Nanoparticles and their effects on differentiation of mesenchymal stem cells

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Key Words:
adipogenic differentiation; mesenchymal stem cells; nanoparticles; osteogenic differentiation; tissue engineering

From the Contents

ABSTRACT

Over the past decades, advancements in nanoscience and nanotechnology have resulted in numerous nanomedicine platforms. Various nanoparticles, which exhibit many unique properties, play increasingly important roles in the field of biomedicine to realize the potential of nanomedicine. Due to the capacity of self-renewal and multilineage mesenchymal differentiation, mesenchymal stem cells (MSCs) have been widely used in the area of regenerative medicine and in clinical applications due to their potential to differentiate into various lineages. There are several factors that impact the differentiation of MSCs into different lineages. Many types of biomaterials such as polymers, ceramics, and metals are commonly applied in tissue engineering and regenerative therapies, and they are continuously refined over time. In recent years, along with the rapid development of nanotechnology and nanomedicine, nanoparticles have been playing more and more important roles in the fields of biomedicine and bioengineering. The combined use of nanoparticles and MSCs in biomedicine requires greater knowledge of the effects of nanoparticles on MSCs. This review focuses on the effects of four inorganic or metallic nanoparticles (hydroxyapatite, silica, silver, and calcium carbonate), which are widely used as biomaterials, on the osteogenic and adipogenic differentiation of MSCs. In this review, the cytotoxicity of these four nanoparticles, their effects on osteogenic/adipogenic differentiation of MSCs and the signalling pathways or transcription factors involved are summarized. In addition, the chemical composition, size, shape, surface area, surface charge and surface chemistry of nanoparticles, have been reported to impact cellular behaviours. In this review, we particularly emphasize the influence of their size on cellular responses. We envision our review will provide a theoretical basis for the combined application of MSCs and nanoparticles in biomedicine.

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Introduction

Nowadays, stem cell-based tissue engineering is a promising technology for use in clinical applications for the repair of damaged or diseased tissue.[1] Mesenchymal stem cells (MSCs) are multipotent cells capable of self-renewal and multilineage mesenchymal differentiation, thus they play important roles in the fields of tissue engineering and regenerative medicine.[1,2] MSCs can differentiate into a variety of cell lineages including osteoblasts, chondrocytes, adipocytes, tenocytes and neurocytes. Originally identified in the bone marrow, MSCs can also be isolated from various other sources including adipose tissue, muscle, amniotic fluid and placenta.[3-5] In particular, bone marrow and adipose tissue are two attractive sources for MSC isolation, and human bone marrow/adipose-derived MSCs have been proven to have great potential in tissue engineering applications.

A number of signalling pathways and transcription factors regulate the osteogenic and adipogenic lineage commitment and differentiation of MSCs. Several signalling cascades including Wnt/β-catenin signalling, Hedgehog signalling, and NEL-like protein 1 signalling play important roles in both adipogenic and osteogenic differentiation.[6-8] In terms of transcription factors, runt-related transcription factor 2 (Runx2), the initial and most specific osteogenic marker, can activate and regulate osteogenesis by increasing the expression of downstream genes.[9]

Alkaline phosphatase (ALP) is an early marker of osteogenic differentiation, continuously correlating with the area of high ossification.[10] Osteocalcin (OCN) is a specific marker of mature osteoblasts, synthesized only by fully differentiated osteoblasts,[11] while osteopontin, another marker of osteogenic differentiation, can enhance mineralization.[12] In the case of adipogenic differentiation, peroxisome proliferator activated receptor gamma (PPARγ) is generally regarded as a master regulator, which can trigger the entire program of adipogenesis.[13] CCAAT/enhancer binding protein alpha, another main transcription factor for adipogenesis, enhances sensitivity to insulin and increases the expression of PPARγ.[14] Adiponectin is exclusively expressed in adipocytes and involved in glucose metabolism.[15] Among them, Runx2 and PPARγ act as the master regulators of osteogenesis and adipogenesis, respectively. The signalling cascades promoting osteogenic and adipogenic differentiation of MSCs generally converge on these two key transcription factors.[2]

Many types of biomaterials such as polymers, ceramics, and metals are commonly applied in tissue engineering and regenerative therapies, and they are continuously refined over time.[16] In recent years, along with the rapid development of nanotechnology and nanomedicine, nanoparticles (NPs) have been playing more and more important roles in the fields of biomedicine and bioengineering. NPs are normally divided into two categories according to their chemical structures: inorganic particles including ceramics and metals (such as hydroxyapatite, silica, gold and silver), and organic particles (such as polymeric particles).[17] They have great potential for various applications including drug/gene delivery, bio-imaging, cell labelling, pathologic diagnosis and disease treatment.[17-19] Alternatively, NPs can be immobilized and used in tissue engineering scaffolds or surface coatings on implants.[20, 21] Consequently, considering the interaction between NPs and target cells, many researchers have focused on the safety of NPs. There have been a large number of studies investigating the effects of NPs on cell viability and cell functions including proliferation and differentiation.

