Review Article

Properties improvement of titanium alloys scaffolds in bone tissue engineering: a literature review

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Abstract: Owing to their excellent biocompatibility and corrosion-resistant properties, titanium (Ti) (and its alloy) are essential artificial substitute biomaterials for orthopedics. However, flaws, such as weak osteogenic induction ability and higher Young's modulus, have been observed during clinical application. As a result, short- and long-term postoperative follow-up has found that several complications have occurred. For decades, scientists have exerted efforts to compensate for these deficiencies. Different modification methods have been investigated, including changing alloy contents, surface structure transformation, three-dimensional (3D) structure transformation, coating, and surface functionalization technologies. The cell-surface interaction effect and imitation of the natural 3D bone structure are the two main mechanisms of these improved methods. In recent years, significant progress has been made in materials science research methods, including thorough research of titanium alloys of different compositions, precise surface pattern control technology, controllable 3D structure construction technology, improvement of coating technologies, and novel concepts of surface functionalization. These improvements facilitate the possibility for further research in the field of bone tissue engineering. Although the underlying mechanism is still not fully understood, these studies still have some implications for clinical practice. Therefore, for the direction of further research, it is beneficial to summarize these studies according to the basal method used. This literature review aimed to classify these technologies, thereby providing beginners with a preliminary understanding of the field.

Keywords: Titanium (Ti); 3D structure; biomaterials; orthopedics

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Introduction

Since the mid-nineteenth century, the repair of mass bone defects has become a focal point of research in orthopedics and oral and maxillofacial surgery. The most commonly used materials are metal, biological ceramics, and high-molecular polymers. With modern medicine and materials development, some novel biological ceramics and bio-glass are superior to traditional metals in some aspects. However, given their tenacity, durability, and cost-effectiveness, metallic prostheses are still the first choice for orthopedic surgery.

As the most commonly used material, titanium (Ti) is a silver-white metal that exhibits the following characteristics: high strength, lightweight, strong plasticity, corrosion resistance, and good biocompatibility (1). Since the invention of the magnesium reduction process to produce pure titanium, it has been widely used in medicine,

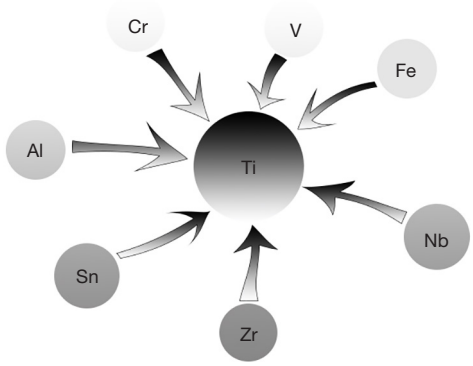

Figure 1 Component change of the titanium alloy. The materials science researchers dedicate to add elements in different proportions into titanium, which could construct different alloy phases and get different physical or bioactive properties.

aerospace, manufacturing, and other industries. In the healthcare industry, titanium is non-toxic, biocompatible, and corrosion resistant, making it a common material for manufacture artificial bone defect fillers and internal fixation screw systems (2). However, further development of research and application has gradually revealed some disadvantages. Firstly, the osteogenic induction ability of conventional titanium alloys is weak, which may lead to the formation of fibrous connective tissue film on the bone-metal contact surface, thus affecting bone regeneration and integration (3). Secondly, Young's modulus of titanium is higher than that of natural bone, which directly causes a degree of bone resorption around the prosthesis during daily activities postoperatively, thereby increasing the risk of osteolysis, resorption, postoperative complications, and secondary surgery (4,5). Thirdly, recent research has shown that the wear debris of titanium could induce local biological reactions, including inflammation, apoptosis, and necrosis, resulting in associated complications (6,7). Moreover, although its excellent durability is an essential feature as a bone-tissue-engineering material, this characteristic means that a second operation may be required for removal to avoid complications like osteolysis and non-union.

Over the last decade, several methodologies have been explored to reform the properties of titanium alloys. In this study, we reviewed relevant studies from the PubMed database from the last 5 years and attempted to predict the development prospects for the property improvement technology of titanium alloys.

