Biocompatibility and corrosion behavior of the shape memory NiTi alloy in the physiological environments simulated with body fluids for medical applications

Jafar Khalil-Allafi a,⁎, Behnam Amin-Ahmadi b, Mehrnoush Zare a

a Research Center for Advance Materials, Faculty of Materials Engineering, Sahand University of Technology, Tabriz, Iran
b Department of Materials Science and Engineering, Sharif University of Technology, Tehran, Iran

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A B S T R A C T

Due to unique properties of NiTi shape memory alloys such as high corrosion resistance, biocompatibility, super elasticity and shape memory behavior, NiTi shape memory alloys are suitable materials for medical applications. Although TiO₂ passive layer in these alloys can prevent releasing of nickel to the environment, high nickel content and stability of passive layer in these alloys are very debatable subjects. In this study a NiTi shape memory alloy with nominal composition of 50.7 atom% Ni was investigated by corrosion tests. Electrochemical tests were performed in two physiological environments of Ringer solution and NaCl 0.9% solution. Results indicate that the breakdown potential of the NiTi alloy in NaCl 0.9% solution is higher than that in Ringer solution. The results of Scanning Electron Microscope (SEM) reveal that low pitting corrosion occurred in Ringer solution compared with NaCl solution at potentiostatic tests. The pH value of the solutions increases after the electrochemical tests. The existence of hydride products in the X-ray diffraction analysis confirms the decrease of the concentration of hydrogen ion in solutions. Topographical evaluations show that corrosion products are nearly same in all samples. The biocompatibility tests were performed by reaction of mouse fibroblast cells (L929). The growth and development of cells for different times were measured by numbering the cells or statistics investigations. The figures of cells for different times showed natural growth of cells. The different of the cell numbers between the test specimen and control specimen was negligible; therefore it may be concluded that the NiTi shape memory alloy is not toxic in the physiological environments simulated with body fluids.

1. Introduction

The NiTi binary shape memory alloys are suitable materials for medical applications. This is due to unique characteristics of shape memory NiTi alloys such as high corrosion resistance, biocompatibility, super elastic and shape memory behavior. One of the significant factors of biomaterials for medical application is the elastic modulus compared with body tissues and bone. It is well known that biomaterials such as titanium alloys and stainless steels have high elastic modulus compared with NiTi shape memory alloys. Besides, NiTi shape memory alloys have super elastic behavior (up to 10% reversible strain). These excellent properties have made NiTi alloys a good candidate for medical applications. Formation of Titanium oxide (TiO₂) layer on the surface of the alloy enhances good biocompatibility with regard to high Ni content (above 50%) of NiTi shape memory alloys. Corrosion and release of Ni ion into the body environment lead to the decrease of biocompatibility in NiTi alloys; but many investigations approve long performance of NiTi implants [1–6], so NiTi alloys should present good corrosion resistance in contact with body fluids. Many factors affect corrosion resistance and biocompatibility of NiTi alloys such as quality of finished surface, remained elements on the surface and homogeneity of microstructure [7]; therefore corrosion investigation of NiTi alloys in the body environment can determine the biocompatibility of NiTi alloys. The body fluid consists of 1% sodium chloride and scarce value of different salts and organic components at 37 °C. Thus corrosion in the body environment is similar to warm sea water which leads to many types of corrosions such as galvanic, pitting and grooving corrosion. Many studies have been performed by researchers related to the corrosion behavior of NiTi alloys in physiological environments. Many researchers presented the corrosion resistance of Nitinol alloys is excellent and others reported the poor corrosion resistance of NiTi shape memory alloys in the literature [7]. Good corrosion resistance is due to formation of titanium dioxide on the alloy surface at natural conditions. TiO₂ has low free energy formation in comparison with TiO and NiO [8], thus titanium dioxide forms thermodynamically on the surface of NiTi alloys. Self healing of titanium dioxide in scratch test is low. It is due to existence of Ni in oxide composition. Heat treatment and surface composition of NiTi alloys affect the stability of titanium oxides.

