



# Nano-Modified Titanium Implant Materials: A Way Toward Improved Antibacterial Properties

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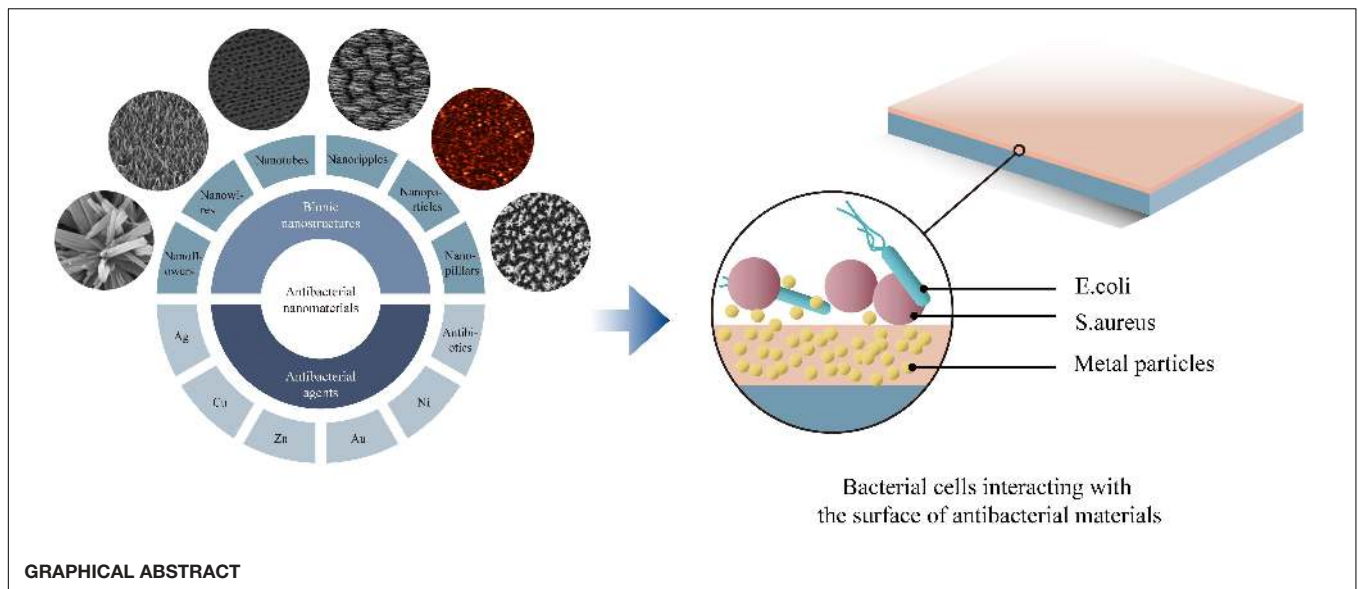
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Titanium and its alloys have superb biocompatibility, low elastic modulus, and favorable corrosion resistance. These exceptional properties lead to its wide use as a medical implant material. Titanium itself does not have antibacterial properties, so bacteria can gather and adhere to its surface resulting in infection issues. The infection is among the main reasons for implant failure in orthopedic surgeries. Nano-modification, as one of the good options, has the potential to induce different degrees of antibacterial effect on the surface of implant materials. At the same time, the nano-modification procedure and the produced nanostructures should not adversely affect the osteogenic activity, and it should simultaneously lead to favorable antibacterial properties on the surface of the implant. This article scrutinizes and deals with the surface nano-modification of titanium implant materials from three aspects: nanostructures formation procedures, nanomaterials loading, and nano-morphology. In this regard, the research progress on the antibacterial properties of various surface nano-modification of titanium implant materials and the related procedures are introduced, and the new trends will be discussed in order to improve the related materials and methods.

**Keywords:** bactericidal, nanostructure, antibacterial, nanoparticles, titanium-implants

## INTRODUCTION

Currently, with the rapid development of materials science and biotechnology, titanium and its alloys as orthopedic implant materials (Sam Froes, 2018) have been widely used in applications such as skeleton structure fixation and joint function repair implants (Gode et al., 2015; Liang et al., 2016; Kaur and Singh, 2019). Many new types of titanium alloys with high-quality performance have been invented through in-depth research on titanium alloy preparation (Zhang C. et al., 2017; Wang et al., 2018; Attarilar et al., 2019a,b; Hafeez et al., 2019) and optimization of titanium alloy composition (Liu et al., 2015a; Wang et al., 2016; Rabadia et al., 2019a,b). These new titanium alloys show outstanding application value in mechanical properties (Guo et al., 2013; Wang et al., 2015; Jawed et al., 2019; Hafeez et al., 2020), corrosion resistance (Lee et al., 2015; Zhu W.Q. et al., 2019; Malhotra et al., 2020), and osteogenic action (Zhu et al., 2016; Li H.F. et al., 2019; Lei et al., 2020).



It is undeniable that the implant material plays a vital role in orthopedic diseases (Hanawa, 2018; Qian et al., 2020), and its infection risk which is directly related to the material condition cannot be ignored (Montanaro et al., 2011; Kumar and Misra, 2018; Li and Webster, 2018). The infection of the tissue in the periphery of implant material is one of the most severe complications in orthopedic surgery (Pfang et al., 2019). The occurrence of infection not only leads to the failure of the implant and the surgery but also increases the patients' recovery period and makes an economic burden on both patients and the medical system. The use of antibiotics is a common and effective way to control this issue, but it also has some disadvantages (Klein et al., 2016; Holleyman et al., 2019). Bacterial infection on the surface of the implanted material may eventually form a biofilm and reduce or completely inhibit the beneficial effects of the bactericidal drugs. Besides, the system-administered anti-infection method can also result in a low concentration of drugs in the surgical area due to scars or fibrosis of the surrounding tissues, which affects the antibacterial efficiency (Andersson and Hughes, 2012, 2014). For this reason, the preparation of titanium-based implant materials with antibacterial properties can effectively solve this problem. This infection issue can be solved by adding metallic bactericidal elements or the addition of antibacterial coatings on the surface of implants (Morones-Ramirez et al., 2005; Ben-Knaz Wakshlak et al., 2016; Pareek et al., 2018).

The emergence of nanotechnology has caused significant changes in many fields of science, such as physics, chemistry, materials science, biology, computer science. Compared with the conventional materials (Wang et al., 2008), nanoscale materials have many new and unique properties, including medical, mechanical, chemical, magnetic, optical (Whitesides, 2005; Dyakonov et al., 2017; Semenova et al., 2017). Some of the nanoscale materials have appeared as new antibacterial agents, and current studies have confirmed that antimicrobial nanoparticles (NPs) and nanocarriers that aimed to deliver antibiotics can effectively treat infectious

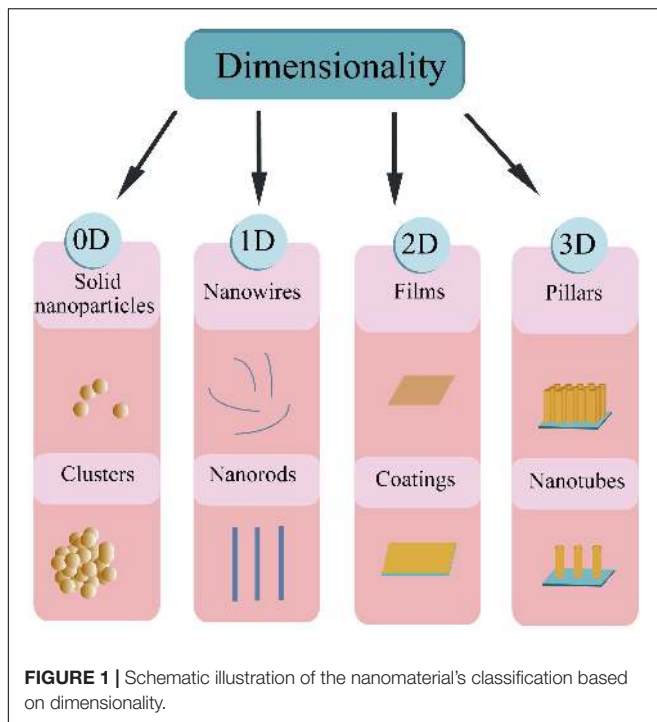
diseases (Huh and Kwon, 2011). Nanoscale materials have higher antibacterial properties compared to traditional antibacterial counterparts due to their high surface area to volume ratio. Therefore, it maintains more active area for biological interactions thus this subject seems to have outstanding research value in biomedical applications (Xia, 2008; Avila et al., 2018). This article summarizes and analyzes the advantages and disadvantages, procedures, antibacterial mechanisms, and possible improvement mechanisms for various nanoscale antibacterial materials, in order to provide a guideline for the modern nano-antibacterial materials with improved design and optimum properties for implant applications.

## CLASSIFICATION OF NANOMATERIALS

Based on the dimension criteria, antibacterial nanomaterials can be divided into four categories: zero-dimensional—nanoparticles, one-dimensional—nanowires, two-dimensional—nanofilms, and three-dimensional—nanoblocks (Figure 1) (Saleh, 2020). Besides, antibacterial nanomaterials can also be classified according to the structural form or antibacterial active ingredients (Gleiter, 2000).

### Classification by the Structural Form

Antibacterial nanomaterials based on the structural form can be classified into antibacterial NPs, antibacterial nanosolids, and antibacterial nano-assembled structures. NPs are known as tiny particles in the size range of 1~100 nm. Their specific structures induce some sort of surface and interface effects such as small size effect, macroscopic quantum tunneling and quantum size effect (Morris, 2018). As a result, nanomaterials with a series of excellent properties enhance the proficiency of nano-antibacterial agents. Compared with ordinary materials, nanomaterials have irreplaceable characteristics, especially in the antibacterial field. Hence it is worthwhile to expand



the scope of their applications. Antibacterial nanosolids can be formed by the aggregation of nano-sized antibacterial particles. They can be further divided into bulk, thin-film, and nanofibrous nanomaterials. Antibacterial nano-assembled structures refer to the artificially assembled and synthesized antibacterial nanomaterial systems. These systems are composed of antibacterial nanoparticles, nanofilaments, or tubes as the basic unit. These various nanostructures can be assembled and arranged in one dimensional, two or three-dimensional space to form the desired nanomaterial structures (Mageswari et al., 2016; Khan, 2020).

Nanomaterials can be synthesized through various methods such as construction and destruction (Figure 2) (Saleh, 2020). On the one hand, NPs can be obtained from the atomic level and then integrated into the desired materials. The methods of this kind of synthesis include self assembly (Zhang et al., 2019b; Deng et al., 2020), laser pyrolysis (Laurent et al., 2010; Dumitrache et al., 2019), condensation (Sano et al., 2020), CVD (Gutés et al., 2012; Tyurikova et al., 2020), sol-gel method (Gonçalves, 2018), soft lithography (Fu et al., 2018), hydrothermal methods (Zhen et al., 2019; Moreira et al., 2020), microwave methods (Henam et al., 2019), sonochemical (Gupta and Srivastava, 2019; Moreira et al., 2020), synthesis using plant extracts (Ogunyemi et al., 2019; Ransozek-Soliwoda et al., 2019), and green synthesis (Gour and Jain, 2019; Irshad et al., 2020). On the other hand, macroscopic level materials can be trimmed down to NPs by different methods, including mechanical grinding (Sviridov et al., 2017; Haque et al., 2018), ball milling (Li Y. et al., 2020), lithography (Fu et al., 2018), vapor deposition (Choi et al., 2018), arc-plasma deposition (Ito et al., 2012; Takahashi et al., 2015), ion beam technique (Heo and Gwag, 2014; Yang J. et al., 2018), severe

plastic deformation (Cui et al., 2018; Sarkari Khorrami et al., 2019), chemical etching (Wareing et al., 2017; Pinna et al., 2020), sputtering (Pišlová et al., 2020), and laser ablation (Pandey et al., 2014; Abid et al., 2020).

