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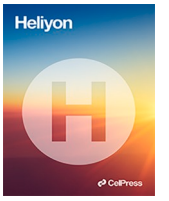

Review article

Surface-modified titanium and titanium-based alloys for improved osteogenesis: A critical review

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ABSTRACT

As implantable materials, titanium, and its alloys have garnered enormous interest from researchers for dental and orthopedic procedures. Despite their success in wide clinical applications, titanium, and its alloys fail to stimulate osteogenesis, resulting in poor bonding strength with surrounding bone tissue. Optimizing the surface topology and altered compositions of titanium and titanium-based alloys substantially promotes peri-implant bone regeneration. This review summarizes the utilization and importance of various osteogenesis components loaded onto titanium and its alloys. Further, different surface-modification methods and the release efficacy of loaded substances are emphasized. Finally, we summarize the article with prospects. We believe that further investigation studies must focus on identifying novel loading components, exploring various innovative, optimized surface-modification methods, and developing a sustained-release system on implant surfaces to improve peri-implant bone formation.

1. Introduction

With the increasingly aging population, there is a growing demand for substituting dysfunctional hard tissues with biomaterials. Titanium and its alloys, with desirable features as biocompatible materials, have been remarkably applied in repairing bone defects in various fields, including orthopedics, oral surgery, and dentistry [1–4]. Despite the excellent biocompatibility and strong corrosion resistance, titanium-based implants are bio-inert, usually resulting in poor bonding strength with surrounding bone tissue and leading to implantation failures [5–9].

The alteration of surface properties of titanium and its alloys is a promising strategy for facilitating bone healing and bone-implant

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integration. In the last decade, plenty of research focused on creating novel surfaces with exceptional components to improve osteogenesis [10,11]. Many substances with osteogenic abilities have been loaded onto titanium surfaces *via* several surface modification methods and then released to regulate reactions in the interface of implants and host tissues (Fig. 1). The loaded substances can stimulate osteoblast proliferation and improve osseointegration between the adjacent bone and the implant, including metals (e.g., zinc (Zn), magnesium (Mg), strontium (Sr), lithium (Li)) and organic substances (e.g., bone morphogenetic proteins (BMPs), microRNAs (miRNAs), statins and phytocompounds).

The surface modification techniques for loading inorganic and organic substances vary due to their different characteristics. As metals can be molten or oxidized, several techniques, such as terminal spraying, magnetron sputtering, and micro-arc oxidation, have been commonly applied [12,13]. On the other hand, organic materials are much more sensitive to temperature than metals. Hence, it is preferable to connect organic materials with implant surfaces by adhesion, electrostatic interaction, or chemical bonds [14–16]. In addition to incorporating osteogenic substances, surface modification methods can promote the physiochemical properties of implants. These methods offer osseointegration improvement and extension of the implant life expectancy in the human body. Depending on the chosen techniques, different biological functions and physicochemical properties of implants can be ameliorated by generating roughness topographies and improving wettability, wear resistance, and corrosion resistance [17,18]. Despite the excellent osteogenic activity, high concentrations of some loading substances are considered hazardous to the human body. Therefore, regulating the release of loading substances is of tremendous significance.

This review concentrates on sustained-released substances incorporated in titanium-based materials and their biological activities, especially osteogenic properties. Moreover, surface-modification methods applied in incorporating sustained-released components are also summarized, along with their influence on the physicochemical and biological properties of titanium-based implants. The article also discussed the release kinetics of substances and strategies of release regulation to provide a reference for subsequent investigations.

2. Osteogenic components loaded onto titanium and its alloys

Modifying titanium surfaces with osteogenic components is a promising strategy to improve the biological activity of an implant. Several recent studies have focused on incorporating novel components onto titanium surfaces, including metals and organic substances. In this part, the article mainly focuses on osteogenic components loaded onto titanium and its alloys. Notably, multiple factors that influence the success of implantation and other properties correlated with bone formation are mentioned, including anti-inflammation, angiogenesis, and anti-bacteria. After the implantation of biomaterials, there must be immune responses at the host-implant interface. Immune cells would secrete inflammatory cytokines, such as interleukin 1 (IL-1) and tumor necrosis factor α (TNFα), which have been proven to promote the proliferation of osteoclasts and bone resorption, leading to implantation failure. Infection-related inflammatory reactions also prevent osteogenesis. Accordingly, inflammatory and bacterial growth inhibitions exert an advantageous effect on bone formation. Besides, more blood vessels forming around pro-angiogenic implants contribute to a better bone metabolic microenvironment by providing oxygen and nutrients [19]. Therefore, besides focusing on the osteogenic property of loaded substances, other biological properties of the osteogenic components are also summarized.

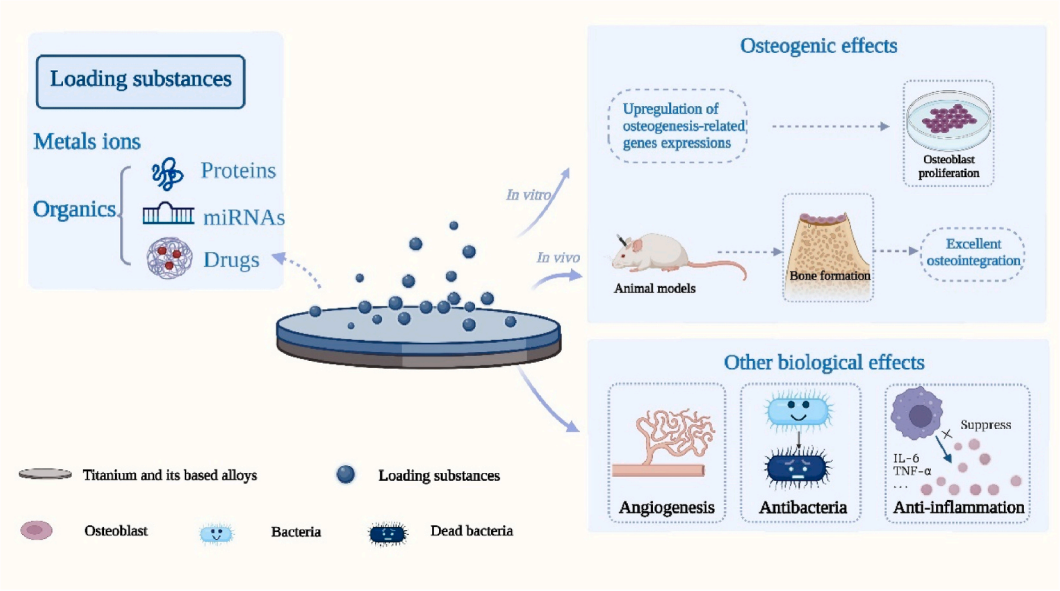

Fig. 1. Substances incorporated in titanium and its alloys and the biological effects they exert.

Table 1
Overview of metals doped on titanium and its alloy, surface modification strategies, and outcomes.

Doping elements	Strategies	Substrates	Release time	Species of cells/animal models	Biological characteristics		Ref.
					Osteogenic activities	Anti-bacteria, angiogenesis, anti-inflammation	
Zn	MAO	Ti	7 d	MC3T3-E1 cells,	Enhanced calcification of MC3T3-E1 cells	Antibacterial activity against *E. coli*	[20]
		Ti	60 d	hFOB1.19 osteoblasts, RAW264.7 cells	Improved proliferation, adhesion, and mitochondrial activity of hFOB1.19 osteoblasts and RAW264.7 macrophages	Inhibited proliferation, growth and adhesion of *S. aureus* and *E. coli*	[21]
				Rats	Facilitated bone formation in rats	Alleviated bacterial infection in rats	
	PIII	Ti	28 d	rBMSCs	Improved adhesion, spreading, proliferation, vitality, ALP activity, and ECM mineralization of rBMSCs	Inhibition of *E. coli* and *S. aureus*	[22]
				Rats	Enhanced bone formation in rats	Reduced bacterial infection in rats	
		Ti	Not available	MC3T3-E1 cells, rMSCs	Increased proliferation of MC3T3-E1 cells; Enhanced initial adhesion, spreading activity, ALP activity, collagen secretion and extracellular matrix mineralization of rMSCs	Inhibition of *E. coli* and *S. aureus*	[23]
	FSP	TNTZ	14 d	BMSCs	Promoted adhesion, proliferation, ALP activity and ECM mineralization of BMSCs	Good bactericidal ability; Inhibited colonization of *S. aureus*	[24]
	Sol-gel	Ti	28 d	MSCs, RAW264.7 cells	Increased adhesion, proliferation, ALP activity, mineralization of MSCs; Inhibited differentiation of RAW264.7 cells	Decreased colony formation and viability of *S. aureus* and *P. aeruginosa*	[25]
				Rabbits	Enhanced new bone formation, interfacial strength and bone maturation in rabbits	–	
	MAO and sol-gel	Ti	Not available	BMSCs	Facilitated proliferation and ALP activity of BMSCs	Reduced adhesion and colonization of *Pg*	[26]
Mg	Electron beam melting	Ti6Al4V	Not available	MC3T3-E1 cells, HUVECs	Enhanced proliferation, viability, adhesion, ALP activity and ECM mineralization of MC3T3-E1 cells	Enhanced proliferation, viability, adhesion, migration, and angiogenesis of HUVECs	[27]
				Rabbits	Accelerated bone formation in rabbits	Increased blood vessel formation in rabbits	
	Mechanical alloying and spark plasma sintering	Ti-xMg alloy	28 d	SaOS-2 cells	Enhanced cell adhesion, proliferation and differentiation in Ti-0.312 Mg, Ti-0.625 Mg and Ti-1.25 Mg	–	[28]
				Rats	Increased BMD and BV/TV around Ti-0.625 Mg alloys	Decreased inflammatory cells proliferation around Ti-0.625 Mg alloys	
	Anodization and hydrothermal treatment	Ti	28 d	rBMSCs, RAW 264.7 cells	Enhanced proliferation, attachment and ALP activity of rBMSCs	RAW 264.7 cells tended to express M2 marker (CD206) in the MgN group	[29]
				Wistar rats	Increased existence of pre-osteoblasts and small vessels; Improved new bone formation	The MgN group tissue showed the largest area of CD206- positive sites	
	PIII	Ti	12 d	rBMSCs	Improved proliferation, spreading and proliferation of rBMSCs	–	[30]
		Ti	Not available	rBMSCs, HUVECs	Enhanced initial adhesion, spreading, osteogenic differentiation ALP activity and OCN protein expression of rBMSCs	Facilitated viability of HUVECs and expression of HIF-1α induced by Mg^{2+}; Inhibition of oral anaerobes induced by Zn^{2+}	[31]
				Rabbits	Increased new bone formation, mineralization and BIC	Enhanced blood vessel growth	

(*continued on next page*)

Table 1 (*continued*)

Doping elements	Strategies	Substrates	Release time	Species of cells/animal models	Biological characteristics		Ref.
					Osteogenic activities	Anti-bacteria, angiogenesis, anti-inflammation	
Sr	Hydrothermal reactions	Ti	21 d	Rabbits	Improved bone formation and bonding strength between bone and implant in rabbits	–	[32]
		Ti	7 d	BMSCs	Enhanced spreading, attachment, proliferation and ALP activity of BMSCs	–	[33]
Li	Plasma electrolytic oxidation	Ti	7 d	rBMSCs	Increased viability, adhesion, proliferation, ALP activity and ECM mineralization of rBMSCs	Enhanced VEGF expression	[34]
				Rats	–	Improved bone formation in rats	
	Electrochemical deposition	Ti	Not available	BMSCs	Promoted adhesion and proliferation of BMSCs	–	[35]
				Dogs	Enhanced bone regeneration and bone-to-implant contact in dogs		
	sandblasting acid etching	Ti	Not available	BMSCs	Improved spreading, attachment and ALP activity of BMSCs	–	[36]
Ag	Anodizing, hydrothermal synthesis, LbL assembly	Ti	14 d	MC3T3-E1 cells	Promoted adhesion and ALP activity of MC3T3-E1 cells	Inhibition of *E. coli* and *S. aureus*	[37]
				Rabbits	Excellent osteoinductive activity	–	
	Magnetron sputtering	Ti	30 h	–	–	Antibacterial activity against *S. aureus*	[38]
	MAO	Ti	120 d	MC3T3-E1 cells	Enhanced cell adhesion, spreading, proliferation and ALP activity when Ag was not excessive	Inhibited growth of *S. aureus*	[39]
Mn	MAO	Ti	28 d	MC3T3-E1 cells	Promoted adhesion, proliferation, differentiation, ALP level and ECM mineralization of MC3T3-E1 cells	Inhibited growth of *E. coli*	[40]
				Rabbits	Enhanced bone formation, density and early osseointegration	–	
Ta	Anodization	Ti6Al4V	Not available	hMSCs	Promoted spreading, extension, differentiation and mineralization of hMSCs	–	[41]
	Sol-gel method	Ti6Al4V	24 d	BMSCs	Improved adhesion, proliferation, ALP expression and mineralization of BMSCs	–	[42]
		Ti	24 d	BMSCs	Enhanced adhesion, proliferation, ALP expression and calcium deposition of BMSCs	–	[43]
	Plasma spraying	Ti	Not available	BMSCs	Facilitated adhesion, proliferation, ALP activity, ECM mineralization and osteogenic differentiation of BMSCs	–	[44]
Co	MAO	Ti	28 d	MSCs	Enhanced adhesion, proliferation and spreading of MSCs	Increased expression of angiogenic factors (VEGF and HIF-1α)	[45]
Cu	Selective laser melting	Ti	Not available	RAW 264.7 cells	Inhibited osteogenic differentiation of RAW 264.7 cells	–	[46]

