial cells. It has been shown that activation of the EGF receptor increases MSC proliferation, migration and survival [30]. Thus, some studies focused on TGF-α application in improvement of MSC-based therapy for cardiovascular diseases. It has been shown that TGF-α can stimulate VEGF secretion by MSCs in vitro. Moreover, transplantation of TGF-α-pretreated MSCs directly into myocardium in an ex vivo model of coronary artery ligation resulted in improved LV function, reduced infarct size, lowered production of inflammatory cytokines and apoptosis [31]. Similar results were obtained later in an in vivo model of myocardial infarction [32]. One of the mechanisms of TGF-α-enhanced cardioprotection may involve augmentation of VEGF production by TGF-α-pretreated MSCs. TGF-α can also equalize VEGF production by adult and neonatal MSCs that have different cardioprotective potential. It has been shown that neonatal MSCs are less effective in the treatment of cardiovascular diseases than their adult counterparts. Taken together, TGF-α treatment of MSCs can be beneficial for heart disease treatment, especially when the number of available autologous, adult MSCs is limited, such as in congenital heart disease [33].

1.2.2.5 Bone Morphogenetic Protein-2, Fibroblast Growth Factor-2 and Insulin-like Growth Factor-1 Cocktail

Bone morphogenetic protein-2 (BMP-2), fibroblast growth factor-2 (FGF-2) and insulin-like growth factor-1 (IGF-1) are well known growth factors involved in heart development, cell differentiation into cardiomyocytes and regulation of survival and proliferation of cardiac stem cells. Hahn and colleagues demonstrated that MSCs cocultured with cardiomyocytes and pretreated with these growth factors better differentiated into cardiomyocytes, had higher level of connexin 43 expression and created better intracellular connections with cardiomyocytes. Moreover, transplantation of BMP-2, FGF-2 and IGF-1-pretreated MSCs enhanced formation of gap junctions, improved cell survival in infarcted myocardium, reduced infarct size and improved LV function [34]. Taken together, pretreatment of MSCs with this cocktail of growth factors might increase efficacy of MSC transplantation and its therapeutic effect in the infarcted myocardium.

1.2.2.6 Interleukin-1β and Transforming Growth Factor-β Combination

Interleukin-1β (IL-1β) is a proinflammatory cytokine involved in immune defense against infection. It has been shown that IL-1β is also involved in angiogenesis as it promotes VEGF secretion from various types of cells including MSCs. The effect of IL-1β on VEGF secretion from MSCs can be enhanced by cotreatment of MSCs with IL-1β and transforming growth factor-β (TGF-β), a multipotent cytokine associated with angiogenesis and anti-inflammatory effects [35]. Transplantation of

IL-1β and TGF-β-pretreated MSCs into heart improved recovery of myocardial function after acute ischemia and reperfusion injury in the Langendorff model. Importantly, MSC pretreatment with these two cytokines may lower the treatment dose for each of them. This, in turn, keeps the proinflammatory effect of IL-1β application on the cardiac environment at a low level. Taken together, cotreatment of MSCs with IL-1β and TGF-β represent an effective strategy to improve angiogenesis in infarcted heart.

1.2.3 Future Directions

One of the recently tested strategies to improve efficacy of MSC transplantation into infarcted heart is based on coating MSCs with myocardial infarction-specific peptides. These peptides are found by phage display technology and synthesized with a palmitic acid tail to facilitate their integration into cell membrane. It has been shown that "painting" MSCs with myocardial infarction-specific peptides can increase MSC localization in infarcted myocardium [36]. Thus, this strategy may be very efficient to improve efficacy of MSC transplantation.

1.3 Preconditioned Media

Preconditioning media are currently used to improve post-injection cell survival by adopting them to hostile conditions of infarcted myocardium. Factors such as inflammation, acid microenvironment, hypoxia, ischemia and mechanical pressure are deleterious regarding cell function. Increasing stress condition tolerance prior to cells application seems to be an effective method to improve therapy outcomes.

1.3.1 Hypoxia

Stem cells exposed to hypoxic conditions show defective function in the range of proliferation, differentiation and effectiveness. Moreover, during stem cell administration into infarcted myocardium part of transplanted cells die as a consequence of low oxygen level. Reduced concentration of oxygen during stem cells culturing may affect survival of the stem cells after administration into infarcted region [37]. Studies indicate that initial in vitro hypoxic preconditioning of MSCs determine post-application enhanced survival and differentiation capability into cardiovascular lineage in comparison to not preconditioned MSCs. The best results were observed when duration of hypoxic stress least 24 h [37]. Yan et al. reported that hypoxic

preconditioning performed on c-kit$^+$ CPCs culture acts by activation of SDF-1α/CXCR4 axis signaling pathway. In consequence, in vitro, it increases expression of pro-survival genes, improves migration ability and shows anti-apoptotic function, especially after 6 h of hypoxic pressure. Furthermore, improvement in cardiac function was observed after administration of hypoxia preconditioned c-kit$^+$ CPCs into myocardial infarction (MI) area in mice [38]. It was also indicated that hypoxia-induced factor 1 (HIF-1) is activated in response to low concentration of oxygen [39]. Several research groups showed that in highly hypoxic microenvironment HIF-1 concentration increases and promotes releasing of pro-angiogenic and pro-vasodilatory factors: VEGF-α and its receptor Flk-1 (Fetal Liver Kinase 1), Ang-1, Epo, EpoR as well as nitric oxide synthase. The process of myocardial angiogenesis stimulation ensure better blood flow in infarcted region and enhance regenerative abilities [37, 39, 40]. Moreover, in vitro and in vivo data showed that survival rate of hypoxia-preconditioned MSC was considerably improved in comparison with that cultured under normoxic conditions [40]. Chacko et al. [37] demonstrated that MSCs cultivated initially under 0.5% hypoxic conditions were better protected against apoptosis when exposed to 0.1% oxygen comparing to MSCs that were not preconditioned. This fact may be also determined by hypoxia-enhanced release of Bcl-xL and Bcl-2 anti-apoptotic proteins. Moreover, the studies show that hypoxic preconditioning increases level of pro-survival molecule pAkt [37] and up-regulates above-mentioned Bcl-2 and Bcl-xL molecules [40]. The strategy of using hypoxic environment to favorably modify MSCs activity may bring positive results in functional recovery after MI. The effects of hypoxic preconditioning on MSCs may be achieved by: enhancing survival of transplanted MSCs, stimulation of cytokines production, supporting endothelial differentiation of MSCs and enhancement of pro-angiogenic potential [40, 41]. To confirm the hypothesis that hypoxic preconditioning increases the effect of transferred MSCs by reducing apoptosis in vivo study was performed.

1.3.2 Mechanical Stretching

In the physiological conditions cardiac muscle cells are continuously subjected to changeable forces during systole and diastole. Heart contraction cycle is associated with dynamic mechanical stress changes, which influences maturation, differentiation and soluble factors secretion potential of cardiac stem cells. In consequence it regulates cell number ensuring proper cardiac function. The fact of mechanical stretching influence on stem cells activity has direct implications in therapeutical strategies. Current investigations concentrate on determining optimal characteristics of stretch conditions. Studies suggest that stretch acts bi-directionally, both positively and negatively, upon the stem cells through: (1) suppression cells proliferation and induction of apoptosis, (2) promotion of adhesion and inflammatory

molecules expression, (3) induction of pro-angiogenic growth factors and hypertrophy-associated molecules release (4) increasing expression of cardiomyocytes and smooth muscle cells specific markers (5) modulating MSCs fate [42, 43]. Mechanical stretching may strongly inhibit maturation of cardiomyocytes both through raising apoptosis and decreasing proliferation. 24-hour analysis of MSCs culture under mechanical stress stimulation shows considerable reduction of Ki-67$^+$ cells number in comparison to static conditions. As aforementioned, physiological mechanical stretching regulates cardiac cells number, allowing proper functioning of the heart. Nevertheless, from a therapeutical point of view, intra-myocardial contraction forces seems to have negative impact on stem cell therapy outcomes due to impaired survival of transplanted stem cell [43]. On the other hand, cyclic mechanical stress effect localization of gap junctions and increases expression of adhesion molecules [i.e. integrin-β1 and focal adhesion kinase (FAK)]. It may suggest beneficial effect of stretching on MSCs by empowering MSCs stabilization in the cardiac microenvironment [43, 44]. The cytokine molecules profile under stretching stimulation in comparison to static group shows significantly increased concentration of Il-6 and Il-1β, whereas unchanged level of IGF-1, HGF, SDF-1α, and TGF-β1. Moreover, process of stretching may induce angiogenesis, through increasing secretion of VEGF and bFGF. It was indicated that in the group with pre-conditioning stretch factor the level of mentioned molecules arise almost 2-fold and 30-fold, respectively, in comparison to control group [43]. A positive correlation between mechanical stress and level of cardiac troponin-I and smooth muscle actin expression was admitted nevertheless, the number of c-kit$^+$ stem cell was strongly diminished. These results indicates that although survival of cardiac stem/progenitor cells is lower under mechanical pressure, stretching may enhance differentiation and maturation of stem cells towards cardiovascular lineage [43]. It was also demonstrated that mechanical forces may affect stem cell fate, through altering their shape, size and alignment [45, 46]. Engler et al. indicated that different elasticity of the matrix used for cell culture may guide hMSCs fate. It is possible due to mechanosensitive properties of hMSCs, which sense forces acting in surrounding microenvironment. In order to direct hMSCs differentiation towards myogenic cells, 8–10 kPa of matrix elasticity was required, which corresponds to elasticity specific to the heart. This finding shows that proper dynamic stimulation and adjustment of matrix mechanics can maximize differentiation of stem cells into specific lineage and improve regenerative properties hMSCs [45]. It is postulated that stretching regulates hMSCs behavior by modulating integrin signaling pathways responsible for self-renewal and differentiation. Moreover, there are following pathways involved in mechanotransduction: Ras/MAPK, PI3K (phosphatidylinositol 3-kinase)/Akt, RhoA/ROCK, Wnt/β-catenin. Additionally, Margadant and Sonnenberg suggested relationship between mechanical stretch and TGF-β factor activation which, besides its inhibiting properties upon the cell proliferation, affect aforementioned signaling pathways and determine cells destiny [45].

1.4 Application of Injectable Hydrogels for Cardiovascular Disease Treatment

Hydrogels are considerably attractive candidates for direct intra-myocardial delivery systems of cells or/and bio-agents. Chemically, they are a highly hydrated network of synthetic or naturally-derived polymer chains.

Synthesis of clinically-desirable hydrogel requires implementation of medical and bio-engineering concepts. Both physical and chemical parameters must be properly adjusted to obtain effective and biologically compatible final products.

Currently, advanced engineered biomaterials are programmable towards the following properties: (1) elasticity and rigidity, (2) mechanical-force-induced deformability, (3) rheology (4) porosity, (5) electrical charge and conductivity, (6) hydration level, (7) internal void function, (8) molecular weight-dependent cutoff for in-out diffusing particles, (9) gelling dynamics, (10) swelling, (11) stimuli-responsiveness, (12) degradability, (13) oxygen permeability, (14) bio-agent release kinetics, and (15) cell adhesion level.

Due to many advantageous properties they might be a key for successful cardiac regeneration therapy. Preformed in vitro, first generation hydrogels have been tested in animal studies giving encouraging results. Primarily, implantable cell- and agent-free hydrogels showed to be an effective method to support mechanical function of the injured heart. Furthermore, an addition of bioactive compounds as well as cells empowered functionality. Nevertheless, from the clinical perspective, application of the preformed hydrogel requires invasive surgery limiting their usability. In addition, animal tissue-derived hydrogels cannot be translated into humans due to xenographic origin and lack of immunological neutrality.

Overcoming this limitation, naturally-derived and synthetic matrices have a potential to become a clinically-relevant solution. Additionally, an emerging innovative strategy—in situ polymerizing hydrogels have been developed [47–56] allowing injection of the liquid content via a less invasive catheter-based approach. Post-injection, directed by cardiac mechanical forces, they adapt themselves to myocardial shape and polymerize providing mechanical support to the heart and anchorage for injected cells [57]. In context of co-applied bioactive factors, hydrogels are an advantageous delivery system with controllable compound release kinetics. Interestingly, Zouein et al. postulated scavenging function of the locally injected hydrogels. According to the authors, in situ-polymerized scaffolds may entrap apoptotic and necrotic cells limiting the release of ‘pro-inflammatory danger-associated molecular patterns (DAMPS)’. Furthermore, reduction of local molecular stress can increase cardiomyocytes survival and improve contractile function [58]. It is worth to note that a novel, in situ favorable microenvironment/niche generation hypothesis has also been postulated. In this context, hydrogel-induced vascularization accompanied with specific local chemokines release profiles promote circulating stem/progenitor cells homing into damaged myocardium [59].

1.4.1 Biomaterial Library

Basically, there are two types of backbone polymers for hydrogel generation: naturally-derived (i.e. fibrin, alginate, chitosan, hyaluronic acid, gelatin) and synthetic (i.e. pNIPAM, PCL, PLGA, HEMA, MPEG). Moreover, to improve scaffold functionality, mixed natural-synthetic as well as natural/synthetic co-polymers matrices have been synthetized. Selection of polymer type and ratio between hydrogel components seems to play a pivotal role. For example, by incorporating specific biomaterials it is possible to optimize physical and chemical characteristics as well as tune cell adhesion due to material-specific cell responses.

Hydrogels of natural origin possess domains which can guide cellular fate. Furthermore, they already have bio-active function and of more importance they are fully biodegrade under physical conditions. Animal-based studies show that natural injectable hydrogels improve cardiac function and enhance neovascularization [60–64]. The major limitation of this biomaterial group is low physio-chemical modification possibility.

On the other hand, synthetic polymers offer superior control over scaffold composition and structure. Moreover, new generation of so called 'smart biomaterials' is an advanced method for spatio-temporal regulation of cell/bio-agent behavior. Triggered by internal [local myocardial microenvironment (i.e. pH, temperature)] or external (i.e. magnetic field) stimuli these highly functional scaffolds alter properties allowing remote control over the scaffold.

In the following section we would like to review current studies regarding desirable hydrogels for routine clinical use in the field of cardiology.

There are three major approaches in regard to hydrogels for cardiac tissue regeneration: (1) cell containing injectable biomaterials, (2) acellular scaffolds with incorporated bio-agents, (3) co-application of cells and bio-agents.

1.4.2 Cellular Injectable Biomaterials

1.4.2.1 Natural Biomaterials

Fibrin

Fibrin, an insoluble coagulation factor, is an important candidate for an injectable self-assembling biomaterial for heart regeneration. Due to its natural origin, it does not require additional modifications to allow cell adherence and localized action [51]. In vivo, when applied along with cells, it gives homogenous cell distribution, sufficient seeding efficacy and hypoxia protection increasing cell survival rate [65, 66]. Moreover, fibrin's F XIII content enhance stem cell migration and proliferation [67]. In conjunction with skeletal myoblasts it improves cell survival and attenuates harmful left ventricular remodeling [50]. Moreover, in combination with

endothelial [55, 68], bone marrow mononuclear (BMMCs) [56], c-kit$^+$/Sca-1$^+$ bone marrow-derived cardiac stem (BMCSCs) [65] and adipose-derived stem cells [69] fibrin glue appeared to enhance new blood formation within the post-infarction scare [55, 56, 65, 68, 69] as well as have a positive impact on hemodynamic parameters (LVEF) [55, 69]. Importantly, Guo et al. [65] demonstrated that intra-myocardially transplanted BMCSCs express cardiac troponin I and connexin 43 which were located between injected BMCSCs and recipient cardiomyocytes that may suggest graft integration. Furthermore, fibrin gel can be modified and optimized to improve its functionality [51, 70–72]. In future, human autologous plasma-derived fibrinogen and thrombin might be utilized to generate a fully immunocompatible in situ delivery platform.

Chitosan

Chitosan, a chitin-derived biodegradable co-polymer, consists of N-acetyl-glucosamine and N-glucosamine subunits [73]. Co-application of injectable chitosan hydrogel along with stem cells shows promising results towards damaged heart regeneration. Lu et al. [47], and more recently Liu et al. [74] fabricated in situ thermo-gelling scaffolds containing chitosan and embryonic stem cells or adipose-derived mesenchymal stem cells (ADMSCs) respectively. Both groups found beneficial effect on cardiac function, neovascular formation, wall thickness and infarct size. Interestingly, in both studies cardiac phenotype of differentiating stem cells was observed [54, 74]. Moreover, the chitosan-ADMSC construct improved hemodynamic function (left ventricular end-diastolic pressure) and reduced apoptosis. It is noteworthy that self-injected chitosan enhanced c-kit$^+$ cells home into myocardium. Liu et al. proposed that it might be associated with reactive oxygen species scavenging by chitosan hydrogel and increased expression of ICAM1 and VCAM1 on the stem cell surface as well as SDF-1 retention within the scaffold. The same research group suggested another advantage feature of chitosan—its cationic character which allows electrostatic interaction with anionic native ECM's glycosaminoglycans and proteoglycans that may improve myocardium-hydrogel integration [74].