## Classification by Antibacterial Active Ingredients

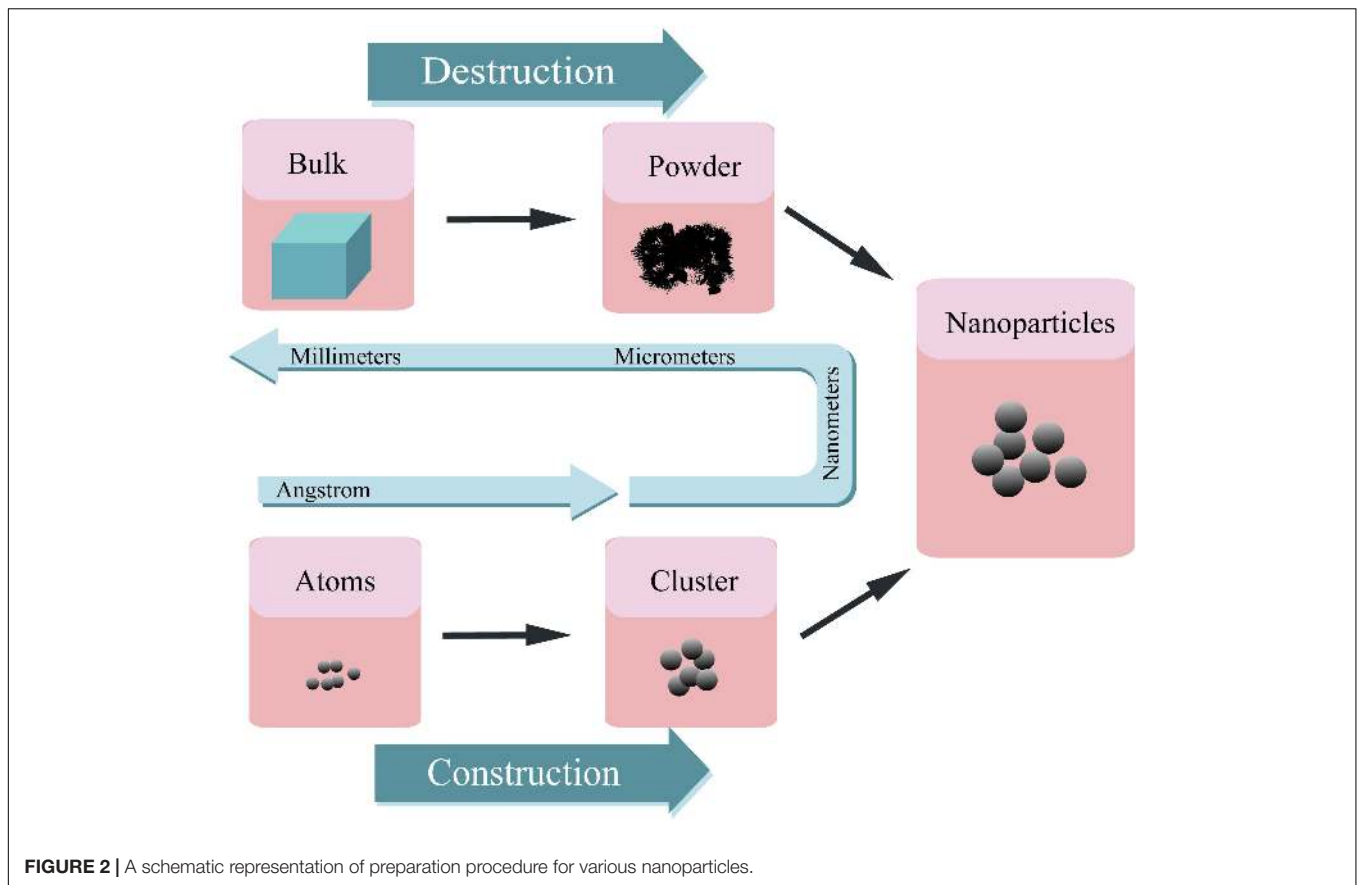
Antibacterial nanomaterials can be categorized into a metal ion and oxide photocatalytic type according to antibacterial active ingredients. Metal ion antibacterial nanomaterials are metallic ions with antibacterial functions (Ag, Cu, Zn, Ni, Co, Al) loaded in a variety of natural or synthetic substrates. They can slowly release the antibacterial ion components to periphery tissues in order to achieve the antibacterial and bactericidal effects. Oxide photocatalytic antimicrobial materials commonly are TiO<sub>2</sub>, ZnO, MgO, CdS, etc. and act in the catalysis of photocatalyst in which OH<sup>-</sup> and H<sub>2</sub>O molecules oxidized to OH free radicals with strong oxidation capacity. Thus, these activated surfaces can inhibit and kill microorganisms that exists in the environment (Buzea and Pacheco, 2017).

## PREPARATION OF NANOSTRUCTURE ON TITANIUM AND ITS ALLOYS

Titanium-based nanostructure (NS) materials have become the focus of current research because of their unique properties in optical, biological, and electrical fields (Miao et al., 2015; Gupta et al., 2018; Wei et al., 2020). Different preparation processes can be used to construct NS titanium surfaces, common nano-morphologies for titanium surfaces with antibacterial functions are nanotube and nano-coating forms. Titanium surfaces with different physical and chemical properties can influence the biological interactions and the adhesion of cells and bacteria (Zhou J. et al., 2018; Elbourne et al., 2019), which in turn affects the ability of early osseointegration and the risk of implant infection.

### Preparation of Nanotubes

Titanium dioxide nanotubes have received extensive attention because of their controllable size and highly ordered surface arrangement. Nanotubes have a larger specific surface area and storage space than other NS forms such as nanorods, nanopikes, and nanowires. These characteristics make it a good candidate among the other NS morphologies for the storage and release of antibacterial agents. The preparation of nanotube structures on the surface of titanium is mainly achieved by three methods: template synthesis (Jung et al., 2002; Lee et al., 2005), electrochemical anodization (Jun et al., 2012; Roman et al., 2014; Cao S. et al., 2018; Shang et al., 2019; Zhang et al., 2019d), and hydrothermal treatment (Tsai and Teng, 2004, 2006), they have different advantages and disadvantages listed in Table 1.

**TABLE 1** | Different nanotube formation processes.

Preparation technique	Shape and size	Characteristics	References
Template synthesis	(1) Tubular arrays or loose aggregates (2) Diameter: 10~500 nm (3) Length: Nanometer to micrometer	(1) Nanotubes with different diameters can be prepared (2) Removing the template may destroy the morphology of the nanotubes	Choi et al., 2017; Luo et al., 2018
Electrochemical anodization	(1) Highly ordered array of nanotubes (2) Diameter: 10~500 nm (3) Length: 100 nm~100 $\mu$ m	(1) Highly ordered (2) Low degree of aggregation	Fathy Fahim et al., 2009; Zhao et al., 2014
Hydrothermal treatment	(1) Single or loose block tube (2) Diameter: 2~20 nm (3) Length: nanometer to micrometer	(1) Simple process (2) It can prepare small diameter nanotubes (3) Difficult to form nanotube arrays	Wang L. et al., 2014; Aal et al., 2015

## Template Synthesis

The template synthesis method can be categorized into a hard template and a soft template method according to the nature of templating agent. The hard template method uses columnar single-crystal anodized aluminum oxide (AAO) (Hoyer, 1996; Ma, 2007; Newland et al., 2018) as a template to prepare nanotube structures by electrochemical deposition. The preparation process for the hard template is complicated, and the shape and size of the nanotubes are dependent on the size and shape of the template hole. Besides, the nanotubes destruction is possible during the separation step from the matrix template so unfortunately, it has poor reproducibility. In the soft template procedure surfactants used as templates

(Bernal et al., 2012; Choi et al., 2017). Firstly, the surfactant should be mixed with water, titanium alkoxide, and other substances, then the polymerization takes place under certain conditions. After drying and calcination, the nanotube structure is prepared. The soft template method overcomes the shape and size dependence to the template holes, which is one of the big limitations of hard templates. However, due to the necessity of high temperatures in removing step of soft templates, the nanotubes may collapse.

Template synthesis can prepare nanomaterials upon design demands and obtain well-formed nanoarrays, but it still has some drawbacks. For example, the AAO template as a commonly used master plate is mechanically weak which makes it difficult to

prepare metal templates with large areas (Masuda and Fukuda, 1995). Moreover, when using solvents to remove the organic compound templates, the structure of the nanomaterials may encounter some damages. Therefore, this synthesis method has the potential to be further optimized to synthesize utility materials with the desired functions using a simple operation (Bera et al., 2004).

### Electrochemical Anodization

Anodizing is one of the most commonly used methods for preparing TiO<sub>2</sub> nanotubes. The nanotube formation with a high aspect ratio and the excellent ordered condition can be attained by this method. The favorable nanotube size can be achieved by changing different factors (electrolyte composition, voltage, pH, anodizing time, etc.) (Gong et al., 2001; Ni et al., 2013; Wang L.N. et al., 2014; Khudhair et al., 2016; Song et al., 2017). Nanotube structures with tube dimensions in the range of 0.2–1000 μm length, 15–250 nm diameters and 10–70 nm thickness can be prepared with different anodic oxidation parameters. The main process of preparing titanium dioxide nanotubes by anodic oxidation are as follows: placing titanium foil or titanium sheet in a reaction cell (electrolytes are usually fluoride ions such as HF, NH<sub>4</sub>F, Macak et al., 2005; Alivov et al., 2009; Minagar et al., 2014) of two- or three-electrode system, and then a constant voltage application. The titanium sheet or foil is oxidized by the combined action of the electric field and fluoride ions. After some time, arrays of titanium dioxide nanotubes with uniform distribution, ordered arrangement and perpendicular to the substrate would be formed. The diameter of the nanotube can be controlled by the oxidation voltage (Bozkurt Çırak et al., 2017); the length of the nanotube is controlled by the combination of the oxidation time (Bhadra et al., 2020), the oxidation temperature (Mohan et al., 2020), and the pH value of the electrolyte (Cipriano et al., 2014); the smoothness of the nanotube, and the cleanliness of the surface are respectively dependent on the type of electrolyte and the water content (Sivaprakash and Narayanan, 2020).

### Hydrothermal Treatment

Hydrothermal treatment is among the momentous methods for nanotube production (Harsha et al., 2011). This method has the potential to produce titanium dioxide nanotubes with suitable crystalline structures (Swami et al., 2010). In this procedure, usually TiO<sub>2</sub> NPs were utilized as a titanium source to carry out a chemical reaction in a concentrated alkaline solution at high temperature. Subsequently, after ion exchange and calcination, the nanotube structure is reached (Wong et al., 2011). The hydrothermal method can synthesize nanotubes with different diameters and lengths by controlling the reaction conditions (Chi et al., 2007; Nakahira et al., 2010; Khoshnood et al., 2017; Mi et al., 2017). Hydrothermal synthesis is performed at high temperatures, and the rate of heating is a critical factor (Seo et al., 2001). Based on the rate of heating, the hydrothermal method can be divided into two categories: conventional and microwave hydrothermal procedures. In the traditional hydrothermal synthesis method, the sample is simply heated in a general

water bath. However, its heating rate is slow and the reaction cycle is long. Additionally, the heating of the reaction system is not uniform. The reaction system is exposed to microwave radiation during the microwave hydrothermal synthesis method. Since microwave heating is a rapid method of heating, the temperature of the reaction system very rapidly increases which results in a significantly reduced reaction period and the more uniform heating (Ribbens et al., 2008; Liu et al., 2014; Meng et al., 2016).

