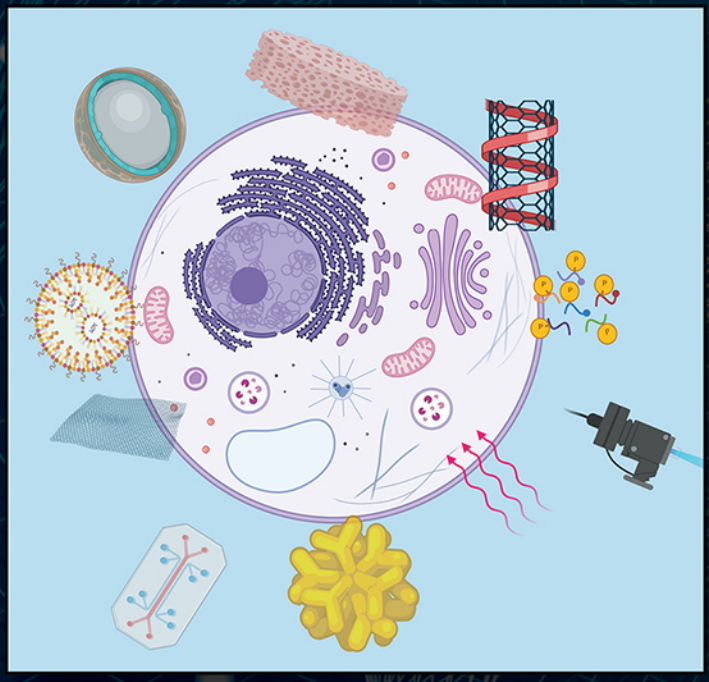
NANOPHARMACEUTICALS IN REGENERATIVE MEDICINE



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Nanopharmaceuticals in Regenerative Medicine

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Edited by
Harishkumar Madhyastha and
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Contents

Foreword

Nanopharmaceutics is a branch of nanobiotechnology with vast applications in diagnostics, regenerative medicine, and drug development in current science of medicine. Within a short span of two decades, the subject has expanded into a promising arena for clinical and translational medicine. The biomedical scientists show immense interest in nanomaterials due to their extraordinary surface to volume area, tunable optical emission, unique electrical, and magnetic behaviour, which particularly helps in drug discovery research. The hybridisation of nanotechnology and tissue regeneration will open a new path of innovation and will have potential application to treat incurable diseases. The book '*Nanopharmaceuticals in regenerative medicine'* is an informative compilation of nanomedicine, combining description of pharmaceutical formulations and their mechanisms of action. The book provides the comprehensive bundle of information and accurate scientific information on nanopharmaceutical use in regenerative medicine and would be epochal to the scientific community, especially clinicians and pharmacists.

I applaud the editors, Dr. Harishkumar Madhyastha who has been my colleague for many years at University of Miyazaki and Smt. Durgesh Nandini Chauhan for the excellent compilation of chapters contributed by well-known scientists and academicians from different countries. All 18 chapters are different from each other in content, but share a single objective of nanopharmaceutical advancement. The most notable chapters include therapeutic applications, technological innovations, and tissue regeneration. The authors successfully navigate the chapter contents with updated literature. I believe '*Nanopharmaceuticals in regenerative medicine'* will remain a valuable resource for years to come.

Prof. Dr. Tsuyomu Ikenoue, MD. Ph.D.
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Preface

Trajectory of scientific thoughts is propelling rapidly through good research communications. Research ideas will be broadcasted through good publications which are mainly dispersed by review manuscripts, book chapters, etc. A comprehensive scientific dissertation serves as a satellite stop reference book for budding academicians, scientists, professionals, and technologists. With extensive and annotated knowledge and information, the book is a gateway for knowledge dispersion and escalation, community curation, and finally betterment of society. With the advancement of scientific knowledge, a new paradigm of science, nanobiotechnology, is emerging in the area of biomedical science and regenerative medicine. In regenerative medicine arena, nanotechnologies have wide and high-impact benefits like drug development, diagnostics, and delivery system. This book provides an in-depth knowledge on applied nanobiomedical contents for university graduates, researchers, and technocrats with striking balance between fundamentals and applications for regenerative medicine. The book contains 18 chapters covering a wide range of topics related to chemistry, pharmacy, and material science. The chapters are broadly classified into three categories; potential insights into smart technologies, interpretations of different modes as delivery systems, and tissue engineering and generation aspects. Each chapter includes multidisciplinary approaches and recommendations to use the nanotechnologies for tissue regeneration with meaningful conclusions and attracts new ideas for future development. Chapters 1–5 emphasize the applications of nanoparticles in regenerative therapy. Chapters 6–12 focus on different technological approaches devoted to tissue recalcitrant engineering. Chapters 13–18 elucidate the updates on nanomaterials in the field of tissue regeneration, with special focus on osteoporosis, cancer, and cardiology with a pharmaceutical angle.

Date: 16 April 2021

Dr. Harishkumar Madhyastha

Durgesh Nandini Chauhan

Editors

Dr. Harishkumar Madhyastha Ph.D., FBRSI. Harishkumar Madhyastha, faculty at Department of Applied Physiology University of Miyazaki, Miyazaki, Japan. With two Ph.D. degrees, he ignited his research career as a scientist in *Spirulina* biotechnology at MCRC-Chennai. Later on, he pursued postdoctoral research at Miyazaki University that culminated in a faculty position in the Department of Applied Physiology at the University of Miyazaki from 2006. His current research interests include generation and delivery of nanosized metallic payloads for regenerative diseases application. His academic credentials are credited with more than 80 *Sci-E* indexed papers; *h*-value of *29*, clarivate analytic cumulative impact factor of *204.5* and RG score of *33.76* with *six* international patents. His research has been presented in conferences more than 100 and has been frequently picked up by national and international media. He is also actively involved in many international projects including ongoing Indo-Japan scientific and academic collaborations. He is Fellow of Biotechnology Research Society of India (FBRSI), Fellow of Royal Biological Society-London (FRBS-UK). He is an officially recognised Indo-Japan academic spokesperson of University of Miyazaki and engaged in outreach programs to further strengthen the cohesive relationship between Indian academicians and University of Miyazaki-Japan.

Mrs. Durgesh Nandini Chauhan, M.Pharma, has completed her B.Pharm degree in Pharmacy from the Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, India and her M.Pharma in pharmaceutics from Uttar Pradesh Technical University, currently known as Dr. A.P.J. Abdul Kalam Technical University, Lucknow in 2006. She is presently working as Assistant Professor in Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India. Mrs. Durgesh Nandini Chauhan has 14 years of academic (teaching) experience from Institutes of India in pharmaceutical sciences. She taught subjects as pharmaceutics, pharmacognosy, traditional concepts of medicinal plants, drug-delivery phytochemistry, cosmetic technology, pharmaceutical engineering, pharmaceutical packaging, quality assurance, dosage form designing and anatomy, and physiology.

She is member of Association of Pharmaceutical Teachers of India (APTI), SILAE: Società Italo-Latinoamericana di Etnomedicina (The Scientific Network on Ethnomedicine, Italy), and so forth. She has written more than 10 publications in national and international journals, 16 book chapters, and has edited 7 books. She is also active as a reviewer for several international scientific journals and active participant in national and international conferences.

