Enhancing regenerative approaches with nanoparticles

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In this review, we discuss recent developments in the field of nanoparticles and their use in tissue regeneration approaches. Owing to their unique chemical properties and flexibility in design, nanoparticles can be used as drug delivery systems, to create novel features within materials or as bioimaging agents, or indeed these properties can be combined to create smart multifunctional structures. This review aims to provide an overview of this research field where the focus will be on nanoparticle-based strategies to stimulate bone regeneration; however, the same principles can be applied for other tissue and organ regeneration strategies. In the first section, nanoparticle-based methods for the delivery of drugs, growth factors and genetic material to promote tissue regeneration are discussed. The second section deals with the addition of nanoparticles to materials to create nanocomposites. Such materials can improve several material properties, including mechanical stability, biocompatibility and biological activity. The third section will deal with the emergence of a relatively new field of research using nanoparticles in advanced cell imaging and stem cell tracking approaches. As the development of nanoparticles continues, incorporation of this technology in the field of regenerative medicine will ultimately lead to new tools that can diagnose, track and stimulate the growth of new tissues and organs.

1. Introduction

The world faces major health challenges due to a combination of an ageing population and lifestyle changes resulting in a rapid increase of chronic disease. In response, current healthcare systems have developed to treat symptoms and to slow disease progression. Regenerative medicine offers an alternative to this regime—a switch from ‘care’ to ‘cure’ by harnessing the body’s own capability to regenerate and delivering this potential to restore health rather than protract decline. A popular strategy within regenerative medicine is the design of materials that can guide endogenous regeneration by making use of the natural regenerative mechanisms present in the human body. The integration of new technologies to create instructive material scaffolds able to exert or trigger a function within the body could improve the biological and mechanical properties and ultimately clinical application of such materials. At the same time, stem cell-based therapies possess an astonishing potential to amplify the body’s natural repair processes. To further progress the potential of cell-based therapies requires the development of techniques that allow the close monitoring of these cells and ways to improve the efficacy of these therapies.

The use of nanotechnology to improve current approaches in tissue and organ regeneration has received increased attention over the years. In particular, nanoparticles offer interesting features to advance the field of regenerative medicine. Nanoparticles are solid colloidal particles with dimensions usually between 10 and 200 nm and offer great versatility in terms of size, surface chemistry and components. Due to their size and surface chemistries, nanoparticles can be exploited as theranostic agents or as carriers for the delivery of drugs, genetic material or growth factors (GFs). Indeed, a variety of nanoparticles has been developed for therapy; among them dendrimers, liposomes, polymer-based nanoparticles, micelles, carbon nanotubes and many more. Several nanoparticle drug delivery systems have undergone rapid clinical development for the treatment of cancer [1].
recent years, increased focus has been on making these nanoparticles ‘smart’ by adding features so that they can react to their biological environment to allow, for example, controlled drug release. This has been achieved by engineering these particles so that they can respond to changing enzyme levels, pH levels or redox levels [2–4]. Other efforts have been directed at creating nanoparticles that can actively target specific (diseased) cell populations by attaching cell targeting ligands to the outer surface of the nanoparticles [5,6]. These and other efforts have resulted in the creation of a wealth of knowledge on how to engineer nanoparticles and increased understanding of their interactions within biological environments. Owing to this knowledge and flexibility in nanoparticle design, this field is at the right stage to allow application in other medical fields such as regenerative medicine and tissue and organ regeneration in particular. This review aims to provide an overview of research in this area focusing on developments in nanotherapeutic strategies to deliver drugs, GFs and genetic material promoting tissue regeneration. Furthermore, the addition of nanoparticles to biomaterials to improve their mechanical stability, biocompatibility and biological activity will be described. The last section will deal with the emergence of a relatively new field of research using nanoparticles in advanced cell imaging and stem cell tracking approaches (figure 1).

