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Local Cellular Responses to Titanium Dioxide from Orthopedic Implants

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Abstract

We evaluated recently published articles relevant to the biological effects of titanium dioxide (TiO₂) particles on local endogenous cells required for normal bone homeostasis, repair, and implant osseointegration. Structural characteristics, size, stability, and agglomeration of TiO₂ particles alter the viability and behavior of multiple bone-related cell types. Resulting shifts in bone homeostasis may increase bone resorption and lead to clinical incidents of osteolysis, implant loosening, and joint pain. TiO₂ particles that enter cells (through endocytosis or Trojan horse mechanism) may further disrupt implant retention. We propose that cellular responses to titanium-based nanoparticles contribute to pathological mechanisms underlying the aseptic loosening of titanium-based metal implants.

Keywords: adverse local tissue reaction; joint replacement; nanoparticle; total hip arthroplasty; total knee arthroplasty

Introduction

Total joint arthroplasty (TJA) is the definitive treatment for severely damaged synovial joints (e.g., symptomatic osteoarthritis^{1,2}). As the number of TJA patients in the United States rises from an estimated 7 million,^{3,4} demands for revision TJA will concurrently increase. Indications for revision TJA include septic and aseptic etiologies (e.g., dislocations, polyethylene wear, wear-induced osteolysis, implant loosening, and adverse local tissue reactions^{5,6}), whereas the causes of such complications often go undetected (e.g., latent periprosthetic joint infections), and mechanisms remain unidentified.⁷ Poor surgical technique and implant design cause implant failure, yet 10–15% of patients experience aseptic implant failure despite well-designed implants and meticulous surgical technique.^{8–10} We propose that the release of titanium nanoparticles may contribute to aseptic implant loosening by negatively influencing periprosthetic skeletal repair cells.

Implant materials can corrode, degrade, and wear (i.e., tribocorrosion), adding particles to the intraarticular joint space that may impede healing of bone-implant interfaces.^{11–13} Metal implants are naturally protected from extensive corrosion by a surface oxide layer, but mechanical stress can overwhelm the protective capacity of this layer and result in the release of harmful metallic particles.¹⁴ Particles that enter the joint space, periprosthetic bone, surrounding soft tissues, and distal tissues^{15–17} cause aseptic implant loosening,¹⁸ adverse local tissue reactions,¹⁹ and/or systemic toxicity,²⁰ all of which can lead to significant patient discomfort and inconvenience. Nonmetal and metal particles likely have a synergistic effect toward implant loosening; however, concentration-dependent patterns of localized tissue/joint damage caused by debris release have not been clearly established and may vary greatly by patient.

The adverse effects of metallic orthopedic particles are of great increased interest, in part, because of the

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large-scale recalls of metal-on-metal implants that have had significant economic costs to the healthcare industry.^{21–23} For example, cobalt and chromium particles disrupt cells locally and have been implicated in systemic disorders such as hypothyroidism, cardiomyopathy, or neuroocular disease.^{19,20,24,25} Although titanium alloys are thought to be biologically inert,²⁶ they may be a source of titanium dioxide (TiO₂) particles that influence human cells. The potential effects of TiO₂ particles need to be further investigated, particularly in the microenvironment of mesenchymal skeletal repair cells within synovial joints, where the largest titanium implants are typically applied.²⁷ Specifically, the biological, physical, and chemical interactions between TiO₂ nanoparticles and endogenous bone-forming cells (e.g., osteoblasts, osteocytes) need to be characterized to better formulate strategies that reduce nanoparticle-based implant loosening. This review examines recent findings on the cellular effects of TiO₂ nanoparticles in the peri-implant joint microenvironment. Specifically, particle size, aggregation, structure, uptake, and endocytosis, as well as the intra- and extracellular effects of titanium particle exposure, are discussed within the context of cell viability, behavior, and phenotypic change.^{28,29} Findings that facilitate a focus on clinically relevant information that could be eventually translated into novel prophylactic and therapeutic options for minimizing aseptic orthopedic implant loosening are highlighted.