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1

An Insight into Advanced Nanoparticles as Multifunctional Biomimetic Systems in Tissue Engineering

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CONTENTS

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Introduction

Tissue engineering (TE) aims to restore living tissues that have been lost or damaged. It was first introduced in the late 20th century. TE holds the potential of engineering damaged tissues and organs with recent technological advancements in stem cell therapy, bioengineering, and nanotechnology (Kim et al., 2013). Since 1997, when Cao et al. displayed the auricular implant grown on the back of a mouse and galvanised the province of biomedical research, the whole-organ engineering has been a proof-of-concept and expanding area of interest in regenerative medicine (Cao et al., 1997). Due to increasing incidences of diabetes, obesity, ageing population, and growing trauma cases, tissue engineering markets have huge potential. In a report, the global tissue engineering market is estimated to grow to approximately 110 billion dollars in the coming years (Tian et al., 2007). Regulatory authorities have recently approved the use of tissue-engineered skin to treat burn wounds and skin ulcers.

The first tissue-engineered skin products that were developed for the treatment of burns include Integra (Integra Lifesciences Corporation, USA), Epicel (Genzyme Biosurgery, USA), and TransCyte (Smith and Nephew, UK). Several products are available for chronic skin ulcer management, such as Apligraf (Organogenesis, USA), Dermagraft (Smith and Nephew, UK and Advanced Tissue Sciences, USA), EpiDex (Euroderm, Germany), Epibase (Laboratories Genévrier, France), and BioSeed-S (BioTissue Technologies, Germany). The tissue-engineered products are also commercially available for cosmetic surgery applications such as BioSeed-M and MelanoSeed (BioTissue Technologies AG, Germany) (Law et al., 2017). Several stem cell engineered products are currently in development and are being tested in clinical and preclinical trials (Wang et al., 2017). Nanoparticles (NP) are solid colloidal particles, and their size range is 10–200 nm. Their small size provides a high surface to volume ratio, one of their most attractive intrinsic properties (Acharya and Sahoo, 2011). Due to their size and surface properties, NPs can be exploited as theranostic agents, drug delivery systems, genetic material, and growth factors. NPs used in biomedicine must be biocompatible, and the stability of nanocarriers in biological media is crucial when formulating nanomedicines (Figure 1.1).

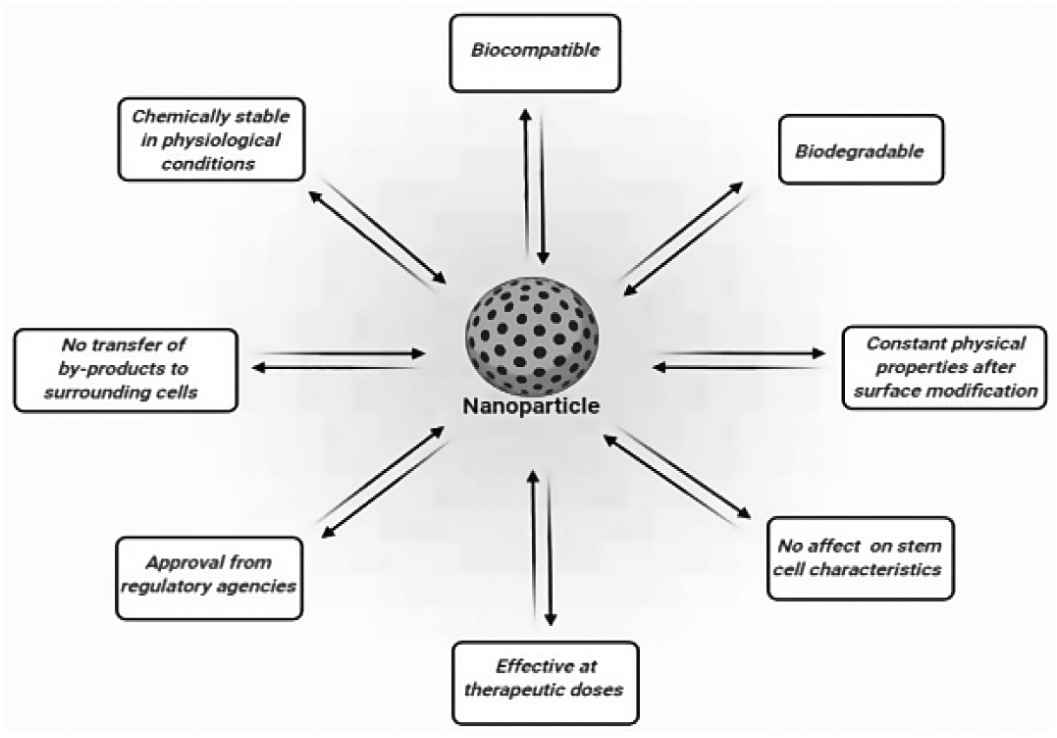

FIGURE 1.1 Desired characteristics of NPs in biomedicine.

The future biomedical applications of NPs are in developing of scaffolds for TE, as carriers of bioactive compounds, cells, and wound dressings. Nanotechnology is being applied in TE to improve the mechanical and biological performances of tissue constructs. The use of nanoparticles is highly advantageous in TE due to their smaller size and large surface area, similar to peptides and small protein molecules. They can smoothly diffuse across cell membranes and facilitate cellular uptake (Green et al., 2008). Furthermore, their sizes and surface characteristics can be easily modified. NPs naturally mimic the nanometre size scale of tissue extracellular matrix (ECM) (Wang et al., 2015). In this chapter, the role of nanoparticles in tissue engineering and regenerative medicine has been discussed.

Over the years, metallic and polymeric NPs have been used as diagnostic and therapeutic agents. The evolution of new ceramic materials for biomedical applications has also grown hastily. Nanoscale ceramics mainly silica (SiO_2) and titanium oxide (TiO_2) are being studied for their significance in regenerative medicine and improving their physical-chemical properties to reduce their cytotoxicity in biological systems.

Metallic Nanoparticles

Iron Oxide Nanoparticles

Iron is a naturally occurring metal in the human body, and the body has evolved to metabolise these particles. Therefore, iron oxide NPs are biocompatible. Due to their superparamagnetic nature resulting in a lack of interparticle attraction, NPs have a minimum risk of accumulation (Chaudhury et al., 2014). In general, magnetite and maghemite crystals with particle sizes 20–150 nm exhibit superparamagnetic order known as superparamagnetic iron oxide NPs (SPIONs). Their physicochemical properties are generally studied using Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy-dispersive X-ray analysis, X-ray diffraction, zeta potential, nanoparticle tracking analysis, and dynamic light scattering (Navaei et al., 2016). Due to their size, magnetic properties, and biocompatibility, iron oxide NPs (SPION) have transpired as exceptional contrast agents in magnetic resonance imaging (MRI). Moreover, the MRI signal intensity can be significantly controlled without altering its in vivo stability. Due to their ideal characteristics, SPOINs have been approved as possible substitutes for Gadolinium (III) contrast agents, among the most widely used contrast agents in MRI (Charlton et al., 2016).