2. Nanoparticles as delivery carriers in bone regeneration

Bone is a versatile tissue as it provides shape, mechanical support and protection for the body while at the same time facilitating movement. The skeletal system also plays an equally important role in mineral homeostasis and participates in the regulation of energy metabolism. Bones can undergo remodelling, allowing them to adapt to mechanical stress, maintain bone health and repair small injuries. Specifically, osteoclasts and osteoblasts are the specialized bone cells that are responsible for the resorption of bone tissue and bone formation, respectively. However, these mechanisms are not able to repair large bone defects caused by injury, disease or congenital malformations. Bone defects or loss of bone, whether caused by congenital disorders, trauma or disease, affect over 20 million people annually. The standard and currently most effective treatment for bone defects consists of transplantation of a patient’s own bone, which has severe drawbacks including limited availability, additional surgical site and time, and donor site morbidity. Despite recent advances in the development of biomaterials as bone graft substitutes for the regeneration of large, clinically relevant defects, most synthetic solutions remain inferior to natural bone grafts in their regenerative potential and are limited to non-weight-bearing applications [7]. Therefore, there is a clinical unmet need for new methods and materials able to stimulate bone formation while providing enough support.

Over the years, there has been increased interest in the development and direct administration of therapeutic agents to promote (bone) tissue regeneration. However, direct administration often has limitations, including degradation, non-specificity and poor cell uptake. This results in the need to use increased doses, which enhances the risk of adverse effects and additionally is not cost-effective. Nanoparticle-based carriers could increase control over the pharmacokinetics of such agents owing to their ability to carry high concentrations of (insoluble) therapeutics, while
protecting them from degradation. In addition, nanoparticles can be modified to allow for controlled drug delivery as well as specific cell and tissue targeting. In the following subsections, the use of nanoparticles as carriers for agents stimulating bone tissue regeneration will be discussed (figure 2). In particular, a large research area is focused on harnessing the use of GFs and other biomolecules in tissue engineering strategies.

2.1. Delivery of growth factors using nanoparticles
The process of bone repair involves a complex cascade of biological events controlled by numerous cytokines and GFs, such as bone morphogenetic proteins, vascular endothelial growth factor (VEGF), platelet-derived growth factor and transforming growth factor-beta 1. The local presence of these GFs at the site of injury is of key importance to trigger healing and regenerative processes [8]. GFs are generally secreted by a wide range of cell types and regulate cellular activities, such as migration, differentiation and proliferation. Over the past few years, there has been increased interest in harnessing the inherent regenerative properties of GFs to stimulate tissue regeneration in patients with large bone defects. Although many recombinant GFs and cytokines are now available, currently employed delivery methods experience insufficient local retention and require high amounts of protein to exert a biological effect, especially in larger animal models and humans [9]. This can be explained by the fact that GFs generally display a short biological half-life in circulation, and they undergo rapid degradation after systemic administration, since they generally show high amounts of protein to exert a biological effect, especially in larger animal models and humans [9]. This can be explained by the fact that GFs generally display a short biological half-life in circulation, and they undergo rapid degradation in vivo [10]. As a result, treatment likely consists of repeated administration but this in turn may lead to undesirable systemic effects and toxicity due to the non-specific distribution and accumulation of GFs throughout the body. Therefore, a delivery system that can protect the GF from degradation while bringing it to the site of injury would greatly benefit GF treatment options. There have been many attempts in recent years to achieve controlled delivery of GFs by the use of carriers. These attempts have been focused on the use of micro- and nanoparticles, injectable gels and composites [11–13].