Characteristics of Tribocorrosion-Produced Titanium Particles

Orthopedic implants form a passivating superficial TiO₂ film that can prevent extensive corrosion.¹⁴ However, implant wear degrades this TiO₂ layer, resulting in depassivation, bare metal exposure, repassivation, and corrosion^{30,31} (Fig. 1). *In vivo* corrosion and wear of titanium implants can, therefore, produce a diverse group of TiO₂, inorganic metallic salts, as well as free metal ions.³² Tribocorrosion-produced TiO₂ particles are mineral oxides that exist in different phases, most commonly rutile and anatase,^{33–35} which can be harmful to some cell types. Similar to concentration and exposure time, critical relationships between size, phase, and stability likely influence the cytotoxicity of particles^{36–38} (Table 1).

Particle sizes need to be considered independently regarding their potential effects on aseptic implant loosening.^{15,34,39,40} In addition, TiO₂ nanoparticles aggregate in the extracellular space or perinuclear cyto-

sol,^{29,41,42} causing agglomerations that may be part of the cytoprotective response of cells to the presence of nanoparticles. Most biological characterizations of nanomaterial–cell interactions have focused on particles smaller than 100 nm and demonstrated surface area-related effects that increase as particle sizes decrease.³⁹ At smaller particle diameters, the surface area-to-volume ratio increases and surface properties exert a more dominant influence on bioreactivity.^{34,40} Therefore, ultrafine particles may exhibit different properties than larger, coarse particles³⁴ or particle agglomerations, which emphasizes the importance of accurately characterizing particle size distributions in clinical samples used for diagnosing aseptic implant loosening. Particle size not only affects nanoparticle–cell interactions but also influences the transportability of particles within a joint and throughout the body.

Although a combination of differently sized particles may be present *in vivo*, their distribution is heterogeneous. For example, Maloney et al.¹⁵ found that >90% of particles were <1000 nm in digested peri-implant tissues. Examination of lymphatic, splenic, and hepatic tissues from TJA patients has also shown that most disseminated particles were <1000 nm in size.¹⁷ Smaller particles enter cells more easily by phagocytosis and should be considered more bioreactive.^{43,44} Furthermore, the ability of nanoparticles to aggregate may increase their observed size.^{29,42,45–47} Agglomerates are often found *in vitro*, but they may be quite unstable and dissociate into their constituent nanoparticles *in vivo*.³⁴ Equally important to aseptic loosening is the recirculation of dissociated particles within synovial joints, causing catalytic cytotoxic effects and/or adverse local tissue reactions (e.g., pseudotumor formation). The cell cycle responds to the presence of nanoparticles (e.g., endocytosis, apoptosis) will provide relevant opportunities for therapeutic intervention strategies that minimize their negative effects.

Cellular Uptake and Cytotoxicity of TiO₂ Nanoparticles

The primary mechanism of TiO₂ nanoparticle cellular uptake is thought to be endocytosis.^{29,48–50} Using pharmacological inhibitors of endocytosis, Cai et al.²⁹ demonstrated a relationship between particle size and mode of endocytosis. Specifically, small (14 nm) particles were taken in by caveolae, whereas larger (74 and 196 nm) particles were taken in by clathrin-coated pits.²⁹ To further confirm the role of endocytosis, an adenosine triphosphate (ATP)-dependent process, these authors inhibited ATP production through application

FIG. 1. Types of wear/corrosion that contribute to TiO_2 nanoparticle release after orthopedic implant placement. Corrosive wear: a corrosive layer forms on the surface of metal implants. Adhesive wear: atomic forces between two surfaces can exceed the strength of one or both surface(s). Relative movement of the two surfaces generates particles of multiple sizes. Abrasive wear: with two materials of different hardness, the softer material will generate particles through mechanical stress exerted by the harder surface. Third-body wear: a third body becomes embedded between two articulating surfaces and additional particles are generated along the path of third body movement. Fatigue: shear stress and/or strain can exceed the fatigue limit of a material, releasing particles.

of NaN_3 in combination with reduced temperature (4°C) and found a significant decrease in the uptake of 14 nm particles (to 16.73% at 4°C ; 26.28% when exposed to NaN_3).²⁹ However, alternative pathways to endocytosis and the entry of nanoparticles into cells still need to be considered, as many other forms of cellular membrane transport are energy dependent and potentially useful for reducing any harmful effects of nanoparticles.

