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CHAPTER 3

Chapter Title:

Encapsulation of bioactive compunds: Role of nanotechnology

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Abstract

Tissue engineering and regenerative medicine, one of the most exciting and emerging fields in the last decade, aim to maintain, restore or improve tissues and thus organ functions. Nanomaterials have attracting unprecedented interest to be applied for such purposes owing to their particular features including biocompatibility, mechanical, optical, electrical, magnetic and catalytic characteristics compared to conventional systems. Nanomaterials have been proved as promising candidates to serve as a carrier system for delivery of growth factors, genes cytokines etc., to control properties of the scaffolds and also to provide contrast for cell imaging. These nanosystems can be made of polymeric, lipid or inorganic materials. In this review paper, the different types of nanomaterials used in tissue engineering and regenerative medicine along with their applications will be covered and discussed.

Key words

Tissue engineering, regenerative medicine, nanomaterials.

3.1 Introduction

Tissue engineering and regenerative medicine are among the fastest evolving interdisciplinary fields in healthcare research aiming to regenerate or replace human cells, tissues or organs enabling to restore or establish normal function (Engel *et al.*, 2007). They have brought high expectations for a great number of current worldwide human health problems like cardiovascular and metabolic diseases, brain and spinal cord injuries, cancer, as well as organ failure (Lee and Walsh, 2016; Anthony and Borlongan, 2017; Curtin, Castaño and O'Brien, 2017; RaquelCosta-Almeida, Reis and Gomes, 2019). The so-called triad of tissue engineering therapies comprises the following essential components: stem cells, biomaterial scaffolds and signaling molecules (Liao, Chan and Ramakrishna, 2008). Commonly, a tissue engineering therapy consists on seeding stem cells over or into porous and biodegradable

biomaterial scaffold, before its implantation, in the aim of restoring a tissue function and/or repopulating a defect site (Dvir *et al.*, 2011). To date, some regenerative medicine products, comprising tissue-engineered biomaterials, scaffolds, implantable devices and cell-based therapies have been applied in clinical trials for diabetes, immune and inflammatory diseases. Unfortunately, the clinical applications of a wide variety of products have been challenged by several scientific and engineering problems such as the biomanufacturing of stem cells or tissues (inability of cells to self-assemble into 3D tissues, effective delivery and instability), the efficacy of biomaterial scaffolds (absence of cellular adhesion and limited ability to fully differentiate transplanted stem cells), the control of micro-environmental signals, the enzymatic degradation of bioactive molecules and the assessment of the efficacy of therapies (Gao *et al.*, 2015). Nanotechnology, despite its immense popularity in biologically-related fields, has been utilized in the field of tissue engineering and regenerative medicine thanks to its promising perspectives to manage these challenges. For instance, the nanomaterials structures are able to control cellular functions like adhesion, differentiation and proliferation. Secondly, the sustained release property and the possibility of functionalizing nanomaterials improve the efficacy of the treatment and reduce the undesirable effects. Furthermore, the nanomaterials, due to their unique optical and magnetic characteristics, are promising contrast agents for monitoring cellular behavior *in vivo* (Santo, Gomes, *et al.*, 2012; Gao *et al.*, 2015; Monteiro *et al.*, 2015; Mostafavi *et al.*, 2020). To substantiate these points, a plethora of research has been investigated, in the last decade, on the applications of nanotechnology in tissue engineering research. It was demonstrated that the fetal bovine serum-loaded liposomes incorporated in poly (2-hydroxyethyl methacrylate) scaffolds significantly improved both chondrocyte adhesion and proliferation (Rampichova, 2012). Similarly, Ertan et al. revealed that the conjugation of insulin-like growth factor I and transform growth factor b1 loaded poly(N-isopropylacrylamide nanoparticles with poly (lactic acid-co-glycolic) acid scaffolds provided more expression of collagen type II and aggrecan than those containing free or single growth factors (Ertan *et al.*, 2013). In addition, ultrasmall paramagnetic iron oxide particles were found efficient in visualizing and tracking human mesenchymal stem cells for up to 14 days after transplantation at injection sites (Mathiasen *et al.*, 2019).

This chapter highlights the very recent advancements in the development of different kinds of nanomaterials (including polymeric, lipid and inorganic materials) combined with biomaterial scaffolds of a specific tissue required for translatable regenerative medicine. Moreover, an overview of the existing technologies for tissue engineering are summarized and discussed.

3.2 Nanomaterials

A nanomaterial is a natural, incidental or manufactured material composed of particles in an unbound, aggregate or agglomerate state, in the nanometer size range (50% or more of the particles should be in a size distribution range from 1 to 100 nm) (Y. Sun *et al.*, 2020). In the last decade, nanomaterials offer an enormous outlook for promoting current therapies due to their particular benefits including targeted drug delivery, reduced side effects, lowered drug concentrations, and increased patient compliance. Nowadays, these systems emerged new treatment tools for tissue engineering therapies and regenerative medicine because of their

unique physical and chemical features (Nemeth, Fine and Fatemi, 2019). Nanomaterials played a paramount role in the protection and the delivery of genetic material biomolecules (growth differentiation factors) and in the determination and the regulation of stem cell behavior through tracing the fate and distribution of stem cells *in vivo*, ascertaining the origin of stem cell diseases, inducing directed differentiation of stem cells and regulating the microenvironment of tissues around stem cells (Arora *et al.*, 2012; Y. Sun *et al.*, 2020). These nanocarriers can be made of polymeric, lipid or inorganic materials (**Figure 1**) (Nemeth, Fine and Fatemi, 2019). In this section, we will review the common types of nanomaterials used in tissue engineering therapies and regenerative medicine.

