# Injectable Scaffolds with Degradable Calcium Alginate Beads as a Cell Delivery System for Tissue Repair

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**Statement of Purpose:** Although mammalian regenerative cells have shown promise for cell therapy, introducing cells directly into the body results in large-scale death as well as the migration of cells from the injection site. Thus, for the application of cell therapy in tissue repair there is a compelling need for suitable polymeric carriers that can provide a scaffold for adhesion of transplanted cells, as well as a template for new tissue formation [1].

Lysine-derived polyurethane scaffolds, which are porous, biodegradable, and biocompatible, have been reported to support cellular infiltration and new tissue formation in subcutaneous, cardiovascular, and bone models [2]. Due to their injectability and ability to cure *in situ* [3], two-component lysine-derived polyurethanes are promising carriers for local delivery of cells using minimally invasive surgical techniques.

**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 protect cells during the process of curing, we encapsulated MC3T3-E1 cells in calcium alginate hydrogel beads with diameter ranging from 300-800μm. Cell viability was assessed using a Live/Dead viability kit (Invitrogen). In order to increase the degradation rate of the alginate, which was designed to protect the cells from chemical reaction and subsequently degrade over 1-2 days, we investigated oxidized alginate [4] for encapsulating cells. The degradation of alginate beads embedded in the scaffolds was evaluated by SEM.

**Results:** Cells were encapsulated in alginate beads with high viability (>90%). However, cell viability decreased with decreasing bead diameter, suggesting that the chemical reaction may adversely affect the cells. A time-course experiment (Fig. A) indicated that the combination of initial reaction shock and limited diffusion afterwards caused cell death inside the scaffolds. Live/Dead staining images showed a color change of some cells from yellow to green after removing beads from the scaffolds, suggesting that the cells recovered from the thermal stress (Fig. C). Since the results indicate that cells could survive the process with abundant supply of nutrients, to improve diffusion after the formation of polyurethane scaffolds as well as to obtain proper physical properties, scaffolds generated from polycaprolactone 300 (Mw300) with 70% bead loading were selected. SEM images revealed the presence of both the beads and interconnected micro pores (50-70 μm, Fig. E) inside the cured polyurethane scaffolds. Moreover, compared to the scaffolds loaded with 50% beads, the scaffolds with 70% beads showed more interconnected pores for transport of oxygen, fluid, and nutrients into the interior of the scaffold (Fig. D, E). As a result, cell viability remained high in the interior of the cured scaffolds (Fig. B).

In order to release cells after final cure of the scaffold, partially oxidized sodium alginate was used for encapsulation. Cell viability was unchanged when encapsulated in the partially oxidized alginate. SEM images reveal that the partially oxidized alginate embedded within the polyurethane scaffold degraded after 3-4 days in dynamic culture, thereby creating more pores within the scaffold as well (Fig. F).

**Conclusions:** MC3T3 cells encapsulated in oxidized alginate beads comparable to the pore size in 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. Future work will focus on cell proliferation and differentiation *in vitro* and the application of this cell carrier for wound healing *in vivo*.

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

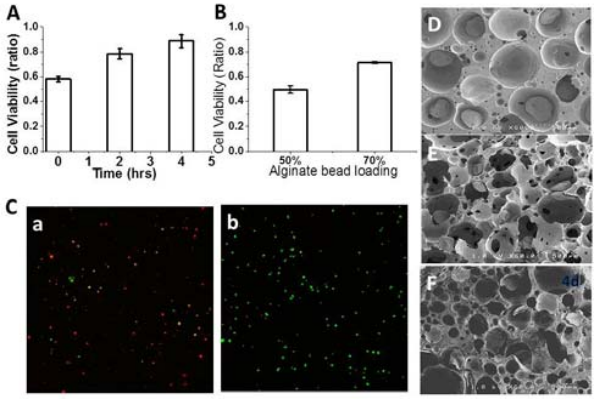


**Fig.** A. Time-course viability of cells recovered after scaffold formation process. B. Viability of cells embedded in scaffolds as a function of bead loading. C. Confocol images of cells embedded in 50% (a) and 70% (b) loading scaffolds. D. E. SEM images of scaffolds with 50% (D) and 70% (E) loadings. F. SEM image of scaffold incorporating partially oxidized alginate beads after dynamic culture.

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## References

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