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**Approaches to promoting bone marrow mesenchymal stem cell osteogenesis on orthopedic implant surface**

Huo S *et al.* MSC osteogenesis on orthopedic implant surface

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**Abstract**

Bone marrow-derived mesenchymal stem cells (BMSCs) play a critical role in the osseointegration of bone and orthopedic implant. However, osseointegration between the Ti-based implants and the surrounding bone tissue must be improved due to titanium’s inherent defects. Surface modification stands out as a versatile technique to create instructive biomaterials that can actively direct stem cell fate. Here, we summarize the current approaches to promoting BMSC osteogenesis on the surface of titanium and its alloys. We will highlight the utilization of the unique properties of titanium and its alloys in promoting tissue regeneration, and discuss recent advances in understanding their role in regenerative medicine. We aim to provide a systematic and comprehensive review of approaches to promoting BMSC osteogenesis on the orthopedic implant surface.

**Key words:** Bone marrow mesenchymal stem cells; Osseointegration; Orthopedic implant; Biofunctionalization

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**Core tip:** Bone marrow-derived mesenchymal stem cells (BMSCs) play a key role in tissue repair after bone and joint injures. The effects of the surface treatment of the orthopedic implants on the osteogenic differentiation of BMSCs are worthy of attention. In this paper, we review recent advances in approaches that promote osseointegration of BMSCs on the surface of orthopedic implants.

**INTRODUCTION**

Since Friedenstein *et al*[1] isolated bone marrow mesenchymal stromal cells from for the first time and regarded them as bone tissue progenitor cells, they have played an increasingly important role in orthopedics. Bone marrow-derived mesenchymal stem cells (BMSCs) are ideal candidates for tissue repair after traumatic injury because they are relatively easy to harvest *in vitro* and can undergo self-renewal and multi-directional differentiation into several mesodermal and non-mesodermal cell lineages including osteoblasts, chondrocytes, and adipocytes[2-6]. Degenerative diseases of bone such as osteoarthritis can lead to bone fractures and immobility, compromising quality of life. In the treatment of osteomyelitis, after effectively controlling the symptoms of infection using local or systemic antibacterial drugs, BMSCs differentiate into osteoblasts and lipoblasts, and finally differentiate into mature bone adipose tissue for repair local injury[7]. However, although much attention has been paid to the engineering of biomaterials that regulate BMSC commitment to specific lineages, like the chondrogenic and osteoblastic lineages, harnessing BMSC fate remains a major challenge[8,9]. Therefore, overcoming these challenges would be very significant in the field of orthopedics, where the ability to stimulate osteogenic BMSC differentiation on biomaterials like titanium and its alloys would translate into higher rates of implant osseointegration and improved long-term functionality. In addition, it is necessary to stimulate the *in vivo* environment using BMSCs to study the cellular response at the bone-implant interface since BMSCs are in direct contact with the implant after surgery[10].

The term “osteointegration” has been used since Professor Branemark first reported the phenomenon of “osteointegration” to describe the stable combination of biomaterials and bone tissue. Osseointegration refers to the direct contact of the bone with the implant without an intermediate layer of connective tissue. This biological fixation is a prerequisite for implantable prostheses and their long-term success.

Titanium and its alloys have been widely used in biomedical areas in recent decades for cardiovascular, orthopedic, and dental applications due to their resistance to fatigue, superior mechanical properties, and load-bearing capabilities[11,12]. For example, the elastic modulus of nitinol is 40 GPa, compared to 30 GPa for bone[13]. However, there are major disadvantages to using Ti-based implants, including inert biomaterials and poor biological activity[14,15]. In addition, they fail to achieve sufficient osseointegration, leading to increased aseptic loosening and premature implant failure[16,17]. Therefore, these problems with Ti-based implant materials have hindered to some extent their development as orthopedic implants. Campoccia *et al*[18] believed that the surface of an ideal osteo-compatible biomaterial should possess the following characteristics *in vitro*: (1) Allow good and tight initial adhesion; (2) support cell attachment and viability; and (3) have a positive influence on the osteogenic differentiation process. Given that the interaction between the implant materials and bone tissue first occurs on the implant surface, it is necessary to modify the implant surface to solve the problems in titanium and its alloys. BMSCs have the critical role to achieve bone and implant osseointegration. Surface composition, hydrophilicity, and roughness of the orthopedic implant can affect BMSC differentiation and affect osseointegration. Thus, the surface of the implant must be biomodified to create a bioactive surface that is helpful to promote cell-material interactions and improve osseointegration of titanium and its alloys[19-21].

Many surface modification techniques like physical vapor deposition, sol-gel, ion implantation, anodization, and micro-arc oxidation have been investigated to improve the surface properties of titanium and its alloys[22-25]. Although many researchers pay attention to the effect of titanium surface modification on its biological activity, there are still few studies on the effect of modification of titanium and its alloys on the behavior of mesenchymal stem cells. The main aim of this review study is to report the state of art on the technological advancements of titanium implant surfaces to promote osteogenic differentiation of BMSCs on orthopedic implants. This review article deals with the titanium properties, innovative physicochemical procedures to modify titanium surfaces, biomimetic functionalization, promotion of BMSC osteogenesis, and inhibition of biofilm accumulation. We hope that it can provide some ideas for better methods to improve osseointegration efficiency.

**Biofunctionalization of orthopedic implant with bioactive** **ceramic to regulate bone marrow mesenchymal stem cell behavior**

Bioactive ceramic materials have a certain degree of solubility in the body, releasing certain ions that are not harmful to the body, participating in organic metabolism, stimulating or inducing bone hyperplasia, promoting defective bone tissue repair, and showing other good biological properties. This type of material may contain hydroxyapatite, or it can produce hydroxyapatite after reacting with body fluids, which can be integrated with bone tissue to form a bone binding interface. This method belongs to chemical combination with high strength and good stability. In this section, we will review the effects of different methods of bioactive ceramic coating on the behavior of BMSCs. The overall situation is shown in Table 1.

***Plasma spraying***

Plasma spraying technology is a method in which a plasma electric arc driven by a direct current is used as a heat source to heat materials like ceramics, alloys, and metals to a molten or semi-fused state before spraying the surface of a pretreated workpiece at a high speed to form a firmly adhered surface layer. Plasma spraying is an effective method to prepare bioactive ceramic coatings. Hydroxyapatite [Ca10(PO4)6(OH)2, HA] is a calcium hydroxide and tricalcium phosphate compound salt with a chemical composition and crystalline structure similar to the main minerals in human bones and teeth. It is also the main inorganic component of human bone tissue, and a typical bioactive material with good biocompatibility and chemical stability. It has been reported that spraying a hydroxyapatite ceramic coating on the surface of titanium-based implants leads to good cellular compatibility, promotes adhesion, proliferation, and osteogenic differentiation of BMSCs, and improves the implant’s bond to surrounding bone tissue. In one study, Dimitrievska *et al*[26] fabricated a new type of titanium alloy that possesses a layer of hydroxyapatite on titanium dioxide by plasma spraying. They studied the behavior of BMSCs on this titanium-based material. The results show that cells have stronger initial adhesion (improved by 20% after 2 h) and higher metabolic activity (improved by 20% after 2 h) on TiO2-HA compared to the titanium dioxide group. Furthermore, the differentiation of BMSCs is evidenced by alkaline phosphatase (ALP) and osteocalcin (OCN), early indicators of osteogenic differentiation, which are significantly increased on TiO2-HA. However, the pure HA coating also has some serious defects: High brittleness, poor fatigue resistance, and weak bonding strength with metal substrates. Porous tantalum has attracted much attention for its good biocompatibility and microstructure similar to cancellous bone[27]. In a recent study, Ta-incorporated HA coatings were developed by Lu *et al*[28] using the plasma spray technique on a titanium substrate. The result demonstrated that Ta-incorporated HA coating could promote initial adhesion and faster cell proliferation after incubation for 3 and 5 days, but it also promotes osteogenic differentiation of BMSCs compared to HA coatings. Akermanite ceramics can induce apatite mineralization. They also have moderate stability in simulated body fluid (SBF) and generally good mechanical properties, and support BMSC attachment[29,30]. The researchers found that the bonding strength between the plasma-sprayed akermanite bioactive coatings and Ti substrates is higher than hydroxyapatite (HA) coatings, and BMSC attachment and proliferation were more significant on akermanite coatings than on HA coatings[31].

***Sol-gel method***

Sol–gel process first described 150 years ago is still receiving great attention as one of the easiest ways to develop modified materials which possess required properties and are characterized by durability and stability[32]. Hence, sol-gel process is another method for preparing bioactive ceramic coatings. The sol-gel technology has some advantages compared to plasma spraying methods, including chemical uniformity, fine grain structure, and lower processing temperature[33]. In a study, a micro/nano-layered structure was prepared on a micro-structured titanium (Micro-Ti) substrate using a sol-gel method with a spin coating technique. The results confirmed that the micro/nano-level structure of large particles (80 nm) significantly promoted MSC proliferation and differentiation compared to other small particles (20 nm and 40 nm)[23]. Inzunza *et al*[34] prepared nanoporous silica coatings on Ti using the sol-gel method and evaporation-induced self-assembly method. The silica coatings with highly ordered sub-10 nm porosity accelerate the adhesive response of early BMSCs and promote BMSC osteogenic differentiation.

**Surface topography to regulate bone marrow mesenchymal stem cell behavior**

A bioactive ceramic layer is coated on the surface of porous titanium, and its osteoconduction supports the new bone to grow into the pore along the pore wall, which can effectively improve biological fixation of the porous titanium coating. However, this method also has some shortcomings: (1) After applying the bioactive ceramic coating, the pores of bone tissue are reduced, so the contact area with bone tissue is reduced; and (2) bioactive ceramic coating still has degradation, poor combination with titanium, and other problems[35]. A variety of surface modification methods have been developed to improve titanium bioactivity. In this section, we will review the different surface modification methods to provide a reference for clinical use (Table 2).

***Chemical treatments***

Chemical methods can be used to increase the thickness of the oxide film to improve the biocompatibility and bioactivity of titanium and its alloys. The surface chemical treatment of titanium and titanium alloys mainly includes alkali treatment, acid treatment, and acid-base two-step treatment. Alkali solution is used to modify the titanium surface to obtain sodium titanate gel with rich Ti-OH groups on the surface, endowing it with biological activity[36,37]. For this purpose, Cai and his team employed potassium hydroxide to modify the surfaces of titanium substrates; the formed potassium titanate layer enhances titanium’s corrosion resistance. The proliferation and differentiation levels of alkaline phosphatase and osteocalcin were significantly increased in MSCs cultured on alkaline-treated titanium after 7 and 14 d of culture, respectively[38].