The SPION-based MRI imaging probes are accepted as T2-weighted contrast agents. Such contrast agents are applied to label, track, and activate stem cells and other cell types. It is a promising non-invasive imaging technique that provides the opportunity to monitor cell status after transplantation and accelerate progress in regenerative medicine (Cromer Berman et al., 2013) (Figure 1.2).

Two such MRI agents are ferumoxides (Feridex/Endorem) of 120–180 nm particle size and ferucarbotran (Resovist) of about 60 nm particle size. These are clinically approved for liver MRI. Resovist can be administered as a bolus, while Feridex must be administered as a slow infusion and is only used in delayed phase imaging. The iron nanoparticles are opsonised and taken up by phagocytic Kupffer cells of normal RES. In Kupffer cells, following the phagocytosis, the NPs exert significant T2/T2* relaxation effects. The hepatocytes negatively improve T2 or T2*-weighted images leading to enhanced exposure of pathological lesions that hampers the spotting of reticuloendothelial cells. The degree of SPIO uptake and the consecutive level of signal intensity drop is used to identify and characterise lesions (Wang et al., 2015). In a multicentric clinical study, Feridex-enhanced T2-weighted images were reported to reveal more lesions, which were not observed in unenhanced images in 27% of cases. This additional information can change therapy in more than 50% of cases (Sahle et al., 2019).

Gold Nanoparticles

Gold nanoparticles (AuNPs) are colloids consisting of nanometre-sized particles of gold. Due to their strong affinity to gold, conjugation of different ligands such as polypeptides, antibodies, and proteins containing moieties such as amines, phosphines, and thiols is possible (González-Béjar et al., 2016). In biomedicine, colloidal AuNPs are potential candidates due to their non-reactive and non-immunogenic

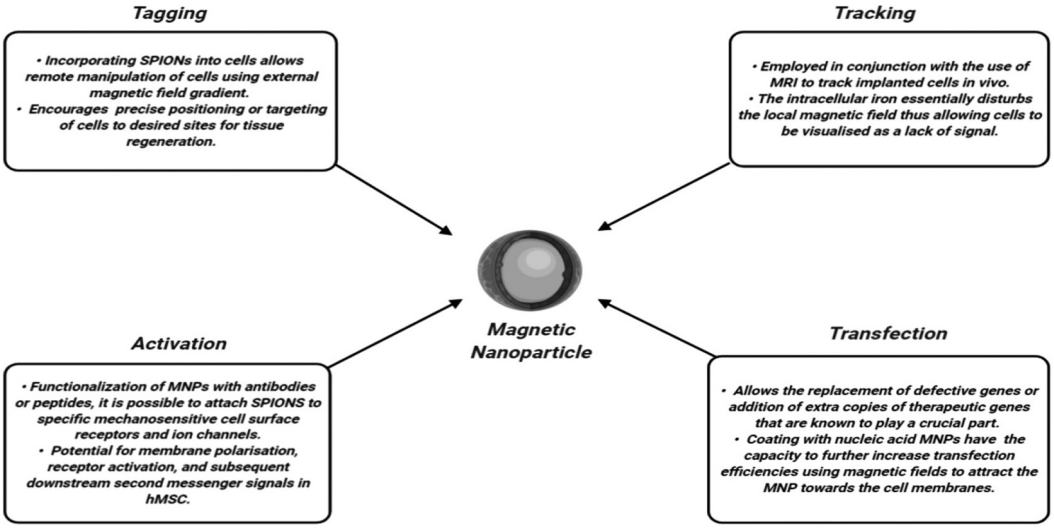

FIGURE 1.2 Applications of magnetic nanoparticles in the field of regenerative medicine.

attributes, biocompatibility, and biodistribution that can be prepared and altered easily. AuNPs can be effectively coupled with other biomolecules without changing their biological function. The gold nanoparticles are promising drug delivery candidates when formulated using compatible tissue reagents (Vacanti, 2006). PEG-functionalised AuNPs, prepared by chemically linking PEG to AuNPs, have been reported to enhance post-SCI recovery by reducing acute phase damage without harmful reactions such as body weight loss and health problems (Paul and Sharma, 2006).

Owing to desired properties, including less expense, non-toxicity, osteogenic differentiation induction, AuNPs are potential candidates as osteogenic agents. Heo et al., 2014 have developed a hybrid hydrogel made of gelatin and AuNPs (Gel-AuNP) as a regeneration strategy for bone tissue repair (Heo et al., 2014). The in vitro and in vivo evaluation of the Gel–AuNP composite hydrogel was effective in bone tissue engineering. The cellular experiments showed that the Gel–AuNP system promoted proliferation, viability, osteogenic differentiation, and significantly high-alkaline phosphate activities in human adipose-derived stem cells, dose dependently. AuNPs were also reported to promote the osteogenic transcriptional profile of mesenchymal stem cells (MSCs) to facilitate the differentiation of MSCs into functional osteoblast cells (Yu et al., 2014). Over the years, AuNPs have emerged as potential X-ray contrast agents due to their biocompatibility, ability to target tumours, high X-ray absorption coefficient and their competence to replace iodine in CT imaging. Since gold is a metal with a high atomic number, it offers better X-ray diminution and is considered ideal for CT imaging (Ahn et al., 2013). Due to their longer circulation time and better contrast, AuNPs are examined as substitutes to gadolinium, barium, or iodine-based agents (Markides et al., 2012).

Silver Nanoparticles

Applications of silver nanoparticles (AgNPs) have been explored in regenerative medicine and widely for wound dressings. Silver ions were found to disrupt the integrity of the bacterial cell by binding to various bacterial enzymes and proteins. Silver can alter critical functions in the bacterial cell, including permeability, enzymatic activity, cellular oxidation and respiratory processes, causing bacterial death (De la Riva et al., 2010). Many studies on the use of AgNPs in tissue engineering scaffolds reported lower infection rates with high biocompatibility. AgNPs have been tested on scaffolds made of diverse materials, including cellulose acetate, polyvinyl alcohol, chitosan-alginate, gelatin and PCL, resulting in

strong antimicrobial activity (Ferreira, 2009). A study reported the cytotoxic and osteogenic differentiation effects of AgNPs and $AgNO_3$ in urine-derived stem cells (USCs). The AgNPs were found to induce actin polymerisation, improve cytoskeletal tension, and activate RhoA, which facilitated the osteogenic differentiation of USCs. AgNPs are sufficient for integration into tissue-engineered coatings that use urine-derived stem cells as seed cells (Rahmani Del Bakhshayesh et al., 2018).

Ceramic Nanoparticles

Titanium Oxide Nanoparticles

Research on the possible applications of titanium oxide nanoparticles biomedicine was first carried out owing to its potential role as photosensitising agents that could be utilised in cancer treatment and photodynamic inactivation of antibiotic-resistant bacteria (Sundaramurthi et al., 2014). Owing to its inert nature, TiO_2 scaffolds are being studied for several biomedical applications, including the development of implants in bone tissue engineering. The addition of TiO_2 to the chitosan scaffold was aimed for bone regeneration and was reported to decrease the scaffold's fast degradation. The connection between chitosan and nano-TiO_2 made it extremely porous, making it an effective replacement for bone tissue engineering (Kutsuzawa et al., 2006).

