Injectable Alginate Microcapsule/Polyurethane Composite Scaffolds for Cell Therapy

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Statement of Purpose: Various therapeutic delivery systems have been investigated as treatments for diseases and tissue regeneration [1]. Recently, there has been extensive research in the transplantation of living cells into damaged tissues for tissue repair. Two general cell delivery approaches have been investigated, including direct injection of cells and delivery of cells within an implanted scaffold. However, cells injected directly into the tissue defect can migrate away from the wound, and implantation of scaffolds seeded with cells requires invasive surgical techniques. In addition, massive cell death induced by the hypoxic environment and nutrient limitation, as well as poor incorporation and integration, are significant limitations of conventional cell delivery systems [2]. The objective of the present study was to develop an injectable two-component polyurethane (PUR) cell delivery system that immobilizes the cells in an *in situ* setting scaffold. MC3T3 cells were encapsulated in alginate beads prior to incorporation in the injectable PUR to protect them from the urethane curing reaction. We hypothesized that encapsulating cells in alginate beads prepared using α-MEM would protect the cells from the chemical reaction and enhance their *in vitro* long-term viability in the PUR scaffold.

Methods: An O/W emulsion technique was used for encapsulation of cells in alginate beads. MC3T3-E1 embryonic mouse osteoblast precursor cells were encapsulated with $1x10^6$cells/ml in alginate beads. Alginate beads were prepared using two kinds of Ca-catalysts: (a) $CaCl_2$ in Di-water (AB1), and (b) $CaCl_2$ in α-MEM (AB2). The loading of cell-encapsulated beads in the reactive PUR scaffold was 50 wt%. Cell survivability in alginate beads was determined using live/dead staining for two types of Ca-catalysts and for α-MEM and buffer. We anticipated that using α-MEM to prepare Ca-alginate beads would improve viability by supplying nutrients and oxygen for the cells. Cell differentiation in the alginate beads, beads alone and beads incorporated in PUR, was characterized using alkaline phosphatase (ALP) as an early marker.

Results: Cell-encapsulated alginate beads were successfully synthesized using the O/W emulsion technique. The average sizes of small (S) and large (B) alginate beads were about 0.75 (±0.03) and 2.00 (±0.03) mm, respectively. Cell viability of AB1 cell-alginate beads quickly decreased with immersion time in $CaCl_2$ DIW solution, particularly for the S beads where cell viability was about 10% in $CaCl_2$ DIW solution after 1 day. However, AB2 cell-alginate beads prepared using α-MEM showed >60% viability for up to 5 days when incubated in $CaCl_2$/α-MEM solution and >80% viability when incubated in α-MEM. These high cell viabilities were preserved when the alginate beads were embedded in the injectable PUR scaffolds, suggesting that encapsulation of the cells in alginate protected them from the chemical reaction. As shown in Fig. 1, ALP activity of encapsulated cells was significantly increased at day 15.

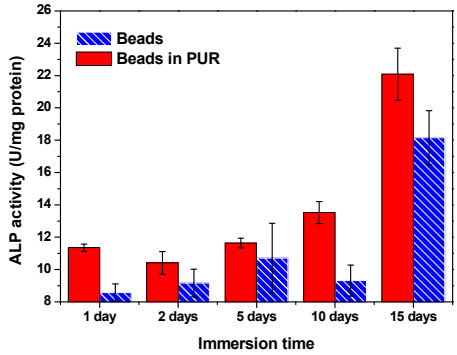

Fig. 1. ALP activity per total protein in alginate beads alone and alginate beads incorporated in injectable PUR as a function of immersion time in α-MEM.

Conclusions: We have shown that encapsulating MC3T3-E1 cells in α-MEM alginate solutions protects the cells from the urethane setting reaction and improves cell viability relative to DIW. The injectable alginate microcapsule/ polyurethane composite scaffold also supports differentiation of MC3T3-E1 preosteoblast cells. It is conjectured that using α-MEM as the solvent to prepare the alginate beads initially supplied nutrients and oxygen for the encapsulated cells. At longer time points, the combination of the relatively high (50 wt%) loading of alginate beads in the scaffold with micropores generated by the urethane foaming reaction resulted in a continuous pathway through which α-MEM medium diffused into the beads, thus supplying fresh nutrients for the cells. This approach may be potentially developed to design injectable cell transplantation vehicles that both localize and maintain the viability of the transplanted cells, as well as support cell proliferation and differentiation.

References

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