Review article

Classification and research progress of implant surface antimicrobial techniques

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Abstract Due to the good biocompatibility and ideal mechanical property, titanium implants have been widely used in dental clinic and orthopedic surgery. However, bacteria induced infection can cause per-implant inflammation and decrease the success rate of implant surgery. Therefore, developing antimicrobial techniques is essential to successful application of titanium implants. Many surface antimicrobial techniques, including antimicrobial coating and surface modifications, have been explored and they always exert antimicrobial effect by reducing bacterial adhesion, inhibiting their metabolism, or destructing cell structure. In this paper, different surface antimicrobial techniques and their recent research progress are reviewed to provide a brief insight on this area.

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Introduction

Since Brånmark implant system was developed in 1965, dental implants have been widely investigated and fast developed to become a mature technique for replace of lost teeth. Now, dental implants has been known as “the third pair of human teeth”. In history, a variety of implant materials have been used, including different metals and their alloys, ceramic materials, polymer materials, composite materials and so on. Among them, pure titanium (containing more than 99% titanium content) and titanium alloy have become the best choice due to their good biocompatibility, ideal mechanical property and high corrosion resistance ability. Therefore, titanium and titanium alloy have become the widely used implant materials in medicine.

One main complication of titanium implants is bacteria-related infection for their long-term use. Despite of their good biocompatibility, titanium and titanium alloy are bio-
inert materials, which have no antimicrobial properties. Bacteria are easy to adhere on their surface to form bacteria clone in oral environment and then lead to peri-implant infection. Therefore, it is necessary to endow the implant surface with antimicrobial property to resist bacteria induced infection. At same time, the excellent biocompatibility for osseointegration of titanium implants should be maintained.

Many techniques have been explored to endow titanium implant surface with considerable antimicrobial property and they can be roughly classified into two categories, surface modification and surface coating. Antimicrobial surface coating usually refers to the additional layer on implant surface which can effectively prevent bacteria adhesion on implant surface, or kill bacteria by releasing antimicrobial substances or ions, and then achieve antimicrobial effect. The layers include ionic antibacterial coating, antibiotic and organic antimicrobial coating, and antibacterial carriers. Whereas antimicrobial surface modification usually refers to changing the biological characteristics of the original implant surface to exert antimicrobial capability by either physical or chemical methods, but with no additional layer on implant surface. The biological characteristics to be changed include surface free energy, surface charge, hydrophobicity, roughness and other characteristics. To obtain ideal surface antimicrobial property, above techniques are applied singly or with a combination of two or more methods. According to above classification, recent research progress on implant surface antibacterial techniques is discussed in this review.

Antimicrobial coatings

The effects of antimicrobial coatings on titanium implant surface mainly rely on antimicrobial elements or compounds in the coating, which can be divided into three groups: ionic antibacterial coating, antibiotic and organic antimicrobial coating, and antibacterial carriers.

Ionic antibacterial coating

Silver antimicrobial coating

Silver (Ag) is the most powerful metal element against microbes and it exerts antimicrobial effect either in form of Ag⁺ or through direct "contact killing". Ag⁺ can enter cytoplasm of bacteria, interact with a variety of proteins, disturb enzymes’ function, inhibit cellular metabolism and cell division, and eventually lead to bacterial death. While direct contact killing is another important mechanism for Ag to kill bacteria. The contact of Ag with bacteria will disrupt cell wall and plasma membrane, leading to leakage of cytoplasm and essential cellular substances, and eventually the cell death.

Many methods have been applied to prepare silver-containing antimicrobial coatings. These methods include plasma spraying, electrochemical deposition, sol–gel method, thermal spraying method, co-sputtering method and plasma immersion ion implantation. In addition, some composite silver-containing antimicrobial coatings are prepared by combination of above methods. Silver-loaded coral hydroxyapatites (SLCHAs) were used as scaffolds for bone tissue engineering. In this study, silver ions were successfully introduced to the CHA scaffolds by ion–exchange reaction and precipitation process to improve its antibacterial property of CHA scaffolds.

