Nitrogen plasma-implanted nickel titanium alloys for orthopedic use

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Abstract

Nickel–titanium shape memory alloys (NiTi) have attracted much attention as orthopedic materials due to their shape memory effect and super-elasticity. However, this alloy consists of equal amounts of nickel and titanium and Ni is well known to cause allergy or other deleterious effects in living tissues. To improve the surface corrosion resistance and mitigate Ni leaching, we have modified the surface chemistry of this alloy with the aid of nitrogen plasma immersion ion implantation (PIII). The implanted surfaces were characterized by X-ray photoelectron spectroscopy (XPS). Electrochemical corrosion and nano-indentation tests were conducted to assess the corrosion resistance and surface hardness. Immersion tests were carried to investigate the extent of Ni leaching under simulated human body conditions and cell cultures employing enhanced green fluorescent protein mice osteoblasts were used to evaluate the cyto-compatibility of the materials. The XPS results reveal that a thin layer of TiN with higher hardness is formed on the surface after nitrogen-PIII. The corrosion resistance of the implanted sample is also superior to that of the untreated NiTi and SS. The release of Ni ions is significantly reduced compared to the untreated NiTi and both the treated and untreated NiTi alloys favor osteoblast attachment and proliferation. The sample with surface titanium nitride exhibits the largest degree of cell proliferation whereas stainless steel fares the worst.

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1. Introduction

Stainless steels and titanium alloys are the most widely used orthopedic materials because of their good mechanical properties, low costs, and acceptable biocompatibility [1]. Nickel titanium (NiTi) shape memory alloys have recently attracted much attention due to their distinctive shape memory effect (SME) and super-elasticity (SE) that may not be found in Ti and stainless steels [2,3]. In spite of scattered reports on the biocompatibility of NiTi [4,5], adverse effects such as unfavorable osteogenesis process and osteonectin synthesis activity [6] as well as high cell death rate [7] have been reported. This problem is suspected to stem from the poor corrosion resistance leading to the release of toxic Ni ions and consequently increased cyto-toxicity and strong allergic reactions in patients [8]. Tantalum and oxygen have been implanted using plasma technology to improve the surface mechanical properties of the materials [9]. Our previous studies have demonstrated that the use of acetylene, nitrogen, and oxygen plasma immersion ion implantation (PIII) can significantly improve the corrosion and wear properties of NiTi [10–15]. However, a comparative study of PIII treated NiTi with other common medical grade metals has not been conducted. In our work reported here, we aim at comparing the properties of nitrogen-PIII NiTi, untreated NiTi and medical grade stainless steels.
2. Experimental details

Circular nickel–titanium (NiTi) shape memory bars with 50.8% Ni (SE508, Nitinol Device Company, Fremont, USA) were cut into 1 mm thick disks 5 mm in diameter. They were polished to a shiny surface and ultrasonically cleaned with acetone and ethanol before implantation was conducted in our plasma immersion ion implanter [16–18]. The implantation parameters are displayed in Table 1. Medical grade stainless steel spinal rods (ISOLA System, DePuy Spine Inc.) 6 mm in diameter were trimmed down to 5 mm in diameter and then sliced into 1 mm thick disks. The samples were polished and cleaned similarly before characterization and cell culturing.

The surface chemical compositions were determined by X-ray photoelectron spectroscopy (XPS) (Physical electronics PHI 5802 system, Minnesota, USA). Surface contaminations were first removed by Ar ion sputtering. A monochromatic aluminum X-ray source was used and the sampled area was 0.8 mm in diameter. The scanning step size was 0.8 eV. The energy scale was calibrated using the Cu2p3 (932.67 eV) and Cu3p (75.14 eV) peaks from a pure copper standard. The electrochemical tests [19] in accordance with ASTM G5-94 (1999) and G61-86 (1998) were performed on a potentiostat (VersaStat II, Minnesota, USA). Surface contaminations were cleaned similarly before characterization and cell culturing. The surface chemical compositions were determined by X-ray photoelectron spectroscopy (XPS) (Physical electronics PHI 5802 system, Minnesota, USA). Surface contaminations were first removed by Ar ion sputtering. A monochromatic aluminum X-ray source was used and the sampled area was 0.8 mm in diameter. The scanning step size was 0.8 eV. The energy scale was calibrated using the Cu2p3 (932.67 eV) and Cu3p (75.14 eV) peaks from a pure copper standard. The electrochemical tests [19] in accordance with ASTM G5-94 (1999) and G61-86 (1998) were performed on a potentiostat (VersaStat II, Minnesota, USA). Surface contaminations were cleaned similarly before characterization and cell culturing.