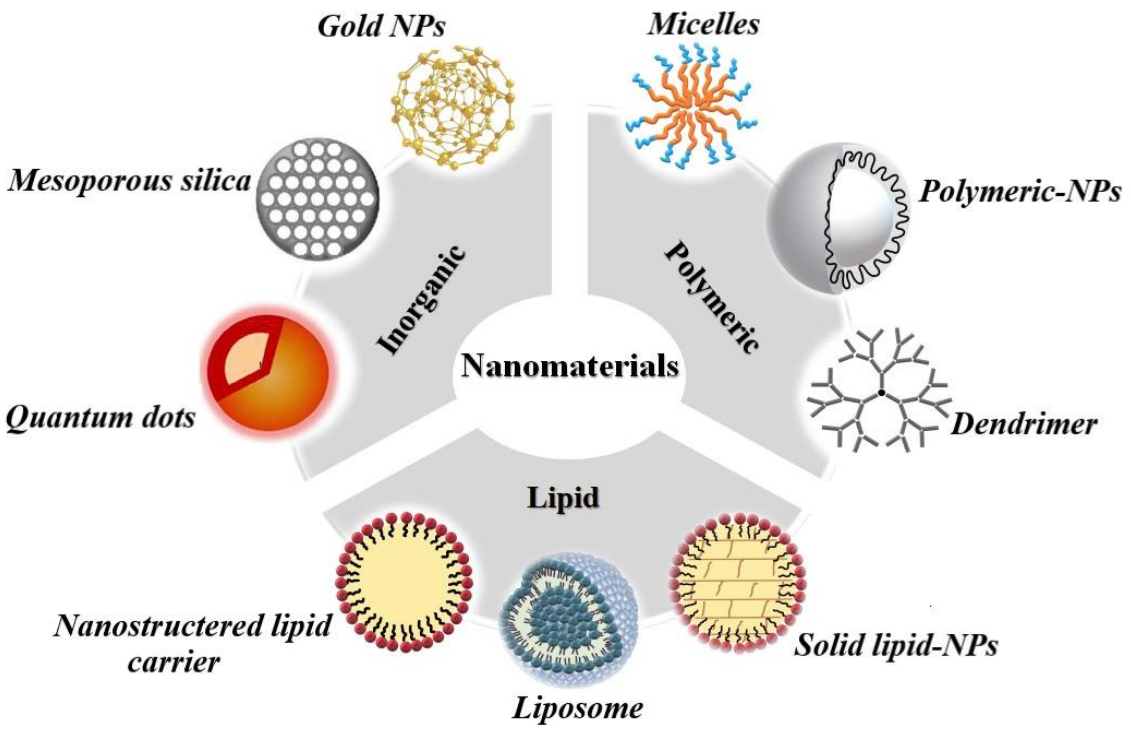

Figure 1: Example of nanomaterials used in tissue engineering.

3.2.1 Polymeric based-nanomaterials

For polymeric based-nanoparticles (NPs), properties like size, shape, surface charge, mechanical strength, porosity, degradation rate and solubility, can be adjusted. In addition, theses nanomaterials are known for their low cytotoxicity, good biocompatibility, improved targeting specificity and high permeation and retention effect (Fathi-achachelouei, Knopf-marques and Evelise, 2019). All these factors make polymeric based-NPs god tools for versatile purposes in tissue engineering and regenerative medicine. Various types of polymer based-NPs exist using biocompatible natural or synthetic polymers. Proteins (gelatin, elastin, albumin, silk), polysaccharides (alginate, dextran, chitosan, heparin, hyaluronic acid,), and synthetic polymers like polyesters, polyanhydrides, polyamides, polyacrylates and polyurethanes have been used single form or in conjugation with specific functional moieties or materials to provide functionalities in tissue engineering field. The macroscopic behavior of NPs is related to their morphology. Thus, depending on the application, choosing suitable type of NPs is a critical point in research (Fathi-

achachelouei, Knopf-marques and Evelise, 2019). Several techniques have been designed for the synthesis of NPs such as self-assembly, nanoprecipitation, single and double emulsion/solvent evaporation, emulsion polymerization, supercritical fluid, salting-out, emulsion/solvent diffusion, dialysis and microfluidic systems (Froiioa *et al.*, 2020). Various types of polymer based-NPs exist:

A. Polymeric nanoparticle

Polymeric nanoparticles are spherical polymeric colloidal particles with a size ranging from 10 to 1000 nm. They can be present as nanospheres or nanocapsules depending on the preparation method. The formers comprise a polymeric matrix extended from the core to the surface, while the latter consist on a polymeric layer surrounding a hollow core (Lammari *et al.*, 2020).

B. Dendrimer

Dendrimers are highly branched, globular polymeric materials with nanometer-scale size much smaller than many organic counterparts. They are composed of a central core, an interior dendritic structure (the branches), and an exterior surface with functional surface groups like additional polymers or ligands enabling improved payload and targeting delivery (Gillies and Fréechet, 2005). Dendrimers, due to their structure, enable precise control of size and shape. The high surface area, neutral surface charge and water solubility, make polymeric dendrimers promising candidates for incorporation into tissue engineering scaffolds either as auxiliaries to an existing scaffold, or as primary scaffold components (Joshi and Grinstaff, 2008).

C. Micelle

Polymeric micelles Polymeric micelles are nanosized molecules of core–shell structure that are formed by the self-assembly of amphiphilic block copolymers in an aqueous media (ML Adams, Lavasanifar and Kwon, 2003). Depending on the self-assembly principle, two forms of polymeric micelles exist: (i) block polymer micelles, which have a unique core-shell structure, with hydrophobic core and surrounding hydrophilic shells in aqueous solutions, and (ii) graft copolymer micelles, where different monomers are applied as main and branch chains enabling two or more opposite properties like oil/water affinity, plasticity/elasticity, and acidity/alkalinity (Bai *et al.*, 2020).

3.2.2 Inorganic based-nanomaterials

Inorganic nanoparticles are also used for tissue engineering applications due to their benefits over organic nanoparticles including low toxicity, good storage stability, no microbial attack and easy preparation. However, they show only moderate transfection efficiencies (Sokolova and Epple, 2008). Many classes of inorganic-NPs have been used as carriers:

A. Metallic nanoparticle

Metallic nanoparticles have a metal core made up of inorganic metal or metal oxide, generally covered with a shell composed of organic or inorganic material or metal oxide (AhmadKhan, 2020). Several methods are used to synthesize metallic-NPs like sol-gel process,spray pyrolysis, electrodeposition, rapid solidification, chemical vapour deposition, etc. Ag and Au are mainly used metal-NPs associated with many synthetic and natural polymers like chitosan, polyvinyl alcohol, polycaprolactone, etc. Several other metallic-NPs like Fe, Al, Cu, Ni, Zr and also magnetic nanoparticles are also applied (Wijesinghe *et al.*, 2020). Although some studies reported that some metal-NPs like nickel are toxic, it has been found that with the proper size and dosage, these NPs are mostly advantageous. Metallic-NPs find immense applications in tissue engineering and regenerative medicine areas due to their particular features including high surface area, antibacterial effect, electrical conductivity and biocompatibility (Eivazzadeh-keihan, Noruzi and Chenab, 2020). Metallic-NPs have also their advantage in therapy as drug carriers or as bioimaging agents (Ardoiwala, Kaundal and Choudhury, 2018). Furthermore, earlier studies reported that the ease of functionalization of metallic-NPs could enhance their biomedical applications and improve their performance in tissue engineering (Eivazzadeh-keihan, Noruzi and Chenab, 2020).

