

# Nanoparticles and their potential for application in bone

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**Abstract:** Biomaterials are commonly applied in regenerative therapy and tissue engineering in bone, and have been substantially refined in recent years. Thereby, research approaches focus more and more on nanoparticles, which have great potential for a variety of applications. Generally, nanoparticles interact distinctively with bone cells and tissue, depending on their composition, size, and shape. Therefore, detailed analyses of nanoparticle effects on cellular functions have been performed to select the most suitable candidates for supporting bone regeneration. This review will highlight potential nanoparticle applications in bone, focusing on cell labeling as well as drug and gene delivery. Labeling, eg, of mesenchymal stem cells, which display exceptional regenerative potential, makes monitoring and evaluation of cell therapy approaches possible. By including bioactive molecules in nanoparticles, locally and temporally controlled support of tissue regeneration is feasible, eg, to directly influence osteoblast differentiation or excessive osteoclast behavior. In addition, the delivery of genetic material with nanoparticulate carriers offers the possibility of overcoming certain disadvantages of standard protein delivery approaches, such as aggregation in the bloodstream during systemic therapy. Moreover, nanoparticles are already clinically applied in cancer treatment. Thus, corresponding efforts could lead to new therapeutic strategies to improve bone regeneration or to treat bone disorders.

**Keywords:** nanoparticles, applications, bone, cell labeling, drug delivery, gene delivery

## Introduction

Biomaterials such as polymers, ceramics, and metals are widely used in bone for regenerative therapies, including in bone grafts and in tissue engineering as well as for temporary or permanent implants to stabilize fractures or replace joints.<sup>1</sup> In recent years, biomaterials in general and bone-related implant materials in particular have been considerably refined,<sup>2</sup> with the objective of developing functionalized materials, so-called smart materials, containing bioactive molecules to directly influence cell behavior.<sup>3</sup>

In this context, nanoparticles that are in the same size range as integral parts of natural bone, such as hydroxyapatite crystals or cellular compartments,<sup>4</sup> are promising candidates for local applications. They form the basis of modular systems, which provide the opportunity to elicit cell responses in a spatially and temporally controlled manner by the defined release of physiologically active substances.<sup>5</sup> Alternatively, nanoparticles can be immobilized and applied as coatings on implant surfaces or can be used for transmembrane transport for cell labeling<sup>6</sup> or gene therapy.<sup>7</sup> In bone, locally applied nanoparticles may be suitable for numerous potential uses with respect to the improvement of tissue regeneration, the enhanced osseointegration of implants, and the prevention of infections.

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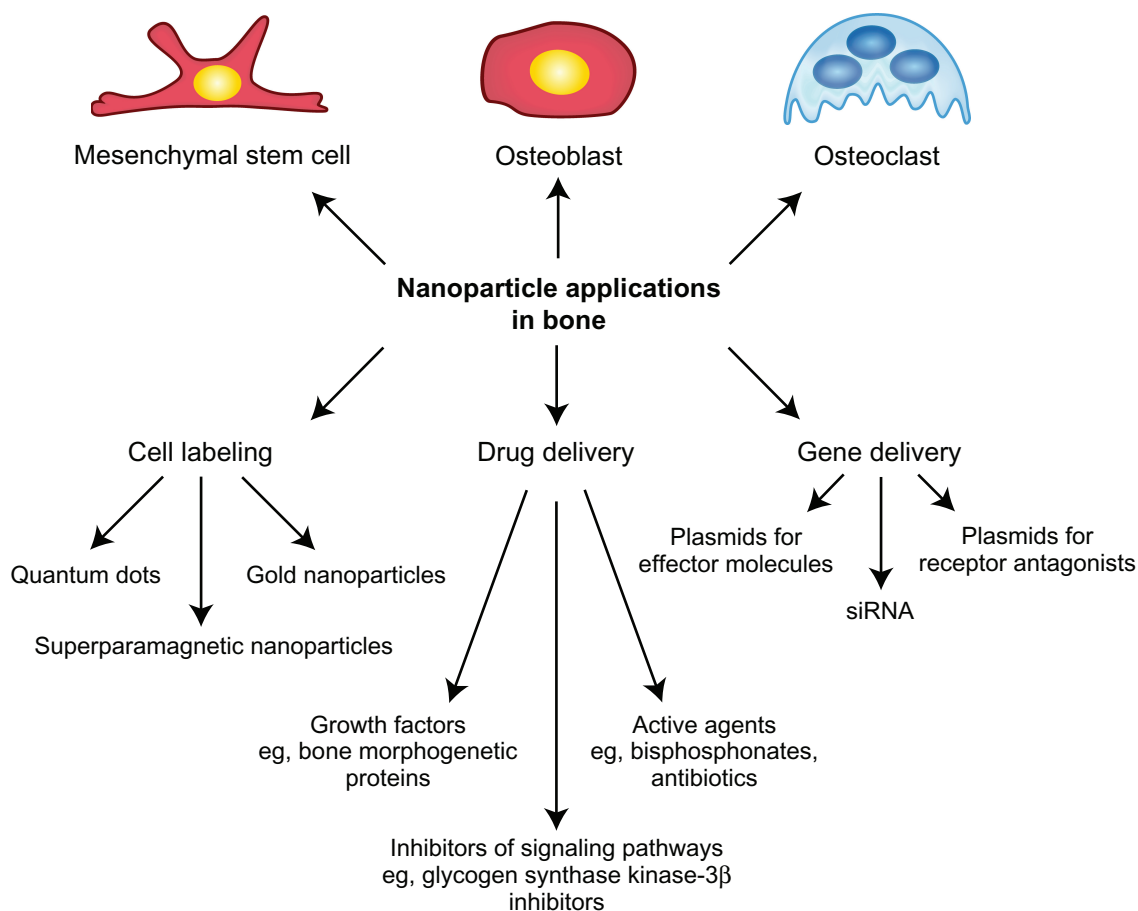
Moreover, systemic application of nanoparticles in bone in a way analogous to cancer therapy is conceivable. However, tissue-specific targeting still represents a great challenge. Specific cell targeting of nanoparticles has been successfully developed for hyperthermia treatment of cancer<sup>8</sup> as well as for drug delivery of paclitaxel.<sup>9,10</sup> Similarly, nanoparticle treatment of systemic bone diseases such as osteoporosis might be feasible in the future. To date, several molecules to target bone have been identified, such as bisphosphonates and their derivatives,<sup>11–13</sup> as well as oligopeptides targeting specifically bone-resorption<sup>14</sup> or bone-formation surfaces.<sup>15</sup>

