3-D PEM Coatings to Deliver FGF-2 and Promote *In vitro* and *In vivo* Osteoprogenitor Cell Proliferation

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Statement of Purpose: Bone regeneration and repair is markedly reduced in elderly mammals. Fibroblast growth factor-2 (FGF-2) is a potent stimulator of preosteoblast proliferation [1]. We hypothesize that FGF-2 is one of the missing factors that can rejuvenate elderly osteoblast progenitors because of its effects on proliferation and its signaling pathway that leads to increased production of bone morphogenetic protein-2 (BMP-2) [2]. A synergistic FGF-2/BMP-2 delivery system could be highly efficacious, especially if it can be achieved via a three-dimensional (3-D) bone graft material. The long-term goal of our research is to develop a sequential 3-D polyelectrolyte multilayer (PEM) delivery system capable of release of FGF-2 and then BMP-2 to stimulate proliferation and differentiation of osteoprogenitors in the elderly. Towards that aim, we report here a novel technique to apply PEM coatings to 3-D scaffolds for successful *in vitro* and *in vivo* FGF-2 delivery.

Methods: PEM coatings were prepared on 3.5 mm diameter scaffolds made of collagen/hydroxyapatite (Healos®, DePuy Spine Inc., Raynham, MA), by alternate 10 min bindings in 300 ul of 1 mg/ml poly L glutamic acid (PG-) or poly-L lysine (PL+) solutions in saline with 3 saline rinses between each, (Sigma, St. Louis, MO). Three bilayers were adsorbed. Recombinant Human FGF-2 (R & D Systems, Minneapolis, MN) (0.5 or 5 ng/ml for *in vitro* studies and 40 ng/ml for *in vivo* studies) was adsorbed on the PEM for 60 min. After each binding or rinse step the samples were centrifuged at 3000 rpm on a filter device to ensure removal of fluid from the inner most pores of the Healos® thereby allowing complete infiltration of the next solution. Scaffolds were UV sterilized before cell culture or implantation. Binding of FGF-2 to Healos® or Healos®/PEM was determined via ELISA (R & D Systems, Minneapolis, MN). *In vitro* cell attachment and proliferation on the 3-D Healos®/PEM/FGF-2 coatings were assessed with primary Col2.3GFP calvarial mouse cells [3] seeded at 10 x 10^3 cells/cm^2. Proliferative effects of the Healos®/PEM/FGF-2 coatings were determined using Alamar Blue assay (Invitrogen™ Life Technologies, Grand Island, NY). Statistical significances were determined by one-way ANOVA with Tukey post-tests. Scanning electron microscope (SEM) images and confocal images of the prepared scaffolds were acquired. For confocal imaging, a layer of BSA-Alexa Fluor® 488 was deposited rather than FGF-2. For *in vivo* assessment of the Healos®/PEM/FGF-2, the scaffolds were implanted into 3.5 mm calvarial defects in four month old CD-1 mice. Implanted scaffolds were removed after three days, cryosectioned, and immunostained with CD166 (ALCAM, marker for mesenchymal progenitor cells), using previously reported techniques [1].

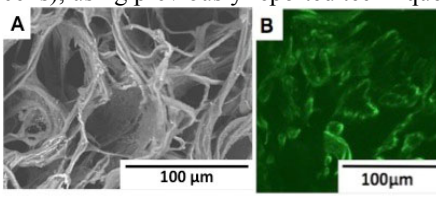

Figure 1: SEM (A) and confocal (B) images of Healos®/ PEM.

Results: A uniform PEM coating can be achieved throughout porous 3-D Healos® without blocking the pores via centrifugation prior to each binding and rinse step (SEM of Fig. 1A). The PEM/BSA-488 coating was deposited around each fiber throughout the thickness of the Healos®, (confocal, Fig. 1B). By ELISA testing, FGF-2 binding efficiency to Healos® alone and to Healos®/PEM was 86.7% ± 3.0, and 91.5% ± 2.1 respectively. Col2.3GFP cells can successfully attach and proliferate *in vitro* on Healos®/PEM similarly to Healos® alone (Fig 2). The addition of FGF-2 to the PEM coating significantly increases proliferation, as seen by the Alamar Blue assay, (Fig. 2). Data shown is normalized to day 1 proliferation.

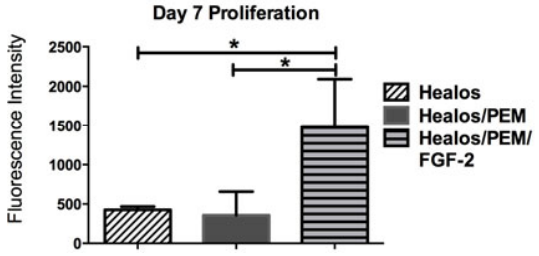

Figure 2: Day 7 Alamar Blue proliferation assay results.

Implantation of Healos®/PEM/FGF-2 increased the number of CD166+ cells in the defect site and marrow relative to Healos® implantation alone (Fig. 3, 2 vs 1). The dashed lines in the images indicate the outline of the host bone. (1) and (2) show DAPI staining while (1') and (2') show CD166 staining, (asterisk is the Healos® autofluorescence). Thin arrows point to marrow cavity of the host bone that responds strongly to FGF-2 exposure (high CD166+). Larger arrows point to corresponding areas in the adjacent sections.

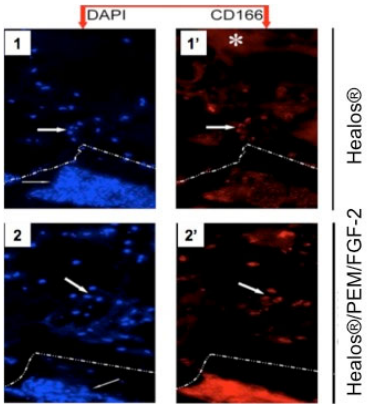

Figure 3: DAPI and CD166 staining of in vivo Healos®/PEM/FGF-2.

Conclusions: A new strategy for applying controlled release PEM coatings to 3-D fibrous scaffolds has been demonstrated. Addition of PEM/FGF-2 to Healos® significantly increases proliferation of progenitor cells *in vitro* and *in vivo* indicating activity of the growth factor is retained.

References: [1] Ou et al. J Gerontol A Biol Sci Med Sci. 2010: 65(10): 1051-1059. [2] Naganawa et al. J Cell Biochem. 2008: 103: 1975–1988. [3] Kalajzic et al. J Bone Miner Res. 2002: 17(1): 15-25.

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