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Cytotoxicity of Metal Ions Released from Nitinol Alloys on Endothelial Cells

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Abstract

Most implantable medical devices are expected to function in the body over an extended period of time. Therefore, immersion tests under simulated conditions can be useful for assessing the amount of metal ions released in situ. In this investigation, dissolved ions from as-received binary and ternary Nitinol alloys in cell culture media were periodically measured under static and dynamic conditions. Endothelial cells were grown in aliquots of culture media obtained and the effect of dissolved ions on cell proliferation and viability of endothelial cells (HUVEC) was studied by cytotoxicity assays. The concentration of metal ions in the media was measured by inductively coupled plasma mass spectrometry.

Keywords

biomaterials; intermetallics; material selection

1. Introduction

Nitinol usage for biomedical implant devices has received significant attention due to its superior corrosion resistance and biocompatibility. The potential problem with Nitinol implant devices is the release of nickel in the human body, which has stimulated a significant amount of research in this area. The Nickel ions can act as cofactors in enzymatic processes involved in protein synthesis and cell replication, change cell morphology, vanquish cell organelles, and even decrease cell numbers. Ni^{+2} can act as an antagonist to

the essential metal ions such as Mg^{+2} , Ca^{+2} , and Zn^{+2} and thereby disturb the biological processes (Ref 1–4). In order to extend the specific usage of Nitinol in biomedical applications, some ternary Nitinol alloys (NiTiX) have been developed and studied (Ref 1, 2).

2. Materials and Methods

Ni-49 at.%Ti, Ni-44.1 at.%Ti-10 at.%Cu, Ni-44.1 at.%Ti-10 at.%Ta, and Ni-44.1 at.%Ti-10 at.%Cr alloys were prepared by arc melting at the National Institute of Standards and Technology (NIST). Samples were prepared by cutting the cylindrical ingots with a high-speed saw into disks of dimension (1 cm × 2 mm). Samples were ultrasonically cleaned with acetone for 5 min.

3. Immersion Tests

The cell culture media was prepared by adding 10% Fetal Bovine Serum (FBS), 1% penicillin, 0.1 mg/mL heparin, and 0.03 mg/mL ECGS into F-12K medium. Nitinol alloys (NiTi, NiTiCr, NiTiCu, NiTiTa) were immersed in six-well cell culture plate in 15 mL of cell culture media (Static Immersion). The cell culture plate was placed in an incubator at 37 °C having 5% CO₂. Similarly, the second set of Nitinol alloys (NiTi, NiTiCr, NiTiCu, NiTiTa) were also immersed in 15-mL cell culture media in the polypropylene centrifuge bottles. Each sample was placed at the bottom of the bottle, which was tightly closed before placement on the shaking plate (Dynamic Immersion). Bottles along with the shaking plate were placed in the incubator at 37 °C having 5% CO₂. After every 1, 3, 7, 15, 28, and 42 days, cell culture media was collected (from static and dynamic immersion tests) and the Nitinol alloys were placed in fresh cell culture media. The collected cell culture media was used for the determination of dissolved metal ions content and also for the sulforhodamine B (SRB) assays.

3.1 Metal Ion Concentration

Metal ion concentration in the cell culture media was determined by ICPMS analysis. As the cell culture media contained amino acids, sugars, vitamins, and lipoic acid, these samples were digested (Ref 1) prior to ICPMS analysis, following the procedure described below:

First, 2 mL of HNO₃-optima grade, (diluted with distilled water in a 1:1 dilution) was added to 2 mL of the each sample. Samples were covered with reflux caps and were heated in a hot block (open vessel digestion system) at 100 °C for 20 min. Later, samples were allowed to cool down and then 2 mL of concentrated HNO₃-optima grade was added. Samples were then heated to 100 °C for 30 min. They were heated again for an additional 30 min without allowing the sample to evaporate. Samples were taken out from the hot block and allowed to cool down. 1.0 mL of 30% H₂O₂-optima grade was added drop-wise. An exothermic reaction took place for approximately 5 min. The samples were again placed in a hot block and 1.0 mL of 30% H₂O₂-optima grade was added to each sample. Samples were allowed to evaporate. Each sample was analyzed in three replicates by ICP-MS analysis.

3.2 Sulforhodamine B (SRB) Assays

Sulforhodamine B (SRB) assays were performed to elucidate the effect of metal ions on the growth of human umbilical vein endothelial cells (HUVEC) cells. HUVEC cells were cultured in a 75 mL cell culture flask. 200 µL of cell culture media were placed into each well of 96-well cell culture plate at a concentration of 1×10^5 cells/mL (approx. 20,000 cells/well). The culture plate was placed in an incubator at 37 °C that was maintained at a high humidity and an atmosphere of 5% CO₂. The HUVEC cells were allowed to grow for 24 h. Cell culture media collected after static and dynamic immersion tests were added to the cells

to conduct SRB analyses. The cells were allowed to incubate for 24 h, after which, SRB assay was performed.