Summarizing, to achieve the desired effect, nanoparticles should fulfill the following criteria: they should be nontoxic for cells, ie, bioinert or biodegradable; they should effectively carry the molecule of interest, eg, labeling agent or drug; and they should exert their actions specifically on their target, without evoking side effects in other tissues. Here, the application of targeting molecules or local application of nanoparticles in the form of bioactive coatings or cements is very promising.

This review focuses on nanoparticles and their potential with regard to bone. In this field of research, no clinical trials as yet have been initiated. However, large numbers of in vitro and in vivo studies emphasize the great potential for nanoparticle applications in bone. Nanoparticles feature various modifiable facets, such as particle chemistry, functionalization, and attachment to certain surfaces. In this review, recent research approaches concerning cell labeling, drug delivery, and gene therapy to influence bone cells, as well as the interactions of different types of nanoparticles with bone cells, will be highlighted.

## Applications of nanoparticles in bone

Increasingly refined nanoparticles are being developed for a wide range of applications (Figure 1). These include cell labeling to broaden research possibilities as well as to improve and noninvasively monitor cell therapy approaches.<sup>6</sup> Moreover, drug delivery systems with improved pharmacologic characteristics are being developed. They promote enhanced therapeutic outcome by providing controlled



**Figure 1** Overview of nanoparticle applications in bone, as highlighted in this review.

release of bioactive molecules, such as growth factors or anticancer drugs.<sup>16</sup> In addition, gene therapy concepts with good prospects are required for future treatment options based on intracellular manipulation.<sup>17</sup> Due to the great potential for nanoparticles in the applications indicated here, these topics are discussed against the background of bone cells and tissue.

## Cell labeling

Nanoparticles offer great potential for cell labeling during regenerative therapies. Depending on the therapeutic approach, labeling agents are applied *in vivo* or the cells are labeled *ex vivo* and are subsequently applied locally or systemically. Thereby, cell labeling allows for the practicable detection of transplanted cells, eg, via magnetic resonance imaging (MRI).<sup>18</sup> Thus, cell labeling provides the opportunity to visualize and track cell transport to the area of the defect *in vivo* and to assess the fate and participation of the transplanted cells in tissue regeneration. This is essential for a reliable evaluation of cell therapy outcome.

Due to their exceptional regenerative potential, mesenchymal stem cells (MSCs) are thought to support tissue regeneration in stem cell therapy. The promising fields for MSC application range from promoting bone regeneration<sup>19,20</sup> to improving fracture healing.<sup>21–24</sup> To monitor these processes, MSCs have been labeled with diverse nanoparticles, such as quantum dots, which are small semiconductor nanocrystals,<sup>25,26</sup> fluorescence-labeled mesoporous silica nanoparticles,<sup>27</sup> gold nanoparticles,<sup>28</sup> or superparamagnetic iron oxide (SPIO) nanoparticles.<sup>29–31</sup> Several types of SPIO nanoparticles have already been clinically approved for use as contrast agents in MRI, eg, of bowel or liver.<sup>32</sup> It should be noted that bone represents a formidable target organ, which poses a particular challenge with regard to cell labeling, due to its high mineralization grade, making the visualization of labeled cells in MRI difficult.