Abbreviations: Ti, titanium; MAO, micro-arc oxidation; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *Pg, Porphyromonas gingivalis; P. aeruginosa, Pseudomonas aeruginosa;* PIII, plasma-immersion ion implantation; ALP, alkaline phosphatase; ECM, extracellular matrix; rBMSCs, rat bone-marrow mesenchymal stem cells; rMSCs, rat mesenchymal stem cells; OCN, osteocalcin; MSCs, mesenchymal stem cells; TRAP, tartrate-resistant acid phosphatase; BMSCs, bone-marrow mesenchymal stem cells; Ti6Al4V, TC4 titanium alloy; HUVECs, human umbilical vein endothelial cells; VEGF, vascular endothelial growth factor; LbL, layer-by-layer. BMD, bone mineral density; BV/TV, bone volume/tissue volume; HIF-1α, hypoxia-inducable factor-1α; BIC, bone-implant contact.

2.1. Inorganic metal ions

Several studies have focused on loading different metals onto titanium surfaces in anticipation of improving the osteogenic effect of implants, reducing osteolytic activity in surrounding bone tissue, or improving the peri-implant immune microenvironment. Such studies have aimed at creating a relatively stable and anabolic microenvironment, which would be more conducive to bone healing and enhance the stability of the implant-bone combination (Table 1).

2.1.1. Zinc

Zinc (Zn) is an essential trace element in the human body, mainly bound to enzymes and proteins as a structural component [47]. Bone tissue contains ~30 % of the total body burden of Zn, which has a critical role in bone metabolism [48,49]. Studies have shown that an influx of zinc ions (Zn^{2+}) to cells mediated by Zrt/Irt-like protein-14 can increase the basal level of cyclic adenosine monophosphate, thus promoting endochondral ossification and production of growth hormone [50,51]. At low concentrations (1 and 10 μM), Zn promoted the mineralization and differentiation of SaOS-2 human osteoblast-like cells [52]. However, compared to the control group, a decrease in ALP activity could be found after 4 d at the concentration of 25 μM. The 50 μM-group showed statistical decreases in both ALP activity after 8 d ($p < 0.001$) and the number of bone nodules after 9 d ($p < 0.001$). These results indicated that a high concentration of Zn (above 25 μM) would inhibit osteogenesis.

Researchers have implanted Zn onto titanium alloys by MAO and PIII [20,22,23]. Incorporation of Zn into titanium could promote bone formation by upregulating the gene expression of runt-related transcription factor-2 (Runx2) and OCN [23]. In the Zn1.0 group (implantation time of Zn ion was 1.0 h), the result showed that there were statistical improvements in ALP activity ($p < 0.001$) and matrix mineralization ($p < 0.05$) compared to acid-etched titanium after being cultured for 14 d. In addition to osteogenic ability, Zn implanted into titanium exerted more potent antibacterial activity against *E. coli* than acid-etched titanium, with a reduction percentage of 31.49 % in the Zn2.0 group.

2.1.2. Magnesium

Approximately 50 % of magnesium (Mg) in the human body is stored in bones. Mg deficiency is associated with impaired growth of bone, low bone mass, and osteoporosis [53]. The additional supply of magnesium ions (Mg^{2+}) around alloys has enhanced bone regeneration [54,55]. Besides stimulating bone formation, Mg^{2+} also exerts anti-inflammatory, antibacterial, and angiogenesis activities [53,56,27].

Due to multiple biological functions, Mg has been incorporated into titanium and its alloys to enhance their bioactivities and functions. Liang et al. fabricated Ti-xMg alloys and found that the appropriate content of Mg (0.312–0.625 wt%) could facilitate the adhesion, proliferation, and differentiation of SaOS-2 cells [28]. However, the high concentration of Mg^{2+} released from alloys with 2.5 wt% Mg was detrimental to cells. Therefore, controlling the incorporation amount of Mg or the release rate of Mg ions is vital. In addition to osteogenic properties, researchers found that Mg^{2+} could exert anti-inflammatory effects and alleviate host immune responses, further promoting osteogenesis [29]. Compared with control groups (TiO_2 nanotubes and blank Ti groups), the MgN coating on the surface of TiO_2 nanotubes downregulated the inflammation-related genes expression (*TNF-α, IL-6, IL-1β*) while significantly upregulated anti-inflammatory genes (*IL-1ra* and *IL-10*). They also found that the ALP activity of rBMSCs was promoted in macrophage-generated conditioned media on 7 d ($p < 0.05$), along with other osteogenesis-related genes (*ALP, RUNX2, OCN, COL1*). The result indicated that the Mg ions released could facilitate macrophage polarization toward the M2 phenotype.

Besides the ability of osteogenesis and anti-inflammatory, another study demonstrated that magnesium deposited on a Ti6Al4V scaffold could upregulate the expression of hypoxia-inducible factor (HIF)-1α and VEGF in HUVECs [55], thereby improving the angiogenesis ability of Ti6Al4V. Yu and colleagues explored the mechanisms of enhanced cell adhesion, osteogenesis, and angiogenesis of Zn/Mg co-incorporated titanium. They postulated that incorporating Zn and Mg could improve the hydrophilicity of titanium surfaces and upregulate the gene expression of integrin-α1 and integrin-β1 in rBMSCs. The concentration of Zn^{2+} and Mg^{2+} in rBMSCs was increased by enhancement of the influx of Zn^{2+} and Mg^{2+} and suppression of Zn^{2+} outflow, which resulted in upregulation of expression of Runx2, ALP, and OCN at the transcriptional level. Also, Mg^{2+} could activate HIF-1α and increase VEGF expression in HUVECs, thereby inducing angiogenesis [31].

2.1.3. Strontium

Strontium (Sr) is active in the *in vivo* regulation of bone metabolism. It accelerates bone formation by promoting osteoblast proliferation while suppressing the activity of osteoclasts involved in bone resorption [57,58]. Sr exerts osteogenic activity by regulating several signaling pathways, such as receptor activator of nuclear factor-kappa B (RANK)/RANKL/osteoprotegerin (OPG) pathway and wingless-type (Wnt) pathway [59]. However, high concentrations of strontium ions (Sr^{2+}) can lead to osteoporosis, so controlling Sr^{2+} release from the titanium surface is crucial [60]. Through a hydrothermal reaction, strontium can be doped onto titanium plates and form $SrTiO_3$ layers with a nanoscale surface architecture. In a case, the authors observed that appropriate amounts of Sr^{2+} from the titanium surface could be released into physiological solutions within 7 d. With the sustained release of Sr^{2+}, the Sr-doped surface displayed higher ALP activities ($p < 0.05$) compared to the surface without Sr after 7 d of culture. Moreover, the expression of critical integrins and genes with an osteoblastic phenotype are upregulated significantly in Sr-incorporated surface [33]. In addition to *in vitro* tests, several reports demonstrated the insertion of Sr-modified implants into osteoporosis-induced rabbits to explore the implants' effects on early bone osseointegration [32]. The percentage of bone-implant contact (BIC%) was much higher in Sr-doped groups than in control groups, both in cancellous bone (31.6 ± 7.4 vs. 19.9 ± 5.5) and cortical bone (80.2 ± 6.6 vs. 70.4 ± 4.6) 3 weeks after implantation. In addition to osteogenic properties, it has been proved that an appropriate dose of Sr ions (0.2–1 mM) could promote angiogenesis by inducing the secretion of VEFG and angiogenin-1 of HUVECs.

2.1.4. Lithium

Lithium (Li) is a non-essential micronutrient in the human body. It has been used for decades as a medication to treat bipolar disorder. Recently, researchers found that Li also displayed other biological effects, enhancing cartilage regeneration and inhibiting osteoclastogenesis [61,62]. To improve the osteogenesis of titanium implants, Qiu et al. doped Li onto the implant surface by MAO [34]. In addition to the significant enhancement in ALP activity, quantitative results showed that the formation of mineralized nodules

Table 2
Overview of organics doped on titanium and its alloys, surface modification strategies, and outcome.

Doping organics elements	Coatings	Substrates	Release time	Species of cells/animal models	Biological characteristics		Ref.
					Osteogenic activities	Anti-bacteria, angiogenesis, anti-inflammation	
BMP-2	BMP-2/(Heparin/Chitosan)$_9$/TiO_2 nanotubes	Ti	28 d	rBMCs	Improved ALP activity, calcium deposition, counts, attachment and spreading of rBMCs	–	[64]
	BMP-2/Heparin	Ti	28 d	MG-63 cells	Improved proliferation, ALP activity and calcium deposition of MG-63 cells		[65]
				Dogs	Increased bone formation and bone remodeling in dogs		
	BMP-2/PDA	Ti	Not available	PDLSCs	Intensified ALP activity, calcium deposition and adhesion of PDLSCs	–	[66]
BMP-7	(Adenovirus-BMP-7)/(avidin–collagen gel)	Ti	Not available	Osteoblasts	Enhanced ALP activity of osteoblasts	–	[67]
	BMP-7/fibronectin/poly (ethyl acrylate)	Ti	14 d	hMSCs	Improved flexibility, mineralization and differentiation of hMSCs using lower BMP-7 amount	–	[68]
miR-21	miR-21 nanocapsules/Ocarboxymethyl chitosan	Ti	100 h	MSCs, BMMCs	Promoted proliferation and osteogenic differentiation of MSCs; Accelerated BMMCs activity	Promoted angiogenic differentiation of MSCs	[69]
				Rabbits	Improved bone-to-implant contact, bone remodeling, bone maturation, bone mineralization and bone-implant bonding strength in rabbits	–	
Other miRs	(Chitosan-antimiR-138)/HA/TiO_2 nanotubes	Ti	14 d	MSCs	Facilitated attachment, ECM mineralization, ALP production and collagen secretion of MSCs	–	[70]
				SD rats	Enhanced bone regeneration, bone-to-implant contact in rats		
	(miR-335–5p/Lipidoid Nanoparticles	Ti	Not available	BMSCs	Improved viability, proliferation and ALP activity of BMSCs	–	[71]
Simvastatin	Simvastatin/TiO_2 nanotubes	Ti	14 d	Wistar rats.	Accelerated bone formation and mineralization; Enhanced BIC and bone maturation in rats	–	[72]
	Simvastatin/HAp/β-CD	Ti	28 d	MC3T3-E1 cells	Increased proliferation, ALP activity and calcium deposition of MC3T3-E1 cells	–	[73]
				Rabbits	Facilitated bone formation and bone-implant bonding strength in rabbits		
Lovastatin	Lovastatin/β-CD/PDA	Ti	30 d	MC3T3-E1 cells	Improved proliferation, ALP activity and calcium deposition of MC3T3-E1 cells	–	[74]
				Rabbits	Enhanced bone formation, bone-implant bonding strength and bone remodeling in rabbits		
Pitavastatin	Pitavastatin/β-CD/chitosan/gelatin	Ti	21 d	MSCs, HUVECs	Accelerated osteogenic differentiation; Improved spreading, migration, ALP activity, collagen secretion and ECM mineralization of MSCs	Increased proliferation, migration, and tube formation ability of HUVECs	[75]

(*continued on next page*)

Table 2 (*continued*)

