Incorporation of Particulate Hydroxyapatite into Collagen-based, Cell-laden Microbeads

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Purpose: To create cell-containing microbeads that mimic the natural matrix composition of bone using collagen type I and hydroxyapatite (HA). Such modular materials can be used to deliver cells for bone repair applications. Microbeads were fabricated using a water-in-oil emulsification process. Pure collagen microbeads are difficult to fabricate using this process because they remain in the oil phase and are fragile. In this study, we added a particulate HA to increase the density of microbead preparations, thereby facilitating harvesting from the oil phase. In addition, HA is similar to the mineral component of bone and may have osteogenic effects. The small aldehyde glyoxal was used to crosslink the collagen matrix. Our goal was to fabricate robust microbeads that maintained the viability of embedded cells.

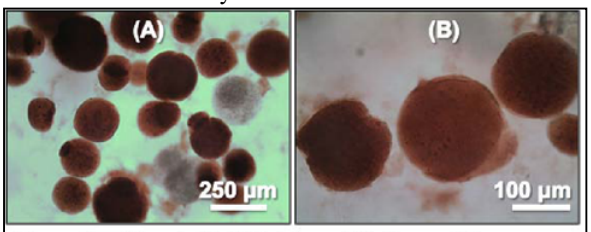

Figure 1 – Alizarin Red Staining of Collagen-HA Microbeads. Scale bar - 250 µm (A) and 100 µm (B).

Methods:. Nano-HA (>200 nm) in DMEM, fetal bovine serum (FBS), 0.1 N NaOH, 1 mM glyoxal, and cold acid collagen type I (6 mg/ml) were sequentially mixed to achieve final collagen and hydroxyapatite concentrations of 3 mg/ml and 5 mg/ml, respectively. The mixture was emulsified in a PDMS bath by an impeller and formed microbeads were collected by centrifugation. Bead morphology, diameter size distribution, and calcium distribution were examined. Human neonatal dermal fibroblasts (hFB) were used as a model cell type, and were added at a density 1.5 x 10^6 cells/ml. Cell viability was monitored directly after microbead fabrication.

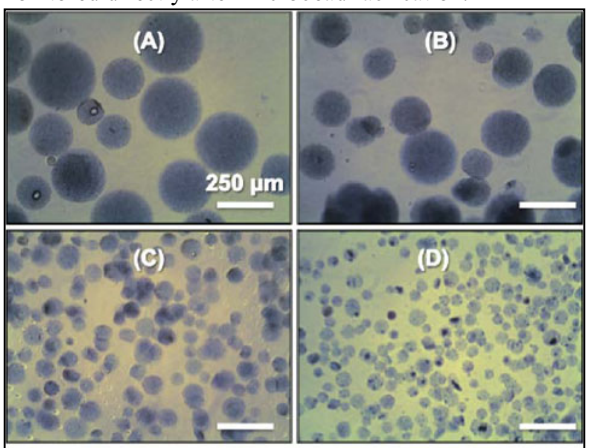

Figure 2. Coomasie stains of Collagen-HA Microbeads fabricated at different speeds. 600 RPM (A), 800 RPM (B), 100 RPM (C), 1200 RPM (D). Scale bar - 250 µm.

Results: The emulsification process produced spherical microbeads with well-dispersed hydroxyapatite as demonstrated by Alizarin Red staining (Fig. 1). Average bead size decreased from 196 to 51 µm with impeller speeds increasing from 600 to 1200 rpm (Fig. 2 A-D). The size distribution was controllable and became more uniform while increasing the impeller speeds, (Fig. 3 A-D) as reflected by the small standard deviation of the bead size at high speeds.

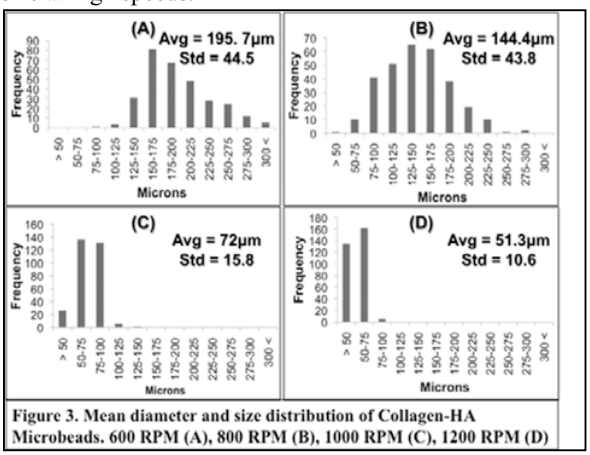

Figure 3. Mean diameter and size distribution of Collagen-HA Microbeads. 600 RPM (A), 800 RPM (B), 1000 RPM (C), 1200 RPM (D)

Cell viability was analyzed and demonstrated that over 95% of encapsulated cells were viable after glyoxal crosslinking (Fig. 4). Cells were embedded into the microbead matrix but were not significantly spread immediately after microbead fabrication.

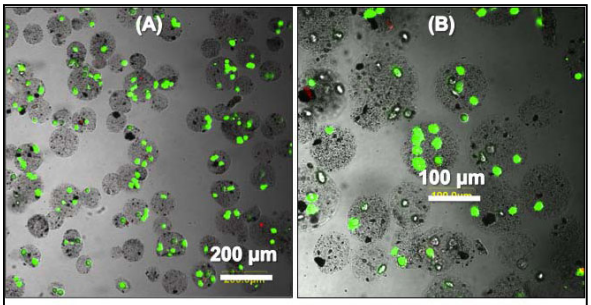

Figure 4. Live/Dead stains of Collagen-HA Microbeads containing human neonatal dermal fibroblasts. Scale bar – 200 µm (A) and 100 µm (B).

Conclusions: Defined biomimetic microbeads were fabricated with collagen and HA as matrix materials, using a water-in-oil emulsification process. Microbead size was controllable and bead morphology was uniform. The process and required reagents were cytocompatible, as evidenced by survival of hFb post-fabrication. The presence of both collagen I and HA may provide osteogenic stimuli to embedded cells, and future work will examine the effects of the microbead matrix on progenitor cell phenotype. Microbeads made from biomimetic materials are an attractive way to control the function of embedded cells, and can also serve as a delivery vehicle to sites that require bone regeneration.