Development and Investigation of a New Generation Matrix for Osteochondral Tissue Engineering

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Statement of Purpose: Osteochondral (OC) defect repair is a major challenge in orthopedic surgery. If left untreated, OC defects can lead to the development of osteoarthritis[1]. Currently clinical treatment methods for osteochondral tissue repair are palliative and do not provide a permanent solution to the problem. In the hopes of providing a better treatment for this condition, tissue engineering has recently been employed. Current scaffolds for OC tissue engineering, which include single phase and biphasic scaffold structures, are inadequate. For this reason, we have developed a gradient matrix system in which the bone and cartilage forming phases co-exist in a continuous fashion. The uniquely designed and advanced matrix system has the potential to promote hierarchically structured osteochondral (OC) tissue regeneration along with smooth OC interface formation.

Methods: Poly (85 lactide-*co*-15 glycolide) (PLGA) microsphere scaffolds with a gradient pore structure were fabricated using a "thermal sintering and porogen leaching" method. In brief, PLGA microspheres (355-425 μm) and sodium chloride (NaCl) porogen (106-212 μm) mixtures (with increasing salt content) were layered into a steel scaffold mold (10mm x 5mm) and thermally sintered, followed by porogen leaching. Porosity of the resulting structure was analyzed by Micro-Computed Topography (μCT). After testing the osteogenic and chondrogenic abilities of the polymer and polymer-hydrogel grafts respectively on disk scaffolds (2mm x 10mm), the biomimetic structure was tested *in vitro* by embedding $5x10^5$ human bone marrow derived stem cells (hBMSCs) into a self assembling peptide gel (PuraMatrix) and infusing it into the pore spaces of the graft. Additionally, $1.3x10^5$ hBMSCs were added to the cell culture media and agitated until they adhered to the scaffold. After 21 days of culture (in co-differentiation media) immunofluorescence staining was performed to determine the osteogenic and chondrogenic differentiation ability of the scaffold.

Results and Discussion: Using the process detailed a gradient scaffold containing 0%, 5%, 10%, 20%, and 40% NaCl porogen was constructed. The combination of thermal sintering and porogen leaching of the layered samples led to the formation of a gradient graft with porosity ranging from 30% to 60% as determined by μCT (Figure 1). The lateral increase in porosity allows the graft to better mimic the native tissue structure. The 30% porous end mimics bone, while the 60% porous end, when infused with the hydrogel phase, mimics the cartilaginous region. Furthermore, the sintered graft when infused with a hydrogel maintains a continuous interface region which is capable of both osteogenic and chondrogenic attributes.

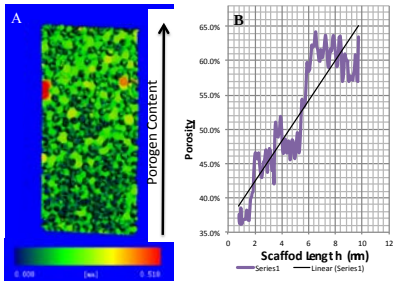

Figure 1: (A) MicroCT image of gradient scaffold showing increasing porosity from bottom to top with (B) quantification of porosity.

Preliminary *in vitro* studies show that osteogenesis and chondrogenesis can be accomplished on the PLGA matrix in the absence of and with PuraMatrix hydrogel respectively, Figure 2.

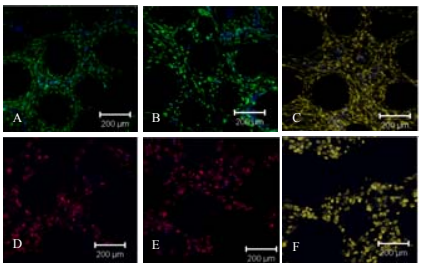

Figure 2: Bone (A,B,C) and cartilage (D,E,F) scaffold layers in separate cultures: seeded with hMSCs displayed (A) Collagen I, (B) Runx2 , and (C) Tubulin as well as (D) Collagen II, (E) Sox9, and (F) Tubulin.

After combining both osteogenic and chondrogenic media in a 50:50 ratio to create a co-differentiation media, it was found that the hBMSCs are capable of osteochondral differentiation as seen by the cells simultaneous display of osteogenic matrix marker, Collagen I, and chondrogenic matrix marker, Collagen II, as shown in Figure 3.

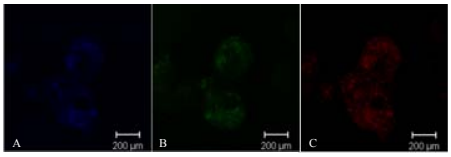

Figure 3: hMSCs cultured for 21 days in co-differentiation media in gradient scaffold. (A) Nuclei Stain alone, (B) Col II, (C) Col I

Conclusions: Our studies, for the first time, have led to the development of a gradient matrix system that promotes hierarchically structured osteochondral tissue regeneration. Bone and cartilage phases of the scaffolds seeded with human BMSCs independently confirmed their potential to support osteogenic and chondrogenic differentiation, respectively. This study also developed a co-differentiation media for OC graft culture *in vitro*. Our long-term goal of this study is to investigate the unique OC matrix system in combination with the intra-operatively enriched human bone marrow aspirate towards the development of an intra-operative tissue engineering strategy for osteochondral defect repair and regeneration.

References: 1. Nukavarapu *et al.* 2013 Biotechnol Adv. 31, 706-21.