For application as cell-labeling agents, nanoparticles need to fulfill certain criteria. On the one hand, the number of nanoparticles per cell has to be high enough to be detectable. On the other hand, this number should be low enough to avoid any interference with cellular functions. With respect to bone cells, and particularly MSCs, the particles ideally should not compromise the differentiation potential. *In vitro* analyses of MSC differentiation capacity in the presence of nanoparticles demonstrated the innocuousness of several SPIO nanoparticles,<sup>29–31,33</sup> as well as of certain gold nanoparticles<sup>28</sup> that were optimized for efficient MSC labeling and MRI visualization. As the MSC differentiation potential *in vitro*

does not necessarily correlate with the *in vivo* situation, a study investigating the stemness of MSCs exposed to SPIO nanoparticles went one step further by verifying the differentiation capacity *in vivo* based on ossicle formation by labeled human MSCs in immunocompromised mice.<sup>34</sup> However, depending on the experimental setting, quantum dots<sup>35</sup> as well as SPIO nanoparticles<sup>36–38</sup> were observed to alter osteogenic differentiation of MSCs *in vitro* and *in vivo*. For SPIO nanoparticles, the iron component was found to be one of the factors responsible for the impairment of the differentiation potential, possibly due to its increased intracellular content caused by the presence of the particles.<sup>36,38</sup> With regard to clinical application, various SPIO nanoparticles were applied subcutaneously in mice, eg, via the incorporation of labeled MSCs in collagen scaffolds, and were demonstrated to be suitable for efficient *in vivo* long-term labeling and for producing convincing visualization by MRI.<sup>30,31</sup> However, one has to keep in mind that with this technique, primarily the particles themselves are tracked and not the cells.

Further efforts are being made to expand the possibilities for detecting nanoparticle-labeled cells. For a considerable time, mostly bifunctional systems such as fluorescent, iron-containing nanoparticles were used.<sup>30</sup> By adding a third functionality via the incorporation of gold for the visualization of the nanoparticles through computer tomography, a refinement of cell-labeling nanoparticles could be achieved.<sup>39</sup>

As it appears, biocompatibility poses a great challenge regarding stem cell labeling due to the natural differentiation potential of the cells and essential preservation *in vivo*. Another issue to be addressed in the near future will be the successful applicability of cell labeling in bone, as visualization of labeled MSCs in mineralized tissue is still challenging. However, ongoing research will further improve and widen the application of nanoparticles for cell labeling, seeking the best possible way to image and track cells in musculoskeletal therapy.

## Drug delivery

Due to the unsatisfactory performance of several biomaterials currently used for bone replacement or tissue engineering, improvements such as the introduction of bioactive molecules are being determinedly pursued.<sup>40</sup> Proteins of interest can either be directly injected at the respective site or adsorbed to a biomaterial surface. In contrast to injected proteins, which are usually rapidly cleared from the body, locally adsorbed proteins are released by desorption or diffusion and may be retained longer.<sup>41</sup> Increasingly, nanoparticles are being explored as finely adjustable delivery systems with regard

to the location and time period of drug release. Local drug delivery is favorable in comparison with systemic application to minimize unwanted side effects.<sup>42</sup> Moreover, adequate tuning of the nanoparticles allows for a temporally controlled, sustained delivery according to requirements.<sup>42,43</sup> The delivery of inhibitory factors of signaling pathways has been reported as a promising tool to elucidate so far unknown pathway functions for research purposes.<sup>44</sup> In addition, nanoparticle refinement with respect to clinical application in cancer treatment has been demonstrated by loading particles with the drug paclitaxel.<sup>9,10</sup>

The following paragraph focuses on the use of nanoparticles as an effective protein or drug delivery system to support bone tissue regeneration. For this purpose, several nondegradable particles, such as silica, lipid, dendrimer, hydroxyapatite, or gold nanoparticles,<sup>43–50</sup> as well as degradable particles made of poly(L-lactide)<sup>51,52</sup> or poly(L-lactide-co-glycolide) (PLGA),<sup>11,42,52–56</sup> have been used. Frequently, nanoparticles were combined with scaffolds such as proteinaceous hydrogels or degradable polymeric matrices to facilitate application in bone.<sup>47,48,53,55,56</sup>

As bone contains bone-forming cells, the osteoblasts, and bone-resorbing cells, the osteoclasts, which act in concert to guarantee bone homeostasis,<sup>57,58</sup> different strategies can be envisioned that could promote the regeneration of bone tissue. On the one hand, osteoblasts could be supported by nanoparticle-based growth factor delivery. On the other hand, osteoclast resorption could be modulated by nanoparticles locally releasing specific inhibitors.

For the enhancement of osteogenic differentiation, bone morphogenetic proteins (BMPs), members of the transforming growth factor (TGF)- $\beta$  superfamily, proved to be very suitable. Although TGF- $\beta$  itself was mainly encapsulated in nanoparticles to potentially improve cartilage regeneration,<sup>59–61</sup> the clinically approved BMP-2 was incorporated in nanoparticles to support bone formation.<sup>50–53,62</sup> Biodegradable<sup>51,53</sup> and nondegradable nanoparticles<sup>50,62</sup> displayed sustained BMP-2 release for up to 2 weeks. Analyses of the viability of osteoblastic cells in the presence of BMP-2-loaded nanoparticles revealed that, depending on the material, cell viability could be negatively affected, thus requiring further material development.<sup>51,62</sup> It is essential that the nanoparticles do not influence the biological activity of released growth factors so that osteogenic differentiation can be successfully supported by locally, sustainably released BMP-2.<sup>51,62</sup> In relation to this criterion, encapsulated BMP-2 implemented into a hydrogel promoted *in vivo* bone remodeling in a rat calvarial critical-size defect.<sup>53</sup> Another clinically

approved member of the TGF- $\beta$  protein family, BMP-7, was investigated regarding its potential for inducing ectopic, subcutaneous bone formation in a rat model. Like BMP-2, BMP-7 encapsulated in PLGA nanospheres and immobilized in a degradable scaffold supported bone formation.<sup>56</sup>

