Material-Directed Human Bone Marrow Stromal Cell Differentiation in 2D and 3D Hydrogel Cultures

Kaushik Chatterjee,[1,2] Nicole M. Moore,[1] Sheng Lin-Gibson,[1] Marian F. Young,[2] 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

Statement of Purpose: Cells cultured within three-dimensional (3D) scaffolds can behave more physiologically than those cultured in two-dimensional (2D) formats.[1] However, few studies have been reported that compare and contrast the long-term cell response in 2D and 3D culture for a given biomaterial. In this study, we present preliminary work on the development of a poly(ethylene glcyol) (PEG) hydrogel-based scaffold system to enable the study of cell response to the biomaterial in the absence of exogenous cues in 2D and 3D culture formats. Comparison of primary human bone marrow stromal cell (hBMSC) differentiation for cells cultured on (2D) and within (3D) PEG scaffolds is presented.

Methods: 4-arm PEG (total relative molecular mass 20000 g/mole, each arm of 5000 g/mole, Jemken Technology) was reacted with 40× molar excess of methacrylic anhydride in a consumer microwave (GE, 110 W) for 10 min to prepare PEG-tetramethacrylate (PEGTM) (Fig. 1a)[2]. RGD peptides were crosslinked into scaffolds to provide cell adhesion sites. RGD peptide (GCGGGRGDS) was synthesized with an Apex 396 peptide synthesizer (Aapptec, Louisville, KY) using standard solid phase Fmoc chemistry. In order to provide a spacer for the peptide, peptide (10 mM in 0.1 M phosphate buffer, pH 8.0) was first reacted with PEG-dimethacrylate (PEGDM, relative molecular mass 4000 g/mole, 2 h reaction time, 1.1 X molar excess) (Fig. 1b). Next, PEGDM-GCGGGRGDS solution was added at 1 mM theoretical concentration to 10 mass % PEGTM in 0.1 M phosphate-buffered saline (PBS) containing 0.05 mass % of Irgacure 2959 (Ciba Chemicals). Gels were prepared by curing the pre-polymer solutions in Teflon molds for 15 min at 2 mW/cm^2 (365 nm light). hBMSC (29 year old female) were obtained from Tulane University Center for Gene Therapy. For 2D, hBMSC were seeded on gel surfaces (4 x 10^4 cells/cm^2). For 3D, hBMSC were suspended in pre-polymer solution prior to photopolymerization (10^6 cells/mL). All samples were cultured in growth media (α-modification of minimum essential medium, 16.5 volume % fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 ug/mL streptomycin). Samples were fixed with formalin at 21 d and stained for mineralization with 1 mass % Alizarin Red S (Sigma) solution.

Results: hBMSC on the gels (2D) attained a mix of rounded and elongated morphologies and remained adherent to the gel surface during 21 d of culture (Fig. 2a). The cells maintained a spherical morphology within the gels (3D) after 21 d. When stained with Alizarin Red S for mineral deposits after 21 d, only a trace amount of staining was seen for cells on the 2D gel surface whereas heavy staining was observed for cells in 3D gel scaffolds. Work is currently underway to quantify proliferation and differentiation/mineralization of hBMSC in 2D and 3D.

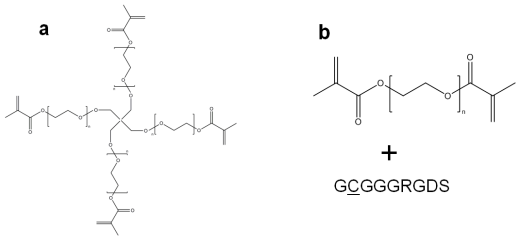

Fig. 1: Chemical structures of scaffold components. (a) Chemical structure of PEGTM. (b) Schematic for peptide conjugation where the Cys residue (underlined) is reacted with the acrylate group of a PEGDM spacer.

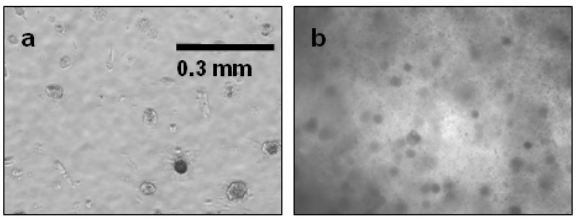

Fig. 2: Phase contrast micrographs of hBMSC on (2D) (a) and within (3D) (b) peptide-functionalized PEGTM hydrogels stained with Alizarin Red S after 21 d culture. Dark patches indicate mineral deposits. Also, note the cloudy background in (b) indicative of heavy mineralization relative to the clear background in (a).

Conclusions: We have prepared a RGD-functionalized PEG hydrogel scaffold to enable measurement of cell response in 2D and 3D culture format. Preliminary work indicates that the scaffold induces osteogenic differentiation of hBMSC in the absence of exogenous biochemical cues in both 2D and 3D culture. Heavy mineralization was observed for cells within the gels (3D) relative to low mineralization for cells on the gels (2D). These results indicate that hBMSC osteogenic differentiation is enhanced in 3D culture compared to 2D.

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References:

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