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Surface immobilization of heparin and chitosan on titanium to improve hemocompatibility and antibacterial activities

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ABSTRACT

Biomaterial-related thrombus formation and bacterial infections are still the most common causes for the failure of medical devices. We report a facile and a highly efficient strategy to inhibit thrombosis and bacteria attachment, by immobilizing heparin (HA) and chitosan (CS) on titanium (Ti). Alkali-treatment was firstly performed on Ti to form nanoporous network structures containing hydroxyl radical (−OH), followed by immobilizing HA and CS on alkali-treated Ti in turn by layer-by-layer assembly. HA was immobilized on alkali-treated surface by covalent immobilization and CS was immobilized on heparinized surface by electrostatic bonding. The successful immobilization of both HA and CS on Ti was confirmed by analyses of scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and water contact angles. The antithrombotic activities of the immobilized surfaces were demonstrated by a reduction in protein absorption, blood clot mass and platelet adhesion. Additionally, the immobilized surfaces also exhibited excellent antibacterial activities against both *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*. The modified surfaces on Ti was established as an effective and promising method to simultaneous improve the hemocompatibility and antibacterial performances of blood contact medical device.

1. Introduction

Titanium (Ti) and its alloys have been widely used in medical devices because of their excellent biocompatibility, mechanical performance and corrosion resistance. However, in some cases (e.g. intravascular stents, blood contacting devices and biosensing), thrombus formation and bacterial infections are still challenges for their application [1–4].

Surface modification is an effective strategy for endowing biomaterials with special surface functions, including osteoconductivity, biocompatibility, wear resistance, corrosion resistance as well as antibacterial activity [5–11]. Layer-by-layer assembly by covalent immobilization has been proven to be a suitable approach for deposition of functional groups, and is becoming a topic of great interest for the surface modification of various substrate biomaterials. The surface modification by covalent immobilization of anticoagulant biomolecules has been applied on biomedical materials to prevent plasmatic coagulation [12–14].

In this work, alkali-based treatment was adopted to generate hydroxyl radical (−OH) on Ti surface, which build a bridge between the functional species like heparin (HA), chitosan (CS) and metal substrate. As a surface modification technique, alkali-based treatments have been shown to be able to enhance the bioactivity of Ti implants by stimulate the deposition of an apatite-like layer [15]. Additionally, alkali surface modification has been suggested as a useful method to allow immobilization of drugs and osteogenic biomolecules onto the implant surfaces [16].

HA is the most commonly used anticoagulant clinically and the anticoagulant mechanism is that HA can interact with antithrombin III (ATIII) and accelerate the inactivation of thrombin and other coagulation factors [17–21]. Therefore, surface heparinization has been proven to be an efficient strategy to prevent thrombus formation of blood-contacting biomaterials.

CS have been widely used in biomedical applications due to its excellent biocompatibility and biodegradability. It has been reported that CS-based biomaterials have been used to promote the reconstruction of bone defects and improve the osteoconductivity in living bodies [22]. As a cationic natural biological macromolecule, CS is positively charged and can bind to negatively charged bacterial cell wall, inhibiting the replication of bacteria [23–26]. Although CS possesses a relatively weaker antibacterial activity compare to metal ions such as silver and copper, it is safer to the human body.

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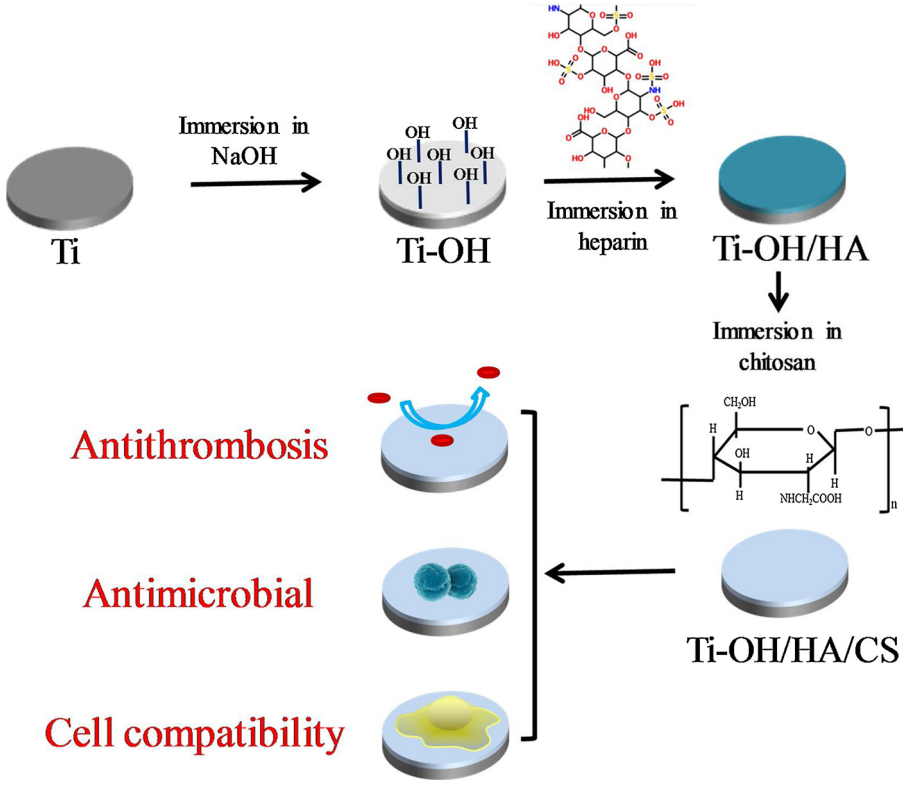