In addition to growth factors, the extracellular matrix molecule osteopontin was incorporated in hydroxyapatite nanoparticles located in a degradable matrix and was analyzed for its osteoinductive potential in a canine endosseous gap implant model. However, other than new bone formation within the matrix, no positive effects were observed in the gap itself.<sup>47</sup>

In contrast to naturally occurring proteins, medium supplements for osteogenic differentiation *in vitro*, such as the synthetic glucocorticoid dexamethasone, are known to feature osteoinductive properties.<sup>63</sup> Accordingly, dexamethasone-loaded dendrimer nanoparticles in a scaffold supported the osteogenic differentiation of rat MSCs *in vitro*, like free dexamethasone.<sup>48</sup> However, due to possible unwanted side effects of the drug itself *in vivo*, such as secondary osteoporosis,<sup>64</sup> a clinical application appears questionable.

In addition to the manipulation of osteoblast behavior, the aforementioned second strategy of drug-loaded nanoparticle application in bone regeneration to inhibit osteoclast resorption activity has also been pursued. In a study using surface-immobilized, dexamethasone-containing nanoparticles, their potential to decrease the number of osteoclast precursors was tested. The released dexamethasone did in fact lead to a local growth inhibition of such progenitors.<sup>55</sup> General excessive osteoclast activity, which eventually leads to bone loss or insufficient regeneration in the case of bone defects, is often treated with bisphosphonates, a class of osteoporosis-antagonizing drugs. However, orally administered bisphosphonates display poor bioavailability (only up to 3% of the ingested amount).<sup>65</sup> In contrast, controlled, sustained delivery in the case of local bone regeneration might be feasible by using bisphosphonate-loaded nanoparticles. As a model drug, alendronate was used in *in vitro* studies, and the inhibitory effect of the particle-associated bisphosphonate on osteoclasts (and their precursors) was confirmed for nondegradable gold nanoparticles as well as for biodegradable nanoparticles.<sup>46,54</sup> Alendronate-modified nanoparticles were localized in the cytosol and, as expected, were observed to reduce the number of multinucleated, tartrate-resistant acid phosphatase-positive cells.<sup>46</sup> However, based on the inhomogeneity of the osteoclast cell culture due to the differing stages of osteoclast development at a given point in time, the effects

of encapsulated, osteoclast-influencing substances are difficult to identify. Because of the high affinity of bisphosphonates to bone, and in particular to hydroxyapatite, bisphosphonate-loaded nanoparticles could be targeted to the bone surface, which might help to specifically address and treat, for example, skeletal tumors.<sup>11,12</sup> Additionally, bisphosphonate-loaded nanocarriers were strongly retained in mineral-comprising implants, eg, hydroxyapatite-containing scaffolds, providing the opportunity to accurately adjust the delivery system.<sup>49</sup> Again, the chemical composition of nanoparticle constructs is of great importance to minimize potential material toxicity and to avoid cellular responses to the material itself rather than to the incorporated drug.<sup>11,62</sup>

Thus, drug delivery in bone has considerable potential. Regarding prospective progress in the area of biomaterials development, improved release kinetics of nanoparticles will contribute to an even more strictly controlled and sustained drug delivery. Moreover, the constantly increasing understanding of bone tissue in general and the regulation of osteogenic differentiation in particular will be the basis for the identification of further proteins of interest and will lead to promising alternatives besides, eg, BMP-2. In addition to the concepts discussed previously to enhance bone formation, eg, with osteoblast growth factors, or to reduce bone resorption with established drugs, such as bisphosphonates, further applications, including the nanoparticle-mediated delivery of analgesics<sup>42</sup> or the application of bone cement comprising antibiotic-loaded nanoparticles,<sup>43</sup> will be pursued in the future.

## Gene delivery

Although the therapeutic effect of many proteins is often obvious in terms of bone metabolism, the delivery of proteins of interest or growth factors still represents a great challenge due to aggregation, short lifetime in the bloodstream, and, moreover, very low efficiency due to short and abrupt release. In respect to this, the application of nanoparticles as gene carriers represents a wide and promising field, because the transfection approach potentially allows long-term expression and therefore a longer therapeutic effect.

Many proof-of-principle studies have already been performed to demonstrate that nanoparticles of an inorganic as well as an organic nature are able to deliver plasmids into bone cells. In such studies, a green fluorescent protein-encoding reporter plasmid combined with different carriers was normally used, such as calcium-doped organosilicate nanoparticles,<sup>66</sup> calcium phosphate nanoparticles,<sup>67</sup> arginine-functionalized hydroxyapatite nanorods,<sup>68</sup> or polymers.<sup>69</sup>

The alternative approach, an application of nanoparticles as oligonucleotide (eg, small interfering ribonucleic acid [siRNA]) carriers, was also effective in other studies, using gold,<sup>70</sup> calcium phosphate,<sup>71</sup> and cationic polymer/lipid TransIT-TKO<sup>®</sup> nanoparticles.<sup>72</sup> A very promising system was developed by Zhang et al<sup>15</sup> and consisted of siRNA-loaded cationic liposomes attached to oligopeptides. Here, oligopeptide (AspSerSer<sub>n</sub>) provided efficient bone targeting to bone-formation surfaces, whereas siRNA that targeted casein kinase-2 interacting protein-1 promoted osteoblast activity, resulting in increased bone formation and enhanced bone microarchitecture.<sup>15</sup>

