Combinatorial Screening of Hydrogel Properties for 3-D Cell Culture: Effect of Stiffness on Encapsulated Osteoblasts

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INTRODUCTION

Billions have been invested in developing tissue engineered products and yet there are few successful products in the market. There is a need to accelerate the pace of research in this field. To this end, high-throughput platforms to rapidly screen cell-biomaterial interactions to optimize tissue regeneration offer a plausible solution.

Combinatorial methods for screening biomaterials have typically involved screening cellular response on material surfaces in two-dimensional (2-D) cell culture format[1]. However, biomaterials are being increasingly used to fabricate scaffolds for regenerating three-dimensional (3-D) tissues. Moreover, cells cultured in 3-D behave more physiologically than those cultured on 2-D surfaces[2]. Therefore, in this work, cell-material interactions were studied using combinatorial methods where cells were cultured in 3-D scaffolds.

Polyethylene glycol (PEG)-based hydrogels have emerged as promising scaffolds for tissue engineering in recent years[3]. The objective here was to develop a simple combinatorial platform to screen properties of these photo-polymerizable gels. Previous research has demonstrated that stiffness of the underlying substrate influences cell behavior[4]. Thus, we examined the effect of mechanical properties (compressive modulus) on osteoblasts encapsulated within hydrogels with continuous gradients of modulus. Preliminary data presented herein indicate that survival and differentiation of encapsulated mouse preosteoblast MC3T3-E1 cells were profoundly influenced by changes in the gel modulus.

EXPERIMENTAL METHODS

Poly(ethylene glycol) dimethacrylate (PEGDM) was prepared from PEG (relative molecular mass=M_w=4000) by a microwave-assisted reaction[5] and characterized by mass spectroscopy. To fabricate gradients, 5.8 mL of buffer solutions containing either 5 % or 20 % (mass fraction) PEGDM and 0.05 % Irgacure 2959 were added to the mixing column and the stock column of a gradient maker (Amersham Biosciences), respectively. The output of the gradient maker was pumped into a mold prepared from Teflon sheets and a glass slide. The cast solution was cured with a 365 nm lamp for 15 min at 2 mW/cm^2. Circular disks were punched along the gradient to measure compressive modulus of the gels (Enduratec, Bose).

For cell studies, MC3T3-E1 cells were suspended at 2.5 X 10^6/mL of the PEGDM solutions above. Gels were transferred to culture media (alpha-minimum essential medium with 10 % by volume of fetal bovine serum) immediately after curing. Cell number was determined from the DNA content assayed using the Picogreen DNA kit (Invitrogen). Differentiation was determined by measuring alkaline phosphatase activity (Stanbio lab) and alizarin red S staining.

RESULTS

Fig. 1 presents a cast gradient solution of 5 % to 20 % (by mass) PEGDM. For easy visualization, trypan blue dye was added to the 20 % solution. Values for compressive modulus for 2.5 % strain are plotted. DNA content measured at 6 weeks indicated a progressive decrease along the gradient with increase in modulus, whereas alkaline phosphatase expression increased with modulus (data not shown). Moreover, alizarin red staining for mineral deposits increased with gel stiffness. Fig. 2 presents the gradient in mineral deposits induced by the encapsulated cells at 6 weeks.

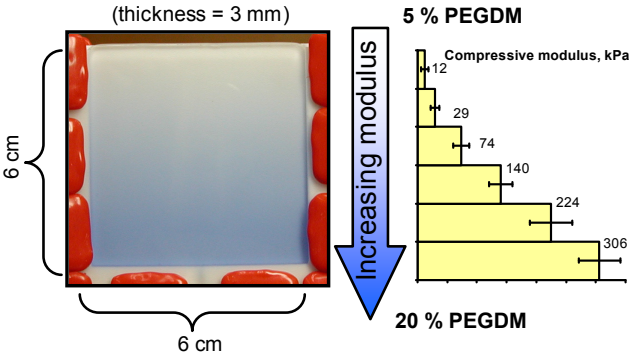

Figure 1. PEGDM hydrogels with gradients in compressive modulus.

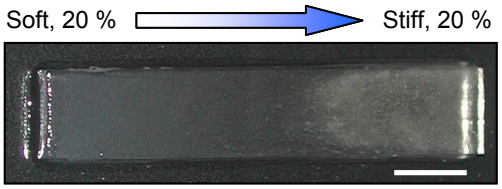

Figure 2. Gradient of induced mineral deposits in the hydrogels with gradients in modulus at 6 weeks (scale bar = 1 cm).

DISCUSSION

A simple, low-cost combinatorial platform was successfully assembled to fabricate hydrogels with gradients in mechanical stiffness spanning nearly a 25-fold change in compressive modulus. Survival of encapsulated murine pre-osteoblasts was higher in the softer ends than in the stiffer ends of the gradients. Increase in matrix stiffness induced spontaneous differentiation of encapsulated cells. Prolonged cell culture led to gradients of visible mineral deposits in the gels. These spatially-graded tissues are ideal for seamless interfacing of tissue-engineered mineralized bone with softer tissues.

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