To investigate the cyto-compatibility of the plasma-treated and untreated samples, osteoblasts isolated from calvarial bones of 2-day-old mice that ubiquitously expressed an enhanced green fluorescent protein (EGFP) were used in our culture in a Dulbecco’s Modified Eagle Medium (DMEM) (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Biowest, France), antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin), and 2 mM L-glutamine at 37 °C in an atmosphere of 5% CO₂ and 95% air. The specimens (1 mm thick and 5 mm in diameter) were fixed onto the bottom of a 24-well tissue culture plate (Falcon) using 1% (w/v) agarose. A cell suspension consisting of 5000 cells was seeded onto the sample surfaces as well as wells without any metal disk serving as a control. Cells were grown in 1 ml of medium and changed every 3 days. Cell attachment was examined after the second day of culture, and cell proliferation examined after 4, 6 and 8 days of culture. Four samples were taken at each time point to obtain better statistics. In our study, cells were allowed to reach confluence during the examination period. To determine the cell number, the attached cells were released by digestion with trypsin-EDTA (Invitrogen) and counted using a haematocytometer (Tiefe Depth Profondeur, Marienfeld, Germany). Cell viability was assessed by staining with 0.2% Trypan blue (Sigma). The number of cells was expressed as a mean value ± standard deviation (S.D.). The data were analyzed by using unpaired two-sample t-test and the statistical analysis was performed using the SPSS program (SPSS for Windows, Release 11.0.0). Cell proliferation was observed by using a fluorescent microscope (Axioplan 2, Carl Zeiss, Germany). The attached living EGFP-expressing osteoblasts were visualized using a 450–490 nm incident filter and the fluorescence images emitted at 510 nm captured using a Sony DKS-ST5 digital camera.

3. Results and discussion

Table 2 lists the detected elements or compounds from the sample surface derived from their binding energies. The major compounds found on the untreated NiTi surface include TiO₂, TiO, and NiO. The N-PIII surface consists of TiN, TiO₂ and small amounts of NiO. The depth profile (data not shown) of the

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nitrogen-PIII</th>
</tr>
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<tbody>
<tr>
<td>Gas type</td>
<td>N₂</td>
</tr>
<tr>
<td>RF power</td>
<td>1000 W</td>
</tr>
<tr>
<td>High voltage</td>
<td>−40 kV</td>
</tr>
<tr>
<td>Pulse width</td>
<td>30 µs</td>
</tr>
<tr>
<td>Frequency</td>
<td>50 Hz</td>
</tr>
<tr>
<td>Implantation time (min)</td>
<td>240</td>
</tr>
<tr>
<td>Base pressure</td>
<td>7.0×10⁻⁵ Torr</td>
</tr>
<tr>
<td>Working pressure</td>
<td>6.4×10⁻⁴ Torr</td>
</tr>
<tr>
<td>Dose</td>
<td>1.4×10¹⁶ cm⁻²</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Elements/compounds formed on surface (binding energy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated NiTi</td>
<td>TiO (455 eV), TiO₂ (458.8 eV), NiO (853.8 eV)</td>
</tr>
<tr>
<td>N-PIII NiTi</td>
<td>TiN (456 eV), TiO₂ (458.8 eV), NiO (853.8 eV)</td>
</tr>
<tr>
<td>SS</td>
<td>Mo (228 eV), Fe (707 eV), carbide (282 eV), Cr (574.4 eV), NiO (853.8 eV)</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Samples</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated NiTi</td>
<td>461</td>
</tr>
<tr>
<td>N-PIII NiTi</td>
<td>1080</td>
</tr>
<tr>
<td>SS</td>
<td>422</td>
</tr>
</tbody>
</table>
N-PIII sample suggests that the NiO concentration is quite low as compared to that of the untreated NiTi sample. Iron is the major element on medical grade stainless steel surface (depth profile data not shown). In addition to small amounts of Cr and Mo, some carbides and NiO are present.

