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# Surface modification of NiTi alloy via a TiN coating functionalized with biomimic multilayer films

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ABSTRACT

In this study, dextran sulfate (DS)/chitosan (CHI) multilayer films were fabricated on TiN-coated NiTi alloy to reduce Ni ion release and improve biocompatibility. The successful formation of TiN coating and DS/CHI multilayer films was demonstrated by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). Inductively coupled plasma mass spectrometry (ICP-MS) test showed that the Ni ion release from NiTi alloy significantly decreased after surface modification. Cytocompatibility including osteoblasts adhesion and differentiation function was evaluated *in vitro*. The results suggest that this surface modification process is beneficial for osteoblasts growth on NiTi alloy substrate. The approach presented here provides an attractive way for surface modification of NiTi alloy to meet the requirements of biomedical applications.



## 1. Introduction

Recently, NiTi alloy has been extensively used in bone-implant applications, such as dental implants and bone tissue engineering, mainly due to its unique shape memory effect, super-elastic property, high corrosion resistance and good biocompatibility [1–3]. Despite the good performance of NiTi alloy, the Ni ion release of NiTi alloy substrate has caused some concern of its safe use as an implant material. Ni ion can be released to the surrounding tissue when NiTi alloy is implanted, which may induce allergic and toxic effects [4,5]. In addition, the interfacial situations of the NiTi alloy implant cannot highly induce the bone-forming cells response or effectively integrate with the surrounding bone tissue, especially at the early stage of implantation. To improve the performance of NiTi alloy implant, one needs to reduce Ni ion release and improve biocompatibility. In this respect, surface modification plays an important role by providing a means to reduce Ni ion release and improve biocompatibility without affecting the desirable bulk attributes of NiTi alloy [6–9].

In this work, a titanium nitride (TiN) coating combined with dextran sulfate (DS)/chitosan (CHI) multilayer films was employed to reduce Ni ions release and enhance osteoblast response of NiTi alloy. Previous study has demonstrated that it is an effective method to fabricate a TiN coating on NiTi alloy for reducing Ni ions release because of its chemical stability, abrasion resistance and corrosion resistance [10]. Recently, layer-by-layer (LBL) self-assembly technique has emerged as a versatile, inexpensive yet efficient technique to build biologically active surfaces [11–13]. For instance, LBL self-assembly was employed to modify titanium alloy surface with bioactive molecules, which showed a positive effect on mechanical implant anchorage in normal bone *in vivo* [13]. Here, LBL self-assembly technique was used to fabricate DS/CHI multilayer films on TiN-coated NiTi substrate. The rationale to select DS and CHI to construct biomimic multilayer films is that DS and CHI are similar to glycosaminoglycans (GAGs) which is present in the extracellular matrix. Since GAGs properties include many specific interactions with growth factors, receptors and adhesion proteins [14], DS and CHI with similar analogous structure may share some characteristics and have related bioactivities. Therefore, we hope that this surface modification approach would be helpful for reducing Ni ion release and improving osteoblasts growth on NiTi substrate.

## 2. Materials and methods

NiTi alloy substrates ($Ti_{49.2}Ni_{50.8}$, at%) were kindly supplied by Northwest Institute for Non-ferrous Metal Research (China) and machined into cylindrical discs with thickness of 5 mm and diameter of 15 mm. The TiN coating on NiTi alloy was prepared by Lifetech Scientific Co., Ltd., (Shenzhen, China) via a filtered

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arcing ion plate technique for the present study. TiN coating modified NiTi alloy was denoted as NiTi–TiN substrate.

Dextran sulfate (DS), chitosan (CHI, low molecular weight, degree of deacetylation 75–85%), and poly(ethylene imine) (PEI) were purchased from Sigma Chemical Co. (MO, USA). The NiTi–TiN sample was initially immersed in 5 mg/ml PEI solution for 20 min. Polyethylene imine (PEI) was used to obtain a precursor layer with a stable positive charge to initiate the LBL self-assembly process. Then, the treated substrate was immersed into DS solution and CHI solution each for 10 min. The process was repeated until the desired layers of DS/CHI films were deposited onto the substrates. Finally, thirteen layers, PEI/$(DS/CHI)_6$ were achieved by such LBL self-assembly technique in the present study. For thirteen layers of PEI/$(DS/CHI)_6$ on the samples, it was related to the fact that the substrate surface was not fully covered by the outermost layer component such as CHI or DS during the first several-layer coating [15]. Thirteen layers of PEI/$(DS/CHI)_6$ were necessary to build-up a constant interface and cover the whole substrate. The NiTi–TiN sample functionalized with DS/CHI multilayer films was denoted as NiTi–TiN-LBL substrate.

Scanning electron microscopy (SEM) (Quanta 200 Philips-FEI Corporation, Netherlands) with energy-dispersive spectroscopy (EDS) was used to investigate the morphologies and chemical compositions of the coatings. Inductively coupled plasma mass spectrometry (ICP-MS) (Elan DRC-e, Perkin Elmer, USA) was used to determine the amount of Ni ion leached from the specimen. The samples were immersed in 2 ml simulated body fluid (SBF) solution and incubated in a thermostatic chamber at $37 \pm 0.1$ °C for 7, 21 and 35 days. At each time point for each group, the SBF solution was taken out and analyzed by ICP-MS.