Data sources: PubMed database searches were completed using the following MeSH terms: Titanium, Bone Formation, Ossification, Ossifications, Osteoclastogenesis, Osteoclastogeneses. The search included randomized controlled trials, meta-analyses, clinical trials, systematic reviews, clinical practice guidelines, evidence-based medicine, and review articles. Search dates: January 2015 through February 2020.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/atm-20-8175).

Alloy composition change

In 1940, Bothe *et al.* (8) first used titanium in medical experiments and confirmed its excellent biocompatibility. This study directly led to the primary use of pure titanium as the research object of bone tissue engineering over the next 30 years, with advantages such as good biocompatibility and strong corrosion-resistance being discovered (9). In 1952, Brånemark *et al.* (10) used pure titanium to construct a metal cage spiral that was inserted into a rabbit bone. A few months later, they found that it was closely bound to the bone and proposed the concept of "osseointegration", which made titanium a popular material for surgical operations.

In the following decade, researchers identified that the high Young's modulus of pure titanium could result in the shade with bone stress, leading to the dissolution of bone around the implant, aseptic necrosis as fiber, and coated formation which causes loosening of the prosthesis. Moreover, metal fatigue occurred in the body's fluid environment due to the lower static stress strength of pure titanium. Adding other metal or non-metal elements to compensate for these shortcomings became a popular material science research direction (*Figure 1*). In 1982, Ti6Al4V was used to produce an artificial joint prosthesis and provided a new idea for using a novel titanium alloy. Initially, niobium (Nb) became a substitute metal element for vanadium (V) and aluminum (Al) in numerous studies due to its lower inherent cytotoxicity (11-15).

Next, although the α+β type titanium alloy has reduced Young's modulus to some extent, Young's modulus of human bones is still lower than that of the α+β type titanium alloy. Therefore, the β-type titanium alloy (about 40–60 Giga Pascal, GPA) has attracted extensive attention as a new research object (16-18). Also, the impact of the thermal effect on the alloy phase has been studied for decades. Malinov *et al.* (19) found that the alloy phase changed from

α to β in a temperature- and proper chemical-controlled environment. Kopova *et al.* added a small amount of Ferrum (Fe) (0–2 wt.%) and Silicon (Si) (0–1 wt.%) to Ti-35Nb-7Zr-6Ta in order to regulate Young's modulus and biological properties, and thus, developed a novel β type titanium alloy (Ti-35Nb-7Zr-6Ta-2Fe-0.5Si) (17,20).

Due to the different element compositions of the three types of titanium alloy, their manufacturing and casting processes are also markedly different. It is essential to examine the treatment mechanism and method of the β type titanium alloy. For example, Plasma Electrolytic Oxidation (PEO) is a traditional surface microstructure treatment technology. In a recent study, Tanase *et al.* (21) selected various kinds of titanium alloy. They divided them into different experimental groups according to their alloy phase, including pure titanium (CP, α type), titanium (Ti6Al4V, α+β type), titanium (Ti13Nb13Zr, close β type), and titanium alloy (Ti45Nb, completely β type) groups. They managed the surface microstructure treatment process for each group by PEO and found that this technology could effectively construct a better rough surface on the β type alloy compared to the α type alloy. However, the complexity of the manufacturing process and high production price limits its mass production and clinical application. More cost-efficient and convenient production methods need to be explored.

Besides changing the physical properties of the alloy, some metal elements have also been added to the alloy composition to reduce the occurrence of postoperative short- or long-term complications. Xu *et al.* (22) incorporated copper into the titanium alloy (Ti6Al4V) using Cross Shading and Selective Laser Melting (SLM) technology, which reduced the inflammatory response around the prosthesis and the formation of fibrous tissue capsules. This method played a specific role in promoting bone and angiogenic differentiation. To reduce the probability of bacterial infection around the prosthesis after surgery, Liu *et al.* (23) added silver to titanium. They obtained an optimal ratio between antibacterial properties and biocompatibility by altering the silver content, which resulted in excellent antibacterial properties for the titanium without damaging human cells.

Over the last decade, many novel medical material engineering technologies that could be combined with other non-metal substances have been developed, which are expected to solve complex problems associated with the metal content of titanium alloys. Spark Plasma Sintering (SPS) was further explored by Fernandez-Garcia *et al.* (24). Bioactive ceramics could be combined with titanium to construct novel scaffolds that promote the osteogenic differentiation of stem cells and accelerate the process of bone integration through SPS technology. Kokubun *et al.* (25) conducted animal experiments with titania-based biological glass alloy (Ti40Zr10Cu34Pd14Sn2) and found that it could effectively minimize the release of metal ions, thus avoiding related cell damage while reducing Young's modulus.

