Chapter 9
Applications of Nanomaterials in Tissue Engineering and Regenerative Medicine

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9.1 Overview of Nanomaterials: Tissue Manufacturing and Regenerative Medicine

Tissue engineering is a rapidly growing domain which involves and which is used to repair, replace, and/or create artificial cells, tissues and organs by utilizing the amalgamation of biological cells and biomaterials (Saha 2013). To regenerate, damaged or diseased cells, tissues, and organs, regenerative medicine provide remarkable insights by the combination of tissue engineering and life science principles (Maoa and Mooney 2015).

Nanotechnology is playing a promising role in the success of tissue engineering and regenerative medicine. Nanotechnology has several applications involving creation of nanofibers, nanostructured scaffolds, and nanopatterns in tissue engineering and regenerative medicine (Saha 2013).

Moreover, utilization of nanofabrication methods has numerous advantages in tissue engineering (Fig. 9.1). The formation of nanopatterns, nanofibers, as well as controlled release nanoparticles by using nanotechnology introduces many applications in tissue engineering including imitating local tissues as biomaterials to be built is of the size of nanometer, for example, cardiovascular tissue, bone marrow, extracellular liquids, and so on (Chung et al. 2007).

This chapter aims to throw light on the applications of nanomaterials in tissue engineering and regenerative medicine, highlighting the most promising and widely used nanomaterials used for the purpose.

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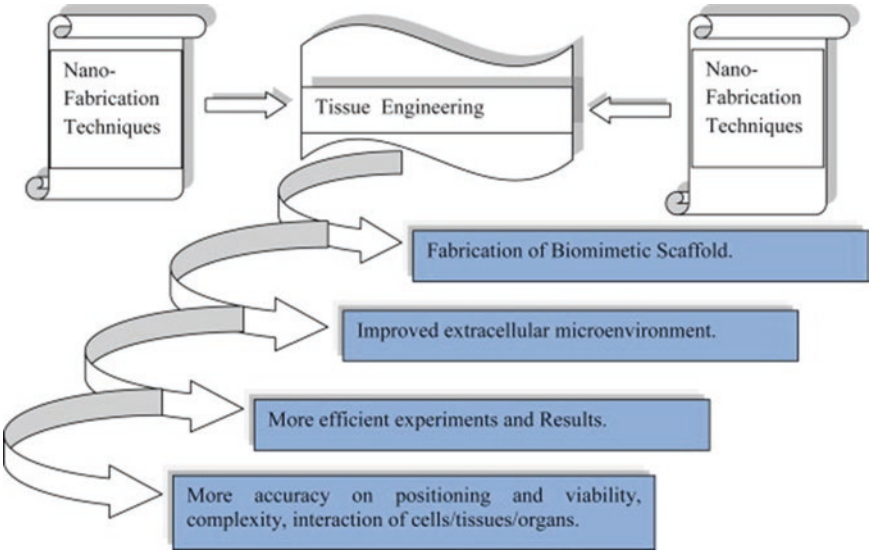

Fig. 9.1 Schematic representation of benefits of using micro- and nanofabrication for tissue engineering (Saha 2013)

9.2 Application of Nanoparticles in Gene Delivery

Gene delivery is a promising technology for the explicit therapy of various gene-related maladies going from hemophilia, cancer, neurodegenerative diseases, hypercholesterolemia, autoimmune disorder to cancer (Choi et al. 2014). To prevent or cure the advancement of the relevant disease, this technique involves the introduction of genes to the destination cells or tissues by the modification of endogenous gene expression (Rapti et al. 2011; Ando et al. 2014).

With the extraordinary advancement of nanotechnology and bioscience, gene therapy depicts a tremendous aptitude in clinical implementation for several severe incurable human diseases (Ibraheem et al. 2014) (Fig. 9.2).

CALLA-01, a focused nanoparticle framework dependent on cyclodextrins, has been produced for the first in-human stage 1 clinical trial (Davis et al. 2010). Another biodegradable and biocompatible polycation is chitosan, which would be filled as a favorable transporter for gene therapy (Lee et al. 2014). One of the widely investigated nanoparticles for gene delivery are lipid-based nanoparticles having greater biocompatibility as well as close similarity with lipidic membranes that encourage their entrance into the cells (Hadinoto et al. 2013; Chitkara et al. 2015). Several other biocompatible nanoparticles pulled in incredible considerations as gene delivery systems, such as low molecular weight polyethylenimine (Dong et al. 2011), poly(β-amino ester)s (Deng et al. 2014), disulfide cross-connected polymers (Tai et al. 2015), polyamidoamine (Xu et al. 2014),and polyphosphoesters (Xu et al. 2015).