With the development of nanotechnology, silver nanoparticles (AgNPs) have become a new choice for silver antimicrobial coatings. Silver nanoparticles have more surface area to exert stronger antimicrobial effect. Furthermore, they show long time and stable antimicrobial ability by oxidative stress reaction and production of reactive oxygen free radicals. Radtke A et al. prepared TiO₂ nanotube array coating doped with silver metal (Ag) by immersing the alloy in silver nitrate solution. The growth of biofilm was effectively inhibited by hydroxyl radicals produced by the coating and the bacterial inhibition rate reached 97.62%. A study by Zongming Xiu explored the antimicrobial effect of Ag on Escherichia coli to distinguish the effect of fixed Ag on nanoparticles and Ag⁺ release from them. The results showed that the antimicrobial effect of silver nanoparticles was indirect and the antimicrobial activity derived from the Ag⁺ released from them.

Zinc antimicrobial coating

Zinc is one of essential elements in human body. When used as antimicrobial agent, it shows many advantages including its safety, less side effect, long-lasting antimicrobial effect and a variety of compounds can be used. Like Ag, Zinc exert antimicrobial effect through three mechanisms: ion antimicrobial theory, contact antimicrobial theory and photocatalytic antimicrobial theory. Zinc oxide can inhibit bacteria by decreasing their adhesion or disrupt bacterial cell membranes.

Ruoyun Wang et al. prepared Zinc-doped ZrO₂/TiO₂ coating on Ti₆Al₄V surface with a combination method of magnetron sputtering and microarc oxidation. The coating showed excellent antimicrobial property against Staphylococcus aureus, and the biocompatibility and corrosion resistance were not influenced. Liu et al. studied the antimicrobial effect of nano-zinc oxide on E. coli and found that nano-zinc oxide could disrupt the bacterial cell membrane and cause leakage of intracellular substance. The antimicrobial ability was positively correlated with the concentration of nano-zinc oxide.

Copper antimicrobial coating

Copper has been used in the form of pure metals and metal compounds since ancient times to achieve anti-inflammatory, antimicrobial and antiproliferative effects. The metal is cheap and easy to obtain. Appropriate amount of copper is non-toxic to human body. A study by Marziyeh Jannesari et al. showed that copper ions and its superoxide compound can inhibit bacterial respiration and lead to the decomposition of DNA, thus exert good antimicrobial property. Antimicrobial stainless steel implants can be prepared by mixing a certain amount copper evenly into 316L stainless steel. Natalie Gugala et al. found that Cu was the most effective metal for preventing the formation of Pseudomonas aeruginosa biofilms. Furthermore, Cu is the most efficacious metal against S. aureus and E. coli biofilms. From these studies, Cu is found to have extended activity against
planktonic cell growth, attachment of biofilms and biofilm proliferation.\textsuperscript{21}

**Fluorine antimicrobial coating**

A study by Nurhaerani et al. indicated that plasma-based fluorine ion implantation into SUS with CF4 gas provided surface antibacterial activity which was useful in inhibiting bacterial adhesion.\textsuperscript{22} Skartveit L et al. studied the antimicrobial effect of TiF\textsubscript{4} in vitro and their results showed that TiF\textsubscript{4} can efficiently inhibited proliferation of *Streptococcus mutans* and *Bacteroides gingivalis* on tooth surface.\textsuperscript{23} Yoshinari et al. introduced fluoride ions into surface of pure titanium with a method of ion accelerators and achieved a good effect on inhibiting pathogenic bacteria of periimplantitis.\textsuperscript{24}

**Composite antimicrobial coating**

Each antimicrobial element or agent shows its unique antimicrobial property and at same time has both advantages and disadvantages. To obtain ideal antimicrobial effect, together with good biocompatibility and osteogenic property, composite coatings are explored by a combination of above antimicrobial elements or agents.

Jin Jianfeng et al. prepared a Ti-GO-Ag multiphase composite antimicrobial coating by constructing a silver particles-containing graphene oxide films (Go) on the surface of pure titanium. Silver in the coating showed good antimicrobial property on *S. aureus* and *Porphyromonas Gingivalis* and graphene oxide can induce ectopic osteogenesis in vivo.\textsuperscript{25} Similar to titanium, Tantalum metal has good biocompatibility and chemical stability. Furthermore, Tantalum compounds shows higher hardness and better corrosion resistant. Heng-Liu Huang et al. prepared Tantalum nitride-silver (TaN-Ag) coating on titanium implants by magnetron sputtering deposition system and found that the antimicrobial effect increased with the content of silver in the coating and ideal size of silver particles for best antimicrobial effect were at 15 ~ 53 nm.\textsuperscript{26}

**Antibiotics and organic antimicrobial coating**

Organic agents are also valuable candidates to prepare antimicrobial coatings on titanium implants and exciting results have been obtained in recent researches. Commonly explored antimicrobial organic agents include chlorhexidine, antimicrobial peptide, antibiotics and so on. Their combinations are also studied to obtain better results.