Titanium dioxide nanotubes can be synthesized by template synthesis, electrochemical anodization, and hydrothermal treatment. Additionally, there are some studies on the fabrication of nanotube structures by plasma electrolytic oxidation. Among the various possible methods, electrochemical anodization is the most commonly used method. By controlling the variables of the electrochemical anodization method, it is feasible to fabricate nanotube structures that satisfy the research needs. It is of great importance to delicately control the release of antibacterial agents in nanotubes and regulate the optimal size of nanotubes in order to promote cell adhesion, proliferation, and differentiation.

### Preparation of Nano Coating

The formation of nano-scale coatings on the surface of titanium can endow new functionalities to the surface. There are various methods for nano-coatings preparation. The conventional surface coating technologies are chemical vapor deposition (CVD) and physical vapor deposition (PVD). Newly developed methods include sol-gel, spin coating, plasma spraying, layer-by-layer self-assembly, and electrophoretic deposition, these methods are listed in **Table 2**.

#### Chemical Vapor Deposition

In the CVD method a single substance or compound containing one or more gas phases of elements is utilized to perform a chemical reaction on the substrate surface and produce a coating. In recent decades, even inorganic coatings can be produced through CVD technologies. Moreover, these methods can be used to purify various substances and precipitates, single-crystal, polycrystalline, and other inorganic thin-film materials (Jin et al., 2013; Manawi et al., 2018). Based on the influential parameters and chemical interactions, CVD methods can be divided into ambient pressure (Jang et al., 2015), low-pressure (Gao et al., 2011; Umrao et al., 2017), thermal (Zeng et al., 2018), ultra-high vacuum (Multone et al., 2008), and organic metal CVD methods (Maury and Senocq, 2003; Gong et al., 2013). Various oxide coatings, nitrides, and metal nano-coatings can be prepared by CVD methods based on the material and the required properties through its varied techniques (Delfini et al., 2017).

#### Physical Vapor Deposition

In the PVD technology, a physical phenomenon is used to vaporize the surface of source material (solid or liquid) into gaseous atoms, molecules, or into ions by ionization under vacuum condition. After the vaporization step, a low-pressure gas (or plasma) is implemented and a functional thin film is

**TABLE 2** | Various nano-coating preparation methods and the related characteristics.

Preparation technique	Characteristics	References
Chemical vapor deposition	(1) Lower equipment cost (2) Controllable coating density and purity (3) Coatings deposition on complex shapes (4) The coating is uniform and dense (5) Firmly combined with the base materials	Son et al., 2016
Physical vapor deposition	(1) Simple production process (2) No pollution, less consumables (3) The coating is uniform and dense (4) Firmly combined with the base materials	Hübsch et al., 2015
Spin-on deposition	(1) Simple production process (2) Low preparation cost and low pollution (3) Accurate and controllable coating thickness	Nguyen et al., 2020
Sol-gel method	(1) Simple production process and low equipment requirements (2) Can be prepared at room temperature (3) Large area coating (4) High purity and homogeneous coating	Hübsch et al., 2015
Plasma spraying	(1) Simple production process (2) Suitable for multiple materials (3) Coating with low porosity, high density and smooth feature	Mahade et al., 2019
Layer-by-layer self-assembly	(1) Simple production process without any need to special equipment (2) Suitable for multiple materials, including polymer materials (3) Can precisely control the coating structure and size	Elmi et al., 2019
Electrophoretic deposition	(1) Simple production process and convenient operation (2) Low preparation cost (3) Accurate thickness control, chemical composition, and porosity (4) Low temperature requirements	Pishbin et al., 2013

deposited on the substrate surface (Chouirfa et al., 2019). The PVD technology can deposit not only metallic and alloy films but also it is capable of depositing ceramics and polymers. The main PVD methods are vacuum evaporation, sputtering deposition, and ion plating.

Vacuum evaporation uses laser and electron beam heating to evaporate the material source into atoms or ions, and subsequently deposits atoms or ions onto the surface of the substrate to form a coating (Kumar et al., 2009; Garbacz et al., 2010). The resultant coating from this method has relatively large pores and poor adhesion to the substrate (Xingfang et al., 1988; Scott et al., 2003; Krysina et al., 2020). Sputter plating uses the base material as the anode and the target material as the cathode. By using the sputtering deposition effect, the argon ions generated by argon ionization knocks out the target material atoms and deposits them on the surface of the base material. The characteristic of this kind of coating is the presence of a few pores, but it can combine with the substrate more efficiently (Ratova et al., 2017; Makówka et al., 2019; Zhang et al., 2019a). Ion plating is involved with the ionization of gasses or vaporized substances under vacuum conditions, during the bombardment of gas ions or vaporized material ions, evaporates, or other reaction products are deposited on the substrate. The coating prepared by this method is uniform and dense, basically free of pores with a strong binding with the substrate (Li et al., 2017c; Zhang et al., 2018a; Tian et al., 2019).

With the development of PVD methods, many advanced PVD-based technologies have been derived which facilitate the production of high-quality nano-coatings. These new developed technologies include activated reactive evaporation (Bulla et al., 2004; Biju et al., 2009; Yuvaraj et al., 2010), activated reactive sputtering (Alajlani et al., 2016, 2017), activated reactive ion plating (Xin et al., 2000), magnetron sputtering (Lelis et al., 2019; Avino et al., 2020; Vuchkov et al., 2020), magnetron sputtering pulsed laser deposition (MSPLD) (Endrino et al., 2002; Jones and Voevodin, 2004), ionized magnetron sputtering (Kusano et al., 1999; Tranchant et al., 2006), pulsed laser deposition (PLD) (Paneerselvam et al., 2020; Wang et al., 2020), and etc.

### Sol-Gel Method

Sol-gel technology uses some compounds with high chemical activity as precursors. After the raw materials are uniformly mixed in the liquid phase, hydrolysis and condensation chemical reactions are carried out to form a stable sol. It reacts with water in a certain solvent and forms a sol through hydrolysis and polycondensation interactions. After the sol is aged, the colloidal particles slowly polymerize to form a gel with a three-dimensional grid structure. After the gel is dried, sintered, and solidified on the surface of the substrate, the NS coating would be achieved (Antonelli and Ying, 1995; Kim et al., 2004).

## Spin-on Deposition

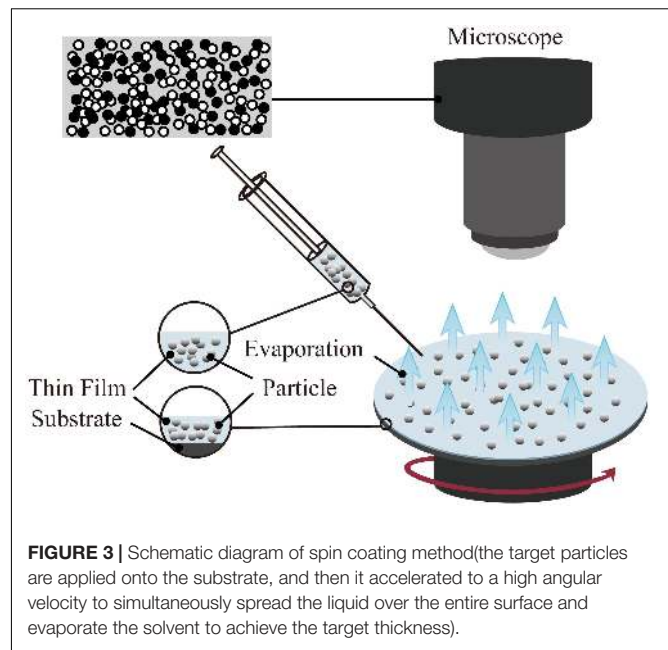
One of the coating preparation methods entitled as the spin coating is able to precisely control the thickness. However, the size of the substrate is limited by the size of the spinning device (Abu-Thabit and Makhlof, 2020). The thickness of the coating prepared by the spin coating method is in the range of 30 and 2000 nm. The typical spin coating method is mainly divided into three steps: glue dispensing, high-speed rotation, and drying. First, the spin-coating droplets are injected onto the surface of the substrate. Then, the spin coating solution is spread on the surface of the substrate through high-speed rotation to form a uniform film. Finally, the remaining solvent is removed by drying; hence the stable coating is obtained. In the process of preparing the coating by spin coating, high-speed rotation, and drying are the key steps to control the thickness, structure, and performance of the coating. The schematic of the spin coating method is shown in **Figure 3**.

Spin coating technology has been successfully applied in the fields of optics (Chtouki et al., 2017; Al-Douri et al., 2018) and electricity (Oytun and Basarir, 2019; Yildiz et al., 2019). Simultaneously, the spin coating method is also used in the preparation of functional thin films in the fields of biology and medicine. For example, a hydrophilic or hydrophobic film is produced on the surface of the base material to achieve the purpose of antibiosis (Kaviyarasu et al., 2017; Huang Y. et al., 2019; Li H. et al., 2020) and anti-corrosion (Kim et al., 2013; Akram et al., 2020).

## Plasma Spraying

Plasma spraying technology uses a plasma arc that driven by direct current as a heat source to heat ceramics, alloys, metals, and other materials to a molten or semi-molten state (Fauchais and Montavon, 2007; Mostaghimi and Chandra, 2007). These materials are sprayed onto the surface of the base material at high speed, and a firmly attached surface coating is formed, this process is schematically presented in **Figure 4**. Plasma spraying is a fundamental nano-coating preparation process. This process has high stability and excellent controllability, and a variety of materials can be used to prepare the coating. At the same time, the prepared coating has low porosity and high deposition efficiency and it is suitable for preparing high melting point metal and ceramic coatings (Cheang and Khor, 1996; Huang et al., 2014; Goudarzi et al., 2018).

The plasma sprayed functional coatings can improve the thermal insulation, anti-oxidation, and surface optical performance of the base material. With further research on functional coatings prepared by plasma spraying, some advances have made in the biomedical field. For example, the preparation of silver-containing coatings with antibacterial effect on the surface of CoCr alloys using vacuum plasma spraying technique (Liang et al., 2020). Also, the HAp coating on the Ti6Al4V surface using the axial suspension plasma spraying method (Hameed et al., 2019), and the HAp coating prepared by the micro-plasma spray method (Wang et al., 2017), both presents enhanced biological performance than the traditional plasma spraying methods.