This review focuses on several commonly used ceramic and metallic NPs (hydroxyapatite, silica, silver, and calcium carbonate) and their potential effects on the cell behaviours of MSCs. Many factors, including chemical composition, size, shape, surface area, surface charge and surface chemistry of NPs, have been reported to impact cellular behaviours, including cell viability, proliferation, and differentiation[22-26] (**Figure 1**). In this review, we highlight the influence of NP size on their cytotoxicity and the activation of signalling pathways or transcription factors during differentiation of MSCs into osteoblasts and adipocytes. Based on the reviewed information, the influence of these NPs on MSCs is summarized, and will provide a theoretical basis for the combined application of MSCs and NPs in biomedicine.

An electronic search of the Web of Science database for literature about NPs and their effects on MSCs differentiation of Science Citation Index from 1990 to 2020 was performed using the following conditions: ((nanoparticles) AND (mesenchymal stem cells) AND (cell behaviours) OR (cell differentiation) OR (cytotoxicity)). The results were further screened by title and abstract to only present hydroxyapatite, silica, silver, and calcium carbonate NPs. Non-Science Citation Index articles were also excluded.

Hydroxyapatite Nanoparticles

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is the major mineral composition of human hard tissue (bone and teeth).[19] There is a long history of the use of HA-based biomaterials to replace or repair bone, due to its excellent biocompatibility. With the broad and rapid development of nanoscience and nanotechnology, nanoscaled hydroxyapatite has drawn much attention for a variety of applications in the field of biomedicine. In dispersed form as NPs, HA NPs can be used as carriers in biological systems to deliver drugs, proteins or DNA to intracellular organelles.[17, 27, 28] HA NPs can also be functionalized with fluorescent dyes for imaging or photodynamic therapy.[29, 30] Additionally, HA NPs can simulate the mineral compartments of bone, thus they have

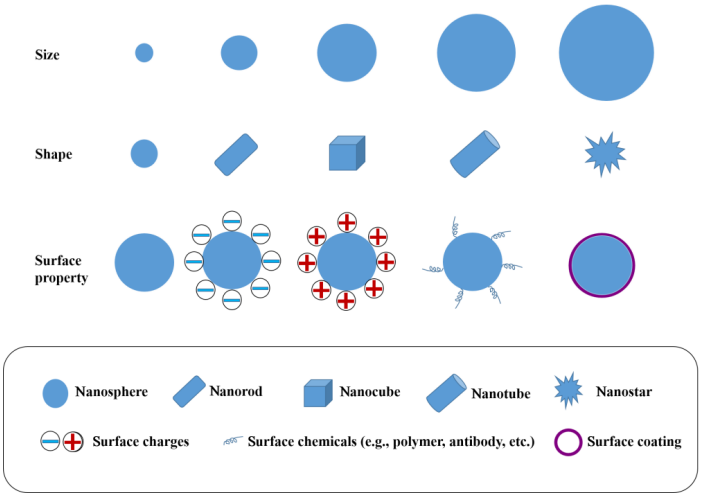

Figure 1. Schematic illustration of the characteristics of nanoparticles impacting cell behaviours.

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potential applications in freeze-cast porous scaffolds and artificial bone regrowth.[31, 32] With the aim of developing successful applications of HA NPs in these areas, many researchers have investigated the bioactivity of various types of HA NPs. In this section, we highlight only the cytotoxicity of HA NPs and their effects on stem cell differentiation.

Cytotoxicity

Internalization of NPs may lead to a variety of effects on cells. Cellular uptake of NPs may bring about changes in cell viability, morphology, differentiation and some other cell functions.[17] Among these effects, one of the most concerning is the cytotoxicity caused by NPs.

