EFFECT OF 3D HYDOGEL SCAFFOLD MODULUS AND TOPOLOGY ON HUMAN BONE MARROW STROMAL CELL FATE

Kaushik Chatterjee[1], Sheng Lin-Gibson[1], Sapun H. Parekh[1], Nicole M. Moore[1], Marcus T. Cicerone[1], Marian F. Young[2] and Carl G. Simon, Jr.[1]

[1]Polymers Division
National Institute of Standards and Technology
Gaithersburg, MD 20899 USA.

[2]National Institute of Dental and Craniofacial Research
National Institutes of Health
Bethesda, MD 20892 USA.

Introduction

There is growing recognition that cells can sense and respond to the physical cues from their environment, such as stiffness, mechanical loading and topology. Physical properties of the matrix can direct cellular response and are critical in the design of scaffolds for tissue engineering. In this work we examined the effect of matrix modulus on differentiation of human bone marrow stromal cells (hBMSCs) encapsulated within three-dimensional (3D) photo-polymerizable polyethylene glycol (PEG) hydrogels. Further, to investigate the topological effects of the hydrogel scaffolds on cellular differentiation we studied the response of hBMSCs on the gel surface (2D) and those encapsulated within the hydrogel (3D).

Experimental

Preparation of acrylated-PEG molecules. Poly(ethylene glycol) dimethacrylate (PEGDM) was prepared from PEG (relative molecular mass= M_w= 4000) by a microwave-assisted reaction by reaction with excess methacrylic anhydride[1]. Four-arm PEG (total relative molecular mass 20000, each arm of 5000, Jemken Technology) was reacted with excess of methacrylic anhydride to prepare poly(ethylene glycol) tetramethacrylate (PEGTM)[1].

PEGTM hydrogels to study modulus effects. hBMSCs from a 29 year old female donor were obtained from Tulane University Center for Gene Therapy. Passage 4 cells were suspended (10^6 cells/mL) in pre-polymer solutions containing different mass fractions (2 %, 3 %, 5 % and 10 %) of PEGTM and 0.05 mass % of Irgacure 2959 (Ciba Chemicals) in 0.1 mol/L phosphate-buffered saline (PBS). Gels were prepared by curing 50 µL of solution in Teflon molds (5 mm diameter and 3 mm height) covered by a glass slide for 15 min at 2 mW/cm^2 using a 365 nm lamp. The gels were transferred to growth media (α-modification of minimum essential medium supplemented with 16.5 volume % of fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin). Gel stiffness was characterized by measuring the compressive modulus determined from the linear fit of the stress-strain curve (5 % to 10 % strain). Cell viability was determined using the live/dead stain (Molecular Probes). Mineralization was determined by staining with 1 mass % Alizarin Red S (Sigma) solution.

Peptide-functionalized PEGDM hydrogels to study topology effects. To provide cell adhesion sites, RGD peptides were crosslinked into a hydrogel network. RGD peptide (GCGGGRGDS) was synthesized with an Apex 396 peptide synthesizer (Aapptec, Louisville, KY) using standard solid phase Fmoc chemistry. Peptide (10 mmol/L in 0.1 mmol/L phosphate buffer, pH 8.0) was first reacted with PEG-dimethacrylate (PEGDM, relative molecular mass 4000, 2 h reaction time, 1.1 X molar excess) to provide a spacer in the network. Next, PEGDM-GCGGGRGDS solution was added at 1 mmol/L theoretical concentration to 10 mass % PEGTM in PBS containing 0.05 mass % of Irgacure 2959. Gels were prepared by curing the pre-polymer solutions in Teflon molds for 15 min at 2 mW/cm^2 (365 nm light). For 2D cell-culture format, 4 x 10^4 cells/cm^2 hBMSCs were seeded on gel surfaces. In the case of 3D scaffolds, 10^6 cells/mL hBMSCs were suspended in pre-polymer solution prior to photo-polymerization. All cells were cultured in growth media and stained for minerals, as described above.

Results and Discussion

Effect of hydrogel stiffness in 3D scaffolds. A series of 3D hydrogel scaffolds were prepared by varying the mass fraction of PEGTM to span a wide range of compressive moduli: 0.2 kPa , 0.5 kPa, 11 kPa and 60 kPa (**Figure 1**) to match the moduli of bone marrow, brain, muscle and soft collagenous bone, respectively. Viability of encapsulated hBMSC increased with increasing gel stiffness (not shown). Mineral deposits were observed in all gels at 21 d, although the extent of mineralization increased with increase of gel modulus (**Figure 1**). Osteogenic differentiation was further confirmed by staining for alkaline phosphatase and osteocalcin expression of encapsulated cells (not shown). These results are in sharp contrast to previous reports in 2D culture (cells on gels) where changes in modulus of the underlying gel led to changes in cell lineage specification[2,3].

