

# Fabrication of Chemically Crosslinked Gelatin Microspheres with Tunable Degradation Profiles

Monica A. Serban, Toyin Knight, Richard Payne, Joydeep Basu, Elias A. Rivera, Neil Robbins, Darell McCoy, Craig Halberstadt, Deepak Jain, Timothy A. Bertram  
Tengion, Inc. Science & Technology, Winston-Salem, NC

## EXPERIMENTAL PROCESS AND RESULTS

1. **Production of gelatin beads.** Spherical, hollow, porous gelatin beads were obtained by spraying a concentrated gelatin solution into liquid nitrogen followed by lyophilization. The majority of the beads had diameters < 100  $\mu\text{m}$ .

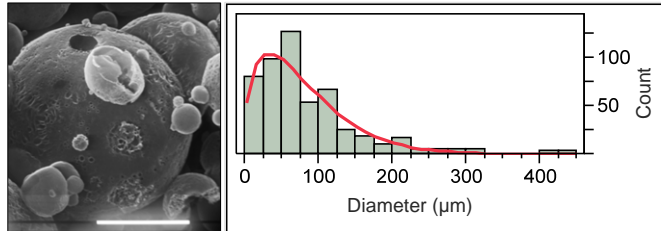


Figure 1. Left -Scanning electron microscopy image showing the overall morphology of gelatin beads (scale bar 100  $\mu\text{m}$ ). Right - Size distribution analysis of beads (n = 500).

2. **Chemical crosslinking.** Gelatin beads were differentially crosslinked by exposure to 10-100 mM EDC (carbodiimide) under otherwise identical reaction conditions. The success of crosslinking was confirmed by colorimetrically quantifying the primary amine groups left on gelatin after EDC reaction using picrylsulfonic acid.

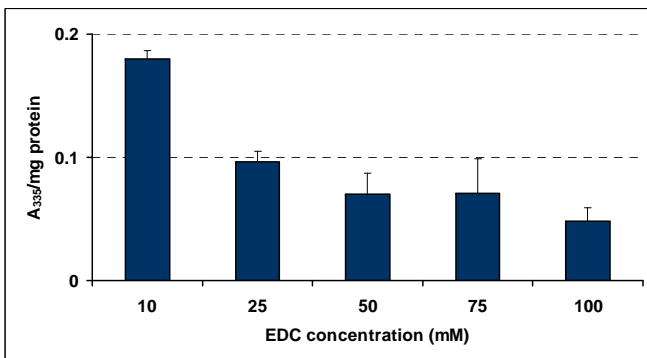


Figure 2. Colorimetric quantification of primary amines present in differentially crosslinked gelatin beads. ANOVA statistical analysis P = 0.0008.

3. **Cytocompatibility of crosslinked beads.** The viability of primary rat kidney cells seeded under dynamic conditions on crosslinked beads was evaluated after 24 hours. The seeding density was  $94.5 \times 10^6$  cells/ml packed bead volume.

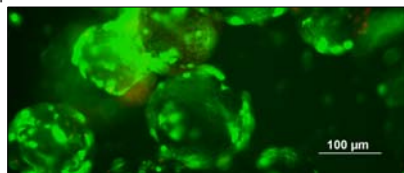


Figure 3. Live/dead staining of cells on gelatin beads crosslinked with 25 mM EDC. The image was randomly chosen as representative for the differentially crosslinked bead series.

## INTRODUCTION

The goal of this work was to investigate the tunability of the enzymatic degradation of gelatin-based biomaterials through the control of the extent of crosslinking. For this, we chose to use a well characterized and widely used reagent, *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC). This zero-length crosslinker promotes the formation of an amide bond between spatially adjacent carboxyl and primary amine functionalities located either intra- or intermolecularly. As indicated by our results, the extent of crosslinking directly correlates to the rate of collagenase-mediated degradation of gelatin beads. Commercially available counterparts provide versatility relative to the size of the beads and possess the porosity desired for cell attachment but offer limited control over the enzymatic degradation rates. For applications where the material biodegradability needs to be finely modulated, the process described herein offers a simple, time and cost efficient alternative.