Many previous studies have already reported the dose-dependent cytotoxicity of HA NPs to a variety of cell types.[33-35] The cytotoxicity of NPs correlated strongly with the number of internalized particles, with higher cytotoxicity associated with a higher degree of particle–cell interaction.[36] Müller et al.[33] found that HA NPs (~30 nm in size) caused significant cytotoxicity to human monocyte-derived macrophages at concentrations ranging from 31.25 to 500 μg/mL. Similarly, HA NPs with varied physicochemical properties showed some cytotoxicity to human monocyte-derived macrophages when added at concentrations higher than 250 μg/mL.[34] Meena et al.[35] also reported that the inhibition of proliferation of human breast cancer cells (MCF-7) by HA NPs (with an average diameter of 10–20 nm) showed a concentration-dependent effect from 50–250 μg/mL. In our recent studies, we investigated the influence of HA NPs of different sizes (~50, 100 and 150 nm) on the cell viability of human MSCs (hMSCs) and found that each size of HA NP exhibited cytotoxicity at concentrations above 50 μg/mL.[37, 38] Meanwhile, in another study, synthesized HA NPs (with a particle size < 50 nm) did not induce cytotoxicity in mouse bone marrow MSCs at concentrations up to 800 μg/mL.[39] Collectively, these data show that the starting cytotoxic concentration of HA NPs varies between cell types. These data may provide useful information in choosing appropriate concentrations of HA NPs for biomedical applications.

HA NPs can escape the phagocytic pathway and a few have even been seen to enter the nuclei through nuclear pores, thus inducing cell apoptosis or necrosis.[25, 34] Cell death is closely related to the HA NP load, which may correlate with the concentration of calcium ions (Ca^{2+}) released form HA NPs after cellular uptake. The increased intracellular Ca^{2+} could cause lysosomal ruptures, resulting in cell necrosis and initiation of apoptosis.[40] Moreover, at higher exposure concentrations, the anticipated agglomeration and subsequent precipitation of HA NPs might cause mechanical damage to cells, explaining their cytotoxicity.[39] Another study has suggested that HA NPs may induce cytotoxicity through reactive oxygen species generation or cytokine production.[36] These mechanisms may be the reasons for the dose-dependent cytotoxic effect of HA NPs.

Cell differentiation

Internalization of NPs can induce modifications in cell function including differentiation of target cells. The effects of HA NPs on osteogenic differentiation of MSCs have been well researched. A number of studies have reported a positive role of HA NPs in promoting osteogenesis.[25, 37, 41-43] HA NPs with an average diameter of 20 nm promoted proliferation and osteogenic differentiation of rabbit MSCs.[41] HA NPs also enhanced mineralization and the expression of collagen I, ALP and core binding factor alpha l in rat MSCs.[25] Wang et al.[42] recently compared the impact of HA NPs of different shapes (nanospheres and nanorods) on MSCs, and found that both shaped NPs promoted osteogenic differentiation of MSCs but that HA nanospheres had stronger stimulatory effects in comparison to HA nanorods. Similar results were found in another study, in which HA NPs accelerated osteogenic differentiation of hMSCs by increasing ALP activity and enhancing mineralization in a similar manner to a short peptide of bone morphogenetic protein-7.[44] In our previous study we reported the *in vitro* uptake of HA NPs and their effects on osteogenic differentiation of hMSCs. HA NPs of three different sizes (~50, 100 and 150 nm) promoted the osteogenic differentiation of hMSCs by enhancing the expression of ALP, osteopontin, OCN and Runx2 (**Figure 2**). [37]

HA NPs might also change the properties of the culture medium and thus affect osteogenic differentiation.[25] The osteogenic differentiation of MSCs was enhanced in HA NP-conditioned culture medium, which was obtained by soaking sterilized HA NPs in complete medium for 3 days and then centrifuging to obtain the supernatant.[45] Since both calcium and phosphate ions have effects on bone cells, changes in their concentrations in the cell culture microenvironment caused by HA NPs may influence osteogenesis.[46] Calcium and phosphate can positively modulate several signalling pathways including extracellular signal-regulated kinases 1/2, which play essential roles in inducing cell differentiation.[46, 47] The appropriate concentrations of calcium and phosphate ions have positive effects on cell proliferation, mineralization and osteogenic differentiation of MSCs.[48] Moreover, small shifts in

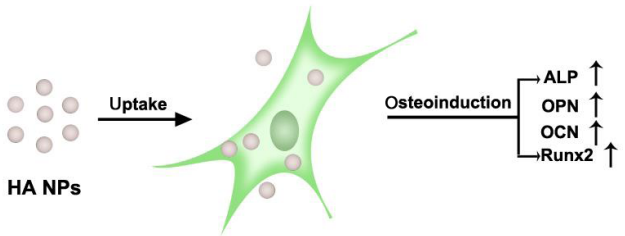

Figure 2. Schematic illustration of osteogenic stimulation of MSCs by HA NPs. ALP: alkaline phosphatase; HA NPs: hydroxyapatite nanoparticles; MSCs: mesenchymal stem cells; OCN: osteocalcin; OPN: osteopontin; Runx2: runt-related transcription factor 2.