Doping organics elements	Coatings	Substrates	Release time	Species of cells/animal models	Biological characteristics		Ref.
					Osteogenic activities	Anti-bacteria, angiogenesis, anti-inflammation	
				SDrats	Promoted MSCs recruitment surrounding implants, bone formation, BIC and bone maturity in rats		
Icariin	PLGA/icariin/TiO_2 nanotubes	Ti	14 d	MC3T3-E1 cells	Enhanced proliferation, and differentiation of MC3T3-E1 cells	–	[76, 77]
				SDrats	Accelerated early osseointegration in rats		
Quercitrin	Quercitrin/ aminosilanized surface	Ti	Not available	hMSCs, RAW264.7 cells	Accelerated adhesion, improved metabolic viability and mineralization of hMSCs; Decreased expression of functional osteoclastic markers	–	[78, 79]
				Rabbits	Decreased expression of Rank and osteoclastic related markers		
	Quercitrin/ aminosilanized surface	Ti6Al4V	Not available	MC3T3-E1 cells	Facilitated ALP activity and mineralization of MC3T3-E1 cells	Decreased survival and adhesion of *S. epidermidis*	[80]
Naringin	(Chitosan/gelatin/ Naringin)$_6$(Chitosan/ gelatin)$_2$	Ti	10 d	ME3T3-E1 cells, RAW 264.7 cells	Improved attachment, ALP activity, mineralization and differentiation of ME3T3-E1 cells; Inhibited formation and generation of RAW 264.7 cells	–	[81]
	Chitosan/Naringin/TiO_2 nanotubes	Ti	10 d	Osteoblasts	Increased spreading, proliferation, ALP activity and mineralization of osteoblasts	–	[82]
EGCG	EGCG/Mg^{2+}	Ti	Not available	hADSCs, RAW 264.7 cells	Enhanced osteogenic differentiation, ALP activity calcium deposition of hADSCs; Decreased TRAP activity, generation of RAW 264.7 cells	–	[83]
				Rabbits	Improved bone-implant integration and bonding strength of rabbits		
Puerarin	Puerarin/calcium phosphate	Ti	Not available	MC3T3-E1 cells	Facilitated proliferation, ALP activity and type I collagen synthesis (dose-dependently) of MC3T3-E1 cells	–	[84]
Proanthocyanidin	Proanthocyanidin/ chitosan/hyaluronic acid/Polyethyleneimine	Ti	14 d	MC3T3-E1 cells	Increased ALP activity, and proliferation; Inhibited P53-induced apoptosis of MC3T3-E1 cells	–	[85]
				SD rats	Enhanced bone formation and osseointegration of rats		
Melatonin	(Chitosan/gelatin)$_8$/ Melatonin/TiO_2 nanotubes	Ti	7 d	MSCs	Promoted spreading, adhesion, proliferation, ALP activity and mineralization of MSCs	–	[86]
Andrographolide	Andrographolide/TiO_2 nanotubes	Ti	72 h	MBCs	Facilitated adhesion and proliferation of MBCs	Inhibited adhesion and biofilm formation of *S. epidermidis* and *S. aureus*	[87]
FHBP	FHBP/PDA	Ti	Not available	MG63 cells	Enhanced ALP activity, oxidative metabolism and	–	[88]

(*continued on next page*)

Table 2 (*continued*)

Doping organics elements	Coatings	Substrates	Release time	Species of cells/animal models	Biological characteristics		Ref.
					Osteogenic activities	Anti-bacteria, angiogenesis, anti-inflammation	
					maturation response of MG63 cells		
SAL	SAL/PDA	Ti	30 d	MC3T3-E1 cells, HUVECs Rats	Increased proliferation, mineralization and ALP content of MC3T3-E1 cells Decreased inflammatory response, bone formation and maturation in rats	Promoted migration and tube-forming capacity of HUVECs Improved blood vessel generation in rats	[89]
Indomethacin.	Chitosan or PLGA/ indomethacin- loaded TiO_2 nanotubes	Ti	9 d (chitosan thin), 30 d (chitosan thick), 19 d (PLGA thin), 31 d (PLGA thick)	HOS cells	Enhanced adhesion, spreading and proliferation of HOS cells	–	[90]
Vancomycin	PDA/Hya/vancomycin-loaded TiO_2 nanotubes	Ti6Al4V	7 d	rBMSCs	Promoted adhesion, ALP activity, ECM mineralization of rBMSCs	Inhibition of *S. aureus*	[91]
				SD rats	Increased BV/TV and bone density in rats	No infection signs in rats	

Abbreviations: PMAA, poly(methacrylic acid sodium salt); PH, poly-L-histidine hydrochloride; PDLSCs, periodontal ligament stem cells; hMSCs, human mesenchymal stem cells; BMMCs, bone marrow mononuclear cells; HA, hyaluronate; HAp, hydroxyapatite; β-CD, β-cyclodextrin; PDA, polydopamine; PLGA, polylactic acid-glycolic acid copolymer; *S. epidermidis*, *Staphylococcus epidermidis*; EGCG, Epigallocatechin-3-gallate; hADSCs, human adipose-derived stem cells; FHBP, (3 S)1-fluoro-3-hydroxy-4-(oleoyloxy) butyl-1-phosphonate or $C_{22}H_{41}FNaO_6P$; HOS, human osteosarcoma; Hya, hyaluronic acid.

in Li-doped titanium was 1.2-fold greater than in the titanium and MAO groups. Moreover, the expression level of OCN and COL I was 1.8 and 6.2 times higher than in the titanium group. Several reports explored the mechanism of the enhanced osteogenesis elicited by Li-doped titanium. They found that it might be achieved by inhibiting the activity of β-catenin phosphorylation and increasing the phosphorylation of glycogen synthase kinase-3 [36]. In addition, sustained release of Li from titanium surfaces could stimulate bone formation *in vivo* [35]. According to micro-CT analysis, BIC and the values of bone volume/total volume of the Li-modified group were 0.831 ± 0.025 and 0.374 ± 0.015, respectively, were much higher than the values of the pure titanium group (0.700 ± 0.023 and 0.302 ± 0.009).

2.1.5. Other metals

Other metals, such as manganese (Mn) [40], tantalum (Ta) [41,42], and cobalt (Co) [45], can also be loaded onto the surface of titanium or its alloys *via* various methods. Such metal-modified surfaces promote cell adhesion, osteoblastogenesis, and bone regeneration primarily by altering the surface structure of the material (formation of a nano-porous microstructure) or upregulating the expression of osteogenesis-related genes. Copper [63] and argentum [38,37] have also been loaded onto titanium alloys due to their excellent antibacterial properties. Through selective laser melting in three-dimensional printing, Xu and colleagues doped copper into Ti6Al4V to form a Ti6Al4V–6Cu alloy, which also exerted good biocompatibility and biosafety. Although the Ti6Al4V–6Cu alloy could not promote osteogenesis effectively, it inhibited the differentiation of monocytes (RAW264.7 cells) to osteoclasts, as well as inhibiting the gene expression of proinflammatory factors such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α [46]. Researchers concluded that the incorporation of copper reduced the activity of osteoclasts around the implant and resulted in a milder inflammatory microenvironment, facilitating tissue healing and bone regeneration.

2.2. Organic substances

In addition to incorporating inorganic metal, organics, including bioactive molecules and medicines, have been doped on titanium and its alloys (Table 2).

2.2.1. Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-beta superfamily and have essential roles in osteogenesis because they regulate BMSC differentiation [92]. Among various BMPs, BMP-2 and BMP-7 derivatives are the most effective in inducing bone morphogenesis [93]. However, several studies have shown no significant osteogenic effect after the release of BMP-2 or BMP-7 from carriers. This phenomenon may be caused by the early "burst" release of BMPs from carriers or an insufficient dose of BMP release in long-term delivery [92,94–96]. Therefore, constructing slow-release systems is essential for BMPs to exert their

biological effects. Recent studies indicated that sustained release of BMP-2 could be achieved by preparing polyelectrolyte multilayers on titanium surfaces [64]. In an instance, BMP-2 was entrapped within the polyelectrolyte coating and could diffuse from thin polyelectrolyte layers, improving pre-osteoblast proliferation and differentiation *in vitro*. In another study, BMP-2 was immobilized on heparinized titanium substrates with a release period of 28 d. Compared with pure titanium, an increase in calcium deposition ($p < 0.001$) and expressions of OCN ($p < 0.05$) as well as osteopontin (OPN) ($p < 0.001$) were discovered on BMP-immobilized heparinized titanium after 21 d of culture [65]. Meanwhile, an *in vivo* study demonstrated that the immobilization of BMP enhanced the formation of vertical bone and the binding between bone and the implant. Similar studies found that BMP-7 immobilized on titanium surfaces also exerted similar biological effects [67,68].

2.2.2. Micro RNAs

miRNAs can regulate gene expression at the post-transcriptional level and have an essential role in homeostasis and bone development [97,98]. However, miRNAs are unstable and readily degraded by RNA enzymes in the environment. Hence, in most cases, miRNAs must be encapsulated to carriers to improve their stability. As one of the most studied miRNAs, miR-21 takes part in the immune response, osteogenic differentiation, and bone regeneration and is used as a marker for diagnosing and treating cancer [99]. Geng and collaborators modified a micro-rough titanium surface with miR-21 nanocapsules embedded within micropores, which led to the sustained release of miR-21 [69]. The confocal images and MTT assay results indicated that miR-21 released from the titanium surface facilitated the adhesion, spreading, and proliferation of mesenchymal stem cells (MSCs). They promoted ALP activity after 3 d ($p < 0.05$), 7 d ($p < 0.05$), and 14 d ($p < 0.01$) of culture. Titanium modified by miR-21 also accelerated bone remodeling by upregulating the expression of osteoclast-(*RANKL*) and osteogenesis-related (*Col-1, Runx2, OCN, and OPN*) proteins. Besides, the angiogenic activity of MSCs was enhanced in miR-21-incorporated titanium groups compared with that in the pure-titanium and acid-treated titanium groups. miR-21-incorporated titanium increased both capillary tube length ($p < 0.01$) and the number of branch points per field ($p < 0.01$). *In vivo* studies demonstrated that miR-21-coated titanium could promote osseointegration. The BIC% of the miR-21 group was 25.1 ± 2.3 %, which was much higher than acid-treated titanium (15 ± 1.6 %) after 1 month of implantation in rabbits. In addition to the ability of osteogenesis and angiogenesis, miR-21 could inhibit the pro-inflammatory cytokine produced by macrophages [100].

Other miRNAs have been reported to exert a regulatory effect on osteogenesis. miR-138 was discovered to be a negative modulator of osteogenic differentiation. Based on electrostatic interactions between chitosan and *anti*-miR-138, Wu et al. fabricated polyelectrolyte multilayers of sodium hyaluronate and chitosan–miR complexes on a titanium surface [70]. miR-138 expression of MSCs on the functionalized titanium surface was downregulated by ~70 % compared with that in samples of pure titanium, thereby promoting bone regeneration *via* upregulation of expression of BMP-2, OCN, collagen 1 (Col1), ALP, osterix (OSX), and Runx2. An *in vivo* study also demonstrated that slow-released antimiR-138 nanoparticles could augment osseointegration. miR-335–5p (another regulator involved in bone remodeling) was shown to stimulate osteogenic differentiation and bone regeneration by suppressing the expression of Dickkopf-1 (the antagonist in the Wnt pathway) [101,102]. The osteogenic capacity of titanium modified with miR-335–5p/lipid nanoparticles was increased by upregulation of expression of the osteogenesis-related genes *ALP*, *Col1*, bone sialoprotein (*BSP*), and *OCN* [71].

2.2.3. Statins

Loading bioactive drugs onto titanium and its alloys to improve the biological properties of implants has garnered considerable attention in recent years. Among lipid-lowering drugs, statins are the first-line treatment option. They are used mainly for plaque stabilization and lipid modulation in patients with coronary artery disease, myocardial infarction, or for people who have suffered a stroke. However, studies indicated that statins could regulate bone metabolism [103]. In addition to promoting osteoblastogenesis and inhibiting osteoclastogenesis by modulating multiple signaling pathways [104], proper doses of statins facilitate bone regeneration indirectly *via* their anti-inflammation and angiogenesis properties [105,106]. However, high local concentrations of statins can trigger inflammation, inhibiting osteogenesis [107].

Nyan et al. loaded simvastatin on micro-arc-oxidized titanium and found that simvastatin-modified implants displayed higher bone-implant contact values ($p < 0.05$) and peri-implant bone volume ($p < 0.05$) than micro-arc oxidized and control groups after 2 weeks [72]. Kwon and colleagues modified the titanium surface with 1,1′-carbonyl diimidazole and β-CD as an intermediate coating to deliver simvastatin. Inclusion complexation formed between β-CD and simvastatin, thereby controlling the release of the drug [108]. Sustained-release simvastatin could upregulate OSX, Runx2, Col1, and BSP expressions at the transcriptional level in MC3T3-E1 cells, promoting osteogenic differentiation.

