Development of a novel injectable alginate-collagen-apatite hydrogel for bone tissue regeneration

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Statement of purpose: Although there has been recent success in the field of bone tissue engineering, regeneration has been limited by the size and shape of the scaffolds implanted to heal bone defects. Thus, injectable hydrogel materials have been developed as promising alternatives as their sol-gel properties allow them to completely fill irregular defects and enhance bone regeneration while being minimally invasive. The rate in which this transition takes place must be favorable in a surgical setting, so that the surgeon has enough time to handle and inject the material yet it sets quickly enough to achieve *in vivo* stability. This time period has been defined by surgeons as between 5-30 minutes.[1] Here, a novel combination of alginate, a polysaccharide derived from algae, and the main components of natural bone, collagen or mineralized collagen fibers, has been developed into an injectable hydrogel with capabilities to promote new bone growth.

Methods: To prepare the injectable alginate hydrogels, alginic acid sodium salt (medium viscosity) was first added to deionized water to form alginate suspensions. Sodium phosphate solutions of varying concentrations were then added to the alginate and sat for one hour at room temperature. Calcium sulfate solutions of varying concentrations were added and mixed for 30 seconds. The gelation time was recorded as the time in which the gel meniscus no longer moved when the vial was tilted 90°.[3] To form alginate-collagen hydrogels, pure collagen fibers were added to the suspension after the alginate. Alternatively, to form alginate-mineralized collagen hydrogels, mineralized collagen fibers were first formed in situ using our m-SBF method. They were added to deionized water before the alginate to ensure homogenous suspension. The gelation process then continued for both composites as previously described. Characterization techniques to analyze the mechanical strength and composition of the hydrogels were completed using dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), x-ray diffraction (XRD) and fourier transform spectroscopy (FTIR).

Results: Various concentrations of calcium and phosphate ions were added to alginate hydrogels to achieve the desired gelation time within the 5-10 minute range (Data not shown here). When pure collagen fibers (C) and mineralized collagen fibers (MC) were added to the hydrogels, the gelation times remained within the desired range, as seen in Fig. 1. Analysis of the mechanical strength of the hydrogels obtained from DMA testing showed that the addition of pure and mineralized collagen fibers increased the compressive moduli of the alginate hydrogels.

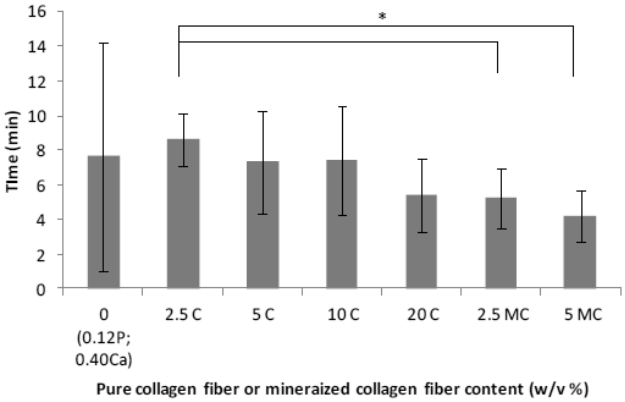

Figure. 1: Gelation times of alginate hydrogels with varying collagen/mineralized collagen fiber content

TGA, XRD and FTIR characterization techniques proved that the hydrogels were composed of alginate, collagen, and inorganic calcium phosphate phases similar to those of natural bone.

Conclusions: A novel, injectable hydrogel for bone tissue regeneration has been developed. The gelation rate, or the rate in which the calcium ions crosslink the G blocks of the alginate, can be tailored by adjusting the alginate, phosphate and calcium concentrations. Phosphate ions are added to the hydrogel to slow down the gelation rate via chelation of the calcium ions to form calcium phosphate.[2] Here, the gelation time has been optimized so that the injectability of the alginiate-mineralized collagen hydrogel is appropriate in a surgical setting, resulting in a less invasive alternative for bone repair. An in situ collagen mineralization process was used to develop an alginate hydrogel comprised of the two main components of natural bone, collagen and calcium phosphate. Therefore, the biocompatibility of the system is expected to be better than that of alginate alone. *In vitro* and *in vivo* studies will be conducted in future studies to evaluate the bone forming capabilities of the injectable hydrogel. Additionally, the mineralized collagen fibers increased the mechanical strength of the alginate hydrogel. Thus, this novel hydrogel composition has great potential to be injected into bone defects or areas of weakened bone to support and enhance natural bone repair.

References: 1: (Tan R. J Mater Sci: Mater Med. 2009;20:1245-1253) 2: (Cho SH. J Biomater Sci: Polym Ed. 2009;20:7-8, 863-876) 3: (Kuo CK. Biomaterials: 2001; 22: 511-521)