Hyaluronic acid

Hyaluronic acid (HA), a non-proteoglycan polysaccharide, is an important element of extracellular matrix providing tissue turgor force which resists compression. In context of cardiac ECM, it regulates cell adhesion and activity (motility, gene expression, intracellular signaling) [75] by cell surface HA receptor (CD44) interaction [76]. Recently, Chang et al. reported applicability of intra-myocardially injected hyaluronic acid-human blood hydrogel containing cardiosphere-derived cells. Both scaffolds components have adhesion motifs for co-applied cells triggering pro-survival pathways. Moreover, HA and lysed blood are able to enhance angiogenesis, vasculogenesis and cardiogenesis. Noteworthy, HA is enzymatically degraded by hyaluronidases and proteases naturally present in the cardiac tissue [77, 78]. In another study, MSC-hyaluronian hydrogel was pre-formed and

cultivated for 3 weeks prior to intramyocardial injection into an infarcted region. Interestingly, after 14 days post application, cells migrated to the border zone increasing neovascularization and reducing fibrosis within scar area [79]. It is important to note however that HA-based carrier system might not provide favorable environment for encapsulated cells due to high hydrophilicity that alters native cell morphology [80].

Collagen

Collagen I and III are major components of myocardial ECM. Respectively, they provide rigidity and elasticity contributing to proper heart contractility. Preformed rat-tail collagen-based hydrogel in combination with human MSCs was shown to be cell-protective and to support MSCs growth and migration when injected subcutaneously. Matrigel, a mouse sarcoma cell-derived ECM composed of collagen, mixed with ESCs and injected intra-myocardially increases fractional shortening and regional contractility. Moreover, it promotes ESCs differentiation into a cardiomyocyte-like phenotype. Nevertheless, from a clinical perspective, Matrigel cannot be used in humans due to its origin but studies indicate collagen functionality. The observation from animal-based comparative studies regarding collagen I hydrogels carried out by Dai et al. [81] and Huang et al. [62] show strong influence of substrate concentration (65 mg/ml and 1 mg/ml, respectively) on new blood vessel formation processes. The first group found almost no angiogenesis, whereas the second increased vascular formation. To avoid immunogenic complications, commercially available recombinant and human tissue-derived collagens have a potential to be used as injectable scaffolds for cell transplantation in humans.

Alginate

Alginate is another one potential substrate which has favorable characteristics for an injectable delivery platform. Chemically, it is the anionic polysaccharide biopolymer which gels in the presence of divalent cations. Injected alone into infarcted region helped maintain ventricular diameter and function up to 2 months post-administration [49, 52]. Recently, Acarregui et al. [82] presented in situ applicable alginate-based hydrogel for erythropoietin-secreting C_2C_{12} myoblasts delivery. Apart from stimulation of hematopoietic system, erythropoietin (Epo) enhances angiogenesis and chemotaxis, inhibits apoptosis, and acts as a pro-survival factor [83]. Injected subcutaneously, in situ formed gel shows pro-angiogenic function but, unfavorably, acting systemically, increased hematocrit level [82].

Naturally-derived Composites

Mixture of hyaluronic acid and gelatin in the commercially available HyStem product was demonstrated to be an effective approach for cardiac regenerative medicine. Prestwich et al. co-injected HyStem hydrogel and human cardiosphere-derived cells (CDCs) that resulted in improved left ventricle ejection fraction, wall thickness and cell engraftment (6 vs. 1% for CDCs + hydrogel and CDCs + buffer at 3 weeks, respectively) [84]. MSCs incorporated in self-forming HA-collagen

hydrogel (conjugated by electrostatic interaction), was shown to support cell survival and proliferation with stable protein release at 1 month in vitro. These results indicate applicability of the system for intra-myocardial delivery of cells as well as therapeutic agents. Noteworthy, physical properties (i.e. tensile module) can be adjusted using carbodiimide chemistry that is important regarding heart muscle contraction support [85]. Dahlmann et al. [86] reported fully defined cross-linkable alginate-HA hydrogel for myocardial tissue engineering. Addition of human collagen I and utilization of low cell-damaging cross-linking strategy based on hydrazine bonds created (1) cytocompatible, non-toxic and biodegradable, (2) physically and mechanically stable, (3) individually shapeable, and (4) easy adjustable hydrogel for therapeutic application in cardiology. In the same study, co-application of HA and collagen I gave preferable functional constructs regarding biophysicochemical properties—active contraction forces, improved active and passive tension, individual shapeability, cytocompatibility [86]. Nevertheless, pre-clinical study is needed to characterize its properties in living organisms. More complex, in situ thermo-sensitive chitosan/nano-hydroxyapatite/collagen composite was mixed with BMSCs and injected subcutaneously showing biocompatibility, and supporting cells proliferation. Moreover, incorporation of stem cells reduced local inflammatory response in comparison with hydrogel alone [87]. According to Le Visage et al. application of pullulan/dextran injectable hydrogel with MSCs promotes cellular engraftment and scaffold integration, inhibits fibrosis, and reduces left ventricular dilatation [88]. Furthermore, dextran microspheres incorporated in hyaluronian-based hydrogel showed to improve adipose tissue-derived stem cell survival in vitro [80]. Adipose tissue-derived stem cells (ASCs) mixed with genipin cross-linked alginate-chitosan hydrogel was demonstrated by Paul et al. As reported, the scaffold supports cell viability and growth, gives immunoprotection and is favorable for long-term storage. In is worth to note that ASCs showed constant VEGF secretion of ∼4.6 ng over 2 weeks with further 3.8-fold increase when preconditioned with hypoxia conditions [89].

1.4.3 Synthetic Biomaterials

Towards creating a biomimetic delivery system, metalloproteinases-degradable p (NIPAAm-co-AAc hydrogel was mixed with BMSCs showing positive mechanical support of LV and enhanced cell survival when applied intra-myocardially (38% of injected cells were present after 6 months). When cells were applied alone there was only transient LV function improvement and cells were not present in myocardium in long-term observation [90]. Regarding LV hemodynamic function, BMSCs were shown to be effective when co-utilized with Dex-PCL-HEMA/PNIPAAm. Additionally, cell-hydrogel construct triggered neovascularization and reduced scar expansion, thus attenuating LV dilatation [91]. In another study Wang et al. demonstrated oligo(poly(ethylene glycol) fumarate—OPF functionality when the hydrogel was injected into myocardium with ESCs. Biomaterial alone as well as

hydrogel + ESCs showed to be advantageous, improving heart function and reducing infarct area. Furthermore, addition of cells had the most potent impact on local neovascularization and reduction of MMP-2 and MMP-9 level comparing to hydrogel or ESCs applied individually [92]. To test cyto-toxicity and biocompatibility, Wu et al. designed MPEG-PCL-MPEG triblock polymer which was combined with MSCs. However not tested in vivo, rapid solidification of the hydrogel makes it clinically-relevant for in situ cell delivery because the scaffold showed to be well tolerated after injection [93]. Applicability of similar construct (αCD/MPEG-PCL-MPEG) hydrogel prevents LV remodeling and improves LV hemodynamic function [94].

Apart from above described synthetic polymers, self-assembling peptides are an emerging source for in situ cardiac tissue engineering. Peptide hydrogels are comprised of ionic self-organized oligopeptides forming interlacing nanofibers (diameter 10–30 nm) [95]. Spontaneous chemical-free sol-gel transition occurs in physiological conditions thus giving an opportunity to deliver cells in harmless manner. Self-assembling peptide degradation products are natural amino acids which can be easily metabolized. Importantly, this new class of scaffolds are non-toxic, non-immunogenic, pathogen-free, biocompatible, and cardiac ECM size-adequate making them ideal candidates for cardiac tissue engineering [96]. It has been shown that c-kit^{+}/Nkx2.5^{+} rat bone-marrow derived mesenchymal stem cells (BMMSCs) mixed with self-assembling RAD16-II nano-polypeptide significantly improve heart hemodynamic parameters and decrease infarct size comparing to BMMSCs alone in acute myocardial infarction model [97]. In addition, incorporation of adhesion motifs (i.e. RGD, RGDSP, IKVAV, YIGSR) allows further post-injection cell behavior control, improves hydrogel integration with native tissue, and has cyto-protective function [48, 98]. Guo et al. [48] demonstrated co-application of c-kit^{+}/Nkx2.5^{low}/GATA4^{low} BMMSCs with RGDSP-attached RADA16 peptide hydrogel. An applied cell-nanofiber construct was beneficial regarding cardiac function and infarct size.

1.4.4 Acellular Injectable Biomaterials

The body of studies strongly suggests that paracrine effect is responsible for stem cell therapeutic effects. Injectable gels containing favorable biological agents seem to be a promising toolbox for the treatment of cardiovascular diseases.

1.4.4.1 Natural Biomaterials

Alginate

Ruvinov et al. introduced sulfate-modified alginate hydrogel enriched with HGF and IGF-1 obtaining a well-controlled intra-myocardial delivery system.

Interestingly, different matrix binding affinity of bio-agents and release kinetics (IGF-1 > HGF) resulted in a sequential factor delivery into surrounding cardiac tissue. Moreover, electrostatic interaction between negatively charged sulfate compounds and incorporated GFs provided localized and prolonged release of the active bio-agents. Sulfate-modified alginate gel also had protein protective function against proteases enhancing HGF/IGF-1 action in situ. Comparing with separately administered factors, a mixture of HGF and IGF-1 was superior regarding formation of mature vessel within the infarcted area and stabilization of post-infarction scar. Furthermore, IGF-1-mediated GATA-4^{+} cell cluster number increase might suggest cardiogenesis [99]. Similarly, Hao et al. composed VEGF-A_{165} (angiogenesis initiator) and PDGF-BB (responsible for vessel functionality) containing hydrogel which was delivered in the peri-infarct region. Increased number of new and important mature blood vessels was reported as contributing to improved cardiac function [100]. In the presented study, a dual growth factor delivery system seems to regulate the angiogenesis process in a more physiological fashion and, importantly, counteracts angioma formation in myocardium as observed when VEGF is applied individually [101].

Fibrin

Reported by Christman et al. incorporation of pleiotrophin plasmid into preformed fibrin hydrogel showed to be an effective method to enhance neovasculature formation in the ischemic heart in comparison with injection of plasmid only [102].

An interesting solution was demonstrated by Jeon et al. [103] where stable and prolonged bFGF delivery was achieved by generation of heparin-fibrin gel using fibrinogen and thrombin. It was shown in vitro that bFGF release kinetics is inversely proportional to the aforementioned component concentration. Moreover, using ischemic limb model, bFGF-loaded fibrin gel contributed to more than a 2-fold capillary density increase, in coparision with control. Similarly, Yang et al. introduced heparin-conjugated fibrin hydrogel loaded with bFGF in an ischemic limb model. Results show significant neovascularization, reduced muscle fibrosis and limitation of local inflammation intensity [104]. Applicability of genetically modified VEGF along with fibrin glue was also shown to be an effective scaffold for in vitro human endothelial cell growth. Even though it's not been tested in vivo, authors indicate that fibrin-VEGF as a delivery system could be useful for ischemic heart disease treatment [105].

Gelatin

Gelatin-bFGF injectable gels were used in chronic [106–108] and acute [109] MI animal models. Results show enhanced angiogenesis in the infarct region [106–108, 110] as well as improved hemodynamic function of the heart and reduced left ventricular remodeling [106, 108, 109]. Moreover, data presented by Sakakibara et al. [110] suggest bFGF-induced favorable micro-environment formation for sequentially injected cardiomyocytes where preformed local vascular niche improved cell survival. To induce new and mature blood vessel formation gelatin

microcapsules were combined with IGF-1 and VEGF. Furthermore, a significant reduction of apoptosis, increase of eNOS synthesis, and improved heart contractile function and diminished left ventricular remodeling was observed in comparison with individually applied gelatin, gelatin + IGF-1 and gelatin + VEGF [111].

Chitosan

Temperature-responsive chitosan-bFGF hydrogel was used as an in situ delivery system for MI treatment in rats. Post intra-myocardial administration biomaterial helped to enhance angiogenesis as well as improved ventricular hemodynamics parameters and reduced deleterious LV remodeling process. The presented example shows human-applicable gel with a triggering mechanism. At body temperature, bFGF-containing chitosan solutions polymerize forming in situ a gel which allows local and slow-release of the bio-agent [112]. Angiogenic potential of chitosan-heparin-bFGF-2 hydrogel was also demonstrated by Fujita and colleagues. Improved blood flow was observed in an ischemic limb model after matrix injection [113].

Naturally-derived Composites

Co-application of chitosan and alignate with placental growth factor (PlGF) was presented by Binsalamah et al. PlGF showed to be an angiogenesis stimulant resulting in improved cardiac function. In the settings of myocardial infarction PlGF-enriched alginate-chitosan nanoparticles were injected into myocardium with the following results: improved left ventricle function, increased vascular density, decreased scar area, reduced serum pro-inflammatory cytokines concentration (mainly TNF-α, IL-6), and increased serum anti-inflammatory IL-10 concentration [114]. Recently, heparin-based coacervate (including nontoxic polycation synthetized from alginate, aspartic acid, glycerol and ethylene glycol) was applied in conjugation with FGF-2 for myocardial infarction treatment. In contrary to FGF-2 delivered alone, coacervate-FGF-2 construct had anti-fibrotic, cardiomyocyte pro-survival and a neovascularization effect at 6 weeks post-injection. Moreover, echocardiographically, a combination of bio-agent and coacervate was superior regarding heart contractibility and remodeling inhibition [115]. Intra-myocardial injectability of chitosan-collagen construct along with angiopoietin-1 derived peptide, QHREDG, was recently proven by Reis et al. [116] however, no functional studies upon injection were carried out.

1.4.4.2 Synthetic Biomaterials

Hydrogels based on thermo-sensitive poly(N-isopropylacrylamide), pNIPAM, are promising candidates for in situ delivery systems. *In* aqueous solution below $\sim$32 °C (LCST; *lower critical solution temperature*) pNIPAM *remains in a soluble state* but above the LCST it forms a hydrogel. Interestingly, thermally

induced phase transition can be adjusted by the incorporation of hydrophilic and hydrophobic monomers [117], providing an opportunity to create a human body temperature-responsive biomaterial. Apart from gelling temperature control, incorporation of other polymers allows mechanical properties tuning. Recently, temperature- and pH-responsive injectable hydrogel (p[NIPAAm-co-PAA-co-BA]) was demonstrated by Garbern et al. The construct was designed to form a gel at 37 °C and pH 6.8 thus, applied into acidic microenvironment of ischemic myocardium allowed prolonged, spatio-temporal controllable delivery of bFGF. Within 1 week, bio-agent retention was 10 times higher when co-injected with hydrogel in comparison to saline. In addition, there was a significant increase of neovascularization ($\sim$35%), relative blood flow (2-fold), and fractional shortening in the infarct region observed in polymer + bFGF treated animals as compared to separately applied ingredients [118]. Implanted intra-myocardially, preformed supramolecular hydrogel composed of αCD/MPEG-PCL-MPEG and recombinated human erythropoietin (rhEPO) was presented by Wang et al. Inhibition of apoptosis, infarct size limitation, empowered cardiac function and increased neovascularization was reported in comparison with the results of using either bio-agent and placebo alone. Importantly, there was no post-application [119]. In the study carried out by He and colleagues, HMBG1 (RAGE receptor binding cytokine) was used in combination with thermo-sensitive Dex-PCL-HEMA/PNIPAAm hydrogel. Analyzing the results of applying either agent individually, HMBG1 + copolymer was superior in α-sarcomeric/MEF2C$^+$, c-kit$^+$/Ki67$^+$ and α-sarcomeric/Ki67$^+$ cell number increase. Moreover, cardiac function improvement and collagen content reduction was observed within infarction area when cytokine was applied with hydrogel [120]. Another in situ thermo-responsive gelling biomaterial designed for cardiac repair is PVL-b-PEG-b-PVL hydrogel along with VEGF. With phase transition point set at 37 °C this delivery system was beneficial regarding hemodynamic heart function as well as improved new blood supply within infarcted area after 1 month. Likewise thermo-responsive polymers described above, metalloproteinase-sensitive PEG-vinyl sulfone-functionalized hydrogel with adhesion ligands for $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins and thymosin β4 (Tβ4) was designed for cardiac regeneration. The adhesion ligands and Tβ4 play pivotal roles in cardiovascular development as well as have pro-survival function, respectively. MMP-triggered scaffold degradation resulted in full biomaterial disappearing 6 weeks post-injection with no immunological reaction. When compared with placebo and gel-only group, gel + Tβ4 showed to be significantly advantageous regarding LVEF improvement [121].