In addition to TiO_2 nanoparticles observed within endosomes, unbound nanoparticles have been discovered in the cytosol,^{48,51} likely from degraded endosomes. Alternatively, nanoparticles may be transported through membrane pores, specific transporters, or a Trojan horse effect^{48,49,52} (Fig. 2). Regarding a possible Trojan horse effect, TiO_2 nanoparticles form a core of biocomplexes rich in calcium and phosphorus that in principle remain

Table 1. Recent Studies Investigating the Effects of TiO₂ Nanoparticles on Musculoskeletal Cells and Tissues Are Summarized, as Well as Examples of Relevant Animal Models and Systems

Citation	Particle characteristics	Cell line	Cellular uptake	Cytotoxicity	Nonfatal shifts in intracellular behavior
Bernier et al. ⁶²	Anatase TiO ₂ (<25 nm)	Mice preosteoblast (MC-3T3)		Dose and time dependent cytotoxicity as measured by LDH release in all cell types	Found no effect on cellular adhesion as measured by number of cells attached.
	Agglomerations measured by dynamic light scattering to be 200–500 nm.	Rat fibroblast (L929)		Dose-dependent inhibition of cell proliferation in MC3T3 cells. Dose-dependent inhibition of cell proliferation in L929 cells but only at very high levels (1000 µg/mL)	IL-6 secretion is increased by TiO ₂ in MC3T3 cells but not L929 cells. Neither cell increased TNF-α secretion
Cai et al. ²⁹	Anatase TiO ₂ (14, 74, and 196 nm)	Neonatal rat calvarial osteoblasts	14 nm particles endocytosed by caveolae and 74 nm and 196 nm particles taken up by clathrin and caveolae. Inhibition of ATP led to significant decrease in uptake of 14 nm particles.	Concentration-dependent effect on proliferation with 74 nm particles.	ALP activity highest in smallest NP, lowest in largest NP.
	Intracellular agglomerations seen through SEM in cytoplasm, endosomes, and lysosomes. Cells exposed to larger 196 nm size particles had more intracellular agglomerations.	Human embryonic kidney cells (HEK 293) Human liver cells (L-02)		Flow cytometry showed larger particles to trigger apoptosis. Decreasing particle size decreased cell apoptosis percentages.	RANKL mRNA highest at largest sizes and lowest at smallest sizes. 100 nm jump point for activity of MMP9
Huang et al. ⁷²	TiO ₂ (15 nm)	Mouse fibroblasts (3T3) Human fibroblasts (HFW)	Demonstrated time-dependent increase in the cellular uptake of nano-TiO ₂	NIH 3T3 fibroblasts showed increase in MTT assay activity after TiO ₂ nanoparticle exposure. Found increase in number of cells through MTT assay, colony forming ability assay, and trypan blue exclusion assay.	NIH 3T3 cells increased ERK 1/2 activation but total levels remained unchanged. Observed increased chromosomal instability with more multinuclei and multipolar spindles. Found that deregulation of <i>PLK1</i> functions on cytokinesis affected spindle assembly and centrosome maturation.
Niska et al. ⁶⁸	Anatase TiO ₂ (5–15 nm, “predominant size 10–15 nm”)	Human fetal osteoblasts (hFOB 1.19)	Invaginations and vacuole formation.	TiO ₂ NP decreased viability. Observed condensed configurations within the mitochondria. Also identified autophagolysosomes	Decreased ALP activity observed. Lipid peroxidation seen through TEM and elevated MDA levels. Increased superoxide anion generation without an increase in <i>SOD1</i> and <i>SOD2</i> .

(continued)

Table 1. (Continued)