Acid treatment is often used to remove the oxide layer and contaminants on the surface of the medical titanium material to obtain a clean and uniform surface. The acid treatment results in a 10-nm thick oxide layer, while the titanium oxide in the air is only 3-6 nm thick[39]. Maekawa *et al*[40] treated titanium with polyphosphoric acid solution for 24 h at 37 °C. Surface texture measurement results show that the maximum surface roughness of the treated titanium surface significantly increased. Significantly higher cell attachment and proliferation were also found on titanium treated with polyphosphoric acid in contrast to untreated titanium (control). By comparing the effects of acid-treated titanium and pure titanium on osteogenic differentiation of bone MSCs, Perrotti *et al*[41] concluded that 1 wk of treatment was more than enough for osteoblast differentiation on acid-treated titanium. Silva and his group suggested that rough surfaces submitted to acid-etching favor undifferentiated mesenchymal cell differentiation into osteogenic lineage cells compared to smooth titanium surfaces without acid treatment[42]. Although many studies have shown that surface acidification can increase the degree of roughening and improve the biological activity of titanium implants, acid treatment may cause hydrogen to penetrate below the oxide layer, thereby triggering hydrogen embrittlement[43].

The acid-alkali two-step method is also used for titanium surface modification. Strong acid erosion could cause micropores on the surface of titanium and titanium alloys to increase surface area. Meanwhile, alkaline solution can form a thicker microporous titanium oxide layer on the titanium surface, improving the titanium implant’s biological activity[44-46]. Li *et al* [47] first placed titanium in oxalic acid solution (5 wt%) at 100 °C for 2 h to remove the oxide layer and acquire a homogeneous micropit surface. Each pretreated titanium plate was treated in 5 mmol/L NaOH solution at 80 °C for 24 h. An *in vitro* cell experiment demonstrated that BMSC adhesion and osteogenesis can be better promoted on a micro/nanoporous surface than on an acid etched titanium surface. However, BMSC proliferation was significantly inhibited on treated surfaces after culturing for 4 and 7 days, which may due to the high pH around the implant. The high pH at the cell/material interface may cause alkalosis and inhibit BMSC proliferation and viability[48].

Hydrogen peroxide can also be used for activation treatment of titanium. Hydrogen peroxide treatment of titanium is a chemical dissolution and oxidation process, which could alter surface roughness, thickness, and hydrophilicity, with improvements in titanium osteoconductivity[49-51]. In one study, titanium was treated with 30% volume (v/v) of H2O2 (5 mL H2O2/g disc) for different times in an unsealed covered container under darkness at room temperature. The modifications induced by 6-24 h H2O2-treated surfaces are most beneficial for maintaining or promoting the attachment, proliferation, and osteogenic differentiation of BMSCs[52].

***Electrochemical anodization***

Anodization refers to the use of an electric field and various dilute acids as electrolyte solutions. A series of REDOX chemical reactions take place on the anode surface to form an oxidation layer. Due to anodization’s simplicity, versatility, and low cost, it has gained widespread attention in the surface treatment of titanium implants. In a study, Xu *et al*[24] found that tube diameter had a significant effect on adhesion, proliferation, and differentiation of MSCs. Titanium was used as the working electrode, platinum sheet was used as the cathode, and 0.50 wt% NH4F + 10 vol% H2O mixture was used as the electrolyte. The anodic oxidation was carried out at 10, 30, and 60 V, which were designated as NT10, NT30, and NT60, respectively. Finally, NT10, NT30, and NT60 were obtained with pore diameters of 30, 100, and 200 nm, respectively. By comparison, although NT60 can promote osteogenic differentiation to the greatest extent, it significantly inhibits cell adhesion and proliferation. NT10 can promote cell proliferation and adhesion, but it is useless for osteogenic differentiation of cells. NT30 supported adhesion and proliferation of BMSCs, and the cells on NT30 became increasingly elongated with increased diameter and showed a large number of prominent filamentous pseudopods. Moreover, it showed better osteogenesis-inducing ability. In another study, Grimalt *et al*[53] produced a nanonets structure on titanium discs. BMSCs cultured on nanonet structured titanium surfaces present a high frequency of alignment and promote osteogenic differentiation of the cells, while cells on untreated titanium surfaces exhibited a random orientation.

Micro-arc oxidation (MAO) is a new type of anodic oxidation technology that deposits a ceramic coating on the metal surface, and it has been widely applied in the surface modification of titanium and its alloys to enhance biological activity and osteogenic capacity. Based on ordinary anodization, arc discharge is used to enhance and activate the reaction occurring on the anode, thereby forming a ceramic film *in situ* on the surface of titanium[54-56]. Zhou *et al*[25] reported that porous coatings prepared by MAO promote BMSC adhesion and osteogenic differentiation. In addition, the larger the pore size, the more conducive to BMSC adhesion and osteogenic differentiation when the pore size is in the range of 3-10 μm. A similar phenomenon was observed in BMSCs in another study. Li *et al*[57] developed two kinds of coatings (MAO and MAO-Alkali coatings) with similar micro-morphologies, both of which significantly promote BMSC adhesion and osteogenic differentiation by mediating the integrin β1 signaling pathway.

***Plasma ion implantation and deposition***

Plasma ion implantation (PIII) is known to modify the surface and near surface regions of materials, and it has many advantages for surface modification of materials, including the following: (1) Changing the surface characteristics of the material alone without affecting the properties of the material; (2) the modified layer will not fall off or fail in combination; and (3) PIII is a low-temperature process (approximately 100 °C), and there is no change in the size of the workpiece due to thermal distortion.

PIII surface modification mainly uses plasma generated after Ar, N2, O2, and other gases or metal gasification to treat the material surface. Under the action of plasma, the surface of the material is bombarded with high-energy particles in the plasma. Chemical bond breakage occurs, and large molecular radicals are generated. At the same time, the material is etched to change the surface properties. PIII of metal materials can effectively improve the mechanical properties, wear resistance, and corrosion resistance of orthopedic implants, thus enhancing their biocompatibility[58]. Yang *et al*[59] explored the effect of titanium treated with oxygen plasma immersion ion implantation (O-PIII) on the behavior of BMSCs with different oxygen doses. The results showed that O-PIII treatment could enhance BMSC adhesion, and there was no significant difference in the titanium surface treated with O-PIII when the oxygen ion dose differed. In their later study, Yang *et al*[60] compared the effects of three doses of oxygen ion implantation into titanium on BMSC behavior. Among these treated titanium disks, the group treated with the highest concentration of oxygen ions has the best effect on cell adhesion, migration, proliferation, mineralization, and differentiation of BMSCs. It has been reported that calcium-ion-implanted titanium also remarkably improved BMSC adhesion and proliferation compared to the untreated sample[61]. Similarly, other studies have evaluated the response of BMSCs to titanium surfaces that had been implanted with Ca and Mg ions using the PIIID technique. The results showed that initial cell attachment on a titanium surface can be improved by Ca and Mg ion implantation. Cells on the Mg ion-implanted surface showed more extended filopodia after 4 and 24 h of cultivation. In addition, the expression of genes associated with osteogenic differentiation like RUNX2 and type I collagen was higher in the Mg ion-implanted surface[62]. These results are consistent with previous studies showing that significant cytotoxicity was not observed after Mg ion implantation into a titanium implant, and initial BMSC adhesion was improved with resulting osteoblast differentiation enhancement[63].

***Laser beam treatment***

Laser beam treatment is a controllable and flexible approach to modifying surfaces, which results in surfaces with increased surface area and enhanced wettability, and it displays negligible corrosion and high removal torques of established implants in preclinical bone models[64,65]. Laser-modified titanium surfaces could enhance upregulation of expression of the osteogenic markers and enhance alkaline phosphatase activity of BMSCs[66]. A recent investigation on the direct metal laser sintering (DMLS) titanium surface found that topographical cues of DMLS surfaces could enhance both protein adsorption ability and BMSC adhesion performance. Moreover, DMLS titanium surface could efficiently induce osteogenesis-associated gene expression in BMSCs *via* H3K27 demethylation and increases in H3K4me3 levels at gene promoters after osteogenic differentiation[67]. In another study, dynamic analyses of early cellular events showed that BMSCs exhibited a more elongated, spindle-like morphology and higher spreading speeds on femtosecond laser-modified surfaces compared to commercially pure titanium[68].

**Covalent immobilization** **bioactive molecules to promote bone marrow mesenchymal stem cell** **adhesion, proliferation, and osteogenic differentiation**

The basic principle of the above physical and chemical methods is to change the physical and chemical characteristics of the metal matrix surface to improve the biocompatibility of the material and BMSC growth inductivity, which is an indirect surface modification method. However, the application of biochemical technology proposed by David A Puleo to improve the surface activity of implants provides a different approach to surface modification from the traditional physical and chemical methods[69]. Contrary to topography-based approaches, biochemical surface modification utilizes macromolecules like extracellular matrix components, peptides, cell growth factors, and others to be fixed on the surface of biomaterials to act as receptors for adjacent cells, matrices, and soluble factors, which form a transition layer suitable for living organisms to control the tissue-implant interface[70]. In this section, we list different types of titanium-implant-bound macromolecules that have been shown to influence BMSC behavior. The overall data are listed in Table 3.

***Extracellular matrix components***

The extracellular matrix (ECM) is composed of several molecules secreted by cells. In addition to providing structural and mechanical support for tissues to interact with cells, these molecules can also bind to soluble molecules like growth factors that are present in extracellular fluid and regulate the occurrence of tissues and physiological activities of cells. The ECM provides a framework for tissue construction and plays an important role in regulating the survival, migration, proliferation, morphology, and other functions of cells in contact with it. Therefore, ECM components are the first choice for the biochemical surface modification of titanium-based bone implant materials.

**TYPE I COLLAGEN**

Collagen type I, one of the main organic components of bone ECM, is known to play an important role during adhesion, proliferation, and mineralization processes and osteogenic differentiation of cells, and it is an intriguing candidate for surface immobilization[71]. Dolder *et al*[72] showed that the modification of titanium alloy by type I collagen can promote BMSC osteogenic differentiation.

Morra *et al*[73] fixed collagen I to the surface of titanium (denoted as Col-Ti), finding that enhanced BMSC adhesion and cell density on Col-Ti, together with increased cell spreading areas on the microscopic surface morphology. RT-PCR analysis of several osteogenic related genes showed that the titanium surface immobilized on type I collagen could significantly promote BMSC osteogenic differentiation.

