Electrochemically deposited chitosan/Ag complex coatings on biomedical NiTi alloy for antibacterial application

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Biomedical NiTi-based shape memory alloys suffer from post-surgery bacterial infection issues and it is of great importance to prevent bacteria adhesion and growth on the implants by using the proper surface modification techniques. In this study, a chitosan/Ag complex coating is deposited on NiTi alloy electrochemically to enhance the antibacterial characteristics. The composition and properties of the coatings are determined systematically. The thickness of the chitosan/Ag complex coating is around 7.5 μm and it adheres well to the NiTi substrate. The chemical state of silver in the chitosan/Ag complex coating is revealed to be different from that in deposited silver without chitosan by X-ray photoelectron spectroscopy (XPS) and leaching tests. Furthermore, the antibacterial properties of the chitosan/Ag complex coatings are assessed and there are significant effects against the model bacterial Escherichia coli (E. coli). The present study discloses that the complex antibacterial coating is promising in orthopedics, dentistry, and other biomedical applications.

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1. Introduction
The shape memory effect, superelasticity, and good biocompatibility render NiTi alloys suitable for dental and medical devices such as stents, orthodontic components, orthopedic implants, and other surgical instruments [1,2]. However, similar to other medical materials, serious implant-related bacterial infection still induces possible post-surgical complications [3] and approaches like surface modification and antibacterial coatings have been proposed to enhance the antibacterial ability of the biomedical NiTi alloys. Many nanoparticles such as Ag, Cu, and ZnO have been studied as antibacterial agents [4–8] and silver was reported to have the highest antibacterial activity [9]. Hence, coatings containing silver as an antibacterial component are of great interest [10,11] having potential applications in food packaging, textile, and medical fields [12–15].

To enhance the biocompatibility of the silver nanoparticles or reduce the toxicity as a result of burst release of silver ions, there have been attempts to incorporate silver into biological friendly polymers to produce antibacterial coatings [16–18]. Chitosan is commonly employed in these antibacterial composite or complex coatings. Chitosan is synthesized by deacetylation of chitin, which is the second most abundant natural polysaccharide. The materials are available in nature and the biocompatible structure is similar to those of hyaluronic acid and glycosaminoglycan extracellular matrix molecules [19,20]. Another major advantage originates from deacetylation in which protonation of the amine groups of chitosan in an acidic solution at pH < 6 forms positively charged groups and becomes soluble [21]. The pH dependent solubility makes chitosan highly versatile and flexible in surface modification and coatings.

Techniques to prepare chitosan coatings include solution casting, layer-by-layer deposition, and electrochemical deposition [22,23]. In electrochemical deposition, charged particles or polymer macromolecules move towards an electrode under the influence of the electric field. This green technique offers the possibility to achieve adjustable and homogeneous deposition of coatings on various substrates at room temperature [24,25] and electrochemical deposition of chitosan or chitosan-based coatings on metal substrate has recently gained interest [26,27]. The cationic property of the chitosan and chitosan/Ag complex in an acidic medium enables electrochemical precipitation of the coatings on a metal substrate thus providing a simple process to fabricate antibacterial coatings on medical devices. In this work, chitosan/Ag complex coatings are prepared on biomedical NiTi alloys by electrochemical deposition with the aim to enhance the surface antibacterial activity.

2. Experimental details
Chitosan (MW = 100 kDa, degree of deacetylation of about 90%) was supplied by Golden-Shell Biochemical Co., Ltd. and AgNO₃ and acetic acid were purchased from Sigma Aldrich and used as received. Chitosan was dissolved in a 2 vol.% acetic acid solution and used in
subsequent electrochemical deposition. NiTi alloy plates (50.8 at.% Ni, Nitinol Devices & Components Inc.) were cut into dimensions of 10 mm × 10 mm × 2 mm, mechanically ground by SiC sandpaper to grade 1200, ultrasonically cleaned with acetone and deionized water, and then air dried.

Electrochemical deposition was carried out in the 1 mM AgNO₃ solution and 1 g·L⁻¹ chitosan acetic acid (pH value 3.7) to obtain the Ag coated NiTi (Ag_NiTi) and chitosan coated NiTi (chitosan_NiTi), respectively. The chitosan/Ag coated NiTi (chitosan/Ag_NiTi) samples were prepared in a mixed solution containing both AgNO₃ and chitosan acetic acid. The NiTi plate was used as the cathode and carbon plate was the counter electrode. There was a 15 mm gap between the two electrodes. Cathodic deposition was performed by connecting the cathode and anode to a direct current power supply (IT6123, ITECH Electronic CO., LTD) and applying a constant voltage of 6 V·cm⁻² for 10 min. Gentle stirring was performed during deposition and afterwards, the coated NiTi plates were rinsed with deionized water and dried in air at room temperature.