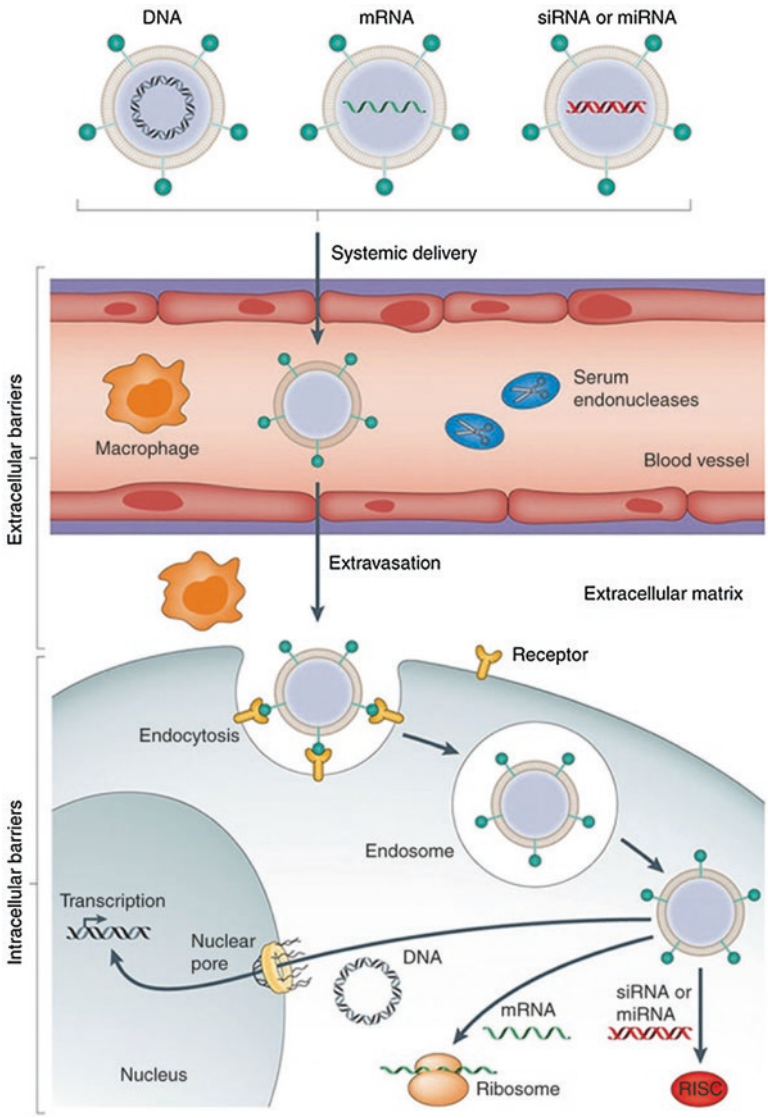

Fig. 9.2 Schematic representation of gene therapy based on nanoparticles (Chen et al. 2016)

9.3 Transfection Agents Due to Nanoparticles

The process of introduction of nucleic acids into the living cells via non-viral means is termed as transfection (Neuhaus et al. 2016). Nowadays, nanoparticles are an effective alternative to insert non-viral DNA to the eukaryotic cells. Nanoparticles help DNA to efficiently link with proteins, ligands and lipids present in the cells by

breaking the endosomal barriers and crossing the membranes in an effective manner (Dzięgiel 2016).

The crossing of membranes and breaking endosomal barriers can be done easily by the use of nanoparticles because despite having smaller size, they provide larger surfaced for adhesion as well as have high stability than other particles (Barkalina et al. 2014). There are many kinds of nanoparticles with different properties and traits and are proved as efficient transfection agents are described below.