The use of nanoparticles is an interesting approach as they may offer improved transport properties and pharmacokinetics profiles in vivo after systemic administration, since they generally display deep tissue penetration allowing more efficient delivery of therapeutic agents to target sites [14]. Moreover, their nanoscale dimensions convey remarkable physiochemical properties allowing the ability to optimize many fundamental properties, such as solubility, diffusivity, biodistribution, release characteristics and immunogenicity. Several types of nanoparticulate compositions have been investigated as delivery systems for tissue regeneration. One of the most commonly used nanoparticle systems are synthetic polymers such as poly(ε-caprolactone) (PLA) or poly(L-lactide-co-glycolide) (PLGA). These are biodegradable, easy to manufacture and importantly, their release profiles can be tailored. Several studies have indicated that such systems are useful delivery systems to promote fracture healing [15]. For example, Park et al. recently demonstrated an interesting approach where they manufactured PLAGA biodegradable nanoparticles for co-delivery of runt-related transcription factor 2 protein and bone morphogenetic protein 2 (BMP-2) plasmid DNA to human mesenchymal stem cells (hMSCs). They showed that hMSC transfection of such PLAGA nanoparticles significantly enhanced osteogenesis [16]. Also natural polymers such as collagen, gelatin, albumin and chitosan have been investigated for this purpose. For example, chitosan nanoparticles were found to have enhanced rhBMP-2 loading and improved morphogen release kinetics due to the good affinity of chitosan for proteins and the large surface area of the nanospheres [17]. In a similar vein, PEgylated and polyethyleneimine (PEI)-modified bovine serum albumin nanoparticles were able to successfully deliver rhBMP-2 and promote new bone formation after implantation in rats [18]. Apart from organic biodegradable nanoparticles also inorganic nanoparticles, such as ceramic nanoparticles (silica, alumina), metals, metal oxides and metal sulfides, have been investigated as drug delivery systems for tissue engineering. Perhaps the most researched particles in this class within the field of bone regeneration are calcium phosphate nanocarriers due to their close resemblance to human bone [19]. For example, in one study, silk-coated hydroxyapatite (HA) nanocarriers were able to show in vitro sustained release of encapsulated GF BMP-2 over a period of 21 days with increased proliferation and osteogenesis compared to non-encapsulated BMP-2 [20].

Inorganic mesoporous silica nanoparticles (MSNs) have received increased interest over recent years due to their favourable chemical properties, thermal stability and biocompatibility. In addition, silica can be easily functionalized, for instance, to modify surface properties and link therapeutic molecules. Indeed, MSNs containing immobilized bone growth factor (BMP-2) on their surface promoted osteogenic differentiation of hMSCs [21]. Zhou et al. expanded this approach by in addition to immobilizing BMP-2 on the surface also loading dexamethasone (DEX) into the pores of the particle. They showed that the dual-incorporated BMP-2 peptide and DEX MSNs could act synergistically to enhance osteogenic differentiation of bone marrow stromal cells [22].

2.2. Delivery of synthetic molecules and other approaches in bone tissue engineering
Where the delivery of GFs is based on stimulating the bone-forming cells, the osteoblasts, another therapeutic strategy could be to inhibit the bone-resorbing cells, the osteoclasts. The function of these cells is closely coupled to that of the osteoblasts and are critical in the maintenance, repair and remodelling of bones. Bisphosphonates, a class of osteoporosis-antagonizing drugs, are used to treat osteoporosis by promoting osteoclast apoptosis; however, they display poor bioavailability. Several types of nanoparticles, such as biodegradable PLAGA and gold nanoparticles have been used to deliver bisphosphonate drugs [23,24]. For example, in a recent study, gold nanoparticles were conjugated to alendronate, a bisphosphonate drug used in the clinic. The gold nanoparticles themselves, alendronate and gold nanoparticles conjugated with alendronate all suppressed osteoclast formation in a dose-dependent manner. Furthermore, in an in vivo model, mice treated with conjugated gold nanoparticles had higher bone density compared with mice groups treated with the drug or gold particles alone [25]. Interestingly, because bisphosphonates bind strongly to bone, these molecules can act both as drug and as a bone-targeting agent. This has been exploited for several approaches including bisphosphonate conjugated nanoparticles for bone tumour targeting [26].

A biomaterial, when implanted, can mediate a variety of adverse reactions including inflammation, and infection,
which may eventually cause implant failure. In addition, inflammation is known to impair bone formation especially in large wounds. For this reason, some researchers have looked into reducing inflammation with the use of nanoparticles. In one such study, chitosan nanoparticles were used as a vehicle for a non-steroid anti-inflammatory drug, diclofenac. These systems could inhibit the production of a principal mediator of inflammation (prostaglandin E2) in activated macrophages [27]. DEX steroid is an anti-inflammatory agent but it also inhibits osteoblast resorption [28]. Nanoparticles have been researched to allow for controlled local release of DEX. For example, local growth inhibition of osteoclast precursors could be accomplished by using surface-immobilized, DEX-containing nanoparticles [29].