B. Ceramic nanoparticle

Ceramic nanoparticle or nanoceramics are primarily composed of oxides, carbides, carbonates and phosphates of metals and metalloids like calcium, titanium, silicon, etc. They are classified as heat-resistant, inorganic and nonmetallic solids (Thomas, Harshita and Talegaonkar, 2015). They can be biodegradable (e.g. carcium phosphate and silica) or non-biodegradable (e.g. iron oxide, aluminum oxide). They are porous, less than 50 nm in size and can be found in various forms like polycrystalline, amorphous, porous, dense, or hollow (Sigmund *et al.*, 2006; Chen, Chen and Shi, 2013). Additionally, since they are not affected by pH fluctuations and temperature, they are suitable for protecting different biomacromolecules like enzymes against denaturation. Furthermore, due to their porous structure, they have a large surface area for incorporating bioactive molecules, and simultaneously they can be functionalized to include connective ligands. However, like the metallic-NPs, they are not biodegradable, which may cause side effects**.** Ceramic-NPs have a wide range of applications across the world. In the biomedical field, they are considered to be excellent carriers for drugs, proteins, genes, imaging agents etc. (Thomas, Harshita and Talegaonkar, 2015).

C. Quantum dots

Quantum dots (QDs) are inorganic semiconductor nanocrystals that have a core/shell structure of 1-10 nm in diameter. They can be covered with amphiphilic polymers and conjugated with different ligands for targeted imaging approach (Bentolila, Ebenstein and Weiss, 2009). Cadmium selenide and cadmium telluride are some examples of QDs widely used for *in vivo* fluorescence imaging (Liu *et al.*, 2011). QDs are one of the most promising inorganic materials for stem cell research due to the fact that they can be synthesized easily in large quantities from various materials using moderately simple techniques. Moreover, they are good candidates for cell labeling, cell tracking and *in vivo* imaging, owing to their potential in imaging

and detection applications. For instance, QDs are robust fluorescent nanocrystals with narrow emission (Kim, 2008).

3.2.3 Lipid based-nanomaterials

Lipid nanoparticles are particularly promising non-viral vectors used for tissue engineering and regenerative medicine applications especially for *in vivo* RNA delivery. Indeed, they can be synthesized easily in a scalable manner, facilitate endosomal escape, functionalized with specific ligands to target the desired or codelivered with adjuvants. In addition, most lipid NPs formulations rely on cationic lipids that efficiently complex the negatively charged RNA (Reichmuth *et al.*, 2016). In this review, we spotlight two types of lipid-NPs: liposomes and solid lipid nanoparticles.

A. Liposome

Liposomes generally consist of an aqueous core surrounded by a lipid bilayer shell. They were first described by Bangham et al. in the 1960s and were the first drug carriers used for targeted drug delivery (1965). The liposome bilayer consists of natural and/or synthetic phospholipids such as phosphatidylserine, phosphatidylcholline, phosphatidylinositol, phosphatidylglycerol and phosphatidylethanolamine (Laouini *et al.*, 2012). The phospholipid composition of the bilayer determines the properties of the liposome, such as surface charge, permeability, and hydrodynamics. Cholesterol may also be incorporated to improve the membrane fluidity and stability (Laouini *et al.*, 2012). Currently, liposomes have been approved for multiple clinical trials, and some liposome formulation products are already on the market (Malam, Loizidou and Seifalian, 2009; Yang *et al.*, 2011). The interest of liposomes is related to their ability to encapsulate both hydrophilic and hydrophobic drugs thanks to the amphiphilic structure of their consisting lipids. Furthermore, their small nano-size, biodegradability, biocompatibility, multi-route options for injection and their good cell affinity make them attractive carriers (Yang *et al.*, 2011).

B. Solid lipid nanoparticles

Despite the clinical success, the major limitations of liposomes as drug carriers are instability in bloodstream and uncontrolled and premature drug release (Yang *et al.*, 2011). Alternatively, solid lipid nanoparticles can instead be used for improved stability [23]. They are composed of surfactants and a lipid matrix that is solid at ambient temperature (Bayón-Cordero, Alkorta and Arana, 2019). The commonly used lipids are fatty acids (palmitic acid, stearic acid and benzoic acid), triacylglycerols (tristearin, trimyristin and tripalmitin) and acyl glycerols (glycerol monostearate, glycerol benzoate and glycerol palmitostearate) (Kaliamurthi and Selvaraj, 2016). These lipids are characterized by their purity, good tolerability, low toxicity index, low price and biodegradability (Lin *et al.*, 2017). However, solid lipid nanoparticles show some limitations like reduced loading capacity of hydrophilic drugs (Mukherjee, Ray and Thakur, 2009). A second generation of lipid based NPs was developed in the aim of improving the loading capacity of solid lipid NPs; so called nanostructured lipid carriers in which the matrix is composed of a blend of a solid and a liquid lipid oil (Patel, 2013).

3.3 Scaffolds

A scaffolds is a structural support engineered to mimic the function of a natural extracellular matrix contributing to the development of functional tissues for medical purposes (Monteiro *et al.*, 2015). Tissue engineering scaffolds can be made of natural or synthetic polymers. Natural polymers hold particular advantages over synthetic ones like commercial availability, superior biocompatibility, easy processing and the possibility to tune their degradation by changing the concentration of the polymer and/ or the cross-linking material (Garg *et al.*, 2011; Monteiro *et al.*, 2015). Natural polymers include collagens, albumin, dextran, starch, fibrin, alginate, gelatin, galactan, elastin, curdlan, chitosan, hyanuronic acid, cellulose, etc. Some limitations are attended when using these biomaterials like the price, the batch-to-batch variation and the cross-contamination (Garg *et al.*, 2011). Synthetic based-scaffolds are classified into two main groups: biodegradable polymers like polylactide, polyglycolide, polycaprolactone, polyurethane, polycyanoacrylate, etc. and non-biodegradable polymers like polyvinyl alcohol, poly (N-isopropylacrylamide) and polyhydroxyethylmethacrylate. Synthetic polymers have been suggested due to their processing flexibility and their ability to be manufactured in many sizes and shapes (Dawson *et al.*, 2008; Kulkarni *et al.*, 2010). The preparation methods of polymeric scaffold have been widely reviewed previously (Engel *et al.*, 2007). In addition to polymeric compounds, certain inorganic materials have also been used as scaffolds for tissue engineering applications. They are categorized into three main groups: bioactive, bioresorbable and bioinert depending on their ability to bond directly with native tissues once implanted. Bioactive materials (e.g., glass-ceramics and bioglasses) exhibit direct bonds with living tissues, and have been applied to fill small tissue defects and irregularities. On the other hand, bioresorbable materials (e.g., calcium phosphate, calcium phosphate cements, and calcium silicates or calcium carbonates) are gradually absorbed *in vivo* and are replaced by new tissues over time. Bioinert materials (e.g., titanium, zirconia, alumina and its alloys) applied as structural-support implants and have no interaction with their adjacent tissue after implantation (Pina *et al.*, 2019). The inorganic materials are frequently difficult to process into highly porous structures and are mechanically brittle. To solve these problems, combinations with natural or synthetic polymers have been proposed for tissue engineering research (Xie *et al.*, 2010; Haaparanta *et al.*, 2015). **Table 3.1** displayed some example of scaffold materials used for tissue engineering.