9.3.1 Mesoporous Silica

The nanoparticles of mesoporous silica are formulated in the form of structures like that of honeycomb. Different channels are present that enables the encapsulation of molecules and their delivery within the cells. They can also work in combination with micelles, magnetic nanoparticles, as well as polymers for the creation of delivery platforms within cells with high stability and biocompatibility (Slowing et al. 2008). We can also obtain nanoparticles of mesoporous silica with different morphology and pore sizes. These properties influence the procedure of loading the molecules to the pores (Wang et al. 2015).

9.3.2 Polymers

Polyamidoamine (PAMAM), chitosan, poly-L-lactic acid (PLA), poly-L-lactide-coglycolide (PLGA), gelatine, and many other polymers are used as transfection agents in regenerative medicine. They can form diverse shapes of nanoparticles, such as dendrimers (Barkalina et al. 2014). In order to attain the efficient and accurately targeted delivery platforms, they can link with other functional groups by either using their natural shapes or mixed (i.e., natural and synthetic polymers) (Nitta and Numata 2013).

9.3.3 Lipids

Another important type of biomimetic molecules are the lipid nanoparticles. Mono- and bi-layered structures are formed by phospholipid nanoparticles while nanospheres are formed by solid lipid nanoparticles. Polymers or surfactants are used to stabilize the lipid cores of such structures (Barkalina et al. 2014). Negatively charged nucleic acids can bind with cationic lipids by ionic reactions. Nanosphere is hydrophobic from inside which provides better water solubility and encapsulation of substations conveyed by them (Carmona-Ribeiro 2010). Still, nanocarriers made with polymers are more stable than this kind (Wang et al. 2015).

9.3.4 Carbon-Based Nanoparticles

Carbon nanotubes (CNT) and graphene oxide are the successfully used forms of carbon-based nanoparticles as transfection agents. In order to overlook the agglomeration and precipitation, these are used with water solvents as they are not dispersible in water. Functional groups are added along with carbon nanotubes to reduce the cytotoxicity caused by CNTs (Zanin et al. 2014).

9.3.5 Metals

Due to their less reactivity, nanoparticles of noble metals are of great interest as transfection agents (Austin et al. 2014). Gold nanoparticles are also successfully used for gene and drug delivery to the cells as their inner core is inert in nature. This allows molecules to bind with them by either covalent or non-covalent conjugation (Ghosh et al. 2008). Moreover, silver nanoparticles can also be used because of their anti-bacterial properties, but they can also produce toxicity (Austin et al. 2014).

9.4 Cell Patterning Via Nanoparticles

Cell patterning is one of the most significant areas of tissue engineering. Fabrication of artificial tissues as a replacement of damaged tissues can be done by different conventional methods and provide promising results, e.g., microcontact printing (Dike et al. 1999) and lithography (Bhatia et al. 1997). These methods provide precise results but are time consuming and expensive. Therefore, several physical methods are introduced for cell patterning involving inkjet printing (Xu et al. 2006) and cell spraying (Nahmias et al. 2005a, b). These methods also possess several shortcomings, for instance, cells got damaged due to high temperature or pressure in inkjet printing as well as deposition of cells is much slow in laser-guided direct writing (Nahmias et al. 2005a, b).

In order to reduce the drawbacks of the aforementioned methods, novel approaches are designed for cell patterning which involves the use of nanoparticles. Mostly, magnetite nanoparticles such as magnetite cationic liposomes (MCLs) are used along with magnetic force for the fabrication of cell pattens on the unspecialized surfaces and provide high resolution (Ino et al. 2007).

9.5 Elastin-Based Nanoparticles for Targeted Gene Treatment

Several genetic diseases can be cured by the gene therapy that aims to transport a specific gene of interest to inactivate, replace or correct the faulty gene. In viral gene delivery methods, viral vectors are used for the delivery of DNA into the host cells,

usually, viruses, for instance, lentivirus (Escors and Breckpot 2010), adeno-associated viruses (Kotterman and Schaffer 2014), adeno viruses (Wold and Toth 2013), and herpes simplex viruses (Manservigi et al. 2010).

Nowadays, Elastin-like polypeptides (ELPs) are becoming popular as vectors for the delivery of drugs and genes because of genetic encodability and phase transition. These are basically protein-based polymers and used as new gene carriers. The property of self-assembly of nanostructures of ELPs into nanoparticles makes them a good choice as the viral gene delivery vectors (Monfort and Koria 2017).

