Effect of Scaffold Stiffness on Human Bone Marrow Stromal Cell Differentiation in 3D Culture

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Statement of Purpose: Cells are known to sense and respond to the mechanical stiffness of their surroundings *in vivo* and those of tissue scaffolds in culture. Scaffold stiffness has been shown to influence a number of different biological responses such as cell morphology, migration, proliferation, differentiation, etc. In recent works, it was reported that primary human bone marrow stromal cells (hBMSC) respond to changes in stiffness of the underlying matrix.[1,2] However, cellular response in three-dimensional (3D) scaffolds is often observed to be different from that in two-dimensional (2D) culture.[3] In this study, we examined the effect of changes in stiffness of poly(ethylene glycol) (PEG) hydrogel scaffolds on fates of encapsulated hBMSC (3D culture).

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 poly(ethylene glycol) tetramethacrylate (PEGTM).[4] hBMSC from a 29 year old female donor were obtained from Tulane University Center for Gene Therapy. Passage 4 cells were suspended at 10^6 cells/ml of pre-polymer solution containing different mass fractions (2 %, 3 %, 4 % and 10 %) of PEGTM and 0.05 mass % of Irgacure 2959 (Ciba Chemicals) in 0.1 M phosphate-buffered saline (PBS). Gels were prepared by curing 50 μL solution in Teflon molds (5 mm diameter and 3 mm height) covered by a glass slide for 15 min at 2 mW/cm^2. 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 ug/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.

Results: Compressive moduli for the gels prepared by varying the mass fraction of PEGTM were found to span a wide range including 0.2 kPa, 0.6 kPa, 5 kPa and 59 kPa (Fig. 1) to match the moduli of bone marrow, brain , muscle and soft collagenous bone, respectively. Viability of encapsulated hBMSC increased with increasing gel stiffness and reached ≈ 50 % for gels of 10 % PEGTM (not shown). Mineral deposits were observed in all gels at 21 d, though only trace amounts of mineral deposits were observed for 2 % gels (Fig. 1). Mineralization was highest in 10 % and decreased at lower gels (Fig. 2). Work is currently underway to quantify mineralization and to confirm osteogenic differentiation by measuring other osteogenic markers.

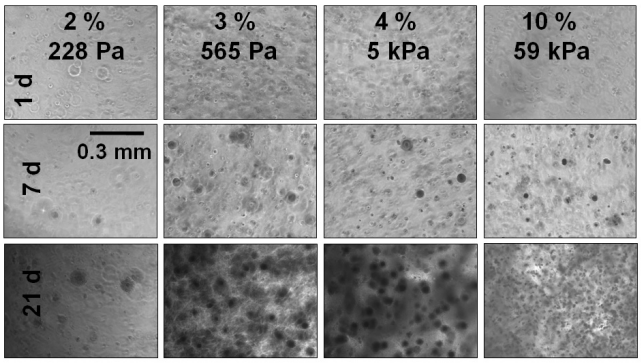

Fig. 1: Phase contrast micrographs of hBMSC encapsulated in gels with different moduli and cultured 1 d, 7 d and 21 d. Mineral deposits indicative of osteogenic differentiation are stained with Alizarin Red S (dark spots).

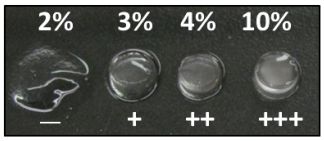

Fig. 2: Photograph of gels encapsulating hBMSC after 21 d in culture indicating visible (white) mineral deposits. Symbols indicate qualitative degree of mineralization ("+") or absence of mineralization ("-").

Conclusions: We examined the effect of scaffold stiffness on hBMSC differentiation within 3D PEG scaffolds over a wide range of compressive modulus (0.2 kPa to 60 kPa). In contrast to previous work in 2D culture, we observed hBMSC osteogenic differentiation at all moduli although it was maximum at 60 kPa (10 %) and was diminished at lower moduli (2 %, 3 %, 4 %). These results suggest that osteogenic differentiation of hBMSC is sensitive to 3D scaffold stiffness.

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