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N-halamine-based multilayers on titanium substrates for antibacterial application

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ABSTRACT

Bacterial infection is one of the most severe postoperative complications leading to clinical orthopedic implants failure. To improve the antibacterial property of titanium (Ti) substrates, a bioactive coating composed of chitosan-1-(hydroxymethyl)- 5,5-dimethylhydantoin (Chi–HDH-Cl) and gelatin (Gel) was fabricated via layer-by-layer (LBL) assembly technique. The results of Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (^{1}HNMR) and X-ray photoelectron spectroscopy (XPS) showed that Chi − HHD-Cl conjugate was successfully synthesized. Scanning electron microscopy (SEM), atomic force microscope (AFM) and water contact angle measurements were employed to monitor the morphology, roughness changes and surface wettability of Ti substrates, which proved the multilayers coating formation. Antibacterial assay against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) revealed that the Gel/Chi–HDH-Cl modified Ti substrates most efficiently inhibited the adhesion and growth of bacteria. Meanwhile, *in vitro* cellular tests confirmed that Gel/Chi–HDH-Cl multilayers had no obvious cytotoxicity to osteoblasts. The study thus provides a promising method to fabricate antibacterial Ti-based substrates for potential orthopedic application.

1. Introduction

Titanium (Ti) and its alloys have drawn great attention in orthopedic field owing to their remarkable mechanical property and good biological performances [1–3]. However, implant-associated infection remains one of the most severe postoperative complications, leading to the failure of implant surgery along with increasing economic and health-related costs [4,5]. The formation of biofilm on the surfaces of implants following by bacterial adhesion, which has been proved to be the key factor to make the bacteria more invasive and competitive against host defenses and antimicrobial therapy, resulting in implant loosening and even implant failure [6–9]. Thus, the ability to prohibit initial bacterial adhesion and biofilm formation would give more surgical success to Ti-based implants [10–13].

To address Ti-implants associated infections in clinical application, many efforts were devoted to altering their surface properties, such as electrochemical anodization [14,15], plasma immersion ion implantation [16,17], electrochemical deposition [18], and target molecules grafting [19,20]. Moreover, layer-by-layer (LbL) assembly technique also employed for surface modification of related implants, since it was considered as an effective approach under mild conditions [3,21]. Some bioactive multilayers (*e.g.* anti-bacteria, anti-osteoporosis, and anti-inflammation) were successfully constructed on substrates via the LbL technique, which was based on alternative deposition of polyanion and polycation via electrostatic interaction [22,23]. In recent decade, chitosan (Chi), a natural cationic polyelectrolyte, was the most common used polycation for orthopedic and drug delivery carrier applications, due to its good biocompatibility, non-toxicity and non-antigenicity properties [24–27]. It also presents antimicrobial property since their positively charged amine groups of glucosamine could disrupt microbial cell membrane and cause the leakage of intracellular components [28]. Gelatin is a derivative of collagen and frequently used to structure multilayers because of its excellent biocompatibility and biodegradability. Nevertheless, the Chi alone is insufficient to inhibit the Ti-implants bacterial infections in biomedical applications [9,28], owing to its poor solubility in water according to the previous studies [29,30]. Therefore, improving the antibacterial ability of Chi would further extend its clinical antibacterial application.

N-halamines composed oxidation-type antibacterial agents had a characteristic structure of one or more N–X (X = Cl, Br, or I) bond. The antiseptic agent of organic N-halamine is generally divided into three types: amine- (RR'-NX), amide- [-C(O)-NX-R] and imide- [(-C(O)-NX-C(O)-] N-halamines [31,32]. Previous studies verified that the stability (against hydrolysis) of halogen from N-halamine structures in aqueous