The applied studies often used a transfection approach to enhance bone regeneration in the case of fractures, delivering plasmids encoding transcription factors, growth factors, and even hormones. Studies performed in this direction described a broad range of carriers, including viral particles,<sup>73,74</sup> folate-chitosan nanoparticles,<sup>75</sup> PLGA nanospheres<sup>76</sup> and nanoparticles,<sup>77</sup> liposomes,<sup>78</sup> and even nonnanoparticulate, polymeric matrixes for the delivery of plasmid, encoding human parathyroid hormone.<sup>79</sup>

The most well-known and widely investigated proteins for bone regeneration are the BMPs, which are known to possess the greatest *in vivo* bone stimulatory capacity and to stimulate the differentiation of MSCs along osteoblastic and chondrogenic lineages.<sup>41</sup> For example, Krebs et al<sup>80</sup> reported a gene delivery system composed of calcium phosphate nanoparticles carrying a BMP-2-encoding plasmid embedded in injectable alginate hydrogels. Hosseinkhani et al<sup>81</sup> used polyethylenimine/DNA nanoparticles encapsulated into poly(glycolic acid) scaffolds to transfect MSCs and promote ectopic bone formation in these scaffolds *in vivo*.

Among other growth factors, potential stimulators of bone regeneration are vascular endothelial growth factor (VEGF) and TGF- $\beta$ . It was demonstrated that adeno-associated viral vectors carrying a VEGF plasmid were able to induce neo-vascularization as well as bone remodeling and resorption in autografts and allografts,<sup>73</sup> whereas retroviral VEGF transduction resulted in enhanced MSC recruitment and accelerated bone formation.<sup>74</sup> TGF- $\beta$  was described as stimulating bone healing after injection of viral carriers functionalized with a TGF- $\beta$ -encoded plasmid.<sup>78</sup> Deng et al<sup>82</sup> demonstrated effective gene delivery of plasmid TGF- $\beta_1$  to rat MSCs when combined with ethylenediamine-modified polysaccharide from mulberry leaves.

Among transcription factors, the osteoblast-specific transcriptional activator Runx2 and LIM mineralization protein-1 (named after protein domains Lin-11, Isl-1, Mec-3)



were considered in particular because of their ability to stimulate bone formation.<sup>83</sup>

All these strategies normally include a spatial application, thus avoiding systemic toxicity and undesirable side effects. Nevertheless, in some cases the systemic application of nanoparticles (naturally in combination with desirable bone-targeting properties) is preferable, eg, in osteoporosis, arthritis, or bone tumors. Here, the combination of nanoparticles with an “osteoprotective” gene is an obvious strategy. For example, Fernandes et al<sup>75</sup> used folate-chitosan nanoparticles in combination with an interleukin-1 receptor antagonist (IL-1Ra) gene to decrease inflammation and reverse alterations in bone turnover in an arthritic rat model.

Such a protective effect could also be achieved via the introduction of tumor inhibitors, eg, IL-18.<sup>84</sup> Cytokine IL-18 is normally described as a T-cell and NK-cell activator and was previously shown to inhibit sarcoma growth via activation of the immune response and antiangiogenic activity *in vivo*. Nie et al<sup>84</sup> observed the inhibition of tumorigenesis in mice after the application of polymeric monomethoxy poly(ethylene glycol) (PEG)-PLGA-PEG nanoparticles complexed with IL-18 plasmid.

The alternative approach is to downregulate factors that enhance tumor formation. It is known that a certain expression profile in some tumors, eg, mammary carcinoma, is associated with metastases formation in the skeleton. With respect to this, Elazar et al<sup>85</sup> demonstrated that silencing of osteopontin and bone sialoprotein, which belong to the “abnormal” expression set described previously, with siRNA-functionalized PLGA nanoparticles led to the inhibition of metastatic bone lysis.

Thus, nanoparticle-based gene therapy offers great opportunities for fine modulation and treatment of bone diseases of different origin – from fractures to malignant tumors. Various possibilities for the delivery of enhancing as well as inhibitory genetic material have been explored over the past years, and several disadvantages of protein delivery, such as inadequate release kinetics, might be overcome by gene delivery approaches. Thereby, polymeric and inorganic nanoparticles represent a more promising field than viral vectors, due to their safety, degradability, and possible surface modifications, allowing nanoparticle targeting to cells of interest. The major advantages are potentially easy spatial application of particles (in terms of implants, cements, or direct bone injections), which minimizes systemic toxicity and potential side effects, and a prolonged therapeutic effect compared with pure proteins, thus making gene therapy a very promising strategy for the future.

## Interactions of nanoparticles with bone cells

During bone regeneration, bone cells such as MSCs, which can differentiate into bone-forming osteoblasts, osteoblasts themselves, and bone-resorbing osteoclasts,<sup>57,58</sup> tightly interact to ensure successful tissue reconstruction. Accordingly, it is important not to disturb such regenerative processes, eg, by eliciting undesired inflammatory responses induced by nanoparticles. Therefore, investigations on possible particle uptake as well as on the potential effects of nanoparticles on bone cell functions, such as limitation of MSC differentiation potential, bone mineralization by osteoblasts, or modulation of osteoclastic resorption activity, are required prior to any nanoparticle application in the field of bone research. Thus, the interactions of bone cells with diverse nanoparticles are discussed here.