extracellular pH induced by the addition of HA NPs could lead to significant changes in the ability of stem cells to express markers of the osteoblast phenotype, such as ALP, OCN and collagen I.[49]

With regard to adipogenesis, several previous studies have shown that HA in the form of coatings or discs suppressed adipogenic differentiation of stem cells.[50-53] For example, HA coating and synthetic HA discs were reported to inhibit adipogenic differentiation of hMSCs, as determined by oil red O staining and the expression of PPARγ and CCAAT/enhancer binding protein alpha.[50] Zhang et al.[53] demonstrated that disc-shaped HA regulated several microRNAs in the adipogenesis pathway, inhibiting adipogenic differentiation of MSCs. However, little is known about the effects of HA NPs on adipogenesis. HA NPs can cross the cell membrane and enter the cytoplasm and cellular organelles, which may cause different effects on adipogenesis compared with bulk HA materials. Our recent study showed that HA NPs did not influence the adipogenic differentiation of hMSCs at non-toxic concentrations, but inhibited differentiation at high concentrations due to their cytotoxicity.[38]

Silica Nanoparticles

Over the past few decades, silica NPs have attracted significant interest for their potential biomedical and biotechnological applications, such as drug delivery,[18] cancer therapy,[54] DNA/gene transfection[55] and cell tracking.[56] Silica NPs used in these applications can be categorized as mesoporous or nonporous NPs, both of which exhibit many unique properties, for instance, excellent biocompatibility, hydrophilic surface, versatile silane chemistry, low cost of production and ease of synthesis.[57] Due to the close interaction between silica NPs and cells, more knowledge of the effects of silica NPs on cells is required. In this section, we focus on the influence of silica NPs on cell viability, proliferation and differentiation. We first discuss the safety and toxicity of silica NPs *in vitro*, which is a prerequisite for their potential applications in medicine. The effects of silica NPs on cell differentiation are subsequently discussed.

Cytotoxicity

Fruijtier-Pölloth[58] and Napierska et al.[59] have comprehensively reviewed the toxicity of various forms of silica *in vitro* and *in vivo*. The toxicity of silica NPs strongly depends on their physical and chemical properties, for instance, surface chemistry, particle size, morphology, and solubility.[60, 61] On the other hand, Chang et al.[62] found that the influence of the physicochemical properties of silica NPs on *in vitro* cytotoxicity are also dependent on the type of cell lines. Thus, considering the potential applications of silica NPs and MSCs in tissue engineering, we mainly discuss the *in vitro* cytotoxicity of silica NPs on MSCs in this section.

There have been many investigations into the *in vitro* cytotoxicity of silica NPs on different cell lines.[63-66] In the case of stem cells, most of the studies reported a general lack of toxicity of silica NPs. For example, Ha et al.[67] demonstrated that the median 50% lethal concentration and 90% lethal concentration of ~50 nm spherical silica NPs for hMSCs and mouse bone marrow stromal cells were higher than 1000 μg/mL, and both types of cells showed at least 75% cell viability compared to controls (without NP treatment) after incubation for 3 days at 1000 μg/mL. Our previous study also reported that silica NPs of different sizes (~50, ~200 and ~400 nm) showed no significant influence on the cell viability of hMSCs after incubation for 24 hours at concentrations ranging from 0–500 μg/mL.[68] Similar results have been reported by Shi et al.,[69] where silica NPs with a diameter of 90 nm showed no cytotoxicity to hMSCs at concentrations of 31.25, 125 or 500 μg/mL. These results revealed little toxicity of silica NPs on stem cells. In another study, silica NPs were reported to be able to increase cell proliferation of human adipose-derived stem cells.[70] Taken together, these results show that silica NPs are noncytotoxic to MSCs at suitable concentrations and thus provide a possible combined application of silica NPs and MSCs.

Cell differentiation

Due to their pluripotent nature, MSCs play important roles in the field of regenerative medicine and have potential clinical applications for the repair of damaged or diseased tissue.[1] There are many factors that regulate the differentiation of MSCs. A variety of NPs have been investigated for their capacity to influence the differentiation of MSCs.