Silica Nanoparticles

Silica nanoparticles (SNPs) received great attention owing to their extensive biomedical applications and their exceptional intrinsic properties such as large surface area, biocompatibility and biodegradability. Due to their extraordinary biocompatible properties, SNPs may be used as ligands to monitor stem cell therapy. Their ability to integrate multiple contrast agents into a single factor can provide clinicians with multiple monitoring controls (Qin et al., 2014). The application of MSNs in tissue engineering allows the development of advanced technologies that enable guidance and tracking of stem cell transplantation to achieve a controlled and targeted stem cell delivery (Jiang et al., 2010). In one of the previous works, 89Zr4+ was immobilised by covalent attachment with p-isothiocyanatobenzyldesferrioxamine (DFO-NCS), a complexing agent to make large-pore MSNs. Quantitative 89Zr4+ labelling was achieved within just a short time due to the high concentration of DFO material on MSNs, and the high signal strength of the nanoparticles was demonstrated by effective PET imaging using a mouse model, indicating that 89Zr4+-DFO MSNs are potential PET imaging agents for long term in vivo imaging (Min et al., 2015). It is important for a tissue engineering scaffold to measure the pore size, distribution and interconnectivity in three dimensions. The characterisation of MSNs is generally done using mercury intrusion porosimetry and, most recently, by X-ray microtomography, which assesses the material dimensions non-destructively. MSNs exhibit tunable pore structures and high surface areas suitable for developing MR-enhancing hybrid materials. The porous nanostructures provide a worthy alternative to integrating many Gd chelates while maintaining all of them open to the media around them (Cao et al., 1997).

Zinc Oxide Nanoparticles

One of the promising applications of zinc oxide (ZnO) nanoparticles for TE owns to the biocompatibility and antibacterial properties of ZnO along with favourable effects arising from the interaction of living cells with ZnO nanoparticulate surfaces (Gritsch et al., 2019). A study by Colon et al., (2006) demonstrated the potential of ZnO and TiO_2 NPs in reducing *S. epidermidis* adhesion and increasing osteoblast functions essential for enhancing the efficiency of orthopaedic implantations. ZnO and TiO_2 nanoparticles also increased the osteoblast adhesion and alkaline phosphatase activity (ALP) compared to their respective microparticles (Colon et al., 2006). Foroutan, 2015 studied the effects of ZnO NP on human MSC differentiation into osteoblasts and reported that ZnO nanoparticles significantly upregulated the gene expressions of osteogenic indicators such as ALP, osteocalcin, and osteopontin (Foroutan, 2014).

Polymeric Nanoparticles

Polymeric NPs are composed of biocompatible and nontoxic biodegradable polymers, including polysaccharides (e.g., alginate, chitosan, dextran, heparin, hyaluronic acid, and pullulan), proteins (e.g., albumin, elastin, gelatin, and silk) and synthetic polymers (e.g., polyamides, polyesters, polyanhydrides, polyacrylates, and polyurethanes) alone or in conjugation in combination with other materials to provide specific functionalities (Mosselhy et al., 2021, Choi et al., 2017). The characterisation of polymeric NPs characterisation is important to ascertain their applicability and evaluate nanotoxicology and exposure assessment. The composition and concentration, surface properties, size, shape, crystallinity, and dispersion state are characterised using several analytical techniques, including chromatography, dynamic light scattering, electrophoresis, electron microscopy, near-infrared spectroscopy, and photon correlation spectroscopy (Zong et al., 2005). The NPs' surface area can be determined by the adsorption of inert gas (such as N_2) by changing the pressure conditions to form a gas monolayer.

Recently, biodegradable polymeric NPs loaded with ciprofloxacin were studied as drug delivery systems aimed for TE to prevent bacterial infection followed by implantation. The prepared poly (DL-lactide-coglycolide) NPs showed predictable, controlled release properties, ensuring a sustained release of the drug at the site of action to improve the therapy. Besides, NPs were more efficient and safer against *S. aureus* and *P. aeruginosa*, indicating that NPs loaded with antibiotics can be effectively used as carriers to deliver antibiotics (Gaspar et al., 2018). In another study, the potential of biodegradable polymer–DNA nanoparticles was demonstrated using poly (β-amino esters) to produce modified stem cells that can efficiently express angiogenic factors. Nanoparticles made of biodegradable polymers can carry foreign DNA into a cell. Nanoparticles loaded with a gene responsible for vascular endothelial growth factor (VEGF) production successfully transformed human mesenchymal stem cells (hMSCs) and human embryonic stem cell-derived cells (hESdCs), which resulted in increased hVEGF, cell viability, and promoted tissue vascularisation upon engraftment into target cells (Dakal et al., 2016).

Applications of Nanotechnology in Different Fields of TE

Stem Cell Engineering

NPs have many favourable properties, including large surface area, cell membrane activity, and increased intracellular uptake, making them suitable carriers for the delivery of genes and growth factors into stem cells. Some potential applications of NPs in stem cell research include non-invasive tracking of transplanted stem cells and intracellular delivery of small drug molecules, nucleic acid products (DNA, RNA, miRNA, and siRNA), and protein peptides. NPs also facilitate the observation of stem cell differentiation and the physiological condition of stem cells. Usually, all of these applications involve the cellular uptake of NPs. Owing to their nanoscale diameter, NPs can enter the stem cells. In most situations, the charged nanoparticles facilitate their cellular uptake (Grenho et al., 2015).

Several clinical trials involving the use of stem cells are being conducted to treat severe wounds (*via* tissue regeneration), cardiovascular, tissue/organ graft-related diseases (Ferreira, 2009). Continuous tracking of stem cells is critical to study the therapeutic effects or adverse events at the graft site. Magnetic resonance imaging is the best available non-invasive technique for monitoring the transplanted stem cells.

SPIONs have shown their potential for utilisation in MRI. SPIONs respond to external magnetic field gradients, and there is no residual magnetisation after their removal, which reduces the risk of aggregation. SPION labelling is used to observe the transplanted neural stem cells in rat brains after seven weeks of transplantation, providing information about stem cell migration in the nervous system (Cruz-Maya et al., 2019). Non-invasive detection tools that can monitor stem cell differentiation in situ are highly needed to study the degree of stem cell differentiation. NPs can be employed as biosensors on stem cells by covalent or non-covalent tagging of some immobilised biological substrate molecules onto the NP surface (Dias et al., 2017). Based on surface-enhanced Raman spectroscopy (SERS) findings, a study reported that 3D graphene oxide loaded in AuNP efficiently detected neural stem cell differentiation.

The substrate-laden NPs facilitated the effective detection of a single MSC differentiation stage by using electrochemical and electrical techniques (Kitsara et al., 2017).

Gene delivery can be a powerful strategy to study stem cell biology and advance tissue engineering therapies. Nanoparticle-based delivery systems could play an important role in gene transfection in stem cell-centred regenerative medicine. NPs immobilise the vector at the regeneration site, minimise damage, and provide a wide area for cell membrane interaction for receptor-mediated endocytosis (Huang et al., 2020). A recent study developed biodegradable polymeric NPs for non-viral gene transfer to human embryonic stem cells (hESC), which showed four times higher gene delivery efficacy than a leading transfection agent, Lipofectamine 2000. The nanoparticles were reported to have minimal toxicity on hESC morphology or showed any non-specific differentiation (Kutsuzawa et al., 2006).