Multiblock copolymer (MBCP-2) synthetized from tripblock PEG-PEG-PEG polymer (Pluronic® P104) was conjugated with VEGF plasmid. Dual pH and temperature triggering system allowed formation of the gel at physiological conditions and scaffold degradation in an acidic environment of ischemic myocardium. Furthermore, VEGF gene transfer in a polymeric hydrogel resulted in enhanced angiogenesis by increasing capillary density with larger vessel formation [122, 123]. Application of Pluronic® F-127 (PEO-PPO-PEO triblock copolymer) in addition of Capryol 90 (propylene glycol monocaprylate) generated injectable thermogelling copolymer with nanoparticle-combined VEGF. Presented delivery

platform applied in the subacute MI rat model resulted in ejection fraction and cardiac output improvement [124]. Injectable starPEG-based heparin hydrogel in combination with a proteolytic-resistant SDF-1α variant, AAV-[S4V]-SDF-1α, is also a potential candidate for infarcted myocardium regeneration treatment via recruiting $CXCR4^+$ stem cells. So far, an in vitro experiment shows enhanced early endothelial progenitor cell migration guided by a chemoattractant gradient [125].

1.4.4.3 Self-assembling Peptides

RAD16-II self-assembling peptide was shown to improve cardiac parameters when conjugated with biotinylated IGF-1 [126] and PDGF-BB [127] via Akt signaling activation associated with pro-survival and anti-apoptotic function. Furthermore, application of protease resistant SDF-1α induced neovascularization, creating local supportive milieu with a following $CXCR4^+$/c-kit^+ stem cell recruitment. $VEGF_{165}$ chemokine incorporated into self-assembling nanofibers significantly increased neovascularization and importantly, created vascular niche attracting cardiomyocyte-like cells [59]. Additionally, Guo et al. used peptides which self-assemble under physiological conditions and fused them with heparin-binding domain sequence LRKKLGKA to obtain prolonged (1 month) VEGF delivery system. This novel strategy enhanced angiogenesis and improved cardiac function comparing with components applied individually [128]. Regarding utilization of heparin-presenting self-assembling injectable nanofibers, Webber et al. demonstrated incorporation of VEGF and bFGF chemokines into a heparin-presenting nanoscaffold. The results show that in a rat ischemic limb model, the presented strategy was effective to induce neovascularization [129].

1.4.4.4 Natural-Synthetic Biomaterials

Bio-conjugation and subcutaneous application of hydrogel composed of PEGDA, thiol-modified analog of heparin, HA or chondroitin along with bFGF resulted in enhanced neovascularization. Noteworthy, heparin concentration was an important factor determining bFGF release kinetics (slower release with higher concentration). Regarding adjustment of gel physical properties, biopolymer composed of chitosan mixed with multi-walled carbon nanotubes (MWNTs) had significantly improved hydrogel strength (by 99% by addition of only 0.8 wt% of MWNTs) [130]. However not tested in vivo, Lee et al. used injectable alginate hydrogel along with PLGA microspheres bioconjugated with heat shock protein 27 (HSP27) fused to a transcriptional activator TAT. The researchers showed that this delivery platform allowed controllable and prolonged HSP27-TAT release kinetics. Furthermore, under hypoxic conditions the construct had potential to restore cardiomyoblasts proliferation [131].

Preliminary in vitro studies on thermo-sensitive PNIPAAm/alginate mixture with human BMSCs showed that the bio-construct is capable of preserving the

viability of entrapped cells and in promoting cell proliferation. In addition, the hydrogel degradation rate could be modified and adjusted by PNIPAAm modification. Recently, Purcell et al. demonstrated HEMA-modified in situ-forming HA hydrogel with recombinant SDF-1α to promote engraftment of circulating BMCs into infarcted myocardium. In vivo, comparing to SDF-1α alone, addition of scaffold resulted in ∼8.5-fold increase in cell retention into injection area [132]. The presented data gives a promising preview of injectable cell delivery tools for cardiac regenerative medicine.

1.4.4.5 All-in-One

An all-in-one toolbox containing cell and bio-active factors entrapped in hydrogel seems to be the preferable method for cardiac tissue regeneration. In a rat MI model, injectable PEGylated fibrin scaffold with hepatocyte growth factor (HGF) and bone marrow-derived mononuclear cells (BMNCs) showed to improve cell prevalence rate 15 times at 4 weeks when comparing to cell-only group. Furthermore, LV ejection fraction was highest among the tested configurations (approximately 40%, 30%, and 25% for scaffold + HGF + BMNCs, scaffold + HGF, and saline respectively). Importantly, the scaffold + HGF + BMNCs group was characterized by the most significant neovascularization, apoptosis reduction, and lowest fibrosis indexes. In another study, cardiac progenitor cells (CPCs) and neonatal cardiomyocytes mixed with biotinylated IGF-1 self-assembling nanofibers showed to enhance angiogenesis and to improve recovery of myocardium (myocyte cross-section increase by 25% comparing to scaffold + cells and untethered IGF-1). Moreover, improved heart function and activation of resident CPCs was observed [126, 133].

References

1. Bao C et al (2010) Enhancement of the survival of engrafted mesenchymal stem cells in the ischemic heart by TNFR gene transfection. Biochem Cell Biol 88(4):629–634
2. Suzuki M et al (2003) Cardioprotective effect of diazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. Circulation 107(5):682–685
3. Wakahara N et al (2004) Difference in the cardioprotective mechanisms between ischemic preconditioning and pharmacological preconditioning by diazoxide in rat hearts. Circ J 68 (2):156–162
4. Cui X et al (2010) Transplantation of mesenchymal stem cells preconditioned with diazoxide, a mitochondrial ATP-sensitive potassium channel opener, promotes repair of myocardial infarction in rats. Tohoku J Exp Med 220(2):139–147
5. Dubey RK, Tofovic SP, Jackson EK (2004) Cardiovascular pharmacology of estradiol metabolites. J Pharmacol Exp Ther 308(2):403–409
6. Dubey RK, Jackson EK (2001) Cardiovascular protective effects of 17beta-estradiol metabolites. J Appl Physiol 91(4):1868–1883

7. Erwin GS et al (2009) Estradiol-treated mesenchymal stem cells improve myocardial recovery after ischemia. J Surg Res 152(2):319–324
8. Morris AJ et al (2009) Regulation of blood and vascular cell function by bioactive lysophospholipids. J Thromb Haemost 1:38–43
9. Lin ME, Herr DR, Chun J (2010) Lysophosphatidic acid (LPA) receptors: signaling properties and disease relevance. Prostaglandins Other Lipid Mediat 91(3–4):130–138
10. Liu X et al (2009) Lysophosphatidic acid protects mesenchymal stem cells against ischemia-induced apoptosis in vivo. Stem Cells Dev 18(7):947–954
11. Kazemipour M et al (2012) Simultaneous determination of lovastatin and niacin in tablet by first and third derivative spectrophotometry and H-point standard addition methods. Res Pharm Sci 7(2):95–102
12. Alberts AW (1988) Discovery, biochemistry and biology of lovastatin. Am J Cardiol 62 (15):10J–15J
13. Xu R et al (2008) Lovastatin protects mesenchymal stem cells against hypoxia-and serum deprivation-induced apoptosis by activation of PI3K/Akt and ERK1/2. J Cell Biochem 103 (1):256–269
14. Jankowski M et al (2010) Cardiac oxytocin receptor blockade stimulates adverse cardiac remodeling in ovariectomized spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 299(2)
15. Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiol Rev 81(2):629–683
16. Florian M, Jankowski M, Gutkowska J (2010) Oxytocin increases glucose uptake in neonatal rat cardiomyocytes. Endocrinology 151(2):482–491
17. Kim YS et al (2010) Promigratory activity of oxytocin on umbilical cord blood-derived mesenchymal stem cells. Artif Organs 34(6):453–461
18. Kim YS et al (2012) Priming of mesenchymal stem cells with oxytocin enhances the cardiac repair in ischemia/reperfusion injury. Cells Tissues Organs 195(5):428–442
19. Bae CH et al (2012) Phorbol 12-Myristate 13-Acetate induces MUC16 expression via PKCdelta and p38 in human airway epithelial cells. Clin Exp Otorhinolaryngol 5 (3):161–169
20. Song H et al (2011) Cardiomyocytes from phorbol myristate acetate-activated mesenchymal stem cells restore electromechanical function in infarcted rat hearts. Proc Natl Acad Sci USA 108(1):296–301
21. Rao YJ, Xi L (2009) Pivotal effects of phosphodiesterase inhibitors on myocyte contractility and viability in normal and ischemic hearts. Acta Pharmacol Sin 30(1):1–24
22. Haider H et al (2010) Phosphodiesterase inhibition with tadalafil provides longer and sustained protection of stem cells. Am J Physiol Heart Circ Physiol 299(5):10
23. Lopaschuk GD et al (2003) Beneficial effects of trimetazidine in ex vivo working ischemic hearts are due to a stimulation of glucose oxidation secondary to inhibition of long-chain 3-ketoacyl coenzyme a thiolase. Circ Res 93(3):17
24. Wisel S et al (2009) Pharmacological preconditioning of mesenchymal stem cells with trimetazidine (1-[2,3,4-trimethoxybenzyl]piperazine) protects hypoxic cells against oxidative stress and enhances recovery of myocardial function in infarcted heart through Bcl-2 expression. J Pharmacol Exp Ther 329(2):543–550
25. Xu X et al (2013) Stromal cell-derived factor-1 enhances wound healing through recruiting bone marrow-derived mesenchymal stem cells to the wound area and promoting neovascularization. Cells Tissues Organs 197(2):103–113
26. Pasha Z et al (2008) Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. Cardiovasc Res 77(1):134–142
27. Liu XB et al (2012) Angiopoietin-1 preconditioning enhances survival and functional recovery of mesenchymal stem cell transplantation. J Zhejiang Univ Sci B 13(8):616–623
28. Conti E et al (2011) IGF-1 and atherothrombosis: relevance to pathophysiology and therapy. Clin Sci 120(9):377–402

29. Enoki C et al (2010) Enhanced mesenchymal cell engraftment by IGF-1 improves left ventricular function in rats undergoing myocardial infarction. Int J Cardiol 138(1):9–18
30. Tamama K et al (2006) Epidermal growth factor as a candidate for ex vivo expansion of bone marrow-derived mesenchymal stem cells. Stem Cells 24(3):686–695
31. Herrmann JL et al (2010) Postinfarct intramyocardial injection of mesenchymal stem cells pretreated with TGF-alpha improves acute myocardial function. Am J Physiol Regul Integr Comp Physiol 299(1):19
32. Herrmann JL et al (2011) Transforming growth factor-alpha enhances stem cell-mediated postischemic myocardial protection. Ann Thorac Surg 92(5):1719–1725
33. Herrmann JL et al (2012) TGF-alpha equalizes age disparities in stem cell-mediated cardioprotection. J Surg Res 176(2):386–394
34. Hahn JY et al (2008) Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. J Am Coll Cardiol 51(9):933–943
35. Luo Y et al (2012) Pretreating mesenchymal stem cells with interleukin-1beta and transforming growth factor-beta synergistically increases vascular endothelial growth factor production and improves mesenchymal stem cell-mediated myocardial protection after acute ischemia. Surgery 151(3):353–363
36. Kean TJ et al (2012) Development of a peptide-targeted, myocardial ischemia-homing, mesenchymal stem cell. J Drug Target 20(1):23–32
37. Chacko SM et al (2010) Hypoxic preconditioning induces the expression of prosurvival and proangiogenic markers in mesenchymal stem cells. Am J Physiol Cell Physiol 299(6):22
38. Yan F et al (2012) Hypoxic preconditioning improves survival of cardiac progenitor cells: role of stromal cell derived factor-1alpha-CXCR4 axis. PLoS ONE 7(7):18
39. Pereira MJ et al (2011) Sensing the cardiac environment: exploiting cues for regeneration. J Cardiovasc Transl Res 4(5):616–630
40. Hu X et al (2008) Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J Thorac Cardiovasc Surg 135(4):799–808
41. Wang JA et al (2009) Anoxic preconditioning: a way to enhance the cardioprotection of mesenchymal stem cells. Int J Cardiol 133(3):410–412. doi:10.1016/j.ijcard.2007.11.096 Epub: 29 Jan 2008, Wydanie
42. Ruwhof C et al (2000) Cyclic stretch induces the release of growth promoting factors from cultured neonatal cardiomyocytes and cardiac fibroblasts. Mol Cell Biochem 208(1–2): 89–98
43. Kurazumi H et al (2011) The effects of mechanical stress on the growth, differentiation, and paracrine factor production of cardiac stem cells. PLoS ONE 6(12):28
44. Salameh A et al (2010) Cyclic mechanical stretch induces cardiomyocyte orientation and polarization of the gap junction protein connexin43. Circ Res 106(10):1592–1602
45. Sun Y, Chen CS, Fu J (2012) Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. Annu Rev Biophys 41:519–542
46. Maul TM et al (2011) Mechanical stimuli differentially control stem cell behavior: morphology, proliferation, and differentiation. Biomech Model Mechanobiol 10(6):939–953
47. Lu WN et al (2009) Functional improvement of infarcted heart by co-injection of embryonic stem cells with temperature-responsive chitosan hydrogel. Tissue Eng Part A 15(6):1437–1447
48. Guo HD et al (2010) Transplantation of marrow-derived cardiac stem cells carried in designer self-assembling peptide nanofibers improves cardiac function after myocardial infarction. Biochem Biophys Res Commun 399(1):42–48
49. Landa N et al (2008) Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat. Circulation 117(11):1388–1396
50. Christman KL et al (2004) Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. Tissue Eng 10(3–4):403–409

51. Martens TP et al (2009) Percutaneous cell delivery into the heart using hydrogels polymerizing in situ. Cell Transplant 18(3):297–304
52. Tsur-Gang O et al (2009) The effects of peptide-based modification of alginate on left ventricular remodeling and function after myocardial infarction. Biomaterials 30(2):189–195
53. Kim IY et al (2008) Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv 26(1):1–21
54. Lu S et al (2010) Both the transplantation of somatic cell nuclear transfer- and fertilization-derived mouse embryonic stem cells with temperature-responsive chitosan hydrogel improve myocardial performance in infarcted rat hearts. Tissue Eng Part A 16 (4):1303–1315
55. Chekanov V et al (2003) Transplantation of autologous endothelial cells induces angiogenesis. Pacing Clin Electrophysiol 26(1 Pt 2):496–499
56. Ryu JH et al (2005) Implantation of bone marrow mononuclear cells using injectable fibrin matrix enhances neovascularization in infarcted myocardium. Biomaterials 26(3):319–326
57. Castells-Sala C, Semino CE (2012) Biomaterials for stem cell culture and seeding for the generation and delivery of cardiac myocytes. Curr Opin Organ Transplant 17(6):681–687
58. Zouein FA et al (2012) Post-infarct biomaterials, left ventricular remodeling, and heart failure: is good good enough? Congest Heart Fail 18(5):284–290
59. Lin YD et al (2012) Instructive nanofiber scaffolds with VEGF create a microenvironment for arteriogenesis and cardiac repair. Sci Transl Med 4(146):3003841
60. LaNasa SM, Bryant SJ (2009) Influence of ECM proteins and their analogs on cells cultured on 2-D hydrogels for cardiac muscle tissue engineering. Acta Biomater 5(8):2929–2938
61. Mihardja SS, Sievers RE, Lee RJ (2008) The effect of polypyrrole on arteriogenesis in an acute rat infarct model. Biomaterials 29(31):4205–4210
62. Huang NF et al (2005) Injectable biopolymers enhance angiogenesis after myocardial infarction. Tissue Eng 11(11–12):1860–1866
63. Shen X, Tanaka K, Takamori A (2009) Coronary arteries angiogenesis in ischemic myocardium: biocompatibility and biodegradability of various hydrogels. Artif Organs 33 (10):781–787
64. Tulloch NL et al (2011) Growth of engineered human myocardium with mechanical loading and vascular coculture. Circ Res 109(1):47–59
65. Guo HD et al (2011) Transplantation of marrow-derived cardiac stem cells carried in fibrin improves cardiac function after myocardial infarction. Tissue Eng Part A 17(1–2):45–58
66. Swartz DD, Russell JA, Andreadis ST (2005) Engineering of fibrin-based functional and implantable small-diameter blood vessels. Am J Physiol Heart Circ Physiol 288(3):14
67. Lee OK (2008) Fibrin glue as a vehicle for mesenchymal stem cell delivery in bone regeneration. J Chin Med Assoc 71(2):59–61
68. Bach TL et al (1998) VE-Cadherin mediates endothelial cell capillary tube formation in fibrin and collagen gels. Exp Cell Res 238(2):324–334
69. Zhang X et al (2010) Preservation of the cardiac function in infarcted rat hearts by the transplantation of adipose-derived stem cells with injectable fibrin scaffolds. Exp Biol Med 235(12):1505–1515
70. Zhang G et al (2006) A PEGylated fibrin patch for mesenchymal stem cell delivery. Tissue Eng 12(1):9–19
71. Zhang G et al (2008) Enhancing efficacy of stem cell transplantation to the heart with a PEGylated fibrin biomatrix. Tissue Eng Part A 14(6):1025–1036
72. Taylor SJ, McDonald JW 3rd, Sakiyama-Elbert SE (2004) Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J Control Release 98(2):281–294
73. Shi C et al (2006) Therapeutic potential of chitosan and its derivatives in regenerative medicine. J Surg Res 133(2):185–192
74. Liu Z et al (2012) The influence of chitosan hydrogel on stem cell engraftment, survival and homing in the ischemic myocardial microenvironment. Biomaterials 33(11):3093–3106

