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Gel microstructure regulates proliferation and differentiation of MC3T3-E1 cells encapsulated in alginate beads

Baek-Hee Lee [a,b], Bing Li [a,b,1], Scott A. Guelcher [a,b,c,*]

[a] Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, USA
[b] Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN, USA
[c] Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA

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ABSTRACT

For cell transplantation into damaged tissues, viable cells must be delivered to the defect site in a suitable carrier. However, the hypoxic and nutrient-limited environment in the carrier can induce massive cell death. The aims of this study were to increase the viability and regulate the behavior of osteoprogenitor cells encapsulated in alginate hydrogels through control of the gel microstructure. Cell survivability in alginate beads was improved through the use of α-MEM as the solvent for alginic acid sodium salt, and by $CaCl_2$ solutions, which supplied additional nutrients for the cells compared to water or buffer. The mesh size and shear modulus of the hydrogel were hypothesized to regulate proliferation and differentiation of osteoprogenitor cells. MC3T3-E1 cells demonstrated enhanced osteoblast differentiation when encapsulated in high-density alginate with smaller mesh size and more rigid mechanical properties, as confirmed by increased alkaline phosphatase activity and osteocalcin secretion. However, MC3T3-E1 cells encapsulated in low-density alginate beads with a larger mesh size and more compliant mechanical properties exhibited increased proliferation. These results demonstrate that the microstructure of alginate hydrogels can regulate the behavior of osteoprogenitor cells, thus suggesting that the tuning the properties of the gel may be a useful approach for enhancing new bone formation.

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1. Introduction

Various therapeutic delivery systems have been investigated as treatments for diseases and for tissue regeneration [1–9]. Recently, there has been extensive research in the transplantation of living cells into damaged tissues for tissue repair [4–7]. Cell delivery approaches currently under investigation include both direct injection of cells and also delivery of cells within an implanted scaffold [4–7]. 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 [6–9]. In addition, massive cell death induced by the hypoxic and nutrient-limited environment, as well as poor incorporation and integration of the delivered cells, are significant limitations of conventional cell delivery systems [2,7].

Cell encapsulation in alginate hydrogels represents one of the most widely investigated approaches for cell therapy [10,11]. Alginate consists of a three-dimensional (3-D) polymeric network with high water content, which imparts its structural and mechanical characteristics to macromolecular-based components in natural tissues [11]. The 3-D structure of alginate not only facilitates the diffusion of body fluids, including nutrients, oxygen and metabolites, but also protects the encapsulated cells against shear forces, chemical reactions and attack by inflammatory cells [10–14].

Cell–cell contact generally arrests cell growth through contact inhibition [14–17]. For example, relatively large islands of 2-D substrates coated with the extracellular matrix (ECM) protein laminin promoted proliferation, while relatively small islands induced apoptosis [15,17]. Similarly, cell–substrate and cell–cell interactions within 3-D gel networks have been suggested to play a critical role in regulating cell proliferation, differentiation and organ size [18–20]. Encapsulation of cells in alginate beads has been investigated extensively due in part to the flexibility of the process, wherein the physicochemical properties of the gels such as biodegradability, bead size, swelling, gel mesh size, mechanical properties and cell seeding density can be controlled by varying the chemical composition and processing parameters [11,21–24].

To promote cell adhesion, proliferation and differentiation, alginate is frequently modified with cell adhesion peptides and proteins [1,25–28]. Specifically, the peptide arginine–glycine–aspartic acid (RGD), which is derived from the ECM protein fibronectin, has been coupled to alginate gels at tunable densities

* Corresponding author at: Department of Chemical and Biomolecular Engineering, Vanderbilt University, PMB 351604, 2301 Vanderbilt Place, VU Station B #351604, Nashville, TN 3723, USA. Tel.: +1 615 322 9097; fax: +1 615 343 7951.
E-mail address: scott.guelcher@vanderbilt.edu (S.A. Guelcher).
[1] Currently at GE Global Research Center, One Research Circle, CEB 1545, Niskayuna, NY 12309, USA.

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