Material	Application	Reference
Natural polymers		
Hyanuronic acid	Brain regeneration	(Wang and Spector, 2009)
Collagen	Skin tissue regeneration	(Ahn *et al.*, 2010)
Salmon fibrinogen and chitosan	Wound healing	(Laidmae *et al.*, 2018)
Synthetic polymers		
Poly (latide-co-glicolide)	Bone regeneration	(Go *et al.*, 2020)
Poly-L-lactic acid	Neural tissue engineering	(Yang *et al.*, 2005)
Polycaprolactone	Cartilage tissue engineering	(Li *et al.*, 2005)
Polyglycolic acid	Urinary tissue engineering	(Knight and Payne, 2013)
Inorganic materials		
Calcium silicate	Bone regeneration	(Shuai *et al.*, 2014)

Calcium phosphate	Cartilage tissue engineering	(H. Sun *et al.*, 2020)
Glass ceramic	-	(Mortera *et al.*, 2008)
Hybrid materials		
Poly (lactide-co-glycolide) + glass fibre	Bone regeneration	(Haaparanta *et al.*, 2015)
poly(l-lactide-co-glycolide) + tricalcium phosphate	Bone regeneration	(Xie *et al.*, 2010)
Polycaprolactone + chitosan	Skin tissue regeneration	(Levengood *et al.*, 2017)
Polyglycolic acid + collagen	Bone regeneration	(Levengood *et al.*, 2017)

Table 1- Example of scaffold materials for tissue engineering applications.

3.4 Applications of nanomaterials in tissue engineering and regenerative medicine

A scaffold can be used as a cell support device upon which cells are implanted into an artificial structure to support three dimensional tissue formation or as a drug delivery device by which drug release is sustained for longer period (Garg *et al.*, 2011). The combination of biomaterial scaffolds with bioactive agent-loaded nanomaterials has opened up a new avenue for tissue regeneration and has proven its efficiency in overcoming the limitations of traditional scaffold systems (Wang *et al.*, 2016). For instance, a traditional scaffold, when applied alone, shows limited ability to fully differentiate transplanted stem cells (Kulkarni *et al.*, 2010). In addition, the incorporation of bioactive agents directly into the scaffold induces poor bioavailability, high enzymatic degradation and possible self-aggregation (Lee *et al.*, 2008). The benefits of combining scaffolds with bioactive agent-loaded nanomaterials are: (i) the reduction of side effects, (ii) the protection of the bioactive agent from the degradation (iii) multiple bioactive agent delivery and (iv) the possibility of a sustained release (Santo, Gomes, *et al.*, 2012; Monteiro *et al.*, 2015). **Figure 2** demonstrated the combination of nanomaterials with scaffold systems. The nanomatrials can be confined within the scaffold and become free upon degradation of the scaffold by the cell (Yamauchi, Kato and Iwata, 2005). In another concept, nanomatrials can be immobilized onto the surface of scaffold. There are two ways of immobilization: (i) non-specific immobilization, which means that the nanosystems are adsorbed at the scaffold and are easily released during the cell culture at each medium exchange; (ii) specific immobilization where the nanomaterials are covalently bound at the surface of the scaffold, improving their stability (Nelson Monteiro *et al.*, 2014). The strategies used for nanomaterials-scaffolds combination have been already reviewed elsewhere (Rampichova, 2012; Wang *et al.*, 2012; N Monteiro, Martins, *et al.*, 2014; N Monteiro, Ribeiro, *et al.*, 2014)(Engel *et al.*, 2007). The bioactive agent delivered can be of two types: (i) growth/differentiation factor, by the introduction of the appropriate bioactive agent-loaded nanomaterials into the scaffold; and (ii) nucleic acid delivery, by cell delivery or by the introduction of the DNA (or RNAi) into a specific nanomaterial that silences (or encodes) a specific protein (Nelson Monteiro *et al.*, 2014). **Table 3.2** demonstrates the applications of

several types of nanomaterials combined with scaffolds for tissue engineering approaches.