Several studies have also involved the application of similar intermediate coatings to load pitavastatin or lovastatin onto titanium surfaces [74,75]. Multilayer films were fabricated onto titanium surfaces by LbL assembly of gelatin and chitosan grafted with pitavastatin-loaded β-CD. Released pitavastatin could promote the osteogenic efficacy of MSCs and the angiogenic efficacy of endothelial cells *via* the regulation of paracrine signaling. Moreover, *in vivo* experiments demonstrated that pitavastatin released locally improved peri-implant osseointegration.

2.2.4. Flavonoids and other polyphenolics compounds

In addition to the synthetic drugs mentioned above, some phytocompounds extracted from medicinal plants have been incorporated onto titanium surfaces. Flavonoids are the best-studied compounds among plant polyphenolics because they can be acquired readily and have excellent biocompatibility [109]. Flavonoids can promote bone regeneration and treat osteoporosis by inhibiting osteoclasts [110,111]. The flavonoids incorporated onto the titanium surface include icariin, quercetin, and naringin. Icariin is an

important bioactive component isolated from horny goat weed, which is inexpensive and stable [112]. It has been proven that icariin could protect against cardiovascular diseases and increase chondrocyte vitality by suppressing inflammation [73]. Wang et al. loaded icariin on TiO_2 nanotubes and covered the surface with PLGA layers to promote the sustained release of icariin [76]. Osteoblasts implanted on icariin-doped surfaces displayed enhanced adhesion and proliferation ($p < 0.05$) of MC3T3-E1 osteoblast cells compared to the pristine titanium at each time point of detection [77]. An *in vivo* experiment confirmed that titanium with a coating of PLGA/icariin/TiO_2 nanotubes exhibited higher bone formation area percentage (36.63 % ± 0.8 %) than pure titanium (14.6 % ± 1.0 %) and titanium modified with TiO_2 nanotubes (17.4 % ± 1.0 %).

Quercitrin is a flavonoid monomer compound present in many types of plants. It has anti-oxidation, anti-inflammation, and antibacterial properties and promotes wound healing [113]. Previous reports indicated that the concentration of quercitrin should be controlled lower than 22.4 μg/mL to avoid cytotoxicity [114]. To slow down the drug release, Quercitrin was grafted onto an amino-silanized titanium surface, and this functionalized titanium exerted excellent biocompatibility [78]. Several studies have demonstrated that quercetin released from a titanium surface could promote osteoblastogenesis *via* upregulating the activity of ALP and OCN, inhibit osteoclast activities and bone resorption by downregulating the expression of osteoclast-related genes *in vivo* (*H + ATPase*, *matrix metalloproteinase 9*, cathepsin K (*Ctsk*)) and *in vitro* (*Calcitonin Receptor*, *Ctsk*, *H + ATPase*, *TRAP*, *MMP9*), as well as suppress the adhesion and viability of *S. mutans* and prevent peri-implant infection [79,80].

Similar to quercitin, naringin is a type of polyethoxylated flavonoid extracted from citrus fruits. Naringin with appropriate concentrations has been proven to exert anti-inflammatory effects on macrophages and inhibit osteoclastogenesis [115]. However, the drug exhibited cytotoxicity at a concentration of over 100 μM [116]. Shen et al. developed a slow-release system in which naringin was loaded onto micro-structured titanium *via* an LbL assembly [82]. Results indicated that naringin could upregulate the expression of Runx2, Col I, ALP, OCN, OPN, and OPG, thereby promoting osteoblast proliferation. Besides, naringin inhibited osteoclast formation by decreasing the activation of CTSK, nuclear factor of activated T cells (NFAT), TRAP, and *Vacuolar ATPases* [82,81].

EGCG is a type of polyphenol found in green tea. It has been shown to display osteogenic properties by regulating the Wnt/β-catenin signaling pathway [117,118]. Studies indicated that a stable metal–polyphenol network could form between polyphenols and metal ions through cation–π interaction, π–π stacking of gallol groups and catechol, and hydrogen bonding. Based on these interactions, Lee et al. fabricated an Mg^{2+}–EGCG coating on the surface of Ti6Al4V [83]. Besides inhibiting osteoclast formation, EGCG and Mg^{2+} possessed synergistic roles in promoting osteogenesis. The *in vivo* experiment also suggested that titanium alloys with an Mg^{2+}–EGCG coating stimulated osseointegration. The BIC% of the Mg^{2+}–EGCG group (8.1 ± 4.3) was significantly higher than uncoated implants (4.4 ± 2.0). In addition, other polyphenols, such as puerarin [84], proanthocyanidin [85], and melatonin [86], have been loaded onto titanium surfaces and displayed excellent efficacy for bone regeneration.

3. Surface modification techniques for improved osteogenesis

The alteration of the surface properties of titanium and its alloys, including surface topography and composition, is regarded as a method for facilitating bone healing and bone-implant integration. This chapter mainly discusses methods for modifying surface morphology, as well as methods for loading organic and inorganic components.

3.1. Methods for modifying surface morphology

Typically, titanium implants with rough topographies function better in cell adhesion, osseointegration, and implant success compared to implants with smooth surfaces [119,120]. Grit-blasting, various etching techniques, and anodization have been applied to enhance roughness on implant surfaces on the micro-scale, nanoscale, or micro/nano-hierarchical scale. Micro-structures and nano-structures have advantages and limitations. Although microstructural surfaces could stimulate osteoblasts differentiation, an apparent decrease in cell population would be found on micron-roughed surfaces [121,122]. In addition, nano-roughed titanium surfaces are beneficial to cell adhesion and proliferation, whereas their low roughness results in insufficient mechanical locking with bone tissues [123]. Hence, recent studies focused on preparing titanium and its alloys with micro/nano-scale hierarchical topography in anticipation of better biological performance [124,125]. This section discusses conventional techniques for roughening the implant surface and novel methods for constructing micro/nano-scale hierarchical topography.

3.1.1. Grit-blasting and etching procedures

Grit blasting can modify the roughness of titanium and its alloys through the projection of abrasive particles with various sizes (110–250 μm), such as silicon oxide, titanium oxide, and aluminum oxide [126,127]. Reportedly, adjusting the particle sizes and the spraying rate could change the topography and roughness of titanium implant surfaces, thus affecting cellular behaviors [128]. Compared with smooth surfaces and surfaces with small grits (100 μm in size), sandblasted large grits (300 μm in size) on Ti6Al4V further increased MC3T3-E1 cell attachment and osteogenesis-related gene expressions.

Another method for roughening the implant surface is through etching procedures, such as acid etching, alkali etching, hydrothermal etching, and electrochemical etching. Among them, acid etching is one of the most applied techniques to produce micro-roughness on the implant surface, which has been proven to facilitate cell attachment and growth [129,130]. Unlike the grit-blast technique, acid etching creates lower surface roughness than the surface prepared with grit blasting [131]. Some reports demonstrated that, after combining with sandblast treatment, large grits with acid etching (called SLA) could result in an improved surface topography with macro roughness (5–20 μm in diameter) and micro pits (0.5–3 μm in diameter) [132,133]. Moreover, the acid etching could remove surface contamination caused by grit-blasting and further enhance surface roughness [131,134]. The SLA-treated surface

showed the maximum number of MG63 cells (2798.89 ± 310.21/cm^2) than grit-blasted groups (2398.22 ± 346.32/cm^2) and acid-etched groups (1255.66 ± 185.45) after culturing for 1 h [131]. The result indicated that the increased surface roughness could improve cell proliferation.

However, the microscale structures fabricated by sandblasting, acid etching, and SLA, are usually irregular, playing a negative role in the further improvement of biological activities [135–139]. Contrarily, electrochemical etching can precisely construct the surface with uniform and ordered topography by adjusting electrolyte conditions, etching time, and voltage. Liang et al. fabricated etched titanium plates in an electrolyte composed of HF and NaCl [140]. The SEM images showed homogeneous bowl-liked microholes formed on a titanium surface.

3.1.2. Combined utilization of techniques to construct micro/nano-scale hierarchical topography

In the last decade, micro/nano-scale hierarchical structures have been constructed on titanium and its alloys, aiming to integrate the superiorities of micro and nano topography. In general, electrochemical-etched surfaces with uniform topography at the microscale can serve as a platform for constructing micro/nano-scale surface structures. Accordingly, nano-structures are fabricated through anodization or alkali-heat treatment. Zhang et al. constructed micro-submicron-nano hierarchical structures on titanium surfaces by combining electrochemical etching, chemical etching, and anodization. Micro-pits, micro-pores, and nanotubes were formed sequentially (Fig. 2) [141]. The multi-level structured titanium displayed super-hydrophilicity on the surface, resulting in a significantly higher cell adhesion and proliferation than pristine titanium. Eventually, the Wnt/β-catenin pathway could be activated, inducing osteoclast differentiation.

To further improve the biological performance of multilevel structured titanium, inorganic and organic components could be incorporated. In a case, a Sr-substituted HAp layer was coated on titanium with a novel kind of micro/nano-scale bionic topography [142]. Through electrochemical etching followed by anodizing and ultrasonic treatment, the surface displayed pit structures both on the microscale and nanoscale, which were similar to the structure of snail operculum, thus exerting strong bond strength with tissues. The nano/micro roughed titanium doped with Sr and HAp could further enhance osteoblast adhesion, proliferation, and mineralization, as well as suppressing osteoclast differentiation. In addition to metals, miR-21 was doped on nano/micro roughed surfaces to promote osteogenesis and angiogenesis [8].

3.2. Methods for loading inorganic components

In this section, we discuss several applied surface modification methods for loading inorganic components, their processes, physicochemical properties, and pros and cons, including plasma spraying, PIII, magnetron sputtering, friction stir processing, anodic oxidation, MAO, and sol-gel technique (Fig. 3).

3.2.1. Plasma spraying

Plasma spraying is a kind of thermal spraying technology, in which powdered coating materials are accelerated, molten by a plasma jet, and deposited onto the implant surface to form layers of coating [148,149] (Fig. 4). Plasma spraying contains vacuum plasma spraying (VPS), atmospheric plasma spraying (APS), and controlled atmospheric plasma spraying (CAPS) [150]. Among them, conventional atmospheric plasma spraying (APS), an economical and safe method, is the most employed method for producing hydroxyapatite (Hap) for clinical application. HAp, similar to the inorganic component of the human bone and hard tissue, is widely used as a coating material for bone repair to promote bone regeneration [151]. In the APS process, molten HAp particles impact the substrate surface at high velocity, deform and cool promptly, then solidify and overlap in splat forms [152]. The microstructural and mechanical characteristics of HAp coatings are directly influenced by feedstock properties and plasma spray parameters [153–155].

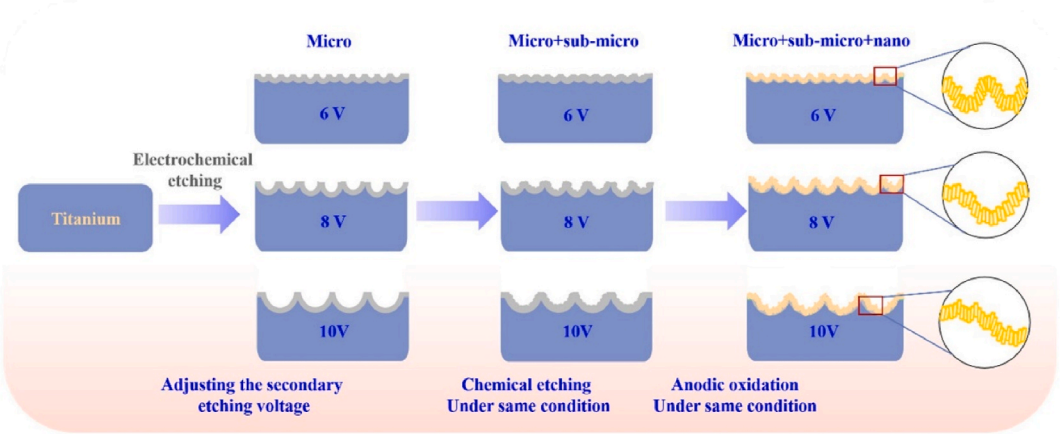

Fig. 2. Schematic diagram of the fabrication of micro–submicron–nano hierarchical structures on titanium surfaces. Reproduced from Ref. [141] with permission.