NPs have a promising role in stem cell engineering. However, NPs can also show adverse effects or cytotoxicity and might alter the self-renewal and differentiation abilities of the stem cells. Impending research should carefully assess the risk to benefit ratio involved with the use of nanoparticles. For example, several studies have not reported the cytotoxic or adverse effects associated with the use of AuNPs. NPs can exert unpredictable molecular responses after a breakdown, which should be considered seriously before the stem cell therapies.

Ocular Regeneration

Several degenerative ocular diseases require the advancement of non-invasive regenerative techniques, including acute macular degeneration (AMD), cataracts, diabetic retinopathy, glaucoma, and other congenital retinal defects such as Leber amaurosis, retinitis pigmentosa and X-linked retinoschisis (San Thian et al., 2006). The current treatment options include laser surgery, photodynamic therapy, vitrectomy, and delivery of angiostatic steroids. Some current therapies fail to address the challenge, whereas others are associated with severe adverse effects (Langer, 2000). So, increasing efforts have been undertaken in the last decade to apply nanotechnology-based systems for the regeneration of lost or damaged eye tissues. Several biocompatible materials are being designed into nanoscaffolds that have a significant role in treating retinal and ocular regeneration (Kaul and Ventikos, 2015). The nanofibrous membranes can imitate natural substrate for retinal pigment epithelium to a great extent, leading to the engineering of in vivo human retinal pigment epithelium monolayer while maintaining its biofunctional characteristics. For corneal tissue regeneration, the scaffolds should be optimised to have sufficient porosity along with mechanical and optical properties (Nukavarapu et al., 2008, Rodriguez-Contreras et al., 2018). A variety of natural and synthetic polymers were used for the fabrication of nanoscaffolds, including a mixture of polyvinyl acetate with collagen type I, collagen with PLGA, PCL with PGS, collagen type I with chondroitin sulphate, and silk fibroin with poly (L-lactic acid-co-ε-caprolactone) (Cruz-Maya et al., 2019, Pellegrini et al., 1997). Nanoscaffolds are currently being developed for lens regeneration, which is generally injected into the lens capsule following the removal of its content (Alaminos et al., 2006).

Nanoparticles in Dental Regeneration

TE is considered an optimal approach for repairing craniofacial bone defects and cartilage destruction resulting from congenital disorders, cancer, infection, and trauma. Due to their morphological similarity to bone tissue and their exceptional surface properties such as surface chemistry, energy, topography, and wettability, nanophase ceramics are widely used as bone replacements, filling and coating, and materials (Webster et al., 2000, Webster et al., 2001, Webster et al., 2005). Nanohydroxyapatite (HA) has been reported to enhance osteoblast adhesion and function and improve mineralisation, indicating their role as a suitable candidate for clinical use. Several studies suggested nanosized HA to have better bioactivity than the micro-sized HA (Nukavarapu et al., 2008, Sato et al., 2006). The same theory applies to other nanoceramics, particularly aluminium, titanium, and zinc oxide (Sarao et al., 2014). A previous study reported that osteoblast adhesion was improved by 146% with 23 nm zinc oxide and 200% with 32 nm of titanium nanoparticle than their respective micro-sized counterparts (Webster et al., 2005).

The clinical use of NanOss (Angstrom Medica Inc), a bone void filler that is the first nanotechnological medical device, was approved by the US Food and Drug Administration (USFDA) in 2005. It consists of calcium orthophosphate NPs that resemble the composition and performance of human bones. Currently, it is being used to treat various general bone injuries (Paul and Sharma, 2006). Ostim is another commercial injectable formulation (synthetic nano-HA in water suspension). It is used to treat alveolar ridge augmentation, metaphyseal fracture, and osteotomies (Smeets et al., 2008). Nanotechnology plays an important role construction of biofunctionalised biomaterials for periodontal tissue repair. Tissue bioengineering with genetically designed NP, a biomimetic of mineralised tissues, leads to the manufacturing of in vitro teeth tissues (Yuan et al., 2011). In a previous attempt, Chen et al., 2005 developed HA nanorod surface with monolayer surfactants, which gave them exact surface characteristics for self-assembling enamel into a prism-like structure at the water-air interface. The size of synthesised HA nanorods was similar to human enamel crystals. Biomimetic approaches are being used for developing nanomaterials to remineralise enamel lesions. Researchers have anticipated that that nano-HA could encourage remineralisation of bone tissue through adhesion (Chen et al., 2005). Some studies also suggest that nano-HA may be employed to deliver the calcium to the mouth to facilitate enamel remineralisation. By acting as suitable biomimetic tools, NPs could improve dental tissue rejuvenation. Several nanomaterials are being engineered to address dental issues such as periodontal defects, bone degeneration, brittle teeth, and lost teeth (Huang et al., 2009, Huang et al., 2019).

Nanoparticles in Skeletal Regeneration

Current options to treat bone defects include replacing lost bone with allogeneic or similar bone grafts. Nevertheless, bone grafting is restricted due to high failure rates, donor site morbidity, risks of surgical complications, and limited availability of free bone tissue to be harvested (Wang and Yeung, 2017). Biomaterials under development could simulate the natural extracellular matrix of the bone structure. These biomaterials are being engineered with the application of nanotechnology (Yao et al., 2019). Because the ECM structure has nanoscale properties, nanomaterials are studied as biomimetics for the ECM structure (Barnes et al., 2007).

Owing to its ability to link cells and ECM formation, collagen is a widely studied material for developing a scaffold to back the bone growth (Liu et al., 2018). In a study, SEM and micro-computed tomography images were used to assess the encouraging role of poly-lactic acid (PLA)/collagen-I nanofibrous scaffold in osteogenic differentiation of hMSCs. Chitosan-based biomaterials promoted bone regeneration owing to their biocompatibility and low immune response. In a recent study, chitosan/HA electrospun scaffolds were reported to encourage cell proliferation and mineral deposition and provide osteoinductivity (Huang et al., 2020).

In addition to mixing polymers to supply 3D scaffolds, NPs have been recently investigated to promote regeneration by delivering bioactive molecules (e.g., growth factors) to support bone formation (Gritsch et al., 2019). De la Riva et al. (2010) developed an abrushite–chitosan system to control the release kinetics and localisation of VEGF and PDGF at the bone defect site, thereby promoting bone healing. The abrushite–chitosan system also protects the growth factors from systemic exposure (De la Riva et al., 2010). Owing to its inert nature, TiO_2 scaffolds are being studied for several biomedical applications, including the development of implants in bone tissue engineering. The addition of TiO_2 in chitosan scaffold aimed for bone regeneration was reported to decrease the fast degradation of the scaffold, and the interaction between chitosan and nano-TiO_2 made it highly porous, rendering it an efficient alternative for bone tissue engineering (Ikono et al., 2019).