In another study, Ao and his team also found that immobilizing type I collagen on a titanium coating could enhance interactions between cells and materials and improve BMSC functions like adhesion, proliferation, and osteogenic differentiation. Furthermore, they compared the effects of different type I collagen fixation methods on BMSC behavior. They concluded that covalent immobilized collagen on titanium coating has a greater regulation effect on BMSC osteogenesis in contrast to adsorptive immobilization, which can be explained from the perspective of increasing the amount of covalently connected collagen and improving stability[74].

**HYALURONIC ACID**

Hyaluronic acid (HyA) is rich in carboxyl groups, and it is another major ECM component that possesses good biocompatibility, degradability, and low antigenicity, In addition, HyA could enhance cell migration and proliferation[75-77]. Based on HyA’s excellent properties, Ao *et al*[78] fabricated a titanium coating modified with HyA by covalent immobilization. They confirmed that BMSCs had more lamellipodia and adhered more closely to the covalent immobilized HyA surface than untreated samples. Other *in vitro* cell experiments have also shown that HyA immobilization on titanium coatings could significantly enhance BMSC attachment, proliferation, and differentiation. Furthermore, Ao *et al*[79] prepared a stable collagen/HyA (Col/HyA) polyelectrolyte multilayer (PEM) film on a titanium coating using a combination of the layer-by-layer self-assembly technique and covalent immobilization. The results showed that BMSCs displayed a polygonal and fusiform-shaped morphology, and cell adhesion and proliferation on the material were also improved. In other words, the construction of Col/HyA PEMs on TCs improved the cell–material interaction. The induction of osteogenic differentiation was further determined using qPCR, and the results confirmed that stable Col/HyA PEM could significantly enhance BMSC osteogenic differentiation.

***Peptide sequence***

It has been found that some short peptides in ECM proteins play important roles in cell behavior regulation[80,81]. Among different ECM proteins, fibronectin (FN), a multifunctional cell adhesive glycoprotein, is one of the most well-known and commonly used to functionalize biological materials. It contains several domains that mediate many cellular processes like cell adhesion, migration, growth, and differentiation. The use of FN-functionalized titanium implants has been shown to improve bone conduction capacity for its ability to attach cells to ECM components *via* integrin receptor interactions[82]. Chen *et al*[83] fixed FN on the surface of titanium, and BMSCs exhibited substantial actin polymerization, in the form of lamellipodia, pseudopodia, and actin stress fiber. However, the cells retained a rounded morphology on untreated surface. Besides, FN-functionalized titanium had a significant positive effect on BMSC proliferation compared to the control. However, its use for clinical applications is hampered due to poor stability, high production costs, and poor ECM protein immunogenicity, which have reduced their biomedical potential[84]. The use of ECM-derived synthetic peptides containing the functional domains of ECM proteins is an effective method to overcome these problems. Therefore, the synthesis of short peptide fragments representing ECM proteins and the modification of titanium-based implants have been gradually developed[85,86]. The most commonly used peptide sequence for surface modification is the arginine-glycine-aspartic acid (RGD) motif. RGD-functionalized titanium can improve early bone growth and matrix mineralization, and it can enhance the combination of materials and new bone[87]. There have been several reports on the effects of RGD on BMSCs. In a study, Karaman *et al*[88] covalently attached RGD peptide to titanium discs. The results indicated that RGD peptide treatment significantly enhanced BMSC adhesion and proliferation. Furthermore, this effect was enhanced by combining cold temperature plasma treatment and RGD peptide coating. Consistent with this, Herranz *et al*[89] concluded that the RGD motif was more favorable for BMSC adhesion, proliferation, and osteogenic differentiation in contrast to fibronectin. In another study, Jordi and his group covalently attached a novel molecule on the titanium surface. The novel molecule possesses adhesion capacity by an RGD gain-of function DNA mutation installed to the heparin binding II (HBII) fragment. The presence of RGD in the HBII domain stimulated focal adhesion formation at BMSC edges where filopodia were spikier compared to bare titanium samples with completely round cells. In addition, HBII-RGD-functionalized titanium surfaces could also stimulate BMSC differentiation and mineralization[90].

***Growth factors***

Growth factors are a class of proteins secreted by cells that act as signaling mediators for the relevant target cells to perform specific behaviors. Growth factors can promote cell proliferation, differentiation, protein synthesis, and migration of specific cells. Growth factors released from the implant surface can increase osteoblast activity and facilitate bone tissue regeneration[91]. Many researchers have been depositing growth factors on biomaterials to affect cell behavior. In one study, Bauer *et al*[92] showed the covalent immobilization of two growth factors, epidermal growth factor (EGF) and bone morphogenetic protein-2 (BMP-2), on the surface of TiO2 nanotubes and their effects on BMSC behavior. Cell adhesion and proliferation were dramatically increased by covalently grafting EGF on a surface of a 100 nm nanotube, but covalently grafted BMP-2 did not. The result was consistent with the finding of previous studies that BMP-2 promotes BMSC differentiation into osteoblast lineages but does not contribute to the cell attachment, adhesion, or proliferation like EGF[93]. Studies on BMP-2’s effect on BMSC differentiation have shown that BMP-2 has a significant effect on osteoblast differentiation potential[94].

Platelet derived growth factor (PDGF) has been shown to play critical roles in bone regeneration after injury, and it significantly contributes to all stages of bone regeneration after trauma[95,96]. Among three types of dimerism, PDGF-AA, -BB, and –AB, PDGF-BB exerts the most potent chemotactic effects on BMSCs[97]. Ma *et al*[98] fabricated a nano-micro hierarchical TiO2 clustered nanotubular structure using anodization, and PDGF-BB was functionalized with PhoA (11-hydroxyphosphonic acid)/CDI (carbonyldiimidazole). The resulting new material had almost no cytotoxicity to host cells, and it significantly enhanced BMSC attachment and osteogenesis-related functions (early proliferation, extracellular matrix synthesis, and mineralization).

**Local** **control release of bioactive molecules to promote** **bone marrow mesenchymal stem cell adhesion, proliferation, and osteogenic differentiation**

Recently, many researchers have focused on biomolecule-controlled release. This controlled release system overcomes the limitation of rapid degeneration and diffusion of biomolecules in the body, which may decrease biomolecule doses, reduce costs, and more importantly, minimize side effects of high-dose biomolecules. An effective controlled-release system can encapsulate bioactive cues in biocompatible and biodegradable microparticles. As the microparticles gradually degrade, biological molecules are released with predesigned dose kinetics over time[99-101]. The key to making bioactive molecules work is their release so that they can induce the required biological response. Many bioactive molecules can be used in this kind of sustained-release system, including growth factors, short peptides, clinical drugs, and others. By sustained release on the implant surface, cell adhesion, proliferation, differentiation, and other behaviors can be regulated, thus improving the implant’s biocompatibility. Table 4 lists the commonly used bioactive molecules and their cellular responses reported in the recent literature.

Coating biodegradable polymers is an effective method to control the drug release kinetics from titanium. In a study, Kim *et al*[102] prepared a new dopamine coating that enhances the initial cell adhesion, mitochondrial activity, and proliferation of BMSCs on the titanium surface. Son *et al*[103] successfully developed hydroxyapatite (HA)-titanium disc surfaces immobilized with dexamethasone (DEX)-loaded poly(lactic-co-glycolic acid) (PLGA) particles using a low-temperature high-speed collision method. The evaluation of HA-titanium surfaces with a particle carrier system potently induced BMSC differentiation *in vitro*. This showed that the gene expression levels of *ALP*, *OPN*, *BSP*, and *OC* were enhanced, and these functional surfaces showed greater osteoinductivity than pure-Ti and HA-Ti surfaces. Cheng *et al*[104] used catechol as a template to modify a photo-crosslinked gel-based hydrogel to enhance its adhesion to the titanium surface, thereby improving the coating’s stability. Synthetic silicate nanoparticles (SNs) were introduced into the hydrogel formulation. The results showed that the addition of SNs to the hydrogel formulation can promote bone formation when co-cultured with BMSCs, suggesting the potential to promote new bone formation in surrounding tissues.

**Approach to indirectly affect bone marrow mesenchymal stem cell adhesion, proliferation, and osteogenic differentiation**

In fact, once implanted, metallic implants would adsorb various proteins, elicit a clotting reaction, trigger an innate inflammatory response, and induce the bone regeneration process[105-107]. Intrinsic inflammation is undesirable but inevitable, and the result of the inflammatory response plays a vital role in the formation of new bone in and material around after implantation[108]. Therefore, it is important to take into account the immunomodulatory effects of biological materials[109]. Specifically, macrophages are involved in almost all-natural wound healing processes. Macrophage polarization has an important effect on wound healing and the biological properties of biological materials[110]. As the key participants of innate host immunity, classically (M1) and alternatively (M2) activated macrophages, the two main phenotypes, are pro-inflammatory and anti-inflammatory, respectively[111]. M1 macrophages express high levels of interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1, inducible nitric oxide synthase, and others. M2 macrophages synthesize IL-10, arginase-1, vascular endothelial growth factor A, and platelet-derived growth factor-BB (PDGF-BB), which support the homing, proliferation, and osteogenic differentiation of BMSCs[112].

Successful biomaterial implantation can be achieved by controlling immune system activation. Hence, many researchers have focused on indirectly regulating the behavior of BMSCs by regulating macrophage polarization. In a study, patterned titanium coatings were prepared by combining grit-blasting, ultrasonic washing, and atmosphere plasma spray which copper meshes were applied to block the molten titanium droplet when spraying. Macrophages tend to polarize to M2 on a patterned titanium surface, while macrophages on traditional titanium coatings exhibit higher M1 polarization.

Up-regulation of osteoinductive cytokines was detected, suggesting that macrophages provide a favorable osteogenic microenvironment[113]. In our previous study, a multi-biofunctional titanium implant was fabricated by covalently immobilizing titanium with the bacitracin. *In vitro* cell biology experiments showed that bacitracin-immobilized titanium could inhibit the secretion of inflammatory factors like TNF-α, IL-6, IL-8, and others, which represent M1 polarization of macrophages, and significantly promote the adhesion, proliferation, and osteogenic differentiation of BMSCs[114]. In another study, Ma *et al*[115] evaluated the osteogenic behavior of BMSCs on TiO2 nanotubular (NT) surfaces in conditioned medium (CM) generated by macrophages. BMSC morphology in CM from macrophages cultured on the NT surfaces was aligned in a consistent direction, while an unordered distribution was observed on the pure titanium surface. In addition, the modified titanium dioxide surface and CM in monocytes cultured on the surface jointly promoted the proliferation, migration, and osteogenic differentiation of BMSCs. The transition of macrophages from M1 to M2 at specific time points is very important for wound healing and tissue regeneration. In a recent study, a dual system hydrogel layer (chitosan/β-glycerophosphate disodium and carboxymethyl chitosan/genipin) of titanium dioxide nanotubes was fabricated to regulate the release of IL-4 and interferon-γ (IFN-γ). In the culture with BMSCs and macrophages, the system showed good cell compatibility and significantly promoted cell proliferation[116].