2.3. Gene delivery
Another approach to modify the local concentration of biomolecules is by influencing the production of these proteins (protein expression levels) within the cells by means of gene delivery. This may have advantages over GF delivery as a sustained production of GFs can be achieved by gene transfection. In addition, gene therapy also allows the down-regulation of undesirable genes as well as the upregulation of, for instance, osteogenic genes. However, main concerns with this approach are the delivery of exogenous DNA to the nucleus while maintaining integrity and stability. In addition, many difficult steps need to be taken until protein expression is achieved; cell membrane internalization, cytosolic release, nuclear uptake, vector dissociation and protein expression. To achieve this, many nanoparticle-based vectors have been designed, including polymeric nanoparticle DNA encapsulation, DNA PEGylation, micelles, liposomes, dendrimers and nanosized inorganic material systems [30]. In the field of bone tissue engineering, several proof-of-principle studies using green fluorescent protein-encoding reporter plasmids have been performed, demonstrating that different nanoparticle carriers are able to deliver plasmids into bone cells [31–33]. Moreover, some studies have shown successful delivery of GF genes. For example, a study using PEI, a cationic polymer which can interact with negatively charged DNA, for the delivery of plasmids containing the BMP-2 gene has been described [34]. In addition, PEI-conjugated gold nanoparticles complexed with plasmids containing the BMP-7 gene have been administered in rabbits to alter wound healing and fibrosis [35]. These studies illustrate that this may be a feasible approach for bone tissue regeneration.

Similarly, small interfering ribonucleic acid (siRNA) can be used to silence undesirable genes. Zhang et al. developed a targeting system based on cationic liposomes attached to oligopeptide ((AspSerSer)) for the delivery of osteogenic siRNA that targets casein kinase-2 interacting protein-1 specifically to bone-formation surfaces. The authors showed that this approach markedly promoted bone formation, enhanced the bone micro-architecture and increased the bone mass in both healthy and osteoporotic rats [36]. In another study, the same authors reported the fabrication of a site-specific bone-targeting drug delivery system from polymeric nanoparticles incorporating sema4D siRNA and demonstrated their cellular uptake and intracellular trafficking within osteoclasts, which prevented the suppression of osteoblast activity [37].

3. Biomaterials functionalized with nanoparticles
In the past few decades, a large part of the field of tissue regeneration has focused on preparing biomaterials that can replace, regenerate or repair damaged cells or tissues. Such materials should provide enough support, while serving as a lattice for cell adhesion, movement and tissue ingrowth. In the context of bone tissue regeneration, these biomaterials need to be biocompatible and biodegradable within a certain time-frame, provide enough mechanical support and possess osteoinductive and osteoconductive properties. Materials such as biodegradable polymeric materials and ceramics are actively investigated as bone tissue engineering materials; however, they have not been able to fully address all types of bone defects, especially large bone defects. Nanoparticles could help improve the regenerative capabilities of these and other biomaterials by offering ways to closely control surface and mechanical properties. Moreover, incorporation of nanoparticles within biomaterials can also improve their biological performance, such as increased cellular adhesion, differentiation and integration of stem cells into the surrounding environment. In addition, the drug delivery capabilities of nanoparticles offer additional possibilities to increase the biological performance of biomaterials. In the next subsections, these and other properties of nanocomposites will be discussed (figure 3).

3.1. Nanocomposite materials
One way to improve the tissue regenerating capabilities of biomaterials, both mechanically and biologically, is by combining materials to create composites. For example, ceramic–polymer composites have long been investigated in order to marry the...