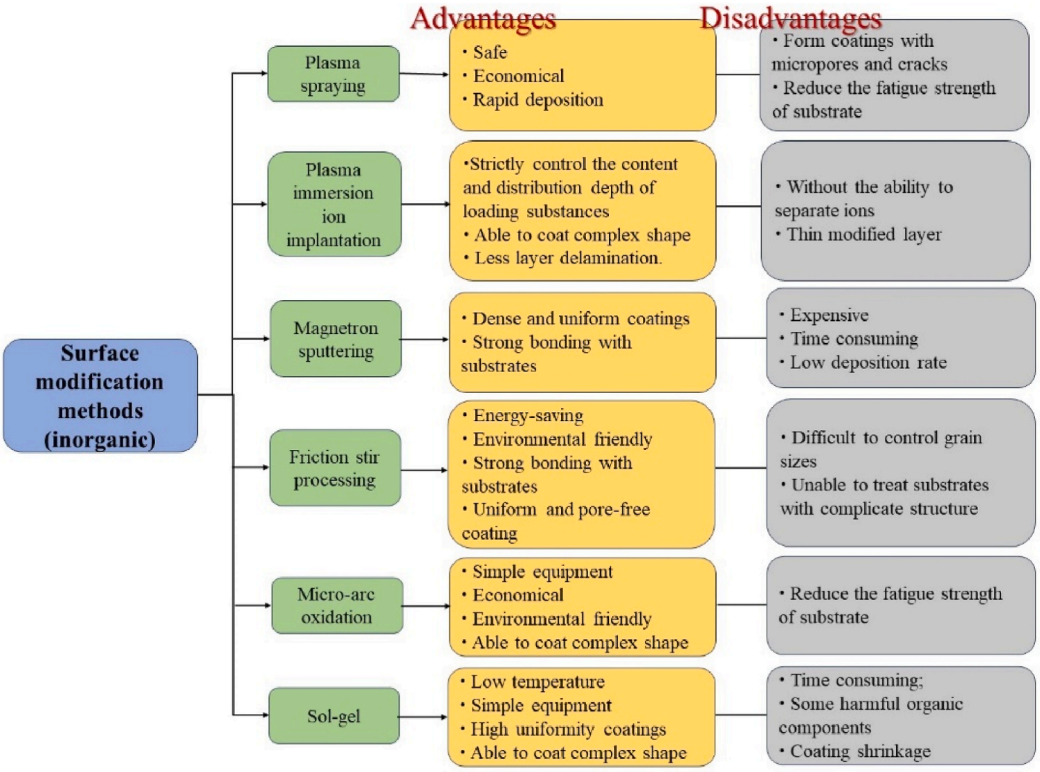

Fig. 3. Surface modification methods for loading inorganic materials, as well as their advantages and disadvantages [143–146,147].

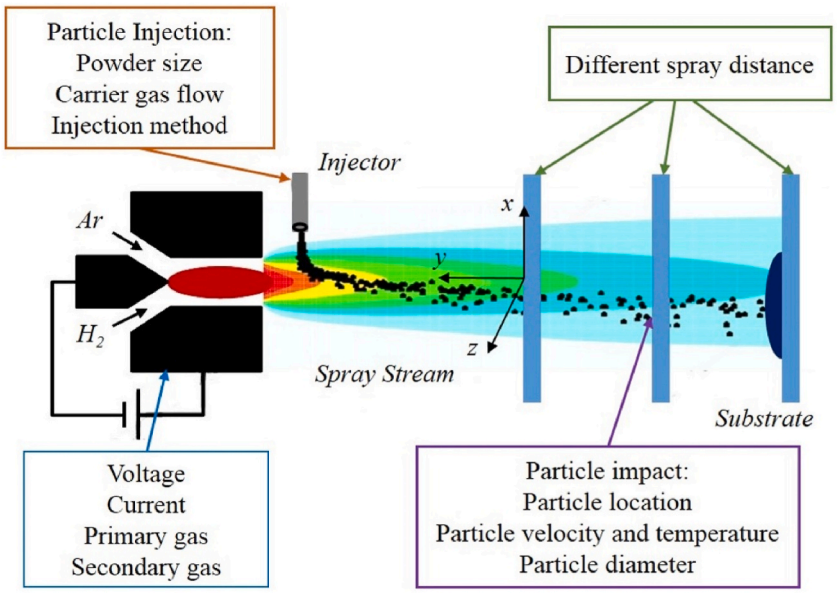

Fig. 4. Schematic illustration and related parameters of plasma spraying. Reproduced from Ref. [148] with permission.

Kweh et al. demonstrated that, with the increase in spay distances (10, 12, and 14 cm) and particle sizes (20–45 μm, 45–75 μm, and 75–125 μm), the mechanical characteristics and structure stability of HAp coatings were weakened, including modulus, hardness, and bond strength. The influences of heat temperatures (ranging from 600 °C to 900 °C) on the mechanical characteristics of coatings were also investigated. Compared to heat treatment of 600 °C, 800 °C-treated HAp coatings showed higher microhardness and elastic modulus, whereas no discernible improvement of mechanical properties was found in the 900 °C-treated group. The higher the

temperature in heat treatment, the more cracks on the surface and the lower crystallinity of Hap were evident, resulting in the deterioration of mechanical properties over 900 °C [153,156]. Therefore, the process with smaller particle sizes, as well as suitable spraying distance and temperature, could fabricate coatings with excellent mechanical properties.

Apart from optimizing the mechanical properties of the plasma-sprayed HAp coating, other research focused on improving its biological performance. Lu et al. incorporated Ta into HA coating, resulting in the enhanced adhesion, proliferation, ALP activity, as well as calcium deposition of rBMSCs, compared to other groups (uncoated Ti, Ti with HA coating, and Ta coating implants) [157]. Some other researchers focused on preparing novel coatings as an alternative to HAp coatings. The CaO–MgO–SiO_2-based bioactive glass-ceramic coating (named M2) was deposited on Ti6Al4V alloy, effectively facilitating osteoblast activities *in vitro* and enhancing osteogenesis and vascularization *in vivo*. M2 coating induced bone-like carbonated apatite formation, possessing stronger bonding strength to the substrate than commercial HA coating, which might help repair load-bearing bone defects [158–160].

Although APS exerts high deposition rates and short coating time, which is superior to other surface modification techniques available in the market, the process still has some drawbacks. The defects, such as porosity and microcracks, always appeared in plasma-sprayed coatings, resulting in the degradation of implant materials. In addition, the crystallinity of incorporated materials and bond strength between coatings and substrates still need to be improved. Several efforts have been dedicated to overcoming those shortcomings, including reinforcing the second layer of materials and optimizing techniques. To further increase the corrosion resistance of titanium alloys (Ti6Al4V), researchers deposited graphene nanoplatelets on HAp coatings to fill gaps between HAp splats, thus reducing porosity and microcracks [161]. The result showed that adding 2 wt% graphene nanoplatelets in HA coatings could decrease the porosity from 15 % to 10 % and reduce the corrosion rate by 87 % in stimulated body fluid (SBF). Another research applied axial suspension plasma spraying (SPS) in the HAp coatings fabrication [152]. This novel technique utilized nanometric powder suspension to prepare coatings, exhibiting a better ability to control the spraying temperature and velocity of flying particles. Compared to APS coating, coatings fabricated by SPS showed 9.5 times corrosion resistance and 1.3 times adhesion strength.

3.2.2. Plasma immersion ion implantation

Unlike plasma spraying, PIII is carried out in a low-pressure system, where substrates are immersed in plasma, and accelerated ions from the plasma are injected into the substrate by applying a glow discharge [30]. One of the advantages of PIII is that it can strictly control the amount of loading components and the depth of distribution by adjusting implantation parameters [22,147]. In addition, the technique enables various elements to be embedded in the surface of substrates in different shapes, enhancing their mechanical and biological properties [162,163]. Carbon plasma immersion ion implantation (C-PIII) and nitrogen plasma immersion ion implantation (N-PIII) have been widely utilized in fabricating coatings. Through PIII, the implanted carbon and nitrogen ions can improve corrosion resistance, wear resistance, and surface roughness without significantly altering the hydrophilicity [164]. Previous reports demonstrated that incorporating nitrogen into micro-arc oxidized titanium *via* PIII could improve the antibacterial ability against *E. coli* and *S. aureus* [165]. In addition to *C*-PIII and *N*-PIII, oxygen plasma immersion ion implantation (O-PIII) treatment with a high oxygen ions dose (4×10^{17} ions/cm^2) could promote the osteogenic activity of titanium both *in vitro* and *in vivo* [163].

In addition, some metallic substrates can be implanted on the surfaces of titanium and its alloys to better improve biological effects. In an instance, Li et al. fabricated Cu/Zn-titanium nitride (TiN) coating on Ti6Al4V alloy *via* PIII, indicating that hydrophilicity, corrosion resistance, and protein adsorption ability were enhanced [165]. Considering the biological properties, Cu/Zn–TiN coatings promoted cytocompatibility and exerted stronger antibacterial activity against *E. coli* than the TiN group. In another study, C and Cu were co-implanted on titanium surfaces, resulting in an improvement in mechanical performance, corrosion resistance, and antibacterial properties [166].

Compared to other surface modification techniques, PIII has some unique advantages: (i) unlike plasma spraying, PIII overcomes the line-of-sight limitation, which is suitable for modifying a complex-shaped substrate; (ii) there is no distinct interface between the modified layer and the substrate; (iii) being carried out in a vacuum system, PIII is an eco-friendly surface modification technique. However, without the ability to separate ions, all the positive ions in plasma will be injected into the substrate surface, which is the main drawback of PIII.

3.2.3. Physical vapor deposition magnetron sputtering

Physical vapor deposition techniques can be categorized into two groups of the magnetron sputtering process and the evaporation technique [167]. Magnetron sputtering is an extensively studied method and has been explored for commercial application. During the magnetron sputtering process, the charged ions generated by inert gases are accelerated by an electric field to strike on target material surface, causing the target atoms to fly out and condense onto the substrate as a thin film, thus building a thin nano-layer that attaches firmly to the substrate [168,169]. The properties of the targeted coating can be controlled during magnetron sputtering by adjusting the atmosphere, bias voltage, discharge power, substrate temperature, deposition time, and post-heat treatment. By changing the nitrogen partial pressure of the working atmosphere in the magnetron sputtering system, Nemati et al. fabricated coatings with different N/Ti ratios (0.4–1.2) on the Ti6Al4V surface [170]. Compared to Ti6Al4V and other groups, the group with equal proportions of N and Ti showed the maximum hardness value (28 GPa), high Young's modulus (about 460 GPa), the best corrosion protection performance in SBF solution, with protection efficiency of 99.4 %, and the highest biocompatibility. However, Ti_xN_y thin films show the decreased wettability of titanium alloys, limiting their applicability due to reduced protein adsorption and cell adhesion [171]. These may be solved by adding hydrophilic coatings, such as PDA coating [172]. In addition to nitrogen, metals can be incorporated into titanium and its alloys *via* magnetron sputtering. To enhance the activity of osteoblasts on the implant surface, Shi et al. combined acid etching and magnetron sputtering to load tantalum onto acid-etched titanium [41]. The microstructure of acid-etched titanium and the sustained release of tantalum could synergistically affect bone formation.

Similar to plasma spraying, magnetron sputtering has also been applied in the fabrication of HAp coating and ion-substituted HAp coating to improve biocompatibility and osteogenesis [173–175]. Lenis et al. incorporated SiO_2/TiN/Ti as an intermediate layer between a Ti6Al4V alloy and a HAp-Ag coating. The intermediate coating further promoted bond strength between metal substrates and HAp coatings. Antibacterial properties and biocompatibility were improved by the fabrication of the HA-Ag/SiO_2/TiN/Ti coating. Although both plasma spraying and magnetron sputtering have been commonly applied in preparing HAp coatings, there are differences between the layers made by the two techniques. The coating thicknesses fabricated by plasma spraying and magnetron sputtering are about 75–150 μm and 20–2000 nm, respectively [167,176]. In contrast with plasma spraying, magnetron sputtering can control the coating nature precisely and prepare denser films that bond better to the substrate, thereby avoiding loading failure due to delamination and cracks of the coating [177]. However, magnetron sputtering is a relatively expensive, time-consuming method with a low deposition rate.

In summary, magnetron sputtering has been broadly used in surface modification and developing novel layers owing to high bond strength and the dense and uniform coating it can prepare. However, the uneven distribution of the magnetic field under normal operating conditions leads to an unstable rate of generated charged particles, which results in additional consumption of materials when the particles bombard the target.

3.2.4. Friction stir processing

The friction stir processing (FSP) technique, having attracted broad attention in the past two decades, is commonly applied in regulating material microstructure and enhancing mechanical behavior [178]. With the tool pin inserted into the material during processing, the rotating tool mechanically stirs the substrate and moves along the path (Fig. 5) [178,179]. Under the stirring heat and intense plastic deformation, grains are refined and homogenized, thus improving the microstructure of materials and mechanical properties [180,181].