## Mesenchymal stem cells

MSCs are multipotent cells, which can be isolated, for example, from bone marrow. MSCs feature *in vitro* plastic adherence and can be characterized by the expression of a defined set of surface markers as well as by their differentiation potential into mesenchymal tissue lineages such as bone, cartilage, or adipose tissue.<sup>86,87</sup> In contrast to osteoclasts, which are known to phagocytize particles due to their relationship to monocytes/macrophages,<sup>88</sup> MSCs do not necessarily take up particles effectively.

With respect to the cellular internalization of nanoparticles or foreign material in general, the uptake behavior depends on the cell type, particle chemistry, charge, and shape, as well as particle carrier systems and cellular microenvironment. Therefore, it is difficult to deduce universally valid rules for the prediction of particle uptake. In terms of charge, positively charged substances such as poly(L-lysine) generally promote the internalization of genetic material.<sup>89</sup> Equally, positive charges on the surface of polymeric nanoparticles were found to be beneficial for particle uptake by MSCs.<sup>90,91</sup> Negatively charged polymeric nanoparticles such as carboxyl- or phosphonate-functionalized particles were also readily internalized by MSCs.<sup>92,93</sup> These observations suggest specific interactions of both, positive and negative functional groups, with the cell surface. Most of the particles were then localized in the cytosol, often in membrane-enclosed clusters.<sup>91,93</sup> For up to 3 weeks in long-term culture, these particles did not hamper the viability of undifferentiated and differentiated MSCs.<sup>91,93</sup> With regard to differing particle chemistry, metallic silver nanoparticles, too, were taken up into endolysosomal structures in the cytosol.<sup>94</sup> Although certain

silver nanoparticles did not compromise MSC viability,<sup>94</sup> others of approximately the same size caused serious DNA damage even at lower concentrations in a different study.<sup>95</sup> In addition to polymeric and metallic nanoparticles, quantum dots of just a few nanometers play an important role in MSC research, based on their excellent cell-labeling qualities.<sup>25</sup> Quantum dots, either unfunctionalized or provided with, eg, arginine-glycine-aspartic acid peptides, were spontaneously taken up by MSCs and could be detected inside the cells over a period of 3 weeks.<sup>35,96</sup>

With respect to the mechanisms for nanoparticle uptake, cells can rely on diverse endocytotic internalization pathways such as clathrin- or caveolae-mediated endocytosis and macropinocytosis.<sup>97</sup> Studies with specific inhibitors for the different uptake routes revealed a heterogeneous picture. Although polymeric and metallic particles were in part taken up via the clathrin-mediated pathway,<sup>90,92,94</sup> macropinocytosis was also involved to some extent.<sup>94</sup>

Following the elucidation of nanoparticle uptake and the underlying mechanisms, the analysis of potential influences on MSC characteristics and function is essential. Again, the literature is quite heterogeneous on this topic, and nanoparticles of different chemical nature and shape will be considered here.

Calcium phosphate nanoparticles present an interesting alternative when attempting to imitate bone structure and function with regard to the *in vivo* crystallinity of calcium phosphates.<sup>98</sup> MSC proliferation was affected in a size-dependent manner by the exposure of these cells to calcium phosphate nanoparticles, with larger particles being more harmful.<sup>99</sup> The same research group further investigated a potential dependency on particle concentration and their form of appearance. It was observed that with increasing concentration as well as with amorphous particles in contrast to crystalline calcium phosphate particles, osteogenic cell differentiation and matrix mineralization decreased.<sup>100,101</sup>

Likewise, metallic particles, eg, commercially pure titanium nanoparticles, decreased adhesion and suppressed MSC differentiation.<sup>102</sup> In contrast, other metallic particles, such as gold nanotracers, did not affect MSC differentiation into the osteogenic and adipogenic lineages.<sup>28</sup>

Similarly, polymeric phosphonate-functionalized particles, which exhibited excellent binding properties to metallic surfaces such as titanium dioxide,<sup>103</sup> were investigated with regard to their effect on MSC differentiation. Qualitative characteristic staining as well as comprehensive quantitative analyses of marker gene expression for the osteogenic, adipogenic, and chondrogenic lineages revealed no

detrimental effect arising from the phosphonate-functionalized particles.<sup>93</sup>

The MSC differentiation potential was investigated in the presence of various other particle types. Quantum dots, eg, consisting of CdSe/ZnS, restrained osteogenic differentiation in terms of alkaline phosphatase activity as well as osteopontin and osteocalcin expression.<sup>35</sup> In contrast, arginine-glycine-aspartic acid-conjugated quantum dots did not affect the differentiation potential, as assessed by characteristic staining for the osteogenic, adipogenic, and chondrogenic lineages,<sup>96</sup> and thus might be better suited with regard to biocompatibility with MSCs. Equally, mesoporous silica nanoparticles, either functionalized with different amounts of positively charged functional groups or with gadolinium, did not influence MSC differentiation, as confirmed by qualitative analyses after short-term exposure.<sup>104,105</sup>