## Layer-by-Layer Self-Assembly

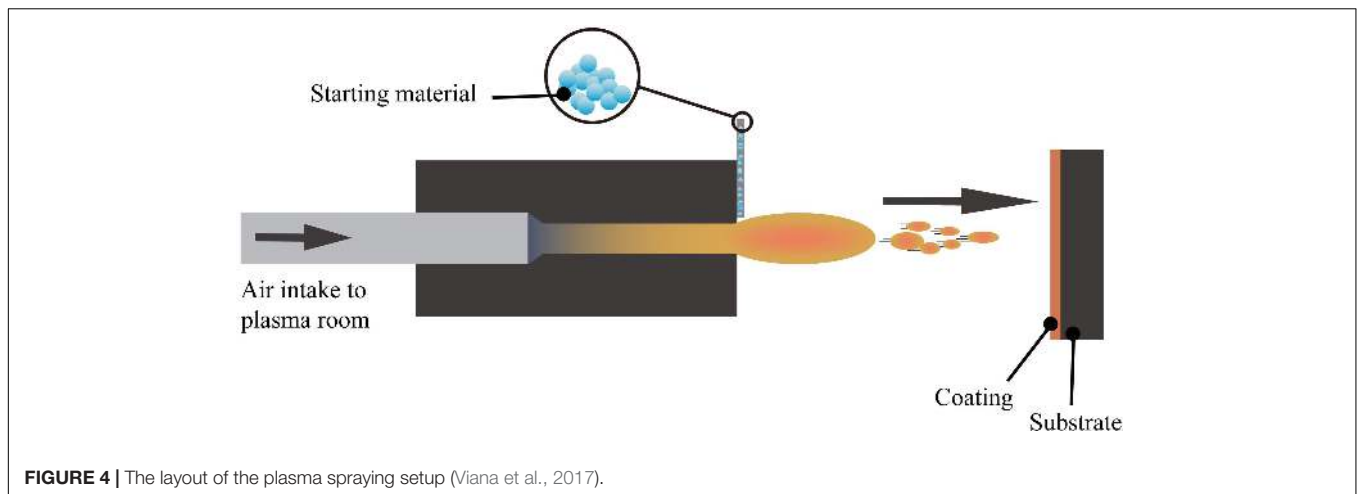
Layer-by-layer self-assembly technology is a relatively new technology in recent years and is widely used in the biology, materials, and nanoscience fields. As shown in **Figure 5** (Zhang et al., 2018d), this technology can assemble a variety of materials (polyelectrolytes, small organic molecules, NPs, etc.) and can precisely control the surface structure and size of the coating. Nanomaterials with ordered structure prepared by self-assembly technology show unique properties. Self-assembly technology is currently a hotspot in the field of nanomaterial research.

Layer-by-layer self-assembly technology can be used to fabricate filtration membranes (Rajesh et al., 2016), sensors (Fernandes et al., 2011), and optoelectronic devices (Eom et al., 2017). Besides, it is able to produce antibacterial coatings (Wu et al., 2015; Huang J. et al., 2019; Li D. et al., 2019; Xia et al., 2019) or drug controlled release coatings (Cao M. et al., 2018; Silva et al., 2018; Zhou W. et al., 2018; Sun et al., 2020; Wu et al., 2020). Hence, this technology seems to would have broad application prospects in the biomedicine field.

## Electrophoretic Deposition

Electrophoretic deposition is the directional movement of charged particles in the direction of the electrode under the action of an electric field. The outer layer of ions exerts pressure on the charged particles, forcing the particles to gather near the electrode and lead them to deposit (Besra and Liu, 2007). The method can prepare a coating with a thickness of 0.1–100  $\mu\text{m}$ , which can meet the coating thickness requirements of various medical implant materials. Electrophoretic deposition techniques with many advantages can be used to prepare bioceramic coatings on metallic substrates (Boccaccini et al., 2010; Avcu et al., 2019; Alaei et al., 2020).

Mahlooji et al. (2019) prepared the chitosan-bioactive glass (CS-BG) nanocomposite coating on the surface of Ti-6Al-4V



**FIGURE 4 |** The layout of the plasma spraying setup (Viana et al., 2017).

alloy by the electrophoretic deposition. The coating has excellent adhesive strength with the base material which can effectively promote the formation of apatite, and also it has a favorable biological activity. In addition, there are several related studies on the application of electrophoretic deposition technology to prepare bioactive glass coatings with various biological activities (Ur Rehman et al., 2018; Ghalayani Esfahani et al., 2019). There are also studies about the fabrication of antibacterial coatings by this method (Braem et al., 2017; Bakhshandeh and Amin Yavari, 2018; Ning et al., 2019; Thinakaran et al., 2020). A study used copper and chitosan to synthesize copper(II)-chitosan[Cu(II)-CS] complex coating, which has an excellent antibacterial effect on both Gram-positive and Gram-negative bacteria. In addition, Human osteoblast-like cells were cultured on the coating surface, which confirmed that the Cu (II) -CS coating had no cytotoxic effect (Akhtar et al., 2020). Furthermore, the results of a study about the chitosan hydrogel membrane (CHM) production by electrophoretic deposition show that the resultant coating can effectively promote the adhesion and growth of L-929 mouse fibroblast cells, and has good biocompatibility. It can also be loaded with suitable cells as a graft and is valuable from the application point of view (Li et al., 2017d).

Nanocoatings can be manufactured by various methods such as vapor deposition, different kinds of spraying, electrodeposition, etc. By considering the existing coating technologies, different manufacturing methods can be selected according to different needs. Adding the appropriate nanomaterials and precise adjustment of the coating parameters leads to manufacturing the desired nano-coatings. Compared with conventional coatings, nano-coatings have excellent mechanical properties, such as lower porosity, higher bond strength, higher hardness, oxidation resistance, corrosion resistance, etc. (Taylor and Sieradzki, 2003; Ranjbar and Rastegar, 2011; Lin et al., 2013). Therefore, the application of surface coatings in different fields will be very beneficial. In addition, the nanosurface coatings with antibacterial properties will be the great help in solving the implant-related infections and antibiotic resistance issues in clinical practice (Kose and Ayse Kose, 2015; Yilmaz and Yorgancioglu, 2018). There are

still many problems related to nanocoatings that need further research and discussion, such as the dispersion technique and stability of nanoparticles in the coating medium, the different properties of various types of nanoparticles, and their possible applications.

## NANOMATERIALS LOADING

Nanomaterials can be used as antibacterial agents on the surface of titanium or titanium alloys and they have a potential to effectively improve the bactericidal properties (Chen et al., 2014; Xin et al., 2019; Xu J.W. et al., 2019). Most of the studies were focused on the metallic nano-antibacterial agents on the surface of titanium and its alloys (Liu et al., 2015b; Gunputh et al., 2018; Cheng et al., 2019). In this regard, the common metal particles are silver, zinc, copper, etc. (Vimbela et al., 2017). The NPs are tiny particles with a particle size in the range of 1–100 nm. The specific properties of NPs like large specific surface area, small size effect, and quantum size effect makes them an ideal option. Therefore, nanomaterials have a series of excellent properties, which improves the bactericidal effects of antibacterial agents in comparison to traditional antibacterial agents (Mi et al., 2018; Guo et al., 2020).

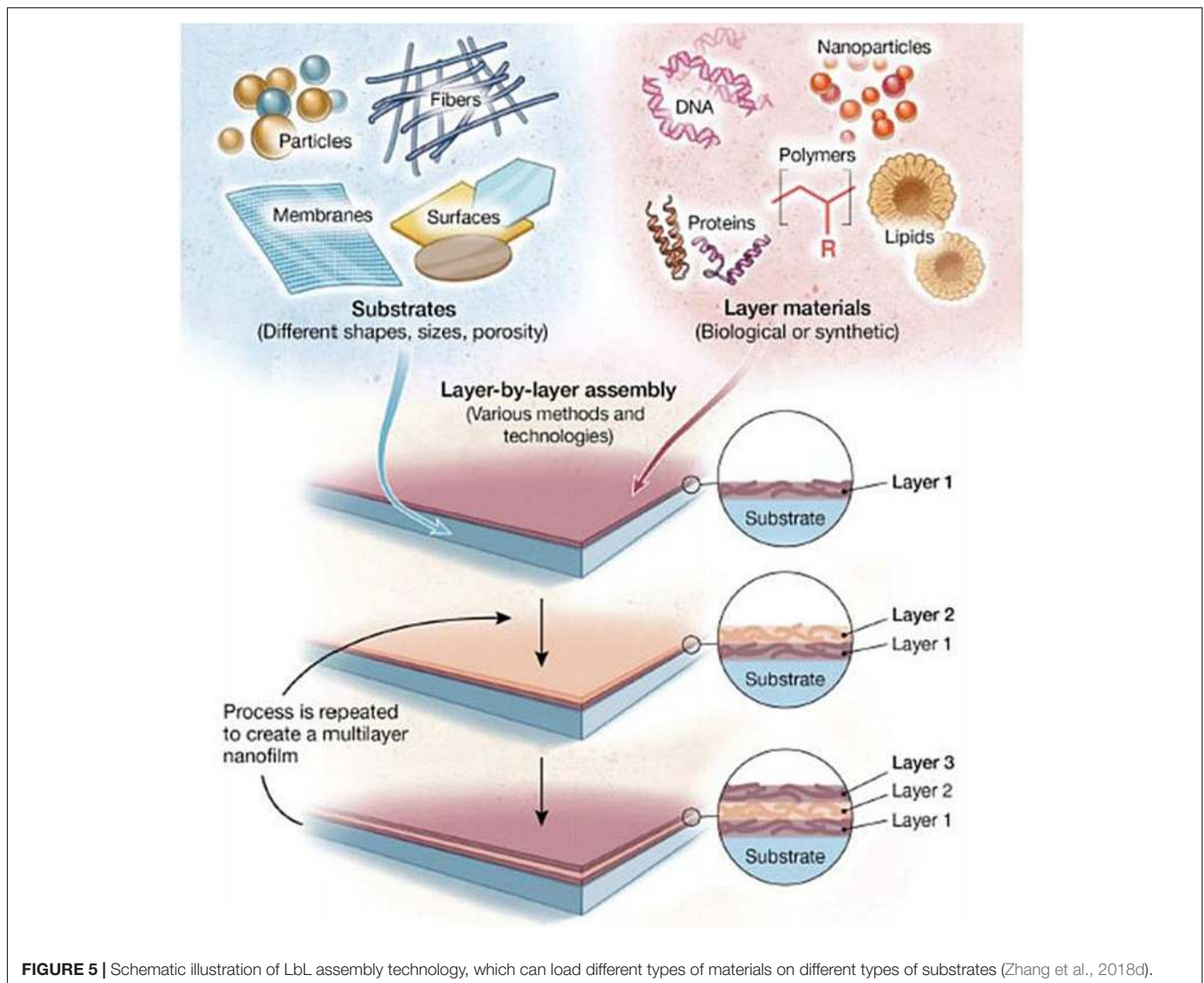
## Metallic Antibacterial Agents

Metallic antibacterial agents have exceptional research value because of their strong antibacterial ability, good biocompatibility, and excellent stability (Ahmed et al., 2016). Inorganic antibacterial agents are usually present on the surface of titanium substrates in the form of NPs. They can be fixed on the surface of the titanium substrate by using a carrier or coated on the titanium substrate's surface to prepare a nano-coating (Kheiri et al., 2019).

### Ag

Silver has many advantages in a broad-spectrum of antibacterial activity (Yang Z. et al., 2018; Wang L. et al., 2019). These advantages make it the most studied and widely used metal-based





**FIGURE 5** | Schematic illustration of LbL assembly technology, which can load different types of materials on different types of substrates (Zhang et al., 2018d).

antibacterial agent. Compared with traditional silver, silver NPs have a larger specific surface area, which significantly enhances their antibacterial ability. In the existing reports, the antibacterial mechanism of silver NPs mainly includes: destroying the structure of bacterial membranes, releasing silver ions and generating ROS to destroy enzymes in the oxidation respiratory chain, and regulating the signal transduction pathway of bacteria (Park et al., 2009; Tang and Zheng, 2018). Unfortunately, silver NPs can cause significant cytotoxicity above a specific dose range. The silver ions released by the silver NPs are highly mobile, and their entrance into living cells with high concentrations can kill healthy cells (AshaRani et al., 2009). The silver element forms which served as antibacterial are Ag<sub>2</sub>O NPs (Chen et al., 2017; Sarraf et al., 2018a; Lv et al., 2019) and Ag NPs (Cheng et al., 2019; Pruchova et al., 2019; Surmeneva et al., 2019). The silver NPs that were loaded on the surface of the titanium substrate to produce NS, showing different antibacterial effects and performance that is listed in **Table 3**.