Surface and three-dimensional (3D) structure transformation

Surface and 3D structure transformation are considered two effective methods to increase the osteogenic induction ability of titanium metal alloy scaffolds, which do not involve changing metal components (*Figure 2*). Given the mechanism of osteogenic differentiation, compared with the smooth surface, the rough surface can increase the contact surface area between membranes and pseudopodia. The specific morphology could provide various fitting angles, create various peaks or valleys to increase surface hydrophilicity, and reconstruct the structure of proteins to promote absorption, thereby optimizing cell adhesion and proliferation ability. A study on human osteoblasts has shown that rough surfaces have more anchor points and increase the adhesion ability of cells, which could provide better conditions for cell proliferation and increase cell activity (26). Moreover, due to the numerous anchors on the rough surface, cell polarity changes could control the expression of structural proteins, including extracellular matrix protein, cytoskeleton protein, integrin protein, and calcic adhesion protein, etc. Cell polarity changes could also promote the differentiation of bone marrow stem cells into osteoblasts to a certain extent (3). The commonly used methods include groove structure, laser sintering, sandblasting, chemical etching, and anodic oxidation. Based on the acid-etching method, Zheng *et al.* (27) compared the differences between direct laser sintering and surface sandblasting and showed that direct laser sintering technology was superior to surface sandblasting treatment in terms of epigenetic regulation of osteogenic differentiation.

In the early stages of research, surface treatment methods often fail to control the shape of the generated surface patterns and only use simple physical/chemical methods to generate rough surfaces. With the improvement of technology, it is possible to control the geometric parameters

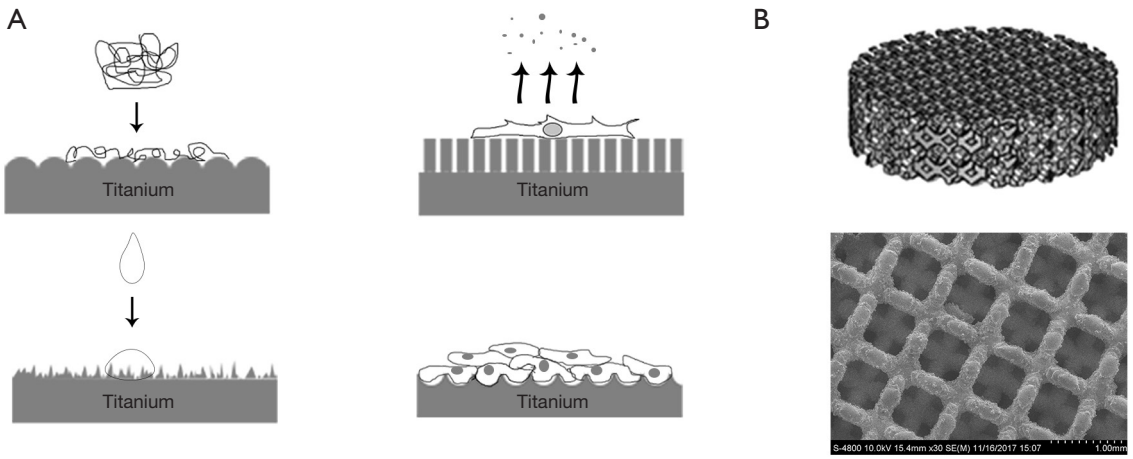

Figure 2 Nano-scale surface modification (A) and 3D structure formation (B). The main structures of nanoscale surface modification are classified as nano-rod, nano-particle, nano-hemisphere and nano-amorphous structure. It could improve titanium alloy properties by promoting protein absorption, cell differentiation, cell proliferation as well as increasing wettability. 3D structure can simulate natural bone structure thus promoting osseointegration, osteogenesis, angiogenesis and reducing Young's modulus of titanium alloy.

and surface microstructure accurately. Zhu *et al.* (28) reported a combination method of photolithography. They inductively coupled plasma-based dry etching for surface design, which can precisely control the microstructure pattern of the titanium surface and screen appropriate patterns and parameters to achieve the best osteogenesis effect.