Currently, many studies have applied FSP in modifying titanium alloys to improve mechanical and biological properties [44,181, 182]. A novel kind of Ti–35Nb–2Ta–3Zr (TNTZ) alloy processed by FSP exerted high strength and suitable elastic modulus similar to bone tissue [182]. The improvement mechanisms are the addition of Nb and Zr, affecting the elasticity, and the plastic deformation of the material under optimal rolling temperature (900 °C) and deformation ratio (35 %). In addition, TNTZ presented similar biocompatibility as Ti6Al4V implants *in vitro*, and the finite element analysis indicated that compared with Ti6Al4V, TNTZ implants improved loading transduction and bone remodeling. In addition to the fabrication of novel titanium alloys, FSP can modify material by introducing secondary-phase particles. Moreover, there is no interfacial reaction between new coatings and the substrate [24,182, 183]. Fang et al. incorporated Zn nanoparticles homogeneously onto the surface of TNTZ by FSP [183]. Grain refinement caused by plastic deformation and high temperature during processing led to an obvious increase in microhardness. Zn ions could be released slowly because of the solid metallurgical bonding between the substrate and Zn nanoparticles. Besides improving mechanical properties, TNTZ/Zn could promote the osteogenic differentiation of BMSCs and antibacterial properties against *S. aureus* compared to TNTZ.

FSP provides an energy-saving, green, and reliable approach for fabricating micro/nano-composites. Compared with other surface modification techniques, the secondary-phase layers prepared by FSP can bond stronger with substrates. Besides, surface corrosion can be inhibited due to the uniform, fine, and pore-free wrought structure [181]. Despite all these advantages, several problems remain unsolved. First, it is difficult to control grain sizes subtly since the size of grains depends on various factors, including processing parameters, cooling time, and basic properties of the material, which change continually during the process. Second, FSP finds it hard to treat curve surfaces or materials with complex structures, which limits its application in commercial processing. In conclusion, FSP is a promising surface modification technique, while further development of the technique and a better understanding of mechanisms is desired and expected.

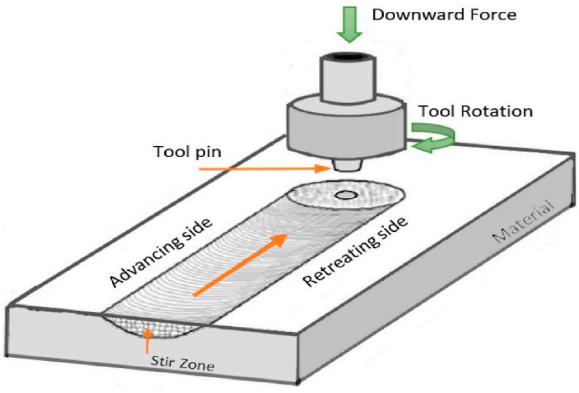

Fig. 5. The schematic diagram of the FSP process. Reproduced from Ref. [179] with permission.

3.2.5. Anodic oxidation and micro-arc oxidation

Anodic oxidation is an effective anodic electrochemical technique to fabricate nanoporous surfaces. Under an externally applied electric field, metal atoms are oxidized and combined with oxygen anions from the electrolyte, thus forming an oxide coating on titanium surfaces. The high temperature and pressure generated by anodic oxidation cause oxidation of the titanium surface to form TiO_2 nanotubes [184,185], which can also be applied as an intermediate layer to connect titanium and organic materials (as described in detail below).

MAO was developed from anodic oxidation technology and allows the formation of a coating superior to that obtained by simple anodic oxidation. By regulating electrolyte compositions and electrical parameters, MAO can fabricate a coating comprising titanium oxides and targeted electrolyte components under an instantaneous high pressure and high temperature created by arc discharge. After MAO treatment, the hardness of titanium increases significantly, resulting in a rough and porous structure of the titanium surfaces. These surfaces show enhanced hydrophilicity and corrosion resistance, aiding the adhesion and proliferation of osteoblasts [39]. Utilizing MAO, Zhang et al. fabricated Sr/Ag-containing TiO_2 coating with nano-structures on titanium. Results indicated that the Sr/Ag incorporated TiO_2 coating facilitated the adhesion, attachment, and proliferation of MC3T3-E1 cells. However, excessive Ag content (1.29 wt%) exerted toxic effects on preosteoblasts [186].

Besides metal incorporation, MAO can also be used to prepare micro-porous TiO_2 layers on titanium surfaces when there are no extra metal ions on the electrolyte. The TiO_2 film produced by MAO can enhance the hydrophilicity of the implant surface and promote cell adhesion due to the porous, rough surfaces produced [39,187,21]. Besides, calcium and phosphorus present in the electrolyte can be incorporated into the coating and promote osteogenesis by increasing the activity of ALP [188,26]. Due to its excellent bioactivity, TiO2 films can be used as an intermediate layer to assist in loading inorganic materials. Li et al. combined MAO and a sol-gel method to prepare a thin hyaluronic acid layer on top of the TiO_2 coating [39]. The proliferation and ALP activity of osteoblasts on the sol–gel-treated MAO-Ti was enhanced significantly compared with that of pure titanium and MAO-Ti. Overall, MAO uses relatively simple equipment with high production efficiency, which can fabricate a rough and porous surface and improve the hydrophilic and bone-promotion activity of the material. However, if using MAO, attention should be paid to controlling the voltage. When implants were anodized under 300 V, the adhesive strength between the film and the substrate could not meet the clinical requirement. Increasing the voltage up to 550 V can contribute to a thicker layer with reduced mechanical strength, and the bond between the coating and substrate is also weakened [189]. Therefore, the voltage should be regulated within an optimal range.

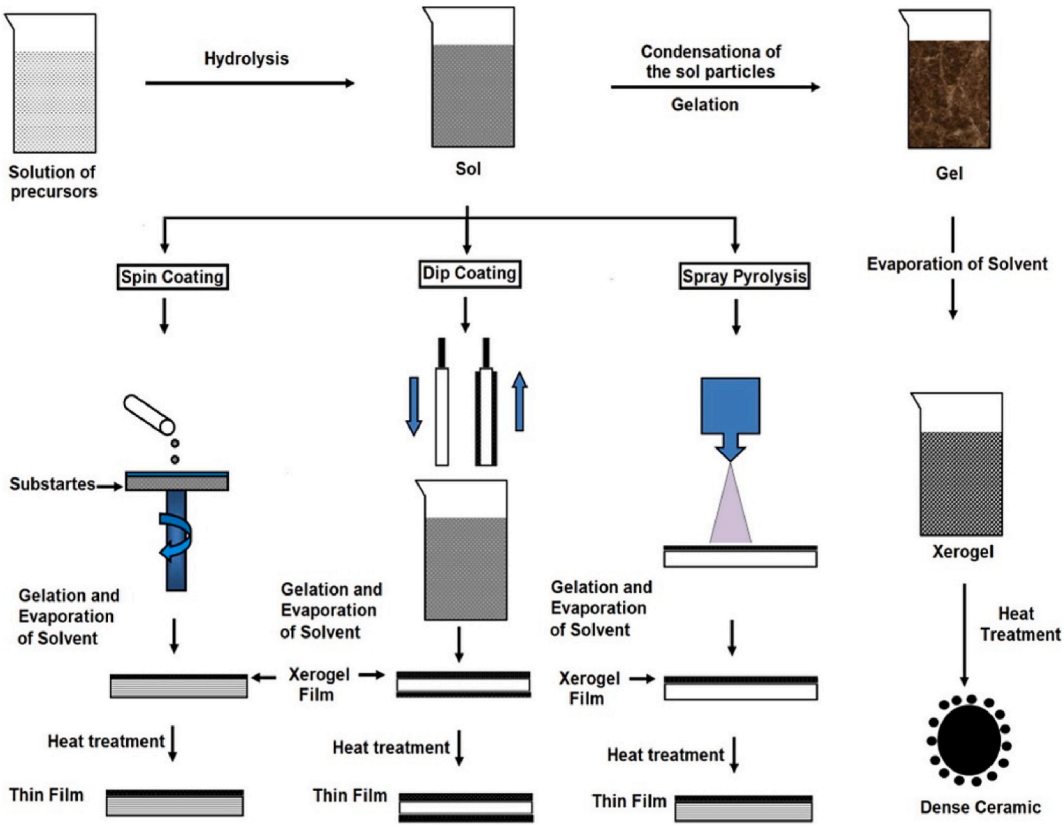

Fig. 6. The preparation process of sol-gel approach. Reproduced from Ref. [191] with permission.

3.2.6. Sol-gel

The sol–gel method is a common method for preparing various functional oxide films. It can be employed to build coatings on titanium and its alloys in a relatively gentle manner [190,191]. Often, researchers modify titanium surfaces by acid etching or electrolytic oxidation to obtain a rough titanium surface before applying the sol–gel method, thus increasing the binding force between titanium and sol–gel layers [192–194]. The target components are initially dissolved in organic solvents to form a sol, which then adheres to the material surface by dipping, spinning, or spray-pyrolysis process. After cooling and evaporation, the sol on the material is transformed into a gel. Finally, the material undergoes low-temperature heat treatment to prepare the target coating [191,192,43] (Fig. 6). It has been shown that the application of the sol–gel technology can improve the physicochemical properties of titanium. Calcium-phosphorous coatings prepared by the sol–gel method have strong interfacial shear strength and bond well to the interface [192]. Another study used sol–gel technology to load tantalum onto acid-etched titanium surfaces and found that titanium's corrosion resistance and water wettability were improved, along with the enhanced adhesion and proliferation of rBMSCs [25]. The coating fabricated by sol-gel also exerts excellent biological characteristics. Shen and colleagues employed the sol–gel method to generate Zn-incorporated titanium. They found that the sol–gel coating was bound to titanium surfaces without delamination. In addition, the Zn-containing layer promoted osteoblastogenesis at the transcriptional level, inhibiting bacteria adhesion and osteoclast differentiation *in vitro*, and promoting osseointegration *in vivo* [195]. Compared to other surface modification techniques, the sol–gel method, with its simple fabrication process and low requirements for equipment, can modify complex-shaped substrates and prepare films with high uniformity. This method avoids the drawbacks of high temperature or high pressure, such as cracks appearing during heat treatment [21,190,195]. However, the adhesion strength of coatings fabricated by the sol-gel technique is relatively low [165,167]. To solve this problem, Zhang et al. found that the bonding strength between the coating and substrate could be significantly improved by the addition of fluoride ions, resulting in stronger chemical bonds in the interface [196]. Besides, the biosafety of the organic compounds remaining in the organic solvent warrants further investigation.

3.3. Methods for loading organic components

Organic materials are much more sensitive to temperature than inorganic materials because they are prone to denaturation at a high temperature or pressure. Thus, the surface-modification methods mentioned above may denature organic materials and impair their biological properties. Therefore, organic materials must be bound to implant surfaces at a moderate temperature by mechanical embedding, adhesion, electrostatic interaction, or chemical bonds, which ensures the structural integrity of the organic materials. Methods for loading organic components and their pros and cons are summarized in Fig. 7.

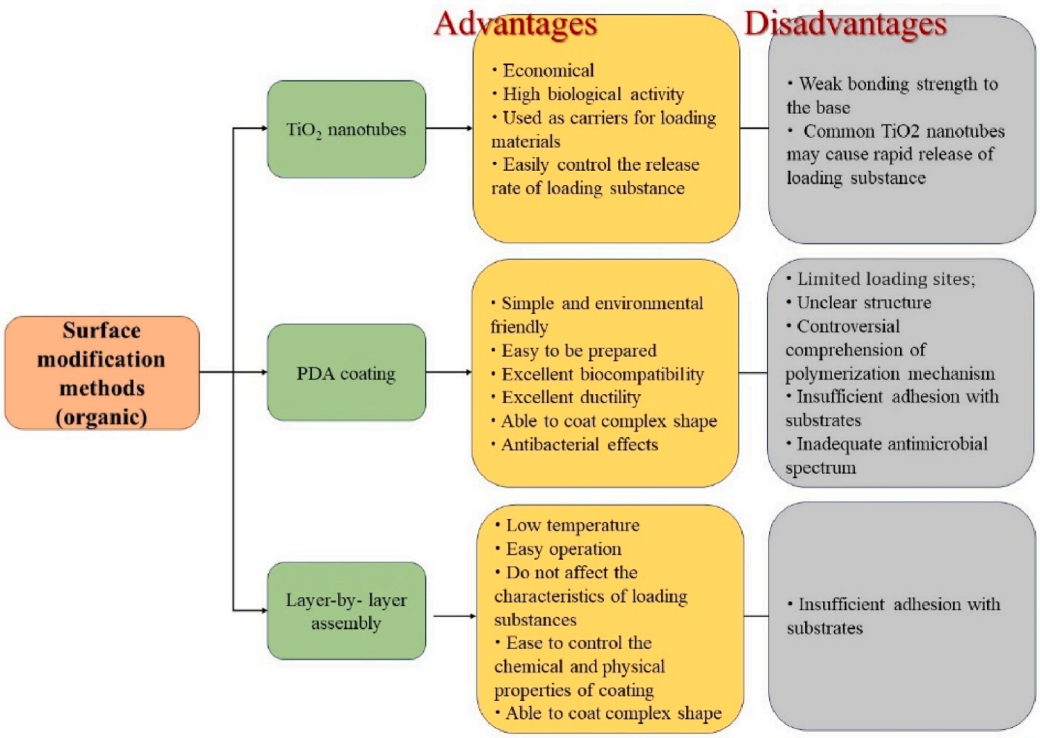

Fig. 7. Surface modification methods for loading organic materials, their advantages and disadvantages [145,197–202].