Citation	Particle characteristics	Cell line	Cellular uptake	Cytotoxicity	Nonfatal shifts in intracellular behavior
Ribeiro et al. ⁵²	Anatase TiO ₂ (<25 nm) Agglomerations formed of biological ions and proteins as determined through elemental maps, electron diffraction pattern, electron energy loss spectrum, and gel electrophoresis.	Primary bone cells from cancellous bone explants	TEM showed osteoblast internalization of biocomplexes. Some cells even had >50% of cytoplasm filled with nanoparticles inside vesicles.	Found no effect on viability with anatase biocomplexes through apoptosis assay TEM showed some cells with swollen mitochondria, autophagolysosome-like structures.	
Valles et al. ²⁸	Rutile TiO ₂ (0.9–1.6 µm) Intracellular agglomerations seen through SEM.	Primary culture of human peripheral blood mononuclear cells THP-1 cells driven to macrophage/monocytic lineage Human primary osteoblasts	Confocal microscopy showed intracellular aggregates suggesting that cells may internalize aggregates rather than individual particles.	No significant changes in LDH release	TiO ₂ stimulated TNF-α, IL6, and IL-1β release from THP-1 macrophages. THP-1 macrophages cocultured with osteoblasts only saw increased IL-1β
Vamanu et al. ⁶⁴	Anatase and rutile mixture TiO ₂ (<100 nm) Extracellular and intracellular agglomerations (>100 nm) seen through TEM and SEM.	Human histiocytic lymphoma cells (U937)	Pseudopodia seen engulfing small groups of nanosized electron dense particles, likely nanoparticles.	Increased percentage of apoptotic cells as measured by DNA quantification. Found increased membrane permeability with time and dose.	
Zhang et al. ⁵⁸	Anatase TiO ₂ (5 and 32 nm) Extracellular agglomerations seen through TEM	Mice preosteoblasts (MC3T3-E1)	TEM showed coated cellular vesicle formation and nanoparticle endocytosis.	Viability reduced at higher nanoparticle concentrations around 100 µg/mL as measured by LDH release assay.	GM-CSF, G-CSF, and IL-1 expression increased. No change in TNF-α

Methods and conclusions are outlined to highlight the need for a future studies that comprehensively identify nanoparticle–cell interactions within the context of improving orthopedic implant retention.

ATP, alkaline phosphatase; ATP, adenosine triphosphate; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte-colony stimulating factor; LDH, lactate dehydrogenase; MDA, malondialdehyde; MTT, tetrazolium dye; NP, nanoparticles; RANKL, receptor activator of nuclear factor kappa-B ligand; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

FIG. 2. A Trojan horse mechanism as a proposed mechanism for the internalization of TiO_2 nanoparticles. **(A)** TiO_2 nanoparticles can form biocomplexes consisting of a TiO_2 core surrounded by ions such as calcium and phosphorus that associate with proteins. **(B)** The surrounding shell of calcium, phosphorus, and protein hides TiO_2 nanoparticles from cell surface receptors. Once internalized, biocomplexes dissociate and TiO_2 nanoparticles can spread throughout the cytosol to effect cellular functions and homeostasis. This Trojan horse mechanism may also be involved in clathrin- and caveolae-mediated endocytosis of TiO_2 , although it remains unclear whether TiO_2 nanoparticles can enter cells without a Trojan horse (or similar) mechanism (indicated by ? in figure).

undetected by cells. Association of biocomplexes with glycoproteins such as fetuin A (alpha-2-HS-glycoprotein [AHSG]) would further facilitate endocytosis. As biocomplexes dissociate within cells, the TiO_2 nanoparticles are released into the cytosol where they may provoke cytotoxic responses.⁵² Although membrane ATPases and transporters (e.g., natural resistance-associated macrophage proteins) have been characterized for other metal ions,^{53,54} titanium-specific transporters are not known. Upon entry into cells, TiO_2 nanoparticles have size-dependent mechanisms (extracellular and intracellular processes) that influence cell growth, protein production, and cellular phenotypes that may initiate (or accelerate) aseptic loosening.

A size-toxicity relationship has been described for some nanoparticles (e.g., CuO , Ag) and cells, such that smaller nanoparticles are more harmful, yet for TiO_2 the evidence remains inconclusive. A study of rat neonatal calvarial osteoblasts compared the effects of anatase phase TiO_2 of various sizes (ranging from 14 to 196 nm) and found no significant differences in cellular morphology among treatments.²⁹ Larger particles and aggregations may remain in the cytosol and interfere with cellular processes.^{29,55} A later investigation of rat mesenchymal stem/stromal cells (MSCs) compared 14, 108, and 196 nm diameter particles and found that cell viability, proliferation, cell cycle progression, cell adhesion, and cell migration decreased with