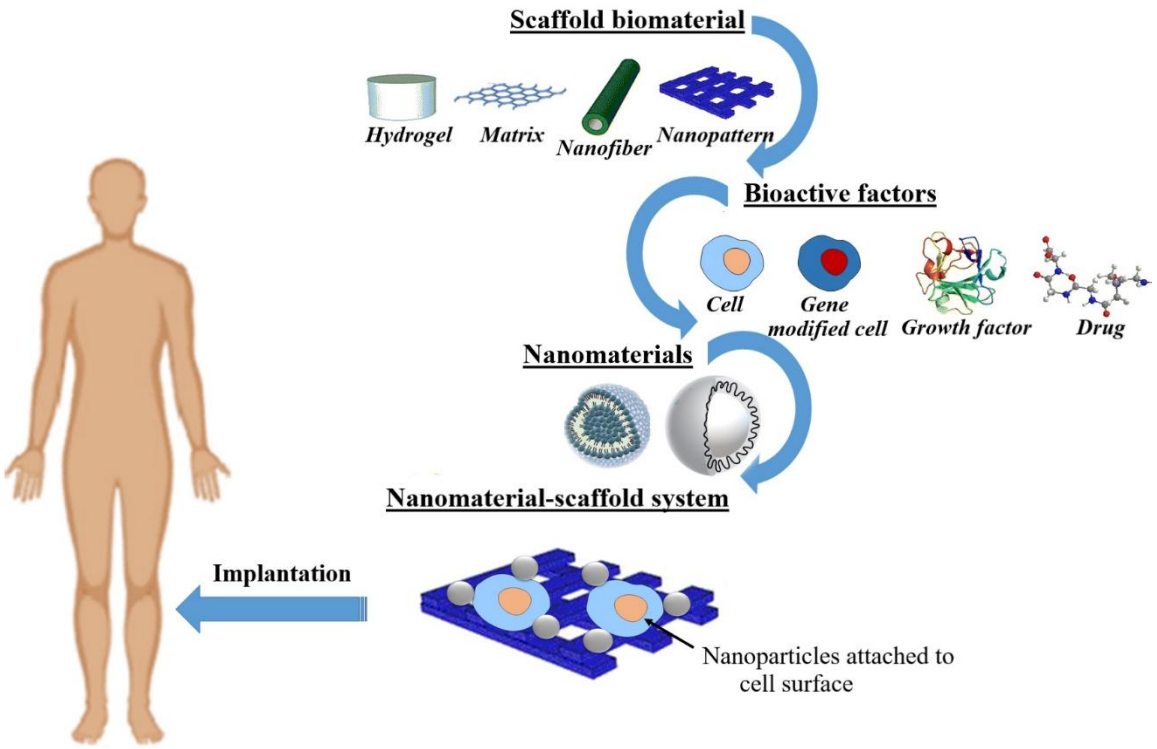

Figure 2: Combination of nanomaterials with scaffold systems.

Nanocarrier	Scaffold	Bioactive agent	Application	Reference
Liposomes	scaffold	TGF- β1	Cartilage regeneration	(P Giannoni and Hunziker, 2003)
	PCL-scaffold	Dex	Bone regeneration	(N Monteiro, Martins, *et al.*, 2014)
	PCL-scaffold	Runx2-plasmid	Bone regeneration	(N Monteiro, Ribeiro, *et al.*, 2014)
	Nanofibrous scaffold	TGF- β, bFGF, IGF-I	Cartilage regeneration	(Mickova *et al.*, 2012)
Polymeric nanoparticles	Collagen/chitosan scaffolds	TGF- β1 plasmid	Cartilage regeneration	(Cao *et al.*, 2012)
	Collagen sponge scaffold	BMP-2	Bone regeneration	(Zhang *et al.*, 2010)
	Chitosan Scaffolds with hyaluronic acid	TGF- β1 plasmid	Cartilage regeneration	(Lu *et al.*, 2013)
	PLGA scaffold	Anterior cruciate ligament	Ligament replacement	(Cooper, 2005)
	PCL scaffold	siRNA	Bone regeneration	(Andersen *et al.*, 2010)
	PEG and PCL	Rabbit	Chondrogenic	(Park, 2007)

	scaffold	chondrocytes	differentiation	
Dendrimer	Polyurethane sponge HA scaffolds	Dex	Bone regeneration	(Oliveira *et al.*, 2010)
Micelles	Gelatin scaffold	Triptolide and BMP-2	Bone regeneration	(Ratanavaraporn, Juruya and Tabata, 2012)
	Gelatin hydrogel with lactic acid oligomer	Dex	Bone regeneration	(Santo, Sato, *et al.*, 2012)
Inorganic nanoparticles	Magnetic NPs containing liposomes	hMSCs	Ischemic diseases	(Ishii *et al.*, 2011)
	Gold NPs	MSCs	Vascular regeneration	(Chen *et al.*, 2018)
	Cerium oxide NPs	HUVECs	Angiogenesis treatment	(Das *et al.*, 2012)
	ZnO NPs	HUVECs	Angiogenesis treatment	(Augustine *et al.*, 2014)

Table 2- Examples of nanomaterials combined with scaffolds for tissue engineering approaches.

TGF-b1 transform growth factor b; PCL-polycaprolactone; Dex-dexamethasone; Runx2- runt-related transcription factor 2; FGF-fibroblast growth factor; IGF-I-insulin-like growth factor I; BMP-bone m orphogenetic protein; PLGA – poly (lactic acid-co-glycolic acid); siRNA e small interfering RNA; PEG – poly (ethylene glycol); NPs- nanoparticles; hMSCs- human mesenchymal stem cells; MSC- mesenchymal stem cells; HUVECs - human umbilical vein endothelial cells; ZnO-zinc oxide.

3.4.1 Growth/differentiation factors delivery

Cell activities such as differentiation, proliferation, migration, and protein expression are mediated by growth factors. Growth factors are endogenous polypeptides, normally produced by healthy cells and act through the cell-surface receptors regulating cellular activities which in turn lead to tissue regeneration (Quaglia, 2008). In certain cases, where tissue regeneration does not occur naturally, growth factors need to be delivered exogenously (**Figure 3.a**) (Vasita and Katti, 2014). Numerous studies have demonstrated the use of nanomaterial-scaffold systems as carriers for the controlled delivery of growth factors improving stem cell proliferation and differentiation *in vitro* (**Table 3.3**). Rampichova et al. noticed that the conjugation of fetal bovine serum loaded liposomes with poly (2-hydroxyethyl methacrylate) scaffold enhanced the adhesion and the proliferation of chondrocyte (Rampichova, 2012). The *in vitro* and *in vivo* studies carried by Park et al. revealed that transplanted human mesenchymal stem cells encapsulated in a fibrin hydrogel containing heparinized nanoparticles loaded with TGF-β3 constitute a clinically efficient tool for the regeneration of hyaline articular cartilage (Park *et al.*, 2011). Furthermore, it was found that PLGA scaffolds containing IGF-I and TGF-b1 loaded PNIPAM nanoparticles provided more expression of collagen type II and aggrecan than those containing free or single growth factors (Ertan *et al.*, 2013). Several studies revealed that the encapsulation of growth factors in nanomaterials provides slow delivery in tissue engineering approaches (Paolo Giannoni and Hunziker, 2003; Jung *et al.*, 2009). Jung et al. noticed that the in situ chondrogenic differentiation of the hASCs was induced and sustained by the continuous release of TGF-b1 from

pluronic heparin NPs seeded onto PCL scaffolds (Jung *et al.*, 2009). Similarly, Giannoni et al. stated that the conjugation of TGF-b1-loaded liposomes with scaffolds represents a suitable means of establishing a slow release over a period of some weeks, enhancing its release kinetics and local efficacy and avoiding the typical side effects upon systemic administration. For instance, they found that only 40% of TGF-beta1 had been released from fibrin clots containing liposome, as compared with those containing the free molecules, where 68% were released after 25 days (Paolo Giannoni and Hunziker, 2003).