9.6 Stem Cell Therapy by Nanoparticles

The treatment of several incurable and degenerative diseases has been made possible due to stem cell therapy Hasan et al. (2018). Nanotechnology is playing a promising role in the success of stem cell therapy due to their incredible properties. Several kinds of nanoparticles are used in stem cell therapy, which are described below.

9.6.1 Metal Nanoparticles

Metal nanoparticles have accumulated great interest to be used in stem cell therapy. Nanoparticles of several metals including gold, silver, and some metal oxides can be used in the process. With regard to foundational microorganism treatment, specialists mean to follow transplanted cells stacked with AuNPs. An ongoing report effectively complexed 40 nm AuNPs along with the two ligands, including rhodamine B isothiocyanate (RITC) and poly-L-lysine (PLL), to rise nanoparticle uptake by human mesenchymal stem cells (hMSC). AuNP uptake did not restrain cell differentiation, and marked human mesenchymal stem cells demonstrated solid constriction, or perceivability, during an in vitro miniature CT imaging (Kim et al. 2016).

Silver nanoparticles possess anti-bacterial properties and can give promising results, but they also create neurodegenerative gene expression and inflammatory responses (Huang et al. 2015). Nanoparticles of cerium oxide (CeO), iron oxide (Fe_3O_4), and zinc oxide (ZnO) are used and due to their magnetic properties gave good results in human stem cells. For instance, modified nanoparticles of superparamagnetic iron oxide (SPIO) are used in human neural stem cells (hNSC) without hindering proliferation and viability of the cells (Yuan et al. 2018) and to decrease nitrosative stress and reactive oxygen species, ceramic and zinc oxides are used (Dowding et al. 2014).

9.6.2 Silica Nanoparticles

Silica nanoparticles are inert and transparent in nature, that is why can be linked to the different kinds of fluorescent probes. In Drosophila, silica nanoparticles penetrated to the neurons without causing cytotoxic effects in vivo and made them an exciting target for the treatment of neurodegeneration (Qian et al. 2008).

9.6.3 Polymeric Nanoparticles

Due to their flexible physical properties, rate of degradation in vivo and synthesis techniques, polymeric nanoparticles are of great importance in the stem cell therapy. Nanoparticles of poly lactic acid (PLA), poly aspartic acid, poly D,L-lactic-*co*-glycolic acid (PLGA), poly butylcyanoacrylate (PBCA), and poly glycolic acid (PGA) are commonly used and are promising materials for the treatment of neurodegeneration (Bhatt et al. 2017).

9.7 Nanofabrication Technology in Tissue Engineering

Nanofabrication refers to the formation of artifacts that can be measured in a nanoscale (Harvey and Ghantasala 2006). Nanofabrication can be divided in two basic approaches:

1. Top-down approach
2. Bottom-up approach

9.7.1 Top-Down Approach

The conventional top-down methodology includes cultivating cells into fully measured porous scaffolds to form tissue constructs. This methodology has numerous impediments such like slow vascularization, limitations of diffusion, lower density of cell, and heterogenous distribution of cells (Tiruvannamalai-Annamalai et al. 2014).

9.7.2 Bottom-Up Approach

The bottom-up or modular approach involves the engineering of complex tissues and organs from the microscale modules. This approach eliminates all the shortcomings of the conventional approach (Tiruvannamalai-Annamalai et al. 2014) (Fig. 9.3).

