Controllable Effects of Mechanical Moduli on Osteoblast Differentiation of Mesenchymal Stem Cells on Polyurethane Substrates

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Statement of Purpose: In order to design cell carriers for the application of cell therapy in tissue repair, there is a compelling need to understand the mechanisms by which regenerative stem cells sense and respond to the physical properties, such as elastic modulus and pore size, of 3D scaffolds. Hydrogels have been extensively investigated as carriers for stem cells, and matrix rigidity has been shown to regulate stem cell differentiation for elastic moduli up to 1 MPa[i]. However, the effects of matrix rigidity on osteogenic differentiation in 3D scaffolds with moduli exceeding 1 MPa, which is representative of the mineralized extracellular matrix in bone, has been investigated in only a limited number of studies. Polyurethane scaffolds, which are porous, biodegradable, and biocompatible, have been reported to support the migration of cells and ingrowth of new tissue *in vitro* and as well as in bone models, with non-toxic degradation product [ii]. Moreover, the mechanical properties can be modified by changing the structures of hard and soft segments, which can be easily achieved by controlling the chain length of polyol and isocyanate in the reaction [iii].

Method: In this study, we have synthesized both 2D films and 3D scaffolds from polyester triol, COSCAT® 83 catalyst, and hexamethylene diisocyanate trimer (HDIt) to ensure the same surface chemistry. To precisely control the porosity, morphology, and pore size of 3D scaffolds, we used 3D molds from 3D-Biotek to fabricate polyurethane scaffolds with completely interconnected pores. Different chain lengths of polyester triol (e.g., from 300 Da to 3000 Da) were used to target the elastic moduli of the synthesized polyurethanes to values representative of trabecular bone. Longer chain (higher molecular weight) polyester triols yielded scaffolds with lower moduli (noted as "compliant"), while lower molecular weight polyesters yielded "rigid" scaffolds. Stem cell osteogenic differentiation was then studied on the polyurethane materials *in vitro*. Bone-marrow derived mesenchymal stem cells from mice were utilized in this study, and alkaline phosphatase (ALP) activity from cell lysate was selected as the early marker of osteoblast differentiation while secreted osteocalcin was selected as the late marker of osteogenesis. Measurements of total protein from cell lysates were used to quantify cell proliferation in this study. Real-time PCR of bone differentiation markers including ALP, osteocalcin and Runx2 were also measured to further compare the differentiation of stem cells on polyurethane materials.

Results: The elastic moduli at the cellular interface of the material of all scaffolds were analyzed to be ranged from 20 to 3800 MPa. MSCs plated on PUR materials showed cellular response to substrate moduli (Fig. A). Total protein from harvested cell lysates indicated that MSCs were able to attach to and proliferate on the polyurethane materials for up to 2 weeks. Time-course ALP measurements were conducted on 2D PUR films *in vitro* showed that ALP activity of stem cells cultured in osteoinductive medium peaked between D7 and D10 on the rigid substrate, while ALP peaked at later time points (D15) on the compliant substrate (Fig. B). A similar result was also obtained for 3D structured PUR scaffolds. Furthermore, SEM images of osteogenic differentiated cells inside PUR scaffolds showed more mineral deposition by cells cultured on the rigid substrate, which is consistent with the ALP activity measurement (Fig. C).

Conclusions: PUR scaffolds with tunable mechanical properties (20 to 3800 MPa) were investigated as a potential polymer carriers for cell therapy in regeneration of bone defects. Bone-marrow derived mesenchymal stem cells responded to the mechanical properties of substrates at elastic moduli >20 MPa. Future work will focus on the study of the effects of other physical properties of this polyurethane carrier (i.e. pore size and surface chemistry) on stem cell behavior and cell fate, as well as identifying the underlying mechanisms of the cell-substrate interactions and the application of this tunable polymer carrier in cell therapy.

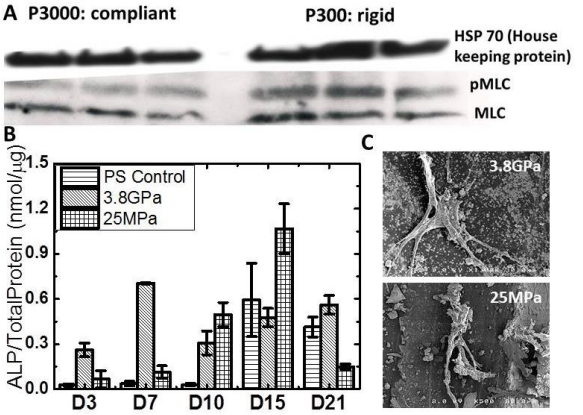

Fig. A. Western blotting of pMLC from cells plated on polyurethane films of different moduli. B. Time-course ALP activity measurements of murine MSCs on PUR films. C. Osteogenic differentiation of MSCs inside the melt cast scaffolds

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