A large number of experiments have proved that silver NPs can effectively exert antibacterial properties. Silver NPs have a good killing effect on Gram-positive cocci represented by *Staphylococcus aureus* and Gram-negative bacilli represented by *Escherichia coli* (Deshmukh et al., 2018; Subramaniyan et al., 2019). Currently, the biggest challenge is to enable the stable release of silver at a suitable concentration on the surface of metal implants (Yang Z. et al., 2019; Zhu Y. et al., 2019; Lai et al., 2020). The nanotube structure, which is prepared on the surface of the titanium substrate and loaded with silver or Ag<sub>2</sub>O NPs, is one of the solutions. Then, based on this structure, a controlled release coating (such as a polydopamine coating) is prepared, the controlled release phenomenon helps to achieve a long-term antibacterial effect (Gao et al., 2019). Compared with silver NPs, the amount of Ag<sup>+</sup> released from Ag<sub>2</sub>O NPs is lower, which may be due to the role of the oxide barrier. According to reports, Ag<sub>2</sub>O NPs have a larger total surface-to-volume ratio, which increases their contact area with

**TABLE 3** | Antibacterial effects and other related information about different silver-containing nanomaterials.

Materials	Base materials	Manufacturing technique	Nanostructure and Size	Antibacterial effect /Antibacterial rate	Cytotoxicity	Cells	References
Ag-CACS-Ti	Cp-Ti	<i>In situ</i> reduction	Nanoparticles	<i>E. coli</i> : 99.8% <i>S. aureus</i> : 99.9%	No	L929 cells	Cheng et al., 2019
Ag-TNTs	Ti-6Al-4V	Anodization+Chemical reduction	Nanoparticles: 102 ± 21 nm	<i>S. aureus</i> : 99.15%	–	–	Gunpath et al., 2018
Ag-HA	Ti-6Al-4V	Laser process	Nanoparticles: 20~30 nm	<i>S. aureus</i> : 77.59%	–	–	Liu and Man, 2017
Ag-HA-CS	Cp-Ti	Pulse electrochemical deposition	Nanoparticles: 303–321 nm	<i>E. coli</i> : 100% <i>S. aureus</i> : 100% <i>C. albicans</i> : 100% <i>P. aeruginosa</i> : 100%	No	BMSCs	Wang X. et al., 2019
Ag-PDA-TNTs	Cp-Ti	electrochemical anodization + <i>in situ</i> reduction	Nanoparticles: 13.5 ± 4.8 nm	<i>E. coli</i> : 54 ± 3.7%/14 days	–	–	Xu et al., 2017
Ag-PDA-TNTs	Ti-7.5Mo	Anodization + polydopamine assisted immobilization technique	Nanoparticles	<i>C. albicans</i> : 100%/48 h <i>S. aureus</i> : 100%/48 h	No	ADSCs	Rosifini Alves Claro et al., 2018
Ag-GO	Ti-67IMP	Physical vapor deposition magnetron sputtering + electrochemical anodization + spin coating	Nanoparticles	<i>E. coli</i> : 97.56%/24 h <i>S. aureus</i> : 98.15%/24 h	No	hFOB cells	Rafieerad et al., 2019
Ag-Ti	Cp-Ti	Target-ion induced plasma sputtering + Ag sputtering	Nanoparticles: 25 ± 5 nm	<i>E. coli</i> : 100%/12 h <i>S. aureus</i> : 100%/12 h	Yes	L929 fibroblast cells	Kim et al., 2018
TAN/TAP	Ti-Si	Vacuum arc remelting + sol-gel method	Nanoparticles: ~2 μm	<i>E. coli</i> : 98-100%/24 h <i>S. aureus</i> : 100%/24 h	No	L929 fibroblast cells; U-2OS human osteosarcoma cells	Horkavcová et al., 2017
Ag-Sr-HA coating	Cp-Ti	Hydrothermal method	Nanoparticles: ~100 nm	<i>E. coli</i> : 99%/24 h <i>S. aureus</i> : 95%/24 h	No	MG63 cells	Geng et al., 2016
Ag-HA coating	Cp-Ti	Electrostatic spraying	Nanorods: length: 50 nm, diameter: 20 nm	<i>E. coli</i> : 100%/24 h	No	Osteoblast	Gokcekaya et al., 2017
Ag film	Ti-6Al-4V	Thermal annealing + DC sputtering	Thickness: 20 nm	<i>E. coli</i> : 100%/24 h <i>S. aureus</i> : 100%/24 h	Yes	NIH3T3 fibroblast cells	Patil et al., 2019
Ag-TiN multilayers	Titanium alloy	Multi-arc ion plating	Thickness: 120 nm	<i>E. coli</i> : 99.88%/24h	Yes	MC3T3-E1 cells	Zhao et al., 2019
NiTiAg coating	Cp-Ti	Electrodeposition + anodization	–	<i>S. aureus</i> : 64.52% <i>S. epidermidis</i> : 92.35%	–	–	Huang et al., 2017

(Continued)

TABLE 3 | Continued

Materials	Base materials	Manufacturing technique	Nanostructure and Size	Antibacterial effect /Antibacterial rate	Cytotoxicity	Cells	References
Ag-DLC coating	Cp-Ti	Thermionic vacuum arc	Nanoparticles: 20 ± 3 nm	<i>S. aureus</i> : 94.2%/24 h	-	-	Mazare et al., 2018
Ag-CDHA	Ti-6Al-4V	Microwave assisted technique	Nanoparticles: 25.1–40.4 nm	<i>E. coli</i> : 83.68%/24 h <i>S. aureus</i> : 82.31%/24 h	No	MC3T3-E1 cells	Sikder et al., 2018
Ag-Pdop hybrid films	Ni-Ti	One-step immersion method	Nanoparticles: average 11.75 nm	<i>S. aureus</i> : >99%/24 h	No	hBMSCs	Yin et al., 2019
CS-Ag-MoS <sub>2</sub> -Ti	Cp-Ti	Ultraviolet light induced reduction	-	<i>E. coli</i> : 99.77%/24 h <i>S. aureus</i> : 98.66%/24 h	No	NIH3T3-E1 cells	Zhu et al., 2020
Ag <sub>2</sub> O-Ta <sub>2</sub> O <sub>5</sub> NTs	Ti-6Al-4V	Anodization + physical vapor deposition	-	<i>E. coli</i> : 100%/2 h	No	Osteoblast	Sarraf et al., 2018b
Ag <sub>2</sub> O-TNTs	Ti-6Al-4V	Anodization + physical vapor deposition	-	<i>E. coli</i> : 100%/2 h	No	Osteoblast	Sarraf et al., 2018a
Ag <sub>2</sub> O-TiO <sub>2</sub> coating	Cp-Ti	Micro-arc oxidation	-	<i>S. aureus</i> : 99%/24 h	No	NIH3T3-E1 cells	Lv et al., 2019
Ag <sub>2</sub> O-TNTs	Cp-Ti	Hydrothermal treatment	-	<i>S. aureus</i> : 100%/10 days; 84%/60 days	No	NIH3T3-E1 cells	Chen et al., 2017

TAN, *Titanium sol* + AgNO<sub>3</sub>; TAP, *Titanium sol* + Ag<sub>3</sub>PO<sub>4</sub>; DLC, diamond-like carbon; CDHA, calcium deficient hydroxyapatite.

microorganisms (Morones-Ramirez et al., 2005). Compared with Ag NPs, Ag<sub>2</sub>O NPs can also permeate or attach Ag<sup>+</sup> ions to the bacterial membrane to achieve better killing of bacteria, while reducing the negative impact on mammalian cells (Sarraf et al., 2018b). In addition, the antibacterial effect of silver NPs *in vivo* is noteworthy. Guan et al. (2019). develop a novel surface strategy involving the formation of polydopamine (PDA) and silver (Ag) nanoparticle-loaded TiO<sub>2</sub> nanorods (NRDs) coatings on Ti alloy. *In vitro* antibacterial experiments showed that Ag-TiO<sub>2</sub>@PDA NRDs coatings have antibacterial effects on Methicillin-resistant *Staphylococcus aureus* on days 7 and 14 according to the bacterial counting method. The efficiencies were 88.6 ± 1.5% and 80.1 ± 1.1%, respectively. The material was then implanted into the tibia of a rat model of osteomyelitis. After four weeks, the results of X-ray, micro-CT, and H&E staining showed that MRSA in the tibia of rats could be killed by Ag<sup>+</sup>, confirming that the material also had good antibacterial activity *in vivo*.

Moreover, with the release of silver NPs, the antibacterial properties of the implant material surface will gradually weaken hence it would be unable to have a long-term and stable antibacterial effect. Therefore, controlling the silver NPs release process and reducing the cytotoxic reactions caused by high concentrations of silver ions is one of the main research directions.

## Cu

Copper is an essential trace element for the human body. Copper plays an important role in maintaining the normal hematopoietic function of the human body, promoting the formation of connective tissue and maintaining the health of the central nervous system. At the same time, copper has strong antibacterial properties and is not prone to drug resistance. Copper NPs can play an essential role in inhibiting infection and forming bone matrix by releasing copper ions (Liu et al., 2019; Mou et al., 2019; Anitha and Muthukumaran, 2020; Lv et al., 2020). As a redox metal (Tripathi and Gaur, 2004; Rauf et al., 2019), it can catalyze the formation of ROS, and at the same time, can destroy the permeability of bacterial membranes, resulting in the leakage of reducing sugars and proteins from cells. These mechanisms caused fatal damage to the bacteria. The antibacterial properties and characteristics of Cu NPs and NS containing Cu NPs are shown in Table 4.

## Zn

Zinc is one of the important trace elements in the human body and plays a vital role in the growth and development of bones (Zhu et al., 2018). It was known that Zn can enhance the expression of M2 marker genes and proteins in macrophages. The adhesion, proliferation, and expression of osteoblast-related genes are increased by Zn (Zhang et al., 2018c; Chen et al., 2020). Zinc NPs are non-toxic and have a higher affinity to bacteria than ordinary zinc, which can lead to better antibacterial effect. In the current research on the antibacterial mechanism of zinc NPs, it has been found that zinc ions can destroy bacterial membranes and promote the production of ROS, thereby achieving antibacterial effects. Related research and results are shown in Table 5. To confirm the antibacterial effects and the

TABLE 4 | Antibacterial effects and other information of different copper-containing nanomaterials.