Biomimetic Nanoscaffolds for Nerve Regeneration

Currently, nerve autografts and allografts are principally used in nerve regeneration; however, their application is associated with problems such as donor site morbidity, loss of function or formation of painful neuromas at the donor site, a limited amount of donor nerves, and the risk of immunorejection (Fathi-Achachelouei et al., 2019). Thus, research is being conducted to develop artificial nerve conduits to enhance nerve regeneration techniques. Various synthetic materials such as polycarbonate, poly(lactic

acid), poly(lactic-co-glycolic acid) (PLGA) and silicon are widely being studied for fabricating nerve conduits as chemical changes to these polymers allow changes in the porosity, biocompatibility, geometric configuration, mechanical strength and degradation (Nectow et al., 2012).

Natural ECM contains collagen fibres of nanometre diameter; efforts are being made to fabricate ideal ECM scaffolds for nerve regeneration using electrospinning. The application of electrospun nerve fibres in nerve regeneration has been reported by many researchers (Liu et al., 2011, Yu et al., 2014). In a study, poly(L-lactide-co-glycolide) polymer fibres were used to fabricate the nerve conduits and implanted into the sciatic nerve of the rat. The study reported no inflammatory response, and rats showed successful nerve regeneration during one month of study. The developed scaffolds were reported to be flexible, permeable, and with no inflammation symptoms (Lee et al., 2006). In a study, cultures of neuronal stem cells (NSCs) with porous polymeric nanofibrous scaffold have been attempted. The nanostructured PLLA scaffold was fabricated using a biodegradable poly (l-lactic acid) (PLLA) and mimic a natural extracellular matrix. The NSCs have shown differentiation on the nanostructured scaffold in cell cultural tests indicating its potential use as a carrier in nerve TE (Yang et al., 2004).

Nanoparticles in Cardiac Regeneration

Currently, both synthetic and natural hydrogels are being used widely in cardiac TE, including poly (N-isopropyl acrylamide, poly-L-lactide, alginate, gelatine, and poly (ethylene glycol) (PEG). The native myocardium achieves heart contraction, allowing conductivity between cardiomyocytes, promoting the dissemination of the action potential. However, in traditional scaffolding materials, conductivity is limited due to the many large pores within the structures (Kamaleddin and Medicine, 2017). Different scaffolding materials have been integrated into NPs to improve the electrical conductivity and imitate the nanofibrous structures of the native myocardium ECM (Kitsara et al., 2017).

In an investigation, a UV-cross-linkable gold (GNR)-incorporated gelatin methacrylate (GelMA) hybrid was fabricated for its utilisation in cardiac TE (Cromer Berman et al., 2011). The incorporated nanomaterial was reported to promote the hydrogel matrix's mechanical stiffness and electrical conductivity, and the hybrid hydrogels seeded cardiomyocytes represented excellent viability, cell retention, and metabolic activity. Similarly, carbon-based nanomaterials, such as carbon nanotubes (CNTs), have enhanced conductivity-related characteristics. For instance, a study reported the synthesis of cardiac patches by planting neonatal rat cardiomyocytes onto CNT-incorporated photo-cross-linkable gelatin methacrylate hydrogels. The electrically conductive and nanofibrous complexes formed by CNT were reported to improve cardiac cell adhesion and organisation and provide outstanding mechanical integrity and improved electrophysiological functions.

Microscale scaffolds cannot effectively replicate the ECM atmosphere, so nanoscale scaffolds such as nanofibres are being studied widely to develop heart tissue constructs. Both synthetic and natural polymers (e.g., collagen, protein, and fibrinogen), synthetic polymers [e.g., poly(lactide), poly(glycolide)], and their copolymers [(PLGA), poly(ε-caprolactone), poly(ethylene-co-vinyl alcohol)] are being spun into nanofibres using electrospinning (Cao et al., 1997). In a study, nanofibres were fabricated using biodegradable non-woven poly(lactide)- and poly(glycolide)-based (PLGA) platforms for cardiac TE applications (Zong et al., 2005). The results suggested that the nanofibrous construction of the matrix permitted the cardiomyocytes to use external hints for isotropic or anisotropic growth. The cardiomyocytes also interacted with the surrounding nanofibrous network to shape their growth to follow the scaffold-prescribed direction. Similarly, a nanofibrous assembly comprised of poly(l-lactic acid) and polyaniline, designed for cardiac TE, was described to show better biocompatibility and a significant outcome on cell differentiation while improved cellular distribution, arrangement, and cell–cell interactions (Wang et al., 2017).

Nanoparticles in Skin Regeneration

Acute and chronic wounds are one of the major causes of morbidity and mortality, affecting many populations across the world, leading to increased research in skin regeneration. However, the development of composite epidermal and dermal layers that can efficiently mimic in vivo skin, greater reviving ability, and negligible scarring is a huge challenge in this field (Singer and Boyce, 2017). Nanomaterials have an

imperative role in skin regeneration owing to their high surface-area-to mass ratio promoting cell–matrix interactions. The main advantage of NPs is their competence to carry, protect, and control the release of drugs. Drug-associated side effects could be reduced due to their ability to deliver directly in a controlled manner, thus reducing the drug dose to a wound bed (Rahmani Del Bakhshayesh et al., 2018). Chronic wounds resulting from burns and injuries are at great risk of microbial infections. The increased incidence of multi-drug resistant microbial (e.g., methicillin-resistant *Staphylococcus aureus*) infections requires novel delivery systems that incorporate antimicrobial components in the scaffold for effective treatments (Mosselhy et al., 2021). Currently, the applicability of antibacterial agent-loaded NPs is widely assessed in skin tissue engineering. AgNPs naturally possess antibacterial and antifungal properties and are incorporated in various antibiotic therapies. The application of AgNPs in treating burn and diabetic wounds was investigated by Tian et al., 2007. It was found that NPs have also accelerated the rate of healing in animal studies along with showing antimicrobial effect. In another study, metallic nanosilver particles-collagen/chitosan hybrid scaffold was reported to act as a bactericidal, anti-inflammatory agent and promote wound healing by regulating macrophage activation and fibroblast migration (Mo et al., 2017).

Severe skin wounds require engineered biomaterials to cover and facilitate tissue revival. An ideal tissue-engineered skin scaffold should act like the native tissue to function and secure the wound bed from blood/fluid loss, infection, and promote exudate discharge. Various systems are being studied as dermal scaffolds, i.e., gels, nanofibres, microfibres, films, and membranes (Sundaramurthi et al., 2012). Nanofibres are ideal candidates for tissue engineering due to their skin-like mechanical properties, simulating the local extracellular matrix, high porosity, and large surface area. The nanofibres were electrospun into nanometre diameters (Figure 1.3).

A wide range of natural and synthetic polymers were electrospun and assessed for their efficiency to support skin regeneration (Table 1.1).

