Injectable and Moldable Chitosan-Collagen Microbead Formulations for Bone Repair

Limin Wang, Rameshwar Rao, and Jan P. Stegemann
Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

Purpose: To deliver cells encased in an appropriate extracellular matrix in a minimally invasive manner for bone repair. Our approach is to embed human bone marrow stem cells (hBMSC) directly in hydrogel microbeads (100-300 μm diameter) consisting of chitosan and collagen. Microbeads can be cultured to promote desired differentiation, and then can be delivered either as a paste or as a pre-formed solid. In this study, we examined the used of β-glycerophosphate (β-GP) and glyoxal as physical and chemical crosslinkers to promote co-polymerization of the matrix materials during the emulsification process used to fabricate the microbeads. Our goal was to preserve cell viability and demonstrate osteogenic differentiation of hBMSC embedded in chitosan-collagen composite microbeads.

Methods: To fabricate microbeads, chitosan and collagen I solutions, 5% β-GP, 0.5 mM glyoxal, and hBMSC were sequentially mixed to achieve a cell density of 1.0×10^6 cells/ml. The mixture was emulsified in a PDMS bath by an impeller and formed microbeads were collected by centrifugation. Microbead diameter, size distribution, morphology, cell viability, and osteogenic differentiation were assessed. Osteogenic differentiation was conducted in a medium containing dexamethasone and β-GP as osteogenic supplements and calcium deposition and osteogenic gene expression were examined. To test effects of shear stress during injection on cell viability, microbeads were passed through 15G, 21G, and 25G needles at rates of 0.1 and 1 ml/s.

Results: The process resulted in spherical microbeads, and the average size decreased from 291 to 82 μm with impeller speeds increasing from 600 to 1200 rpm (Fig. 1A-B). The size distribution also became narrower with increasing impeller speeds. Coomassie staining confirmed the presence of collagen protein matrix (Fig. 1C).

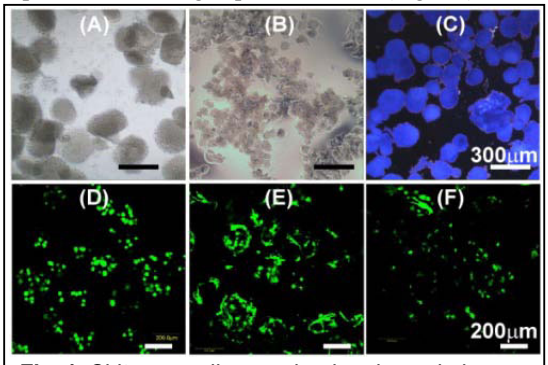

Fig. 1: Chitosan-collagen microbead morphology (A-B), protein content (C), and cell viability (D-F).

Vital staining showed that cell viability was high immediately after microbead fabrication (Fig. 1D), and was maintained over a 10-day culture period. Cell morphology in microbeads with 50/50 chitosan/collagen ratios (Fig. 1E) had a spread morphology, while most cells in microbeads with higher chitosan content retained a rounded morphology (63/37, Fig. 1F).

When exposed to osteogenic medium, hBMSC in microbeads expressed higher levels of OCN and OSX at day 15, relative to the control group (Fig. 2A). In controls, OCN expression decreased from day 0 to 15 while OSX gene expression increased. There was no significant difference in BSP and CI expression between groups. The osteogenic group at day 15 showed significantly higher calcium deposition than the control group at both days 0 and 15. Calcium content in the control group was low throughout the culture period.

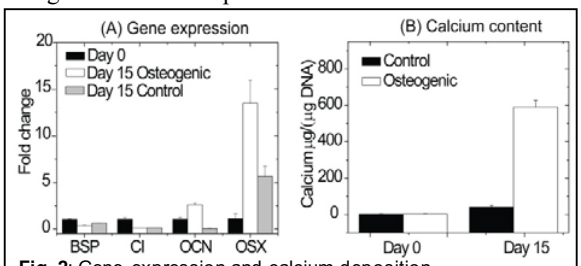

Fig. 2: Gene expression and calcium deposition.

Extrusion of microbead pastes from standard syringes and needles (Fig. 3A-C) did not adversely affect cell viability. Concentrated microbead preparations also could be molded into prescribed geometric shapes (Fig. 3D-F), and maintained their integrity when manipulated with surgical forceps. Cohesion in microbead preparations is probably due to electrostatic forces.

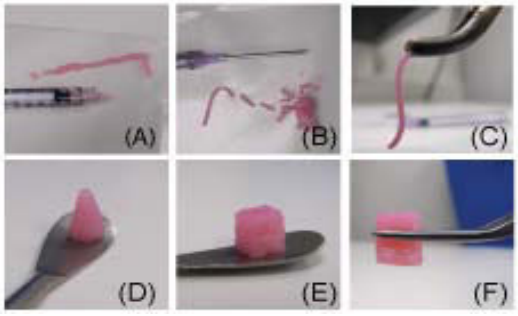

Fig. 3: Images of injected (A-C) and molded (D-F) microbead preparations. Scale bar is 1 cm.

Conclusions: Chitosan-collagen microbeads produced in this study were robust and suitable for use as cell delivery vehicles. Viability of embedded hBMSC was maintained, and the matrix supported osteogenic differentiation, as demonstrated by gene expression and calcium deposition. Lower chitosan/collagen ratios enhanced cell-matrix interactions as indicated by hBMSC morphology. In addition, it was demonstrated that cultured microbead preparations could be concentrated into pastes and delivered through needles or molded into desired shapes, without loss of cell viability. Such injectable and moldable cell-seeded materials have utility as bone void fillers to enhance healing in challenging applications.