Epigenetically Directed Differentiation on Drug-Eluting Scaffold for Osteochondral Implant

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Statement of Purpose: Osteoarthritis affects more than 46 million Americans, and the rate is predicted to rise (MacLean C. J Am Acad Orth Surg. 2017;25:S55-S59). Instead of artificial implants to replace a diseased joint, a more biocompatible solution to restore full function is to utilize the patient's own cells to generate tissue for healing. Human mesenchymal stem cells (hMSCs) possess the potential to differentiate through osteogenesis and chondrogenesis to cell types necessary for joint treatment under requisite cues. However, there is a challenge in generating complex tissue, such as an osteochondral (OC) construct, with only these soluble cues. Through systematic screening, our lab has identified small molecules that specifically enhance or inhibit osteogenesis through epigenetic modulation. We believe that the identified molecules can be employed to obtain spatially organized bone and cartilage through locally controlled differentiation of hMSCs within a drug-eluting scaffold. The OC joint is a 3-D tissue with lineage zonation, and our goal is to recapitulate stem cell recruitment and differentiation using a scaffold with extensive interfacial area (e.g. microfibrous geometries) and spatially modulated local cues (epigenetic drugs) that control stem cell lineage phenotypes. A second goal of our work is to elucidate the emergent phenotypes of stem cells within the 3-D scaffolds using single cell high content imaging based on surrogate nuclear reporters to parse and predictably profile the emergent phenotypes.

Methodology: *Scaffold design and characterization:* Scaffolds were constructed with airbrushed E1001(1k) fibers and by 3-D printing using polycaprolactone. Small molecule drugs capable of increasing or inhibiting osteogenesis were loaded in the fibers. The scaffolds have been characterized using a PhenomProX scanning electron microscope and a MTS Syntech mechanical testing frame. *Cell differentiation within scaffold:* Cell seeding, attachment and spreading (morphology) within the scaffolds was assessed through confocal microscopy. hMSC differentiation within the scaffolds without drugs and in presence of drugs will be evaluated through both quantitative assays and immunofluorescence staining. Specifically, osteogenesis will be evaluated by performing fast blue and Alizarin red staining assays, as well as quantified by performing ALP activity assay. Chondrogenesis will be assessed by Alcian blue staining assay and quantified by performing qPCR for Sox9, aggrecan, collagen I, and collagen II. Differentiation within the construct will also be validated by immunofluorescence staining for osteogenic markers such as RUNX2 and osteocalcin, and chondrogenic markers such as aggrecan and collagen II. *Modeling cell fate outcomes:* Differentiation and lineage outcomes within the scaffold, in response to treatment with small molecules and soluble factors, are modelled and characterized through high content image analysis and informatics. This approach involves high resolution imaging and a series of image processing and analysis steps with machine learning capable of defining and parsing cell fates within 24-72 hours of exposure to external cues (Dhaliwal A et al 2016; Treiser MD et al. 2010). An artificial neural network will be developed to estimate the extent of differentiation from the set of features calculated from high-resolution images.

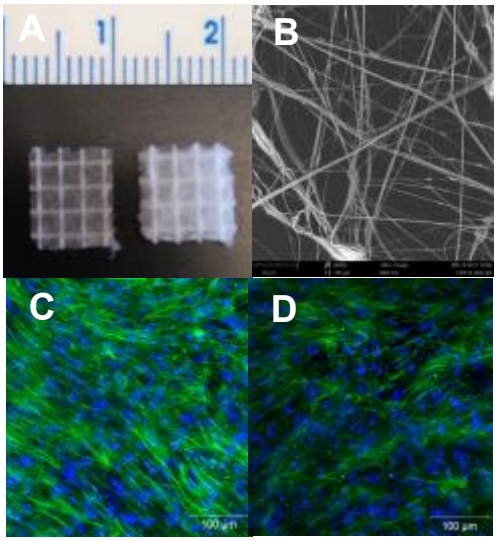

Figure 1. 3-D printed construct with airbrush fibers and drugs for engineering osteochondral tissue. A) Scaffolds constructed with 2 and 5 layers of fibers. **B)** Microscopy of scaffold fibers. **C)** hMSCs on scaffold without drug. **D)** hMSCs on scaffold with drug.

Results: The scaffolds have a tensile modulus of 25 MPa with a porosity of 80%, and the fibers are 2.0 ± 0.5 μm. hMSCs attach and spread well on scaffolds. Furthermore, cell viability and morphology is maintained when the hMSCs are seeded within drug-loaded scaffold, compared to control scaffold. Preliminary data indicates that scaffolds support hMSC differentiation as well.

Conclusions: Overall our preliminary studies indicate that 3D printed scaffolds with airbrushed fibers support hMSC culture with and without drug-loading and hMSC differentiation. Small molecule drugs to locally enhance or inhibit ostogenic differentiation were successfully incorporated within the scaffolds. Studies are ongoing to evaluate the effect of the epigenetic drugs on differentiation within the scaffold.