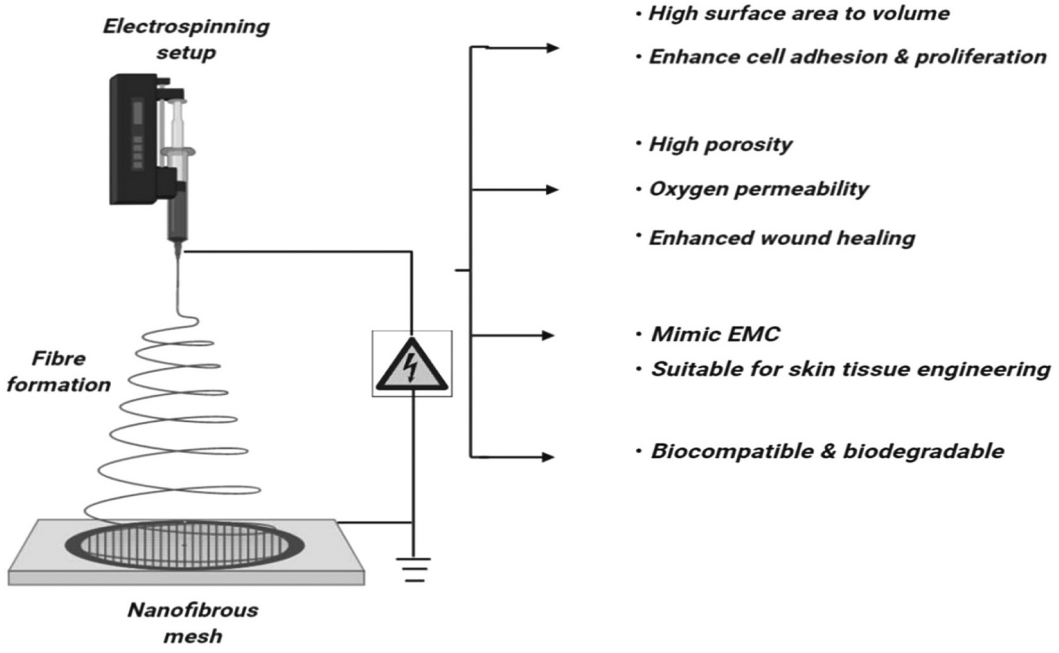

FIGURE 1.3 Electrospun nanofibres for skin regeneration.

TABLE 1.1

List of Studies Conducted on Various Types of Polymeric Nanofibres in the Field of Skin Regeneration

Polymer	Diameter	Active Constituent	Result	Reference
Gelatin and Poly(L-lactic acid)-co-poly-(e-caprolactone) (PLLCL)	Less than 700 nm	Penicillin– 146 streptomycin solution	The nanofibres showed sustained release	(Jung et al., 2013)
Polycaprolactone (PCL)	382 nm ± 140 nm	Potassium phthalimide salt and 2-chlorocyclopentanone	Enhanced fibroblast growth while maintaining normal cell morphology	(Hough et al., 2006)
Poly (D,L-lactic-co-glycolic acid) (PLGA)	400 nm ± 25 nm	Angiogenin and Curcumin	In vivo angiogenesis and anti-infection properties of composite nanofibres were transplanted into the infected full-thickness burn wound	(Mo et al., 2017)
Polycaprolactone (PCL)	130 nm	Gentamicin sulphate	The encapsulation of drug improved the bioactivity of nanofibres	(Ahn et al., 2018)
Bioactive glass	21.87 ± 0.21 Mpa	BG precursor solution	The nanofibres showed antibacterial activity against *S. aureus and* induced the secretion collagen and vascular endothelial growth factor	(Abdul Khodir et al., 2018)
Gelatin	339 ± 91 nm and 276 ± 88 nm	1,4-butanediol diglycidyl ether (BDDGE)	Non-toxic with capability of tailoring gelatin's mechanical and physical properties and ability to synthesis of new extracellular matrix	(Zhou et al., 2017)
Collagen	310 ± 117 Nm	Tilapia skin	Promoted viability, migration and differentiation of HaCaTs	(Dias et al., 2018)
PLGA	398–135 nm	Bone marrow derived mesenchymal stem cells (BM-MSCs)	Implanted BM-MSCs were found to promote epithelial edge in growth and collagen synthesis	(Pilehvar-Soltanahmadi et al., 2016)
Chitosan	177.6–40.5 nm	Poly(caprolactone) (PCL)	Served as provisional matrix to promote wound closure	(Zielińska et al., 2020)
Cellulose acetate	396.66 ± 0.90 nm	Soya protein	Promoted fibroblast proliferation, migration, infiltration, and integrin β1 expression.	(Markides et al., 2012)

(*Continued*)

TABLE 1.1 (Continued)

Polymer	Diameter	Active Constituent	Result	Reference
Gelatin and Polycaprolactone	417 ± 165 nm	Gel powder of pig skin	Provided high porosity which contributed to an ideal water vapour permeability rate and good mechanical and biological properties	(Liu et al., 2011)
Polycaprolactone (PCL)	0.71 ± 0.33 μm	Spirulina	Enhanced viability and proliferation of mouse fibroblasts	(Ahn et al., 2013)
Poly (3-hexylthiophene) (P3HT)	503 ± 116, 421 ± 128 and 314 ± 101 nm	Phalloidin (fluorescein isothiocyanate)	Showed better cell proliferation along with retaining cell morphology	(Fathi-Achachelouei et al., 2019)
Alignate-PCL	1.35 ± 0.45 μm	Spirulina	Capable of holding large amounts of water, to support the backbone of the nanofibres	(Tan and Marra, 2010)
Chitosan & Gelatin	120 &220 nm	Trifluoroacetic acid and Dichloromethane	Chitosan-gelatin-blend nanofibres were found to enhance tensile strength as compared to gelation nanofibres	(Kamaleddin and Medicine, 2017)
3D silk fibroin	18.56 ± 0.1 μm	Cocoons	Use of 3D nanofibre scaffolds presented a high porosity with controlled full thickness	(Jung et al., 2013)
Silk fibroin	250 nm	Human amniotic membrane	Due to fibroin nanofibre coating, mechanical properties of HAM improved significantly	(Colon et al., 2006)
Peptide	6 mm	Heparin	Nanofibre-treated burn wounds formed well-organised and collagen-rich granulation tissue layers with greater density of newly formed blood vessels	(Qin et al., 2014)
Poly(lactic-co-glycolic acid) (PLGA)		Heparin sodium salt	Stimulated cellular activity by enhancing the bioactivity of the released GF	(Levengood et al., 2017)

TABLE 1.2

Recent Patents Representing Utilisation of Nanotechnology in the Field of Tissue Engineering and Regenerative Medicine