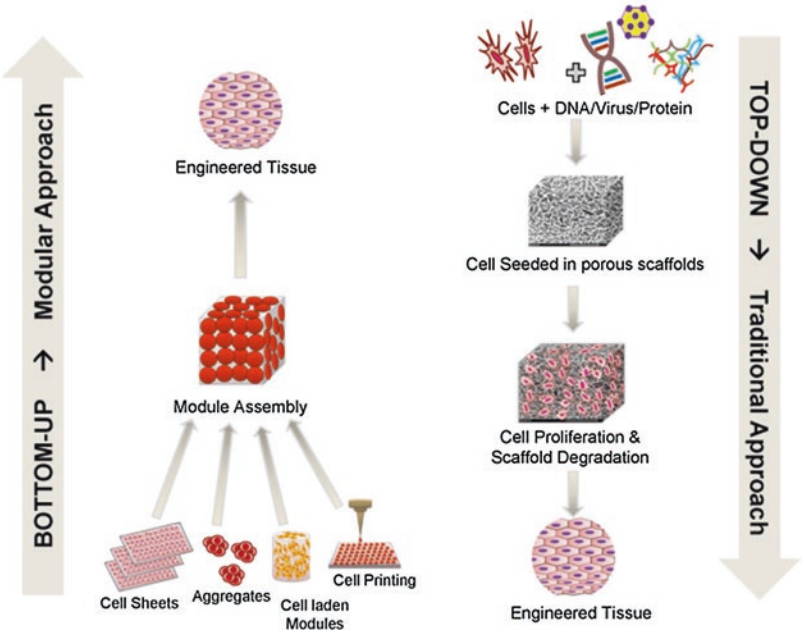

Fig. 9.3 Comparison of top-down and bottom-up approaches for tissue engineering (Tiruvannamalai-Annamalai et al. 2014)

9.8 Nanofibrous Scaffolds in Tissue Engineering

Nanofibrous scaffolds are basically extracellular matrices that are artificially designed to deliver natural environment for the formation of tissues. Due to their greater surface to volume ratio, nanofibrous scaffolds efficiently promote cell proliferation, differentiation, and adhesion (Gupta et al. 2014).

Nanofibrous scaffolds have architectural features like that of extracellular matrix which has a complex 3D network for proliferation, growth, and differentiation of cell and consists of nanofiber-based cellular matrix. Nanofibrous scaffolds containing nanofibers have greater similarity with several extracellular matrix molecules, for instance, matrix proteins involving fibronectin, laminin, and collagen (5–500 nm in size) as well as proteoglycans including hyaluronic acid (450–1000 nm in size) (Lelkesa 2005).

9.9 Scaffolds of Nanostructured Materials for Replacing Damaged Organs

The demand of scaffolds designed from nanostructured materials is increasing tremendously because of their ability to mimic native tissues by configuring their geometry and optimizing biomaterials. Nowadays, the request for replacement of

organs and regeneration of tissues is surging because of growing number of cases associated with tissue damage and organ failure as there is a scarcity of organs for the transplantation (Gupta et al. 2014).

9.9.1 Neural Tissue Generation

The key standard of neural tissue designing is to give a positive situation involving biomimetic scaffolds and cells in vitro, and further to stimulate the capability of body to practically recuperate beforehand irrevocable tissues instead of straightforwardly to embed the artificial tissues (Place et al. 2009). Nerve regeneration approaches involve the use of natural polymers (chitin, chitosan, alginate gelatin, collagen), synthetic biodegradable polymers (PLGA, poly L-lactic acid, poly ε-caprolactone), conducting polymers (polyaniline, polypyrrole) and synthetic no-degradable polymers (silicone). An ideal nerve channel must be flexible, biocompatible, thin, compliant, neuro-conductive, biodegradable, porous, and neuro-inductive (Verreck et al. 2005). Even though the above-mentioned biomaterials fulfill most of the aforementioned criteria, still they possess few drawbacks that have to solved in order to meet neuro regeneration applications (Haile et al. 2007).

To overcome those drawbacks and improve the properties of nerve scaffolds, researchers have incorporated the use of different techniques including electrospinning, polymer blending, and introducing nerve growth factors in the scaffolds (Sua 2005). Table 9.1 depicts the summary of techniques and biomaterials used to enhance nerve regeneration.

9.9.2 Cardiovascular Attempts

The rate of cardiovascular diseases (such as heart failure and myocardial infarction) is increasing day by day. The only solution to these diseases was heart transplantation which was not possible for every patient due to the scarcity of donors. Cardiovascular tissue engineering has revolutionized this concept by introducing injectable gels, artificial implantable blood vessels, cardiac patches, etc. created from biodegradable polymers (Curtis and Russell 2010; Kai et al. 2011). Biodegradable polymers are divided in to two principal classes, i.e., synthetic and natural polymers.

