A Macrodesigned, Acellular Scaffold Promoting Endogenous Cell Influx and Viability for Cartilage Regeneration

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Statement of Purpose: The overall goal of this project is to develop biocompatible, mechanically robust, chondrogenic scaffolds that promote the influx of endogenous progenitor cells, and deliver inductive growth factors to induce chondrogenesis. This paper reports the mechanical characterization of highly porous chitosan-alginate (Ch-Al) scaffolds with interconnected structures and good wetting ability, and quantified the effects of adding chondroitin sulfate (CS) on the stiffness of Ch-Al scaffolds. The effects of adding longitudinally oriented channels into the scaffold on fluid uptake and cell distribution were evaluated, as was cell viability.

Methods: Ch-Al scaffolds were fabricated with 1%, 2%, or 3% w/v chitosan dissolved in 2% v/v acetic acid and 1%, 2%, or 3% w/v alginate dissolved in dH_2O, mixed at various ratios. The solutions were homogenized, titrated to pH 7.4, poured into teflon molds, and frozen at -80 C overnight. The resulting solid scaffolds were lyophilized for 3 days and crosslinked for 15 min with 1% w/v $CaCl_2$ with or without 2% w/v CS. Young's modulus was determined by punch indentation compression (Instron, Norwood, MA) test at a strain rate of 2 mm/s using Sneddon's equation and Poisson's ratio = 0.45. To determine the effects of channels on fluid uptake, a 2% w/v 1:1 ratio Ch-Al solution was poured into cylindrical molds (2 cm height, 1 cm diameter) with or without channels (1 mm diameter) along the longitudinal axis of each scaffold. Then wetting speed was determined by placing the scaffolds onto in a thin liquid film of water containing 0.1% w/v SafraninO to enhance visualization. Scaffolds were weighed immediately after wetting at 1 min and after total submersion for 24 hr. For cell viability, mouse bone marrow stromal cells (mBMSC) cultured under standard conditions were seeded on 0.5%, 1%, and 2% w/v 1:1 ratio Ch-Al and Ch-Al-CS scaffolds. Viability was performed using live/dead stain with calcein AM and ethidium homodimer-1 (Invitrogen, Carlsbad, CA).

Results: Figure 1 shows the formulational dependence of mechanical properties on alginate, chitosan, and chondroitin sulfate content. Comparison between the left (no CS) and right (CS) shows that CS can significantly increased scaffold stiffness. In particular, the 2:1 ratio of Ch-Al experienced the most dramatic effects when CS was incorporated during crosslinking. This may be due to an optimal charge balance at this formulation, where two parts cationic chitosan are balanced by one part anionic alginate and one part anionic CS.
Wetting speed in Ch-Al scaffolds with channels was three fold faster than scaffolds without channels. Further, swelling ratio and equilibrium water uptake were also increased in scaffolds with channels compared to those without. However, these parameters did not vary significantly over time, indicating that scaffold wetting occurred in its entirety within the first minute of fluid exposure. Channeled Ch-Al scaffolds displayed increased influx by a concentrated chondrocyte solution and much more homogeneously distributed cells throughout the height and diameter of the scaffold compared to non-channeled scaffolds. Preliminary studies indicated that lower concentrations of Ch-Al promoted higher mBMSC survival, and Ch-Al-CS scaffolds supported greater cell viability than Ch-Al alone.

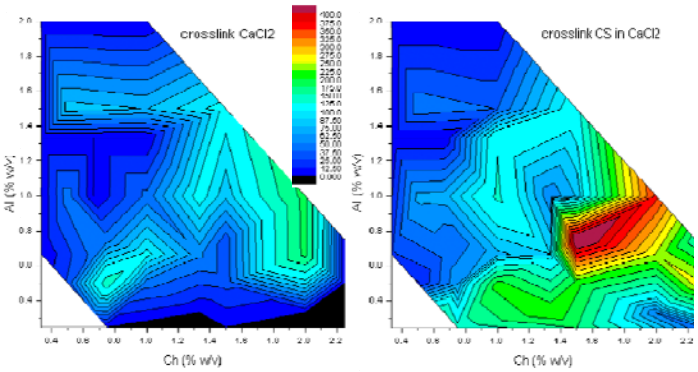

Figure 1. *Heat maps showing dependence of scaffold stiffness (kPa) on alginate, chitosan, and chondroitin sulfate content with $CaCl_2$ alone (left) or CS in $CaCl_2$ (right). Heat map color scale indicates stiffness in kPa.*

Conclusions: The mechanical properties of Ch-Al scaffolds can be varied by changing the ratio and concentration of chitosan and alginate solutions. The addition of CS to a 2:1 Ch:Al formulation resulted in peak stiffness and improved mBMSC viability. Incorporating macroscopic channels into the microscopic porous scaffold architecture drastically increased the wetting speed. This system of capillary action and porous absorption allowed for a much greater uptake flow rate than porous absorption alone. Channeled chitosan-alginate scaffolds imbibing a concentrated cell solution demonstrated homogeneous distribution throughout the scaffold volume. Preliminary cell viability results showed encouraging cell survival on Ch-Al-CS scaffolds. Further research is continuing to assess cell proliferation and spreading on these scaffolds, as well as chondrogenic potential, specifically ECM production and stem cell differentiation. All of these results indicate that Ch-Al-CS scaffolds with longitudinally oriented channels are strong candidates for endogenous cell-based cartilage regeneration.