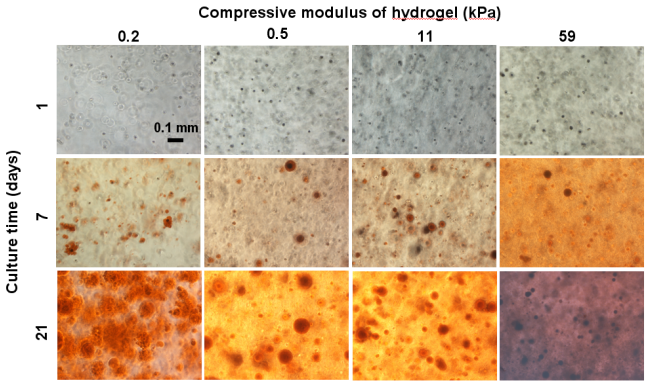

Figure 1: Phase contrast color micrographs hBMSCs encapsulated in gels with different moduli and cultured for 1 d, 7 d and 21 d. Mineral deposits indicative of osteogenic differentiation are stained with Alizarin Red S (orange/ red spots).

Effect of hydrogel topology in 2D and 3D scaffolds. hBMSCs within the hydrogels (3D) attained a spherical morphology immediately after photopolymerization that was maintained throughout 21 d. Cells on the gels (2D) initially attained a spread morphology (at 1 d) that became less spread by 3 d to attain a mix of rounded and partially-spread cells (**Figure 2**). When the gels were stained with Alizarin Red S for mineral deposits after 21 d (**Figure 3**), only a trace amount of staining was observed for cells on the 2D gel surface whereas heavy staining was observed in the 3D hydrogel scaffolds. These mineralization results indicate that 3D topology of the scaffold enhanced osteogenic differentiation of hBMSCs relative to the 2D culture format.

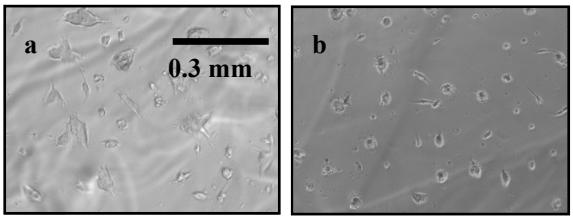

Figure 2: Phase contrast micrographs of hBMSCs on (2D) RGD peptide-functionalized PEGDM hydrogels at (**a**)1 d and (**b**) 4 d.

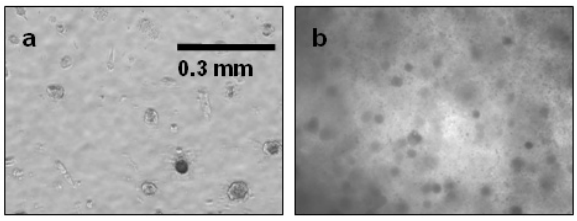

Figure 3: Phase contrast micrographs of (**a**) hBMSCs on (2D) and (**b**) within (3D) RGD peptide-functionalized PEGDM hydrogels stained with Alizarin Red S after 21 d culture. Dark patches indicate mineral deposits.

Conclusions

We have prepared PEG hydrogel scaffolds to study the effect of modulus and topology on encapsulated hBMSCs. Osteogenic differentiation of hBMSC within 3D scaffolds was observed for a wide range of compressive modulus (≈ 0.2 kPa to ≈ 60 kPa) although it was maximum at ≈ 60 kPa and was diminished at lower moduli. These data suggest that osteogenic differentiation of hBMSC is sensitive to 3D scaffold stiffness. Mineralization of hBMSCs cultured on and within RGD-functionalized PEG hydrogel scaffolds was also measured to study cell differentiation in 2D and 3D culture formats. Preliminary work indicates that the scaffolds induced osteogenic differentiation of hBMSC in the absence of exogenous biochemical cues in both 2D and 3D culture. However, increased staining for mineral deposits was observed in 3D relative to 2D indicating that osteogenic differentiation of hBMSCs is enhanced in 3D compared to 2D culture. Taken together, these results elucidate the role of modulus and topology on material-directed osteogenic differentiation in 3D scaffolds.

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