SRB assays measures total biomass (total numbers of cells) based on cellular protein content. Briefly, 50 μL of trichloro acetic acid (TCA), which is used to fix the cells to the bottom of the plate, was added to each well. The well plate was held at room temperature for 30 min. The cell culture plates were then washed using deionized water and allowed to air dry. 100 μL of SRB solution was placed into each well and allowed to sit for 30 min (SRB binds to protein). The plates were then rinsed with 1% acetic acid to remove unbound SRB and again left to air dry. 200 μL of tris base solution were now added to each well to dissolve SRB bound to the protein. The Plates were then placed into a TECAN GENios multiplate reader for reading of the 60 absorbance of the sulforhodamine. Each experiment was repeated three times and the results obtained were normalized with respect to cell grown in fresh cell culture media.

4. Results and Discussions

Table 1 lists the average concentration of dissolved metal ions in the cell culture media. A relatively high concentration of Ni was observed in both static and dynamic test conducted on binary NiTi. NiTiCu, and NiTiCr alloys released higher concentrations of Cu and Cr under static immersion as compared with dynamic immersions. The amount of Ta released from NiTiTa under both static and dynamic immersions was very low.

Passive metals like titanium, chromium, and tantalum develop stable oxide layers on Nitinol surfaces, which contribute to their corrosion resistance in physiological conditions. NiTi and NiTiCu forms a passive TiO_2 layer on their surfaces while other ternary Nitinol alloys, NiTiCr and NiTiTa forms Cr_2O_3 and Ta_2O_5 , respectively, in addition to TiO_2 layer. These oxides films serve as a protective barrier against metal ion leaching. The protectiveness of this passive film is determined by the rate of metal ion transfer through the film and by the stability of the film against dissolution (Ref 4). Therefore, the metal ion release under immersion tests was due to the partial dissolution of the oxide surface layers on Nitinol alloys and the exposure of the nickel and copper enriched sub-layers. The elemental nickel from the surface layers can diffuse through the porous TiO_2 layer and become released into the surrounding environment (Ref 5, 6). Similarly, the release of Cu and Cr ions was attributed to the fact that their tendency to replace Ni atoms in the crystal lattice (Ref 1, 7). The metal ion concentration generally decreases with number of days immersed mainly due to regeneration the oxide layer (Ref 4).

The average values of three SRB assays and the standard deviation error bar are shown in Fig. 1, where (a) is for static immersion and (b) is under dynamic immersion.

All SRB assay results were normalized with respect to fresh media. The SRB assay results revealed that Ni and Cu ions exhibited greater toxicity than Cr and Ta ions on HUVEC cells. It is the known fact that Ni can destroy cell organelles and even decrease cell numbers (Ref 1, 3). Moreover, Ni^{+2} can act as an antagonist to essential metal ions like Mg^{+2} , Ca^{+2} , and Zn^{+2} and disturb the biological processes (Ref 4). Similarly, Cu^{+2} ions also induce oxidative stress within the cell, which result in severe loss of cell viability (Ref 8).

Low cell viability (low SRB values) was observed with binary NiTi alloy after 1 day of immersion under static (0.742) and dynamic (0.695) immersions. However, higher SRB values (high cell viability) were observed with NiTi after 42 days of immersion under static (0.933) and dynamic (0.836) immersions. This was expected due to the low concentration of metal ions released after 42 days of immersion. It should be noted that when high concentration of Ni and Cu ions was released into the culture media (see Table 1) low SRB

values or low cell viability was observed (see Fig. 1). Similarly, the amount of Ta and Cr ions released (see Table 1) does not seem to effect SRB values very much (see Fig. 1).

5. Conclusions

More nickel was released from the binary Nitinol alloy after 1 day of immersion in cell culture media under static and dynamic conditions as compared with that from the ternary Nitinol alloys. However, all Nitinol alloys released relatively less nickel after 42 days of immersion under both conditions. In general, as the relative proportion of dissolved metal ion increased, the greater was the degree of toxicity. The SRB assay revealed that Ni and Cu ions exhibited greater toxicity than Cr and Ta ions on HUVEC cells.

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Fig. 1.
SRB assays under static (a) and dynamic (b) conditions

Table 1

Average concentration ($\mu\text{g/L}$) of dissolved metal ions under static(S) and dynamic (D) conditions

Nitinol alloys	Ni						X (=Cu, Ta, Cr)					
	Day 1	Day 3	Day 7	Day 15	Day 28	Day 42	Day 1	Day 3	Day 7	Day 15	Day 28	Day 42
NiTi-S	12.4	7.1	1.8	3	4.2	0.9	...	...	...	...	...	...
NiTiCu-S	5.2	4.1	7.8	6.9	12.7	0.6	22.2	16.1	26.6	11.5	14.4	...
NiTiTa-S	1.4	0.1	3.9	4.4	2.8	2.9	...	...	...	0.1	0.1	...
NiTiCr-S	5.5	0.4	1.5	5.5	9.6	1.9	22.5	2.7	...	7.1	12.8	...
NiTi-D	23.4	2.51	4.9	5.8	3.2	3	...	...	...	...	...	...
NiTiCu-D	3.2	8.8	3.1	3.6	1.3	1.4	...	5.4	...	7.2	...	1.7
NiTiTa-D	1.1	3.3	4.9	3.6	2.9	2.1	0.3	...	1.6	0.1	0.2	0.2
NiTiCr-D	10.9	3.5	2.5	3.3	4.4	2.3	1.4	4.2	1.3	3.7	2.4	11.3