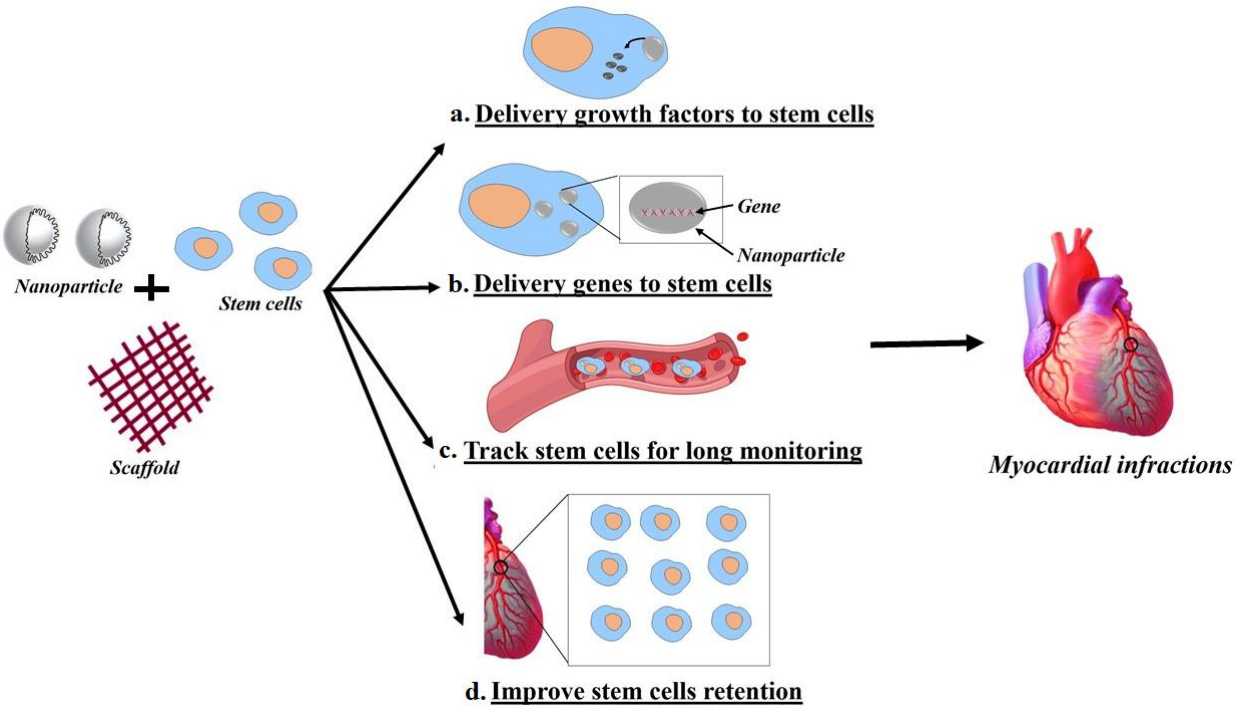

Figure 3: Application of nanomaterial-scaffolds system in tissue engineering and regenerative medicine (Example of myocardial infractions).

Growth factor	Material	Comments	Application	Reference
TGF- β1	Liposome-scaffold	Less side effects upon systemic administration	Cartilage regeneration	(P Giannoni and Hunziker, 2003)
FBS	Poly(2-hydroxyethyl methacrylate) fibrous scaffold - liposomes	Enhanced chondrocyte adhesion and proliferation	-	(Rampichova, 2012)
TGF- β1	PLCL scaffold - PLGA/Pluronic heparin NPs	Differentiation of hASCs to chondrogenic lineage was induced and sustained.	Cartilage regeneration	(Jung *et al.*, 2009)
IGF-I + TGF1	PLGA scaffolds - PLGA and PNIPAM NPs	Improved expression of collagen type II and aggrecan	Cartilage regeneration	(Ertan *et al.*, 2013)
TGF-β3	Fibrin hydrogel - heparin NPs	hMSCs embedded in fhydrogels proliferated more readily in samples containing TGF-β3-loaded nanoparticles compared to those containing the fibrin hydrogel alone.	Cartilage regeneration	(Park *et al.*, 2011)
BMP-2	Fibrin hydrogels with heparin - PLGA NPs	Obtention of more mature and highly-mineralized bone	Bone regeneration	(Chung *et al.*, 2007)
BMP-2	Fibrin hydrogel - heparin NPs	More potent effects of hMSCs on bone regeneration for fibrin containing encapsulated form than those with un-encapsulated form.	Bone regeneration	(Park *et al.*, 2009)
BMP-2 + BMP-7	Chitosan-PEO scaffolds - PLGA and PHBV NPs	Synergistic effect of using two growth factors on alkaline phosphatase expression.	Bone regeneration	(Yilgor *et al.*, 2009)
BMP-2	Collagen sponge scaffold - PEI coated albumin NPs	reduced initial burst release and prolonged retention at implantation site.	Bone regeneration	(Zhang, Doschak and Uludag, 2009)

TGF-b1-transform growth factor b1; FBS-fetal bovine serum; PLCL-poly(lactide-co-caprolactone); PLGA-poly(lactic acid-co-glycolic acid; NPs-nanoparticles; HASCs- human adipose tissue-derived stem cells; IGF-I-insulin-like growth factor I; PNIPAM - poly(N-isopropylacrylamide); hMSCs-human mesenchymal stem cells; BMP- bone morphogenetic protein-2; PEO-poly(ethyleneoxide); PHBV-poly(3-hydroxybutyrateco-3-hydroxyvalerate); PEI- polyethylenimine.

Table 3. Examples of nanomaterials-based strategies for growth factors release in tissue engineering and regenerative medicine approaches.