3.3.1. TiO_2 nanotubes

Organic materials can be loaded into titanium by embedding them within TiO_2 nanotubes. As mentioned above, anodic oxidation can be employed to prepare TiO_2 nanotubes. First, titanium (or its alloy) is immersed in a fluoride-containing electrolyte and oxidized at the anode. Then, the titanium-oxide layer is formed on the metal surface (Fig. 8) . However, the titanium-oxide layer (nanotubes) prepared this way lacks a stable morphology. It can be annealed at 400–600 °C to transform from an amorphous structure to a crystalline structure to maintain its tubular morphology [203,204]. Annealing significantly impacts the physicochemical properties of TiO_2 nanotubes. It can lead to a stronger connection between the TiO_2-nanotube coating and titanium substrate, as well as enhancement of the corrosion resistance and hydrophilicity of the nanotube coating [204,205]. In addition, the crystalline-phase structure of nanotubes is dependent upon the annealing temperature. Annealing at 450 °C results in the formation of an anatase crystalline phase in amorphous nanotubes, whereas increasing the temperature to 600 °C results in the partial transformation of anatase to rutile. The highest cellular activity on TiO_2 nanotubes is observed if the crystalline phase structure is anatase–rutile mixture [203].

In addition, the geometry of TiO_2 nanotubes is also affected by changes in anodization parameters and the electrolyte solution. For example, the diameter and length of nanotubes can be increased by increasing the applied voltage, prolonging the duration of anodization, or regulating the fluoride concentration in the electrolyte. Besides, applying an electrolyte with a low pH contributes to the elongation of nanotubes. There is also a relationship between the geometry of nanotubes and their biological properties. An increase in the diameter of a nanotube within a specific range can facilitate the proliferation of human osteosarcoma cells. Park et al. [206] demonstrated that 15 nm was the optimal pore size to enhance the adhesion, proliferation, migration, and differentiation of MSCs. The cellular activities were significantly improved when the diameter was between 15 nm and 30 nm. However, tubes with a diameter above 50 nm would increase cell apoptosis. On the contrary, a study found that larger nanotubes within a specific range (80 nm, 100 nm, 120 nm) could also facilitate the proliferation of human osteosarcoma cells [204]. Xie et al. pointed out that 70 nm-sized nanotubes were the most appropriate for promoting osteogenesis compared to others (30, 50, 100, 120 nm) [207]. Due to the inconsistency, more investigations are needed on this subject [203,87,208–210].

A tube-like microstructure aids the accommodation of various organic agents (e.g., drugs) at a low temperature (Fig. 9). By controlling the voltage during anodization, Feng et al. prepared TiO_2 nanotubes of a length of 200 nm and diameters of 50 nm and 100 nm, respectively. Subsequently, 2 mg of andrographolide was loaded into these TiO_2 nanotubes [87]. The andrographolide released from nanotubes could inhibit bacterial adhesion and biofilm formation. Andrographolide-loaded TiO_2 nanotubes and unloaded TiO_2 nanotubes could promote the adhesion, proliferation, migration, and differentiation of rBMSCs compared with smooth titanium. TiO_2 nanotubes of diameter 100 nm promoted more excellent adhesion and proliferation of cells than TiO_2 nanotubes of diameter 50 nm. However, organic material was loaded in TiO_2 nanotubes with a weak binding force, resulting in a rapid release. One strategy to solve this problem is increasing the diameter and length of nanotubes by adjusting the parameters in fabricating processes. The longer length and the larger diameter of nanotubes, the more organics can be incorporated, and then the release time is extended [211,212]. Besides, to develop a sustained-release system, polymers such as chitosan, PLGA, and PDA were introduced upon the oxidized layer to cover or "wrap" the organic material [76,90,91]. Ma et al. fabricated a PLGA coating on the surface of icariin-loaded TiO_2 nanotubes. They demonstrated that TiO_2 nanotubes and icariin could synergistically promote the adhesion and proliferation of osteoblasts, as well as improve the combination between titanium and bone [77]. PLGA degradation within 2 weeks resulted in a slow release of icariin from nanotubes.

The stability of drug release within TiO_2 nanotubes is also related to the bonding strength of nanotubes. "Micro-movements" can occur between nanotubes and titanium substrates during implantation, leading to the layer's early "peeling" and detachment. Therefore, intensifying the bonding strength between the nanotube layer and substrate is essential [213]. Secondary oxidation is a way to increase the bonding strength. By applying secondary oxidation, Luo et al. optimized the structure of TiO_2 nanotubes and improved the bonding strength and corrosion resistance between the nanotube coating and substrate significantly [202].

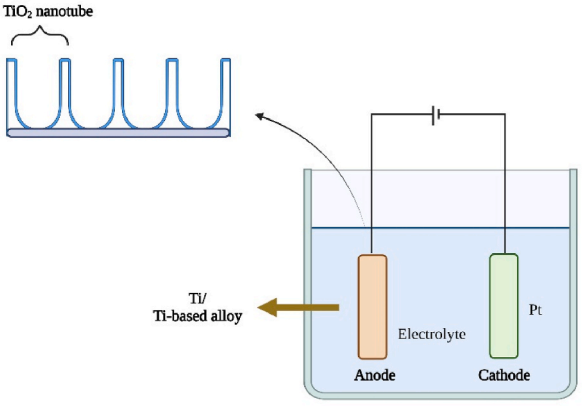

Fig. 8. Experimental apparatus for anodization and the TiO_2 nanotubes fabrication.

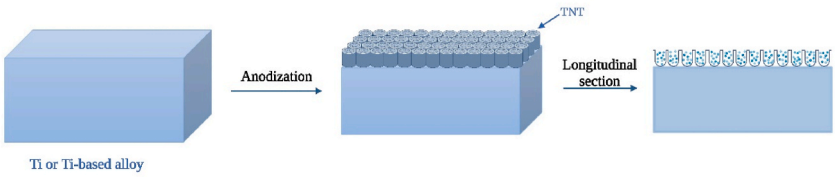

Fig. 9. Schematic illustration of TiO_2 nanotubes drug loading system on titanium surfaces.

3.3.2. PDA coating

PDA is a polymeric substance formed by the oxidative polymerization of dopamine, which has high adhesion and excellent ductility. Because of its auto-polymerization, dopamine can be deposited into almost any material shape to form PDA. The preparation process of this coating is simple and environmentally friendly because it can be done by immersing the substrate in an aqueous solution of dopamine at the room temperature. The thickness of the film is correlated positively with the duration of immersion and dopamine concentration [214]. The PDA coating increases the hydrophilicity of the titanium surface [88,215], which facilitates the adhesion and proliferation of cells [214]. Baldwin et al. co-deposited dopamine hydrochloride (DHC) and FHBP on the titanium surface. They found that PDA addition improved the hydrophilicity of FHBP-Ti and that DHC-FHBP-Ti had a superior effect in promoting osteoblast maturation compared with single FHBP-Ti [215].

The unique chemical groups on PDA endow PDA with various functions. Electron transfer can occur among dopamine molecules due to the catechol present in PDA. This group enables PDA to reduce metal ions, deposit them on the surface, reduce oxidants, scavenges free radicals, and produce reactive oxygen species with antimicrobial effects [216,217]. PDA also contains amino and hydroxyl groups, which are the basis for connecting PDA coatings with other substances [214,215,218]. PDA can adsorb various growth factors and small molecules based on these chemical groups [66,219,220]. Lee et al. loaded BMP-2 onto titanium surfaces using PDA as an intermediate layer. The modified titanium could promote the osteogenic differentiation of PDLSCs through integrin-mediated cell-matrix adhesion [89]. The drug salidroside was loaded onto micro-arc-oxidized titanium *via* PDA. *In vitro*, slow-release salidroside promoted the proliferation of BMSCs and migration of HUVECs. These results implied that titanium modified by PDA and salidroside contributed to bone formation and neovascularization [221]. Overall, the bioactive PDA coating can be prepared readily. In addition, it can be used as an interlayer to assist the loading of metal ions, growth factors, and drugs on titanium, resulting in broad application prospects.

3.3.3. Layer-by-layer assembly

LbL is a self-assembly method for preparing polyelectrolyte multilayers (PEMs) using binding forces such as electrostatic attractions, hydrogen bonds, and covalent bonds between assembled components. The titanium or titanium alloys are charged by physical or chemical methods and is immersed in a polyelectrolyte solution with an opposite charge to form the first monolayer by charge-to-charge interaction. After washing, a second monolayer is constructed on the specimen in a solution containing the opposite charge to the first layer. These steps are repeated until a target-loaded multilayer film is obtained [14] (Fig. 10). Upon application of this method, researchers usually prepare the intermediate layer using common substances that can be charged readily. They are biocompatible (e.g., gelatin, chitosan, hyaluronic acid) to enable the adhesion of the target coating to implants effectively while constructing PEMs. Due to the high polarity of these polyelectrolyte films, the hydrophilicity of titanium increases significantly after being modified, which also increases with the number of stacked layers [85,222].

Besides enhancing the hydrophilicity of titanium, electrolyte membranes applied in LbL methods can improve the biological properties of implants. Cai et al. prepared chitosan–gelatin multilayers on titanium surfaces using an LbL method based on the electrostatic adsorption between chitosan and gelatin [223]. Osteoblast proliferation on the modified titanium surface was enhanced compared to that of the control group. Besides, functional groups in electrolyte membranes can interact with target organic materials, thereby assisting drug incorporation. Most organic materials containing the hydroxyl group (e.g., naringin, procyanidins, icariin) can

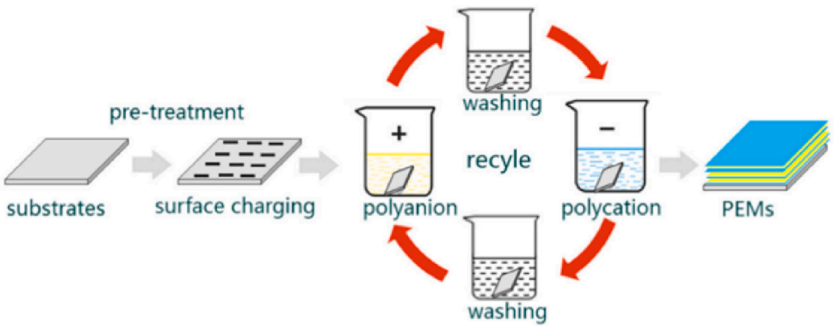

Fig. 10. A schematic representation of the LbL assembly process. Reproduced from Ref. [14] with permission.

attach to the protonated amino groups of chitosan coatings and be encapsulated into the micro-clearance between PEMs [224]. Shen et al. used gelatin and chitosan as interlayers to load naringin onto titanium by combining the amine groups on chitosan and hydroxyl groups on naringin [81]. The slow-released naringin from titanium could increase the expression of osteoblast genes such as *Runx2* and *OCN* and reduce the expression of osteoclast-related genes such as *CTSK* and *NFAT* in MC3T3-E cells at the transcriptional level.

The LbL method facilitates the incorporation of ingredients into titanium at lower temperatures and forms a coating with higher bioactivity. The process is simple and elaborate instrumentation is not required. Researchers can control the reaction time and the number of coating stacks to unify the thickness. This facility ensures the homogeneity of the physicochemical and biological properties of the products, as well as facilitating industrial mass production. In addition, the chemical structure of the intermediate layer and target ingredient must be analyzed precisely when designing the integration to ensure that they can be bonded stably.