Figure 2. Use of nanoparticles for drug delivery in bone tissue regeneration.
biological properties of calcium phosphate ceramics with the mechanical properties of polymers, and this has created some interesting materials [38]. However, such materials have not been able to fully address the clinical need. This may partly be due to the fact that while it is possible to improve the mechanical properties of ceramics by incorporating polymers, the bioactivity of the ceramic can thereby be compromised [39]. The incorporation of nanoparticles within biomaterials to create nanocomposites is an emerging new class of materials for bone fracture repair that can show improved mechanical and/or biological performance compared to analogous composites without nanoparticles. A wide range of nanostructured materials have been tested so far (e.g. using ceramics, polymers and hydrogels). Operating at the nanoscale level gives researchers the possibility to create biomaterials with novel physical properties. Especially the ability to manipulate at scales of around 100 nm is crucial because the classic laws of physics change. Indeed scale seems important in material design as many such nanomaterials have demonstrated superior properties compared to their micrometre-structured counterparts [40]. For example, HA is the native mineral structure of bone, which is a poor material for bone reconstruction in the microscale due to its brittleness and slow degradation rate. However, incorporation of HA nanoparticles into polymeric materials has created promising scaffolds for bone tissue engineering. For example, HA nanoparticles coated on top of polymeric PLGA facilitated bone regeneration in rat bone defects in a concentration-dependent manner, where increased exposure of HA nanoparticles on the surface resulted in accelerated bone deposition [41]. In a similar vein, PLA scaffolds coated with HA nanoparticles could stimulate the expression of osteogenic proteins (e.g. BMP-2, osteopontin) on scaffold-attached rat bone marrow-derived mesenchymal stem cells and facilitated bone regeneration of critical-sized bone defects [42]. Several other studies have shown that incorporation of HA nanoparticles improves cell adhesion, interaction, growth and osteoblast differentiation [43,44]. In addition to HA nanoparticles, incorporation of metallic nanoparticles prepared from, for example, titanium, and iron oxide could increase collagen and calcium deposition by osteoblasts, leading to enhanced tensile strength compared to non-metallic incorporated materials [45,46]. It has been hypothesized that the nanostructural topographical properties (nanotopography) of the materials rather than the chemistry play a critical role in the improved biological performance of nanocomposites. Indeed, natural tissue also is a nanostructured material, consisting of collagen fibrils and proteins with dimensions in the 100 nm size or less. This is also true for bone tissue, which is in fact a nanostructured composite composed of a polymer matrix (mainly collagen) reinforced with nanometre-sized ceramic particles (mainly carbonated HA). Therefore, by incorporating nanoparticles within biomaterials, a surface topography is created that may partly mimic the complex extracellular matrix that bone cells interact with, proliferate in, and differentiate within, which stimulates the bone cells to grow. Several studies, comparing implants with smooth surfaces to nanostructured surfaces and their ability to promote tissue growth, appear to support this claim [47]. For example, a study by Khang et al. showed that bone cells respond differently on submicrometre and nanometre scale titanium surfaces, and that small changes in nanometre surface features can have large consequences towards bone regeneration [48]. Therefore, it seems likely that not only the chemistry of biomaterials but also their topography needs to be considered when designing biomaterials for tissue regeneration.

Besides improving the biological properties of nanocomposites, there are numerous examples where nanoparticles were able to improve the mechanical properties of biomaterials. For example, nanoparticles of biphasic calcium phosphate have been shown to increase the tensile strength of a biomaterial composed of polyvinyl alcohol/gelatin nanomat [49]. Also carbon nanotubes can be used to significantly improve the mechanical properties of biomaterials [50] but are also associated with difficulties in dispersing due to strong van der Waals forces and physical entanglements, limiting their usefulness [51]. An alternative class of nanoparticle that shows high potential for use as polymeric reinforcement are MSNs. Even small loading ratios of silica nanoparticles within polymers have been shown to significantly increase the mechanical
properties compared with the polymer alone [52]. There are many other studies that have shown the advantages of incorporating nanoparticles within materials. Discussion of all these materials is outside of the scope of this review. We refer the reader to several excellent review articles describing different types of nanocomposite materials and their use in tissue engineering [53–57].

3.2. Controlled release of biomolecules from biomaterials

Another strategy to build bioactive materials for tissue regeneration is by incorporating biomolecules directly into the materials that can promote (stem) cell attachment and direct (stem) cell fate *in situ*. Thus, such materials not only act as a scaffold but also as a delivery vehicle for controlled release of bioactive molecules. Many examples exist where this approach has led to improved biological performance of biomaterials. However, the application of bioactive molecules, especially proteins, directly within scaffolds does not come without difficulties: the proteins may denature in the process, are often not completely released, and cannot be released in a sustainable and temporally controlled manner necessary for the long-term formation of bone, which makes their deployment complicated and expensive. There have been many elegant solutions to prevent these problems including chemical immobilization of the proteins or encapsulation within a delivery system based on, for example, networked hydrogels, microparticles or nanoparticles [10]. Using nanoparticles may have some advantages over the other approaches because of their drug delivery capabilities (as discussed in §2), inherent properties that improve the mechanical and biological properties of biomaterials (discussed in §3.1), and ease of functionalization. For example, BMP-2-coated PLGA nanoparticles within a fibrin hydrogel complex were found to be capable of enhancing bone regeneration in large-sized bone defects in rats [58]. And like BMP-2, BMP-7 encapsulated in PLGA nanoparticles within a nanofibrous PLA scaffold showed controlled release of BMP-7 followed by ectopic bone formation in rats [59]. In another approach, block copolymer nanolithography was used to tune the size and spacing of gold nanoparticles on a surface. The selective immobilization of BMP-2 on the gold nanoparticles offered the possibility of exactly controlling the amount of immobilized GFs down to the molecular level [60]. In addition to GFs, other proteins can be incorporated into biomaterials in order to stimulate bone tissue regeneration. For example, the extracellular matrix molecule osteopontin, a protein that plays an important role in bone remodelling, was incorporated in HA nanoparticles and its release from a degradable matrix was analysed for its osteoinductive potential in a dog bone defect model [61].