3.4.2 Therapeutic gene delivery

The use of growth/differentiation factors is challenging as they have short half-lives and may denaturate during the encapsulation processes. Furthermore, the long time periods that cells took to reach the differentiated state, the use of combinations of growth factors and the difficulty to differentiate the cells into one specific lineage are considered as other factors that limit the use of growth/differentiation factors in tissue engineering approaches (Yau *et al.*, 2012). Engineering tissues through gene therapy to synthesize and secrete proteins like growth factors represents an alternative approach to overcome these limitations and to control stem cell differentiation (Galli *et al.*, 2021). Gene therapy can be used for gene augmentation, gene editing, or gene silencing depending on the final goal (Itziar, Rodr and Vicente-pascual, 2020). Gene therapy consists on the insertion of the vector containing the functioning gene (nucleic acid) into the host cell to replace a hereditary genetic defect or, to provide a new function such as overexpressing growth factors or even killing undesirable cells (like cancer cells) (**Figure 3.b**) (Nelson Monteiro *et al.*, 2014). Diverse nucleic acids are being used for this purpose. DNA and messenger RNA (mRNA) provide protein expression, whereas, microRNA, small interfering RNA (siRNA), aptamers or oligonucleotides induce gene silencing (Pozo-Rodríguez *et al.*, 2019). The *ex vivo* technique, generally carried out for the delivery of genes, uses autologous cells that are recovered from the patient's body. The cells are generally transduced with viral or non-viral vectors and re-inserted into the target tissue (Winn *et al.*, 2005). Viral vectors (i.e. lentivirus and retrovirus) are known for their high transfection efficiency and transgene expression. Non-viral vectors (i.e. polymers, liposomes, cationic lipids and proteins) have comparatively lower transduction efficiency, but they have been widely proposed due to their easy production, safety, tissue selectivity, effective delivery, higher pDNA size encapsulation and flexibility in formulation. Nanomaterials–scaffold systems may be effective in tissue engineering applications by delivering genes in a cell-controlled, efficient, and spatially localized manner. The combination may obviate the issues of toxicity, silencing efficiency and long-term expression (Nelson Monteiro *et al.*, 2014). Numerous studies have been conducted to apply these systems for gene delivery improving stem cell proliferation and differentiation *in vitro* (**Table 3.4**). Guo et al. found that the repair of cartilage defects in rabbits' knees was enhanced by TGF-b1 gene-modified-tissue engineering of cartilage on the basis of a strategy using autologous MSCs, chitosan scaffold and liposomal transfection (Guo *et al.*, 2007). Furthermore, Kulkami et al revealed that fibrin microspheres can be utilized for gene delivery to extend the capacity of a fibrin scaffold to preserve the structural integrity and functional integrity of DNA (Kulkarni *et al.*, 2011). In another study, it was observed that BMP-4- transfected ASCs seeded onto PLLGA scaffold significantly improved *in vivo* chondrogenesis in a rabbit articular defect model (Shi *et al.*, 2013). Moreover, the transfection efficiencies of the liposome and PEI complexes were enhanced *in vitro* when released from resorbable gene-activated matrix (Bolliet, Bohn and Spector, 2008). Liu et al. investigated carbon nanotube delivery of siRNA against CXCR4, a cell-surface receptor necessary for HIV entry into human T cells and found that 90 % silencing efficiency was observed upon incubation for 3 days (Liu *et al.*, 2007).

Gene	Material	Comments	Application	Reference
pDNA-NGF	fibronectin-coated PLG	Polymer-mediated transfection of HEK293T with an NGF-encoding plasmid resulted in outgrowth by dorsal root ganglia neurons.	Neural regeneration	(Whittlesey and Sheaa, 2006)
pDNA-bGal-EGFP	fibrin scaffold	the fibrin-lipoplex system produced higher transfection efficiency for two reporter genes at day 7.	Multiple gene delivery	(Kulkarni *et al.*, 2009)
pDNA	fibrin scaffold–fibrin microsphere	fibrin microspheres can extend the capacity of fibrin scaffolds and were able to degrade and release DNA differentially.	Gene delivery	(Kulkarni *et al.*, 2011)
pDNA-encoding BMP4	GAM-liposomes	GAM technology enhances the release duration and functional stability of incorporated DNA.	Bone regeneration	(Bolliet, Bohn and Spector, 2008)
pDNA-TGF-b1	chitosan scaffolds-liposome	defect filled with regenerated hyaline-like cartilage tissue twelve weeks after implantation.	Cartilage regeneration	(Guo *et al.*, 2007)
BMP4 plasmid	PLLGA scaffold- PLGA NPs	improved *in vivo* chondrogenesis in a rabbit articular defect model.	Cartilage regeneration	(Shi *et al.*, 2013)
si-RNA	PCL scaffold-PCL NPs	gene silencing of $TRIB_2$ and BCL_2L_2 in enhancing adipogenic osteogenic and differentiation, respectively.	Bone regeneration	(Andersen *et al.*, 2010)
siRNA	PEG-PAMA-Gold NPs	significant RNAi activity in HuH-7 cells.	Gene delivery	(Motoi *et al.*, 2006)
siRNA	Carbon nanotubes	90 % silencing efficiency was observed upon incubation for 3 days.	HIV treatment	(Liu *et al.*, 2007)
HER_2 siRNA	Quantum-dot based chitosan NPs	gene silencing was occurred after functionalization of the particle surface with HER_2 that target specific receptors on the cell surface	Gene delivery	(Tan, Jiang and Zhang, 2007)
DNA	Silica NPs	the uptake of DNA into cells was shown by adsorbing on the cell surface.	DNA vaccination	(Luo *et al.*, 2004)

pDNA-Luc-EGFP	PLGA-Polyplexe	enhanced reporter gene expression up to 1500-fold, compared to unmodified substrates.	gene delivery	(Bengali *et al.*, 2009)
pDNA-bGal, pDNA-Luc-EGFP	lipoplexe	the presence of the peptide in the lipoplex increased internalization efficiency by up to 4.5-fold.	gene delivery	(Rea *et al.*, 2009)
pDNA-BMP2-VEGF-BMP-2-VEGF	liposome	high levels of BMP-2 and hVEG are secreted.	bone regeneration	(Guo-ping *et al.*, 2010)

GAM- gene-activated matrix; NGF- nerve growth factor; PLG- poly(lactide-co-glycolide; TGF- transforming growth factor; EGFP- enhanced green fluorescent protein; bGal- encoding b-galactosidase; MSCs- mesenchymal stem cells; PLLGA - poly(Llactic-co-glycolic acid); HA- hydroxylapatite; PEG–PAMA, poly(ethylene glycol)-block-poly(2-(N,N-dimethylamino)ethyl methacrylate) copolymer; PLGA- poly(lactide-co-glycolide); Luc-luciferase; BMSCs-Bone marrow mesenchymal stem cells; BMPs- Bone morphogenetic proteins; VEGF- vascular endothelial growth factor; hBMP-2- human bone morphogenetic protein 2; hVEGF165- vascular endothelial growth factor 165.

Table 4. Examples of nanomaterials-based strategies for gene delivery in tissue engineering and regenerative medicine approaches.