The essential readings from our electrochemical tests are shown in Table 3. The breakdown potential is designated by $E_b$. Higher $E_b$ values represent better corrosion resistance. The $E_b$ values measured from the untreated NiTi, N-PIII NiTi, and SS samples are 461 mV, 1080 mV, and 422 mV, respectively. Therefore, the corrosion resistance of the four samples in ascending order is SS < untreated NiTi < nitrogen-PIII treated NiTi. The N-PIII samples exhibit higher $E_b$ values than the untreated NiTi and SS samples, suggesting that the corrosion resistance of the nitrogen-PIII samples is enhanced. The SEM surface morphologies of the samples after the electrochemical tests are shown in Fig. 1. Only tiny holes can be observed on the N-treated surface, whereas much bigger holes with irregular shapes are found on the untreated NiTi and SS sample surface. Our results demonstrate unambiguously that the corrosion resistance of N-treated NiTi is more superior.

The amounts of ions leached from the samples after the immersion tests are listed on Table 4. The amount of Ni leached from the untreated sample is 320 μg/L, whereas that from the N-PIII sample is 57.9 μg/L. It suggests that out-diffusion of Ni ions is significantly reduced by nitrogen-PIII. No Ti is found from the untreated and N-PIII NiTi. With regard to the SS sample, the ions leached from the SS sample are Cr 47 μg/L, Fe 35 μg/L, Ni 22 μg/L, and Mo 3.8 μg/L.

Nano-indentation was employed to examine the surface hardness (H) of the samples. The hardness profiles are shown in Fig. 2. In the nitrogen-PIII sample, the maximum hardness is 7.7 GPa on the top surface. It gradually decreases to 4.5 GPa at a depth of 165 nm. In the untreated NiTi sample, the maximum hardness is 5.2 GPa at around 30 nm from the surface and gradually diminishes to 4.7 GPa at 150 nm. In the SS sample, the hardness exhibits the maximum value of 6.7 GPa at 50 nm from the surface and then decreases gradually to a rather constant value of 5.2 GPa at 200 nm from the surface. Our

### Table 4: Immersion test results

<table>
<thead>
<tr>
<th>Elements samples</th>
<th>Al</th>
<th>Ti</th>
<th>V</th>
<th>Cr</th>
<th>Fe</th>
<th>Ni</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated NiTi</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>320</td>
<td>–</td>
</tr>
<tr>
<td>N-PIII NiTi</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>57.9</td>
<td>–</td>
</tr>
<tr>
<td>SS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>47</td>
<td>35</td>
<td>22</td>
<td>3.8</td>
</tr>
</tbody>
</table>

ND—Not detected.

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Fig. 1. SEM micrographs of the plasma implanted NiTi and other metals after electrochemical tests: (A) NiTi alloy without surface treatment, (B) with nitrogen-PIII implantation and (C) SS.

Fig. 2. Hardness versus depth profiles acquired from the untreated NiTi, N-PIII NiTi, and stainless steel samples.
results suggest that the hardness of the nitrogen-PIII surface is generally higher than that of the untreated NiTi substrate at 0–75 nm. Compared to the SS sample, the hardness of the N-PIII NiTi sample is lower than that of the Ti sample except at the topmost region. In general, the surface hardness of the N-PIII NiTi is higher than that of the untreated NiTi and SS samples.