**PERSPECTIVE OF OSTEOGENESIS on TITANIUM SURFACE**

BMSCs are used as core cells for the renewal and repair of local bone, cartilage, and medullary adipose tissue[117]. BMSCs perceive the titanium surface and become activated during the osteogenesis and osteointegration phases. BMSCs then establish contact with the titanium surface and maintain this relationship until they differentiate into osteoblasts and osteocytes, subsequently embedding in the mineralized matrix[118]. At present, many researchers are mainly focused on the effect of different modification methods on the behavior of BMSCs and have made great progress. However, problems also exist in the modified implants such as poor biological safety and poor stability[119].

In addition, it is important to note that there are great limitations to the existing methods of judging osteogenesis on titanium surface, and the current means of skeletal muscles mainly rely on magnetic resonance imaging (MRI), X-ray computed tomography, and X-rays[120,121]. Nevertheless, there is still no effective method for the bone integration evaluation on metal implants, which can only rely on pathological biopsy examination. Therefore, the evaluation of osteogenesis on the surface of titanium and its alloys *in vivo* may be an important research target in the future. And more in depth basic and clinical research is necessary to develop more products.

**CONCLUSION**

In this article, we have summarized recent advances in the approaches for surface modification of titanium and its alloys, and systematically elaborated these modification methods and their effects on cell behavior. The methods like sol-gel, ion implantation, anodization, and micro-arc oxidation can promote osteogenic differentiation of BMSCs and improve the rate of osseointegration by changing surface roughness and hydrophilicity, or regulate the microenvironment of the bone-implant interface. We recommend that the application of modern surfaces in the clinical practice of orthopedics be encouraged to increase and accelerate the osseointegration of the implant and its alloys. To the best of our knowledge, few researchers have done similar work, so we hope that our work might develop some ideas for better methods to improve osseointegration efficiency.

**REFERENCES**

1 **Friedenstein AJ**. Precursor cells of mechanocytes. *Int Rev Cytol* 1976; **47**: 327-359 [PMID: 11195 DOI: 10.1016/S0074-7696(08)60092-3]

2 **Abou-Ezzi G**, Supakorndej T, Zhang J, Anthony B, Krambs J, Celik H, Karpova D, Craft CS, Link DC. TGF-β Signaling Plays an Essential Role in the Lineage Specification of Mesenchymal Stem/Progenitor Cells in Fetal Bone Marrow. *Stem Cell Reports* 2019; **13**: 48-60 [PMID: 31204302 DOI: 10.1016/j.stemcr.2019.05.017]

3 **Balera Brito VG**, Chaves-Neto AH, Landim de Barros T, Penha Oliveira SH. Soluble yerba mate (Ilex Paraguariensis) extract enhances in vitro osteoblastic differentiation of bone marrow-derived mesenchymal stromal cells. *J Ethnopharmacol* 2019; **244**: 112131 [PMID: 31377259 DOI: 10.1016/j.jep.2019.112131]

4 **Lin Z**, He H, Wang M, Liang J. MicroRNA-130a controls bone marrow mesenchymal stem cell differentiation towards the osteoblastic and adipogenic fate. *Cell Prolif* 2019; **52**: e12688 [PMID: 31557368 DOI: 10.1111/cpr.12688]

5 **Luo ZW**, Li FX, Liu YW, Rao SS, Yin H, Huang J, Chen CY, Hu Y, Zhang Y, Tan YJ, Yuan LQ, Chen TH, Liu HM, Cao J, Liu ZZ, Wang ZX, Xie H. Aptamer-functionalized exosomes from bone marrow stromal cells target bone to promote bone regeneration. *Nanoscale* 2019; **11**: 20884-20892 [PMID: 31660556 DOI: 10.1039/c9nr02791b]

6 **Nehlin JO**, Jafari A, Tencerova M, Kassem M. Aging and lineage allocation changes of bone marrow skeletal (stromal) stem cells. *Bone* 2019; **123**: 265-273 [PMID: 30946971 DOI: 10.1016/j.bone.2019.03.041]

7 **Wu H**, Hu B, Zhou X, Zhou C, Meng J, Yang Y, Zhao X, Shi Z, Yan S. Artemether attenuates LPS-induced inflammatory bone loss by inhibiting osteoclastogenesis and bone resorption via suppression of MAPK signaling pathway. *Cell Death Dis* 2018; **9**: 498 [PMID: 29703893 DOI: 10.1038/s41419-018-0540-y]

8 **Kim IL**, Khetan S, Baker BM, Chen CS, Burdick JA. Fibrous hyaluronic acid hydrogels that direct MSC chondrogenesis through mechanical and adhesive cues. *Biomaterials* 2013; **34**: 5571-5580 [PMID: 23623322 DOI: 10.1016/j.biomaterials.2013.04.004]

9 **Viswanathan P**, Ondeck MG, Chirasatitsin S, Ngamkham K, Reilly GC, Engler AJ, Battaglia G. 3D surface topology guides stem cell adhesion and differentiation. *Biomaterials* 2015; **52**: 140-147 [PMID: 25818420 DOI: 10.1016/j.biomaterials.2015.01.034]

10 **Yin D**, Komasa S, Yoshimine S, Sekino T, Okazaki J. Effect of mussel adhesive protein coating on osteogenesis in vitro and osteointegration in vivo to alkali-treated titanium with nanonetwork structures. *Int J Nanomedicine* 2019; **14**: 3831-3843 [PMID: 31213804 DOI: 10.2147/IJN.S206313]

11 **Gordon LM**, Joester D. Mapping residual organics and carbonate at grain boundaries and the amorphous interphase in mouse incisor enamel. *Front Physiol* 2015; **6**: 57 [PMID: 25852562 DOI: 10.3389/fphys.2015.00057]

12 **Wang C**, Hu H, Li Z, Shen Y, Xu Y, Zhang G, Zeng X, Deng J, Zhao S, Ren T, Zhang Y. Enhanced Osseointegration of Titanium Alloy Implants with Laser Microgrooved Surfaces and Graphene Oxide Coating. *ACS Appl Mater Interfaces* 2019; **11**: 39470-39483 [PMID: 31594306 DOI: 10.1021/acsami.9b12733]

13 **Hanawa T**. Titanium-Tissue Interface Reaction and Its Control With Surface Treatment. *Front Bioeng Biotechnol* 2019; **7**: 170 [PMID: 31380361 DOI: 10.3389/fbioe.2019.00170]

14 **Jiang N**, Guo Z, Sun D, Li Y, Yang Y, Chen C, Zhang L, Zhu S. Promoting Osseointegration of Ti Implants through Micro/Nanoscaled Hierarchical Ti Phosphate/Ti Oxide Hybrid Coating. *ACS Nano* 2018; **12**: 7883-7891 [PMID: 29979574 DOI: 10.1021/acsnano.8b02227]

15 **Oliveira WF**, Arruda IRS, Silva GMM, Machado G, Coelho LCBB, Correia MTS. Functionalization of titanium dioxide nanotubes with biomolecules for biomedical applications. *Mater Sci Eng C Mater Biol Appl* 2017; **81**: 597-606 [PMID: 28888015 DOI: 10.1016/j.msec.2017.08.017]

16 **Huang B**, Yao Q, Huang Y, Zhang L, Yao Y, Gong P, Tang H. Combination Use of BMP2 and VEGF165 Promotes Osseointegration and Stability of Titanium Implants in Irradiated Bone. *Biomed Res Int* 2018; **2018**: 8139424 [PMID: 30627574 DOI: 10.1155/2018/8139424]

17 **Nyan M**, Tsutsumi Y, Oya K, Doi H, Nomura N, Kasugai S, Hanawa T. Synthesis of novel oxide layers on titanium by combination of sputter deposition and micro-arc oxidation techniques. *Dent Mater J* 2011; **30**: 754-761 [PMID: 21946498 DOI: 10.4012/dmj.2011-067]

18 **Campoccia D**, Arciola CR, Cervellati M, Maltarello MC, Montanaro L. In vitro behaviour of bone marrow-derived mesenchymal cells cultured on fluorohydroxyapatite-coated substrata with different roughness. *Biomaterials* 2003; **24**: 587-596 [PMID: 12437953 DOI: 10.1016/s0142-9612(02)00373-3]

19 **Liu P**, Hao Y, Zhao Y, Yuan Z, Ding Y, Cai K. Surface modification of titanium substrates for enhanced osteogenetic and antibacterial properties. *Colloids Surf B Biointerfaces* 2017; **160**: 110-116 [PMID: 28918187 DOI: 10.1016/j.colsurfb.2017.08.044]

20 **Pawlik A**, Socha RP, Hubalek Kalbacova M, Sulka GD. Surface modification of nanoporous anodic titanium dioxide layers for drug delivery systems and enhanced SAOS-2 cell response. *Colloids Surf B Biointerfaces* 2018; **171**: 58-66 [PMID: 30007219 DOI: 10.1016/j.colsurfb.2018.07.012]

21 **Pippenger BE**, Rottmar M, Kopf BS, Stübinger S, Dalla Torre FH, Berner S, Maniura-Weber K. Surface modification of ultrafine-grained titanium: Influence on mechanical properties, cytocompatibility, and osseointegration potential. *Clin Oral Implants Res* 2019; **30**: 99-110 [PMID: 30521101 DOI: 10.1111/clr.13396]

22 **Lampé I**, Beke D, Biri S, Csarnovics I, Csik A, Dombrádi Z, Hajdu P, Hegedűs V, Rácz R, Varga I, Hegedűs C. Investigation of silver nanoparticles on titanium surface created by ion implantation technology. *Int J Nanomedicine* 2019; **14**: 4709-4721 [PMID: 31308654 DOI: 10.2147/IJN.S197782]

23 **Shen X**, Ma P, Hu Y, Xu G, Zhou J, Cai K. Mesenchymal stem cell growth behavior on micro/nano hierarchical surfaces of titanium substrates. *Colloids Surf B Biointerfaces* 2015; **127**: 221-232 [PMID: 25687093 DOI: 10.1016/j.colsurfb.2015.01.048]