3.4.3 Cell delivery

Cell therapy consists on introducing new cells into a tissue in the aim of treating a specific disease. Cell-based tissue engineering is one of the most widely used tissue engineering methodologies in the literature and is linked with particular benefits. These include the direct placement of cells into a defected tissue, which decreases considerably the expected lag phase for progenitor cell recruitment to the defect site (Galli *et al.*, 2021). The most widely used stem cells include periodontal ligament cells, bone marrow stromal cells, endothelial progenitor cells, dental pulp stem cells, mesenchymal stem cells, etc (Hare *et al.*, 2009; Peters, 2018; Galli *et al.*, 2021).

Nanomaterials play a paramount role in regulating stem cell behavior because of their special characteristics, including size, shape, surface charge, and surface area. Many studies have been reported in the literature investigating the role of nanomaterials in stem cells based-therapy, as illustrated in **Table 3.5**. The advantage of applying nanomaterials include tracking the stem cells *in vivo* for long-term (**Figure 3.c**) (Y. Sun *et al.*, 2020). SPIONs mark stem cells by three main ways: (i) attaching NPs to the cell surface, (ii) receptor-mediated endocytosis, and (iii) transfecting agent-mediated endocytosis (Frank *et al.*, 2002). The first approach is always limited due to the fact that the reticuloendothelial system recognizes and clears SPION-labeled cells. However, they can persist in the cytoplasm of stem cells where they are biocompatible through the internalization pathway (Y. Sun *et al.*, 2020). QDs are widely used in long-term monitoring in living cells, as compared to traditional fluorescent probes (Liu *et al.*, 2019). Numerous studies have investigated the possibility of labeling stem cells through several modifications like electroporation (Sun *et al.*, 2014), bioconjugation (Shah and Mao, 2011) and delivery by phospholipid micelles (Dubertret *et al.*, 2002), all of which preserve the safety and stability of the label. Silica dioxide NPs are used as ultrasound contrast agents, widely associated with radionuclides, fluorescein or helium ions to enhance the imaging of the stem cells, hence facilitating stem cell tracking (Accomasso *et al.*, 2012).

Another advantage of applying nanomaterials in cell based therapy is the improvement of the stem cells retention after their transplantation (**Figure 3.d**). Stem cells can be administrated via several routes (Y. Sun *et al.*, 2020). The application of stem cell therapy is generally limited by the low cell retention after transplantation. It has been reported that NPs can increase stem cell retention by creating a cardiac-specific extracellular matrix environment, promoting the interactions with cardiomyocytes and affecting the focal adhesion complex of myocardial MSCs (Popara *et al.*, 2018). In another study, it has been noticed that applying SPION-labeled MSCs with a magnetic field might improve cell homing of MSCs at the site of injury and result in enhancing the cardiac function and attenuating the injury after heart failure (Naseroleslami, Aboutaleb and Parivar, 2018).

Application of nanomaterials in stem cells tracking						
Stem cells	**Nanomaterial**	**Imaging modality**	**Model**	**Application**	**Properties**	**Reference**
hMSCs	Au@BSA@PLL nanotracers	CT	Mouse	Lung repair	labeled MSCs were tracked for up to 23 d after transplantation by CT imaging.	(Ning *et al.*, 2019)
hMSCs	USPIO	MRI	IHD Patient	Heart disease	labeled MSCs could be tracked by semiautomated regional T2 relaxation time analysis 14 days after transplantation at injection sites.	(Mathiasen *et al.*, 2019)
hESC-CPCs	Iron oxide NPs	MRI	Pig	Heart disease	MRI is effective in tracking the location and dispersion of cell grafts.	(Skelton *et al.*, 2016)
oligodendrocyte progenitors	Magnetic NPs	MRI	Rat	Neuron repair	the iron particles remain localized and are not taken up by other cells even after 14 days.	(Bulte *et al.*, 1999)
hESC-CMs	PANPs	PAI	Rat	Heart disease	PANPs provide high efficiency for cells labeling without adverse effects on cell function, structure, and gene expression	(Qin *et al.*, 2018)
Neural stem cells	Au-MION	MRI	Rat	-	MRI signals correlated with gold-positive staining of transplanted cells.	(Wang, Lee and Holmström, 2006)
hMSCs	SiO_2-NPs	Immunofluorescence	Rat	Heart disease	SiO_2-NPs show high compatibility in terms of lack of cyto- and genotoxicity.	(Gallina *et al.*, 2015)
Application of nanomaterials in improving stem cells retention after transplantation						
Stem cells	**Nanomaterial**	**Model**	**Application**	**Properties**		**Reference**
hMSCs	SiO_2-NP	*In vitro*	Heart disease	increased *in vitro* adhesion capacity of hMSCs		(Popara *et al.*, 2018)
MSCs	SPION	Rat	Heart disease	improve cell homing of MSCs at the site of injury and result in enhancing the cardiac function and attenuating the injury after heart failure		(Naseroleslami, Aboutaleb and Parivar, 2018)

hMSCs- ; AuNP-Gold nanoparticles; BSA-bovine serum albumin; PLL- poly-L-lysine; CT-computer tomography; hESC-CPCs - embryonic stem cell-derived cardiac progenitors; USPIO-ultrasmall paramagnetic iron oxide; MRI-magnetic resonance imaging- IHD-ischemic heart disease; hESC-CMs - embryonic stem cell-derived cardiomyocytes ;PAI- photoacoustic imaging; PANPs-photoacoustic nanoparticles; Au-MION-monocrystalline iron oxide nanoparticles; hCPC-human cardiac progenitor cell; SPION- superparamagnetic iron oxide nanoparticles.

Table 5. Examples of nanomaterials-based strategies for cell therapy in tissue engineering and regenerative medicine approaches.

3.5 Conclusion

In this chapter, we spotlight the inimitable and advanced features of nanomaterials for rapid development of nanotechnology-based tissue engineering and regenerative medicine applications. Numerous studies have revealed the potential of nanomaterials–scaffold systems in that field through delivering growth/ differentiation factors and genes in a cell-controlled, efficient, and spatially localized manner and regulating stem cell behavior by improving stem cells tracking and retention after transplantation. Several kinds of nanomaterials are used to protect, control the release of bioactive molecules, decrease the site effects and track the cells. They include polymeric, lipid and inorganic based materials. Each material has its own features; polymers can improve the efficiency, enhance the targeting specificity and reduce the cytotoxicity; lipids have been approved for multiple clinical trials; while, inorganic systems have good storage stability, no microbial attack and simple fabrication requirements. Therefore, the choice of the appropriate system is a crucial key to gain specific requests.

3.6 Reference

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