75. Gerecht S et al (2007) Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proc Natl Acad Sci USA 104(27):11298–11303
76. Yang MC et al (2010) The influence of rat mesenchymal stem cell CD44 surface markers on cell growth, fibronectin expression, and cardiomyogenic differentiation on silk fibroin—hyaluronic acid cardiac patches. Biomaterials 31(5):854–862
77. Chang CY et al (2012) Hyaluronic acid-human blood hydrogels for stem cell transplantation. Biomaterials 33(32):8026–8033
78. Park D et al (2012) Hyaluronic acid promotes angiogenesis by inducing RHAMM-TGFbeta receptor interaction via CD44-PKCdelta. Mol Cells 33(6):563–574
79. Fiumana E et al (2013) Localization of mesenchymal stem cells grafted with a hyaluronan-based scaffold in the infarcted heart. J Surg Res 179(1):27
80. Kim BS et al (2010) Improvement of stem cell viability in hyaluronic acid hydrogels using dextran microspheres. J Biomater Sci Polym Ed 21(13):1701–1711
81. Dai W et al (2005) Thickening of the infarcted wall by collagen injection improves left ventricular function in rats: a novel approach to preserve cardiac function after myocardial infarction. J Am Coll Cardiol 46(4):714–719
82. Acarregui A et al (2012) Hydrogel-based scaffolds for enclosing encapsulated therapeutic cells. Biomacromol 11:11
83. Murua A et al (2011) Emerging technologies in the delivery of erythropoietin for therapeutics. Med Res Rev 31(2):284–309
84. Prestwich GD et al (2012) The translational imperative: making cell therapy simple and effective. Acta Biomater 8(12):4200–4207
85. Chung EJ, Jakus AE, Shah RN (2013) In situ forming collagen-hyaluronic acid membrane structures: mechanism of self-assembly and applications in regenerative medicine. Acta Biomater 9(2):5153–5161
86. Dahlmann J et al (2013) Fully defined in situ cross-linkable alginate and hyaluronic acid hydrogels for myocardial tissue engineering. Biomaterials 34(4):940–951
87. Huang Z et al (2011) In situ-forming chitosan/nano-hydroxyapatite/collagen gel for the delivery of bone marrow mesenchymal stem cells. Carbohyd Polym 85(1):261–267
88. Le Visage C et al (2012) Mesenchymal stem cell delivery into rat infarcted myocardium using a porous polysaccharide-based scaffold: a quantitative comparison with endocardial injection. Tissue Eng Part A 18(1–2):35–44
89. Paul A et al (2011) Superior cell delivery features of genipin crosslinked polymeric microcapsules: preparation, in vitro characterization and pro-angiogenic applications using human adipose stem cells. Mol Biotechnol 48(2):116–127
90. Wall ST et al (2010) Biomimetic matrices for myocardial stabilization and stem cell transplantation. J Biomed Mater Res A 95(4):1055–1066
91. Li XY et al (2010) Injectable hydrogel helps bone marrow-derived mononuclear cells restore infarcted myocardium. Cardiology 115(3):194–199
92. Wang H et al (2012) Injectable biodegradable hydrogels for embryonic stem cell transplantation: improved cardiac remodelling and function of myocardial infarction. J Cell Mol Med 16(6):1310–1320
93. Wu DQ et al (2008) Fabrication of supramolecular hydrogels for drug delivery and stem cell encapsulation. Langmuir 24(18):10306–10312
94. Jiang XJ et al (2009) Injection of a novel synthetic hydrogel preserves left ventricle function after myocardial infarction. J Biomed Mater Res A 90(2):472–477
95. Leon EJ et al (1998) Mechanical properties of a self-assembling oligopeptide matrix. J Biomater Sci Polym Ed 9(3):297–312
96. Koutsopoulos S (2012) Molecular fabrications of smart nanobiomaterials and applications in personalized medicine. Adv Drug Deliv Rev 64(13):1459–1476
97. Cui XJ et al (2010) Transplantation of mesenchymal stem cells with self-assembling polypeptide scaffolds is conducive to treating myocardial infarction in rats. Tohoku J Exp Med 222(4):281–289

98. Jung JP, Moyano JV, Collier JH (2011) Multifactorial optimization of endothelial cell growth using modular synthetic extracellular matrices. Integr Biol 3(3):185–196
99. Ruvinov E, Leor J, Cohen S (2011) The promotion of myocardial repair by the sequential delivery of IGF-1 and HGF from an injectable alginate biomaterial in a model of acute myocardial infarction. Biomaterials 32(2):565–578
100. Hao X et al (2007) Angiogenic effects of sequential release of VEGF-A165 and PDGF-BB with alginate hydrogels after myocardial infarction. Cardiovasc Res 75(1):178–185
101. Schwarz ER et al (2000) Evaluation of the effects of intramyocardial injection of DNA expressing vascular endothelial growth factor (VEGF) in a myocardial infarction model in the rat–angiogenesis and angioma formation. J Am Coll Cardiol 35(5):1323–1330
102. Christman KL et al (2005) Enhanced neovasculature formation in ischemic myocardium following delivery of pleiotrophin plasmid in a biopolymer. Biomaterials 26(10):1139–1144
103. Jeon O et al (2005) Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin. J Control Release 105(3):249–259
104. Yang HS et al (2010) Delivery of basic fibroblast growth factor using heparin-conjugated fibrin for therapeutic angiogenesis. Tissue Eng Part A 16(6):2113–2119
105. Zisch AH et al (2001) Covalently conjugated VEGF–fibrin matrices for endothelialization. J Control Release 72(1–3):101–113
106. Sakakibara Y et al (2003) Toward surgical angiogenesis using slow-released basic fibroblast growth factor. Eur J Cardiothorac Surg 24(1):105–111
107. Yamamoto T et al (2001) Intramyocardial delivery of basic fibroblast growth factor-impregnated gelatin hydrogel microspheres enhances collateral circulation to infarcted canine myocardium. Jpn Circ J 65(5):439–444
108. Iwakura A et al (2003) Intramyocardial sustained delivery of basic fibroblast growth factor improves angiogenesis and ventricular function in a rat infarct model. Heart Vessels 18 (2):93–99
109. Liu Y et al (2006) Effects of basic fibroblast growth factor microspheres on angiogenesis in ischemic myocardium and cardiac function: analysis with dobutamine cardiovascular magnetic resonance tagging. Eur J Cardiothorac Surg 30(1):103–107
110. Sakakibara Y et al (2002) Prevascularization with gelatin microspheres containing basic fibroblast growth factor enhances the benefits of cardiomyocyte transplantation. J Thorac Cardiovasc Surg 124(1):50–56
111. Cittadini A et al (2011) Complementary therapeutic effects of dual delivery of insulin-like growth factor-1 and vascular endothelial growth factor by gelatin microspheres in experimental heart failure. Eur J Heart Fail 13(12):1264–1274
112. Wang H et al (2010) Improved myocardial performance in infarcted rat heart by co-injection of basic fibroblast growth factor with temperature-responsive chitosan hydrogel. J Heart Lung Transplant 29(8):881–887
113. Fujita M et al (2007) Therapeutic angiogenesis induced by controlled release of fibroblast growth factor-2 from injectable chitosan/non-anticoagulant heparin hydrogel in a rat hindlimb ischemia model. Wound Repair Regen 15(1):58–65
114. Binsalamah ZM et al (2011) Intramyocardial sustained delivery of placental growth factor using nanoparticles as a vehicle for delivery in the rat infarct model. Int J Nanomed 6:2667–2678
115. Chu H et al (2013) The effect of a heparin-based coacervate of fibroblast growth factor-2 on scarring in the infarcted myocardium. Biomaterials 34(6):1747–1756
116. Reis LA et al (2012) A peptide-modified chitosan-collagen hydrogel for cardiac cell culture and delivery. Acta Biomater 8(3):1022–1036
117. Hoare T et al (2011) Magnetically triggered nanocomposite membranes: a versatile platform for triggered drug release. Nano Lett 11(3):1395–1400
118. Garbern JC et al (2011) Delivery of basic fibroblast growth factor with a pH-responsive, injectable hydrogel to improve angiogenesis in infarcted myocardium. Biomaterials 32 (9):2407–2416

119. Wang T et al (2009) The inhibition of postinfarct ventricle remodeling without polycythaemia following local sustained intramyocardial delivery of erythropoietin within a supramolecular hydrogel. Biomaterials 30(25):4161–4167
120. He YY et al (2012) Intramyocardial delivery of HMGB1 by a novel thermosensitive hydrogel attenuates cardiac remodeling and improves cardiac function after myocardial infarction. J Cardiovasc Pharmacol 26:26
121. Kraehenbuehl TP et al (2011) Human embryonic stem cell-derived microvascular grafts for cardiac tissue preservation after myocardial infarction. Biomaterials 32(4):1102–1109
122. Kwon JS et al (2009) Enhanced angiogenesis mediated by vascular endothelial growth factor plasmid-loaded thermo-responsive amphiphilic polymer in a rat myocardial infarction model. J Control Release 138(2):168–176
123. Garripelli VK et al (2010) A novel thermosensitive polymer with pH-dependent degradation for drug delivery. Acta Biomater 6(2):477–485
124. Oh KS et al (2010) Temperature-induced gel formation of core/shell nanoparticles for the regeneration of ischemic heart. J Control Release 146(2):207–211
125. Baumann L et al (2012) A novel, biased-like SDF-1 derivative acts synergistically with starPEG-based heparin hydrogels and improves eEPC migration in vitro. J Control Release 162(1):68–75
126. Davis ME et al (2006) Local myocardial insulin-like growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. Proc Natl Acad Sci USA 103(21):8155–8160
127. Hsieh PC et al (2006) Controlled delivery of PDGF-BB for myocardial protection using injectable self-assembling peptide nanofibers. J Clin Invest 116(1):237–248
128. Guo HD et al (2012) Sustained delivery of VEGF from designer self-assembling peptides improves cardiac function after myocardial infarction. Biochem Biophys Res Commun 424 (1):105–111
129. Webber MJ et al (2010) Capturing the stem cell paracrine effect using heparin-presenting nanofibres to treat cardiovascular diseases. J Tissue Eng Regen Med 4(8):600–610
130. Wang SF et al (2005) Preparation and mechanical properties of chitosan/carbon nanotubes composites. Biomacromol 6(6):3067–3072
131. Lee J et al (2009) Controlled delivery of heat shock protein using an injectable microsphere/hydrogel combination system for the treatment of myocardial infarction. J Control Release 137(3):196–202
132. Purcell BP et al (2012) Synergistic effects of SDF-1alpha chemokine and hyaluronic acid release from degradable hydrogels on directing bone marrow derived cell homing to the myocardium. Biomaterials 33(31):7849–7857
133. Padin-Iruegas ME et al (2009) Cardiac progenitor cells and biotinylated insulin-like growth factor-1 nanofibers improve endogenous and exogenous myocardial regeneration after infarction. Circulation 120(10):876–887

Chapter 2
Nanorobotic Agents and Their Biomedical Applications

Abstract Application of nanorobotic agents is one of the most-promising perspective for future development and progress in medicine. Molecular machines gain significant attention with an ultimate goal to create a theranostic platform interacting with biological system and being able to perform atomic-level tasks. Such concept requires advanced technological approach i.e. design and assembly techniques, in vivo real-time navigation system, sensing methods as well as data transfer. Currently, both artificial (carbon nanotubes) and biological (DNA, proteins, bacteria) components are investigated as a building blocks of nanobots. This chapter presents advancements in nanorobotic agents biomedical application.

Keywords Nanomedicine · Nanorobot · Molecular machine

2.1 Nanomedicine and Nanorobotics

One of the concept in nanomedicine, described by Freitas [1], describes an idea of introducing nanorobots and related machines into biological systems for cellular and molecular-level tasks. Due to very challenging character of this perspective, multidirectional and interdisciplinary approach is required including selection of nanorobotic agents type, energy source, control and navigation system. Ongoing theoretical and experimental studies in nanomedicine will give a strong support for advancements in nanodevice design and further clinical application.

Nanorobotic agents, structures with overall dimensions in the nanometers size range, are designed to perform predefined tasks including target molecule delivery for therapeutic or/and diagnostic (theranostic) purpose. Particular physical phenomena associated with nanoscale diameter require specific and advanced nano-composites assembly methods as well as control and actuation strategies [2]. Nevertheless a concept of applying nanobots in clinical medicine is relatively new, molecular robotics gains significant attention and recognition reflected by increasing number of demonstrations and publications. Of note, despite significant advancement in the medical strategies prognosis of patients remains unsatisfactory.

T. Jadczyk et al., *Innovative Diagnostics and Treatment: Nanorobotics and Stem Cells*, Nanotheranostics, DOI 10.1007/978-981-10-4527-1_2

Thus, adoption of new technologies is especially of a great interest. The advancement in the field of nanotechnology with improvement in design, sensing, control, as well as better understanding of molecular biology are important steps towards translational medicine.

2.2 Bio-nanorobotics

It is generally said that bio-nanorobotics reflects and incorporates many observations and natural law aspects, what is represented in design, programming, manufacturing and controlling of the nanomachines as shown in Figs. 2.1 and 2.2 [3].

2.3 Nanorobotic Agents in Biomedical Applications

The consolidation of nanotechnology, medicine and surgery provides an exciting evolution in a healthcare sector and medical technology. In 1959 Richard P. Feynman, Nobel Prize laureate in physics, was the first scientist who proposed a vision of nanorobots and atomic level machines application in the treatment of disease in humans i.e. nanorobot-based therapy for heart disorders [4].