Fig. 1. Schematic illustration of the synthetic route of Ti/OH/HA/CS on Ti via a layer-by-layer method and its hemocompatibility and antimicrobial activities.

Herein, we propose for the first time a facile and a highly efficient strategy to impart antithrombotic and antibacterial properties to Ti substrate by simultaneously immobilizing HA and CS. Before the immobilization, alkali-treatment was firstly performed to produce hydroxyl radical (−OH) on Ti. The rationale preparation and characterization of the modified surfaces is illustrated in Fig. 1. To confirm successful immobilization of HA and CS, the modified surface were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and water contact angle measurements. The amount of immobilized HA and the stability of HA immobilized on the samples were assessed. The in vitro antithrombotic properties of the modified surfaces were analyzed in terms of protein absorption, blood clot mass and platelet adhesion. The antibacterial properties were evaluated by SEM observation, plate-counting method and fluorescence staining. In addition, the biocompatibility was also discussed.

2. Materials and experiments

2.1. Materials

Heparin sodium injection (2ml:12,500 unit) was produced by Tianjin bio-chemical pharmaceutical co. LTD, china. Carboxymethyl chitosan (CMCS) was produced by Pure Chemistry Scientific Inc, USA. Toluidine blue O (TB), paraformaldehyde(PFA), sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), and FITC labeled phalloidin were produced by Sigma-Aldrich, USA. Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), penicillin–streptomycin (P/S), and phosphate-buffered saline (PBS) were produced by Gibco BRL, USA. n-Hexane was produced by AccuStandard Inc, USA. Beef extract and peptone were produced Sangon Biotech,china.

2.2. Preparation of samples

2.2.1. Alkali treatment of Ti samples

Commercially pure titanium (cp Ti-grade 2) was used as the substrate. The samples (φ 14 × 2 mm) were ground with SiC abrasive paper to a mirror finish and were ultrasonically cleaned in acetone, ethanol, and distilled water before alkali treatment. The Ti plates were soaked in 5 M sodium hydroxide (NaOH) for 6 h at 60 °C in a water bath pot. Afterward, the samples were taken out, rinsed with deionized water twice and weathered. The above modified Ti samples were designated as Ti/OH.

2.2.2. Immobilization of biomolecules

Ti/OH samples were soaked in the 20 times diluted heparin sodium solution for 2 h at 37 °C in a water bath pot. Afterward, the samples were taken out, rinsed with deionized water for 1 min and weathered. The modified samples were designated as Ti/OH/HA. Ti/OH/HA samples were immersed in 5 g/L carboxymethyl chitosan solution for 2 h at 37 °C, and then were taken out, rinsed and weathered. The samples were designated as Ti/OH/HA/CS1. Ti/OH samples were soaked in the 20 times diluted heparin sodium solution for 40 min at 37 °C. Afterward, the samples were taken out, rinsed with deionized water, and immersed in 5 g/L carboxymethyl chitosan solution for another 40 min at 37 °C. The experimental procedure was repeated three times. The above samples were designated as Ti/OH/HA/CS2. Similar to the previous preparation method, the immersing time of Ti/OH samples in heparin sodium solution, and then in carboxymethyl chitosan solution change for 24 min. The experimental procedure was repeated five times. The samples were designated as Ti/OH/HA/CS3.

2.3. Surface characterization

The surface morphology of Ti, Ti/OH, Ti/OH/HA, Ti/OH/HA/CS1, Ti/OH/HA/CS2 and Ti/OH/HA/CS3 was examined by field-emission scanning electron microscopy (FE-SEM, JSM-7001 F, JEOL) and atomic