In the present research work biocompatibility and corrosion behavior of NiTi shape memory alloys at simulated body aqueous fluids have been investigated.
2. Materials and methods

NiTi shape memory alloy with 50.7 atomic percent Nickel was produced by VIM (vacuum induction melting) method [9]. Pure titanium slab (99.5%) and pure Nickel plates (99.9%) were used to produce Ni50.7Ti shape memory alloy. The produced slab (15 × 10 × 5 cm) was rolled up to 1.5 mm thickness. The rolled sample was homogenized at 1050 °C for 24 h. This composition of NiTi alloy was chosen since it shows super elastic behavior at room temperature. In other words, the austenite finish temperature (Af) is below 25 °C. In order to investigate the microstructure and evaluate the grain size number using the optical microscope (Olympus PMB3), the samples after grinding and polishing were etched in 2H2O, 2HNO3, and 1HF solution. Grain size number of samples was determined according to ASTM E112 standard.

2.1. DSC experiments

DSC experiments were performed (type 822e Mettler Toledo) according to ASTM F2004 standard in the air in order to determine the transformation temperatures. DSC specimen (48.2 mg) was heated up to 125 °C, where they were held for 5 min. Then the DSC measurements started by cooling the specimens down to −100 °C with a cooling rate of 10 °C min−1. At −100 °C the specimens were again held for 5 min and then heated up to 125 °C with a heating rate of 10 °C min−1.

2.2. Corrosion experiments

Electrochemical tests at physiological environment simulated with body fluids were done according to ISO 10993-15 standard. The electrochemical behavior was investigated in different solutions, such as Ringer solution and NaCl (0.9 weight percent) solution. The composition of Ringer solution has been presented in Table 1. The reference electrode was an Ag/AgCl electrode. The PGSTAT instrument of Autolab Company was used for electrochemical tests. General Purpose of Electrochemical Software (GPES) was used to data analysis and plotting the corrosion curves. Electrolyte solution was degassed by nitrogen for 10 min with 0.3 l/min intensity and then it was poured into the cells. Samples (2 × 1.5 × 0.15 cm) were polished and all of their surfaces except the area of 1 cm2 were covered to preserve from contacting with electrolyte solution. The surface of the samples was degreased by acetone. The electrochemical cells were calibrated.

Table 1

<table>
<thead>
<tr>
<th>Composition</th>
<th>Concentration (g/100 ml)</th>
<th>Equimolar (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.86</td>
<td>147</td>
</tr>
<tr>
<td>KCl</td>
<td>0.03</td>
<td>4.5</td>
</tr>
<tr>
<td>CaCl2, 2H2O</td>
<td>0.03</td>
<td>4.0</td>
</tr>
<tr>
<td>a+ K+ Cl−</td>
<td></td>
<td>156.0</td>
</tr>
</tbody>
</table>

Fig. 1. a) Optical micrograph and b) DSC curve of NiTi shape memory alloy with 50.7 at.% homogenized at 1050 °C for 24 h.

Fig. 2. Potentiodynamic polarization curves of NiTi samples in the a) 0.9% NaCl and b) Ringer solutions.
and 20 mm, respectively. The mouse culture experiments. The diameter and height of each well plate was 25
mm. The samples were degreased by acetone, too. The contact angle was measured by contact angle measuring system type KRUSS G10 to
study the surface wettability of sample surface.

Data were collected from 10° to 90° (2θ) with a step size of 0.05°.

2.3. SEM and XRD experiments

The surface morphology of the samples after corrosion tests was observed by SEM XL30, Philips Co., Holland. Before SEM observation, the
samples were coated with gold by physical vapor deposition method
with BAL-TEC sputter-coater instrument. The surface coating is needed
for none conductive materials to achieve the better image quality.

To evaluate available phases, the XRD profiles were collected on a
Bruker-Axs type D8 Advance diffractometer equipped with a Cu-
Kα tube (wavelength = 1.54 Å) operating at 40 kV and 40 mA.