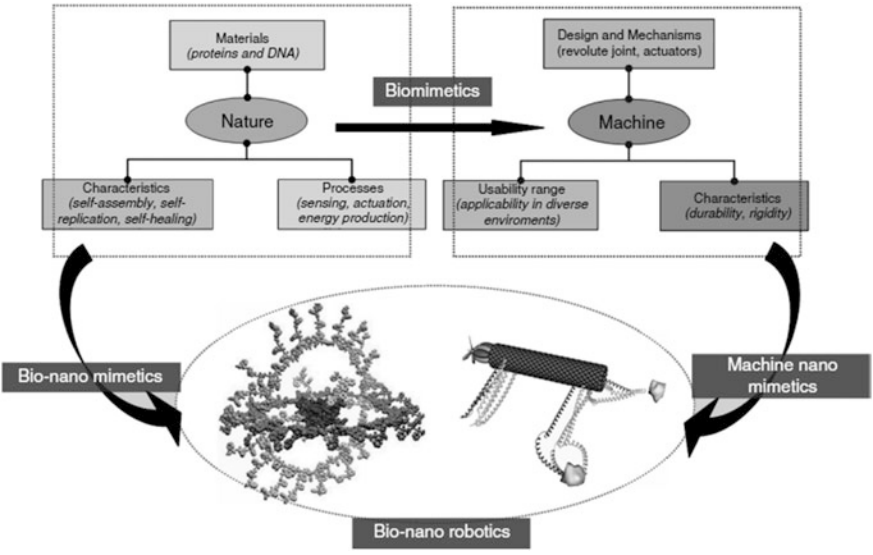


Fig. 2.1 Biomimetics—bio-nano robotics, inspired by nature and machine. Adapted with permission from Ref. [3]

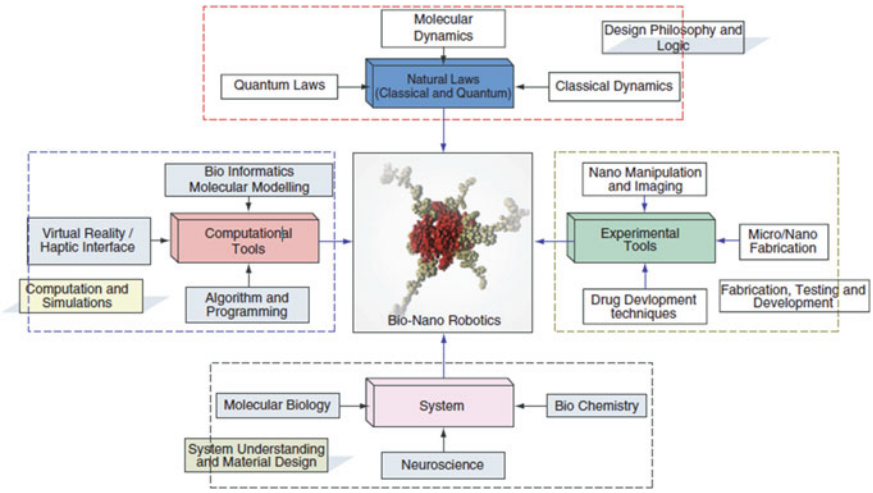


Fig. 2.2 Bio-nano robotics as a multidisciplinary field. Adapted with permission from Ref. [3]

2.4 Biomolecular Machines and Bio-nanorobots

Development in robotic molecular-level complement is a critical step for biomolecular machines evolution, in aim to create a functional motion, force, as well as to sense signal and store information.

Protein and DNA are explored to act as bio-mechanical joints, motors, transmission elements and biochemical sensors. DNA nanotubes can serve as structural elements like links and DNA hinge. Moreover, ATPase protein flagella motors, viral protein motors, DNA-based structures are also being explored as actuators. Interestingly, rhodopsin and Heat Shock Factor proteins are also researched as sensors. These bio-inspired robot components assembled together provide an picture of applicable biomolecular machine.

In aim to functionalize and optimize medical nanorobotic devices, various elemental components like molecular gears, bearings, chemical sensors are required (Fig. 2.3). Thus, complex manufacturing, synthesis techniques and interdisciplinary approaches are incorporated to obtain an operational product. Positional assembly strategy, a concept in molecular manufacturing, relays on combining molecular parts one by one in a controlled fashion. Although the theoretical aspect of atom by atom positional assembly is well established, the nanorobotic experimental researches are still in the early developmental phase [4]. Interestingly, even more futuristic, there are concepts of incorporating biological elements into nanobots (Fig. 2.4).

Cavalcanti et al. worked on application of nanobioelectronics for diabetes mellitus treatment [5]. With computational approach, they proposed a 3D prototype of a device designed to improve glucose management through a external electronic

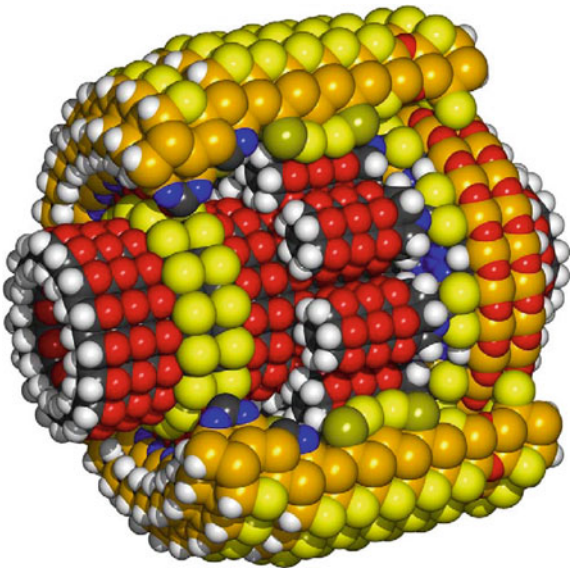


Fig. 2.3 A molecular planetary gear is a mechanical component that might be found inside a medical nanorobot. The gear converts shaft power from one angular frequency to another. The casing is a strained silicon shell with predominantly sulphur termination, with each of the nine planet gears attached to the planet carrier by a carbon-carbon single bond. The planetary gear shown here has not been built experimentally but has been modelled computationally. Adapted with permission from Ref. [4]

device which can communicate with nanorobots. They addressed nanorobotic sensing with chemical signal dispersion using a 3D environment for prototyping and analysis as shown in Fig. 2.5. The model used an embedded chemosensor for glucose monitoring by modulating human SGLT3 protein glucosensor activity and the SG33-P antibody for modelling the integrated circuit. Higher concentrations of proteins can be identified which couples human SGLT3 isoforms to the intracellular bloodstream signalling. Afterwards, the data was transmitted to the external device (i.e. mobile phone).

Martel et al. worked on two complementary systems. The first one is a MRI-based medical nanorobotic platform to access the regions of human body inaccessible to catheterization [6]. The basic principle relies first on exploiting the high homogenous magnetic field inside the tunnel of the clinical MRI scanner (typically 1.5T or 3T) to bring magnetic entities such as superparamagnetic nanoparticles at saturation magnetization. When at saturation magnetization, such magnetic nanoparticles provide the maximum displacement force for a given magnetic gradient (variation of the magnetic field strength over distance). Indeed, when inside the tunnel of a MRI scanner, no displacement occurs since the magnetic field is uniform. To apply 3D directional magnetic gradients to induce a displacement force along a pre-defined trajectory in the vascular network on magnetic nanoparticles encased in therapeutic

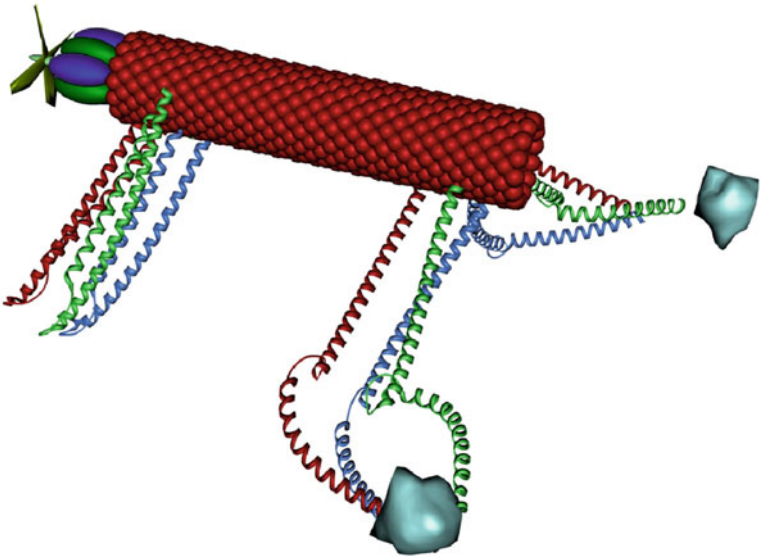


Fig. 2.4 The biological elements will be used to fabricate robotic systems. A vision of a nano-organism: carbon nanotubes (CNT) form the main body; peptide limbs can be used for locomotion and object manipulation, a biomolecular motor located at the head can propel the device in various environments. Adapted with permission from Ref. [3]

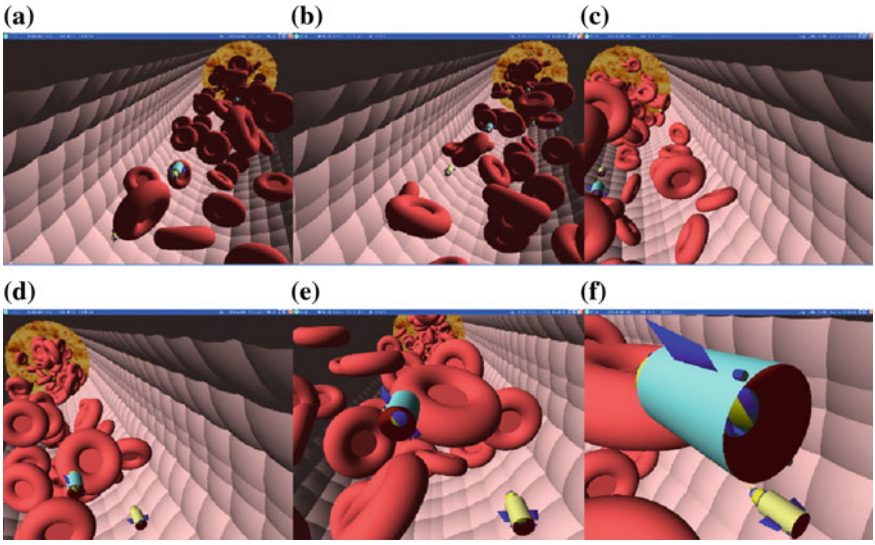


Fig. 2.5 The real-time simulation serves as an advanced tested environment for task-based analysis, providing an extremely useful investigation tool for device prototyping, control, and manufacturing analysis. The nanorobots move through the workspace sensing blood glucose levels. Adapted with permission from Ref. [5]

microcarriers. Such directional gradients can be generated using the gradient imaging coils of the MRI scanner or by distorting the homogenous field of the scanner using ferromagnetic cores introduced at specific locations in the tunnel of the scanner. This is referred to as Magnetic Resonance Navigation (MRN) [7] or Dipole Field Navigation (DFN) [8], respectively. MRN and DFN are more appropriate for naviation in larger blood vessels such as arteries and large arterioles. Technological issues including but not limited to the generation of higher gradients at the human scale and the limit of medical imaging modalities, prevents these technologies to reach the microvascular network.

As such natural nanorobots in the form of flagellated magnetotactic bacteria (Fig. 2.6) have been considered [9]. A special platform dubbed the magnetotaxis platform produces a 3D directional magnetic field that align a chain of intracellular nanoparticles synthesized in the cells during cultivation towards the targeted region to be treated which is typically a solid tumor. This chain acts like a microscopic compass needle that is used to indicate to the bacteria the direction of the tumor. Once inside the tumor, the magnetic field is set to zero to allow the bacteria to seek autonomously regions of low oxygen concentrations which correspond to regions of fast duplicating tumor cells. Each bacterium is previously loaded with the therapeutic agents. These bacteria are very effective in physiological microenvironments such as near and inside the tumoral volume [10]. As such, some types of localized cancers where peritumoral injections are possible such as colorectal and

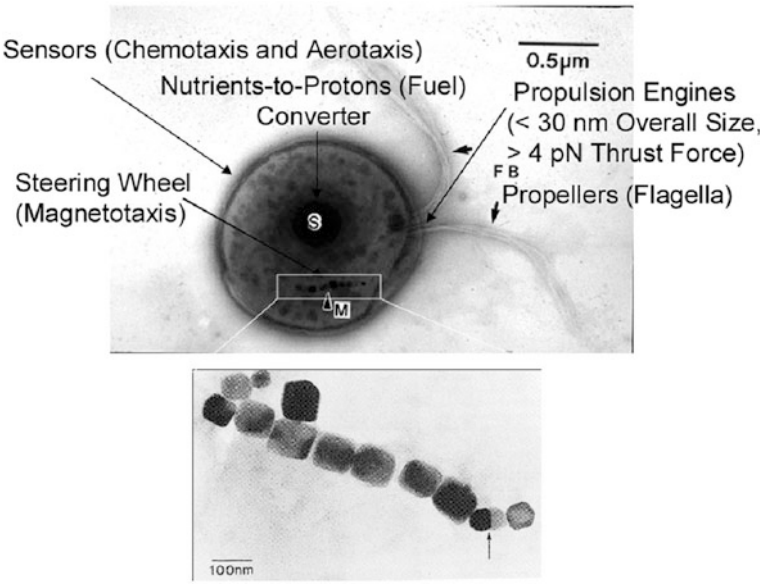


Fig. 2.6 Representation of the MC-1 MTB as a computer controllable bio-actuator with its flagella bundles (FB) for propulsion and its chain of magnetosomes (*bottom*) allowing steering control through magnetotaxis. Adapted with permission from Ref. [9]

prostatic cancers are candidates for this particular approach. But some locations require intraarterial injections. Since the bacteria are ineffective in larger blood vessels due in part to the blood flow and the distance separating the injection site the site of treatment, efforts are also underway to combined MRN and DFN to bring the drug-loaded bacteria encased in special microscopic vesicles closer the tumor where they become more effective.

Mustapha Hamdi proposed an application of DNA for computational designing and multiscale modelling of a encapsulated DNA-multi-walled carbon nanotube actuator, which can be incorporated as a drug delivery carrier and biomolecular transport system [11] as shown in Fig. 2.7. Encapsulated double-stranded DNA was connected to a support electrode and it actuates the sliding inner tube. With variation of temperature in mild acidic medium, the inner nanotube slides due to change in conformation.

Mechanical properties of three nanoactuator structure, A-DNA, Z-DNA and B-DNA were studied through molecular dynamics simulation of DNA denaturation, and Z-DNA showed most powerful and controllable driving performances as described in Fig. 2.8. Encapsulation of DNA inside carbon nanotube was simulated and ionic current feedback sensor was implemented for position determination. In conclusion, the developed system was based on a nanoscale linear servomotor with integrated position sensor.

Hogg and Freitas evaluated numerical models of chemical source power available for nanorobots which oxidize blood glucose [12]. The researchers studied kinetics of oxygen release from red blood cells through haemoglobin saturation as well as the capacity of nanorobots to generate power by processing oxygen. The same authors worked on acoustic-based communication approaches for medical nanorobots [13]. They analysed in vivo acoustic property of body various tissues observing a frequency-dependent changes in attenuation. Based on obtained data, spherical nanorobots designs was proposed. Moreover, the authors showed that a communication rate of 10^4 bits/s is possible with a power supply generated from

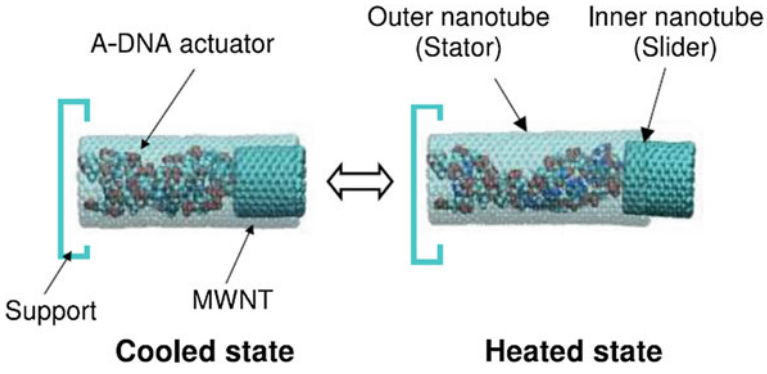


Fig. 2.7 Basic concept of a linear encapsulated DNA-MWNT nanoactuator controlled by the temperature parameter. Adapted with permission from Ref. [11]

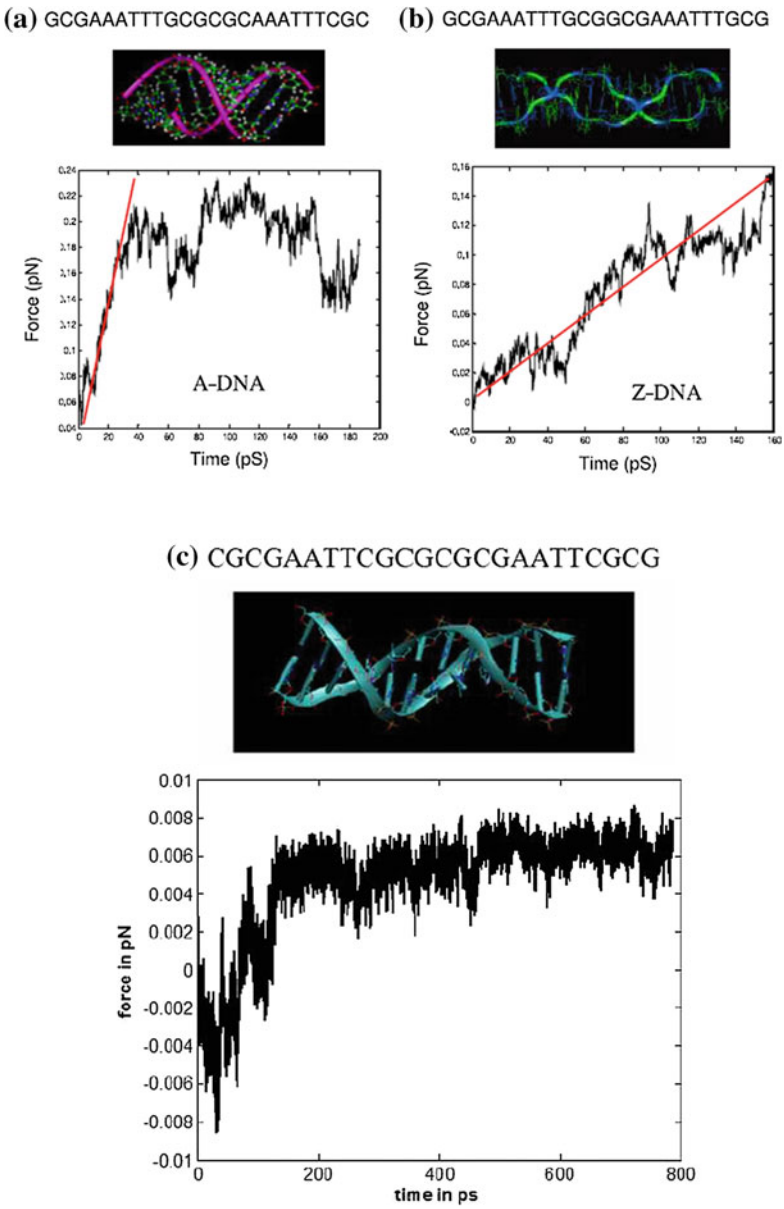


Fig. 2.8 Chemical structure and mechanical force delivered by DNA denaturation for **a** A-DNA, **b** Z-DNA and **c** B-DNA. Adapted with permission from Ref. [11]

oxygen and glucose present in bloodstream. They proposed that the demonstrated system can also be used in a therapy with a short bursts at considerably higher power, without damaging nearby tissues.