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR; Spectrum 100, PerkinElmer, USA) was utilized to determine the surface composition of the coated NiTi samples. The elemental concentrations in the chitosan/Ag_NiTi were determined and the high resolution Ag 3d signals were recorded. The surface topography of the coated NiTi samples was examined by atomic force microscopy (AFM, Auto Probe CP, Park Scientific Instruments) using the tapping mode from an area of 2 × 2 μm². The surface and cross-sectional SEM images were acquired by scanning electronic microscopy (SEM; JEOL JSM-820, JEOL, USA) and the elemental composition of the samples was determined by energy-dispersive X-ray spectroscopy (EDS) equipped on the SEM. The specimens were cleaned in alcohol, dried, and coated with carbon before SEM observation and both the SEM images and EDS data were obtained at an electron accelerating voltage of 20 kV. The phase of the bare and coated NiTi samples was studied with the X-ray diffraction (XRD; Philips X'pert diffractometer) using Cu Kα radiation (λ = 0.154056 nm) at room temperature. The coating adhesion strength of both chitosan_NiTi and chitosan/Ag_NiTi was evaluated according to ISO2409:2007 [28]. Six lines (1 mm spacing) were made in the coatings using a cutting blade and six more lines were cut orthogonal to the original lines forming a grid with 25 squares. A tape was applied to the grid and removed evenly within 5 min.

Fig. 1. Schematic representation of (a) protonation of chitosan, (b) complexing between chitosan and Ag ions, and (c) mechanism of electrochemical deposition.

Fig. 2. FTIR spectra: (a) chitosan_NiTi and (b) chitosan/Ag_NiTi.

Fig. 3. XPS spectra: (a) chitosan_NiTi, (b) chitosan/Ag_NiTi, and (c) Ag_NiTi.

Fig. 4. High-resolution XPS Ag 3d core-level spectra: (a) Ag_NiTi and (b) chitosan/Ag_NiTi.
Fig. 5. AFM images of different surfaces: (a) bare NiTi, (b) Ag_NiTi, (c) chitosan_NiTi, and (d) chitosan/Ag_NiTi.

Fig. 6. SEM images of coated NiTi and corresponding cross-sectional image: (a) Ag_NiTi, (b) chitosan_NiTi, and (c) chitosan/Ag_NiTi and EDS spectra of (d) Ag_NiTi, (e) chitosan_NiTi, and (f) chitosan/Ag_NiTi.
To investigate the Ag release profiles from Ag_NiTi and chitosan/Ag_NiTi, the samples were immersed in 25 mL of deionized water at 37 °C individually and the amounts of leached Ag were determined. 1 mL of the solution was collected at certain intervals and exchanged with 1 mL of fresh water. The Ag concentration in the solution was determined by inductively-coupled plasma atomic emission spectrometry (ICP-AES; Optima 2100DV, Perkin Elmer, USA). The cumulative release concentration of Ag was calculated.

The antibacterial activity of the fabricated samples was assessed using *Escherichia coli* (*E. coli*) by the microplate method\[29\]. Overnight suspensions of *E. coli* were obtained in lysogeny broth (LB) on an orbital shaker platform agitated at 150 rpm at 37 °C. The coated and bare NiTi samples were placed in 1 mL of the 100 times diluted suspension in a 24-well microplate which was then subjected to vigorous shaking and incubated for 12 h. The growth or killing of the bacteria was determined by measuring the optical density (OD) at 600 nm on Microplate reader (Powerwave XS MQX200R). The bacterial suspension without the sample was as the control, while the blank obtained from the LB culture solution was removed from each OD value. The OD measurement was carried out in triplicate and the values obtained were averaged to yield the final data. The antibacterial activity was determined qualitatively by a modified disk diffusion method. The overnight suspensions of *E. coli* were diluted 10⁵ times and 200 μL of the diluted suspension was spread onto the as-prepared LB agar dish separately. The coated surfaces of the samples were covered on the agar and the plates were examined for possible clear zones after overnight incubation at 37 °C. The presence of any clear zone that formed around sample on the agar was observed as an indication of inhibition against the bacteria.