Several NPs have already been used to induce osteogenic differentiation of MSCs (summarized in **Table 1**). Among these NPs, silica NPs were employed in diverse experiments by different research groups to demonstrate their effects on osteogenic differentiation of MSCs. Ha et al.[67] studied the biosafety of ~50 nm silica NPs and their results showed that silica NPs can stimulate mineral deposition activity of hMSCs and MC3T3-E1 pre-osteoblast cells. Huang et al.[71] conducted an experiment on the internalization of silica NPs (~110 nm) and found that silica NPs were able to transiently promote the expression of osteogenic marker proteins by hMSCs. Shi et al.[69] reported that ~90 nm silica NPs could release silicon ions which stimulated the osteogenic differentiation of hMSCs by increasing their ALP activity and enhancing the expression of bone-related genes and proteins (Runx2, OCN and osteopontin). In our recent study,[68] we investigated the influence of silica NPs of different sizes (~50, 200 and 400 nm in diameter) on osteogenic differentiation of hMSCs. These silica NPs were biocompatible and were shown to promote osteogenic differentiation of hMSCs by enhancing ALP expression and mineralization. Interestingly, the larger sized silica NPs (~200 and 400 nm) significantly enhanced the size of bone nodules. It is well known that bone is a highly dynamic tissue which undergoes renewal throughout life via a process in which new bone is synthesized by osteoblasts and worn bone is resorbed by osteoclasts.[72] Silica NPs have been shown to play important roles in skeletal development and bone remodelling.[73] It has also been reported that silica NPs promote formation and mineralization of osteoblasts and negatively influence osteoclast formation.[73]

Several studies have explored the possible mechanisms underlying the positive effects of silica NPs on osteogenic differentiation of bone-related cells. The silicon ions released from degraded silica NPs and the change of cellular mechanical properties caused by the internalization of silica are considered to be the two main contributors. On the one hand, silicon, an important trace element, has been proven to play a significant role in bone repair.[78, 79] The ionic products released from H_4SiO_4 and $Ca_7Si_2P_2O_{16}$ ceramics have been reported to promote osteogenic differentiation of

Table 1. Various nanoparticles used in osteogenic differentiation of MSCs

Nanoparticle	Chemical composition	Size (nm)	Shape	Surface coating	Cytotoxicity	Application and results	Reference
Hydroxyapatite NPs	$Ca_{10}(PO_4)_6(OH)_2$	Diameter: ~20; length: ~50, width: ~8; length: ~100, width: ~43; length: ~150, width: ~23; length: ~200, width: ~20	Nanosphere, nanorod	Without	Size-, dose-dependent cytotoxicity to MSCs	Promoted proliferation and osteogenic differentiation of MSCs	25, 37, 41, 42, 44
Silica NPs	SiO_2	50, 90, 110, 200, 400	nanosphere	Without	A general lack of cytotoxicity to MSCs	Transiently enhanced osteogenic protein expression in hMSCs; Released silicon ions to stimulate the osteogenic differentiation of hMSCs	68, 69, 71
Calcium carbonate NPs	$CaCO_3$	Length: ~240, width: ~90	Nanorod	Poly(acrylic acid)	Showed no cytotoxicity to osteoblasts at concentrations of 1–1000 μg/mL	Enhanced proliferation and expression of osteoblast-related genes	74
Silver NPs	Ag	10, 20, 30	Nanosphere	Poly(vinyl pyrrolidone)	Time-, dose-dependent cytotoxicity to MSCs	Did not influence the osteogenic differentiation of MSCs or osteoblasts	75-77

Note: hMSCs: human mesenchymal stem cells; MSCs: mesenchymal stem cells; NPs: nanoparticles.

several bone-related cell lines by enhancing the expression of osteogenic-related proteins or genes.[80, 81] Some osteogenic-related signalling pathways including the transforming growth factor beta 1 pathway, extracellular signal-regulated kinases pathway, WNT and sonic hedgehog pathways have been found to be activated by silicon ions.[82-84] On the other hand, modulation of cellular mechanics can regulate cell function development including osteogenic differentiation.[85] The internalization of silica NPs causes changes in actin stress fibres, which could stimulate the osteogenic differentiation of hMSCs.[71, 85]

While most studies focus on the effects of silica NPs on osteogenesis, there have only been a few reports investigating their influence on adipogenic differentiation. Generally, there is considered to be an inverse relationship between osteogenic and adipogenic differentiation, i.e., stimulation of osteogenesis appears to suppress adipogenesis, or *vice versa*. Accordingly, silica NPs should have the potential to suppress adipogenesis. In a previous study, silica NPs displayed significant inhibitory effects on adipocyte differentiation via regulation of the phosphorylation of p38.[86] Additionally, our recent study also showed that silica NPs inhibited the adipogenic differentiation of hMSCs, reflected in decreased lipid droplet formation, triglyceride synthesis and adipogenic marker expression.[87]

As mentioned above, silica NPs exhibited significant stimulatory effects on osteogenic differentiation and inhibitory effects on adipogenic differentiation of MSCs *in vitro* (**Figure 3**). Thus, silica NPs may be a promising candidate for application in scaffolds for bone tissue engineering and have potential applications in the treatment of obesity.