Architectures based on self-assembled functional DNA oligonucleotides and interlocked nanomechanical components are also explored [14] (Fig. 2.9). Without any restriction in motion, mechanical interlock allows controlled and specific motion with reduced diffusion. Precise alignment of structural and functional modules of DNA in 2D and 3D is possible through DNA origami technique. This method can be used to synthesise scaffolds for specific integration of multiple functional elements made of DNA. Circular geometrical interlocked DNA nanostructures like dsDNA nanorings (Fig. 2.10) are extensively researched for building artificial nanomachines.

Abi and colleagues worked with 3D DNA nanostructures, self-regulating sensor and other actuator devices [15]. They showed electrochemical switching with these structures self-assembled on gold electrodes. External chemical stimuli-triggered mechanical reconfiguration acts as a nanoswitch, thus, this solution can serve as a functional and operation element in nanorobots and 3D DNA tetrahedral based surface-tethered nanoelectronic devices. Four DNA sequences were annealed followed by immobilization on gold surface through thiol linking at three component strand ends as shown in Fig. 2.11. Chronocoulometric analysis was used for the DNA tetrahedron surface coverage evaluation and nano mechanical switch induced by configuration was electrochemically checked in solutions of high ionic strength. This reconfiguration-based switching induced by molecular stimulus showed high efficiency and for the first time, this research addressed surface immobilization based 3D DNA architecture for electronic nanoswitch.

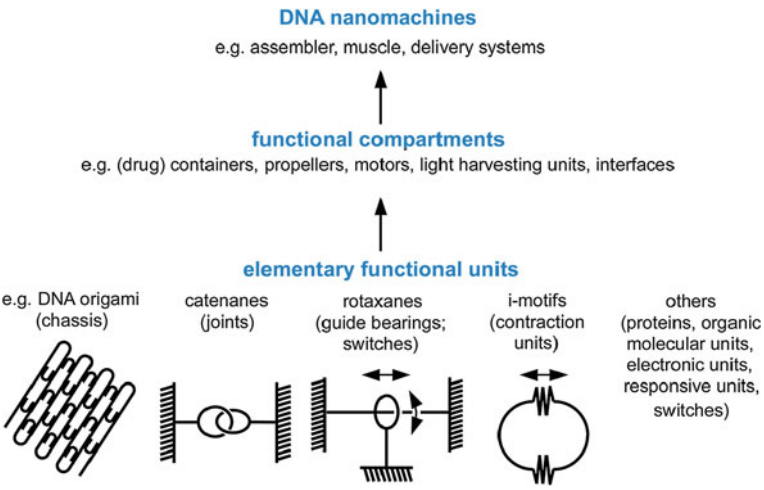


Fig. 2.9 Hierarchical assembly of complex DNA nanomachines from functional compartments. These will consist of elementary units, each featuring a highly specific functionality. Adapted with permission from Ref. [14]

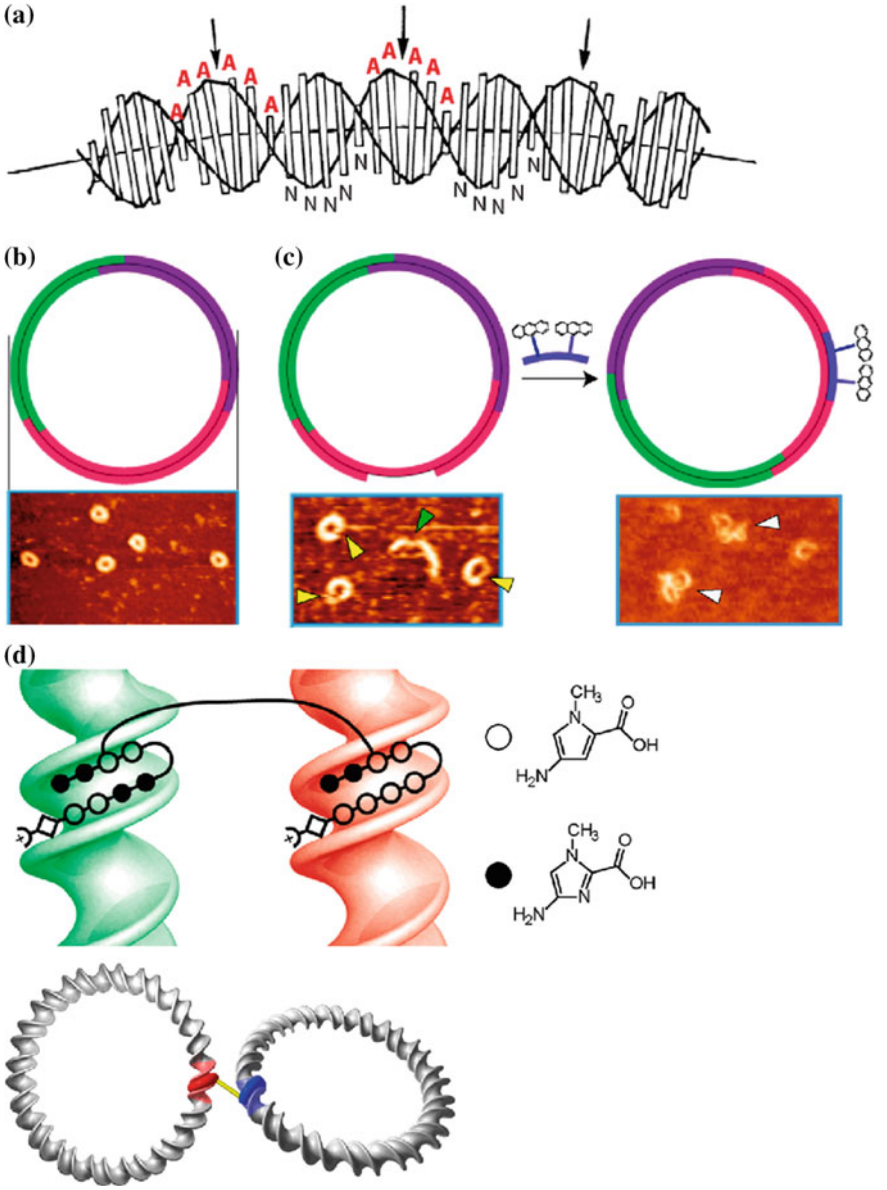


Fig. 2.10 Generation of dsDNA nanorings. **a** Intrinsic dsDNA-bending by repetitive A-tracts. **b** 168-bp dsDNA-nanoring ligated from three 51-mer dsDNAs, flanked by 5-nt ssDNA overhangs (*top*) exhibit a regular, nondistorted shape (*bottom*). **c** Assembly of dsDNA nanocircles into oligomeric aggregates mediated by anthracene intercalation. Adapted with permission from Ref. [14]

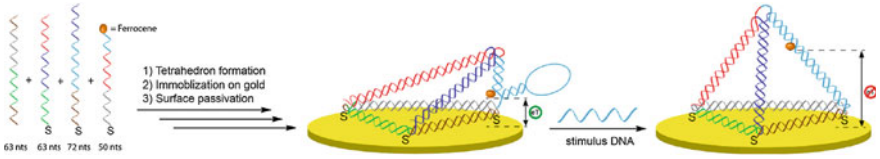


Fig. 2.11 Schematic representation of the gold-tethered DNA tetrahedron containing a reconfigurable edge with a stem-loop (hairpin) structure. Reconfiguration of the tetrahedron takes place after hybridization of complementary DNA (stimulus DNA) to the hairpin region, which results in the change of the distance between the Fc redox probe and the electrode surface and thus in the variation of the ferrocene (Fc) electrochemical signal. Mercapto-1-hexanol (MCH) molecules used to block the gold surface are not shown. Adapted with permission from Ref. [15]

Redox-switchable molecular grippers gain much attention in nanorobotics as they can grab a molecular cargo and translocate it over different location in functionally controlled manner. In this regards, resorcin [4] arene cavitands are known to have two spatial conformations, a contracted vase and an expanded kite, thus, they can act very well as molecular grippers. As an extension, the leg shapes can be used for functionalization and metal surface attachments. Pochorovski and Diederich applied naphthoquinone moiety as a redox-active wall component and cavitands with hydrogen bond acceptor groups as shown in Fig. 2.12 [16]. It was presented that carboxamide moieties can initiate redox-induced conformational switching and alter binding properties. With steric congestion, they found that association constant increased and guest release rates reduced when triptycene-quinone moiety was used instead of naphthoquinone. As the cavitands here were dependent on external proton sources for switching, further research is leading to independent switching.

Zhang et al. demonstrated a contact electrification field-effect transistor (CE-FET) with an external mechanical stimulation-induced charge transfer properties [17]. For this, authors used coupled metal oxide semiconductor field-effect transistor and triboelectric nanogenerator. The CE-FET shown greater sensing range and has a potential application for biomedical nanorobotics (Fig. 2.13).

Hemispherical microstructures of superparamagnetic polymer composite (SPMPCs) with programmed anisotropy was printed by Ergeneman et al. in large array with inkjet printer [18] (Fig. 2.14). With a favourable magnetic properties of embedded nanostructures and added functionality of polymers, SPMPCs can by successfully applied in microsystems construction and nanorobotics. Various operations can be performed using magnetic fields i.e. drug delivery, sensing specific physical and chemical signals. Superparamagnetic magnetite nanoparticles dispersed in SU-8 were used to make magnetic hemispheres. Magnetic field was applied during curing to programme magnetic anisotropy (Fig. 2.15). Authors demonstrated different assembly methods and their independent dynamic control. The fabricated units can be used as microelectromechanical systems and nanorobotic tools for various medical applications, and the magnetic microhemispheres can be easily manipulated at liquid interface.

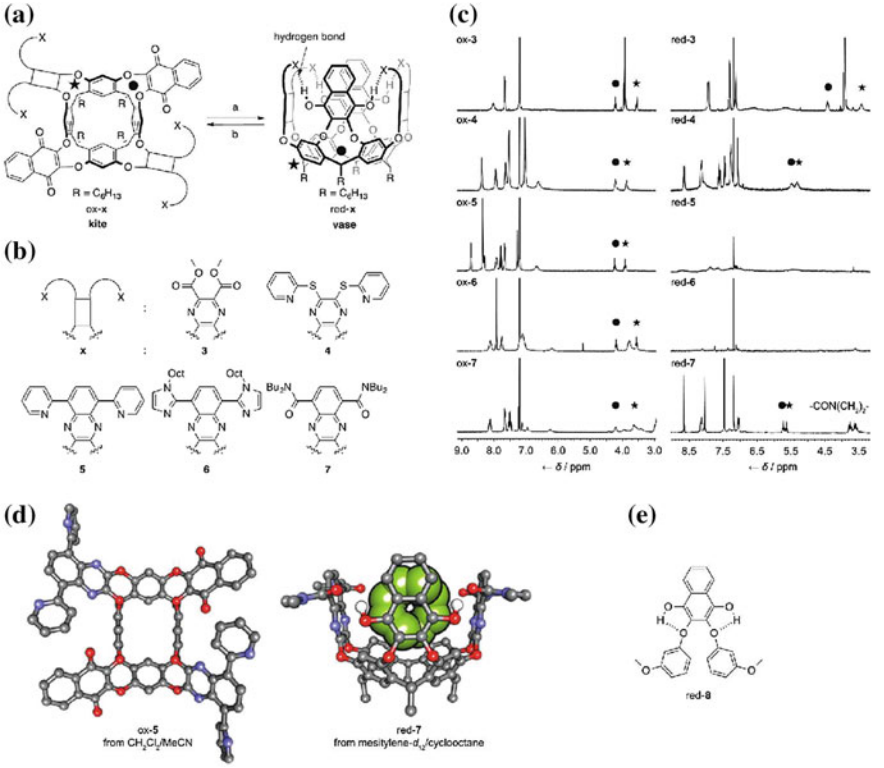


Fig. 2.12 **a** Parent quinoxaline-based cavitand that is switchable by changing temperature, pH, and Zn2+ ion concentration. Cavitands, whose binding properties can be modulated by changes in pH (**b**), by light (**c**), and by metal-ion complexation (**d**). Adapted with permission from Ref. [16]

Kopperger et al. demonstrated the diffusive transport system for molecular cargo, which was tethered to a DNA-based scaffold [19]. With a DNA origami technique, they made a rectangular platform with a molecular tether attached to a flexible hinge preventing diffusive loss of cargo as shown in (Fig. 2.16). AFM images of origami platforms were taken before and after molecule translocation. Authors showed that diffusive motion is much more efficient for easy and quick transfer over long distance.

Vach et al. explored various nanopropellor shapes which can be used in low Reynold number fluid for propulsion in media like biological fluids [20]. Researchers constructed magnetic propellers with a viable optimization strategy, thus getting a higher dimensionless speed than any other previously reported. They studied the correlation between the dimensionless speed and the pseudochirality, and also compared randomly shaped propellers from solution synthesis with previous nanofabricated propellers.