At present, further structural treatment primarily includes 3D printing technology (i.e., selective laser melting and electron beam melting) and nano-level surface structure formation (nanotube array, nano-rod array, etc.). These two technologies both have unique advantages. 3D printing technology provides accurate shape control. Researchers could better simulate the porous structure of human cancellous bone at a 3D level to promote bone growth, thereby helping bone-implant integration. Meanwhile, nano-level surface structure formation can better regulate cell adhesion, proliferation, and differentiation from the nano-level material-cell interaction.

In 2004, Fujibayashi *et al.* (29) discovered that 3D structures provide a similar growth environment as the human body's natural bone structure compared with two-dimensional (2D) structures. Since then, the 3D porous structure has become a particular focus of research. Based on 2D micro-rough surface, the 3D porous structure can effectively promote osteogenesis, bone ingrowth, accelerate bone integration, and reduce Young's modulus, thus reducing aseptic bone dissolution (30-33). Meanwhile, the 3D porous structure promotes the growth of new bone into the scaffold, which promotes bone integration, osteogenesis, and increases angiogenesis, and reduces the occurrence of stent-related complications due to changes in stress and strain (34).

Similarly, Attar *et al.* produced Ti and Ti-TiB alloy porous structures with different porosity levels. The properties test then demonstrated that 37% of the porous group showed elastic moduli close to that of human bone (35). However, another study confirmed that porous scaffolds with different shapes or structures have a minimal effect on cellular osteogenesis than the enhanced osteogenic capacity provided by the porous nature itself (36). Hedayati *et al.* studied the isolated and modulated effects of topological design and material type on mechanical properties. Their results showed that topological modification could significantly influence the porous properties to a greater extent than the material type (37). Also, Zhou *et al.* (38) verified the osteogenic effect of 3D scaffold-based scaffold on adipose stem cells and confirmed that its mechanism was related to the IGF-1R/protein kinase B (AKT)/Mammalian Target of Rapamycin Complex 1 (mTORC1) cell signaling pathway.

3D printing is a new rapid prototyping technology, which has been widely used in the field of tissue engineering research. 3D printing technology has good controllability, making it possible to precisely regulate the porosity and pore diameter of the titanium scaffolds for further research. Some studies have reported that scaffolds with different

pore sizes, porosity, pore shapes, and pore structures were constructed using this technique to achieve the optimal promotion of physical properties, cell adhesion, proliferation, and osteogenic differentiation (39-43). Kapat *et al.* (44) used the powder metallurgy method to build Ti6Al4V with different pore sizes and porosities and then measured the pore distribution, mechanical properties, surface roughness, contact angle, and protein adsorption ability. Subsequent *in vivo* and *in vitro* experiments determined the optimal impact on cell distribution and differentiation. 3D printing technology typically refers to Electron Beam Melting (EBM) and Selective Laser Melting (SLM) in the metallurgy field. There are differences between these two methods. For example, SLM has a higher resolution ratio and costs more than EBM. Although the operational approaches of these technologies differ, some scientists have found no significant difference in the construction of titanium alloy scaffolds (45).

Micro/nano-meter microstructure morphology construction is another new research direction, which include 3D printing technology, novel acid corrosion and anodic oxide technology and nano-level surface morphology formation (nano-meter surface pattern, nanotube, and nano-rod arrays). The hydrophilicity, protein adsorption ability, the surface roughness of scaffold are increased, thus promoting cell viability, proliferation, adhesion, and differentiation. Some studies have reported that various forms of nano-meter surface structure improve the biocompatibility and bioactivity of the titanium alloy scaffolds through nanoscale scaffolds-cells interface interaction (46,47). Results reported by Liu *et al.* (48) may explain the underlying mechanism. Their *in vitro* experiment indicated that the rough titanium surface could accelerate the osteogenic procession by activating the extracellular regulated protein kinases (ERK1/2)- micro ribonucleic acid (miR)-1827-Osterix signaling pathway. Recent research by Clainche *et al.* (49) found that the morphology construction could induce osteogenic differentiation and inhibit bacterial infection. They also indicated that the nanostructured surface with sharp nanosheet protrusions could cut the cell membrane of bacteria and reduce bacterial attachment.