**Nano-chlorhexidine (CHX) antimicrobial coating**

Chlorhexidine is a widely used surface disinfectant with good antimicrobial effects against both Gram-positive and Gram-negative bacteria. As a result, it is commonly used for local cleaning and disinfection during oral surgery and oral care.\textsuperscript{27} Martijn Riool et al. prepared a new epoxy-based coating containing chlorhexidine on titanium implant surface, and 10% content of chlorhexidine within the coating showed strong antimicrobial effect against *S. aureus* and prevented experimental biomaterial-associated infection.\textsuperscript{28} Wood et al. prepared a chlorhexamethy hexametaphosphate nanoparticle coating on the surface of pure titanium and average diameter of the particles were 49 nm. The coating showed sustained release of CHX and exhibited antimicrobial efficacy against oral primary cloning bacterium *Streptococcus gordonii* within 8 h. The antimicrobial efficacy was greater in the presence of an acquired pellicle which is postulated to be due to retention of soluble CHX by the pellicle.\textsuperscript{29}

**Antimicrobial peptide coating**

Swedish scientists G. Boman discovered an antimicrobial peptide (cecropin) in the pupae of Sigibui in 1980. Since then, a variety of antimicrobial peptides have been found by scholars in other insects and mammals and they are uniformly named antimicrobial peptides (AMP). It is generally believed that antimicrobial peptides kill bacteria by destructing the integrity of bacterial cell membrane, but details of bactericidal mechanisms need to be further elucidated.\textsuperscript{30} Guangzheng Gao et al. constructed a polymer brush based implant coating which consisted of covalently grafted hydrophilic polymer chains conjugated with an optimized series of antimicrobial peptides (AMPs). The coating was extremely effective in resisting biofilm formation and had no toxicity to osteoblast-like cells. Therefore such coating is valuable for the development of infection-resistant implants.\textsuperscript{31} Yazici et al. engineered a chimeric peptide coating with both antimicrobial properties and robust solid-surface. The coating exhibited strong antimicrobial efficacy against a variety of bacteria, including *S. mutans*, *Staphylococcus epidermidis*, and *E. coli*, and reduced bacterial colonization onto titanium surfaces below the detectable limit.\textsuperscript{32}

**Antibiotic coating**

Antibiotics are also valuable candidate to prepare antimicrobial coatings on titanium implant surface. Gentamicin, amoxicillin, vancomycin, ceftiofen and other antibiotics are commonly investigated in the preparation of antimicrobial coatings. Shula Radin et al. prepared Vancomycin-containing calcium phosphate ceramic coating on titanium alloy substrate and the sample showed sustained release of vancomycin and effective bacteriostatic effect within 72 h. Therefore, this coating is useful in orthopedic surgery such as hip replacement but its application in dental implants needs further investigations.\textsuperscript{33}

Michael et al. evaluated antibacterial effect of different antibiotics against bacterial isolates recovered from orthopedic implants and they found that ciprofloxacin and vancomycin, representing aminoglycosides which are routinely incorporated into bone cement, were more active than gentamicin, cefamandole and erythromycin.\textsuperscript{34}

**Other organic coatings**

Although antibiotics have better antimicrobial effects, the risk of bacterial resistance limits their clinical use. Therefore, non-antibiotic organic antimicrobial agents, such as chlorophenol and polyhexamethylene biguanidine, exhibit greater clinical value.\textsuperscript{35} A composite antimicrobial coating of chlorhexidine and chloroxylenol was prepared and the coating exhibited broad-spectrum and durable antimicrobial effect against *Staphylococcus epidermidis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *Candida albicans* at baseline and up to 8 weeks. Therefore it is valuable for control of device-related infection.\textsuperscript{36} A poly (hexamethylene biguanide) hydrochloride (PHMB) coating was absorbed on titanium alloy
surface after simple chemical pretreatment and the adsorbed PHMB reacted bactericidally against several oral bacteria, but produced no adverse effects on SaOs-2 cells within 48 h cell culture.\(^{37}\) Therefore the coating is valuable to control implant-associated infections.

**Antibacterial carrier**

Many organic and inorganic antimicrobial elements or agents are attempted to load on titanium implant surface to prevent oral infections. Whereas they need a substrate or carrier to attach or fix them on implant surface. The carriers include the oxidation layer (Titanium dioxide, TiO\(_2\)) on titanium surface or an additional layer composed of either degradable or non-degradable substances, or both.