The majority of the developed nanocomposite materials for bone tissue engineering consider only the release of a single GF or biomolecule. This may be limiting the clinical success of such materials since there are many proteins that play a role in fracture repair [62]. In particular, in the process of bone regeneration, locally produced GFs mediate first the migration of osteoprogenitors to the defect site and subsequently the direct differentiation of osteoprogenitors towards specific cell lineages. In addition, they control cell proliferation, bone re-vascularization and the production of extracellular matrix. These processes are tightly regulated by numerous GFs and cytokines that regulate both the bone-forming process (osteogenesis) and the formation of new vessels (angiogenesis). For this reason, recent focus has been on incorporating a combination of several GFs within biomaterials. For example, chitosan nanocomplexes encapsulating PIGF-2 and BMP-2, GFs that stimulate angiogenesis and osteogenesis, respectively, indicated that the dual delivery of PIGF-2 and BMP-2 has a greater potential to regenerate bone tissue compared with the delivery of either GF alone [63]. In another approach, a dual GF delivery system was designed composed of PLGA nanoparticles and alginate microcapsules encapsulating BMP-2 and VEGF, respectively. The material showed a positive effect on the formation of vascularized bone which indicates a synergistic effect of BMP-2/VEGF [64].

Another important factor is the need for controlled spatial and temporal delivery of signalling molecules, since GF expression levels differ at the different stages of bone healing which can have important consequences for bone growth [65]. In particular, local levels of endogenous VEGF peak at 5 days and decrease towards normal levels 10 days after bone fracture in rodents. Continuous exposure of cells to relatively high levels of VEGF during the repair process was shown to negatively affect bone formation [66]. Therefore, the development of ‘smart’ biomaterials with the ability to spatio-temporally control the dose, sequence and profile of release of several GFs and cytokines so as to regulate cellular fates during tissue regeneration represents one of the next steps within this field of research. There are several examples of carriers that allow for temporal controlled release of a few GFs using nanogels, cross-linked gelatin–polymer composites or gelatin-based coatings [67]. In these systems, the biomaterials are produced by incorporating different layers that serve as matrices enabling internal architecture with controlled release properties.

Temporal controlled release of nanoparticles from biomaterials can be achieved via responsive linkers. For example, in a study by Tokatlian et al. nanoparticles were immobilized to a biomaterial through the use of matrix metalloproteinases (MMPs) sensitive linkers. In this way, cell-secreted MMPs could release the nanoparticles from the biomaterial in a temporally controlled manner [68]. External stimuli such as light have also been used to induce remotely controlled biomolecule release from a hydrogel–nanoparticle hybrid scaffold [69]. When coupled to biomolecules such approaches could give rise to temporally controlled release of signalling biomolecules.

4. Stem cell engineering with the use of nanoparticles

Stem cells can be distinguished from all other cell types by their unique ability to continuously self-renew and differentiate into mature cells of a variety of lineages. In addition, they can contribute directly to therapy owing to their intrinsic ability to secrete therapeutic and/or beneficial factors such as anti-inflammatory cytokines or angiogenic factors. Currently, various stem cells, such as adult stem cells, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are being researched, wherein each presents its own unique advantages and disadvantages. In the context of bone tissue regeneration especially bone marrow-derived MSCs are the most relevant since they have the ability to differentiate into bone, cartilage, adipose, muscle, tendon, ligament and marrow stroma cells. Bone marrow transplantations are
already used clinically in combination with osteoconductive materials to augment bone healing. Moreover, in several animal models of bone loss, MSCs could induce rapid bone regeneration and fracture repair [70]. In order to further enhance their innate abilities, researchers have focused on engineering MSCs as well as other cell sources to provide additional, more precise, control over their differentiation into specialized cells. In addition, there has been considerable interest in the ability for close monitoring of these cells to increase their therapeutic effectiveness.