Silver Nanoparticles

Due to their lethal effects on a wide spectrum of bacterial and fungal species, silver (Ag) NPs are currently one of the most commercialized nanomaterials in medicine.[77, 88] Ag NPs with their unique physicochemical properties are proving to be a promising candidate for a new antibacterial agent. Currently, hundreds of consumer products are available containing Ag NPs, such as wound dressings, bandages, catheters and surgical masks.[89] Moreover, some studies have shown that Ag NPs can act as carriers for drug delivery,[90] while Ag NPs have also been incorporated into scaffolds for tissue engineering.[91] With the increasing use of Ag NPs in the medical field, more and more attention has been paid to the issue of their potential safety. Some researchers have investigated the effects of Ag NPs on cell function and development including viability, proliferation and differentiation. In order to control the size or shape of Ag NPs, surfactants (such as poly(vinyl pyrrolidone), cetyltrimethylammonium bromide, and sodium bis(2-ethylhexyl) sulfosuccinate) are normally used during the synthetic process.[92] These normally-applied surfactants have been reported to play a significant role in the cytotoxicity and cell differentiation potential of the NPs.[93-97] In this section, we focus on the influence of Ag NPs themselves on the cell behaviours of MSCs.

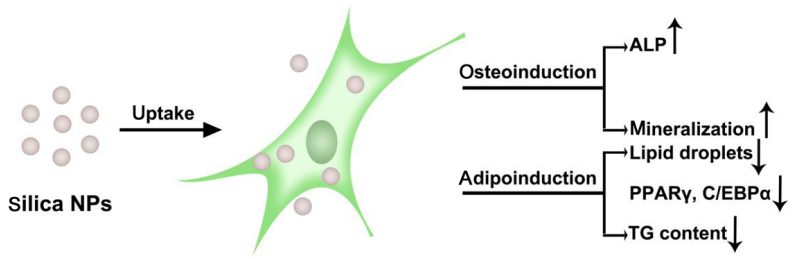

Figure 3. Schematic illustration of stimulation of osteogenesis and inhibition of adipogenesis of MSCs by silica NPs. ALP: alkaline phosphatase; C/EBPα: CCAAT/enhancer binding protein alpha; MSCs: mesenchymal stem cells; NPs: nanoparticles; PPARγ: peroxisome proliferator activated receptor gamma; TG: triglyceride.

Cytotoxicity

The medical applications of Ag NPs have already been demonstrated in many preclinical studies. Thus, it is important to fully evaluate the safety and potential toxicity of Ag NPs. The toxic properties of silver compounds are well known, as observed in the form of argyria when large amounts of silver ions are used for wound dressing.[88] Due to their small size and variable properties, Ag NPs have been shown to induce toxicity in cells derived from various organs.[98] The toxicity of Ag NPs is dependent on particle size, dose and time. Park et al.[99] compared the cytotoxicity of Ag NPs of different sizes (20, 80, 113 nm) and found that Ag NPs of 20 nm were more toxic than larger NPs. Hussain et al.[100] reported that Ag NPs of different sizes (15 and 100 nm) resulted in cellular morphological modifications, cell membrane damage and mitochondrial dysfunction of rat liver cells after an exposure of 24 hours. Braydich et al.[101] studied the cytotoxicity of Ag NPs on C18-4 germline stem cells and found that their cytotoxic effects on mitochondrial activity increased with increasing concentration. Our previous studies have reported dose-dependent toxicity of 30 nm diameter Ag NPs on hMSCs,[77, 98] while Pauksch et al. demonstrated that Ag NPs at a concentration of 10 μg/g impaired cell viability of hMSCs and osteoblasts after incubation for 21 days, although no cytotoxicity was observed at earlier time points and at lower concentrations.[75] These studies revealed that the possible reason for the cytotoxicity caused by Ag NPs is the fact that they are taken up by cells. Within the cells, the Ag NPs can induce excess production of reactive oxygen species, which is considered to be the main contributor to their cytotoxic effects.[99, 102]

Cell differentiation

Bacterial infection, a constant concern in tissue engineering, may contribute to implant failure.[91] Ag NPs are often employed as an antimicrobial agent for fabrication of implant scaffolds which are subsequently seeded with hMSCs. Moreover, hMSCs may come into close contact with Ag NPs after implantation of Ag NP-coated or -incorporated implants.[103] In this context, it is very important to analyse the effects of Ag NPs on stem cell differentiation. For implant scaffolds coated with or incorporating Ag NPs, the negative influence of Ag NPs on hMSCs should be minimal after long-term incubation.

