An injectable and settable cell delivery system for tissue repair derived from in situ chemical polymerization

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Statement of Purpose: Autologous and allogeneic cell-based therapies have emerged as promising approaches for regenerative medicine [1]. The two-component lysine-derived polyurethanes, which can form porous, biodegradable, and biocompatible scaffolds with tunable mechanical properties fitting different tissue defects, are promising carriers for local delivery of cells using minimally invasive surgical techniques due to their injectability and ability to cure *in situ*. In order to overcome the drawback that the highly chemical-reactive environment decreased survivability of the delivered cells, bone marrow-derived stem cells (BMSCs) were encapsulated in oxidized alginate to protect them from heat and mass transfer at early time points, while dissolution of the temporary alginate barrier created macropores at later time points.

Methods: In this study, we used injectable polyurethanes synthesized from a polyester triol, an iron acetylacetonate catalyst, and a lysine triisocyanate-PEG (LTI-PEG) prepolymer, as carriers for cell delivery. Considering that the reactants of polyurethane are highly reactive, cells cannot be encapsulated directly due to the chemical reaction between NCO-terminated prepolymer and the cells. In order to temporarily protect cells during the process of curing, we encapsulated MSCs in the fast degrading oxidized calcium alginate hydrogel [3] beads with diameter ranging from 300-800. Cell viability was assessed using a Live/Dead viability kit (Invitrogen). Fluxes of heat and CO2 during the PUR setting reaction were calculated by a chemical reaction kinetics model [2]. Excessive generation of CO_2 was hypothesized to cause cell death, and thus the scaffold fabrication process was optimized to reduce the exposure of cells to CO_2. The designed cell carrier was then evaluated in a cutaneous defects in rats *in vivo*. Male rat MSCs were transplanted into defects in female rats for SRY (sex-determining region Y) analysis.

Results: Cells were encapsulated in fast degrading oxidized alginate beads with high viability (>90%). However, cell viability decreased with decreasing bead diameter, suggesting that the chemical reaction adversely affects the cells (Fig. A). heat and CO2 generated by the reaction were hypothesized to reduce cell survivability. Live/Dead staining results indicated that viability exceeded 70% for 500 um beads by delaying addition of the beads after mixing the reactive liquid PUR components, which reduced the exposure of cells to generated CO_2. As shown in Fig. B, cell viability significantly increased as CO_2 generation decreased from 0.34 ml/g to 0.26 ml/g. In order to improve diffusion after the formation of polyurethane scaffolds, alginate bead loading was increased to 70 wt% to increase interconnectivity (Fig. C).

Rat BMSCs injected into 10-mm excisional wounds showed viable cells for up to 7 days (Fig. E). Histomorphometric analysis shows that PUR/oxidized alginate scaffolds augmented with rat BMSCs support significantly greater ingrowth of extracellular matrix at days 4 and 7 (Fig. F).

Conclusions: MSCs encapsulated in oxidized alginate beads comparable to the pore size of trabecular bone (~500 μm) were embedded in reactive polyurethane scaffolds with Young's moduli ranging from 80kPa to 120kPa (Compression), followed by rapid dissolution of the alginate gel. In the present study, we reduced CO_2 generation during the PUR setting reaction by delaying the addition of wet alginate beads, thereby increasihg cell viability at early time points. Bead loadings of 70 wt% effectively balanced the requirements of mechanical integrity and interconnected pores to support cell growth and migration at later time points (Fig. D). After cure, the beads degraded to form macropores that supported deposition of new matrix *in vivo*. These observations highlight the potential utility of injectable PUR/Alg scaffolds augmented with cells and biologics for tissue regeneration. Future work will focus on identifying the mechanisms by which the transplanted MSCs enhance tissue regeneration.

Key words: polyurethane; injectable scaffolds; oxidized alginate; cell therapy; tissue engineering

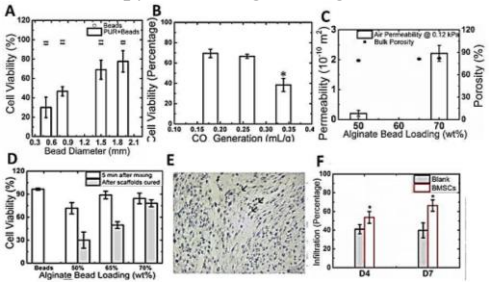

Fig. A. Cell viability was directly correlated to bead size when embedded in PUR scaffolds, suggesting that diffusion of reaction products into the beads is killing the cells. B.Cell viability increased with the decreased generation of CO_2 in the early reaction stage (10min) by delaying the addition of wet beads. C. Porosity and permeability of PUR scaffolds as a function of oxidized alginate bead loading for delayed addition. D. Cell viability in the very early and lateral stages with different loadings. E. SRY immunohistochemical staining reveals the presence of delivered MSCs. F. Histomorpho-metry shows significantly more new matrix at 4 and 7 days for scaffolds augmented with BMSCs

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References

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