Mesenchymal Stem Cell and Gelatin Microparticle Encapsulation in Thermally and Chemically Gelling Injectable Hydrogels for Tissue Engineering

Stephanie N. Tzouanas, Adam K. Ekenseair, F. Kurtis Kasper, Antonios G. Mikos
Department of Bioengineering, Rice University, P.O. Box 1892, MS 142, Houston, TX 77251, USA

Statement of Purpose: Our group has previously developed a thermogelling macromer based on poly(N-isopropylacrylamide) (PNiPAAm) with pendant epoxy rings and a degradable polyamidoamine (PAMAM) crosslinker for dual-hardening, injectable, and hydrolytically degradable hydrogels [1, 2]. In this study, mesenchymal stems cells (MSCs) were encapsulated together with gelatin microparticles (MSCs) in this hydrogel system to investigate the effects of GMP size and loading ratio on (1) encapsulated cell viability and differentiation and (2) hydrogel mineralization.

Methods: A factorial design was implemented to study the effects of GMP size, GMP loading ratio, and cellularity on cell viability, cell differentiation, and hydrogel mineralization. Two GMP size ranges, 50-100 µm and 250-300 µm, were investigated. GMP loading ratios (the ratio between GMP and dry hydrogel mass) were studied (1:20 and 1:5). A fifth group without GMPs was included as a control. The same five GMP size and loading conditions were studied in acellular hydrogels as well. Samples were analyzed after 0, 7, 14, 21, and 28 days. Viability of the MSCs encapsulated in the hydrogel was assessed by means of a Quant-iT Picogreen DNA assay (Molecular Probes, Eugene, OR). Mineralization was assessed by means of a Genzyme calcium assay (Genzyme Diagnostics PEI, Charlottetown, Prince Edward Island, Canada). Alkaline phosphatase (ALP) activity, an early marker of osteogenic differentiation, was measured using the Sigma-Aldrich ALP assay (Sigma, St. Louis, MO). Cellular viability was evaluated in stained sections (Live/Dead Viability/Cytotoxicity Kit, Invitrogen, Eugene, OR) with confocal fluorescence microscopy. Sections of the hydrogels were stained with von Kossa reagent to visualize phosphate, an important component of bone mineralization.

Results: Cells demonstrated long-term viability within the hydrogels dependent on GMP size. Smaller GMPs at higher loading ratios allowed for better viability, likely due to increased surface area for cellular interaction and attachment. However, greater interaction between GMPs and MSCs through higher GMP surface area resulted in reduced ALP expression and hydrogel mineralization, which can be attributed to enhanced MSC differentiation in a suspended rather than attached state. The hydrogel system investigated, both with and without the co-encapsulation of GMPs, allowed for MSC encapsulation and survival for a period up to and including 28 days. Both the epoxy-based chemical gelation pathway (which is rapid, requires no cytotoxic initiator or catalyst molecules, and leaves no residue post- reaction) and the enhanced hydrogel water content (via PAMAM incorporation) provided favorable long-term cell viability. Calcium deposition in hydrogels was enhanced at earlier time-points when GMPs were encapsulated within the system, particularly for larger GMPs presenting greater

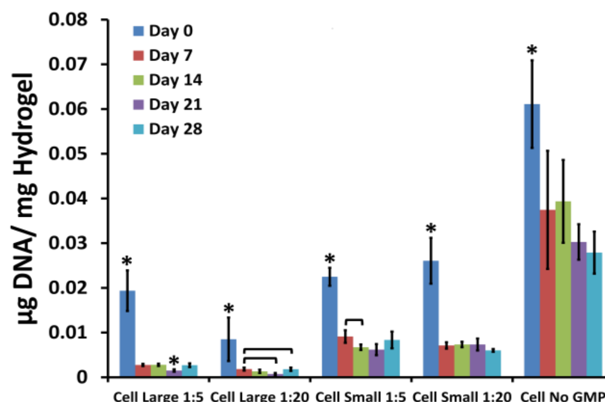

Figure 1. DNA in cellular hydrogels (µg DNA / mg Hydrogel) for GMP loading and size conditions. "Large" refers to particles 250-300 µm in diameter, and "Small" to particles 50-100 µm in diameter. 1:20 and 1:5 designate GMP loading ratios. Within groups, time-points with * differ from all other time-points ($p < 0.05$). Brackets indicate statistically significant differences between time-points within the same group ($p < 0.05$).

available reservoir volume. While the long-term mineralization potential of the hydrogels was reduced by encapsulation of MSCs, hydrogel mineralization was unaffected by GMP loading at the conditions investigated.

Conclusions: The effects of GMP size and loading ratio on the viability, ALP expression, and calcium deposition of MSCs encapsulated within *in situ* dual-gelling injectable hydrogels combining PNiPAAm-based thermogelling macromers and PAMAM-based crosslinkers were studied. Increased interaction of cells and GMPs through greater available GMP surface area, use of an epoxy-based chemical gelation mechanism, and the tunable high water content of the thermogelled hydrogels led to favorable long-term cell viability. Compared to cellular hydrogels without GMPs, hydrogels co-encapsulating cells and GMPs demonstrated greater production of alkaline phosphatase by cells at all time-points and a transient early enhancement of hydrogel mineralization for larger GMPs at higher loading ratios. Such injectable, *in situ* forming, dual-gelling hydrogels capable of delivering and maintaining populations of encapsulated mesenchymal stem cells and promoting mineralization *in vitro* offer promise as novel therapies for applications in tissue engineering and regenerative medicine.

Acknowledgements:
This work was supported by a grant from the National Institutes of Health (R01 DE17441).

References:
1. Ekenseair AK, et al. Biomacromolecules. 2012;13:1908-15.
2. Ekenseair AK, et al. Biomacromolecules. 2012;13:2821-30.

©2014 Society for Biomaterials