Recently, researchers shifted emphasis to the nanometer-micron co-existence mode and found that it was significantly superior for promoting cell osteogenesis than any one of the two structures. Dumas *et al.* (50) used femtosecond laser technology to construct a nanometer-micron notch ripple structure, which significantly increased the cell adhesion and proliferation ability, accelerated cell spread, and increased the cell osteogenesis ability compared to the polished scaffold surface. Gulati *et al.* (51) combined 3D printing technology and anodic oxidation technology to construct a micro-surface structure of co-existence micron-scale spherical particles and a nano-level vertical tube array, which significantly increased the osteogenic differentiation ability of stem cells. Similarly, Fu *et al.* (20) used 3D printing technology to construct a regular micron-level repeated ripple and irregular nanoscale branch structure on pure titanium and confirmed that this structure could promote osteogenic differentiation via the wingless/integrated (Wnt)/β-catenin signal transduction pathway. However, Zhang *et al.* (52) proposed an entirely different opinion. In their experiment, although the titanium 3D structure combined with acid-etched could improve *in vivo* osseointegration ability, the overall osteogenic performance of this surface was not as good as the conventional sandblasted, large-grit, acid-etched surface. These findings suggest that scientists need to explore the mechanisms of cell-surface interaction further.

Coating technology

Coating technology is one of the most popular methods for modifying the properties of titanium alloy scaffold. Owing to its excellent physical properties, researchers used the physical/chemical/biological method to create various bioactive substances for attaching solids on the titanium alloy scaffold. It is expected that this could solve problems such as poor osteogenic differentiation induction ability, bacterial contamination, osteoporosis, or scaffold-related bone destruction and resorption. At present, the commonly used ingredients mainly include hydroxyapatite (HA) coating, metal and metal oxide coating, or drug/bioactive factor-related coating (*Figure 3*).

HA is an essential main inorganic component to natural human bone, which is commonly used as an artificial bone substitute. HA has good biocompatibility, no cytotoxicity, and can be chemically combined with bone tissue. HA can also release calcium, phosphorus, and other osteogenic-related substances in the human body and be assimilated and absorbed by human bone tissue. In clinical application, surgeons directly inject HA into the bone defect. It has been reported that as the surface coating of titanium alloy, HA can promote cell adhesion, proliferation and increase osteogenic differentiation (53).

Techniques for HA coating were initially developed in the 1990s, including plasma spraying, physical

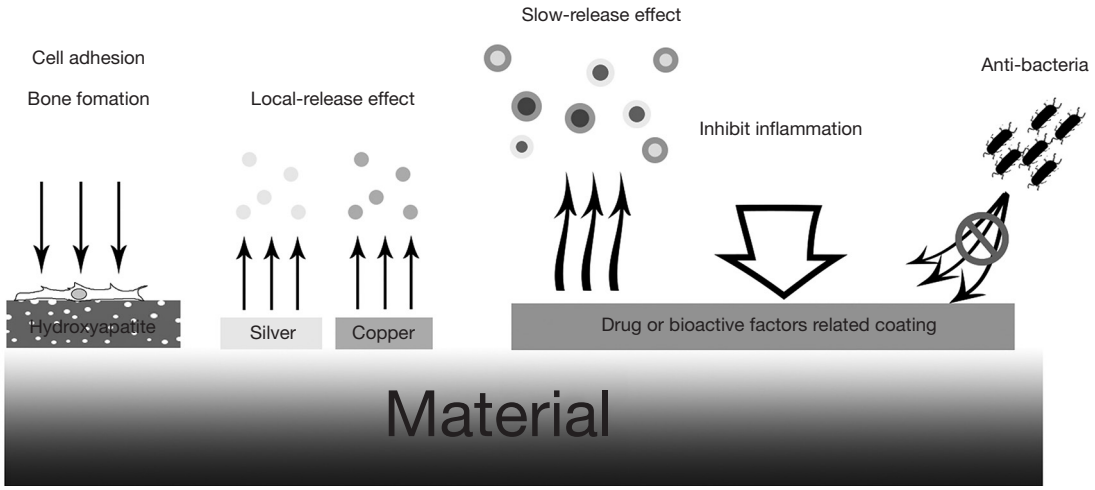

Figure 3 Coating methods of titanium. By selecting the appropriate coating material, the researchers can almost solve any single problem. The hydroxyapatite improves cell adhesion and promotes bone formation. The coating technology can reduce systemic side effects by creating local-release and slow-release effect. The antibiotics or cytokines coating can inhibit bacterial growth and reduce inflammation.

vapor deposition, aerosol-gel technology, and chemical deposition. In recent years, with the development of surface microstructure treatment technology, HA coating can be used with 3D printing and other technologies to construct nano-level microstructures. However, although it has been shown that the HA coating constructed by this microstructure treatment technology has a more vital osteogenic promotion ability, the coating-scaffold interface adhesion force is poorer than the amorphous coating.