Osteoblasts ($10^4$ cells per disk) were seeded onto native and treated NiTi alloys as well as tissue culture polystyrene (TCPS). After 3 days, the cells were stained with rhodamine phalloidin (Invitrogen, USA) at room temperature for 1 h and then stained with Hoechst fluorescent dyes (Sigma, USA) for 5 min. The cytoskeletal actin and cell nuclei were observed with confocal laser scanning microscopy (CLSM, Leica DMI 6000, Germany). After 1, 4 and 7 days, *p*-nitrophenyl phosphate (Sigma) was employed to determine alkaline phosphatase (ALP) activities of osteoblasts. The absorbance at 405 nm wavelength was measured with a spectrophotometric microplate reader (Bio-Rad 680). Total protein content in the cell lysates was determined using a commercially available kit (BCA, Sigma). The ALP activity was normalized by total intracellular protein production.

## 3. Results and discussion

*Preparation and characterizations of TiN coating and DS/CHI multilayer films*: SEM images of native and treated NiTi substrates are shown in Fig. 1. It can be seen that native NiTi alloy surface displayed visible scratches, which was attributed to polishing abrasion (Fig. 1a). After TiN coating was deposited, all scratches disappeared (Fig. 1b). However, the NiTi–TiN sample still appeared to be coarse and rough because of distribution of particles with different sizes (Fig. 1b), which might be derived from the formation of TiN coating. After DS/CHI multilayer films were deposited, the sample appeared smoother, compared with the NiTi–TiN substrate (Fig. 1c). This phenomenon could be interpreted that dense uniform multilayer films were formed onto the NiTi–TiN substrate after LBL self-assembly. This change in surface morphology was also reported in other literature [16], indicating that fully covered and layered coatings are well developed on substrate after the deposition.

Furthermore, to confirm the successful construction of TiN coating and DS/CHI multilayer films onto NiTi alloy, the chemical composition of the surfaces at various stages of surface modification was determined by EDS. Fig. 2 shows the representative EDS spectra of NiTi–TiN and NiTi–TiN-LBL substrates. The NiTi–TiN substrate displayed three elements of Ni, Ti, and N (Fig. 2a). After being coated with DS/CHI multilayer films, two additional peak of C and O were observed (Fig. 2b). The presence of C and O elements derived from DS and CHI molecules provided evidence for successful LBL film formation on the NiTi–TiN substrate.

The Ni ion release from native and modified NiTi alloys in SBF solution was measured by ICP-MS. After 7, 14, and 35 days immersion in SBF solution, Ni ion concentrations of NiTi substrates were 4.48, 15.30 and 65.40 μg/ml. Those of NiTi–TiN substrates were 2.26, 6.41 and 13.30 μg/ml. Furthermore, Ni ion concentrations of NiTi–TiN-LBL substrates were 2.22, 5.86 and 11.80 μg/ml. After 35 days immersion in SBF solution, the amount of Ni ion release from the NiTi–TiN-LBL sample was about 6 times lower than that from the native NiTi alloy, which indicated that this TiN/LBL composite coating can effectively decrease diffusion of Ni ion from NiTi alloy.

*Assessment of osteoblasts growth behaviors on NiTi substrates*: The cellular behavior on the surface of implant is crucial for evaluation of the cytocompatibility of a biomaterial. Cell adhesion on substrate is the first sequential reactions when it contacts with a material surface. To investigate cell adhesion behavior, osteoblasts were cultured on native and treated NiTi alloys and visualized with a double staining of actin (cytoskeleton) and nucleus via CLSM. Osteoblasts grown on NiTi–TiN-LBL substrate showed significantly highest numbers of cells and best spreading, covering almost the entire surface of the sample, compared with NiTi and NiTi–TiN substrates (Fig. 3a–c). Furthermore, ALP activity was evaluated to investigate osteoblastic differentiation of osteoblasts on different substrates (Fig. 3d). ALP activity is one of the most widely used as a transient early osteo-differentiation marker for osteoblasts. After 4 days, obsteoblasts grown on NiTi–TiN-LBL samples showed significantly higher ALP activity than that of corresponding native NiTi alloys ($p < 0.05$). After 7 days, ALP activity of NiTi–TiN-LBL samples showed statistically significant difference compared with that of NiTi–TiN ($p < 0.05$) and native

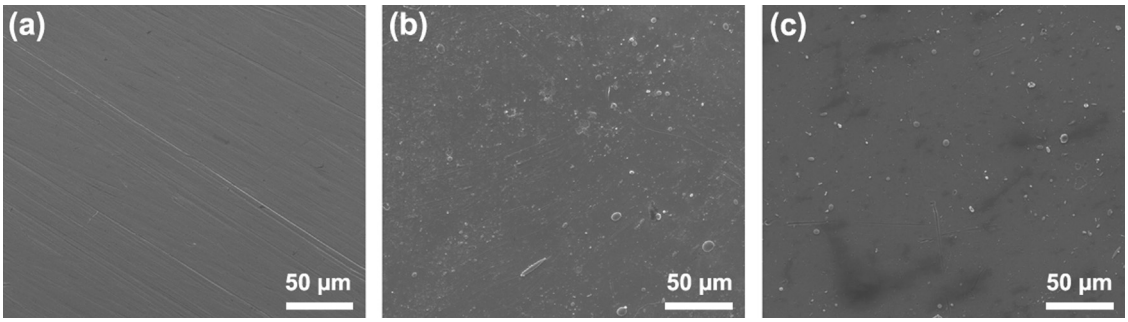


**Fig. 1.** SEM images of (a) NiTi, (b) NiTi–TiN and (c) NiTi–TiN-LBL substrates.