Novel Approach for Anchoring Bioactive Peptides to Bone Regenerative Biomaterials

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Statement of Purpose: Treatment options for filling bone voids and promoting bone regeneration at implant sites are currently limited. Native bone consists primarily of collagen I and hydroxyapatite (HA)[1]; therefore, a biomaterial consisting of both of these components would closely mimic native bone. However, use of intact collagen I can be problematic due to the risk of pathogen transfer and possible immune response.[1] To avoid these complications we are exploring the use of collagen-derived peptides such as DGEA and P15. Our prior studies suggest that these peptides can recapitulate some of the biological effects of collagen I.[2] To generate bone-mimicking matrices, we aim to couple collagen-derived peptides to both pure HA and polymeric electrospun matrices that incorporate HA nanoparticles. However, methods for anchoring peptides to these types of matrices are very limited. To address this issue, we have modeled the mechanism that bone binding proteins use to associate with the HA within native bone. More specifically, bone sialoprotein (BSP) employs a negatively charged heptaglutamate (E7) domain to form an ionic linkage with positively charged Ca^{2+} present within HA[3]. We propose exploiting this interaction to bind DGEA more tightly to the surface of HA-containing biomaterials. In the current study we tested the hypothesis that the addition of an E7 domain to DGEA would allow for a strong ionic linkage of the peptide to HA, and that the E7-modified DGEA peptide would retain bioactivity.

Methods: Peptides were coated onto biomaterials for 2 hours at 37°C. In vitro, retention of E7 modified DGEA relative to unmodified DGEA was evaluated, after extensive washing, by fluorescent microscopy. In vivo, retention of peptides was evaluated after implantation into a subcutaneous rat model for up to 21 days by fluorescent microscopy. Adhesion of Mesenchymal Stem Cells (MSCs) to peptide modified disks was evaluated by labeling cells with a fluorescent dye, allowing them to adhere, lysing, and measuring fluorescence. Cell spreading was monitored by labeling actin cytoskeleton and cell nuclei and imaging after several time points. Osteogenic differentiation was monitored by a colorimetric alkaline phosphatase stain.

Results: HA disks were coated with DGEA or E7DGEA, both modified with a FITC tag to allow detection by fluorescent microscopy. Disks were then washed for 7 days in saline and visualized. These experiments showed that significantly more E7DGEA was bound to HA disks than DGEA (not shown) suggesting tighter coupling. To determine whether the E7 domain facilitated better peptide tethering in vivo, we implanted peptide-coated HA disks into subcutaneous pouches in rats for up to 21 days. Examination of retrieved disks showed significantly greater retention of E7DGEA than DGEA (Fig 1).

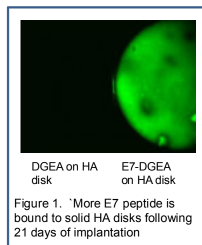

Figure 1. `More E7 peptide is bound to solid HA disks following 21 days of implantation

We next tested whether the E7 domain could be used to anchor peptides to HA-containing electrospun polycaprolactone (PCL/HA) scaffolds. As shown in Figs 2A&B, in vitro studies revealed that more E7-DGEA was retained on PCL/HA than DGEA, and only negligible amounts of E7-DGEA associated with PCL, confirming the specificity of the E7/HA interaction. Similarly, more E7-DGEA was retained than DGEA on scaffolds implanted into skin pouches (Fig 2C).

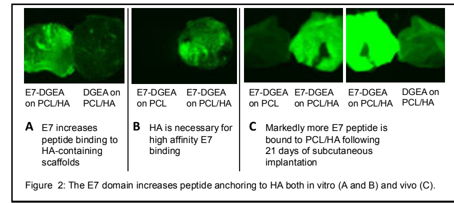

Figure 2: The E7 domain increases peptide anchoring to HA both in vitro (A and B) and vivo (C).

To assess the effects of adding the E7 domain on the bioactivity of DGEA, we evaluated cell responses known to be elicited by the activation of collagen-binding integrins. Adhesion assays showed that E7-DGEA induced greater cell attachment to HA disks than DGEA (Fig 3), and also retained the ability to stimulate cell spreading (not shown). We then monitored the capacity of the peptides to stimulate expression of osteoblast-associated proteins such as alkaline phosphatase (ALP), given that activation of collagen-binding integrins is known to induce osteoblastic differentiation of MSCs. As shown, MSCs grown on E7DGEA-coated disks exhibited greater ALP activity than cells grown on DGEA-coated disks (Fig 4). In future studies we will evaluate additional osteoblastic markers, and also determine whether E7-DGEA promotes differentiation of MSCs grown on HA-containing electrospun scaffolds.

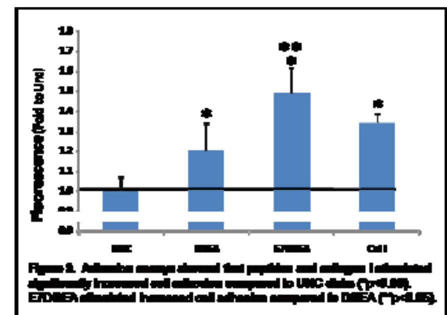
Figure 3. Adhesion assays showed that peptides and collagen I stimulated significantly increased cell adhesion compared to UNC disks (*p<0.05). E7DGEA stimulated increased cell adhesion compared to DGEA (**p<0.05).

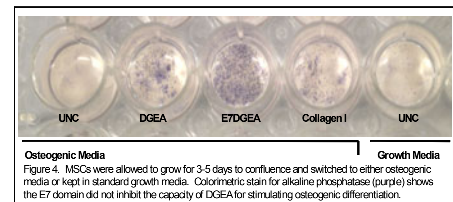

Figure 4. MSCs were allowed to grow for 3-5 days to confluence and switched to either osteogenic media or kept in standard growth media. Colorimetric stain for alkaline phosphatase (purple) shows the E7 domain did not inhibit the capacity of DGEA for stimulating osteogenic differentiation.

Conclusions: Results from this study suggest that modification of DGEA with an E7 domain promotes peptide tethering to HA without compromising bioactivity. We anticipate that the E7 domain will prove to be a powerful new tool for coupling a wide range of bioactive peptides/proteins to any kind of composite biomaterial incorporating HA.

References:

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