Materials	Base materials	Manufacturing technique	Nanostructure	Antibacterial effect/Antibacterial rate	Cytotoxicity	Cells	References
Cu NPs coating	Cp-Ti	Laser ablation	NPs: ~10nm	<i>S. aureus</i> : 83.21%/24 h		-	Fernández-Arias et al., 2020
Cu NPs coating	Cp-Ti	Plasma immersion ion implantation and deposition	Nanoparticles	<i>E. coli</i> : 50 ± 7%/24 h	No	rBMMS	Yu et al., 2016
CuO-TiO <sub>2</sub> coating	Cp-Ti	Magnetron sputtering	NPs: ~100 nm	<i>S. aureus</i> : 90 ± 1%/24 h	No	Osteoblasts	He et al., 2017
3Cu-HA coating	Ti-6Al-4V	Electrophoretic deposition	Nanoparticles: 50~100 nm	<i>S. aureus</i> : >99%/24 h	No	MG63 cells	Hacidi et al., 2017
5Cu-HA coating	Ti-6Al-4V	Electrophoretic deposition	Nanoparticles: 50~100 nm	<i>E. coli</i> : MIC: 700 µg/ml <sup>-1</sup> /20 h <i>S. aureus</i> : MIC: 1400 µg/ml <sup>-1</sup> /20 h	Yes	MG63 cells	Hacidi et al., 2017
2.5Cu-HA coating	Ti-6Al-4V	Laser deposition	Small aggregates: 200~1000 nm	<i>E. coli</i> : reduce growth to 42%/24 h <i>S. aureus</i> : reduce growth to 75%/24 h	No	NIH3T3-E1	Hidalgo-Robatto et al., 2018
Cu-TiO <sub>2</sub> NTAs	Cp-Ti	Anodizing magnetron-sputtered	-	<i>S. aureus</i> : >90%/28 d	No	EA, hy926 cells	Zong et al., 2017

feasibility of clinical applications Zinc-containing nanocoatings were studied, the results have shown excellent antibacterial effects both in vitro and in vivo antimicrobial experiments. Li et al. (2017b) prepared hybrid ZnO/poly-dopamine/arginine-glycine-aspartate-cysteine nanorod arrays on the titanium surface using atomic layer deposition and hydrothermal methods. The material was implanted into the femur of the rabbit model infected with *S. aureus*. After four weeks, femurs from animal models were tested by H&E staining and Giemsa staining, the zinc-containing nanorod arrays were less infected than the control soft tissues and bones, demonstrating that the release of zinc ions can play an effective anti-infective role.

## Au

Gold nanoparticles (Au NPs) as an antibacterial agent have been demonstrated in many researches (Grandi et al., 2011; Samanta et al., 2019). It has also some other surface functions such as photocatalysis, photothermal effect, and ROS-stimulating activity (Xia et al., 2015). Au NPs can also achieve antibacterial effects by destroying the cytoplasmic membrane of bacteria (Li et al., 2016). Au NPs are non-toxic and highly light stable, which can also be used as a probe to accurately locate biological macromolecules on the cell surface and within the cell, and can also be used for immunohistochemical localization.

Yang T. et al. (2019) prepared a gold nanorods (GNR) structure on the Ti surface by layer-by-layer self-assembly method. Also, they evaluate the photothermal antibacterial efficiency of Ti-GNR under near-infrared radiation (NIR) with a wavelength of 808 nm. The alamarBlue<sup>TM</sup> assay was used to detect the number of viable bacteria on different samples. The results showed that the antibacterial rates of Ti-GNR on *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa* were 45.72, 56.94, 14.61, and 20.24%, respectively. The NIR-treated Ti-GNR-NIR surface had antibacterial rates of 26.31, 31.84, 61.82, and 66.74%, respectively. The results show that the Ti-GNR surface after near-infrared radiation has high antibacterial activity against *E. coli* and *P. aeruginosa*. At the same time, the cell culture results showed that the Ti-GNR and Ti-GNR-NIR surfaces had lower cytotoxicity to MC3T3-E1 cells. Xu W. et al. (2019) prepared TiO<sub>2</sub> nanotube arrays (TNT) on Ti plates by anodizing, and then loaded gold NPs (Au NPs) into TNT. Under visible light, the antibacterial ability of nanotubes loaded with gold NPs against anaerobic bacteria was evaluated. The experimental results show that the average antibacterial efficiency of TNT materials loaded with Au NPs is above 85%, and the highest antibacterial rates for *F. nucleatum* and *P. gingivalis* can reach 92.13 and 97.34%.

## Ni

Nickel is an indispensable element in the human body, and its content is in minimal range in the human body. Nickel maintains the structural stability and metabolism of biological macromolecules. Lack of nickel can cause diabetes, uremia, kidney failure, and other diseases. Studies have shown that Ni<sup>2+</sup> can effectively kill bacteria (Yasuyuki et al., 2010), but excessive Ni<sup>2+</sup> will cause cytotoxicity (Lü et al., 2009; Hang et al., 2012). A recent study showed that Ni<sup>2+</sup> released from NiTi alloys could exhibit antibacterial properties (Ohtsu et al.,

**TABLE 5 |** Different antibacterial properties and biocompatibility of zinc-containing coatings.

Materials	Base materials	Manufacturing technique	Nanostructure	Antibacterial effect/Antibacterial rate	Cytotoxicity	Cells	References
ZnO-Sr-OPDA-TNTs	Cp-Ti	Anodization hydrothermal treatment + atomic layer deposition	ZnO film:thickness<2 nm	<i>E. coli</i> : 87%/12 h <i>S. aureus</i> : 91%/24 h	No	MC3T3-E1 cells	Zhang K. et al., 2017
ZnO-CS-CNTs-Ti	Cp-Ti	Electrophoretic deposition atomic layer deposition	ZnO film thickness: <10 nm	<i>E. coli</i> : 73%/24 h <i>S. aureus</i> : 98%/24 h	Yes	MC3T3-E1 cells	Zhu et al., 2017
ZnO-TiO <sub>2</sub> coating	Cp-Ti	Hydrothermal low temperature liquid phase	Nanoparticle diameters: 50 ± 8~80 ± 9 nm	<i>E. coli</i> : 93.2%/12 h <i>S. aureus</i> : 97.5%/24 h	No	MC3T3-E1 cells	Pang et al., 2019
Zn-Ti nanowires	Cp-Ti	Sol-gel + alkali heat	–	<i>S. aureus</i> : 66.58%/3 days <i>P. gingivalis</i> : 45.02%/3 days <i>A. actinomycetemcomitans</i> : 53.42%/3 days	No	MC3T3-E1 cells	Shao et al., 2020
Zn-CHI-GEL multilayer films	Cp-Ti	LBL self-assembly	Film thickness: 14.91 ± 0.97~17.72 ± 0.63 nm	<i>E. coli</i> : 47.37%/24 h <i>S. aureus</i> : 52.94%/24 h	Yes	Osteoblasts	Karbowniczek et al., 2017
ZnO-TiO <sub>2</sub> coating	Cp-Ti	Micro-arc oxidation	Nanoparticles: Average 30 nm	<i>S. aureus</i> : 51.4 ± 14.7%/24 h	No	MC3T3-E1 cells	Zhang et al., 2018b
ZnO-Ti coating	Cp-Ti	Micro-arc oxidation	–	<i>E. coli</i> : 48.08%/24h	–	–	Zhang et al., 2019c
ZnO-PPy-HA coating	Cp-Ti	Electrochemical deposition	Nanoparticle: Average 243 nm	<i>E. coli</i> : 63.5%/12h <i>S. aureus</i> : 72.8%/12h	No	BMSCs	Maimaiti et al., 2020
Zn-HA coating	Ti-6Al-4V	Co-precipitation + flame spraying	50–200 nm	<i>E. coli</i> : 99.9%/3 h	No	WST-1 cells	Yang et al., 2017
Zn-HA coating	Ti-6Al-4V	Plasma spraying	–	<i>E. coli</i> : 63.5%/7 days <i>S. aureus</i> : 36.6%/7 days	No	Saos-2 cells	Sergi et al., 2018

**TABLE 6** | The antibiotics used in various NS titanium and its alloys.

Materials	Antibiotic	Manufacturing technique	Base materials	Antibacterial effect/Antibacterial rate	Cells	Cytotoxicity	References
PCL-HA-Rf coating	Rifampicin	Electrospinning	Cp-Ti	<i>S. aureus</i> : >99.9%/24 h <i>MRSA</i> : >99.9%/24 h <i>S. epidermidis</i> : >99.9%/24 h <i>P. aeruginosa</i> : >99%/24 h	hFOB	No	Kranthi Kiran et al., 2019
HA-GS coating	Gentamicin	Vacuum cold spraying	Ti-6Al-4V	<i>E. coli</i> : 96.4%/31 days	Osteoblasts	No	Fathi et al., 2019
GS-PAA coating	Gentamicin	Layer-by-layer assembly	Ti-6Al-4V	<i>E. coli</i> : 99.93%/16 h <i>S. aureus</i> : 99.86%/16 h	-	-	He et al., 2020
Van-Sr-HAP nanocomposite	Vancomycin	Co-precipitation	Cp-Ti	<i>MRSA</i> : 99.45%/12 h <i>MSSA</i> : 98.22%/12 h	MSCs	No	David and Nallaiyan, 2018
NFX-PLGA coating	Norfloxacin	Nano spray drying	Cp-Ti	<i>E. coli</i> : 95.42~99.93%/24 h	L929	No	Baghdan et al., 2019

2017). However, due to slight  $\text{Ni}^{2+}$  release from the NiTi alloy, its antibacterial ability is relatively weak. Therefore, in nanometric range the specific surface area of the NiTi alloy is increased, and the release amount of  $\text{Ni}^{2+}$  is increased, thereby enhancing its antibacterial ability. Hang et al. (2018) produced Ni-Ti-O NPs with different lengths (0.55–114  $\mu\text{m}$ ) on NiTi alloys by anodization. The antibacterial effect of different samples to *S. aureus* was determined by the plate counting method. It was found that the antibacterial rate increased as the anodizing time increased. When the length of Ni-Ti-O NPs exceeds 11  $\mu\text{m}$ , the antibacterial rate can reach 100% along with its excellent biocompatibility.

Liu et al. (2018) also used anodizing method to prepare Ni-Ti-O NPs on NiTi alloy and studied the effects of different annealing temperatures (200, 400, 600°C) on antibacterial properties and biocompatibility. The length of the NPs is 2.05–2.78  $\mu\text{m}$ . The results show that the antibacterial rate of the smooth NiTi alloy surface is only 36%, and the antibacterial rate of the surface after anodizing reaches 84%. After annealing at 200°C, the antibacterial rate of Ni-Ti-O NPs was close to 100%. Ni-Ti-O NPs annealed at 200–400°C all showed good cell compatibility. Therefore, the preparation of NPs with different sizes on the surface of NiTi alloy can effectively enhance the antibacterial effect of the material by increasing the specific surface area.

An enormous number of studies on different inorganic nanoparticles (Ag, Cu, Zn, Au, Ni) indicate that Ag NPs as antibacterial agents have very efficient antibacterial performance compared to other antibacterial agents. At present, the mechanism of antibacterial actions of silver and silver ions is still under controversy. As mentioned previously, Ag NPs stimulate the generation of ROS and induce high oxidative stress, which is thought to be the foremost antibacterial mechanism (Park et al., 2009; Siritongsuk et al., 2016). Under normal circumstances, ROS generated in the cell receives a restriction that can be eliminated by antioxidants (Ramalingam et al., 2016). The antibacterial effect of Ag NPs stems from the dehydrogenase inactivation in the oxidative respiration chain along with excessive ROS generation. These circumstances inhibit oxidative respiration and the natural growth of the cells (Su et al., 2009; Quinteros et al., 2016). In addition, two antibacterial mechanisms, contact killing, and ion-mediated killing are widely accepted. Ag NPs can anchor to the bacterial cell wall and infiltrate it, which can cause physical changes to the bacterial membrane (bacterial membrane damage, leakage of bacterial contents) and ultimately lead to bacterial death (Khalandi et al., 2017; Seong and Lee, 2017). The primary antibacterial form of Ag NPs is the silver ion ( $\text{Ag}^+$ ) (Liu and Hurt, 2010; Le Ouay and Stellacci, 2015), and the target of  $\text{Ag}^+$  has been identified as a number of molecules [DNA, peptides (membrane-bound or inside the cell) or cofactors] (Le Ouay and Stellacci, 2015). The interaction of Ag NPs with cellular structures or biological molecules will result in impaired bacterial function and ultimately death. Antibiotics are usually used to attack particular molecules of certain bacteria, but silver ions react with all the nearby molecules, thus having a wide-spectrum antibacterial effect. Silver does not react with water, but can easily dissolve in water with the presence of an oxidizing agent

TABLE 7 | Antibacterial NS formation methods and the related properties.