24 **Xu Z**, Lai Y, Wu D, Huang W, Huang S, Zhou L, Chen J. Increased Mesenchymal Stem Cell Response and Decreased Staphylococcus aureus Adhesion on Titania Nanotubes without Pharmaceuticals. *Biomed Res Int* 2015; **2015**: 172898 [PMID: 26640782 DOI: 10.1155/2015/172898]

25 **Zhou W**, Huang O, Gan Y, Li Q, Zhou T, Xi W. Effect of titanium implants with coatings of different pore sizes on adhesion and osteogenic differentiation of BMSCs. *Artif Cells Nanomed Biotechnol* 2019; **47**: 290-299 [PMID: 30688103 DOI: 10.1080/21691401.2018.1553784]

26 **Dimitrievska S**, Bureau MN, Antoniou J, Mwale F, Petit A, Lima RS, Marple BR. Titania-hydroxyapatite nanocomposite coatings support human mesenchymal stem cells osteogenic differentiation. *J Biomed Mater Res A* 2011; **98**: 576-588 [PMID: 21702080 DOI: 10.1002/jbm.a.32964]

27 **Shi LY**, Wang A, Zang FZ, Wang JX, Pan XW, Chen HJ. Tantalum-coated pedicle screws enhance implant integration. *Colloids Surf B Biointerfaces* 2017; **160**: 22-32 [PMID: 28915498 DOI: 10.1016/j.colsurfb.2017.08.059]

28 **Lu RJ**, Wang X, He HX, E LL, Li Y, Zhang GL, Li CJ, Ning CY, Liu HC. Tantalum-incorporated hydroxyapatite coating on titanium implants: its mechanical and in vitro osteogenic properties. *J Mater Sci Mater Med* 2019; **30**: 111 [PMID: 31583537 DOI: 10.1007/s10856-019-6308-9]

29 **Sun H**, Wu C, Dai K, Chang J, Tang T. Proliferation and osteoblastic differentiation of human bone marrow-derived stromal cells on akermanite-bioactive ceramics. *Biomaterials* 2006; **27**: 5651-5657 [PMID: 16904740 DOI: 10.1016/j.biomaterials.2006.07.027]

30 **Wu C**, Chang J, Ni S, Wang J. In vitro bioactivity of akermanite ceramics. *J Biomed Mater Res A* 2006; **76**: 73-80 [PMID: 16224776 DOI: 10.1002/jbm.a.30496]

31 **Yi D**, Wu C, Ma X, Ji H, Zheng X, Chang J. Preparation and in vitro evaluation of plasma-sprayed bioactive akermanite coatings. *Biomed Mater* 2012; **7**: 065004 [PMID: 23159958 DOI: 10.1088/1748-6041/7/6/065004]

32 **Gonçalves MC**. Sol-gel Silica Nanoparticles in Medicine: A Natural Choice. Design, Synthesis and Products. *Molecules* 2018; **23**: [PMID: 30104542 DOI: 10.3390/molecules23082021]

33 **Stepanovska J**, Matejka R, Rosina J, Bacakova L, Kolarova H. Treatments for enhancing the biocompatibility of titanium implants. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2020; **164**: 23-33 [PMID: 31907491 DOI: 10.5507/bp.2019.062]

34 **Inzunza D**, Covarrubias C, Von Marttens A, Leighton Y, Carvajal JC, Valenzuela F, Díaz-Dosque M, Méndez N, Martínez C, Pino AM, Rodríguez JP, Cáceres M, Smith P. Synthesis of nanostructured porous silica coatings on titanium and their cell adhesive and osteogenic differentiation properties. *J Biomed Mater Res A* 2014; **102**: 37-48 [PMID: 23568757 DOI: 10.1002/jbm.a.34673]

35 **Bloebaum RD**, Beeks D, Dorr LD, Savory CG, DuPont JA, Hofmann AA. Complications with hydroxyapatite particulate separation in total hip arthroplasty. *Clin Orthop Relat Res* 1994; : 19-26 [PMID: 8118974 DOI: 10.1097/00003086-199401000-00005]

36 **Jonásová L**, Müller FA, Helebrant A, Strnad J, Greil P. Biomimetic apatite formation on chemically treated titanium. *Biomaterials* 2004; **25**: 1187-1194 [PMID: 14643592 DOI: 10.1016/j.biomaterials.2003.08.009]

37 **Kim HM**, Miyaji F, Kokubo T, Nakamura T. Preparation of bioactive Ti and its alloys via simple chemical surface treatment. *J Biomed Mater Res* 1996; **32**: 409-417 [PMID: 8897146 DOI: 10.1002/(SICI)1097-4636(199611)32:3<409::AID-JBM14>3.0.CO;2-B]

38 **Cai K**, Lai M, Yang W, Hu R, Xin R, Liu Q, Sung KL. Surface engineering of titanium with potassium hydroxide and its effects on the growth behavior of mesenchymal stem cells. *Acta Biomater* 2010; **6**: 2314-2321 [PMID: 19963080 DOI: 10.1016/j.actbio.2009.11.034]

39 **Sittig C**, Textor M, Spencer ND, Wieland M, Vallotton PH. Surface characterization of implant materials c.p. Ti, Ti-6Al-7Nb and Ti-6Al-4V with different pretreatments. *J Mater Sci Mater Med* 1999; **10**: 35-46 [PMID: 15347992 DOI: 10.1023/A:1008840026907]

40 **Maekawa K**, Yoshida Y, Mine A, Fujisawa T, Van Meerbeek B, Suzuki K, Kuboki T. Chemical interaction of polyphosphoric acid with titanium and its effect on human bone marrow derived mesenchymal stem cell behavior. *J Biomed Mater Res A* 2007; **82**: 195-200 [PMID: 17266020 DOI: 10.1002/jbm.a.31139]

41 **Perrotti V**, Palmieri A, Pellati A, Degidi M, Ricci L, Piattelli A, Carinci F. Effect of titanium surface topographies on human bone marrow stem cells differentiation in vitro. *Odontology* 2013; **101**: 133-139 [PMID: 22678711 DOI: 10.1007/s10266-012-0067-0]

42 **Silva TS**, Machado DC, Viezzer C, Silva Júnior AN, Oliveira MG. Effect of titanium surface roughness on human bone marrow cell proliferation and differentiation: an experimental study. *Acta Cir Bras* 2009; **24**: 200-205 [PMID: 19504002 DOI: 10.1590/S0102-86502009000300007]

43 **Taborelli M**, Jobin M, François P, Vaudaux P, Tonetti M, Szmukler-Moncler S, Simpson JP, Descouts P. Influence of surface treatments developed for oral implants on the physical and biological properties of titanium. (I) Surface characterization. *Clin Oral Implants Res* 1997; **8**: 208-216 [PMID: 9586465 DOI: 10.1034/j.1600-0501.1997.080307.x]

44 **Spriano S**, Bosetti M, Bronzoni M, Vernè E, Maina G, Bergo V, Cannas M. Surface properties and cell response of low metal ion release Ti-6Al-7Nb alloy after multi-step chemical and thermal treatments. *Biomaterials* 2005; **26**: 1219-1229 [PMID: 15475051 DOI: 10.1016/j.biomaterials.2004.04.026]

45 **Wen HB**, Liu Q, De Wijn JR, De Groot K, Cui FZ. Preparation of bioactive microporous titanium surface by a new two-step chemical treatment. *J Mater Sci Mater Med* 1998; **9**: 121-128 [PMID: 15348900 DOI: 10.1023/A:1008859417664]

46 **Wen HB**, Wolke JG, de Wijn JR, Liu Q, Cui FZ, de Groot K. Fast precipitation of calcium phosphate layers on titanium induced by simple chemical treatments. *Biomaterials* 1997; **18**: 1471-1478 [PMID: 9426176 DOI: 10.1016/S0142-9612(97)82297-1]

47 **Li J**, Wang G, Wang D, Wu Q, Jiang X, Liu X. Alkali-treated titanium selectively regulating biological behaviors of bacteria, cancer cells and mesenchymal stem cells. *J Colloid Interface Sci* 2014; **436**: 160-170 [PMID: 25268820 DOI: 10.1016/j.jcis.2014.08.053]

48 **Shen Y**, Liu W, Lin K, Pan H, Darvell BW, Peng S, Wen C, Deng L, Lu WW, Chang J. Interfacial pH: a critical factor for osteoporotic bone regeneration. *Langmuir* 2011; **27**: 2701-2708 [PMID: 21309596 DOI: 10.1021/la104876w]

49 **Nagassa ME**, Daw AE, Rowe WG, Carley A, Thomas DW, Moseley R. Optimisation of the hydrogen peroxide pre-treatment of titanium: surface characterisation and protein adsorption. *Clin Oral Implants Res* 2008; **19**: 1317-1326 [PMID: 19040449 DOI: 10.1111/j.1600-0501.2008.01611.x]

50 **Pan J**, Liao H, Leygraf C, Thierry D, Li J. Variation of oxide films on titanium induced by osteoblast-like cell culture and the influence of an H2O2 pretreatment. *J Biomed Mater Res* 1998; **40**: 244-256 [PMID: 9549619 DOI: 10.1002/(SICI)1097-4636(199805)40:2<244::AID-JBM9>3.0.CO;2-L]

51 **Takemoto S**, Yamamoto T, Tsuru K, Hayakawa S, Osaka A, Takashima S. Platelet adhesion on titanium oxide gels: effect of surface oxidation. *Biomaterials* 2004; **25**: 3485-3492 [PMID: 15020122 DOI: 10.1016/j.biomaterials.2003.10.070]

52 **Daw AE**, Kazi HA, Colombo JS, Rowe WG, Williams DW, Waddington RJ, Thomas DW, Moseley R. Differential cellular and microbial responses to nano-/micron-scale titanium surface roughness induced by hydrogen peroxide treatment. *J Biomater Appl* 2013; **28**: 144-160 [PMID: 22457041 DOI: 10.1177/0885328212441495]

53 **Llopis-Grimalt MA**, Amengual-Tugores AM, Monjo M, Ramis JM. Oriented Cell Alignment Induced by a Nanostructured Titanium Surface Enhances Expression of Cell Differentiation Markers. *Nanomaterials (Basel)* 2019; **9**: [PMID: 31766660 DOI: 10.3390/nano9121661]