4. ***In vitro* degradation.** The enzymatic degradation rates of differentially crosslinked beads was evaluated *in vitro* by using a collagenase/dispase mix (at 37°C for 1 hour). The degraded soluble protein was determined colorimetrically (Bradford assay) and normalized to the total amount of protein in the sample (determined after complete digestion).

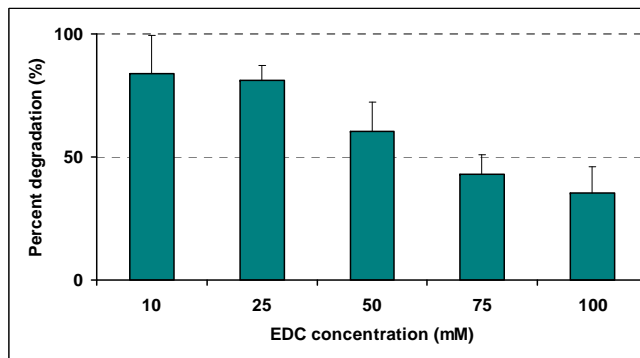


Figure 4. Quantification of resistance to enzymatic digestion of differentially crosslinked gelatin beads (ANOVA statistical analysis P = 0.007).

5. **Biocompatibility and biodegradability evaluation.** Representative differentially crosslinked, cell seeded beads were assessed in 3 months old Lewis rats at 1 week and 4 weeks via injections into the kidney parenchyma. The degradation pattern correlated well with the *in vitro* data and all samples integrated well in the surrounding tissue.

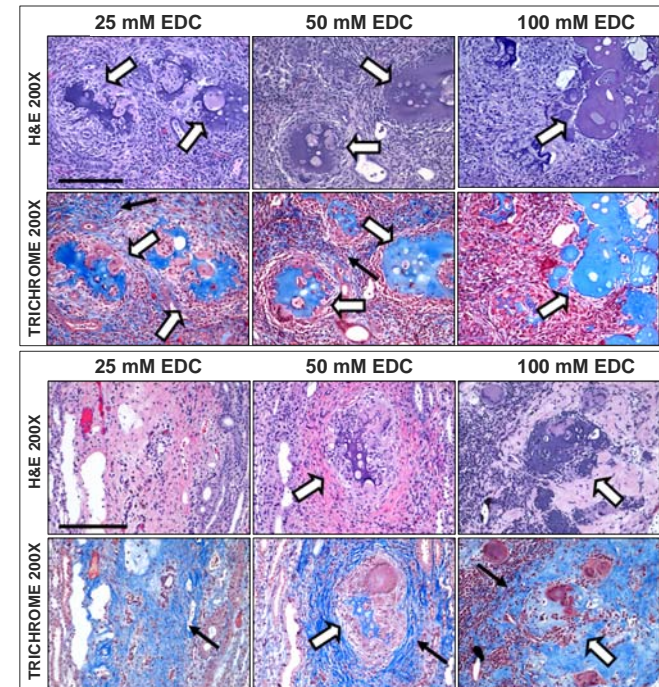


Figure 5. Histological evaluation of kidney sections showing the degradation of gelatin beads at top - 1 week and bottom - 4 weeks post-injection. White arrows indicate the crosslinked beads both in the H&E and Trichrome stained sections. Trichrome stains gelatin blue and allows the visualization of both the beads (white arrows) and gelatin traces (black arrows) resulting from bead degradation (scale bar 200  $\mu\text{m}$ ). This evaluation was conducted to compare the tissue *in situ* degradation of the test articles to *in vitro* profiles, not to evaluate regenerative outcome.

## CONCLUSIONS

- The *in vitro* enzymatic degradation rate of gelatin-based beads can be controlled at synthesis with the concentration of EDC used for crosslinking.
- The method presented here represents a scalable 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 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.

## ACKNOWLEDGEMENTS

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## REFERENCES

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