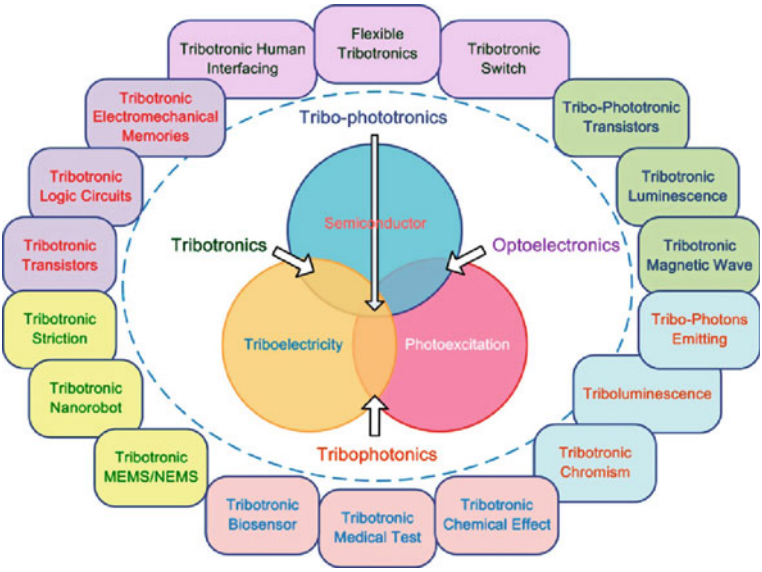


Fig. 2.13 Schematic diagram showing the three-way coupling among triboelectricity, semiconductor, and photoexcitation, which is the basis of tribotronics (triboelectricity semiconductor coupling), tribophotonics (triboelectricity photon excitation coupling), optoelectronics, and tribophototronics (triboelectricity semiconductor photoexcitation). Plenty of potentially important directions and applications are projected and expected to be explored in the near future. Adapted with permission from Ref. [17]

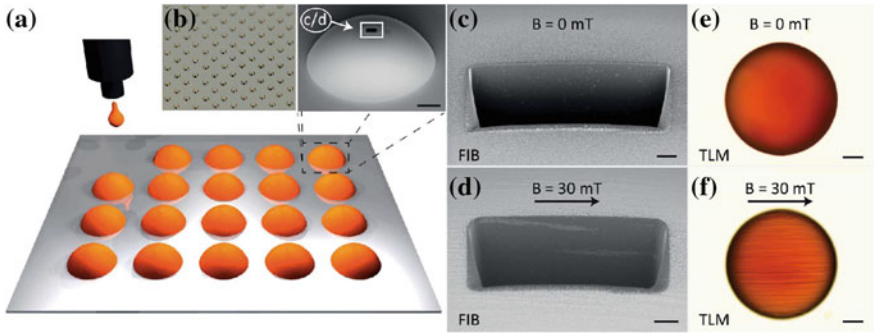


Fig. 2.14 The MPC hemispheres are fabricated by inkjet printing (**a**) (scale bar of the inlet is 50 mm). The photo of an array of MPC hemispheres is shown (**b**). The cross-sectional SEM image of the normal MPC (**c**) and anisotropic MPC (**d**) obtained by an FIB cut (scale bar is 2 mm). In a normal MPC (**c**) the nanoparticles are homogeneously distributed in the polymer matrix. In an MA MPC (**d**) the formed lines are approximately 2 mm apart from each other. The microscope image of the normal hemisphere is shown in (**e**) and the MA hemisphere in (**f**) (scale bar is 50 mm). The nanoparticles cannot be seen normally, but the lines of nanoparticles in the MA hemisphere can be seen easily by an optical microscope. Adapted with permission from Ref. [18]

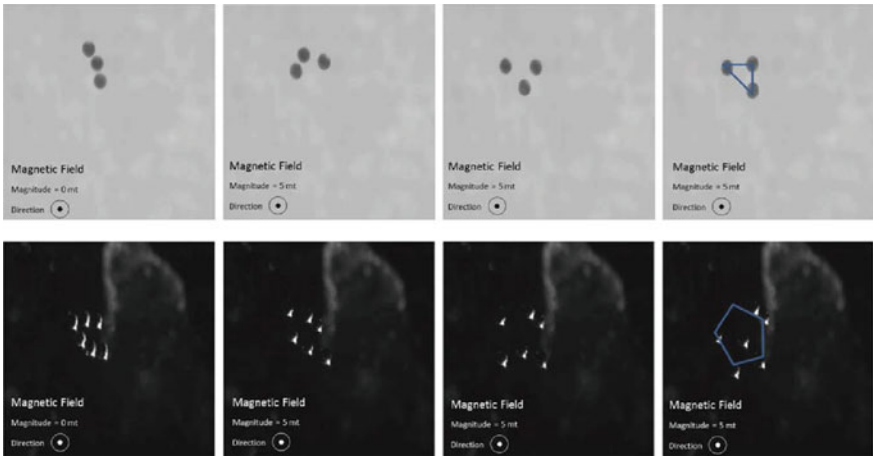


Fig. 2.15 By applying out-of-plane magnetic fields the samples can be magnetized out-of-plane. The dipoles repel each other as they are forced to stay at the interface. A triangle obtained by three samples and a tetragon obtained by five moving parts (4 individual and one couple acting as one). Adapted with permission from Ref. [18]

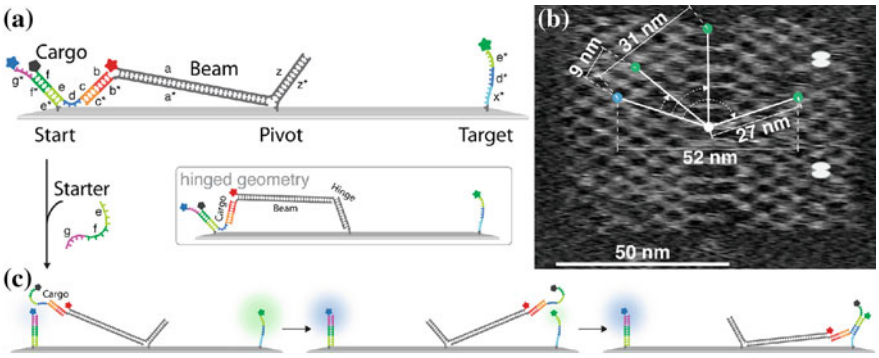


Fig. 2.16 **a** Schematic side view of a DNA origami platform with a rigid tether fixed in the center. The cargo (domains *b*, *c*, *d*, *e*, *f*) can hybridize to sequence domains at the start (*e**, *f**) and a target (*d**, *e**) position, as well as to a single stranded extension (*b**, *c**) of the rigid tether. The inset shows an alternative binding orientation, which results in a more flexible, "hinged" structure. Complementary sequences are indicated by matching colors as well. All sequence domains have a length of 8 nt except for *a*/*a**(88 nt) and *z*/*z**(21 nt). **b** Typical AFM image of the DNA origami rectangle, on which the start position (*blue*) and three different target positions are indicated. The position of two dumbbell hairpin loops (*light gray*) are also shown, which are placed asymmetrically to create height contrast for AFM imaging. **c** Mechanism of cargo transport and fluorescent labeling. The cargo DNA is functionalized with a Black Hole Quencher I molecule. Duplex formation with start or target strand reduces the fluorescence intensity of the Atto 488 dye (*blue*) and Atto 532 dye (*green*), respectively. An additional Atto 655 dye (*red*) used as a marker for gel electrophoresis experiments is permanently attached to the tether and is not affected by quenching. Upon addition of starter strand (domains *g*, *f*, *e*), the beam and cargo are released from the start site, unquenching the *blue* dye. Upon binding to the target site, the *green* dye is quenched. Adapted with permission from Ref. [19]

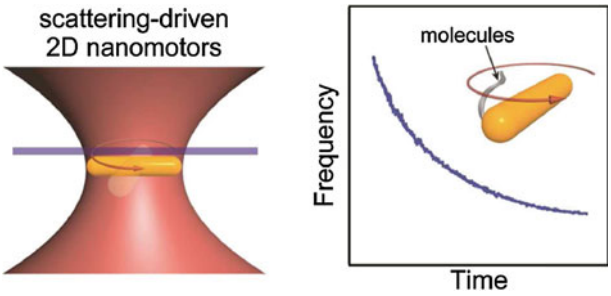


Fig. 2.17 Gold nanorod rotary motors driven by resonant light scattering. Adapted with permission from Ref. [21]

Shao et al. showed that single-crystal gold nanorods can be used as rotary motors in aqueous solutions driven by resonant scattering of polarized laser light [21]. Optical torque is used in this concept (Fig. 2.17). They experimented with rotation of gold nanorods in an optical trap. Against a glass surface, nanorods are trapped in 2D and their surface plasmon resonance property decides the rotational dynamics. CMOS camera was used to monitor rotational dynamics. This motor can be used for viscosity and molecular sensing. The molecular attachment part is highly useful for interaction measurement and conformational change for large bio-macro molecules and polymers.

Xu et al. showed the applications of plasmonically active rotary nanomotors for tunable release of multiplex biochemicals [22]. They showed the active tuning of biochemical releasing rate by rotating nanoparticles. The plasmonic nanomotors were made by assembling nanoparticles. Scanning electron microscopy (SEM) was used to confirm the successful making of the three-layered plasmonic rotors as shown in Fig. 2.18. Raman spectroscopy was used to study the chemistry and releasing rate of each chemicals. Confocal Raman microscope was used at 532 nm excitation with a step size of 250 nm. This confluence of surface enhanced Raman scattering and nanoelectromechanical systems can be easily used for various bio-applications through nanorobotics. They performed both experimentation and analytic modelling and single and demonstrated that multiple analytes can be released at the desirable rates just by controlling the rotation speeds of the nanomotors.

Jabbari et al. reviewed computational approach for complex 3D nanostructures with nucleic acid origami [23]. Through complementary base pairings, these RNA molecules can be designed to form 3D structures. As both, DNA and RNA origami are relatively new research topics in experimental research; there are various challenges and future direction to implement these as biomedical nanorobotic elements.

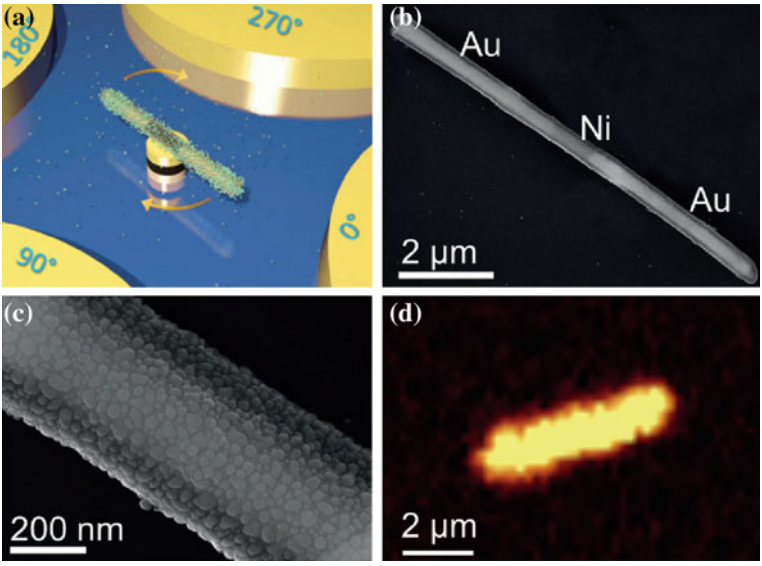


Fig. 2.18 **a** Representation of a rotating nanomotor sensor that releases molecules. **b** Scanning electron microscopy (SEM) images of a plasmonic nanorod for the nanomotor rotor made of a three-layer structure with a Au-Ni-Au nanowire as the core, a silica coating, and a dense layer of Ag nanoparticles on the outer surface of silica. **c** A higher-magnification SEM image shows the dense Ag nanoparticles grown on the silica. **d** Raman mapping of 1 mm R6G dispersed on a plasmonic nanomotor rotor showing essentially uniform SERS intensity on the surface. Adapted with permission from Ref. [22]

Vavassori et al. worked on magnetomechanical nanoactuation which can adopt its shape under remote magnetic external stimulus, and the system works without physical contacts [24]. The 3D magnetic nanostructures were made by focused electron-beam induced deposition (FEBID) (Fig. 2.19) with various nanomedical applications.

Chechetka et al. demonstrated supramolecular nanotransporters which were magnetically and near-infrared light-powered and used for remotely controlling enzymatic reaction [25] (Fig. 2.20). It can be incorporated for precise analysis of biomolecular behaviours in cancer cells at the cellular level. The system was made with magnetic iron nanoparticles, liposomes, and carbon nanohorns, and was highly biocompatible with strong magnetic and photothermal properties. They demonstrated the concept by using a magnet and a NIR laser to release substrates in cells and mouse bodies from the structures at the desired location. This nanorobotic concept has a great potential in cancer treatment.

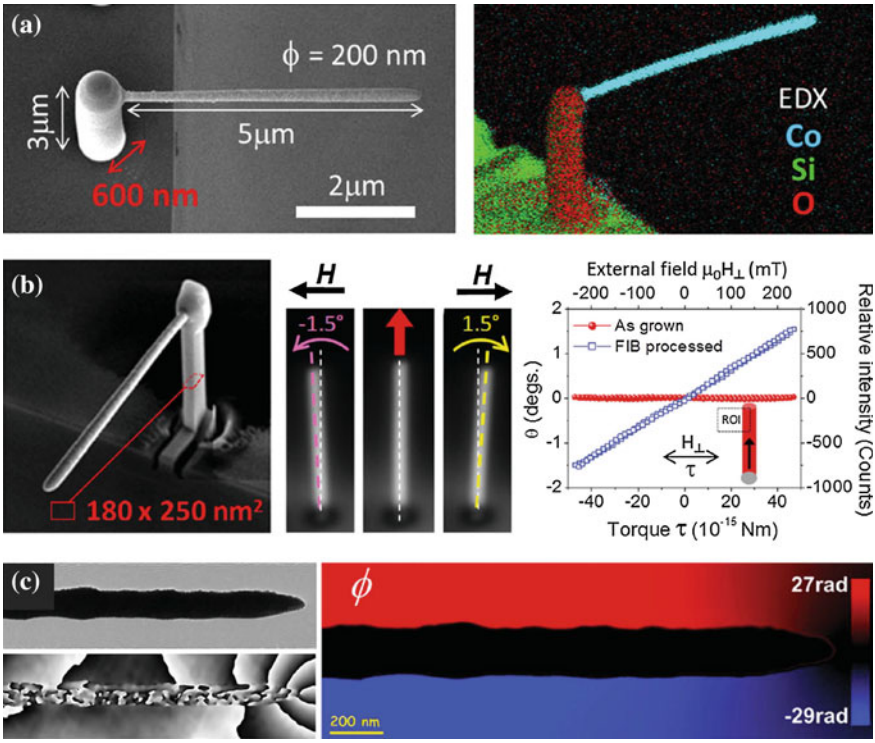


Fig. 2.19 Composite NAMMS cantilever device. **a** SEM image (*left*) and energy-dispersive X-ray analysis (EDX, *right*) showing the elemental mapping of the composite Co/SiO x NAMMS. **b** (*left*) same composite NAMMS device shown in panel (**a**) after having thinned the SiO x pole using FIB milling; (*center*) optical images of the field induced angular swing of the suspended Co nanorod in the FIB thinned composite NAMMS device; (*right*) plots of the field dependence of the angular swing of the original (*red dots*) and FIB thinned (*blue open dots*) composite NAMMS device. **c** Raw hologram (*top-left panel*) and raw phase image reconstructed from the hologram (*bottom-left panel*) of a magnetically saturated Co cantilever; the *right-hand panel* (**c**) displays the unwrapped 2D hologram phase map giving information on the phase difference used for determining the Co saturation magnetization value. Adapted with permission from Ref. [24]

2.5 AFM Based Nanorobotic Systems

Atomic force microscopy has been incorporated as a tool for nanoscale interactions. Li et al. worked with biological samples using a nanorobots system for in situ sensing and manipulations [26] demonstrating that AFM tip, as an end effector, can be used to manipulate objects at nanoscale as shown in Fig. 2.21. In this example, AFM tip was functionalized with specific antibodies, which can identify specific receptors of the cell membrane at high resolution. This method opened the path for studying biological systems at molecular level by conducting in situ imaging, manipulation and sensing at protein and DNA levels.