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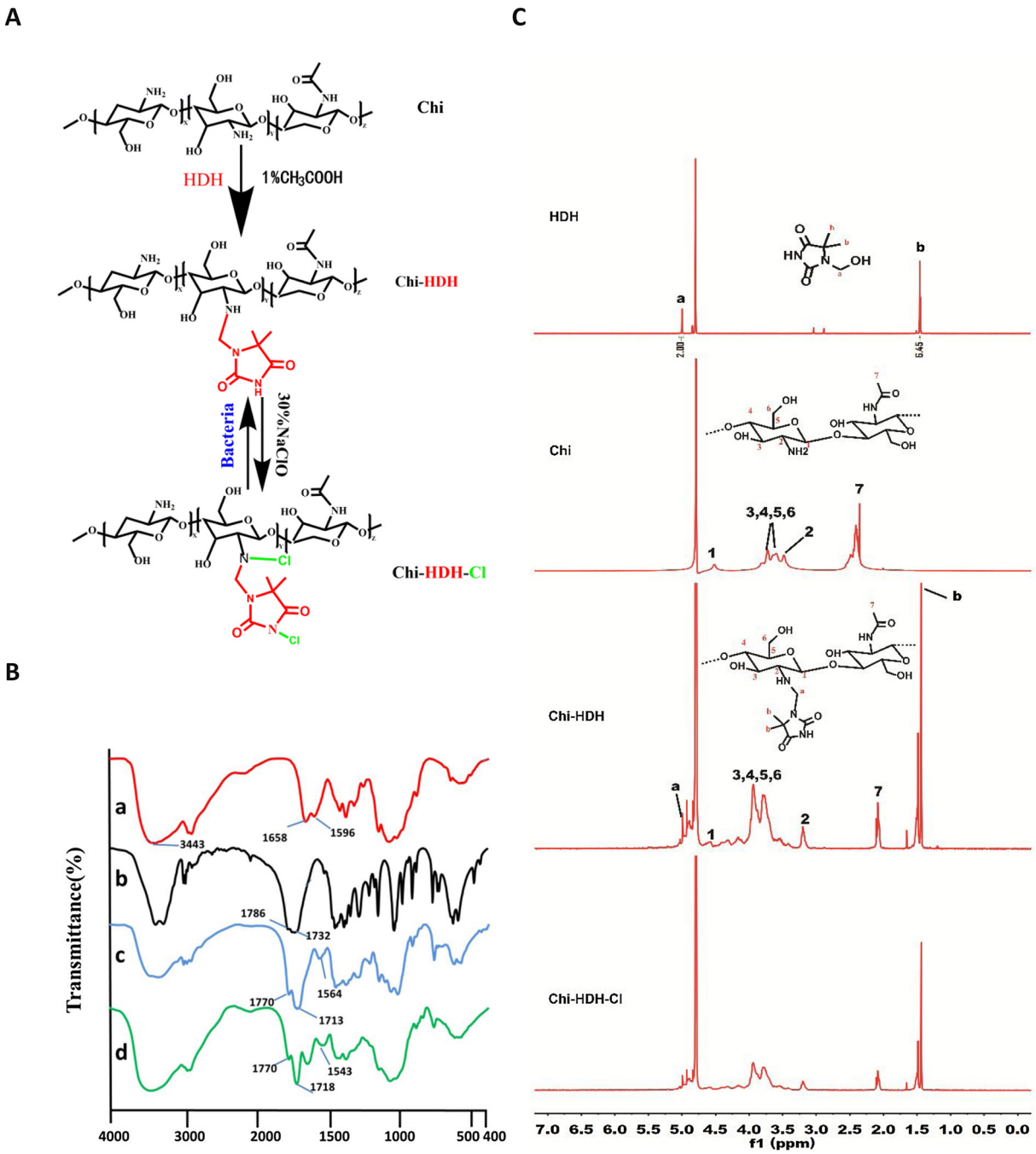

Fig. 1. (A) Synthetic diagram and molecular structure of Chi-HDH and Chi-HDH-Cl conjugate; and (B) FTIR and HNMR spectra (C) of (a) Chi, (b) HDH, (c) Chi–HDH and (d) Chi-HDH-Cl, respectively.

solution was relatively low and followed an order of imide < amide < amine N-halamine. It leads to their antimicrobial activity have an inverse trend: imide > amide ≫ amine N-halamine [33,34]. Thanks to the powerful antibacterial activity of N-halamine, environment-friendly, long-term chemical stability, rechargeability, biosafety, and low cost [31,34], N-halamine compounds attracted much attention for their broad-range spectrum inhibition of microorganisms, such as viruses, fungi, Gram-positive and Gram-negative germs [33,35,36]. For instance, Natan et al. developed N-halamine cross-linked polymethacrylamide nanoparticles to efficiently inhibit bacteria adhesion against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria [37]. Bai et al. fabricated the electrospinning fibrous membranes by mixing with 1,3-dibromo-5,5-dimethylhydantoin and 1,3-dichloro-5,5- dimethylhydantoin to endow it with superior antibacterial property [38]. Li et al. developed a hybrid combined N-halamine with ZnO nanoparticles on the Ti substrates, which exhibited excellent antibacterial activity against *E. coli* and *S. aureus* and good biocompatible toward the MC3T3-E1 preosteoblast [39].

In this study, Chi (low molecular weight) was modified with 1-(hydroxymethyl)-5,5-dimethylhydantoin (HDH) to obtain antibacterial conjugate compound (Chi-HDH-Cl). Then, Chi-HDH-Cl (polycation) and gelatin (polyanion) were used to construct multilayered structure on Ti substrates via LBL technique, respectively. Previous studies demonstrated that the Ti surface modification using Gel/Chi multilayers via LBL technique could mimic the extracellular microenvironment and was beneficial for the cytocompatibility and osteoconductivity [40,41]. The objective of this work is to develop a N-halamine-containing antibacterial coating on Ti substrates and to investigate how the multilayer