In this field of research, nanoparticles provide an interesting platform to modulate stem cell behaviours. Potential applications of nanoparticles in stem cell research include non-invasive stem cell tracking in vivo, stem cell-based delivery systems releasing biologically relevant molecules (e.g. pro-survival, anti-inflammatory), and nanoparticles that deliver biomolecules intracellularly to direct stem cell differentiation and proliferation (e.g. GFs, synthetic peptides, DNA). This field of research is still in its beginning stage with most examples of engineering stem cells represented by loading them with nanoparticles for tracking purposes (figure 4). Several types of nanoparticles have been researched for this purpose with most interest focused on magnetic nanoparticles and quantum dots (QDs) but also several examples of polymeric and silica nanoparticles exist.

Superparamagnetic iron oxide (SPIO) nanoparticles have received particular attention because they act as good contrast agents in magnetic resonance imaging (MRI) and can be coated with an organic layer such as chitosan, gelation or functional groups to infer biocompatibility and colloidal stability to the particles. Indeed, SPIO nanoparticles for stem cell tracking can be a powerful approach, and as few as 1000 MSCs could be detected by MRI when loaded with SPIO nanoparticles, even after one month [71]. Encouragingly, several SPIO nanoparticles have been approved by the US Food and Drug Administration as MRI contrast agents; however, not for stem cell labelling applications because it is currently unclear how SPIO particles affect the function and fate of stem cells. Several reports indicated that the labelling of stem cells with certain types of SPIO nanoparticles does not affect cell viability and the ability of stem cells to differentiate. Specifically, several studies showed that the internalization of SPIOs (ferumoxides) by hMSCs using a liposome transfection agent did not affect their chondrogenic, adipogenic or osteogenic differentiation [72]. However, recent studies suggested that some types of SPIO labelling may affect the metabolism and some functions of stem cells [73,74]. Also more clarity around long-term in vivo toxicity is needed. Moreover, the gradual loss of MRI cell signal due to cell division, as well as the low resolution and therefore the lack of functional information, are limitations. Alternative design strategies could help to overcome these limitations. For example, SPIO nanoparticles with gadolinium-based chelates (GdDTPA) allowed dual labelling of hMSCs, where live cells could be discriminated from dead cells in real time [75].

QDs are frequently used tools for bioimaging applications and have also been investigated for the long-term labelling of stem cells. Although some studies have shown that QDs can affect chondrogenic and osteogenic differentiation potential of stem cells [76,77], other studies using bioconjugated QDs showed that the stem cells could maintain their osteogenic differentiation potential [78,79]. This indicates that next to a probably strong concentration dependency, the biological behaviour of QDs is also dependent on their (outer) structure. Indeed recent studies have not only focused on generating non-cytotoxic QDs, but also on developing multifunctional QDs that in addition to their labelling can also be used as drug or gene delivery vehicles or allow for multimodal imaging [80].

For example, in a recent study multifunctional QDs modified with beta-cyclodextrin and a CKKRGD peptide on the surface were developed for enhancing the differentiation and long-term tracking of hMSCs [81]. This modified QD could deliver DEX and siRNA into hMSCs while allowing for long-term in vivo tracking (three weeks). This is a promising field of research; however, the current cost of QD labelling and issues associated with toxicity and accessibility of whole animal imaging remain barriers for the clinical development of such nanoparticles and need to be addressed in the future.

Silica and polymeric nanoparticles represent newer types of nanoparticles that also show interesting properties for stem cell tracking [82]. For example, there are a few studies which used MSNs conjugated with fluorescent dyes, and showed that they could be internalized in MSCs without affecting cell viability, proliferation and capability to differentiate [83,84]. Moreover, in one of these studies, the labelled silica nanoparticles were able to discriminate between live and early stage apoptotic stem cells [83]. The advantage of using such particles is that the silica matrix chemically and physically confines the fluorescent dye while providing an easy-to-functionalize surface for bioconjugation. Also combinations of organic–inorganic nanocarriers have been investigated to merge the theranostic capabilities of metal oxides with the biocompatibility and drug delivery capacity of polymers [68].

