Endothelial Cell Response to Vascular Endothelial Growth Factor on Hydroxyapatite Surfaces

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Statement of Purpose: The field of bone tissue engineering aims to create osteoconductive, osteoinductive scaffold materials that optimize bony in-growth and integration of scaffolds into the body. A major challenge in accomplishing this goal in critical-sized defects is the absence of vasculature within the scaffold to deliver nutrients to newly forming bone tissue. To address this issue, surface modification techniques may be used, and scaffolds may be seeded with cells or loaded with growth factors. In this study hydroxyapatite (HA), a popular biomaterial due to its similarity to the mineral phase of bone, is modified by the addition of a phosphonic acid self-assembled monolayer (SAM) and is used for the attachment of vascular endothelial growth factor (VEGF). The objective of this study is to characterize VEGF-functionalized SAMs on HA surfaces and to assess their feasibility for encouraging blood vessel infiltration into HA scaffolds. This will be addressed by ensuring biocompatibility through the evaluation of metabolic activity and morphology of human umbilical vein endothelial cells (HUVECs) cultured on the modified HA surfaces.

Methods: Commercially available 9.5 mm diameter HA discs (HiMed, Bethpage, NY) were used in this study. Discs were chemically cleaned and coated with 11-phosphonoundecanoic acid (11-PUDA) and/or 16-phosphonohexadecanoic acid (16-PHDA) SAMs by an established soaking and annealing process [1]. Terminal carboxyl groups were activated using carbodiimide chemistry, allowing covalent bonding to VEGF. Discs were prepared using sterile reagents after the annealing process and SAM attachment was confirmed by contact angle goniometry (CA) and atomic force microscopy (AFM). Primary HUVECs (Life Technologies Corp., CA) were maintained according to the vendor instructions, in Media 200 with low serum growth supplement. When confluent, cells were trypsinized and plated directly on HA surfaces at confluent cell density, as calculated by total cell count of the cell suspension obtained. Control (1-2) and test (3-4) groups were: (1) cells on tissue-culture plastic (2) cells on cleaned and THF treated HA surfaces (3) cells on HA surfaces with 11-PUDA SAMs and (4) cells on HA surfaces with 11-PUDA SAMs and VEGF. Cultures on HA surfaces were performed in suspension culture plates to avoid cell attachment to the well plate. All cultures were done in 48-well plates, with 600 µl media per well. After 3 and 7 days, cultures were assessed for metabolic activity using alamar blue (AbD Serotec, UK) and normalized by dsDNA as determined using Quant-iT PicoGreen (Molecular Probes, OR).

To visualize HUVECs on HA surfaces, cells were fixed in formalin, permeabilized with 0.1% Triton X-100, stained with fluorescent-conjugated phalloidin (actin stain) and counterstained with propidum iodide (nuclear stain). Representative fluorescent microscopy images were obtained to compare cell morphology at 1, 3 and 7 days.

Results: CA (Figure 1) and AFM analyses indicated the presence of SAMs and VEGF on HA surfaces. CA increased with the addition of SAMs and decreased to surface energies ideal for cell adhesion after VEGF attachment. The root mean squared roughness values (nm), as calculated from AFM analysis, were 210.3, 203.2, 145.7, and 148.8 for control, 11-PUDA, 16-PHDA, and mixed SAMs groups, respectively. Since all groups with VEGF attached exhibited similar CA values, and the 11-PUDA group was closest to the control value in terms of roughness (suggesting that SAMs followed the contour of the surface), only the 11-PUDA group was used in the cell studies.

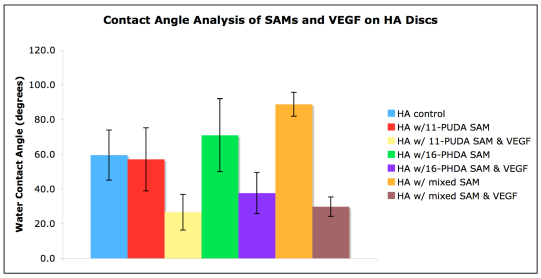

Figure 1: Contact Angle Analysis

At day 7, decreased metabolic activity was observed on all HA groups compared to non-HA control (Figure 2). However, the addition of SAMs decreased metabolic activity, with some recovery seen in the VEGF group. A similar trend was observed at Day 3 (data not shown).

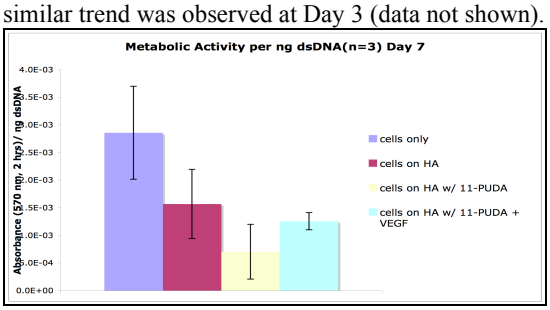

Figure 2: HUVEC Metabolic Activity at Day 7

Little morphological change was seen in the cells over the time course studied.

Conclusions: Material characterization demonstrated successful attachment of SAMs, specifically 11-PUDA, and VEGF to HA surfaces. HUVEC response in terms of metabolic activity over 7 days demonstrated that HA has a detrimental affect, but that VEGF helps to sustain metabolic activity. Further evaluation of HUVEC response is needed to make further conclusions about the effects of these HA surfaces on cell function and activity.

References: 1. Torres, N. Acta biomaterialia. 2010; **6**(8): 3242-3255.