Feature	Manufacturing technique	Base materials	Antibacterial effect and rate	Cells	Cytotoxicity	Wettability	Roughness	Morphology	References
Nanoflowers	Chemical etching + hydrothermal oxidation	Cp-Ti	<i>S. aureus</i> : 43.12%/24 h <i>MRSA</i> : 73.15%/24 h	hFOB	No	Hydrophilicity	Ra = 829 nm	A	Vishnu et al., 2019
Nanowires	Hydrothermal synthesis	Ti-6Al-4V	<i>S. aureus</i> : 7.4%/18 h	Osteoblasts	No	Hydrophilicity	-	B	Jaggessar et al., 2018
Regular nanotubes	Acid etching + anodic oxidation	Ti-6Al-4V	<i>E. coli</i> : 72.6%/2 h <i>S. aureus</i> : 68.2%/2 h	MG63	No	Hydrophilicity	Ra = 120 nm	C	Rahnamaee et al., 2020
Irregular nanotubes	Electrochemical anodization	Ti-6Al-4V	<i>E. coli</i> : 48.7%/2 h <i>S. aureus</i> : 50.8%/2 h	MG63	No	Superhydrophilicity	Ra = 360 nm	D	Rahnamaee et al., 2020
Nanotubes	Electrochemical anodization	Cp-Ti	<i>S. aureus</i> : 36.78%/16 h	-	-	Hydrophilicity	$R_{rms} = 45.60$ nm	E, F	Simi and Rajendran, 2017
Nano-ripples	Femtosecond laser direct writing	Cp-Ti	<i>E. coli</i> : 56%/24 h	MSCs	No	Superhydrophilicity	Ra = 274.6 nm	G	Luo et al., 2020
Nanoparticles	Aerosol flame synthesis	Aluminum alloy	<i>S. aureus</i> : 80%	-	-	Superhydrophilicity	-	H	De Falco et al., 2018
Nanopillars	Plasma etching	Cp-Ti	<i>P. aeruginosa</i> : 87 ± 2%/24 h <i>S. aureus</i> : 72.5 ± 13%/24 h	-	-	Hydrophobicity	-	I	Linklater et al., 2019

(oxygen), which is termed oxidative dissolution. The oxidative dissolution of silver is also an important mechanism for its antibacterial action (Mollema and Hiemstra, 2015), through the above mentioned multiple mechanisms, which directly or indirectly lead to the ability of Ag NPs to exert efficient antibacterial effects.

The issue of cytotoxicity of Ag NPs still needs further studies since it depends on lots of factors, including the shape of NPs, dimension, concentration, etc. (Attarilar et al., 2020), since high concentration of Ag NPs may be cytotoxic. Moreover, some other studies demonstrated the influence of NPs' size on cytotoxicity. Several studies have shown that Ag NPs with the small size range (<20 nm) may cause varying degrees of cytotoxic effects, while large or condensed particles (>100 nm) sometimes do not have any considerable adverse outcomes (Marambio-Jones and Hoek, 2010; Yang et al., 2012; Rizzello and Pompa, 2014; Foldbjerg et al., 2015). Traditionally, antibiotics targeting specific infections or classes of bacteria have been used for implant material infections. In this regard, Ag NPs have a substantial potential to replace with antibiotics due to their favorable antibacterial properties and broad antibacterial activity over other inorganic nanoparticles. Combining Ag NPs with implant materials for in vitro and in vivo studies makes them promising antibacterial implant materials. To reduce the toxicity of silver and to decrease the level of silver in the blood, future research should focus on developing effective techniques for combining silver nanoparticles with the implant materials that can release silver ions in a controlled and harmless way.

## Antibiotics

In addition to using inorganic antibacterial agents to prepare materials with antibacterial properties, antibiotics can also be used on titanium or its alloys' surfaces to prepare nano-coatings or other NS to achieve antibacterial effects (Li et al., 2017a; Liu et al., 2017; Mohan Raj et al., 2018). The chosen antibiotics need to be able to kill Gram-positive cocci and Gram-positive bacillus effectively (Hickok and Shapiro, 2012). Available antibiotics include rifampicin, gentamicin, vancomycin, etc. (Simchi et al., 2011) (Table 6). Under ideal conditions, the antibiotics released by the prepared nanomaterials should reach the effective drug concentration and should maintain a long sufficient sterilization time (Salwiczek et al., 2014; Nguyen-Tri et al., 2019).

## BIONIC NANOSTRUCTURES

In recent years, the wings of insects, such as dragonflies and cicadas, have attracted much attention as a model biological system because of their excellent antibacterial and antifungal properties (Ivanova et al., 2012; Diu et al., 2014; Kelleher et al., 2016). Some studies have shown the presence of physical nano-protrusions on the surface of insect wings. Their antibacterial properties may be due to the fact that when microbial cells come in contact with the protrusions, they possibility enhance the stress and deformation of the membrane structure of the microbial cells, leading to their

destruction. It eventually leads to the dissolution and death of the cells (Ivanova et al., 2013; Nowlin et al., 2014; Bandara et al., 2017). By investigating the surface structure of insect wings as a model and preparing bionic structures according to it, new ideas for preparing modern antibacterial titanium alloys have emerged.

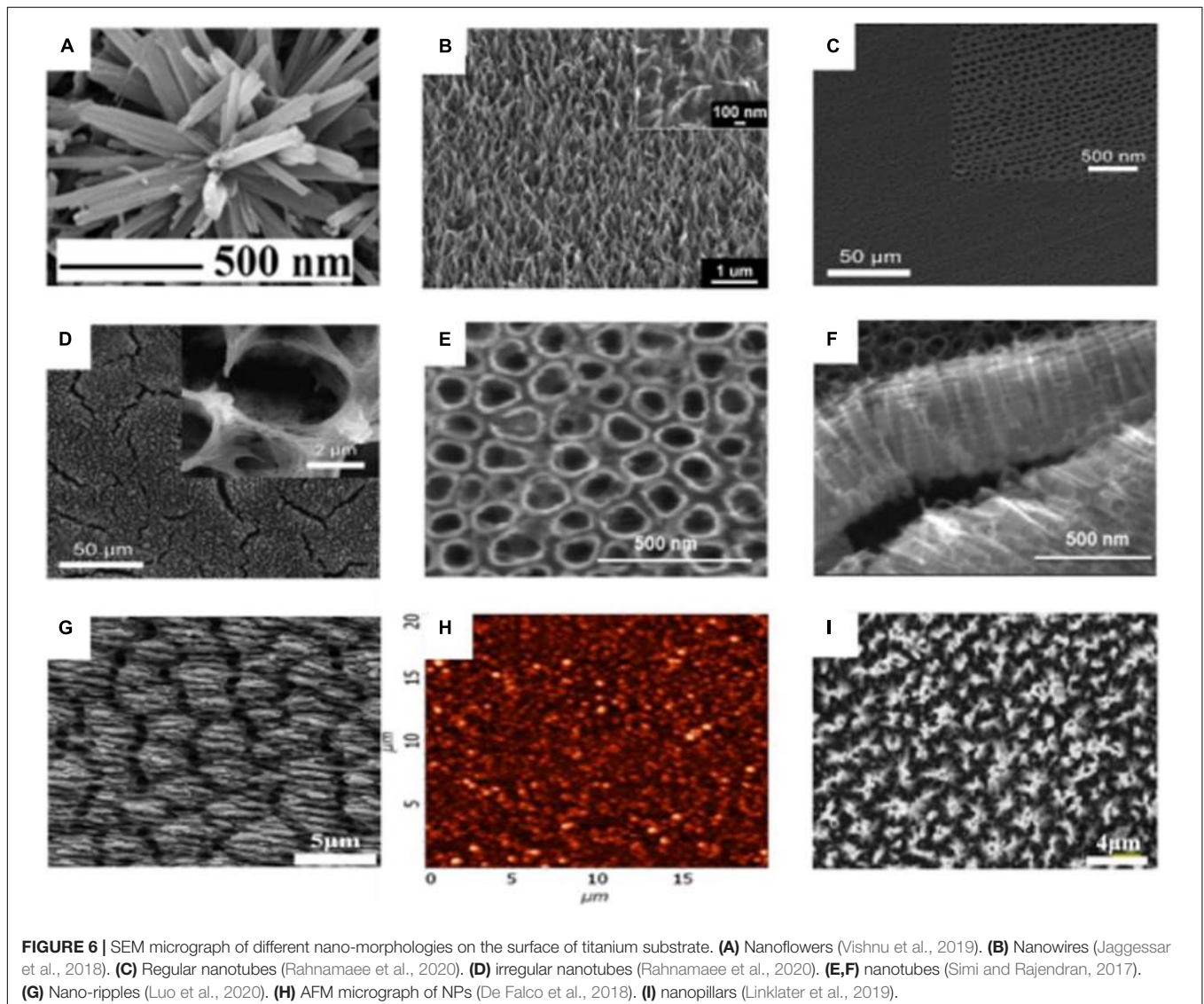
## Antibacterial Nanopatterns and Fabrication Methods

It has been shown that modification of the surface morphology of materials can be used to achieve antibacterial effects and inhibition of biofilm formation (Modaresifar et al., 2019; Stratakis et al., 2020). By changing the surface morphology without adding other chemical reagents, the antibacterial and antibiofilm formation properties can also be achieved (Campoccia et al., 2013; Hasan et al., 2013). Moreover, it has a slight effect on the mechanical strength of the material.

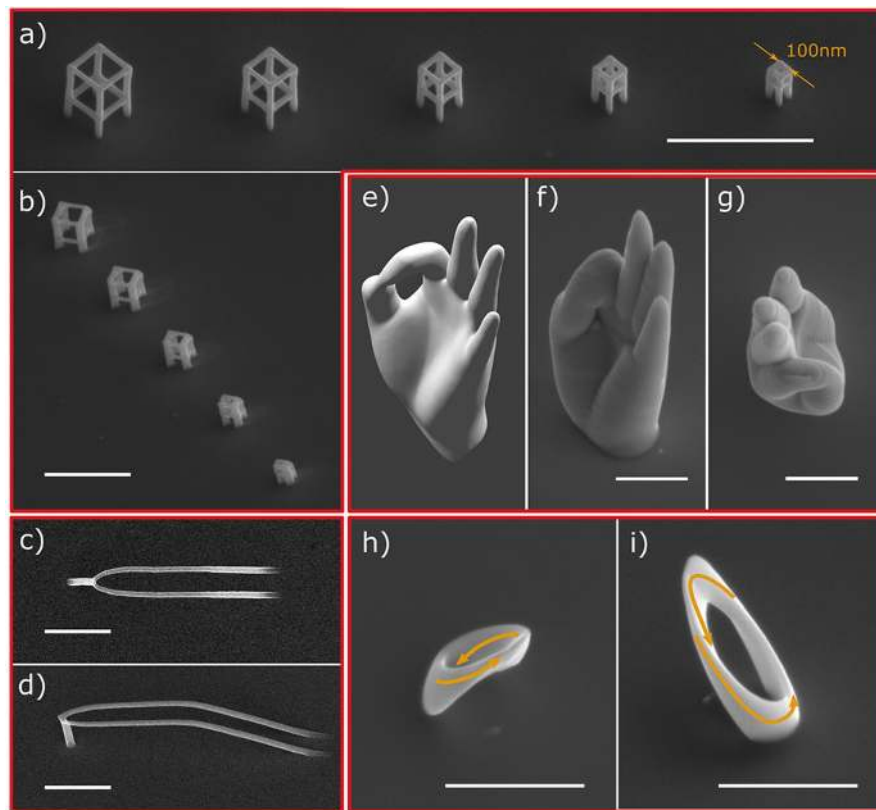
Changing the surface morphology to prepare biomimetic structures with micron and nano-scale surface morphologies, and exploring the effect of surface morphology and size of titanium or its alloys that can effectively attack bacteria are the currently urgent problems to be solved. **Table 7** lists some of the antibacterial NS formation methods and their properties.