**Titanium dioxide (TiO\(_2\))**

Pure titanium will rapidly form a thin layer of TiO\(_2\) in the air and the layer shows both good biocompatibility and chemical stability. Therefore it always functions as the interface material for titanium to form osseointegration with bone. In addition, TiO\(_2\) exhibits antimicrobial property in certain circumstance.\(^{38}\) For example, TiO\(_2\) nanotubes can effectively inhibit bacterial adhesion after ultraviolet radiation. TiO\(_2\) nanoparticles can disrupt bacterial membrane by producing reactive oxygen species.\(^{39}\)

In addition, TiO\(_2\) also serve as a carrier of antimicrobial drugs which can be released persistently from the metal. Petrini et al. constructed a zinc modified TiO\(_2\) surface formed under ultraviolet irradiation and zinc is more easily combined on titanium surface with high ratio of anatase phase. Such Zinc-TiO\(_2\) coating exhibit excellent antimicrobial activity.\(^{40}\) Tang et al. prepared gentamicin loaded TiO\(_2\) nanotubes by vacuum drying and freeze-drying respectively. Such coatings could inhibit bacterial adhesion and biofilm formation, and the antimicrobial effect was positively correlated with the diameter of the nanotubes.\(^{41}\)

**Degradable carriers**

In 2013, Pitarresi et al. proposed biodegradable composite antimicrobial coating on implant surface, in which polymers serve as the carriers of antimicrobial drugs and the release of drugs is controlled by the degradation rate of polymers. Such biodegradable antimicrobial coatings may be valuable to prevent bacteria infection and reduce implant failure.\(^{42}\) The commonly used biodegradable materials include chitosan, polylactic acid, sol–gel and hydrogel films.

Chitosan is a kind of biodegradable polysaccharide and is commonly used as a carrier of antimicrobial drugs. Swanson et al. prepared a chitosan-vancomycin antimicrobial coating on titanium implant surface and the morphology of the coating can be well controlled.\(^{43}\) Chitosan-RGD peptide segment antimicrobial coating was also prepared on Ti–Al–V alloy surface and the coating showed good antimicrobial properties.\(^{44}\) Polylactic acid (PLA) is also a commonly used biodegradable material. Karacan et al. constructed an thin layer of gentamicin (GM) containing polylactic acid and hydroxyapatite (PLA/HAp) composite coating, which could be efficiently applied to titanium implants and the drug release rates can be efficiently controlled.\(^{45}\)

Silica sol–gel thin films can be used as a drug delivery system with potential clinical applications for the prevention or treatment of periprosthetic infections.\(^{46}\) Radin and Ducheyne used silica sol-gels to create coatings on titanium plates for local vancomycin delivery.\(^{47}\) Hydrogel is also used as a degradable antimicrobial carrier. Giglio et al. prepared a new electro-synthesized polyacrylic hydrogel films as bioactive titanium implant coatings in which ciprofloxacin was entrapped. Such coating can effectively inhibit methicillin-resistant \(S.\) aureus (MRSA) growth, but sows good biocompatibility to MG63 human osteoblast-like cells.\(^{48}\) The results of this study suggest that antibiotic modified hydrogel coatings could represent an effective approach to preventing serious bacterial infections frequently related to orthopedic surgery without affecting osteoblast functions pertinent to new bone formation.

**Non-degradable carriers**

In addition to the commonly used biodegradable carriers mentioned above, non-degradable materials such as hydroxyapatite, bone cement and mesoporous materials are also often used as carriers for antimicrobial coatings.

Hydroxyapatite (HA) is the main inorganic component of human skeleton, which can be chemically bonded with organic tissue and shows good biocompatibility.\(^{49}\) HA has no antimicrobial property, but it can be used as a carrier for antimicrobial agents. Micro-nanostructured hydroxyapatite (HA) coating was prepared on Ti surface and then the antibacterial agent of chitosan (CS) was loaded on the HA surface.\(^{50}\) Such HA/CS composite coating can effectively inhibit the bacterial growth and improve biological property. Zinc-modified hydroxyapatite also showed good antimicrobial activity against \(S.\) aureus, \(S.\) mutans, \(S.\) epidermidis, etc.\(^{51}\

Beyth et al. added quaternary ammonium polyethylene imine (QPEI) nanoparticles into bone cement to endow titanium implants with antimicrobial property. A strong antibacterial effect against \(S.\) aureus \(ATCC\) 8325-4 and \(Enterococcus\) faecalis after an ageing period of 4 weeks was evident the antimicrobial effect of bone cement loaded nanoparticles is better than that of antibiotics.\(^{52}\)

**Surface modification**

Surface modification can endow titanium implants with antimicrobial properties and the mechanism underlying it is to influence the adhesion or polymerization of bacteria by changing the surface hydrophilicity, roughness, crystalline phase, potential energy and so on. Many techniques, including ultraviolet (UV) treatment, ion implantation technology, laser cladding technology and anodizing technology, can be used to attain such purpose.