increasing nanoparticle size.⁴² Consistent with work on other cell lines,^{56,57} osteogenic differentiation was reduced in cells exposed to larger nanoparticles,⁴² suggesting that particle size and agglomeration chemistry may influence cell biology and/or aseptic loosening processes. In addition to modulating cell behavior, TiO₂ nanoparticles can directly affect cell viability. Increases in regulated cell death among osteoblast lineage cells have been observed after exposure to nano- and micro-particles.^{45,50,51,58–61} Mitochondrial and cell membrane permeability both increase in the presence of TiO₂ particles, suggesting significant cell stress.^{58,62} Investigations involving human MSCs, osteoclasts, and histiocytic lymphoma cells have confirmed similar responses,^{41,60,63,64} yet the current challenge is to define how these cells interact with other peri-implant cell types such as preosteoblasts and osteoblasts.

TiO₂ nanoparticles likely induce apoptosis through both direct and indirect mechanisms.^{65,66} The induction of apoptosis across a number of cell types suggests a universal mechanism of dose-related DNA damage and oxidative stress.^{41,63,64,67,68} TiO₂ nanoparticles may also react with membrane components, leading to reactive oxygen species, leakage of Ca²⁺, Ca²⁺-dependent endonuclease activation, and apoptosis.⁶³ Documented high concentrations of TiO₂ nanoparticles that cause direct genotoxicity and apoptosis^{20,37,63,69–73} provide a logical starting point for *in vitro* experiments, which should reveal the dose-dependent mechanisms responsible for TiO₂-related processes of aseptic orthopedic implant loosening. TiO₂ nanoparticle localization within the nucleus has not been widely documented, but particle aggregates have been observed in the perinuclear region.^{20,29,37,41,42,46,62,63,69–74} This indicates an indirect mechanism of genotoxicity related to cellular stress. However, localization of TiO₂ has only been reported in one study of periodontal ligament cells,⁷⁵ whereby particles may have transported through nuclear pores or fused vesicles into the nucleus, a phenomenon previously described for Ag nanoparticles.^{75,76} Cell-specific observations of nanoparticle accumulation within the nucleus and organelles will help identify which cells are most vulnerable to TiO₂ exposure and the possible mechanisms of nanoparticle incursion into joints after TJA.

Interpretation of reported nanoparticle sizes can be particularly difficult given the irrepressible tendency of these nanoparticles to agglomerate. Of note is the possibility that interstitial void spaces among loosely aggregated nanoparticles may provide adequate substrates for the attachment and functionalization of or-

ganic molecules (Fig. 2). Nonetheless, reported individual particle sizes may be misleading if aggregations are frequently formed. As bioreactivity is widely believed to be related to surface area, aggregations of nanoparticles may, therefore, exhibit different toxicity and abilities to enter cells than their constituent particles. The potential for nanoparticle aggregations to dissolve *in vivo* complicates the correlation between *in vivo* and *in vitro* data. Studies that incorporate both approaches in conjunction with clinical samples and data will yield useful information to guide clinical approaches that reduce aseptic implant loosening rates.

Indirect Effects of TiO₂ Exposure on Cell Viability

Indirect effects of titanium particle exposure on cell viability have also been proposed. For example, extraction of conditioned media from human MSCs exposed to sub-micron titanium was cytotoxic to naive human MSCs and induced apoptosis in the absence of particles. However, dilution of the conditioned media to a 1:1 ratio abolished this effect, suggesting a critical concentration of signaling factors.⁶⁰ TiO₂ particle-induced apoptosis also exhibits both dose and time dependency. Although the translation of these findings to *in vivo* systems is less obvious, the identification of thresholds indicates dysregulation of cellular regulatory mechanisms after a critical concentration is surpassed.⁶⁰

Importantly, cellular apoptosis in reaction to TiO₂ nanoparticles is the result of both direct and indirect processes. TiO₂ first causes oxidative stress and direct injury to cells, and subsequently accelerates the inflammatory cascade by paracrine cytokine release, which is followed by increased apoptosis. However, TiO₂ particles do not always induce oxidative stress and cell death in stress-tolerant cells (e.g., endothelial cells).^{77,78} Yet in the presence of significant cellular stress, tissue necrosis can contribute to cell death. For example, histiocytic lymphoma cells (U937) show morphological characteristics consistent with both apoptosis and necrosis.⁶⁴

Other researchers have suggested that rutile-structured TiO₂ particles induce apoptosis, whereas anatase-structured TiO₂ particles may increase necrosis.⁷⁹ Importantly, experimental knockouts of key apoptosis molecules (e.g., Bak and Bax) exhibit significant cell death in the presence of TiO₂, clearly highlighting the important contribution of necrosis.^{80–82} Apoptosis (programmed cell death) and necrosis (non-specific cell death) together provide complementary mechanisms to detrimentally impact peri-implant

tissues and cells, and may compound complications of aseptic orthopedic implant loosening.