54 **Voevodin AA,** Yerokhin AL, Lyubimov VV, Donley MS, Zabinski JS. Characterization of wear protective AlSiO coatings formed on Al-based alloys by micro-arc discharge treatment. Surface and Coatings Technology 1996;86-**87**:516-521 [DOI: 10.1016/S0257-8972(96)03069-1]

55 **Khanna R**, Kokubo T, Matsushita T, Nomura Y, Nose N, Oomori Y, Yoshida T, Wakita K, Takadama H. Novel artificial hip joint: A layer of alumina on Ti-6Al-4V alloy formed by micro-arc oxidation. *Mater Sci Eng C Mater Biol Appl* 2015; **55**: 393-400 [PMID: 26117770 DOI: 10.1016/j.msec.2015.05.021]

56 **Li LH**, Kong YM, Kim HW, Kim YW, Kim HE, Heo SJ, Koak JY. Improved biological performance of Ti implants due to surface modification by micro-arc oxidation. *Biomaterials* 2004; **25**: 2867-2875 [PMID: 14962565 DOI: 10.1016/j.biomaterials.2003.09.048]

57 **Li G**, Cao H, Zhang W, Ding X, Yang G, Qiao Y, Liu X, Jiang X. Enhanced Osseointegration of Hierarchical Micro/Nanotopographic Titanium Fabricated by Microarc Oxidation and Electrochemical Treatment. *ACS Appl Mater Interfaces* 2016; **8**: 3840-3852 [PMID: 26789077 DOI: 10.1021/acsami.5b10633]

58 **Manova D**, Gerlach JW, Mändl S. Thin Film Deposition Using Energetic Ions. *Materials (Basel)* 2010; **3**: 4109-4141 [PMID: 28883323 DOI: 10.3390/ma3084109]

59 **Yang CH**, Wang YT, Tsai WF, Ai CF, Lin MC, Huang HH. Effect of oxygen plasma immersion ion implantation treatment on corrosion resistance and cell adhesion of titanium surface. *Clin Oral Implants Res* 2011; **22**: 1426-1432 [PMID: 21457349 DOI: 10.1111/j.1600-0501.2010.02132.x]

60 **Yang CH**, Li YC, Tsai WF, Ai CF, Huang HH. Oxygen plasma immersion ion implantation treatment enhances the human bone marrow mesenchymal stem cells responses to titanium surface for dental implant application. *Clin Oral Implants Res* 2015; **26**: 166-175 [PMID: 24313899 DOI: 10.1111/clr.12293]

61 **Michalska J**, Sowa M, Piotrowska M, Widziołek M, Tylko G, Dercz G, Socha RP, Osyczka AM, Simka W. Incorporation of Ca ions into anodic oxide coatings on the Ti-13Nb-13Zr alloy by plasma electrolytic oxidation. *Mater Sci Eng C Mater Biol Appl* 2019; **104**: 109957 [PMID: 31500028 DOI: 10.1016/j.msec.2019.109957]

62 **Won S**, Huh YH, Cho LR, Lee HS, Byon ES, Park CJ. Cellular Response of Human Bone Marrow Derived Mesenchymal Stem Cells to Titanium Surfaces Implanted with Calcium and Magnesium Ions. *Tissue Eng Regen Med* 2017; **14**: 123-131 [PMID: 30603469 DOI: 10.1007/s13770-017-0028-3]

63 **Kim BS**, Kim JS, Park YM, Choi BY, Lee J. Mg ion implantation on SLA-treated titanium surface and its effects on the behavior of mesenchymal stem cell. *Mater Sci Eng C Mater Biol Appl* 2013; **33**: 1554-1560 [PMID: 23827608 DOI: 10.1016/j.msec.2012.12.061]

64 **Sisti KE**, de Andrés MC, Johnston D, Almeida-Filho E, Guastaldi AC, Oreffo RO. Skeletal stem cell and bone implant interactions are enhanced by LASER titanium modification. *Biochem Biophys Res Commun* 2016; **473**: 719-725 [PMID: 26456647 DOI: 10.1016/j.bbrc.2015.10.013]

65 **Sisti KE**, Piattelli A, Guastaldi AC, Queiroz TP, de Rossi R. Nondecalcified histologic study of bone response to titanium implants topographically modified by laser with and without hydroxyapatite coating. *Int J Periodontics Restorative Dent* 2013; **33**: 689-696 [PMID: 23998165 DOI: 10.11607/prd.1151]

66 **Bressel TAB**, de Queiroz JDF, Gomes Moreira SM, da Fonseca JT, Filho EA, Guastaldi AC, Batistuzzo de Medeiros SR. Laser-modified titanium surfaces enhance the osteogenic differentiation of human mesenchymal stem cells. *Stem Cell Res Ther* 2017; **8**: 269 [PMID: 29179738 DOI: 10.1186/s13287-017-0717-9]

67 **Zheng G**, Guan B, Hu P, Qi X, Wang P, Kong Y, Liu Z, Gao P, Li R, Zhang X, Wu X, Sui L. Topographical cues of direct metal laser sintering titanium surfaces facilitate osteogenic differentiation of bone marrow mesenchymal stem cells through epigenetic regulation. *Cell Prolif* 2018; **51**: e12460 [PMID: 29701270 DOI: 10.1111/cpr.12460]

68 **Dumas V**, Guignandon A, Vico L, Mauclair C, Zapata X, Linossier MT, Bouleftour W, Granier J, Peyroche S, Dumas JC, Zahouani H, Rattner A. Femtosecond laser nano/micro patterning of titanium influences mesenchymal stem cell adhesion and commitment. *Biomed Mater* 2015; **10**: 055002 [PMID: 26334374 DOI: 10.1088/1748-6041/10/5/055002]

69 **Puleo DA**, Nanci A. Understanding and controlling the bone-implant interface. *Biomaterials* 1999; **20**: 2311-2321 [PMID: 10614937 DOI: 10.1016/S0142-9612(99)00160-X]

70 **Morra M**. Biomolecular modification of implant surfaces. *Expert Rev Med Devices* 2007; **4**: 361-372 [PMID: 17488230 DOI: 10.1586/17434440.4.3.361]

71 **Wang Y**, Luo S, Zhang D, Qu X, Tan Y. Sika pilose antler type I collagen promotes BMSC differentiation via the ERK1/2 and p38-MAPK signal pathways. *Pharm Biol* 2017; **55**: 2196-2204 [PMID: 29115171 DOI: 10.1080/13880209.2017.1397177]

72 **van den Dolder J**, Jansen JA. The response of osteoblast-like cells towards collagen type I coating immobilized by p-nitrophenylchloroformate to titanium. *J Biomed Mater Res A* 2007; **83**: 712-719 [PMID: 17559125 DOI: 10.1002/jbm.a.31428]

73 **Morra M**, Cassinelli C, Cascardo G, Bollati D, Baena RR. Gene expression of markers of osteogenic differentiation of human mesenchymal cells on collagen I-modified microrough titanium surfaces. *J Biomed Mater Res A* 2011; **96**: 449-455 [PMID: 21171164 DOI: 10.1002/jbm.a.32948]

74 **Ao H**, Xie Y, Tan H, Wu X, Liu G, Qin A, Zheng X, Tang T. Improved hMSC functions on titanium coatings by type I collagen immobilization. *J Biomed Mater Res A* 2014; **102**: 204-214 [PMID: 23661627 DOI: 10.1002/jbm.a.34682]

75 **Lou J**, Stowers R, Nam S, Xia Y, Chaudhuri O. Stress relaxing hyaluronic acid-collagen hydrogels promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cell culture. *Biomaterials* 2018; **154**: 213-222 [PMID: 29132046 DOI: 10.1016/j.biomaterials.2017.11.004]

76 **López-Ruiz E**, Jiménez G, Álvarez de Cienfuegos L, Antic C, Sabata R, Marchal JA, Gálvez-Martín P. Advances of hyaluronic acid in stem cell therapy and tissue engineering, including current clinical trials. *Eur Cell Mater* 2019; **37**: 186-213 [PMID: 30889270 DOI: 10.22203/eCM.v037a12]

77 **Murakami T**, Otsuki S, Okamoto Y, Nakagawa K, Wakama H, Okuno N, Neo M. Hyaluronic acid promotes proliferation and migration of human meniscus cells via a CD44-dependent mechanism. *Connect Tissue Res* 2019; **60**: 117-127 [PMID: 29658360 DOI: 10.1080/03008207.2018.1465053]

78 **Ao H**, Xie Y, Qin A, Ji H, Yang S, Huang L, Zheng X, Tang T. Immobilization of hyaluronic acid on plasma-sprayed porous titanium coatings for improving biological properties. *J Biomater Sci Polym Ed* 2014; **25**: 1211-1224 [PMID: 24927161 DOI: 10.1080/09205063.2014.926577]

79 **Ao H**, Xie Y, Tan H, Yang S, Li K, Wu X, Zheng X, Tang T. Fabrication and in vitro evaluation of stable collagen/hyaluronic acid biomimetic multilayer on titanium coatings. *J R Soc Interface* 2013; **10**: 20130070 [PMID: 23635490 DOI: 10.1098/rsif.2013.0070]

80 **Rivera-Chacon DM**, Alvarado-Velez M, Acevedo-Morantes CY, Singh SP, Gultepe E, Nagesha D, Sridhar S, Ramirez-Vick JE. Fibronectin and vitronectin promote human fetal osteoblast cell attachment and proliferation on nanoporous titanium surfaces. *J Biomed Nanotechnol* 2013; **9**: 1092-1097 [PMID: 23858975 DOI: 10.1166/jbn.2013.1601]

81 **Sverzut AT**, Crippa GE, Morra M, de Oliveira PT, Beloti MM, Rosa AL. Effects of type I collagen coating on titanium osseointegration: histomorphometric, cellular and molecular analyses. *Biomed Mater* 2012; **7**: 035007 [PMID: 22406648 DOI: 10.1088/1748-6041/7/3/035007]

82 **Schwarzbauer JE**, Sechler JL. Fibronectin fibrillogenesis: a paradigm for extracellular matrix assembly. *Curr Opin Cell Biol* 1999; **11**: 622-627 [PMID: 10508649 DOI: 10.1016/S0955-0674(99)00017-4]

83 **Chen C**, Li H, Kong X, Zhang SM, Lee IS. Immobilizing osteogenic growth peptide with and without fibronectin on a titanium surface: effects of loading methods on mesenchymal stem cell differentiation. *Int J Nanomedicine* 2015; **10**: 283-295 [PMID: 25678785 DOI: 10.2147/IJN.S74746]