In addition, Jang *et al.* (54) treated titanium membranes with calcium-phosphate coating and punched holes in the coating film. Their results indicate that this coating could induce bone formation and facilitate a tight combination between the new bone and the titanium surface. The coating of punch holes could reduce bone formation. Some compounds containing carbon and phosphorus, such as organic glass, tricalcium phosphate, calcium phosphate, alendronate sodium, carbon graphene, and calcium-silicon compounds, have been shown to exhibit a more substantial osteogenic differentiation promotion effect than the traditional HA coating and have become a particular focus of research (55-61).

Another kind of candidate for surface coating is metal or metal oxide. Different metals have different properties; for example, tantalum, gray metal with similar physical properties to titanium, can accelerate the natural mineral deposits in body fluids through the activation of the Wnt/beta and transforming growth factor-β (TGF-β)/drosophila mothers against decapentaplegic protein (Smad) signaling pathways, which could mediate osteogenesis (62). Wang *et al.* (63) manufactured a nanotube array coating with tantalum on the titanium alloy scaffold and then seeded adipose-derived stem cells on the coating. The subsequent animal experiment on a rabbit spinal defect model confirmed that it significantly promoted osteogenesis.

Copper and silver have been shown to exhibit significant anti-microbial activity. However, previous studies demonstrated that these ions also resulted in organ toxicity, inhibition of osteogenesis, and bone integration at high concentrations. To avoid these toxic effects, some researchers used a nanoscale metal ion to produce a kind of slow-release coating that inhibits the growth of bacteria but will not damage the properties of the titanium alloy scaffold (64,65).

In a recent study, Zhao *et al.* (66) reported a novel Tremella-like zinc oxide (ZnO)/type I collagen (Col-I) composite coating on the titanium surface. They improved the properties of ZnO, which allow a safe, visible yellow light to activate it, and made it adhere to the titanium metal surface, and subsequent experiments confirmed its excellent antibacterial properties and osseointegration ability.

Titanium dioxide is one of the most common and convenient metal oxides. It can be easily obtained chemically or anodic oxidation processes on the pure titanium scaffold. Titanium dioxide coating could change the physical properties and form a micro or nanoscale rough surface structure to some extent, thereby improving the

hydrophilia and osteogenic differentiation induction ability of scaffold. Its convenience allows it to be combined with other types of titanium property improvement techniques to promote osteogenesis (67,68) further.

Drug/bioactive factor-related coatings are currently the most popular coating technology. This refers to the creation of a slow-release film that carries different drugs depending on the requirement. With further development, this technology could carry traditional drugs and metal ions or seed stem cells. Using the local slow-releasing method, the traditional oral or intravenous administration side effects of medications can be avoided. However, due to the toxicity of local concentration, achieving a stable and controllable slow-release form and selecting an appropriate type of medium that does not cause damage to the property of the scaffold are vital concerns.

The material choice of the coating mediator medium is crucial. Haversath *et al.* (69) used poly-dl-lactide (PDLLA) carrying rhBMP-2 to form a slow-release coating surface on a titanium alloy, which did not promote bone formation as expected. Researchers hypothesized that PDLLA has a certain degree of inhibition of bone formation, which counteracts the positive effect of rhBMP-2. At present, the commonly used coating media commonly include chitosan, penicillin, calcium-phosphorus compounds, etc., which exhibit good cellular compatibility, no osteogenic inhibition, and could form a slow-releasing structure (70).

Furthermore, the choice of drugs depends on the medical requirement, and the drug dose-effect of controlled-release stents carry side effects and synergy. For example, bone morphogenetic protein-2 (BMP-2) plays a vital role in the process of osteogenesis (71). Recent research has shown that combined with a slow-release coating constructed by chitosan and other medium composition; it can effectively promote the osteogenesis ability of the titanium alloy scaffold (72-74). On the other hand, another study showed that BMP-2 inhibits cell proliferation and adhesion at high concentrations (75).