To date, most studies have mainly focused on the effects of Ag NPs on osteogenic differentiation of bone-related cells. Pauksch et al.[75] reported that Ag NPs had no influence on the expression of ALP and Runx2 (two important markers of osteogenic differentiation) in hMSCs and osteoblasts at noncytotoxic concentrations. Samberg et al.[76] demonstrated that exposure to 10 or 20 nm Ag NPs at concentrations up to 100 μg/mL did not influence the osteogenic differentiation of human adipose-derived stem cells. Similarly, we previously found that 30 nm Ag NPs did not influence the osteogenic differentiation of hMSCs even at cytotoxic concentrations.[77] In another study, 20 nm Ag NPs were reported to promote osteogenic differentiation of human urine-derived stem cells at noncytotoxic concentrations after exposure for 24 hours, reflected by enhanced ALP activity, osteogenesis-related gene expression and mineralization level.[104] Similar results have been reported by Mahmood et al.,[105] who found that Ag NPs promoted osteogenic differentiation of MC3T3-E1 bone cells by enhancing ALP activity and mineralization. However, little is known about the influence of Ag NPs on adipogenic or chondrogenic differentiation of stem cells. There have been few investigations involving the effects of Ag NPs on adipogenesis or chondrogenesis and the conclusions are inconsistent. Our previous reports showed that 30 nm Ag NPs did not influence adipogenic differentiation but promoted chondrogenic differentiation of hMSCs *in vitro*.[106, 107] Also, Samberg et al.[76] found that Ag NPs did not influence the adipogenic differentiation of human adipose-derived stem cells. In contrast, Sengstock et al.[103] reported that Ag NPs impaired the adipogenic differentiation of hMSCs in a dose-dependent manner, whereas chondrogenic differentiation was unaffected after 21 days of incubation. Collectively, Ag NPs showed no negative impact on the capacity of stem cells for osteogenic differentiation, while there is no definite conclusion concerning their effects on adipogenic and chondrogenic differentiation of stem cells. These contradictory results may be explained by the fact that the effects of Ag NPs on cell differentiation are dependent on physicochemical properties and cell type.[108, 109] Moreover, the mechanism of how Ag NPs influence the differentiation process is still not clear. In this regard, more careful *in vitro* studies with well-characterized Ag NPs to elucidate the involved mechanism are highly desired.

Calcium Carbonate Nanoparticles

Calcium derivatives are the most important natural constituents of bone and teeth.[110] Due to their excellent biocompatibility, calcium-based inorganic materials such as calcium carbonate and calcium phosphate have attracted more attention in many biomedical applications. Calcium carbonate ($CaCO_3$), one of the most common minerals on earth, naturally exists as three

different polymorphs: calcite, aragonite and vaterite,[111] and their stability is orderly decreasing.[112] $CaCO_3$ has a long history of applications in industrial fields. It has been used as an additive in plastics, food, paints, paper and inks.[110, 113] Moreover, $CaCO_3$, especially at the nano-scale, also has great potential in biomedical applications, including drug delivery and bone regeneration.[114-116] For better application in biomedical fields, the cellular effects of Calcium carbonate NPs (CC NPs) in various forms should be better understood. In this section, we discuss the effects of CC NPs on cell viability and differentiation.

