Broad application of a heptaglutamate domain to functionalize hydroxyapatite-containing biomaterials

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Statement of Purpose: With more than 500,000 bone grafting procedures performed yearly in the United States alone, the need for improving current treatment options is evident. Hydroxyapatite (HA) is an osteoconductive material that makes up the main component of native bone. Additionally, HA has been historically utilized as coating for metal implants and a bone filler. In recent approaches, HA has been used in composite regenerative matrices incorporating collagen, growth factors, and polymers. A significant challenge associated with the use of HA is the limited methods for functionalizing these surfaces. To circumvent this issue, we have attempted to model the mechanism that native bone binding proteins use to associate with native HA in bone.

Many bone binding proteins such as bone sialoprotein (BSP) contain domains of negatively charged amino acids, specifically glutamate (E) or aspartate (D). These regions carry a net negative charge, presumably interacting with positively charged Ca^{2+} present in native HA in bone. Therefore, we have evaluated a coupling technique using a heptaglutamate domain (E7) to tightly anchor a number of peptides to HA-containing materials.

We propose exploiting the E7-HA interaction to functionalize HA-containing biomaterials with a number of peptide domains including: proteoglycan binding domain FHRRIKA, the RGD integrin binding motif, and the collagen-derived sequence, DGEA. In the current study, we tested the hypothesis that the addition of an E7 domain would facilitate enhanced binding to HA-containing materials. Additionally, we investigated the activity of E7-modified DGEA and BMP-2 peptide in a rat tibial model.

Methods: Peptides were coated onto biomaterials for 2 hours at 37°C. For peptide retention studies, all peptides were conjugated with a fluorescent tag (FITC) to allow for visualization of relative peptide quantities. In vitro, relative peptide retention was evaluated after washing, by fluorescent microscopy. In vivo, retention of peptides was evaluated after implantation into a subcutaneous rat model for up to 2 months by fluorescent microscopy. Peptides ability to enhance % bone integration was measured using BioQuant software after 7 days of implantation into a tibial model (peptides without a FITC tag). Disks coated with appropriate peptides (DGEA, E7DGEA, E7BMP-2) or rBMP-2 protein were implanted for 7 days, collected and stained with Goldner's Trichrome.

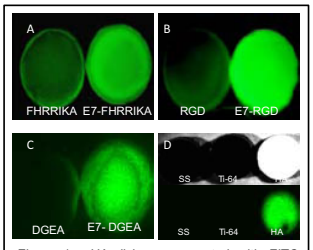

Figure 1. HA disks were coated with FITC conjugated peptides in order to visualize using fluorescent microscopy: FHRRIKA or E7-FHRRIKA (A), RGD or E7-RGD (B), and DGEA or E7DGEA (C). In panel D stainless steel (SS), Titanium alloy (Ti-64) or HA disks were coated with E7-DGEA to emphasize the specificity of the E7 domain for HA-materials.

Results: In Fig. 1 HA disks were coated with FITC tagged peptides: FHRRIKA (A), RGD (B) or DGEA (C) with or without an E7 domain. These data clearly indicate that E7-modified peptides are retained at much higher concentrations than unmodified peptides. Importantly, E7-peptides do not bind to stainless steel or titanium alloy, confirming the high specificity for binding to HA (Fig 1D). Over a 24 hour period tracking peptide depletion from solution, we have shown that the E7 domain actually directs much more peptide to the surface of HA disks than unmodified peptides (not shown). To test this E7-HA linkage in vivo we implanted HA-materials coated with equimolar concentrations of DGEA or E7DGEA into a subcutaneous model for 2 months (Fig 2). It is evident that E7-DGEA is retained in vivo for up to 2 months on HA disks (A) and HA-containing polycaprolactone scaffolds (B). To test whether this increased retention would have any effect in vivo we implanted disks coated with DGEA or E7DGEA into a rat tibiae model for 7 days (Fig 3A). Importantly, not only did E7-DGEA elicit an enhanced % bone integration above that of uncoated disks but also enhanced response above unmodified DGEA (presumably due to enhanced loading and retention directed by the E7 domain). We also evaluated the capacity of an E7-BMP-2 peptide to increase % bone integration compared with rBMP-2 protein (Fig 3B) under these same conditions and found that E7-BMP-2 was able to match the effects of the full length rBMP-2.

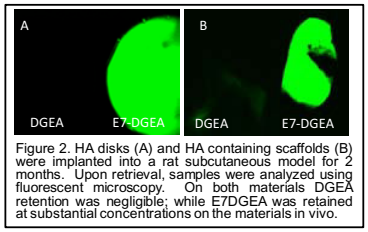

Figure 2. HA disks (A) and HA containing scaffolds (B) were implanted into a rat subcutaneous model for 2 months. Upon retrieval, samples were analyzed using fluorescent microscopy. On both materials DGEA retention was negligible; while E7DGEA was retained at substantial concentrations on the materials in vivo.

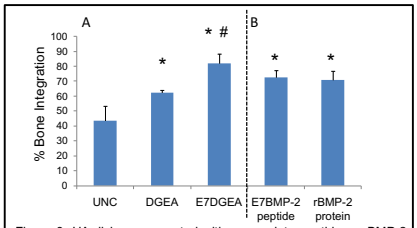

Figure 3. HA disks were coated with appropriate peptide or rBMP-2 protein and implanted into a rat tibiae model for 7 days. Upon retrieval, samples were sectioned and stained with Goldner's Trichrome for bone formation. Bone integration was measure using BioQuant software. Panel A shows that E7-DGEA increased % Bone Integration above that of uncoated (UNC) disks and unmodified DGEA. In Panel B, a comparison of recombinant BMP-2 protein and an E7-modified BMP-2 peptide is made. Importantly both E7-BMP-2 and rBMP-2 increased bone integration over uncoated HA, but there was no significant difference between the two. A p-value <0.05 was considered significant. Significance is indicated by * (comparison to uncoated (UNC)) and # (comparison to DGEA)

Conclusions: Results from this study suggest that modification of peptides with an E7 domain enhances peptide tethering and facilitates retention to HA-containing materials for at least 2 months. We believe that the broad applicability of the E7 domain to many different peptides, as shown here, speaks to the potential benefit this strategy presents for enhancing response to a variety of HA-containing materials.

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