**Ultraviolet (UV) treatment**

The surface of titanium implants can be treated with ultraviolet light to acquire special biological properties. Investigations show that ultraviolet irradiation has no effect
on surface morphology, but can change surface hydrophilicity from hydrophobic to superhydrophilic, and the surface carbon content is also decreased. Furthermore, bacterial adhesion and polymerization on implant surface decreased significantly after 8 h of UV treatment, and the effect of UVC was stronger than that of UVA.53

In another study by Suketa et al. a thin layer of photocatalytic anatase TiO2 was added onto pure titanium surface by plasma source ion implantation followed by annealing. The layer exhibited a strong photocatalytic reaction under UVA illumination and the viability of both Actinobacillus actinomycetemcomitans and Fusobacterium nucleatum cells was suppressed to less than 1% under UVA illumination within 120 min.54

Changing surface roughness

Implant surface roughness can affect bacterial adhesion. Puckett et al. reported that a surface that has higher nano roughness has higher surface energy, which leads to higher protein adsorption, resulting in decreased bacterial adhesion.55 Many techniques are applied to create nano-scale structures on implant surface, which includes ion implantation technology, laser cladding technology, anodizing technology, etc.

Yanzhe Zhang et al. fabricated the Ag–ZnO-HA (silver-zinc oxide-hydroxyapatite) nanocomposite on the Ti6Al4V by laser cladding means and the coating exhibited excellent antibacterial properties against the E. coli and S. aureus in vitro and good osteogenesis properties in vivo.56 Min-Kyung Kang et al. studied the antimicrobial effect of titanium anodized in sodium chloride + calcium acetate (CA) + β-glycerol phosphate disodium pentahydrate (β-GP) mixed solution, and the oxidized titanium surface showed better antimicrobial property against S. mutans when compared with that of pure titanium.57

Surface charge

Many measurements can be applied to engage electrical charges on titanium surface as this may also affect bacterial adhesion.31,32,59 Negatively charged surface is less susceptible to bacterial adhesion than a positively charged surface because most bacteria have negatively charged cell walls,59,60 and the influence of this electrical repulsion on bacterial adhesion increases with increasing surface hydrophilicity.31 Gao discovered that the antibacterial effect of the studied surface was from an electrostatic disturbance where the surface triggered an autolytic and/or cellular death mechanism.31

Surface crystalline phase

Regarding the surface crystalline phase, titanium dioxide can be found in 3 forms: anatase, rutile, and brookite.51,62 The differences in crystallinity is closely related to their capacity to prevent bacterial adherence.63 Anatase, rutile, and brookite all have photocatalytic activity (PCA),5,61,64 which is responsible for their antibacterial capacity. The oxidation or reduction reactions of these crystalline are catalyzed by a light of an appropriate wavelength. The PCA depends on the concentration of OH radicals, crystalline structure, and surface area (particle size).62 Some scholars have succeeded in increasing the anatase phase through various methods, including anodic oxidation, hydrothermal treatment and plasma treatment, and this crystalline phase shows good antimicrobial properties.59,65

Conclusion

In this paper, different surface antimicrobial techniques of titanium implants, including antimicrobial coating and surface modifications, are reviewed and recent research progress are discussed. These techniques usually exert antimicrobial effects by inhibiting bacteria adhesion, decrease biofilm formation, interrupt cellular metabolism and respiration, and disrupt bacterial wall and cell membrane, eventually lead to cell death. Promising results both in vitro and in vivo have been obtained suggesting their great potential for future clinical application. Furthermore, these techniques usually involve composite materials, nanotechnology, bioengineering and multidisciplinary fusion, all of which indicate possible break through in this area in the near future. Although much progress have been obtained in this area, there are still some problems. Most of these techniques are still at laboratory stage and their reliability, biocompatibility, long-term effect against bacterial in vivo, and other potential harm to human body need to be further elucidated before their use for human body.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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