Different nano-morphologies can be prepared through different preparation processes: nanoflowers, nanowires, nanotubes, nano-ripples, NPs, and nanopillars are possible morphologies, **Figure 6**. Among these nano-topographies, nanopillars show good bactericidal effect, which may be related to its high aspect ratio (Linklater et al., 2018). In each nanotopography, cell activity was not significantly inhibited. Furthermore, in some nanotopographies, the cells' metabolic activity tends to increase (Jaggessar et al., 2018).

There are several methods to fabricate nanopatterns, and the commonly used methods are chemical etching (Heidarpour







**FIGURE 7** | SEM images of different 3D geometries prepared by the focused electronbeam-induced deposition (FEBID) method. **(a,b)** A series of cubes. **(c,d)** Smooth nanowires. **(e–g)** Nanoscale replica of a human hand. **(h,i)** Cobalt Möbius strip (Skoric et al., 2020).

et al., 2020), reactive ion etching (Ganjan et al., 2019), plasma etching (He et al., 2013), hydrothermal synthesis (Jaggessar and Yarlagadda, 2020; Wategaonkar et al., 2020), and anodic oxidation (Mohan et al., 2020). The antibacterial nanopatterns with different antibacterial efficiencies have been prepared by these methods, but the antibacterial effect of these patterns is still not very satisfactory, which may be due to the fact that the production of antibacterial surfaces on titanium and its alloys are more difficult than other materials [silicon, polymethylmethacrylate (PMMA), etc.]. The development of nanopatterns with efficient antibacterial properties can enable the better clinical application of titanium-related medical materials and may address the bacterial resistance problem caused by antibiotic abuse. Currently, some novel methods for nanopatterning have been developed, such as two-photon polymerization (2PP) (Liao et al., 2020) and electron beam induced deposition (EBID) (Perentes et al., 2004; Skoric et al., 2020). Two-photon polymerization is a new 3D structure fabrication technology based on CAD/CAM, that precisely constructs 3D geometries with resolutions down to 100 nm. 2PP's high resolution, adaptability to a wide range of materials, and the ability to create true 3D structures make it a very promising technology for the fabrication of medical implants (Doraiswamy et al., 2006; Ovsianikov and Chichkov, 2012). EBID fabrication technology is also gaining much attention (Hirt et al., 2017),

enabling the fabrication of 3D structures in tens of nanometers and the deposition of a wide range of materials (metallic, organic, semiconducting, magnetic, superconducting, etc.) (Utke et al., 2008; Huth et al., 2018). EBID technology currently achieves vertical growth rates of hundreds of nanometers per second (Winkler et al., 2018) and can improve processing efficiency through several methods: optimizations of gas injection systems (GIS) (Friedli and Utke, 2009), deposition at low temperatures (Bresin et al., 2013), and simultaneous deposition of multiple beams (Riedesel et al., 2019). A variety of 3D geometries can be prepared by this method, as shown in **Figure 7**. In the future, the development of high-resolution, efficient, and controllable 3D nanomorphology methods will be a critical challenge to be overcome.

## Antibacterial Mechanism of Nanopatterns

Nanoscale structures with specific dimensions are fabricated on the surface of the substrate material, and this nanoscale structure performs mechanocidal action through different mechanisms. The interactions between nanopatterns and bacteria are multifaceted, and the exact mechanism of bactericidal action and the role of various factors in regulating bactericidal behavior are still controversial (Bandara et al., 2017; Linklater et al.,

2017). Most researchers agree that nanopatterns with high aspect ratio are key factors in causing mechanical deformation of the bacterial cell walls, which in turn causes their rupture and death (Watson et al., 2015; Truong et al., 2017; Cao Y. et al., 2018). Xue et al. (2015) have developed mathematical models to explain the bactericidal properties of nanopatterns on the surface of cicada wings. Gravity as well as non-specific forces (such as van der Waals forces) have been demonstrated to play a role in bacterial cell wall rupture. In addition, extracellular polymeric substance (EPS) has been demonstrated to play an important role in regulating the bactericidal effects of nanopatterns, they are known as natural biopolymeric mixtures of proteins and polysaccharides which excreted by microorganisms. EPS plays an important role in the formation of biological membranes, also it promotes cell signaling and protects bacteria from the harmful effects of environmental factors (Limoli et al., 2015). Bandara et al. (2017) found that some bacteria under the influence of nanopillars secrete EPS with adhesion. Bacteria find the adherent surface unfit to survive and try to move away, and the anchoring action of EPS leads to rupture of the bacterial cell wall and bacterial death. Furthermore, the applied mechanical forces affect the metabolomics and bacterial genome and may also be the mechanism by which bacteria die on their surfaces (Rizzello et al., 2011; Belas, 2014; Persat, 2017; Velic et al., 2019). The mechanism of the antibacterial effect of nanopatterns is still not completely clarified, hence the research on the antibacterial mechanisms is crucial for the preparation of materials with excellent antibacterial effect.

## Factors Affecting the Antibacterial Property of Nanopatterns

The design parameters (height, diameter, and spacing) of the nanopatterns have a significant influence on the antibacterial properties. Nanopatterns with different parameters (height from 100 nm to 900 nm, diameter from 10 nm to 300 nm, and spacing less than 500 nm) have been reported in the literature to exhibit bactericidal properties (Modaresifar et al., 2019). Velic et al. (2019) studied the effect of varied design parameters of nanopatterns on bacteria behavior by developing a two-dimensional finite element model and demonstrated that reducing the pillar diameter could effectively promote bactericidal efficiency. Additionally, preparing nanopillar structures with similar diameters but different densities and heights showed that extra stretched nanopillars with varying heights leads to rupture in the bacterial cell membranes (the membranes exceeded the threshold value of stretching) (Wu et al., 2018).

The occurrence of implant material-related infections often begins with bacterial adhesion to the implant surface subsequently the colonization of bacteria and biofilm formation happens. Biofilms are bacteria produced microbial communities formed by the EPS substrate to inhibit the damage of bacteria. Statistically, about 99% of bacteria could exist with biofilm status (Zimmerli and Sendi, 2017). Biofilms exist as reservoirs

for bacteria, often leading to chronic and systemic infections. Therefore, reducing the opportunity of bacteria to adhere to the surface of the implants is essential to prevent infection. The adhesion of bacteria to a material surface depends on the surface properties of the material, such as surface roughness, wettability, and topography. Bacteria are more likely to adhere to rough rather than smooth surfaces (Sarró et al., 2006; Fan et al., 2013) and more likely to adhere to hydrophobic rather than hydrophilic surfaces (Pagedar et al., 2010). At the same time, the nanoscale surface has a higher resistance to bacterial adhesion than the micron and macroscale surfaces (Singh et al., 2011; Spengler et al., 2019). The severe plastic deformation (SPD) process is a recently developed technique for the fabrication of nanostructures (Dyakonov et al., 2019; Shuitcev et al., 2020). It is noteworthy that by reducing the grain size to the sub-micron range (100–500 nm) through SPD processes, the mechanical properties of commercially pure titanium are comparable to those of conventional Ti-6Al-4V alloys (Singh et al., 2011). It was found that the increase in Ra of the pure titanium surface after SPD processing was followed by an increase in the adhesion density of *S. aureus*, which was independent to *P. aeruginosa*. The effect of surface hydrophilicity on bacterial adhesion can be explained by thermodynamics (Bruinsma et al., 2001), with a higher affinity of hydrophobic bacteria for hydrophobic surfaces and of hydrophilic bacteria for hydrophilic surfaces (Sabirov et al., 2015).

## CONCLUSION

Nanomaterials have the characteristics of small size and large specific surface area which lead them to have more potent antibacterial activity and drug loading capacity than traditional materials, thus showing an excellent antibacterial effect. However, once the safe antimicrobial material exceeds its safe dose during the release process, it will produce cellular cytotoxicity and even affect the osteogenic performance of titanium implant materials' surfaces. Moreover, the antibacterial ability of the implant material surface will gradually weaken with the release of antibacterial substances. The nano-morphology on the surface of the titanium implant material can achieve a long-term antibacterial effect. However, the destructive effect of the nano-morphology surface on different bacteria is quite different, and the antibacterial range is limited. At present, the nanometer modification of implant materials is still in its infancy and lots of improvement must be done in order to make them an ideal option for medical applications both form cytotoxicity and functionality, this study aims to produce a guideline and achieve the as-mentioned goals. In this regard, nanostructure formation methods including template synthesis, electrochemical anodization, and hydrothermal treatment was discussed. In addition, nano coating methods such as CVD, PVD, sol-gel, spin coating, electrophoretic deposition, plasma spraying, and LBL technologies were introduced. Then different antibacterial agent loading on these nanostructured surfaces were analyzed. The loading substances can have metallic nature like Ag, Cu, Zn, Au,

and Ni, even some antibiotics including rifampicin, gentamicin, and vancomycin can be loaded on these nanostructures.

Although, there are many known involved parameters and methods, it seems that more research in this field is from crucial importance since it affect the health issues. Some modern, safe and economic methods and materials should be introduced by researchers. The accurate and complementary guidelines about the safe thresholds and stability of antibacterial agents and procedures must be prepared. The precise mechanisms both from bactericidal and functionality points of view should be understood. The effect of antibacterial schemes on biocompatibility, osteogenic activity, genotoxicity, and their possible influence on periphery tissues should be analyzed. In future, the direction of research shifts to improve the antibacterial properties of non-cytotoxic antibacterial nanoparticles and develop implant materials with stable and long-lasting antibacterial effects. In the study of cytotoxic nanoparticles with antibacterial properties (e.g., silver nanoparticles), further clarifying the factors and mechanisms leading to cytotoxicity and developing controllable and harmless antibacterial materials are the next research focuses. The possible bactericidal mechanisms, the design parameters to achieve the efficient antibacterial performance on different bacteria species, and the development of high-precision nanopattern processing technologies are still among the future problems to be solved in nanopattern antibacterial studies. This review paper can help the investigators to develop the new methods, procedures, and materials to attain a modern scheme in design of nanostructured antibacterial materials with long-last, safe, biocompatible, and effective on broad bacterial infections characteristics.

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## AUTHOR CONTRIBUTIONS

JQL, JL, and SA contributed equally to the design, reorganize the figures, and writing the manuscript. CW, MT, KX, and CY collated the resource. JY, LW, CL, and YT helped with editing the manuscript. YT conceived and designed the outline of this review. All the authors read and approved the final manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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