Chitosan-based Composite Microbeads for Cell Delivery

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Purpose: The goal of this study was to create microbeads (20-1000 μm in diameter) containing both chitosan and protein (collagen and fibrin) for cell delivery applications. It is challenging to fabricate chitosan-based composites containing living cells because most methods to create chitosan gels involve exposure to strong base, which prevents the gelation of protein components and causes cytotoxic effects. In this study, β-glycerophosphate (β-GP) was used to initiate the chitosan gelling process at physiological temperature and pH, thereby providing a suitable environment for type I collagen self-assembly and polymerization of fibrin using thrombin. This method was used to fabricate chitosan-collagen and chitosan-fibrin microbeads containing embedded cells using an emulsification process.

Methods: To fabricate microbeads, cold acid chitosan solution was supplemented with a range of β-GP concentrations from 2.5 to 12.5 wt%, after which either type I collagen or fibrin/thrombin solution was added to obtain different chitosan:protein ratios including 100:0, 50:50, and 75:25. Either human bone marrow stem cells (hBMSC) or neonatal dermal fibroblasts (hNDFB) were then mixed with the solution at a density of 1.0×10^6 cells/ml. The mixture was injected into a pre-cooled PDMS bath and mechanically emulsified by an impeller at 400 to 1400 rpm for 5 min on ice, followed by a continuously stirred gelation step at 37 °C for 30 min. The gelling process was stopped by cooling on ice for 5 min. The formed microbeads were collected by centrifugation for characterization and biological evaluation *in vitro*. Characterization included energy-dispersive X-ray spectroscopy (EDS), diameter distribution, protein loading, and bead recovery efficiency. Biological evaluation included cell viability and cytoskeletal morphology over two weeks in culture.

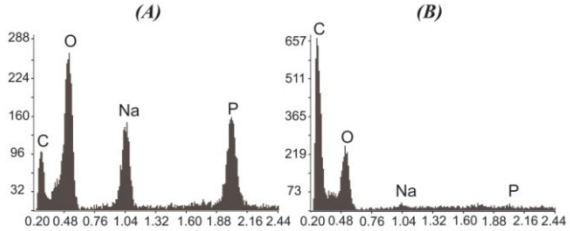

Figure 1: *EDS analysis of composition of unwashed (A) and washed (B) hydrogels.*

Results: Both chitosan-collagen and chitosan-fibrin microbeads were successfully fabricated using a range of β-GP concentrations from 5% to 12.5%. X-ray microanalysis demonstrated that Na and P, the two characteristic elements in β-GP, disappeared after washing with DI water (Fig. 1). By varying emulsification speeds, microbead diameters could be varied from 20 to 1000 μm (Fig. 2). Protein quantification showed that the final protein content in microbeads increased with the amount of protein added to the initial mixture, and the measured protein amounts in beads were similar to the initial protein mass added. Bead recovery from the emulsification bath was highest for protein-containing beads (~90-100% recovery, see Fig. 3A) and lowest for pure chitosan beads (~80%).

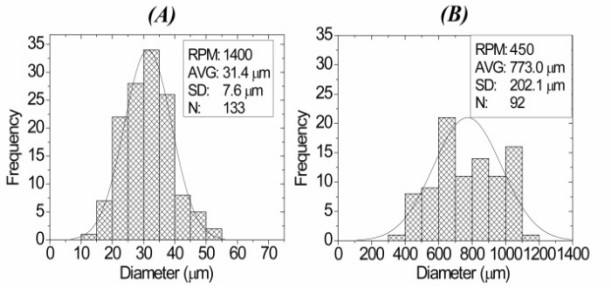

Figure 2: *Diameter distribution of chitosan-collagen beads at 1400 rpm (A) and 450 rpm (B).*

Both hBMSC and hNDFB maintained their viability after microbead fabrication. In protein-containing beads, cells continued to proliferate with increased cell density from days 1 to 8 (Fig. 3B-D). Actin staining clearly demonstrated that cells adhered to and spread over the surrounding protein matrix (Fig. 3E). In contrast, pure chitosan beads did not support cell adhesion though original cell densities were maintained.

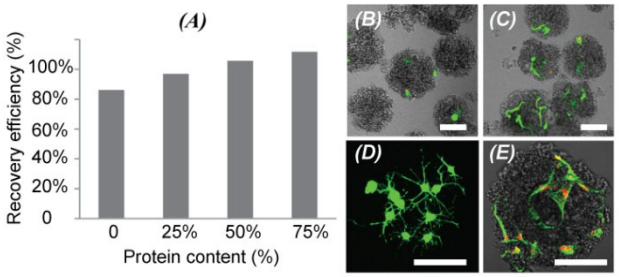

Figure 3: *Recovery efficiency of chitosan-fibrin beads (A). Cell viability at days 1 (B) and 8 (C-D) and cytoskeleton at day 8 (E). Scale bar is 100 μm.*

Conclusions: Chitosan-collagen and chitosan-fibrin microbeads have been fabricated by emulsification using β-GP as a gelling agent. Exposure of cells to β-GP in the emulsification process was not detrimental to hBMSC or hNDFB viability. Incorporation of proteins into chitosan hydrogels significantly enhanced bead recovery and cell-matrix interactions, as indicated by cell adhesion and proliferation. Chitosan materials have been used widely in regenerative medicine applications but are hampered by poor cell-matrix interactions. Supplementing the matrix with a protein component promises to improve cell viability and allow enhanced control of cell function.