84 **Franz S**, Rammelt S, Scharnweber D, Simon JC. Immune responses to implants - a review of the implications for the design of immunomodulatory biomaterials. *Biomaterials* 2011; **32**: 6692-6709 [PMID: 21715002 DOI: 10.1016/j.biomaterials.2011.05.078]

85 **Bierbaum S**, Hempel U, Geissler U, Hanke T, Scharnweber D, Wenzel KW, Worch H. Modification of Ti6AL4V surfaces using collagen I, III, and fibronectin. II. Influence on osteoblast responses. *J Biomed Mater Res A* 2003; **67**: 431-438 [PMID: 14566783 DOI: 10.1002/jbm.a.10084]

86 **Ku Y**, Chung CP, Jang JH. The effect of the surface modification of titanium using a recombinant fragment of fibronectin and vitronectin on cell behavior. *Biomaterials* 2005; **26**: 5153-5157 [PMID: 15792542 DOI: 10.1016/j.biomaterials.2005.01.060]

87 **Elmengaard B**, Bechtold JE, Søballe K. In vivo study of the effect of RGD treatment on bone ongrowth on press-fit titanium alloy implants. *Biomaterials* 2005; **26**: 3521-3526 [PMID: 15621242 DOI: 10.1016/j.biomaterials.2004.09.039]

88 **Karaman O**, Kelebek S, Demirci EA, İbiş F, Ulu M, Ercan UK. Synergistic Effect of Cold Plasma Treatment and RGD Peptide Coating on Cell Proliferation over Titanium Surfaces. *Tissue Eng Regen Med* 2018; **15**: 13-24 [PMID: 30603531 DOI: 10.1007/s13770-017-0087-5]

89 **Herranz-Diez C**, Mas-Moruno C, Neubauer S, Kessler H, Gil FJ, Pegueroles M, Manero JM, Guillem-Marti J. Tuning Mesenchymal Stem Cell Response onto Titanium-Niobium-Hafnium Alloy by Recombinant Fibronectin Fragments. *ACS Appl Mater Interfaces* 2016; **8**: 2517-2525 [PMID: 26735900 DOI: 10.1021/acsami.5b09576]

90 **Guillem-Marti J**, Gelabert M, Heras-Parets A, Pegueroles M, Ginebra MP, Manero JM. RGD Mutation of the Heparin Binding II Fragment of Fibronectin for Guiding Mesenchymal Stem Cell Behavior on Titanium Surfaces. *ACS Appl Mater Interfaces* 2019; **11**: 3666-3678 [PMID: 30607934 DOI: 10.1021/acsami.8b17138]

91 **Lieberman JR**, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. *J Bone Joint Surg Am* 2002; **84**: 1032-1044 [PMID: 12063342 DOI: 10.2106/00004623-200206000-00022]

92 **Bauer S**, Park J, Pittrof A, Song YY, von der Mark K, Schmuki P. Covalent functionalization of TiO2 nanotube arrays with EGF and BMP-2 for modified behavior towards mesenchymal stem cells. *Integr Biol (Camb)* 2011; **3**: 927-936 [PMID: 21829821 DOI: 10.1039/c0ib00155d]

93 **Katagiri T**, Takahashi N. Regulatory mechanisms of osteoblast and osteoclast differentiation. *Oral Dis* 2002; **8**: 147-159 [PMID: 12108759 DOI: 10.1034/j.1601-0825.2002.01829.x]

94 **Kim SH**, Park JK, Hong KS, Jung HS, Seo YK. Immobilization of BMP-2 on a nano-hydroxyapatite-coated titanium surface using a chitosan calcium chelating agent. *Int J Artif Organs* 2013; **36**: 506-517 [PMID: 23661555 DOI: 10.5301/ijao.5000215]

95 **Andrae J**, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008; **22**: 1276-1312 [PMID: 18483217 DOI: 10.1101/gad.1653708]

96 **Hollinger JO**, Hart CE, Hirsch SN, Lynch S, Friedlaender GE. Recombinant human platelet-derived growth factor: biology and clinical applications. *J Bone Joint Surg Am* 2008; **90 Suppl 1**: 48-54 [PMID: 18292357 DOI: 10.2106/JBJS.G.01231]

97 **Fiedler J**, Etzel N, Brenner RE. To go or not to go: Migration of human mesenchymal progenitor cells stimulated by isoforms of PDGF. *J Cell Biochem* 2004; **93**: 990-998 [PMID: 15389881 DOI: 10.1002/jcb.20219]

98 **Ma Q**, Jiang N, Liang S, Chen F, Fang L, Wang X, Wang J, Chen L. Functionalization of a clustered TiO2 nanotubular surface with platelet derived growth factor-BB covalent modification enhances osteogenic differentiation of bone marrow mesenchymal stem cells. *Biomaterials* 2020; **230**: 119650 [PMID: 31806404 DOI: 10.1016/j.biomaterials.2019.119650]

99 **Holland TA**, Bodde EW, Cuijpers VM, Baggett LS, Tabata Y, Mikos AG, Jansen JA. Degradable hydrogel scaffolds for in vivo delivery of single and dual growth factors in cartilage repair. *Osteoarthritis Cartilage* 2007; **15**: 187-197 [PMID: 16965923 DOI: 10.1016/j.joca.2006.07.006]

100 **Moioli EK**, Clark PA, Xin X, Lal S, Mao JJ. Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering. *Adv Drug Deliv Rev* 2007; **59**: 308-324 [PMID: 17499385 DOI: 10.1016/j.addr.2007.03.019]

101 **Moioli EK**, Hong L, Guardado J, Clark PA, Mao JJ. Sustained release of TGFbeta3 from PLGA microspheres and its effect on early osteogenic differentiation of human mesenchymal stem cells. *Tissue Eng* 2006; **12**: 537-546 [PMID: 16579687 DOI: 10.1089/ten.2006.12.537]

102 **Kim SH**, Park JK, Hong JH, Jung HS, Hong KS, Lee JH, Park KB, Choi SK, Seo YK. Increase of BM-MSC proliferation using L-DOPA on titanium surface in vitro. *J Biomater Appl* 2012; **27**: 143-152 [PMID: 21343218 DOI: 10.1177/0885328210397679]

103 **Son JS**, Choi YA, Park EK, Kwon TY, Kim KH, Lee KB. Drug delivery from hydroxyapatite-coated titanium surfaces using biodegradable particle carriers. *J Biomed Mater Res B Appl Biomater* 2013; **101**: 247-257 [PMID: 23143817 DOI: 10.1002/jbm.b.32834]

104 **Cheng H**, Yue K, Kazemzadeh-Narbat M, Liu Y, Khalilpour A, Li B, Zhang YS, Annabi N, Khademhosseini A. Mussel-Inspired Multifunctional Hydrogel Coating for Prevention of Infections and Enhanced Osteogenesis. *ACS Appl Mater Interfaces* 2017; **9**: 11428-11439 [PMID: 28140564 DOI: 10.1021/acsami.6b16779]

105 **Anderson JM**, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Semin Immunol* 2008; **20**: 86-100 [PMID: 18162407 DOI: 10.1016/j.smim.2007.11.004]

106 **Balce DR**, Li B, Allan ER, Rybicka JM, Krohn RM, Yates RM. Alternative activation of macrophages by IL-4 enhances the proteolytic capacity of their phagosomes through synergistic mechanisms. *Blood* 2011; **118**: 4199-4208 [PMID: 21846901 DOI: 10.1182/blood-2011-01-328906]

107 **Mantovani A**, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004; **25**: 677-686 [PMID: 15530839 DOI: 10.1016/j.it.2004.09.015]

108 **Omar O**, Lennerås M, Svensson S, Suska F, Emanuelsson L, Hall J, Nannmark U, Thomsen P. Integrin and chemokine receptor gene expression in implant-adherent cells during early osseointegration. *J Mater Sci Mater Med* 2010; **21**: 969-980 [PMID: 19856201 DOI: 10.1007/s10856-009-3915-x]

109 **Wynn TA**, Vannella KM. Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* 2016; **44**: 450-462 [PMID: 26982353 DOI: 10.1016/j.immuni.2016.02.015]

110 **Kang H**, Kim S, Wong DSH, Jung HJ, Lin S, Zou K, Li R, Li G, Dravid VP, Bian L. Remote Manipulation of Ligand Nano-Oscillations Regulates Adhesion and Polarization of Macrophages in Vivo. *Nano Lett* 2017; **17**: 6415-6427 [PMID: 28875707 DOI: 10.1021/acs.nanolett.7b03405]

111 **Ma QL**, Zhao LZ, Liu RR, Jin BQ, Song W, Wang Y, Zhang YS, Chen LH, Zhang YM. Improved implant osseointegration of a nanostructured titanium surface via mediation of macrophage polarization. *Biomaterials* 2014; **35**: 9853-9867 [PMID: 25201737 DOI: 10.1016/j.biomaterials.2014.08.025]

112 **Klopfleisch R**. Macrophage reaction against biomaterials in the mouse model - Phenotypes, functions and markers. *Acta Biomater* 2016; **43**: 3-13 [PMID: 27395828 DOI: 10.1016/j.actbio.2016.07.003]

113 **Zhang Z**, Xie Y, Pan H, Huang L, Zheng X. Influence of patterned titanium coatings on polarization of macrophage and osteogenic differentiation of bone marrow stem cells. *J Biomater Appl* 2018; **32**: 977-986 [PMID: 29237352 DOI: 10.1177/0885328217746802]

114 **Nie B**, Ao H, Zhou J, Tang T, Yue B. Biofunctionalization of titanium with bacitracin immobilization shows potential for anti-bacteria, osteogenesis and reduction of macrophage inflammation. *Colloids Surf B Biointerfaces* 2016; **145**: 728-739 [PMID: 27289314 DOI: 10.1016/j.colsurfb.2016.05.089]

115 **Ma QL**, Fang L, Jiang N, Zhang L, Wang Y, Zhang YM, Chen LH. Bone mesenchymal stem cell secretion of sRANKL/OPG/M-CSF in response to macrophage-mediated inflammatory response influences osteogenesis on nanostructured Ti surfaces. *Biomaterials* 2018; **154**: 234-247 [PMID: 29144982 DOI: 10.1016/j.biomaterials.2017.11.003]

116 **Chen J**, Li M, Yang C, Yin X, Duan K, Wang J, Feng B. Macrophage phenotype switch by sequential action of immunomodulatory cytokines from hydrogel layers on titania nanotubes. *Colloids Surf B Biointerfaces* 2018; **163**: 336-345 [PMID: 29331905 DOI: 10.1016/j.colsurfb.2018.01.007]