3. Results and discussion

Electrochemical deposition schematically illustrated in Fig. 1 is employed to fabricate the chitosan and chitosan/Ag complex coatings on the NiTi alloy substrate. Chitosan is insoluble in water, but can become a cationic polyelectrolyte and dissolve in an acidic solution due to protonation of the amine groups via the following reaction\[30\]:

\[
\text{Chit–NH}_2 + \text{H}_3\text{O}^+ \rightarrow \text{Chit–NH}_3^+ + \text{H}_2\text{O}.
\]

In the fabrication of the chitosan/Ag complex coatings, the chitosan solution is mixed with a AgNO₃ solution to obtain the cationic complex because the amine groups in chitosan can chelate silver ions according to the following reaction\[31\]:

\[
\text{Ag}^+ + 2\text{Chit–NH}_2 \rightarrow [\text{Ag(Chit–NH}_2)_2]^+.
\]

In the electrochemical deposition system, the electric field produces electrophoretic motion of the cations (Ag⁺, Chit–NH₃⁺ or [Ag(Chit–NH₂)₂]⁺) to the cathode NiTi substrate where they form an insoluble deposit electrochemically. Ag_NiTi, chitosan_NiTi and chitosan/Ag_NiTi samples can be produced by this simple and green technique. The FTIR spectrum of the chitosan coated on the NiTi is depicted in Fig. 2(a). The broad absorption peak near 3300 cm⁻¹ indicates –OH or –NH₂ stretching in chitosan, whereas peaks at 2868 cm⁻¹ and 2917 cm⁻¹ are assigned to the characteristic C–H asymmetric stretch vibrations of –CH₂. The peak at 1648 cm⁻¹ corresponds to the amide I band which is caused by stretching of the C–O–C amide II band at 1591 cm⁻¹ accounts for the NH bending vibrations in the amide group\[32,33\]. The absorption band at 1153 cm⁻¹ was asymmetric stretching of the C–O–C bridge, whereas the peaks at 1070 cm⁻¹ and 1029 cm⁻¹ are assigned to C–O stretching in cyclic alcohol (–CH₂–OH) and primary alcohol (–CH₂–OH) in the polysaccharide structure, respectively\[34\]. Compared to the spectrum of the chitosan coating, the spectrum of the chitosan/Ag complex coating (Fig. 2(b)) exhibits most of the main peaks. However, there is a slight peak shift.

![Fig. 7. XRD patterns: (a) NiTi substrate, (b) chitosan_NiTi, (c) Ag_NiTi, and (d) chitosan/Ag_NiTi.](image)

![Fig. 8. (a) Cumulative release profiles of Ag from Ag_NiTi and chitosan/Ag_NiTi in deionized water at 37 °C and (b) antibacterial activity measured by optical density OD600 of an E. coli suspension in an aqueous LB broth treated with different samples.](image)
from 1591 cm$^{-1}$ to 1556 cm$^{-1}$ due to the co-ordination bond between the heavy metal atom (silver in this case) and electron rich groups (oxygen/nitrogen) [35]. Moreover, a new peak appearing at 825 cm$^{-1}$ is assigned to the stretching vibration of the chitosan/Ag complex [36].

Fig. 3 compares the XPS survey spectra acquired from Ag_NiTi, chitosan_NiTi, and chitosan/Ag_NiTi. The spectra obtained from the chitosan and chitosan/Ag coatings show C, O and N signals which originate from the chitosan molecules in the two coatings. The strong Ag signals in the spectrum of Ag_NiTi suggest the existence of atomic Ag instead of silver oxide. The atomic concentrations of C, O, N, and Ag in chitosan/Ag_NiTi determined by XPS analysis are 56.7%, 33.2%, 8.1%, and 2.1%, respectively, suggesting a chitosan to Ag ratio of 4:1 in chitosan/Ag_NiTi. To confirm and compare the chemical states of Ag in Ag_NiTi and chitosan/Ag_NiTi, the high-resolution Ag 3d spectra are recorded and the results are shown in Fig. 4. The doublet peaks at 368.1 eV and 374.1 eV in Fig. 4(a) are assigned to Ag(0), confirming the atomic nature Ag(0) in Ag_NiTi. Similar peaks are observed from the spectrum of chitosan/Ag_NiTi (Fig. 4(b)), but the peaks shift to 367.4 and 373.3 eV, respectively. The higher electron density of Ag in the complex coating compared to Ag atoms indicates that Ag is in the ionic state and acts as an acceptor for electrons in the complex coating and this phenomenon has been observed [18].

Fig. 5 displays the surface morphology of the coated NiTi substrate over an area of $2 \times 2$ μm$^2$ by AFM. The surface of the NiTi substrate is roughened after deposition. As shown in Fig. 5(b), Ag dendrites with long main branches and parallel branches are formed during electrochemical deposition in the AgNO$_3$ solution as a result of diffusion controlled deposition [37]. Fig. 5(c) indicates that the chitosan coating deposited on the NiTi substrate is dense on the micro-scale. A granular structure is also observed because the diffusion ability during deposition is affected by the intrinsic chain stiffness and high molecular weight [38]. This effect is apparently enhanced from the complex of chitosan and Ag, resulting in bigger granules in the complex coating as shown in Fig. 5(d).