**Figure 4.** Nanoparticles used for stem cell tracking purposes.

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*Note: The reference to an image in the text should be accompanied by a properly formatted citation in the bibliography.*
Nanoparticle-based technology is also a promising approach to genetically modify stem cells, which can be used to provide them with useful functionalities. This is a particularly powerful approach when combined with scaffold-based strategies such as done by 1m and co-workers who engineered MSCs using a PLGA-based non-viral method which allowed slow release of the plasmid to MSCs seeded on the scaffold [85]. Similarly, Park et al. used PEI-modified PGLA nanoparticles to deliver plasmids encoding an SOX trio to guide cartilage formation (chondrogenesis) [86]. The efficient drug delivery capabilities of nanoparticles can also be used for co-delivery to stimulate multiple processes simultaneously such as in a study by Jeon et al., who developed nanoparticles for the co-delivery of Cbfa-1 siRNA and an SOX protein to guide chondrogenesis while inhibiting osteogenesis [87].

5. Concluding remarks
In the past few decades, nanoparticles have revolutionized the field of drug delivery due to their unique physical characteristics and nanostructures that can be designed to fit multiple purposes. Their application in the field of regenerative medicine is still in its beginning phase but is already making important contributions.

Firstly, we have described how nanoparticles can be used to deliver different types of biomolecules to give cues to stimulate or inhibit certain biological processes. Several different types of nanosized systems that could stimulate bone tissue formation processes or that could inhibit harmful processes such as inflammation have been discussed. In many of these processes, the use of a delivery system is preferred or sometimes even needed because the biomolecules in question have short half-lives, degrade fast and/or can be harmful at high doses. Especially when looking at tissue regenerative processes, where the biological processes are tightly controlled by numerous GFs and cytokines, it is important to develop technology that can actively give biological cues to help stimulate the right biological processes at the right time. The wealth of knowledge created in the past few decades on nanoparticles for stimulated release in the field of cancer therapy will aid the development of more sophisticated nanoparticle-based approaches for tissue engineering. Multifunctional nanoparticles that allow for dual drug delivery, temporal control of the cargo and specific cell or protein targeting would further promote the use of nanoparticles within this and related fields of research.

Secondly, another advantage of nanoparticles is that they can be easily integrated in other technologies and materials. In §3, we discussed the benefits of nanoscale surface modification of scaffolds. The drug delivery properties of the nanoparticles as discussed in §2 can be incorporated in the materials, and so creating instructive materials that can positively influence tissue formation. The continued advancement of such ‘smart’ biomaterial systems holds the promise for improved therapies in tissue engineering and regenerative medicine as a whole. Moreover, nanocomposites by themselves show enhanced biological activity, cell survival, and improved regenerative outcomes compared to microscale materials. To improve and perfect such materials the effects of the topography, material and chemistry need to be better understood in future work. This will allow reconstruction of the chemistry and three-dimensional environment up to the nanoscale to create truly biomimetic materials that stem cells can bind to, proliferate and differentiate in.

Thirdly, stem cell therapy has enormous potential to cure a multitude of chronic diseases. Despite its promise, new stem cell therapies have up to now become clinically available. Technology that can determine the biodistribution, mechanism of action and fate of transplanted stem cells is needed to understand and refine stem cell therapies. In §4, we discussed examples of nanoparticles that have been used as a tool to track and modulate stem cell behaviour. The strength of these systems is that the drug delivery capacity can be combined with theranostic capabilities to both track and deliver (genetic) material to modulate stem cell behaviour. Nanoparticles represent promising imaging platforms; however, many aspects such as those related to long-term toxicity and in vivo applicability need to be addressed. Also the ability to perform functional and high-resolution imaging within large animals remains an important hurdle. Nevertheless, the wealth of knowledge that is available of how to manipulate the structure of nanoparticles, and their rapid clinical development for cancer therapy, gives confidence that the above challenges can be met.

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