Rapid Screening of Hydrogel Properties for 3D Tissue Culture: Effect of Modulus on Encapsulated Osteoblasts

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Statement of Purpose: With few successful products in the market despite large investments in tissue engineering research, there is a need to accelerate research. Towards this objective, developing high-throughput platforms to rapidly screen cell-biomaterial interactions offers a plausible solution. Furthermore, cells cultured in 3-dimensions (3D) behave more physiologically than those cultured on 2-dimensional (2D) surfaces[1]. Therefore, in this work cell-material interactions were studied using combinatorial methods where cells were cultured in 3D scaffolds.

Polyethylene glycol (PEG)-based hydrogels have emerged as promising tissue engineering scaffolds in recent years[2]. It is has been demonstrated that modulus of underlying substrate influences cell behavior[3]. The objective of this work was to examine the effect of mechanical properties (compressive modulus) on osteoblasts encapsulated within gradients of hydrogel modulus. Preliminary data presented herein indicate that differentiation and proliferation of encapsulated mouse osteoblasts are profoundly influenced by the stiffness of the hydrogel.

Methods: Poly(ethylene glycol) dimethacrylate (PEGDM) was prepared from PEG 4000 as described[4] and characterized by mass spectrometry. Gradients in gel modulus were prepared using a gradient maker (Amersham Biosciences) filled with 3 mL each of 5 % and 15 % (by mass) PEGDM [in phosphate-buffered saline (PBS) containing 0.05 % photoinitiator Irgacure 2959]. Using a peristaltic pump, the solution was cast into a bottom-filling vertical mold clamped between teflon and glass slides and cured with 365 nm light for 15 min at 2 mW/cm^2. Compressive modulus was measured (Enduratec, Bose) on discs stamped from gradient gels (without cells) soaked in PBS for 24 h post-curing. For cell studies, gradient gels were prepared from PEGDM solutions containing 2.5 X 10^6 MC3T3-E1 cells per mL. Gels were transferred to culture media (α-MEM with 10 % by volume fetal bovine serum) immediately after curing. Osteoblast number and cell differentiation within the gels were assayed using the Picogreen DNA (Invitrogen) and alkaline phosphatase activity kits (Sigma-Aldrich), respectively.

Results: Fig. 1 presents a gradient of 5 % to 15 % (by mass) PEGDM. Trypan blue dye was added to the 15 % solution for visualization of the gradient. Nominal values for compressive modulus at 5 % strain are given and span a wide range from 4 kPa to 155 kPa. After 6 weeks in culture, cell concentration in the gels measured using a Picogreen kit (data not shown) indicated a decrease in DNA content with increasing gel stiffness, whereas alkaline phosphatase activity per nanogram of DNA increased with increasing gel stiffness (data not shown). Additionally, Alizarin red S staining indicated increased calcium deposition with increasing gel stiffness. Beyond 8 weeks of culture, white mineral deposits were visible within the stiffer regions of the gradient hydrogels. Experiments are under way to determine if the mineralization is mediated by osteoblasts.

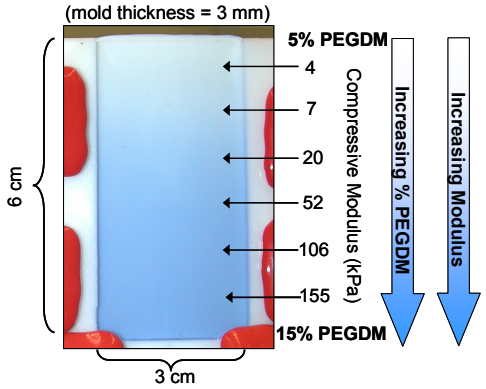

Fig. 1: PEGDM gels with a gradient in compressive modulus fabricated with a gradient maker.

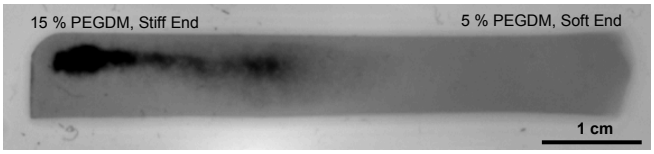

Fig. 2: Photograph of gradient of mineral deposited by osteoblasts after 12 weeks in culture within a hydrogel with a modulus gradient.

Conclusions: The effect of gel modulus in 3D culture was studied using osteoblasts cultured within the gradient hydrogels spanning nearly a 30-fold range in stiffness. Whereas softer gels promoted proliferation of osteoblasts, stiffer gels induced osteoblast differentiation.

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