**RGDC peptide immobilization on titanium nitride-coated Nitinol surface enhances osteprogenitor cell attachment**

G. Zorn[1], R. Adadi[2], I. Gotman[1], E.Y. Gutmanas[1], C.N. Sukenik[2]

[1] Faculty of Materials Engineering, Technion-IIT, Haifa 32000, Israel
[2] Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel

**Introduction:** Nitinol (NiTi) is an attractive material for medical implants due to its unique shape memory/superelasticity property and low elastic modulus. The integration of this alloy in body tissues can be enhanced by attaching specific biomolecules employing self-assembled monolayers (SAM) as covalent anchors. However, the success of such surface biofunctionalization can be compromised by released metal ions, especially when these are the allergenic and carcinogenic Ni ions. It has been previously shown that ion release from Nitinol can be significantly reduced by coating it with titanium nitride (TiN) [1].

The main thrust of this work was to enhance the cell attachment capability of TiN-coated nitinol via immobilization of an RGD peptide using phosphonate anchored self-assembled monolayer (SAM) as a cross-linker [2]. RGD (arginine-glycine-aspartic acid) amino acid sequence is found in many extracellular matrix proteins and is responsible for their cell attachment activity.

**Methods:** Polished 3 mm thick NiTi (Nitinol) plates were PIRAC nitrided at 1000°C, 1 h to obtain a thin TiN coating. In brief, NiTi samples were placed in a sealed stainless steel container that allows selective inward diffusion of atmospheric nitrogen that subsequently reacts with the Ti component of NiTi to form TiN. Being a non line-of-sight process, PIRAC nitriding allows uniform coating of complex-shape implants.

Peptide grafting onto the nitrided NiTi coupons involved three stages: (i) attachment and self-assembly of 11-Chloroacetyl-1-Undecylphosphonic acid (CAUDPA) by immersion into THF-solution of CAUDPA and heating at 100°C for 18 h[3]; (ii) exchanging the terminal chloroacetyl functionality of CAUDPA with iodoacetyl to enhance electrophilicity; and (iii) reaction of iodoacetyl with the thiol group present in the terminal cystein of the RGDC peptide by soaking in an aqueous solution of RGDC at $T_{room}$ for 24 h. The coupons were characterized at different stages of processing employing scanning electron microscope (SEM), contact angle measurements, Auger electron spectroscopy (AES), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM).

Osteopreginitor cells ($10^4$ cells/cm$^2$) were seeded on the RGDC-grafted and bare TiN-coated NiTi coupons. The cells were allowed to attach for 1 h and then additionally cultured for 48 h (all at 37ºC). SEM examination and Alamar blue metabolic assay were used to measure cell adhesion and viability.

**Results/Discussion:** According to XRD and SEM analyses, PIRAC nitriding of NiTi produced a ~ 0.5 µm thick TiN coating with a $Ti_2Ni$ sub-layer underneath. AES/XPS depth profiles confirmed that the TiN layer contained practically no Ni. The shapes and areas of O1s, N1s and Ti2p XPS peaks were consistent with a topmost surface that is a mixture of Ti oxynitride and $TiO_2$, with a TiN inner layer.

The high resolution XPS and contact angle measurements suggest that immersion in the CAUDPA solution resulted in the formation of a uniform organic monolayer with the molecules covalently bound to TiN-coated NiTi in a configuration where the phosphonic groups turn toward the substrate [3] while the acetyl chloride tails face the free surface. Peptide attachment to the SAM-coated NiTi surface was confirmed by analyzing the shape and area of the N1s XPS peak before and after the grafting procedure, Fig 1. The fraction of NiTi surface covered by RGDC was estimated as 60%. After 48 h in cell culture, a significantly higher density of osteoprogenitor cells was observed on RGDC-grafted vs. bare TiN-coated NiTi coupons, Fig. 2. This is in agreement with the approximately two-fold increase in cell number indicted by Alamar blue fluorescence.

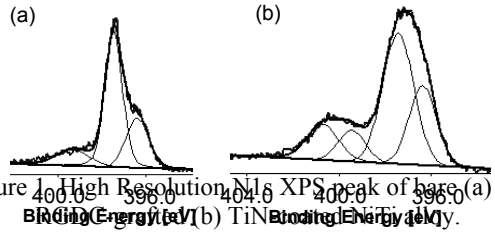


Figure 1. High Resolution N1s XPS peak of bare (a) and RGDC-grafted (b) TiN-coated NiTi alloy.

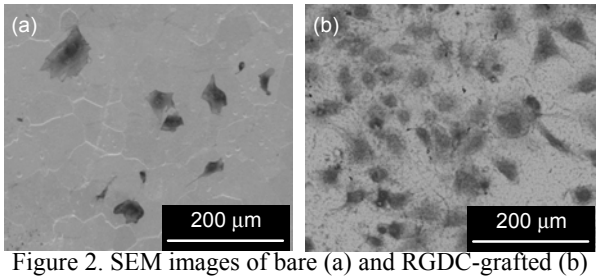


Figure 2. SEM images of bare (a) and RGDC-grafted (b) TiN-coated NiTi alloy after cell culture test.

**Conclusions:** The surface of Nitinol shape memory alloy was coated with TiN to prevent Ni ion release when implanted into the body. This nitrided surface was biofunctionalized by covalent attachment of a phosphonate-anchored SAM that served as a cross-linker for immobilization of RGDC peptide. The peptide grafting procedure resulted in significant enhancement of osteprogenitor cell adhesion to TiN-coated NiTi surface.

**References:**

1. Starosvetsky D. Biomaterials 2001; 22:1853-1859.
2. Gawalt E. S. Langmuir 2003; 19:200-204.
3. Zorn G. Chemistry of Materials 2005; 17:4218-4226.