Metal ions, such as copper, silver, magnesium, strontium, etc., can promote osteogenesis and avoid certain toxicities by being carried with a slow-released coating film (76-79). Van Hengel *et al.* (80) indicated that the synergistic antibacterial effect could prevent infections by antibiotic-resistant bacteria. They combined slow-release silver/zinc nano-particles with a plasma electrolytic oxidation biofunctionalized titanium surface. The results showed that this combination significantly inhibited the growth of methicillin-resistant Staphylococcus aureus and proved to be a fruitful strategy for preventing infection.

Carrying an antibiotic, such as gentamicin, can enhance the accuracy and curative effect of bacterial inhibition to reduce the complications caused by bacterial infection. Furthermore, the bacteria-mediated inflammatory responses could be restrained to avoid the osteogenesis inhibition effect generated by improper inflammatory activation (81,82).

Substances such as alendronate sodium and calcitriol could promote the formation of bone defects around the scaffold and have been shown to play a role in increasing screw stability in animal model experiments of osteoporosis (83-86).

Exosomes are small membranous vesicles containing complex ribose nucleic acid (RNA) and proteins. As a paracrine pathway, they could regulate the function and phenotype of surrounding cells. Zhai *et al.* (87) extracted and purified exosomes secreted by pre-osteogenic differentiational stem cells to avoid the side effect of stem cell therapeutics. These exosomes were then loaded onto titanium scaffolds. Cell and animal experiments showed that this cell-free scaffold could efficiently regenerate bone tissue, which presents a novel bone regeneration method. Lan *et al.* (88) reported a similar result further explained the mechanism that this osteogenic induction was achieved partly by activating the RhoA/ROCK signaling pathway.

The remaining studies of carried medicines involved polydopamine (89,90), stem cells, microRNA nano-particles (91), plasmid deoxyribonucleic acid (DNA) (92), and other drugs that meet the different specific design requirements (93,94).

Functionalization

Functionalization refers to the use of physical/chemical/biological surface treatment methods to obtain activating surface properties of titanium alloy, thus improving the bone induction ability and biocompatibility of the alloy without using additional coating materials (*Figure 4*). The earliest surface functionalization treatment was calcium ion implantation technology reported by Hanawa *et al.* (95) in 1993. The major mechanism of this technology involves artificial simulation of a natural body environment, thereby inducing spontaneous apatite formation. Researchers introduced calcium ions into pure titanium to form a surface-modified layer containing a variety of calcium and titanium oxides to promote osteogenesis.

Recently, different chemical treatment methods were explored. Alkali heat treatment activation modification refers to submerging the titanium scaffold into a strong

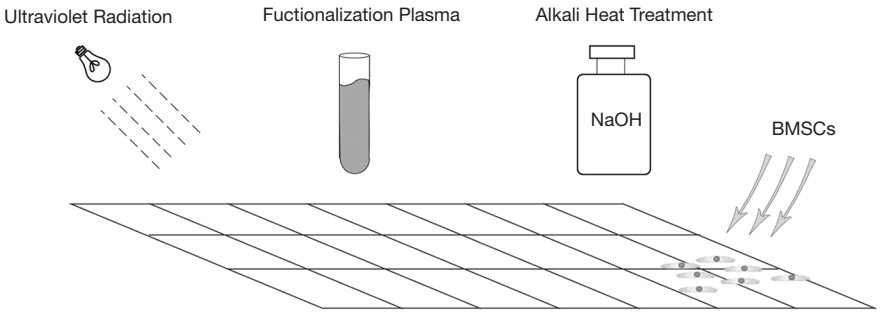

Figure 4 Functionalization. To improve the properties of titanium alloys, the researchers construct activated surfaces by physical/chemical/biological methods. It could induce apatite formation spontaneously and promote bone formation. The co-culture of exogenous stem cell could stimulate osteogenic differentiation of adjacent somatic cells by secret exosomes.

alkaline liquid for a period of time, and then treating it with high temperature to obtain an active surface, which can release alkaline components into body fluids, thus inducing natural HA deposition and an osteogenic effect (96). Cazzola *et al.* (97) used tea polyphenols, which are a kind of green tea extract, to functionalize the surface of titanium alloy to distribute hydroxyl groups on the surface, thus increasing the hydrophilicity of the titanium alloy, facilitating the deposition of HA into body fluids and promoting osteogenesis. Kwon *et al.* innovatively created amine functionalities on the surface of titanium by using a non-thermal atmospheric pressure plasma jet. This method could significantly increase cell adhesion ability and hydrophilicity.

