Covalent Attachment of Self-Assembled Peptide Amphiphile Nanofibers to Metallic Implant Surfaces

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Statement of Purpose: Here we present a general strategy for altering the surface chemistry of nickel-titanium shape memory alloy (NiTi) to facilitate covalent attachment of self-assembled peptide amphiphile (PA) nanofibers. This highly versatile class of self-assembling molecules has been used to template the mineralization of hydroxyapatite (1), promote rapid and selective neural progenitor cell differentiation (2), present bioactive epitopes for integrin-mediated cellular interactions (3-5), and facilitate protein/macromolecule binding (6). As such, PA nanofibers are interesting motifs for surface functionalization of implant materials. Here we show that these self-assembling nanostructures can be covalently attached to Nitinol substrates to create functionalized, nano-textured, bioactive metallic surface. Specifically, a PA with the Arg-Gly-Asp-Ser (RGDS) cellular adhesion sequence (7) has been used to biofunctionalize NiTi substrates via an aminosilane linker.

Methods: Equimolar Nickel-Titanium (NiTi) was polished and chemical treated prior to silane deposition with aminopropyltriethoxysilane (APTES). Peptide amphiphiles (PA) were synthesized by solid phase peptide synthesis (SPPS), purified by high performance liquid chromatography (HPLC). Low weight percent PA solutions were drop-cast onto silanized NiTi to create thin nanofiber coatings. Covalent attachment was achieved by immersion in a solution containing *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) and *N*-Hydroxysulfosuccinimide sodium salt (sulfo-NHS). These functionalized NiTi surfaces have been characterized by X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectroscopy (ToF-SIMS), and atomic force microscopy (AFM). Covalent attachment was probed qualitatively by photoluminescence of fluorescently labeled PA molecules. Biocompatibility was evaluated by proliferation assays with mouse calvarial (MC3T3-E1) cells and bovine pulmonary artery endothelial (CPAE) cells, and by scanning electron microscopy (SEM) images of seeded cells.

Results/Discussion: Measurements by XPS and SIMS show the chemical treatment of NiTi with HF, HNO_3, and then boiling water results in a surface with decreased Nickel concentration and increased TiO_2 over other tested treatments. After silanization, XPS and SIMS confirm the presence of APTES. AFM shows an increase in surface roughness created by acid-treatment, while the aminosilane deposition leads to a nano-scale textured surface. Upon drop-casting of the PA, AFM shows nanofibers formed by concentration effects, which remain intact after covalent attachment. These changes in surface morphology are clearly evident by AFM, as seen in Figure 1. To demonstrate covalent attachment, a PA containing a pyrene moiety was used to allow spectrofluorescence measurements of the surface in reflectance mode. After washing in the appropriate conditions, the covalently attached PA coating retain their characteristic spectra, while the non-covalently attached PA coatings are largely diminished. A proliferation assay was performed to demonstrate biocompatibility of each stage of the surface expected to be exposed to adjacent tissue as degradation occurs in vivo. MC3T3-E1 and CPAE cells were cultured in vitro for one week and were shown to attach and proliferate on all substrates, indicating a level of biocompatibility. SEM images of fixed cells on these substrates were taken to view the cellular morphology. The cells adhered to and were spread similarly on all substrates.

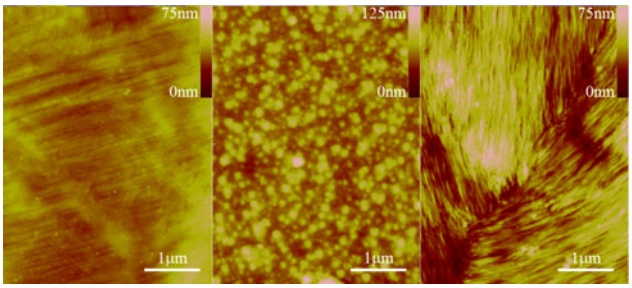

Figure 1. AFM images of NiTi (left), silanized NiTi (middle), and covalently attached PA on silanized NiTi (right)

Conclusions: We have developed novel, facile method to covalently attach self-assembled PA nanofibers using an intermediary aminosilane layer on pre-treated Nitinol substrates with very low Ni and increased TiO_2. Biological assay results showed no toxic effect on endothelial and pre-osteoblast cells by any of the materials or reagents used. Hence, with a combination of several novel techniques, we have developed a general strategy to create bioactive Nitinol material that can be used as biomedical implants with enhanced capabilities to facilitate cell proliferation, adhesion, and potentially a variety of implant-specific cellular responses.

References:

1. Hartgerink JD. *Science*. 2001;294(5547):1684-8
2. Silva GA. *Science*. 2004;303(5662):1352-5.
3. Hartgerink JD. *Proc Natl Acad Sci USA*. 2002;99(8): 5133-8.
4. Behanna H. *J Am. Chem. Soc*. 2005;127(4):1193-200.
5. Niece KL. *J Am. Chem. Soc*. 2003;125(24): 7146-7.
6. Rajangam K. *Nano Letters* 2006;6(9):2086-2090.
7. Ruoslahti E. *Science*. 1987;238(4826):491-7.