3. Results and discussion

3.1. Corrosion behavior

Potentiodynamic polarization curves of NiTi samples in the 0.9% NaCl
and Ringer solutions have been shown in Fig. 2. A same corrosion
behavior does exist for both solutions. With the increase of the potential, the
corrosion current increases with a mean gradient. Further increase of
potential reduces the increasing rate of corrosion current because the
alloy is in the passive region. In the passive region the increase of the
passive potential occurs. With further increase of the potential, the
pitting corrosion happens and the alloy is in the transpassive state, thus
the corrosion current increases. The passive state can be achieved again
in the high potential which is called repassivation. In this region, the
increase of the corrosion current is slight. In the reverse scanning, a shift of whole polarization curve towards the region of
higher corrosion current in a constant potential was achieved. It is clear
from Fig. 2 that the line of the reverse scanning is in the higher position
relative to the direct scanning. This is due to the pitting corrosion of the
NiTi alloy in the 0.9% NaCl and Ringer solutions. The intercept point of
the direct and reverse scanning lines indicates the resistant potential (Ecorr).

Table 2. The values of the corrosion properties of the NiTi alloy in the 0.9% NaCl and ringer
solutions (potential in the form of mV and current in the form of logarithm A).

<table>
<thead>
<tr>
<th>Corrosion parameters</th>
<th>0.9% NaCl</th>
<th>Ringer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosion current (Icorr)</td>
<td>3.357×10⁻⁸</td>
<td>5.1×10⁻⁷</td>
</tr>
<tr>
<td>Corrosion potential (Ecorr)</td>
<td>−404</td>
<td>−492</td>
</tr>
<tr>
<td>Passive potential (Epass)</td>
<td>−270</td>
<td>−481.8</td>
</tr>
<tr>
<td>Passive current (Ipass)</td>
<td>4.7×10⁻⁶</td>
<td>2.12×10⁻⁶</td>
</tr>
<tr>
<td>Pitting potential (Etrans)</td>
<td>230</td>
<td>104.5</td>
</tr>
<tr>
<td>Repassivation potential (Erep)</td>
<td>465</td>
<td>153.1</td>
</tr>
<tr>
<td>Resistant potential (Erp)</td>
<td>−135</td>
<td>−200</td>
</tr>
</tbody>
</table>

The electrolytes were collected after potentiostatic tests to quantify
the amount of Ni ions by Perkin Elmer 2100 spectrometer. It is noticeable that the values of pH for the solutions were measured by
Metrohm 692 instrument before and after all electrochemical tests.

2.4. biocompatibility tests

Sheets of the NiTi alloy with 50.7 atomic percent nickel was cut
into rectangular pieces with a size of 10×10×1.5 mm³. The samples
were ground and polished and then rinsed by distilled water. The samples were degreased by acetone, too. The contact angle was
measured by contact angle measuring system type KRUSS G10 to
study the cellular adhering according to the sessile drop.

All samples used for cell culture tests were ultrasonically rinsed by
acetone: water (1:4) solution for 20 min, washed by distilled water,
air-dried and autoclaved at 120 °C for 10 min prior to the tests.

Samples were separately placed in the bottom of well plates for cell
culture experiments. The diameter and height of each well plate was 25
and 20 mm, respectively. The mouse fibroblasts L929 cells were used to
cell culture experiments. The cell culture medium was R-6504-
Lot096H19580432-SIGMA Medium RPMI-1640. Sodium bicarbonate
and penicillin streptomycin 100 µg/ml were added to the cell culture
medium. The blank well plate was used for negative control.

The samples of cytotoxicity test were wire cut (1×1×1.5 cm), then
ground and polished. The cells were incubated in the medium at 37 °C in
a 5% CO₂ atmosphere with 95% relative humidity and they were held for
2, 5 and 9 days.

After incubation, the unattached cells were removed using a
phosphate buffered saline (PBS) solution. The samples after 5 and
9 days were chosen for SEM analysis.

