Towards Regeneration of the Cranial Suture:
Pore Curvature Regulates Mesenchymal Stem Cell Fate in Macroporous Tissue Engineering Scaffolds
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Statement of Purpose: Craniosynostosis is a debilitating disease characterized by the premature fusion of cranial bones by loss of sutures, resulting in an increase of intracranial pressure, subsequently develops deafness, blindness and in some cases mental disability. Traditionally described as a bone disease, recent data suggests some forms of craniosynostoses are a stem cell disease involving depletion of progenitor cells in the suture mesenchyme due to aberrant growth factor signaling. We aim to use three-dimensional (3D) tissue engineering scaffolds from synthetic materials to design an optimized biomaterial scaffold microenvironment which recapitulates the cranial suture as a stem cell niche.

Materials and Methods: Nanofibrous, macroporous tissue engineering scaffolds are fabricated from poly (L-lactic acid) by a sugar-sphere porogen method and thermally-induced phase separation. Sugar-spheres are selectively isolated in the ranges: 65-120um diameter (small) and 250-425um diameter (large). We isolated bone marrow stromal cells and cranial suture mesenchymal stem-cells from wild-type mice and cultured on scaffolds for up to 3 weeks (growth media, n > 4). Gene expression was assessed by qPCR; protein expression and distribution by IHC. Cytochalasin-D (CytD) was used to inhibit actin polymerization, verteporfin (VP) was used to inhibit YAP signaling (5 uM) and lysophosphatidicacid (LPA) was used to induce YAP signaling (25 uM) in vitro. Image analysis performed with Fiji.

Results: We demonstrate sufficiently steep curvature (small-pores, diameter <125um) caused significantly decreased nuclear aspect ratio (NAR) and increased cell-clustering while shallow curvature (large-pores, diameter >250um) caused significantly increased NAR and increased distance between cells. Alterations in curvature-mediated cell organization correlate to differential modulation of YAP/TAZ signaling, by increased gene expression of YAP-related genes in large-pores: YAP1, CTGF, Cy61, peaking at 48 hours and maintained through 3-weeks culture in vitro, and increased osteogenesis (RUNX2, COL1A1, OSX expression, $p<0.01$). In contrast, small-pores maintain stem-cell marker expression (Gli1, CD44, CD146, $p<0.01$) and minimal YAP activation. By immunofluorescence we demonstrate YAP activation in large-pores by quantitative increases in YAP nuclear translocation and decreased phosphorylation, from 48 hours in vitro ($p < 0.05$); small-pores maintain YAP phosphorylation and cytosolic localization. Cytoskeletal disruption by CytD decreases YAP activity and osteogenic differentiation in large-pore constructs, where cytoskeletal tension may regulate nuclear activity. Additionally, we demonstrate that direct inhibition of YAP signaling by VP in large-pores, and activation of YAP signaling by LPA in small-pores dysregulates curvature-mediated gene expression($p<0.05$).

We additionally propose a proof-of-concept tissue engineering construct that allows for simultaneous differentiation and maintenance of stemness in a spatially controlled manner. We aim to ultimately demonstrate a method to use 3D microenvironments with appropriate pore sizes to recapitulate the complex microenvironment of the bone-suture interface. The cranial suture is an ideal physiologic model for regenerating and restoring a stem cell population in a complex microenvironment, with a compelling clinical need.

Conclusions: Porosity is universally regarded as a key parameter for the success of tissue engineering constructs, across scaffolding systems and materials, including synthetic polymers, natural polymers, metals, and bioceramics, to influence tissue integration, cell migration, and mass transfer. It is also well established that skeletal tissues, such as bone, respond to mechanical stimuli in various ways. In this work we investigate the specific design of macropores and its role in guiding stem cell fate in engineered skeletal tissues, using a well-controlled macroporous platform. We demonstrate that curvature is a key design motif which alters cellular trajectories towards either differentiation to bone, with sufficiently large pores, or maintenance of stemness, with sufficiently small pores, with evidence to support mechanistic insight implicating differential YAP/TAZ regulation. The ultimate goal of tissue engineering is to predictably cause regeneration; optimally tuned biomaterial matrices must be designed for tissue-specific applications which includes an intimate understanding of how pore design influences tissue fate.

References:
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