117 **Fu X**, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal Stem Cell Migration and Tissue Repair. *Cells* 2019; **8**: [PMID: 31357692 DOI: 10.3390/cells8080784]

118 **Duvvuru MK**, Han W, Chowdhury PR, Vahabzadeh S, Sciammarella F, Elsawa SF. Bone marrow stromal cells interaction with titanium; Effects of composition and surface modification. *PLoS One* 2019; **14**: e0216087 [PMID: 31116747 DOI: 10.1371/journal.pone.0216087]

119 **Yeo IL**. Modifications of Dental Implant Surfaces at the Micro- and Nano-Level for Enhanced Osseointegration. *Materials (Basel)* 2019; **13**: [PMID: 31878016 DOI: 10.3390/ma13010089]

120 **Razek AAKA**, Ashmalla GA. Assessment of paraspinal neurogenic tumors with diffusion-weighted MR imaging. *Eur Spine J* 2018; **27**: 841-846 [PMID: 28821978 DOI: 10.1007/s00586-017-5265-6]

121 **Surov A**, Nagata S, Razek AA, Tirumani SH, Wienke A, Kahn T. Comparison of ADC values in different malignancies of the skeletal musculature: a multicentric analysis. *Skeletal Radiol* 2015; **44**: 995-1000 [PMID: 25916616 DOI: 10.1007/s00256-015-2141-5]

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**Table 1 Biofunctionlization of orthopaedic implant with bioactive ceramic to regulate bone marrow mesenchymal stem cell behavior**

|  |  |  |  |
| --- | --- | --- | --- |
| **Method** | **Preparation of bioactive ceramic** | **Cell response** | **Ref.** |
| Plasma spraying | TiO2-HA nanocomposite powders were thermally sprayed *via* the HVOF (high-velocity oxy-fuel) technique. | HBMSCs have stronger initial adhesion and favor osteogenic differentiation. | Dimitrievska *et al*[26] |
| Ta-incorporated HA coatings were fabricated using the plasma spray technique on a titanium substrate. | Ta-incorporated HA coating could promote initial adhesion, faster proliferation, and osteogenic differentiation of BMSCs. | Lu*et al*[28] |
| An atmosphere plasma spray system was applied to spray the synthesized 40-80 μm powders onto the treated substrates. | The attachment and proliferation of BMSCs were more significantly on akermanite coatings than on HA coatings. | Yi*et al*[31] |
| Sol-gel method | Ti disks were etched with the mixed solution of HF and H2SO4. Next, EtOH solutions containing tetrabutyl titanate (TBT) were spin-coated onto samples. | The micro/nano-level structure of large particles (80 nm) significantly promoted MSC proliferation and differentiation. | Shen*et al*[23] |
| Pre-hydrolyzed silica solution was added to a solution containing the pores structure-directing agents dissolved in ethanol. | The silica coatings accelerate the adhesive response of early BMSCs and promote BMSC osteogenic differentiation. | Inzunza *et al*[34] |

HVOF: High-velocity oxy-fuel; HBMSCs: Human bone marrow-derived mesenchymal stem cells; HA: Hydroxyapatite; BMSCs: Bone marrow-derived mesenchymal stem cells; HF: Hydrofluoric acid; H2SO4:Sulfuric acid;TBT: Tetrabutyl titanate; MSC: Mesenchymal stem cell.

**Table 2 Surface topography to regulate bone marrow mesenchymal stem cell behavior**

|  |  |  |  |
| --- | --- | --- | --- |
| **Method** | **Treatment process** | **Cell response** | **Ref.** |
| Chemical treatments | Commercial pure Ti was immersed into KOH solutions | The differentiation levels of ALP and OCN were significantly increased. | Cai*et al*[38] |
| The Ti disks were immersed into solutions of polyphosphoric acid. | Significantly higher cell attachment and proliferation were also found on Ti treated with polyphosphoric acid. | Maekawa *et al*[40] |
| Surfaces submitted to polishing plus etching with 0.8% HF, 13% HNO3 solution. | Rough surfaces submitted to acid-etching favor undifferentiated BMSCs into osteogenic lineage cells. | Silva *et al*[42] |
| The Ti disks were pickled in oxalic acid solution and NaOH, respectively. | Although BMSC adhesion and osteogenesis were promoted, proliferation was significantly inhibited on treated surfaces. | Li *et al*[47] |
| The titanium was treated with H2O2 | H2O2-treated surfaces were beneficial for promoting BMSC attachment, proliferation, and osteogenic differentiation. | Daw *et al*[52] |
| The anodic oxidation was carried out to prepare nanotube on titanium surface. | NT30 supported adhesion, stretching, proliferation, and osteogenic differentiation of BMSCs.  | Xu *et al*[24] |
| Electrochemical anodization | Nanonets on titanium surfaces were prepared.  | BMSC cultured on nanonets structured Ti surfaces present a high frequency of alignment.  | Grimalt *et al*[53] |
| The Ti disks were micro-arc oxidized in an electrolyte solution | The MAO-coating significantly promoted adhesion and osteogenic differentiation of BMSCs by mediating the integrin β1 signaling pathway. | Li*et al*[57] |
| O-PIII treatment was performed in a high-vacuum chamber with a radio frequency plasma source. | O-PIII treatment could enhance the adhesion of BMSCs. | Yang *et al*[59] |
| Plasma ion implantation and deposition | O-PIII treatment was performed in a high-vacuum chamber with a radio frequency plasma source. | The group treated with the highest concentration of oxygen ions has the best effect on adhesion, migration, proliferation, and differentiation of BMSCs. | Yang *et al*[60] |
| The Ti-based alloy was modified by electropolishing and plasma electrolytic oxidation process. | The calcium-ion-implanted titanium remarkably improved BMSC adhesion and proliferation compared to the untreated sample. | Michalska *et al*[61] |
| Highly ionized Ca and Mg plasmas were generated from a filtered vacuum arc source and accelerated within the electric field between a sheath and the substrates. | Initial cell attachment on a titanium surface can be improved by Ca and Mg ion implantation. In addition, the expression of osteogenic-related genes like RUNX2 and type I collagen was higher in the Mg ion-implanted surface. | Won *et al*[62] |
| The Ti discs were polished with abrasive grit (grades 240–600), and then treated with laser radiation at various fluences (132, 210, or 235 J/cm2). | Laser-modified titanium surfaces could enhance upregulation of expression of the osteogenic markers and enhance alkaline phosphatase activity of BMSCs. | Bressel*et al*[66] |
| Laser beam treatment | DMLS discs were fabricated in an argon atmosphere with Yb fibre laser system  | Topographical cues of DMLS surfaces could enhance BMSC adhesion, as well as osteogenesis | Zheng*et al*[67] |
| The laser system was a Ti: Sa laser chain, which delivers 120 fs, 800 nm pulses at a repetition rate of 5 kHz. | BMSCs exhibited a more elongated, spindle-like morphology and higher spreading speeds on FS laser-modified surfaces. | Dumas*et al*[68] |

Ti: Titanium; KOH: Potassium hydroxide; ALP: Alkaline phosphatase; OCN: Osteocalcin; HF: Hydrofluoric acid; HNO3: Nitric acid; BMSC: Bone marrow-derived mesenchymal stem cell; NaOH: Sodium hydroxide; H2O2: Hydrogen peroxide; MAO: Micro-arc oxidation; O-PIII: Oxygen plasma immersion ion implantation; Ca: Calcium; Mg: Magnesium; RUNX2: Runt-related transcription factor 2; DMLS: Direct metal laser sintering: Yb: Ytterbium; FS: Femtosecond.

**Table 3 Covalent immobilization bioactive molecules to promote bone marrow mesenchymal stem cell adhesion, proliferation, and osteogenic differentiation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Bioactive molecules** | **Treatment process** | **Cell response** | **Ref.** |
| Type I collagen | Titanium fiber meshes were treated with NaOH, followed by p-nitrophenyl chloroformate, and coated with collagen type I. | The modification of titanium fiber meshes can promote BMSC osteogenic differentiation. | van den Dolder*et al*[72] |
| Covalent immobilization of collagen on titanium  | Greater regulation effect on BMSC osteogenesis compared to adsorptive immobilization. | Ao*et al*[74] |
| Hyaluronic acid was immobilized on titanium surface by layer-by-layer technique  | BMSCs had more lamellipodia and adhered more closely to the covalently immobilized HyA surface. | Ao*et al*[78] |
| HyA | Covalent immobilization of RGD peptide on titanium surface | RGD-functionalized titanium can improve early bone growth and matrix mineralization. | Elmengaard *et al*[87],Karaman *et al*[88] |
| RGD peptide | HBII-RGD was immobilized on the Ti surface | HBII-RGD-functionalized Ti surfaces could stimulate BMSC differentiation and mineralization. | Guillem-Marti *et al*[90] |
| Growth factors | Covalently graft EGF and BMP-2 onto the oxide surfaces. | BMSC adhesion and proliferation were dramatically increased by covalently grafting EGF, but covalently grafted BMP-2 did not. | Bauer *et al*[92] |
| PDGF-BB loading on titanium nanotube. | PDGF-BB functionalized surfaces significantly enhanced BMSC attachment and osteogenesis-related functions | Ma *et al*[98] |

NaOH: Sodium hydroxide; BMSC: Bone marrow-derived mesenchymal stem cell; HyA: Hyaluronic acid; RGD: Arg-Gly-Asp; HBII-RGD: Heparin binding II-Arg-Gly-Asp; Ti: Titanium; EGF: Epidermal growth factor; BMP-2: Bone morphogenetic protein-2; PDGF-BB: Platelet-derived growth factor BB.

**Table 4 Local control release of bioactive molecules to promote bone marrow mesenchymal stem cell adhesion, proliferation, and osteogenic differentiation**

|  |  |  |
| --- | --- | --- |
| **Bioactive molecule** | **Cell response** | **Ref.** |
| L-DOPA | The new L-DOPA coating enhances the initial cell adhesion, mitochondrial activity, and proliferation of BMSCs on the titanium surface. | Kim *et al*[102] |
| DEX | The HA-Ti surfaces with DEX carrier system potently induce BMSC osteogenic differentiation *in vitro*. | Son *et al*[103] |
| SNs | The addition of SNs to the hydrogel formulation can promote bone formation when co-cultured with BMSCs | Cheng *et al*[104] |

L-DOPA: L-3,4-dihydroxyphenylalanine; BMSCs: Bone marrow-derived mesenchymal stem cells; HA: Hydroxyapatite; Ti: Titanium; DEX: Dexamethasone; SNs: Silicate nanoparticles.