Cytotoxicity

$CaCO_3$ can be moulded by organisms into complex and beautiful shapes as in teeth and bones as well as shells.[114] Thus, it possesses excellent biocompatibility and low toxicity. Kong et al.[117] evaluated the cytotoxicity of $CaCO_3$ microparticles and the result showed that they could be used as noncytotoxic gene carriers. Cell viability tests conducted by He et al.[111] also demonstrated the noncytotoxic nature of $CaCO_3$ ceramic on rat bone MSCs. Different from bulk materials, CC NPs can be internalized into cells, which may cause different impacts. Several previous reports have studied the cytotoxicity of CC NPs. For instance, rod-shaped CC NPs (240 nm in length, 90 nm in width) exhibited no negative effects on cell viability of osteoblasts at concentrations of 1–1000 μg/mL, and tended to promote cell growth as their concentration increased.[74] Huang et al.[118] tested the acute and sub-chronic toxicity of CC NPs and their data suggested that CC NPs are more bioavailable than micro $CaCO_3$. Horie et al.[113] investigated the cellular influences caused by three types of CC NPs with different solubilities and size, and the result showed that none of the NPs caused remarkable cell membrane damage or significant effects on cell viability of two cell lines (human keratinocyte HaCaT cells and human lung carcinoma A549 cells) even up to 1000 μg/mL. However, they also demonstrated that CC NPs could induce some cellular influences via intracellular calcium release. At high concentrations, the generation of reactive oxygen species induced by CC NPs should be considered, which is one of the most important cellular effects caused by NPs.[110]

Cell differentiation

Due to its excellent osteoconductivity and biodegradability, $CaCO_3$ is considered as a promising candidate for bone tissue engineering. It has been used clinically in dental, orthopaedic, maxillofacial and craniofacial surgery.[119] The bone forming response of $CaCO_3$ has been shown to be comparable to that of HA.[120] The basic difference between $CaCO_3$ and HA is their solubility: $CaCO_3$ is biodegradable while HA exhibits poor biodegradability. $CaCO_3$ is mostly used as a matrix or scaffold for regulating the bioactivity of bone-related cells. Indeed, a variety of studies have reported that calcite and aragonite porous scaffolds can be used as bone grafts or active carriers for various cell types, including osteoblasts and MSCs.[121, 122] For instance, an aragonite matrix obtained from coral was reported to show stimulatory effects on the differentiation of MSCs to the osteogenic phenotype.[116] $CaCO_3$ with nanostructure also has positive effects on osteogenesis. Fujihara et al.[123] fabricated a new type of guided bone regeneration membrane composed of polycaprolactone and $CaCO_3$ composite nanofibers, on which human osteoblasts showed good cell attachment and proliferation. Yang et al.[74] cultured CC NPs with MC3T3-E1 cells and found that CC NPs exerted a positive effect on cell proliferation and increased the expression of ALP, OCN and bone sialoprotein. Huang et al.[118] found that administering CC NPs (administered by gavage) could increase the serum calcium concentration and maintain whole-body bone mineral density in ovariectomized mice. Our previous study also reported that CC NPs significantly promoted osteogenic differentiation of hMSCs by increasing ALP activity, collagen secretion and the expression of osteogenic-related genes.[124]

A variety of factors comprehensively regulate the processes of osteogenic differentiation. Calcium ions are considered to be a coupling factor between osteoblasts and osteoclasts, playing an important role in regulating osteogenic differentiation by affecting specific calcium ion-related signalling pathways and expression of calcium-dependent proteins.[125, 126] Therefore, the stimulatory effects of CC NPs on osteogenesis may be caused by the calcium ions they release.

Conclusion

HA, silica, silver and CC NPs, which exhibit many unique properties, have already been widely used in various biomedical applications. In this review, we explain how these NPs, when administered in the culture medium, affect the proliferation and differentiation of MSCs, supporting the common use of NPs and stem cells in tissue engineering and regenerative medicine. However, the exact mechanisms via which they cause cytotoxicity and influence the differentiation process are still not clear. In this regard, more studies are needed to focus on the mechanisms involved. Nevertheless, we summarize the influential rule of these NPs on the cell functions of MSCs. Designing and synthesizing suitably-sized and shaped HA/silica/CC NPs together with appropriate nanostructured scaffolds can be applied as a novel strategy for bone tissue engineering. At suitable concentrations, Ag NPs do not cause cytotoxicity or affect cell differentiation, but kill bacteria. In this context, Ag NPs can be used for wound dressing or coated onto implant surfaces to prevent bacterial infection.

Author contributions

QF determined the topic of the review and modified the manuscript; XY conceived, performed the main data analysis and wrote the manuscript; YL, XL and WH collected related articles; YL performed the data analysis of section "Hydroxyapatite Nanoparticles" and "Silica Nanoparticles"; XL performed the data analysis of section "Silver Nanoparticles"; WH performed the data analysis of section "Calcium Carbonate Nanoparticles"; QH helped perform the data analysis and draw the table and figures. All authors approved the final version of this manuscript.

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Conflicts of interest statement

The authors declare no competing financial interest.

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