Bone-Related Effects of TiO₂ Exposure

Consistent with patterns of osteolysis and/or peri-prosthetic fractures observed in patients with aseptic loosening, TiO₂ particles are known to increase bone resorption.^{83–85} Specific consequences of TiO₂ nanoparticle exposure to osteoblasts include increased cell death, mitochondrial membrane permeability, lactate dehydrogenase release, and gene expression alterations (e.g., *TNFSF11*).^{29,58,62} Preosteoblasts (MC3T3-E1) may be particularly sensitive to TiO₂ nanoparticles, as they exhibit decreased proliferation even at extremely low concentrations (20 µg/mL).^{58,62} In contrast, fibroblastic (L929) cells maintain normal cell proliferation rates until exposure to higher concentrations of TiO₂ (>500 µg/mL).⁶² Heterogeneity in the responses of different cell types to elevated TiO₂ concentrations make the assessment of *in vivo* consequences challenging, particularly when multiple cell types interact to form healthy bone tissue and a well-fixed orthopedic implant.

Osteogenic differentiation of human MSCs into osteoblasts is suppressed by exposure to submicron Ti particles.^{60,61} In addition, human MSCs exposed to TiO₂ exhibit structural alterations (e.g., deregulated actin skeleton formation, decreased integrin-binding sialoprotein expression, diminished collagen Type I (*COL1A1*), and integrin-binding bone sialoprotein (*IBSP*) production, inhibited ECM formation), decreased cellular viability, and slower cellular proliferation.^{45,60,61} TiO₂ may be both cytotoxic to osteoblast precursors and inhibit the formation of new osteoblasts, thus preventing new bone formation and hindering implant fixation. Nonetheless, the identification and timing of events that lead to aseptic implant loosening need to be better contextualized within a framework of measured TiO₂ particle release *in vivo*.

TiO₂ particle exposure may also increase the secretion of matrix proteases by osteoblasts. For example, coarse TiO₂ particles (4.5 µm mean size) increased the expression of genes related to metalloproteinase activity (e.g., *Mmp2* and *Mt10*) in preosteoblasts (MC3T3-E1), but did not alter *Timp2* expression.⁸⁶ Metalloproteinase inhibitors such as TIMP act as negative feedback regulators on the activity of metalloproteinases⁸⁷ and increase bone resorption. Further compounding bone resorption, the proteolytic activity of MMP2 protein may be increased in the presence of TiO₂ particles.⁸³ In summary, larger particles alter extracellular processes, smaller particles enter cells to

disrupt intracellular processes, and the viability of endogenous skeletal repair cells can be modulated by direct and indirect mechanisms of TiO₂ exposure.

Conclusions

Investigations on the effects of tribocorrosion-produced TiO₂ particulate debris on musculoskeletal tissues and cells remain critical given the absolute number of titanium-based surgical implants used in TJA procedures every year. TiO₂ nanoparticles have been demonstrated to alter cell viability, behavior, as well as extracellular and intracellular processes. Resulting shifts in bone homeostasis may increase bone resorption and explain clinical findings of osteolysis, loosening, and pain. Future investigations are necessary to identify effects on cells (intra- and extracellular) and better characterize TiO₂ nanoparticle behavior *in vivo*. This remains especially challenging because of technical limitations that preclude accurate measurements of TiO₂ nanoparticles in human tissues and cells. However, the findings presented in this review suggest that TiO₂ may be an important contributor to aseptic orthopedic implant loosening. Further characterization of inflammatory processes and adverse cellular behaviors upon exposure to multiple sizes of TiO₂ particles should yield targets for clinical interpretation and guide novel intervention strategies.

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Abbreviations Used

MSCs = mesenchymal stem/stromal cells
TiO₂ = titanium dioxide
TJA = total joint arthroplasty

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