Proliferation test was done by Image-Pro Plus version 1.3. The p-value
was measured by student t-test method. Differences were considered
significant at p<0.05.

![Fig. 3](image-url). Potentiostatic polarization curves of NiTi samples in the a) 0.9% NaCl and b) Ringer solutions.
potential of the NiTi alloy is negative which facilitates the corrosion in NiTi alloys; on the other hand, due to formation of passive layer and low passive current in this alloy, a good corrosion resistance can be achieved. It is better to notice that the current density is almost stable just before the pitting corrosion and then increases, suddenly.

It can be concluded from Table 2 that the corrosion resistance of NiTi alloy in 0.9% NaCl solution is better than Ringer solution, because the pitting potential and resistant potential of NiTi alloy in Ringer solution is lower than the 0.9% NaCl solution. This is due to the composition of Ringer solution. The Ringer solution contains potassium chloride, calcium chloride, sodium chloride. The content of chloro ion (Cl\(^{-}\)) in the Ringer solution is 0.03 g/l higher than the 0.9% NaCl solution.

On the other hand, the passivation and repassivation potentials of NiTi alloy in the Ringer solution are lower than 0.9% NaCl solution. This can be due to the higher oxygen content of the Ringer solution [5].

Potentiostatic polarization curves of NiTi samples in the 0.9% NaCl and Ringer solutions have been shown in Fig. 3. It can be seen from Fig. 3 that with increase of time, the current suddenly increases and then a relative stable current is achieved in both solutions. This indicates the formation of the passive layer in the NiTi alloy in the 0.9% NaCl and Ringer solutions. It is clear from Fig. 3 that the mean stable current of NiTi alloy in Ringer solution is 2–2.5 times greater than that in the 0.9% NaCl solution; therefore the corrosion rate of NiTi alloy in Ringer solution is higher than that in the 0.9% NaCl solution. These findings confirm the potentiodynamic results.

The measured pH of solutions before and after electrochemical tests has been represented in Table 3. It is obvious that the pH value of the solutions increases after the electrochemical tests, therefore the concentration of hydrogen ion in both solutions decreases. The existence of hydride products in the XRD analysis confirms the decrease of the concentration of hydrogen ion in solutions.

With increase of the pH, the intensive pitting corrosion can occur instead of homogenous corrosion [4]. In other words, the pitting potential decreases when the pH increases [11,12]. The difference between the original pH with the measured pH after test for the Ringer solution is lower than that in the 0.9% NaCl solution, thus the pitting corrosion of the NiTi alloy in the Ringer solution is lower than that in the 0.9% NaCl solution.

The Ni release was measured by atomic absorption method in high potentials and short times. The mean Ni release of NiTi alloy with 50.7 at.% nickel in the 0.9% NaCl and Ringer solutions were 0.35 and 1.67 ppm, respectively. This value is negligible in comparison with the daily delivered nickel of human body [5].

SEM micrograph of the samples after potentiodynamic tests confirms the pitting corrosion in the surface of NiTi alloy. The SEM micrograph of

### Table 3
The measured pH of solutions before and after electrochemical tests.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Original pH</th>
<th>After potentiodynamic test</th>
<th>After potentiostatic test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>6.8</td>
<td>7.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Ringer</td>
<td>6.9</td>
<td>9.1</td>
<td>8</td>
</tr>
</tbody>
</table>

![Fig. 4](image_url) SEM micrographs of the NiTi sample at different magnifications after potentiodynamic test in the 0.9% NaCl solution.

