Characterization of Crosslinked Gelatin Microspheres for Controlled Growth Factor Delivery

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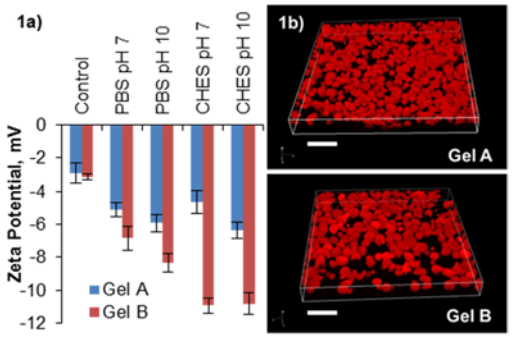
Statement of Purpose: Hydrogel beads, tailored microenvironments engineered to mimic specific physiological targets, have proven potential for translational therapies in regenerative medicine. This process has been demonstrated in beads hosting the organization of precursor blood vessel networks from embedded endothelial cells and fibroblasts and calcium deposition indicative of osteogenesis. Thus, we have complemented the bead systems with entrapped and enzymatically-released growth factors such as BMP-2 and VEGF from crosslinked gelatin microspheres. Challenges posed by drug delivery strategies include therapeutic loading of the desired molecule and sustained release for the duration of tissue remodeling. This work presents our progress characterizing gelatin microspheres to be used for the entrapment and *in vivo* delivery of growth factors.

Methods: *Gelatin Microsphere Emulsification.* Microspheres were fabricated using a water-in-oil emulsification process from gelatin type A and B by suspension in 100cSt PDMS oil. Briefly, a 6 wt% gelatin solution was added to PDMS oil heated to 40°C. The mixture was stirred to form microspheres in the gelatin phase, then rapidly cooled in an ice bath to set the gel spheres. The microspheres were separated from PDMS oil by several washes in PBS with L101 surfactant.

Genipin Crosslinking. Gelatin microspheres were crosslinked with genipin to stabilize their structure under physiological conditions. Briefly, microspheres were suspended in PBS or 0.2M CHES buffer with a pH of 7 or 10 and allowed to react until completion (24h for gel A, 96h for gel B). Crosslinked spheres were washed and deflocculated by sonification prior to freeze-drying.

Microsphere Characterization: The electrical charge, or zeta potential, of crosslinked microspheres was measured using a Zetasizer (Malvern) operating in PBS (pH 7.4) at 37C. Microsphere dimensions were measured using a Mastersizer 2000 (Malvern) in deionized water at 20°C. Microspheres were loaded with a 100 µg/mL solution of FITC-Dextran (Mw 20kD), a model molecule with similar size and zeta potential as growth factors BMP and VEGF, by swelling overnight at 37°C. Loaded spheres were suspended in PBS and the amount of FITC-Dextran retained was determined by measuring FITC emission from unbound FITC-Dex remaining in suspension.

Results: Evaluation of crosslinked microspheres indicated a range of about -4 to -12 mV surface potential (Fig. 1a). Spheres made from gel B and crosslinked in CHES buffer indicated the highest negative potential, a feature beneficial for adhering positively charged growth factors. Altering the gel type and reaction conditions resulted in average hydrated (swelled) sphere sizes ranging from 15 to 30 microns with distributions ranging from about 15 to 50 microns (Fig. 2a). These combinations resulted in FITC-Dextran retention of up to 1 ug/mg microspheres (Fig. 2b). Gelatin microspheres were successfully incorporated and uniformly dispersed in agarose (Fig. 3a) and fibrin (Fig. 3b) hydrogel microbeads designed for therapeutic cell delivery.



Fg. 1a: Zeta potentials of gelatin microspheres. **Fig. 1b**: Representative 3-D confocal micrographs of crosslinked gelatin type A and type B microspheres; 50 µm scale bars.

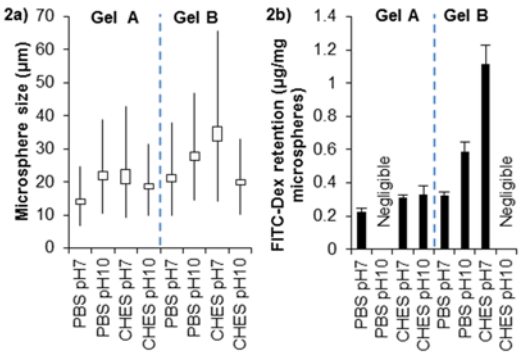


Fig. 2a: Average size and distribution measurements of gelatin microspheres. Box plot shows numerical mean and volume-weighted sphere sizes, whiskers show 10% and 90% population size distributions. **Fig. 2b:** FITC-Dextran loading potential for crosslinked gelatin microspheres.

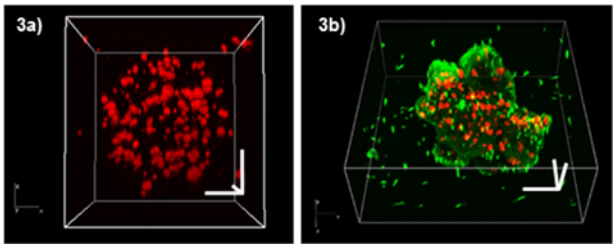


Fig. 3a: Crosslinked gel A microspheres (red) embedded in agarose bead; 50 µm scale bars. **Fig. 3b:** Crosslinked gel B microspheres (red) embedded in FITC-stained fibrin bead (green); 100 µm scale bars.

Conclusions: Analysis of gelatin microspheres showed crosslink reaction conditions to influence material characteristics, including zeta potential and sphere size. Higher zeta potential and larger sphere size was correlated with greater FITC-Dextran retention, although with greater variability in sphere dimensions. Future work will exploit the loading characteristics of gelatin materials to maximize growth factor retention and controlling subsequent release during tissue remodeling of the beads.