The SEM images of the coated surface are shown in Fig. 6. A dendritic structure is observed on Ag_NiTi and a granular structure is observed from chitosan_NiTi and chitosan/Ag_NiTi. These are consistent with the AFM images. EDS confirms the presence of Ag from the Ag_NiTi and that in chitosan/Ag_NiTi is confirmed (Fig. 6(d) and (f)). The respective cross-sectional SEM images of the coated surfaces are shown in the inset. The thicknesses of the deposited Ag, chitosan, and chitosan/Ag coatings are approximately 3 μm, 3.5 μm, and 7.5 μm, respectively. No delamination of the chitosan and chitosan/Ag coatings is observed from the cross-sectional images, suggesting tight bonding between the coatings and NiTi substrate. This is also confirmed by the results of the cross-cut adhesion tests. Both coatings pass the adhesion test, with ratings of 0 and 1 for chitosan_NiTi and chitosan/Ag_NiTi, respectively. The edges of the cuts are smooth and none of the squares of the lattice were detached from chitosan_NiTi. Only several small flakes caused by the cutting are observed from chitosan/Ag_NiTi.

The XRD patterns of the NiTi substrate and the coated samples are shown in Fig. 7. The peaks at 42.8° and 77.6° represent the B2 (110) and B2 (211) of the predominant B2 phase of the NiTi substrate. The broader and weak diffraction peak in the small diffraction angle in Fig. 7(b) arises from the amorphous chitosan coating, and the peaks at 2θ = 38.2° and 44.4° in Fig. 7(c) correspond to the (111) and (200) diffraction of the Ag coated NiTi. The (111) plane is present with a high intensity, indicating that Ag deposits preferably onto the (111) facet under these conditions [39]. The absence of amorphous diffraction of chitosan in Fig. 7(d) results from the crystalline structure change due to the formation of coating. The absence of the Ag (200) signal and weak signal of Ag (111) confirm that Ag is predominantly in the ionic state in the chitosan/Ag complex coating. The small Ag (111) peak may arise from the unintentional deposition of atomic Ag into the chitosan/Ag complex coating.

The amounts of Ag leached from Ag_NiTi and chitosan/Ag_NiTi as a function of immersion time in deionized water are shown in Fig. 8(a) and different Ag release behavior can be clearly observed. The cumulative concentration of Ag ions from atomic Ag in Ag_NiTi is low at 0.01 μg/mL. On the contrary, Ag ions are released gradually from chitosan/Ag_NiTi and the concentration rises to around 1 μg/mL after 24 h. The different Ag release profiles are expected to result in different antibacterial activities of Ag_NiTi and chitosan/Ag_NiTi.

Generally, the optical density at 600 nm (OD$_{600}$) of the bacterial suspension reflects the concentration of the bacteria and a higher OD600 thus infers a larger bacteria concentration [40]. To evaluate the antibacterial activity of the coated NiTi samples against E. coli, OD$_{600}$
of the *E. coli* suspension incubated with the samples is measured using a microplate reader. As shown in Fig. 8(b), the values of *E. coli* suspension after 12 h incubation with the bare NiTi and chitosan_NiTi are similar to the control value, which is obtained from the suspension without samples. The slightly smaller value of Ag_NiTi reveals the ineffective antibacterial activity because of insufficient Ag release from the Ag in the metallic state. The smallest OD600 is observed from chitosan/Ag_NiTi and a high concentration of Ag ions is released. It is only 15% of the control value thus confirming the significant antibacterial property against *E. coli*. Fig. 8 exhibits the typical antibacterial results of the samples by the disk method. The *E. coli* suspension in aqueous LB is spread evenly on the agar plates. The samples are covered and incubated for 12 h. Formation of a clear zone around the samples shows inhibition of bacterial growth as an indication of the antibacterial activity [41]. The Ag_NiTi and chitosan_NiTi samples do not show clear inhibition zone, whereas chitosan/Ag_NiTi sample exhibits distinctive antibacterial inhibition zones. The results are in line with those obtained from the microplate method and clearly demonstrate that the chitosan/Ag complex coating exhibits excellent bacterial inhibition.

### 4. Conclusion

Chitosan/Ag complex coatings are produced on biomedical NiTi alloys by a simple electrochemical deposition technique. As indicated by OD600 test and disk diffusion method, the chitosan/Ag complex coatings exhibit better antibacterial activity against *E. coli* compared to Ag and chitosan-coated NiTi. The complex coatings can be used to enhance the antibacterial property of NiTi implants and have many potential biomedical applications.

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