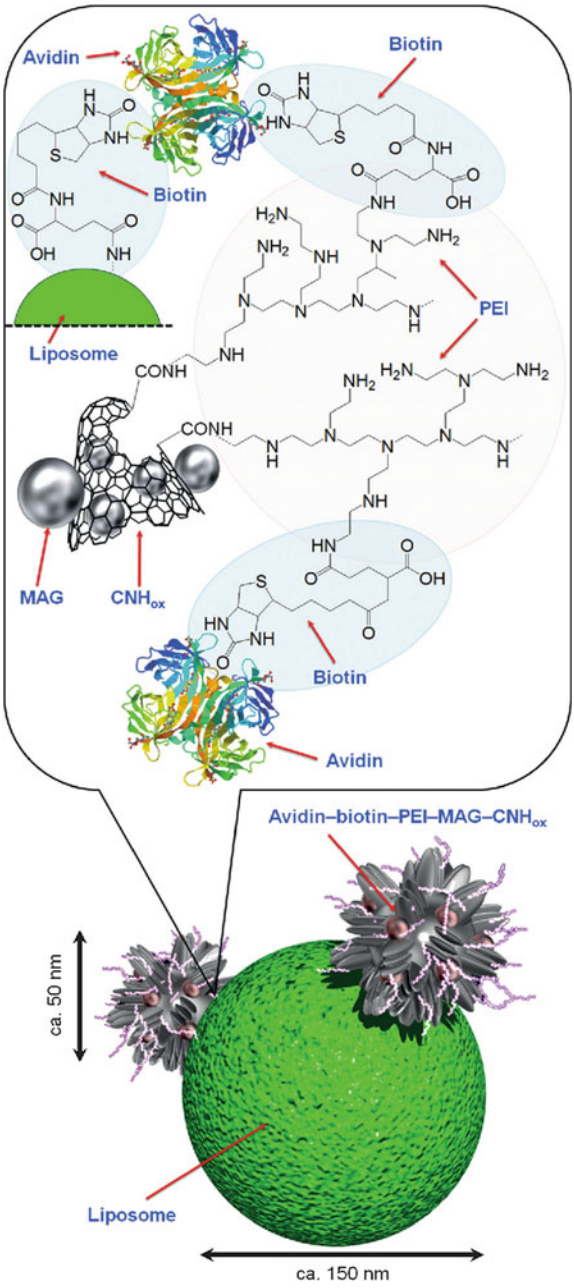


Fig. 2.20 Schematic illustration of the nanotransporters. Adapted with permission from Ref. [25]

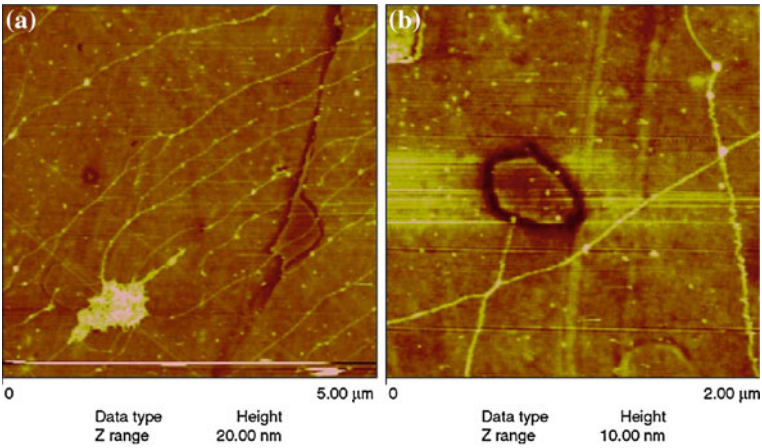


Fig. 2.21 AFM-based nanorobotic system, which includes an AFM system (nanorobot) and the augmented reality environment (visual haptic interface and command generator). Adapted with permission from Ref. [26]

Yang et al. worked with AFM-based nanorobotic manipulator to study biophysical dynamics and ion channel activities in insulinoma β-cells [27]. The nanorobotic platform together with optical observations provided fine dissection of ion channels mechano-property. This provided a novel biomechanical marker with an insight in insulin secretion process for insulin secretion process study.

Similarly, Acosta et al. presented an AFM platform for nanorobotics applications [28]. For manipulation system, protruding tip AFM cantilevers were incorporated in a dual tip system with one as protruding tip and other as quartz oscillator (Fig. 2.22). This settings allows modification of kinematical configurations. The system provided high speed imaging with dynamic force feedback together with efficient nanomanipulation. In comparison to classical AFM, five times faster imaging speed was obtained as the quartz oscillator performs sub-picometer range oscillation.

Chacko et al. developed a biological nanomanipulation system assisted with fast visualization using stimulated emission depletion (STED) superresolution microscope coupled to an AFM [29]. This specific configuration allows single-cell level nanosurgeries and manipulation of cellular structures. Authors carried out a cell manipulation as shown in Fig. 2.23 by testing microtubules labelled with Abberior Star 635P inside the cells of fixed fibroblast. AFM tip was used to probe the cell surface with live monitoring. STED and confocal image were applied for the resolution enhancement.

The summary of main navigation systems and potential nanorobotic agents for medical applications are presented in Tables 2.1 and 2.2, respectively.

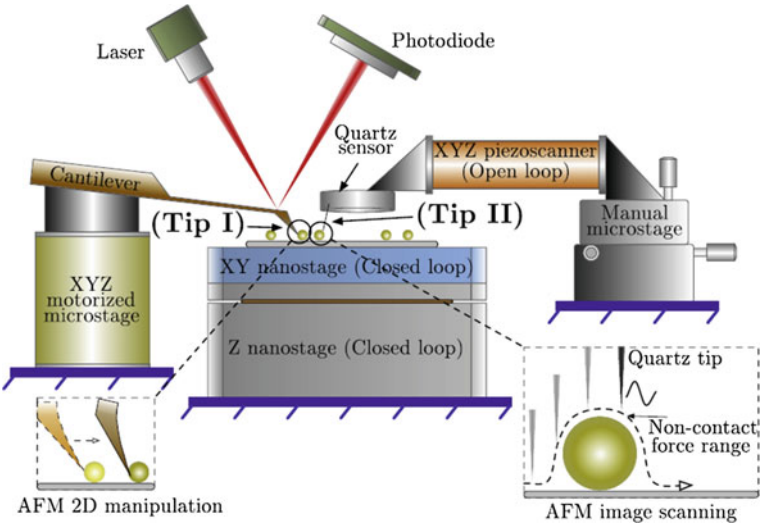


Fig. 2.22 System diagram for the new configuration of the two-tip system. The quartz is integrated into the system for true-non-contact-mode imaging. Adapted with permission from Ref. [28]

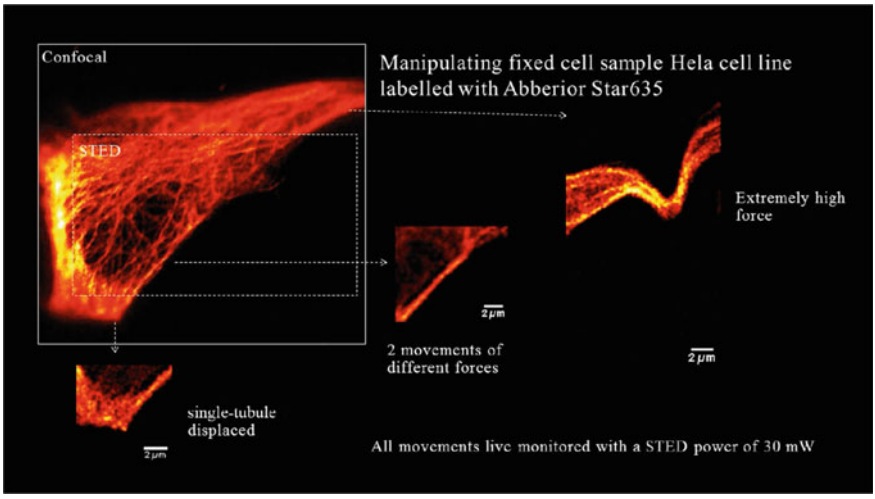


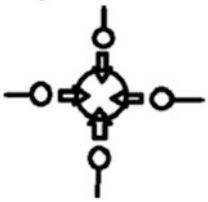
Fig. 2.23 Manipulation performed on a fixed cell: three different movement areas are recorded in this video segment. The confocal and STED images are shown to demonstrate resolution difference. The three different movements shown uses different forces. The still images were done at the lower most part of the cell, and the AFM interaction is recorded at a different height so that movement can be visualized. Adapted with permission from Ref. [29]

Table 2.1 List of main navigation platforms for nanorobotic agents

Technological platforms	Principle	Specifications	Physiological areas
MRN 	Exploits of the magnetic fields inside the tunnel of a clinical MRI scanner to navigate n a no robotic agents, the uniform B_0 field saturates the magnetic agents, while the imaging gradients induce 3D displacement forces on the nanorobotic agents	Highest magnetic field strength (1.5T–3T) to saturate all types of magnetic agents, fast but relatively low directional gradients (0.04T/m), provides real-time tracking of magnetic agents and physiological tissue imaging	Arteries, arterioles
DFN 	Distorts the uniform magnetic field inside the tunnel of a clinical MRI scanner to create high directional gradients	Highest magnetic field strength (1.5T–3T) to saturate all types of magnetic agents, high (0.3T/m preset directional gradients	Arteries, arterioles
FFN 	Mechanically moves the patient typically in the fringe field surrounding a clinical MRI scanner	High magnetic field strengths possible (0.5T–1.5T) to increase the magnetization level of agents slow directional but very high (above 2T/m possible) gradients	Arteries, arterioles with no directional change preferred
EMA 	Electromagnetic directional fields generated by ratios of electrical currents passing in surrounding coils	Very low magnetic field strengths (approximately 0.1T) requiring larger magnetic volume per agent, fast and high (0.3T/m) directional gradients	Arteries and larger physiological areas
MPI-based actuation 	Displacement of the FFP (region of low field strength) by changing the ratios of electrical currents passing in surrounding coils	Relatively low field strength depending on the distance from the surrounding coils, fast and very high (max. 2T/m) directional gradients, provides realtime tracking of magnetic agents	Arteries, arterioles

(continued)

Table 2.1 (continued)

Technological platforms	Principle	Specifications	Physiological areas
Magnetotaxis 	Generation of a 3D zone (known as the aggregation zone) with directional fields generated by surrounding coils capable of directing and constraining the displacement of self-p rope lied magnetically guided (torque-based) agents in such a targeted zone	Typically above approximately 15 gauss outside the aggregation zone and towards zero inside the aggregation zone	Microvascular networks, interstitial and tumoral micro-environments, capillary vessels

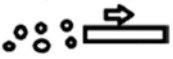
Adapted with permission from Ref. [30]

Table 2.2 Main types of potential medical nanorobotic agents

Medical nanorobotic agents	Description	Compatible platforms and specifications	Physiological areas
TMMCs 	Biodegrade polymeric micro-structures containing magnetic nanoparticles and therapeutic payloads	MRN, DFN. FFN, MPI—can be scaled down to approximately 50 μm in diam. (typ. around 150 μm for hum an interventions such as liver chemoembolization)	Arteries, arterioles
MTB-based agents 	Magnetotactic bacteria cells transporting payloads and being directed to the target site using magnetotaxis and aerotaxis	Magnetotaxis platform—1 to 2 μm in diameter allowing the cells to target tumoral regions, typ. 100–150 body lengths per second in good environmental conditions, MTB-LP carries approximately 70 drug-loaded 170 nm liposomes, chain of MNPs in each cell allows magnetic directional torque to be used, while an oxygen sensor	Microvascular networks, interstitial and tumoral microenvironments, capillary vessels

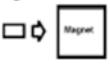
(continued)

Table 2.2 (continued)

Medical nanorobotic agents	Description	Compatible platforms and specifications	Physiological areas
		combined with the microaerophilic behavior of the MC-1 MTB allows the delivery of pay loads in hypoxic regions of tumors	
Magnetic microcarriers 	Special TMMCs capable of transporting typically MTB-based agents towards micro-vascular networks where MTBs are more effective	MRN, DFN, FFN, MPI initially followed by the magnetotaxis platform—Same dimensions as TMMCs with embedded super paramagnetic nanoparticles for the induction of a directional force and MRI-tracking, contains drug-loaded MTB-based agents instead of drug molecules alone to be released at a specific embolization site	All vascular network
Magnetically steerable microorganism-propelled microstructures 	Magnetic microstructure allowing a directional magnetic torque to be induced and being propelled by one or more microorganisms	Magnetotaxis platform—self propelled agent larger than MTB-based agent (typ. tens of micrometers ill diameter)	Typ. too large for capillary networks, interstitial spaces and tumoral micro-environments, no sufficient force for arteries
Catalytic microjets 	Chemically propelled agent capable of being directed through the induction of a magnetic torque	Magnetotaxis platform—present overall lengths in tens of micrometers, self-propelled agents that can be directed using a magnetic torque	Smaller arterioles, too large for capillaries, and not adequate for arteries

(continued)

Table 2.2 (continued)

Medical nanorobotic agents	Description	Compatible platforms and specifications	Physiological areas
Magnetic microrobotic agents 	Capable of high degrees of freedom but much larger than nanorobotic agents	EMA—agents rely on a larger magnetic induction volume in the order of a few tens of micrometers	Arteries and physiological areas allowing larger microrobotic agents
Helical micro- and nano-swimmers 	Biomimetic constructs typically in the form of artificial flagella actuated by a rotating magnetic field that induces a torque for directional displacement	Magnetic platforms capable of generating weak rotational magnetic field—a relatively weak rotating magnetic field is needed to achieve displacements of only a few body lengths per second	Smaller arterioles, not adequate for arteries and tumoral environments

Adapted with permission from Ref. [30]

Acknowledgements The project was supported by Horizon 2020 (SCIENCE), 7th FP (BAMI) and STRETEGMED II (CIRCULATE) grants to WW and TJ.

References

1. Freitas RA Jr (2005) What is nanomedicine? Nanomedicine 1(1):2–9
2. Sierra DP, Weir NA, Jones JF (2005) A review of research in the field of nanorobotics. Sandia National Laboratories, 50 p
3. Ummat A, Dubey AA, Mavroidis C (2006) Bionanorobotics: a field inspired by nature. In: Bar-Cohen Y (ed) Biomimetics: biologically inspired technologies. CRC Press, Boca Raton, pp 201–227
4. Freitas RA Jr (2005) Nanotechnology, nanomedicine and nanosurgery. Int J Surg 3(4): 243–246
5. Cavalcanti A, Shirinzadeh B, Kretly LC (2008) Medical nanorobotics for diabetes control. Nanomedicine 4(2):127–138
6. Martel S et al (2009) MRI-based medical nanorobotic platform for the control of magnetic nanoparticles and flagellated bacteria for target interventions in human capillaries. Int J Rob Res 28(9):1169–1182
7. Martel S et al (2007) Automatic navigation of an untethered device in the artery of a living animal using a conventional clinical magnetic resonance imaging system. Appl Phys Lett 90 (11):114105
8. Latulippe M, Martel S (2015) Dipole field navigation: theory and proof of concept. IEEE Trans Rob 31(6):1353–1363
9. Martel S et al (2009) Flagellated magnetotactic bacteria as controlled MRI-trackable propulsion and steering systems for medical nanorobots operating in the human microvasculature. Int J Rob Res 28(4):571–582

10. Felfoul O, et al (2016) Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions. Nat Nanotechnol
11. Hamdi M (2009) Computational design and multiscale modeling of a nanoactuator using DNA actuation. Nanotechnology 20(48):485501
12. Hogg T, Freitas RA Jr (2010) Chemical power for microscopic robots in capillaries. Nanomedicine 6(2):298–317
13. Hogg T, Freitas RA Jr (2012) Acoustic communication for medical nanorobots. Nano Commun Netw 3(2):83–102
14. Jester SS, Famulok M (2014) Mechanically interlocked DNA nanostructures for functional devices. Acc Chem Res 47(6):1700–1709
15. Abi A et al (2014) Electrochemical switching with 3D DNA tetrahedral nanostructures self-assembled at gold electrodes. ACS Appl Mater Interfaces 6(11):8928–8931
16. Pochorovski I, Diederich F (2014) Development of redox-switchable resorcin[4]arene cavitands. Acc Chem Res 47(7):2096–2105
17. Zhang C et al (2014) Contact electrification field-effect transistor. ACS Nano 8(8):8702–8709
18. Ergeneman O et al (2014) Inkjet printed superparamagnetic polymer composite hemispheres with programmed magnetic anisotropy. Nanoscale 6(18):10495–10499
19. Kopperger E, Pirzer T, Simmel FC (2015) Diffusive transport of molecular cargo tethered to a DNA origami platform. Nano Lett 15(4):2693–2699
20. Vach PJ et al (2015) Fast magnetic micropropellers with random shapes. Nano Lett 15(10):7064–7070
21. Shao L et al (2015) Gold nanorod rotary motors driven by resonant light scattering. ACS Nano 9(12):12542–12551
22. Xu X, Kim K, Fan D (2015) Tunable release of multiplex biochemicals by plasmonically active rotary nanomotors. Angew Chem Int Ed Engl 54(8):2525–2529
23. Jabbari H, Aminpour M, Montemagno C (2015) Computational approaches to nucleic acid origami. ACS Comb Sci 17(10):535–547
24. Vavassori P et al (2016) Remote magnetomechanical nanoactuation. Small 12(8):1013–1023
25. Chechetka SA et al (2016) Magnetically and near-infrared light-powered supramolecular nanotransporters for the remote control of enzymatic reactions. Angew Chem Int Ed Engl 55(22):6476–6481
26. Li G, Xi N, Wang DH (2005) In situ sensing and manipulation of molecules in biological samples using a nanorobotic system. Nanomedicine 1(1):31–40
27. Yang R et al (2013) Cellular biophysical dynamics and ion channel activities detected by AFM-based nanorobotic manipulator in insulinoma beta-cells. Nanomedicine 9(5):636–645
28. Acosta JC et al (2013) Gentle and fast atomic force microscopy with a piezoelectric scanning probe for nanorobotics applications. Nanotechnology 24(6):065502
29. Chacko JV et al (2014) Cellular level nanomanipulation using atomic force microscope aided with superresolution imaging. J Biomed Opt 19(10):105003
30. Martel S (2016) Swimming microorganisms acting as nanorobots versus artificial nanorobotic agents: a perspective view from an historical retrospective on the future of medical nanoro botics in the largest known three-dimensional biomicrofluidic networks. Biomicrofluidics 10(2):021301