In addition to the effects on the differentiation potential, further MSC functions can be modulated by nanoparticles. Ferucarbotran particles with superparamagnetic properties were efficiently taken up by MSCs.<sup>106</sup> Due to the intrinsic peroxidase-like activity of such magnetic nanoparticles,<sup>107</sup> ferucarbotran particles stimulated MSC growth through a reduction in intracellular hydrogen peroxide.<sup>108</sup>

Thus, the heterogeneous picture of research on the interactions of nanoparticles with MSCs makes it difficult to draw general conclusions. However, it becomes clear that parameters such as chemistry, size, and shape in some cases greatly affect the particle uptake behavior of MSCs as well as their natural differentiation potential. Thus, it will be necessary to continue the thorough verification of nanoparticle innocuousness independent of their characteristics to ensure unaffected MSC differentiation.

## Osteoblasts

Osteoblasts are bone-forming cells with the ability to mineralize and continuously remodel bone.<sup>57</sup> Additionally, osteoblasts make an essential contribution to the regulation of osteoclast activity and thus to bone homeostasis by secreting the osteoclast-regulating factors macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor  $\kappa$ B ligand (RANKL), and its decoy receptor osteoprotegerin.<sup>57,58</sup> Therefore, nanoparticles exerting effects on osteoblasts might, in addition, indirectly influence osteoclasts.

Similar to MSCs, osteoblasts were demonstrated to internalize a variety of nanoparticles, such as wear debris,<sup>109</sup> quantum dot/hydroxyapatite composites,<sup>110</sup> calcium phosphate nanoshells,<sup>111</sup> and polymeric particles.<sup>112</sup> Again, various factors influenced nanoparticle uptake and potential cellular effects.

With regard to the particle charge, the internalization of positively charged nanoparticles is generally facilitated. In line with this observation, positive surface groups appeared to enhance internalization by osteoblastic cells when comparing negatively and positively charged hydroxyapatite nanoparticles or mesoporous silica nanoparticles with increasing positive charge.<sup>104,113</sup> However, studies with negatively charged phosphonate-functionalized nanoparticles indicated that these particles, too, were taken up by osteoblasts.<sup>112</sup>

In addition to particle charge, shape plays an important role. Normally, spherical nanoparticles are readily internalized and do not impede cell functions. In accordance with this general observation, spherical zinc oxide nanoparticles were not cytotoxic for osteoblastic cells, in contrast to rod-shaped particles of the same material.<sup>114</sup> However, needle-shaped as well as spherical hydroxyapatite nanoparticles decreased osteoblast cell numbers in a different study.<sup>115</sup>

With respect to nanoparticle size, the findings again diverged. On the one hand, hydroxyapatite and titania nanoparticles of up to 40 nm diameter decreased osteoblastic cell proliferation and viability, respectively.<sup>99,116</sup> On the other hand, in another study, only hydroxyapatite nanoparticles with a diameter of 20 nm enhanced cell growth of osteoblast-like cells compared with larger particles with a diameter of 80 nm.<sup>117</sup>

In addition to particle uptake by osteoblasts and potential effects on proliferation and viability, the consequences of nanoparticle presence on the differentiation and mineralization of osteoblastic cells were assessed in a variety of studies with a wide range of particles. The expression of alkaline phosphatase, a marker for osteoblast differentiation,<sup>118</sup> was found to be reduced by the addition of titanium and silver nanoparticles,<sup>109,119</sup> whereas alkaline phosphatase activity and collagen type I synthesis, as well as calcium deposition and mineralization, were increased in the presence of calcium phosphate nanoshells, hydroxyapatite-coated iron oxide particles, strontium-doped hydroxyapatite nanocrystal surfaces, and nanostructured zinc oxide and titania in a different experimental series.<sup>111,120–122</sup> These collected findings imply the involvement of complex mechanisms in the internalization of differently composed nanoparticles and in the evocation of variable osteoblast responses.

As osteoblasts secrete factors that regulate osteoclastogenesis,<sup>58</sup> the expression of RANKL, M-CSF, and osteoprotegerin was analyzed following incubation with various nanoparticles. A catabolic phenotype was induced by polyethylene particles through alteration of the RANKL/osteoprotegerin ratio.<sup>123</sup> Similar osteoclastogenic effects

were triggered in osteoblastic cells by titania nanoparticles via increased gene expression of granulocyte colony-stimulating factor.<sup>116</sup> In contrast, phosphonate-functionalized nanoparticles did not affect the expression of osteoclast-regulating genes in primary human osteoblasts.<sup>112</sup>

Further examined effects of nanoparticles on osteoblasts comprised DNA damage, confirmed for hydroxyapatite nanoparticles in osteoblastic cells,<sup>115</sup> and titania particles in various murine organs, including bone marrow.<sup>124</sup> Furthermore, a possible improvement in implant fixation by applying nanoparticle coatings was investigated. Contrary to expectations, calcium phosphate nanoparticle coatings did not enhance the osseointegration of implants *in vivo*.<sup>125</sup> In contrast, titanium implants coated with a composite of hydroxyapatite nanoparticles and poly(D,L-lactide) improved the osseointegration in terms of new bone formation and mechanical fixation in sheep compared with implants without hydroxyapatite nanoparticles.<sup>126</sup>

In summary, the number of publications on current research regarding the interactions of nanoparticles with osteoblasts appears manageable. Still, findings with regard to nanoparticle influence on differentiation and mineralization of osteoblasts clearly diverge, depending on particle characteristics. As osteoblast behavior is closely connected to osteoclast formation and activity, it will be important to generally extend respective analyses to factors such as RANKL, which affect bone homeostasis in general, in order to ensure covering different fields of potential nanoparticle influence.