9.9.2.1 Natural Biodegradable Polymers

Natural polymers are referred to as polymers obtained from nature (Vroman and Tighzert 2009). Natural polymers involve fibrin, collagen, gelatin, alginate, Matrigel, and chitosan. Natural biodegradable polymers possess several merits

Table 9.1 Summary of techniques and biomaterials to enhance nerve regeneration (Subramanian et al. 2009)

Biomaterials	Technique of fabrication	Enhanced properties	References
Star-poly(ethylene glycol)	Integration of polysaccharide (e.g., heparin)	Adjustable mechanical and physical properties for the adoption of definite tissue requirements	Freudenberg et al. (2009)
Chitosan	Altered with (γ-glycidoxypropyltri methoxysilane)	Mechanical strength	Amado et al. (2008)
Poly(β-hydroxybutarate)	Sheets infused with the molecules of extracellular matrix	Proliferation and adhesion of cell	Novikova et al. (2008)
Poly(ε-caprolactone)	Thermal fiber bonding and electrospinning	Mechanical strength	Lee et al. (2008)
Poly(ε-caprolactone)	Alignment of fibers by electrospinning	Contact control	Chew et al. (2008)
Poly(sialic acid)	Modification of hydrogel by adsorbed poly-L-ornithine, poly-L-lysine, collagen, or laminin	Mechanical compatibility and cell adhesion	Haile et al. (2008)
Poly(lactic-*co*-glycolic-acid)	Improved immersion precipitation method	Hydrophilicity and selective permeability	Oh et al. (2008)
Poly(D,L-lactide-*co*-ε-caprolactone)	PPy nanoparticle composite and PPy coating substrate	Electrical signal for the assembly of cell functions	Zhang et al. (2007)
Chitosan	Thermo-responsive chitosan-based hydrogel functionalized with polylysine	Surface characteristic (charge density, wettability), mechanical compatibility, injectable scaffold	Crompton et al. (2007)
Poly(ε-caprolactone)	Electrospinning (polymer blending using collagen)	Biological characteristic (adhesion of Schwann cell, differentiation, and migration)	Schnell et al. (2007)
Collagen	Crosslinked hydrogel using YIGSR peptide improved dendrimers	Biological property (enhance the corneal epithelial cell growth as well as outgrowth of neurite)	Duan et al. (2007)
Poly(glycerol-sebacate)	Replica molding	Flexibility, robust contact, control response, micropatterned reactants, surface degradable	Bettinger et al. (2006)
Poly(lactic-*co*-glycolic acid)	Microbraiding process	Porosity, flexibility	Burdick et al. (2006)
Poly(D,L-lactide-*co*-glycolide)	Low pressure injection molding	Lengthwise aligned channels, porosity, imitates the geometry of innate nerves	Bini et al. (2004)
Poly(2-hydroxyethyl methacrylate)	Fiber templating process	Physical properties like soft tissues, oriented scaffold	Sundback et al. (2003)
Poly(2-hydroxyethyl methacrylate)	Liquid-liquid centrifugal casting	Mechanical features like spinal cord	Flynn et al. (2003)

involving ample accessibility, biodegradability, as well as renewability, whereas its demerits include inadequate electrical conductivity, fast degradation, weak mechanical properties, and immunological reaction (Dhandayuthapani 2011).

9.9.2.2 Synthetic Biodegradable Polymers

Synthetic polymers refer to the man-made polymers (Toong et al. 2020). Synthetic polymers include poly(glycolic acid), poly(lactic acid), poly(lactic-*co*-glycolic acid), polycaprolactone, polyurethanes, and poly(ethylene glycol). Synthetic biodegradable polymers also possess some merits involving controlled structure, stable mechanical properties, flexibility, as well as no immunological concerns, whereas some of its demerits are low biocompatibility and absence of cell attachment (BaoLin and Ma 2014).

In order to improve the drawbacks of both natural and synthetic polymers, researchers have made novel natural/synthetic composites (combination of both natural and synthetic polymers). In this manner, properties of composites have improved to the greater extent. These include PLA/chitosan, TiO_2-PEG/chitosan, and gelatin/PCL/graphene. Natural/synthetic composites possess high biocompatibility, strong mechanical strength, increased electrical conductivity, and improved biological properties (Zhuab 2018; Chen et al. 2019).

9.10 Conclusion, Outlook, and Future Aspects

Nanotechnology is the promising tool for the advancement of tissue engineering and regenerative medicine. The amalgamation of nanotechnology along with tissue engineering and regenerative medicine has introduced new insights for the regeneration and repair of damaged or diseased cells, tissues, and organs. In future, it can be predicted that nanotechnology would be helpful in the formation of complex artificial organs such as heart.

Conflict of Interest There are no conflicts of interest.
Ethical Issues There are no concerned ethical issues.

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