![Fig. 5](image_url) The SEM micrographs of the NiTi sample after potentiostatic test in a) 0.9% NaCl solution and b) Ringer solution.
the NiTi sample at different magnifications after potentiodynamic test in the 0.9% NaCl solution has been displayed in Fig. 4. It is clear that the pitting corrosion has been occurred in the NiTi sample in NaCl solution, but no pitting corrosion was observed for NiTi sample in the Ringer solution after potentiodynamic test. Fig. 5 shows the surface of NiTi samples after potentiostatic test in the NaCl and Ringer solutions. As discussed above, the difference between the original values of pH with the measured pH after potentiostatic tests for Ringer solution was low in comparison with NaCl solution, therefore it can be predicted that low pitting corrosion will occur in Ringer solution compared with NaCl solution at potentiostatic tests. SEM micrographs presented in Fig. 5 approve this theory. Besides, a stable current was achieved for NiTi alloy in the Ringer solution according to the potentiostatic curves (Fig. 3), therefore low pitting corrosion can be predicted.

3.2. Biocompatibility tests

Before cell culture experiments, the contact angle of the NiTi alloy was measured. The contact angle with polar liquid (water) was 80.9° and the antipolar liquid (di-iod-methanol) was 60.8°.

Fig. 6 shows the proliferation of the cells for NiTi alloy with 50.7 at.%. A natural cell growth was achieved by comparing the cell growth of the control sample with the cell culture experiments. The cell proliferation is a multi step process including a) Cell attachment, b) formation of Filopodia (for easy attachment), c) webbing and d) stretching [13]. It is clear from Fig. 6 that the cell growth rate was decreased after holding for 9 days. This is due to the destroying of cells.

It can be concluded from Fig. 6 that the number of cells increases when the holding time increases. Besides, it was not obtained any...
disadvantage effect in the growing cells; on the other hand a single cell was achieved after holding for 9 days. The proliferation of cells is controlled by the parameter called the contact inhibition. When the cells grow and their membranes contact with each other, the protein sprinkle of cells prohibit them from further proliferation. The contact inhibition parameter preserves the cells from growth leading to cancer.

Hiromoto et al. [13] investigated the growth of the same cells on the titanium metal. The presented cell growth after 5 days is similar with our results after holding for 9 days.

The SEM micrographs presented in Fig. 7 clearly exhibited the cell response on the metal. The cell adhesion and stretching on the metal have been shown in Fig. 7. After 9 days, we can see a complete cell layer on the surface of metal.

It is known that Ni as an element or combination with other elements has negative effects on the biological tissues due to its poisonous behavior. These effects can be generated by using the alloys as implants or prostests in the human body for long times. It is important to investigate the biological effects of the NiTi alloys due to the high Ni content of the binary NiTi alloys (above 50at.%) [14,15]. Therefore, the Ni release during cell culture was analyzed by atomic absorption spectroscopy. The Ni release of the samples at RPMI environment for 2, 5 and 9 days was 0.22, 0.29 and 0.36 ppm, respectively which is negligible compared with daily delivery of nickel.

The number of created live cells in the RPMI environment for the holding time of 2, 5 and 9 days was 48, 150 and 175, respectively. The p-value was calculated for samples at different holding times and compared with the control sample. A negligible difference with the control sample was achieved because they all were lower than 0.05. Therefore, it may be concluded that the NiTi samples were non-poisonous.

4. Conclusions

1. The pitting corrosion of NiTi alloy with 50.7 atomic percent nickel at potentiodynamic test in Ringer solution was higher than that in the NaCl solution.

2. The difference between the original values of pH with the measured one after potentiostatic tests for Ringer solution was lower than that in the NaCl solution. The SEM micrographs reveal that low pitting corrosion occurred in Ringer solution compared with NaCl solution at potentiostatic tests.

3. The results of Ni release by atomic absorption method showed that the NiTi alloy with 50.7 at.% nickel in the Ringer solution or 0.9% NaCl is a promising candidate for biomedical applications.

4. The number of created live cells in the RPMI environment which were held for 2, 5 and 9 days was 48, 150 and 175, respectively.

5. The Ni release of the samples at RPMI environment for 2, 5 and 9 days was 0.22, 0.29 and 0.36 ppm which is negligible compared with Ni delivery each day.

6. The p-value was calculated for samples at different holding times and compared with the control sample. A negligible difference with the control sample was achieved because they all were lower than 0.05. Therefore, it may be concluded that the NiTi samples were non-poisonous.

References