In addition, ultraviolet light-mediated photofunctionalization, which refers to treating the titanium surface with ultraviolet light radiation in a dark room for several days, is a novel research direction of surface functionalization processing. Scientists have found that photofunctionalization can increase the hydrophilic of scaffold and make it absorb the liquid easily (98). Pham *et al.* (99) treated titanium coins with hydrofluoric acid, thereby creating a fluoride modification F and assessing cell toxicity, cell proliferation, and osteogenic induction ability. The results showed a significant improvement in cell proliferation and enhancement of the firm attachment of the implant surface to the junction epithelium with a proper concentration of fluoride. Titanium alloy screws were functionalized with ultraviolet light and tested in animal experiments by Hirota *et al.* (100). The results showed that they could effectively increase the surface hydrophilicity, reduce pollution of carbonaceous matter, and form a positive conversion electrostatic charge, which played an important role in promoting the recruitment, adhesion, and proliferation of osteoblasts. Similar photo-functional surface treatments had also been reported to promote osteogenesis (101). However, the underlying mechanism is still unknown. Chika *et al.* (98) hypothesized that this phenomenon could be associated with a significant reduction in atomic carbon on titanium microfiber surfaces. Liu *et al.* (102) provided a partial insight into this; they found that with the accumulation of ultraviolet radiant energy, biological activities were increased step-by-step. The surface physicochemical changes induced by ultraviolet radiant energy alternated the functional site exposure of extracellular matrix proteins. They also demonstrated the synergistic effects of the FAK-RhoA and ERK1/2 signaling pathways on mediating the URE-dependent cell behaviors.

Some physical treatments can also promote osteogenesis and inhibit bacterial growth. Magnetoelectric fields have been studied to promote bone tissue regeneration. Sahm *et al.* (103) reported that endogenous electric fields could alternate the direction of differentiation of stem cells. They used an alternating electric field stimulating human osteoblasts that were seeded on titanium electrodes and found that the expression of osteogenic-related proteins was significantly increased.

Moreover, the biological method was used to accelerate osteogenic procession. During the natural bone defect restoration process, stem cells (such as bone marrow mesenchymal stem cells) could differentiate into osteoblasts and osteoclasts, partly regulated by the exosome secretion of neighboring stem cells. Some researchers introduced early-staged osteogenic differentiation of stem cells into the titanium scaffold to demonstrate its osteogenic induction ability (104). Hong *et al.* (105) pretreated the surface of the

titanium alloy with platelet-rich plasma, attached platelets to the surface of rough titanium alloy, and used the growth factor generated by the platelets to promote osteogenesis. The results reported by Ma *et al.* (106) may explain this phenomenon; they treated the TiO_2 nanotubular surface with platelet-derived growth factor-BB and obtained similar osteogenic differentiation improvement results.

Summary

At present, titanium and its alloys are the most commonly used metals in bone tissue engineering. There has been considerable research on improving physical properties such as durability, corrosion resistance, elasticity, hardness, etc. These characteristics are the basis of its application. Considering the economic considerations, feasibility, and physical properties, it seems that the research has reached a bottleneck. Also, the biological activity of the titanium alloy still has significant room for improvement. Many studies have shown that there were still defects in the application of titanium and its alloys, and the mechanisms are still unknown.

At present, research into the improvement of the properties of titanium alloy bone tissue engineering has gradually transformed into research regarding the potential mechanisms. Although titanium is considered to have good bioactivity among metals, compared with other non-metallic materials, such as biological ceramics and high-molecular polymers, it seems to exhibit no advantages. Of course, although organic materials have excellent biological activity, their physical properties still need to be improved. We cannot predict which kind of material will prevail in the future. It can be argued that improving the biological properties of metals is as important as improving the physical properties of organic materials. At the same time, the material-cell interactions on both sides are likely to be intricately connected aspects of the same mechanism.

In conclusion, although the research on improving the properties of titanium alloy bone tissue engineering may have entered a bottleneck stage, further research is still needed.

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