S. No	Patent	Type of Nanoparticles	Summary of Invention	Remarks	Reference
1.	US 6,552,172 B2 (Apr. 22, 2003)	Fibrin nanoparticles	Provides methods for preparing fibrin NPs and microbeads of different sizes with compositions	Provides method for incorporating active agents into a cell, method for isolating stem or progenitor cells from a biological sample and a composition of fibrin particles bound to stem or progenitor cells	(Marx and Gorodetsky, 2003)
2.	US2006/016.5805A1 (Jul. 27, 2006)	Magnetic nanoparticles	Provides method of use of magnetic pole matrices for tissue engineering and targeting systematic therapy for cardiovascular disease using magnetic polymer nanoparticles based gene/drug delivery	The magnetic pole matrices possesses advantages such as distributing the magnetic nanoparticles conjugated with genes or drugs locally and uniformly on the artificial surface which essentially solved the blood vessel blocking problem along with promoting the adhesion of the cells on specific locations	(Steinhoff et al., 2006)
3.	WO2011145963A1 (Nov. 24, 2011)	Non-leaching polymeric nanoparticles	The invention is related to methods of NP preparation particularly for the intracellular delivery of hydrophobic drugs	These nanoparticles can be used to control the activity and differentiation of cells, including stem cells through the intracellular delivery of the hydrophobic drugs	(Ferreira et al., 2011)
4.	US 8,172.997 B2 (May 8, 2012)	Cerium oxide	Provides method of preparation of novel non-agglomerated ultra-fine ceramic oxide nanoparticles by a novel Sol micro emulsion process	Applications of the NPs include benefits for wound healing, treating arthritis and joint diseases, anti-aging and the treating of inflammations	(Seal et al., 2012)
5.	US 2012/0087868 A1 (Apr. 12, 2012)	Mesenchymal stem cells having nanoparticles loaded cells	The invention also provides methods of manufacturing the nanoparticle-loaded cells, and methods for treatment and/or imaging	The interaction of NPs and NP-loaded cells with one or more electromagnetic radiations or magnetic fields provides opportunities for imaging of tissues and/or detection of various diseases, diseased cells, disease states and the like	(Todd and Danilkovitch, 2012)

(*Continued*)

TABLE 1.2 (Continued)

S. No	Patent	Type of Nanoparticles	Summary of Invention	Remarks	Reference
6.	US 2013/0273011 A1 (Oct. 17, 2013)	Stem cell generated nanoparticles (40–100 nm)	Discloses stem cells and derived nanoparticles useful for the treatment of acute radiation syndrome and other inflammatory conditions The endometrial regenerative cells are administered to patients having been exposed to radiation to augment recovery of endogenous hematopoietic stem cells	The invention pertains to stem cell therapeutic activities and treatment of hematopoietic injuries through administration of mesenchymal stem cells that are capable of increasing survival after radiation injury through accelerating hematopoietic recovery. It also pertains to the use of exosomes secreted by said mesenchymal stem cells for achievement of this purpose	(Ichim et al., 2013)
7.	US 8,663,675 B2 (Mar. 4, 2014)	Polymeric nanoparticles	Injectable matrix is used to create a three-dimensional matrix system in an area of the desired tissue or organ	Various embodiments herein relate to an injectable matrix used for regeneration, reconstruction, repair, or replacement of organ or tissue	(Ghoroghchian and Ostertag, 2014)
9.	US 9,109,203 B2 (Aug. 18, 2015)	Magnetic nanoparticles are coated with silica	Silica-coated MNPs were developed using the water-in-oil (w/o) reverse micelle method	The NPs can be utilised for targeting neural stem cells	(Yung et al., 2015)
10.	US 2016/0030600 A1 (Feb. 4, 2016)	Nanoparticle (lipid based and polymer based), has a size from about 100 nm to about 400 nm	This invention relates to nanoparticles for use in the in vivo diagnostics of epicardial derived cells (EPDCs) to nanoparticles for use in the treatment of cardiac injury	Targeted delivery of phagocytable particles to EPDCs can be exploited in the delivery of therapeutic agents to EPDCs, for instance in regenerative therapy approaches for cardiac injury and in the delivery of labelling agents to EPDCs, which is for instance applicable in in vivo imaging of these stem cells	(Schrader, 2016)

11.	US 2016/0067273 A1 (Mar. 10, 2016)	O-gal nanoparticles	Provides compositions and methods for preparation of synthetic O-gal nanoparticles	The present invention gives compositions and methods comprising molecules and nanoparticles that can replace the O-gal nanoparticles generated from natural materials in the induction of repair and regeneration of injuries	(Galili, 2016)
12.	US 9,750,845 B2 (Sep. 5, 2017)	Polymeric nanoparticles	It describes methods and compositions of nanocomposites consisting citric acid copolymers, poly L-lactic acid (PLLA) and polylactic-co-glycolic acid (PLGA)	The present invention describes a composition aimed for strengthen scaffolds for bone tissue engineering applications and, to a lesser extent, for soft tissue regeneration	(Ameer and Webb, 2017)
13.	US 2018/0360768 A1 (Dec. 20, 2018)	Mineral-based nanoparticles	The silicate nanoparticles are synthesised through a process where the precipitate of sodium silicate is mixed with one or more elements and compounds and milled into NPs	NPs comprising silicate that induce human mesenchymal stem cells (hMSCs) into a cartilage lineage through the upregulation of cartilage-specific genes resulting in the transformation of the cell phenotype into that of a chondrocyte, i.e., a cartilage producing cell.	(Gaharwar and Carrow, 2018)

Patents

The pioneer work in the field of TE primarily focused on what materials and structures were needed to be utilised to develop tissue equivalents (Wang et al., 2017). In the coming years, the research was more focused on physiological concepts and mechanisms involved in TE including cell attachment, differentiation and the role of growth and differentiation factors in TE. Next, the research revolved on evolving the knowledge for application of new polymers and different methods for engineering of matrices for creating of new tissues. In the last few decades, advances in the field of nanotechnology have enabled fabrication of biomimetic microenvironment at nanoscale leading to increased efforts in utilisation of engineered NPs as biomimetic nanoscaffolds (Liu et al., 2018, Sundaramurthi et al., 2014). Table 1.2 reviewedsome of the most important patents in this field.

Future Prospects and Limitations

Different biomaterial-based NPs including metals, ceramics, and polymers have demonstrated promising role in TE application. However, there are still many challenges in the field that needs to be addressed. The focus of current research should be on the study of toxicity, carcinogenicity, and teratogenicity of NPs and appropriate evaluation tools need to be developed so as to ensure safety of human health in terms of the use of nanomaterials in regenerative medicine (Yang et al., 2004). NPs are being studied as a promising targeted contrast agent for non-invasive diagnosis; however, so far only iron oxide NPs are being utilised in clinical application (Chen et al., 2005). The reason for this could be the difficulty in achieving desirable pharmacokinetic properties and particle homogeneity with NPs along with the lack of clinical studies related to their elimination, toxicity, and biodegradation. Therefore, there is a dire need for the development of specific guidelines and precautionary measures for applications of NPs in biomedical field.

Summary

Owning to their biocompatibility and surface modification, various types of NPs have been studied to be utilised as a diagnostic and therapeutic agent. NPs for skin have been studied extensively in previous years' regeneration mainly for the treatment of burn victims and for wound healing. At present, several tissue-engineered skins are available for human use. By acting as suitable biomimetic tools, NPs have shown great potential for improving dental, ocular, skeletal, and cardiac tissue regeneration. In this chapter, we have reviewed the use of nanostructures structures in TE in different ways such as by acting as antimicrobial agents, providing constant release of several growth factors, or by acting as contrasting agents for monitoring of stem cells. Although NPs offer a promising role in TE, the lack of teratogenicity, carcinogenicity, and toxicity studies needs to be addressed. Despite the challenges, nanotechnology has potential to stimulate the reconstruction of complex tissue architectures in future.

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