4. The release detection and regulation of substances loaded on titanium

Substances loaded on titanium and its alloys will be released into surroundings, thus exerting their biological characteristics. The concentration of substances and their release periods have essential roles when analyzing the effect of components on modified titanium and its alloys. As mentioned above, various components can be loaded on titanium and its alloys. Some components are released in their original form (e.g., proteins, medicines), whereas others (e.g., metals) are released as ions. Therefore, detection methods to evaluate the release kinetics of those substances vary. To avoid cytotoxicity and expand the release period, several strategies have been introduced to regulate the release components. In this part, release detection methods and regulatory strategies are summarized.

4.1. Detection methods for evaluating the release of substances

The concentration of metal ions in a solution can be measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) or inductively coupled plasma-mass spectrometry (ICP-MS) [20,21,89]. Compared with ICP-MS, ICP-OES is less expensive and easier to operate but has a higher detection limitation. In terms of biologically active proteins, the released amount can be evaluated *via* immunological methods based on antigen-antibody reactions. Typical methods include enzyme-linked immunosorbent assay (ELISA) and western blotting (WB). An ELISA can be carried out readily and exhibits high sensitivity and selectivity due to antigen-antibody reactions. WB can also be used to detect bioactive proteins. However, the complex procedure and lower sensitivity limit the application of this method, which can be used only for semi-quantitative analyses.

Compared with metal ions and bioactive proteins, more methods can be applied in the analyses of drug release, including titration, spectral analysis, and chromatography. Titration has been phased out due to its low detection accuracy and inability to measure trace elements. Among spectral analyses, ultraviolet–visible (UV–Vis) spectrophotometry has been used widely to evaluate the content of components with absorbance in the wavelength between 190 nm and 800 nm. As an inexpensive, simple, and sensitive method, UV–Vis spectrophotometry can detect a compound at a low concentration. However, due to low selectivity, this method is not applicable to evaluate substances with similar structures in mixtures [225]. Unlike spectral analyses, chromatography first separates mixed compounds and then analyzes the target substance. This is an effective method for analyzing the composition and content of a mixture. Most recently, high-performance thin-layer chromatography (HPTLC) has been applied as an alternative to conventional TLC. This method uses a high-pressure infusion system and pumps samples into a column equipped with a stationary phase, thus separating components and detecting substances [226]. With high sensitivity and selectivity advantages, HPTLC is recommended for quantitative analysis of the release of organic components.

4.2. Strategies for regulating the release of substances loaded on titanium and its alloys

There are two drug-release models: burst release and slow continual release. The release of implant components always features an initial burst release followed by a slow and sustained release. Without controlling the burst release, a rapid increase in local drug concentrations at the early stage is observed, which may be detrimental to the human body. As the healing of bone defects is a long process, great attention should be paid to the long-term functionality of surface-modified implants. As loading substances continuously release from surfaces and their concentrations decrease, the osteogenic functionality of the material is also reduced. An ideal situation is that loading substances can be released at effective concentrations for as long as possible. Therefore, regulating the release of substances loaded on implants is crucial for both implant functionality and the avoidance of cytotoxicity. Several strategies to regulate the release of components are summarized.

Currently, the most commonly applied strategy is to add a second layer of polymers that can wrap or cover organic materials, such as chitosan, PLGA, PDA, and collagen [76,206,90,91]. Zhao et al. covered the Ag-doped Ti6Al4V alloy with a PDA layer to prevent Ag^+ from reaching the toxic concentration of 10 mg L^{-1} [43]. Compared to Ti–Ag group, the rate of Ag^+ released from the PDA-covered group was much lower. In addition, the release rate of substances can be regulated by controlling the thickness of polymer layers. Gulati et al. [90] fabricated a simple coating of PLGA to cover indomethacin-loaded TiO_2 nanotubes. Compared with drug-loaded nanotubes without a PLGA coating, the burst release of indomethacin in the group covered with a thin layer of PLGA was reduced from 77 % to 57 %, and the duration of drug release was prolonged from 4 d to 19 d. For a thick PLGA film, the burst release was reduced to 12 %, and the release duration was prolonged to 31 d [211].

Another promising strategy is to increase the bond strength between loading substances and substrates through chemical or physical actions. Tang et al. deposited hyaluronic acid/chitosan multilayers on titanium surfaces to incorporate proanthocyanidins

(PAC) [85]. The connection between HA/CS layers and PAC was achieved through the electrostatic interactions between the amine group of CS and the hydroxyl radical of PAC. PAC released sustainably for up to 14 d, and the burst release phase could not be observed. In addition to electrostatic attractions, inorganic metals could be bonded with surface-modified implants through chelating bonds. Gao et al. fabricated PDA coatings on TiO_2 nanotubes and then loaded Ag nanoparticles on PDA *via* chelation [227]. At the burst release stage (day 1), the concentrations of released Ag^+ were 0.639 ppm for Ag–TiO_2 groups and 0.155 ppm for Ag-PDA-TiO_2 groups. The result indicated that PDA improved the bonding strength between TiO_2 and Ag nanoparticles efficiently, avoiding the cytotoxicity caused by the rapid release of Ag^+.

In recent years, researchers have garnered increasing attention to endow biomaterials with stimuli-responsive characteristics, which are capable of responding to diverse stimuli spatiotemporally, such as enzyme, thermal, and pH [228–231]. The mechanism of enzyme-responsive release is that specific enzymes secreted by bacteria have the ability to degrade and alter the coating structure, thus triggering drug release. Accordingly, researchers covered BMP-2-loaded TiO_2 nanotubes with hyaluronidase-sensitive multilayers containing chitosan and sodium hyaluronate-lauric acid (SL) [228]. The secreted hyaluronidase would degrade SL multilayers on the titanium implant, resulting in the release of lauric acid to kill bacteria. The release of BMP-2 could be accelerated by the degradation of coatings, further inducing osteoblast differentiation. In addition to enzyme-responsive coatings, Li et al. [231] designed a thermo-sensitive chitosan-glycerin-hydroxypropyl methylcellulose hydrogel (CGHH) onto simvastatin-loaded TiO_2 nanotubes. At the normal temperature of the human body, GGHH was in the sol state, which could release of simvastatin to promote the differentiation of MC3T3-E1 osteoblasts. However, as shown in the *in vivo* test with subcutaneous infection animal models, the high temperature induced by an infection could trigger the transition of CGHH from the sol state to the gel state, thus releasing abundant glycerin to kill bacteria. In summary, stimuli-responsive strategies play a vital role in the regulation of drug release, but most applications of stimuli-activated systems focus on antibacterial properties, in which the whole system is triggered by characteristics of infections, such as bacteria-secreted enzymes, high temperature caused by inflammatory, and low pH. Research about applying stimuli-responsive systems to improve osteogenesis is desired and expected.

5. Discussion and future prospects

Despite the successful clinical application of orthopedic implants *in vivo*, most artificial implants have a life span of 10–15 years [232,233]. One of the most significant reasons for implant failure is aseptic loosening, caused by several factors [234,235]. First, the mismatched elastic modulus between adjacent bone and implants can trigger stress shielding, which results in peri-implant bone resorption and aseptic loosening [236]. The modulus of Ti and Ti6Al4V is about 120 GPa and 114 GPa, respectively, much higher than the modulus of cortical bone (10–30 GPa) [18]. Additional elements have been involved in fabricating multi-element alloys to lower the elastic modulus of titanium, such as Mo, Nb, Ta, and Zr [237]. Among all techniques mentioned above, FSP is an energy-saving and promising technique able to prepare novel titanium alloys with low modulus. For instance, Ti–Nb–Ta–Zr alloys fabricated by FSP showed moduli similar to bone tissue, ranging from 40 to 80 GPa, which reduces the possibility of aseptic loosening [182]. Moreover, wear particles produced by the implant can initiate cytokines secretion (TNF-α and IL-1), which activates osteoclast and induce bone resorption and aseptic loosening [238]. The wear resistance of surfaces can be improved by the modification of *C*-PIII, *N*-PIII, and magnetron-sputtered TiN [162,170]. In addition, the corrosion resistance property of titanium is also closely bound to the life expectancy of implants. Even with passive oxide layers, titanium and its alloys undergo corrosion reactions in the body environment [239]. Surface modification methods, such as PIII, magnetron sputtering, FSP, and MAO, can improve corrosion resistance through the fabrication of a dense and uniform coating on the surface that can prevent body fluid from reaching the substrate. In contrast, porosity and microcracks appearing in plasma-sprayed coatings can result in the degradation of implant materials. Briefly, it will be an appealing direction to combine novel low-modulus titanium alloys with advanced surface modification techniques for better physicochemical properties and osseointegration.

In addition to several factors mentioned above, insufficient osseointegration can result in aseptic loosening as well. Optimizing the surface topography of titanium and its alloys is a promising strategy to facilitate osseointegration between implants and bone tissues. The acid etching technique has been widely applied to increase surface roughness before the incorporation of osteogenic substances, which plays a facilitating role in cell adhesion and osseointegration [119,120]. Moreover, recent studies found that implant surfaces with micro/nano-scale hierarchical topography characteristics performed better in enhancing bone formation [124,125]. Microstructural surfaces can stimulate osteoblasts differentiation and facilitate mechanical locking with bone tissues, while nano-roughed titanium surfaces are beneficial to cell adhesion and proliferation. Therefore, it is a promising direction to fabricate surfaces with micro/nano-scale hierarchical topography characteristics. According to recent studies, titanium with a novel kind of micro/nano-scale bionic topography could be fabricated through electrochemical etching followed by anodizing and ultrasonic treatment [8,142,240]. The novel bionic topography inspired by snail operculum could significantly improve bone-implant bonding strength.

In the last decade, a significant number of substances have been incorporated on the surface of titanium and its alloys to create novel surfaces with osteogenesis properties and further enhance peri-implant bone regeneration. This article summarized several osteogenesis components that have been doped on titanium and its alloys. However, part of the research studies is limited to the osteogenic properties of substances. Peri-implant bone defect healing is a complex process involving multiple cell types to play a part coordinately, including MSCs, osteoblasts, osteoclasts, endothelial cells, and immune cells [232,241–243]. Therefore, it is provincial to focus only on the osteogenic efficacy of loading substances. Angiogenesis is also important for functional bone formation since blood vessels can transport oxygen, nutrients, and waste products, as well as originate angiocrine signals to regulate bone formation [8,244–246]. Besides, during the early wound healing, the host immune response would occur and influence the biological activities of cells related to osteogenesis, which affects the final fate of orthopedic implants [247]. The antibacterial property has also been proven

as an important aspect of implantation success [9]. Microbial infection of implants severely inhibits osseointegration, leading to implant failure. Therefore, to further improve bone integration, more osteogenic substances with angiogenesis, anti-inflammatory, and antibacterial effects should be attempted to be co-incorporated into titanium.

In addition, the construction of sustained-release systems, which benefit the long healing process of bone defects, is of great importance. Some loading components are released abruptly in large quantities and have a short release duration, resulting in toxicity and less satisfied long-term clinical anticipation. Promising strategies to extend releasing durations include improving bonding strength between components and substrates and covering released components with bioactive polymers. Recently, an increasing number of studies have focused on designing surfaces with dynamic, responsive characteristics. With stimuli responsiveness, titanium and its alloys can "smartly" respond to the surrounding environment, which satisfies the requirements of dynamic release regulation.

In short, it is a trend of surface modification to produce low-modulus titanium alloys with desirable physicochemical characteristics and micro/nano-scale hierarchical topography. Multi-functional coatings with stimuli-responsive abilities are also recommended to be introduced on titanium alloys, further improving the biological properties of implants. We believe that ideal surface-modified orthopedic implants loaded with multi-functional components can satisfy various clinical requirements in the future.

6. Conclusion

Titanium and its alloys have been applied widely in bone-tissue engineering. Aimed at obtaining better physicochemical properties, biological activity, and more convenient operability, research on optimizing the surface properties of titanium and its alloys is ongoing. Future studies should focus on fabricating novel titanium alloys with proper elastic modulus, enhanced corrosion resistance, wear resistance, as well as micro/nano-scale hierarchical topography through surface modification. In order to further improve biological properties, the selection of ideal coating materials with multifunctional properties and better regulation of the release effects is necessary. With optimizations of loading components and surface modification techniques, surface-modified titanium implants can integrate rapidly and effectively with host tissue in orthopedics and dental procedures.

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Data availability statement

No data was used for the research described in this review article.

CRediT authorship contribution statement

Jingling Li: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Yaxin Zheng:** Writing – review & editing, Writing – original draft, Visualization, Investigation. **Zihe Yu:** Writing – original draft, Investigation. **Ranjith Kumar Kankala:** W. **Qianying Lin:** Investigation. **Jingbo Shi:** Investigation. **Chao Chen:** Writing – review & editing, Funding acquisition. **Kai Luo:** Writing – review & editing, Funding acquisition. **Aizheng Chen:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Quan Zhong:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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