Chemically Crosslinked Gelatin Microspheres with Tunable Degradation Profiles

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Statement of Purpose: Regenerative healing is a temporally dynamic process in which biomaterials can contribute spatial attributes that may be needed for shorter or longer times, depending on the regenerative context. Incorporating control of *in vivo* degradation rate into the production of biomaterials for regenerative medicine applications is therefore highly desirable. We investigated the feasibility of fine-tuning the *in vitro* rate of enzymatic degradation in gelatin-based biomaterials by controlling of the extent of crosslinking with a well-characterized crosslinking reagent (*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbo-diimide hydro-chloride, or EDC) as proof-of-concept for fine-tuning *in viv*o degradation rates. EDC is a zero-length crosslinker used in the manufacture of currently marketed clinical products that catalyzes the formation of an intra- or intermolecular amide bond between spatially adjacent carboxyl and primary amine functionalities. The extent of crosslinking was directly proportional to the rate of collagenase-mediated degradation of gelatin beads. Although commercially-available gelatin beads (e.g., Cultispher® S, G or GL; Sigma-Aldrich, St. Louis, MO) provide versatility relative to the size of the beads and possess the appropriate porosity for cell attachment, they offer limited opportunity to control enzymatic degradation rates. The process described herein offers a time and cost efficient, simple method for optimizing degradation rate.

Methods:

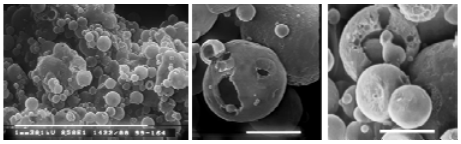
Fabrication of gelatin beads. A 10% w/v gelatin solution (Gelita, Inc., Sioux City, IA) was prepared in water and then air sprayed into liquid nitrogen (LN_2) with a thin layer chromatography reagent sprayer. LN_2 was allowed to evaporate in a chemical hood and beads were collected and lyophilized. Beads were imaged by Scanning Electron Microscopy (SEM).

EDC crosslinking. Lyophilized gelatin beads were suspended in 0.1M MES buffer, pH 4.7 (Thermo Fisher Scientific, Rockford, IL) and rehydrated for 3 hours at room temperature. The buffer was then removed and a 1:1 (v/v) suspension made with EDC/MES pH 4.7 solution, where EDC concentrations ranged from 0.01-1 M, and the suspension was incubated overnight at room temperature. Beads were filtered and washed with distilled water (wash volume = 20X bead volume), then frozen and lyophilized.

Enzymatic degradation. Degradation rates for beads crosslinked with varying concentrations of EDC were assayed by their susceptibility to collagenase/dispase digestion and compared to Cultispher beads. Cultispher and crosslinked gelatin beads were suspended at 20 mg/ml in PBS, pH 7.4. To each sample (0.5 ml) 50 µl of 30 U/ml collagenase/dispase mix (Thermo Fisher Scientific) was added. Samples were vortexed then incubated for 1 h at 37°C on a rocker. Degradation was calculated as the ratio of soluble protein at 1 hr/total soluble protein as measured by a Bradford assay.

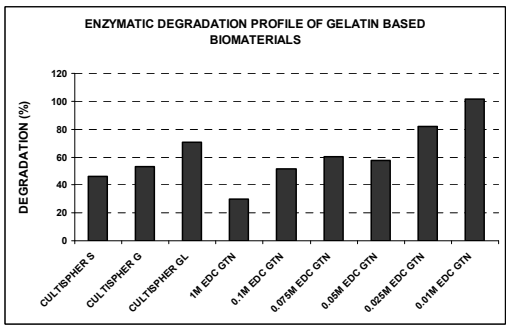
Results: The methods described produced spherical gelatin microbeads with a size distribution range of 10-430 µm in diameter (median diameter ~80 µm). The surface of the spheres appeared porous and the inside hollow.

Figure 1. SEM imaging of gelatin beads illustrating morphology and size distribution of the biomaterial. Scale bar =1mm (left) and 0.1mm (center, right).



Exposure to collagenase/dispase released varying amounts of soluble protein from both Cultispher and crosslinked gelatin beads. For the gelatin beads, these *in vitro* degradation rates correlated well with the EDC concentrations used for crosslinking.

Figure 2. Degradation rate of crosslinked gelatin beads was roughly proportional to concentration of EDC crosslinker after 1 hr exposure to collagenase/dispase enzyme mixture.



Conclusions:

- The *in vitro* enzymatic degradation rate of gelatin-based beads can be controlled at synthesis with the concentration of ECD used for crosslinking.
- The fabrication process presented here represents a straightforward process for manufacturing biodegradable scaffolds with tunable enzymatic susceptibility using a reagent that is currently used in the production of clinical products.
- The translation of tunable *in vitro* degradation to tunable *in vivo* degradation is under active study and could potentially represent a useful platform technology for producing biomaterials where the temporal persistence of the spatial and structural characteristics could be optimized to the specific needs of the organ and/or tissue being regenerated.