## Osteoclasts

Osteoclasts, bone-resorbing cells, are derived from hematopoietic precursor cells and are closely related to macrophages.<sup>127</sup> Because macrophages are known to activate the immune response by phagocytizing pathogens,<sup>128</sup> it is no surprise that osteoclasts, too, are capable of phagocytizing foreign matter such as biomaterial particles.<sup>88</sup> Osteoclasts are part of a complex network coordinating bone homeostasis and regenerative processes, eg, in the case of bone defects. In this regard, the osseointegration of bone substitutes as well as a favored bone formation is highly desired. Osteoclasts, like MSCs and osteoblasts, play a decisive role in fracture healing.<sup>129</sup> If disadvantageously influenced, eg, by metallic wear particles of implants used for fracture stabilization,<sup>130,131</sup> excessive osteoclast activation frequently causes aseptic loosening of implants.<sup>132</sup> Thus, locally applied (drug-loaded) nanoparticles might favorably modulate osteoclast resorption. However, due



to the potential for affected osteoclast activity as a result of contact with foreign matter itself, it is a prerequisite to investigate the basic interactions of osteoclasts with nanoparticles.

In fact, diverse nanoparticles were readily taken up by undifferentiated monocytes and differentiated osteoclasts.<sup>94,112</sup> However, differing subsets of monocytes, eg, those involved in chronic disease and in tissue repair, respectively, differentially incorporated nanoparticles,<sup>133</sup> implying a whole set of parameters influencing nanoparticle uptake in osteoclastic cells. With respect to the intracellular localization of nanoparticles, varying observations were made. Although metallic and polymeric nanoparticles were localized in the cytoplasm, partly surrounded by membrane-like structures,<sup>94,112</sup> hydroxyapatite nanoparticles were detected within specialized cellular cavities with a connection to the extracellular environment, the so-called surface-connected compartments.<sup>134</sup> The uptake of various nanoparticles may lead to changes in osteoclast morphology, eg, a less pronounced ruffled border and modulation of cell proliferation,<sup>120</sup> as well as interference with osteoclast formation and resorption activity. An established characteristic to assess the formation of osteoclasts is the number of tartrate-resistant acid phosphatase-positive, multinucleated cells that are generated in vitro.<sup>135</sup> Based on the analysis of this feature, diverse metallic and silica nanoparticles negatively influenced osteoclast formation.<sup>119,136–138</sup> In contrast, other particles did not compromise the formation of multinucleated cells<sup>112,139</sup> or even promoted osteoclast maturation.<sup>140</sup> Similarly, the influence of nanoparticles on osteoclast resorption activity is heterogeneous. Although the resorption activity of undifferentiated monocytes and osteoclasts could be enhanced by metal ions or nanoparticles,<sup>141,142</sup> other types of particles did not necessarily modulate resorption.<sup>112</sup> In addition to the effects that nanoparticles exert on osteoclast function, inflammatory responses can be induced by nanoparticles, eg, in implant wear, which, in turn, may affect osteoclasts.<sup>140,143</sup> Osteoclast precursors themselves, too, were observed to react to titanium alloy and SPIO nanoparticles with increased cytokine production.<sup>144,145</sup> However, the exposure of osteoclasts and their precursors to nanoparticles does not coercively lead to an upregulation of inflammatory factors in these cells.<sup>66,112</sup> In addition to potential inflammation, further severe effects such as DNA damage or initiation of apoptosis can be elicited by nanoparticles in osteoclast precursors.<sup>116,146</sup>

In short, osteoclasts originate from the same precursors as other phagocytizing cells, such as macrophages, and readily take up nanoparticles. Arising clinical issues include

aseptic implant loosening due to excessive osteoclast activity potentially caused by implant wear in the form of particles. However, only a few studies actually investigated osteoclast resorption activity, even though this osteoclast feature is crucial for eventual changes in bone mass. In general, nanoparticles stimulated osteoclasts. Thus, a careful selection of promising candidates for continuing research is essential.

## Conclusion

Nanoparticles represent a promising tool for research purposes as well as for therapeutic approaches in bone. These could be finely modified, taking into account that the type of interaction between nanoparticle and cell varies depending on nanoparticle composition. Initial studies confirmed the innocuousness of several nanoparticles with respect to MSC differentiation potential and osteoclast function. Thus, different strategies for nanoparticle application in bone (eg, as cell-labeling agents and for drug or gene delivery) have great potential for monitoring and supporting tissue regeneration. In other areas, such as cancer treatment, nanoparticles already contribute to successful clinical approaches, and similar efforts to use nanoparticulate systems to promote bone regeneration will gradually lead to therapeutic success here as well.

## Disclosure

The authors report no conflicts of interest in this work.

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