Fig. 3 shows the cell proliferation versus number of days plots. The nitrogen-PIII sample is well tolerated by the EGFP-expressing osteoblasts. After culturing for 2 days, the cells start to attach to and proliferate on all the samples except for stainless steel. After 4 days, cell proliferation on the untreated NiTi alloy samples is slightly higher than that of the nitrogen-PIII NiTi, SS samples. However, the nitrogen-PIII samples exhibit the highest degree of cell proliferation among the samples after 6 and 8 days of culturing. Cell proliferation on the SS samples is significantly lower than that on the N-treated NiTi and the untreated NiTi control sample after 8 days \((P<0.05)\). The cell proliferation observed on the untreated NiTi, N-PIII NiTi and stainless steel samples after 2 days of culturing are shown in Fig. 4. It is clearly seen that cells are attached to and proliferate on all the samples. The results of cell culturing undoubtedly demonstrate that there is no immediate cyto-toxic effects on the N-PIII NiTi samples. The stainless steel samples show the least degree of cell proliferation after 8 days of cell culturing. An insignificant amount of dead cells emerges after 8 days of culturing perhaps due to cell apoptosis.

Nitrogen-PIII produces a thin layer of TiN on the surface together with a graded interface with the NiTi substrate. In addition to establishing a protective layer against corrosion and wear, this treatment suppresses the surface Ni concentration and reduces the possibility of Ni ion leaching. Moreover, the surface TiN which can be classified as hard ceramic [21] possesses higher surface hardness compared to the untreated NiTi and SS.

Medical grade stainless steels are the most common implantable materials in medicine. These metals are believed to possess good corrosion resistance and deformability as well as good compatibility with living tissues [22]. However, it is known that stainless steels contain small amount of nickel (Ni) and chromium (Cr) for corrosion resistance enhancement. Ni is toxic to living tissues and reported to be carcinogenic as well. Furthermore, Cr may cause impairment of osteoblast proliferation and differentiation in addition to cytokine release [23]. Our XPS results reveal that Cr and Ni are detected on the surface of the medical grade stainless steel samples and the immersion test results also confirm out-leaching of these ions. It has also been reported that stainless steels have poor corrosion resistance under physiological conditions resulting in Ni and Cr release [1]. These findings may explain the insignificant growth of osteoblast on the stainless steel samples in our study [24].

The use of nickel titanium alloys in human implants is still controversial due to its extremely high nickel concentration compared to other medical grade metals such as stainless steels. Adverse effects such as nickel ion leaching from implants has been reported in humans [25]. Previous in vivo and in vitro studies indicate that the rate of cell proliferation is lower on NiTi samples compared to stainless steels [26]. However, our cell culturing experiments show higher proliferation on the untreated NiTi samples than medical grade stainless steels after 8 days of culturing \((p<0.05)\). In addition to the better surface mechanical properties, nitrogen-PIII favors osteoblast proliferation. Based on reports in the literature, the TiN coating is well tolerated by different cells, particularly bone cells [27]. This phenomenon can be attributed to the growth of the calcium phosphate phase on the surface of TiN coated titanium implants, whereas such activities do not take place on the untreated titanium based implants [28]. Our surface composition analysis reveals that the surface treated layer consists of TiO\(_x\)N\(_y\) oxynitride (data not shown). This coating is favorable to the formation of bone-like materials under in vivo conditions. Our cell culturing results also suggest that the N-PIII samples are as good as, if not better than, the untreated NiTi alloy. However, it should be mentioned that this study only reveals the short term
cyto-compatibility effects. A long term cytotoxicity test up to a year is necessary prior to subjecting these surface-treated materials to clinical use.

4. Conclusion

A graded TiN layer is formed on the surface of NiTi alloy after nitrogen plasma immersion ion implantation. The enhanced surface possesses better corrosion and wear properties than the untreated NiTi and medical grade stainless steel. In terms of cyto-compatibility, the cell viability on stainless steel is significantly inferior compared to the untreated NiTi and nitrogen-PIII NiTi. Our data suggest that nitrogen-PIII is favorable to osteoblast proliferation and a viable method to improve the corrosion resistance of NiTi and mitigate nickel out-diffusion from the materials.

Acknowledgements

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