The role of substrate stiffness on stem cell differentiation into osteogenic cells

Xiaowei Li[1, 2], Ashley McClary [3], Ning Zhang [1,2], Anna Scott [4], Glenn D. Prestwich [5], Xuejun Wen [1,2]
[1]Department of Bioengineering, Clemson University, Clemson, SC, United States
[2]Department of Orthopedic Surgery, Cell Biology, Medical University of South Carolina, Charleston, SC, United States
[3] South Carolina Governor School of Mathematics and Science, Orangeburg, SC, United States
[4]Glycosan BioSystems, Inc., Salt Lake City, UT, United States
[5] Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, United States

Statement of Purpose: To effectively repair, regenerate or engineer tissues, bioactive scaffolds may be used to support adhesion, migration, proliferation and differentiation of cells appropriate to the particular tissues. Recently, it has been shown that substrate mechanics can modulate tissue cell phenotype in a way similar to biochemical signals (1). Adult Stem cells derived from human bone marrow are a good source of stem cells due to the highly proliferate and undifferentiated characteristics and also confirmed differentiation potential to cells in bone, cartilage, skin, and fat tissue (2). In this study, we tested the role of substrate stiffness or elastic modulus on the stem cell differentiation into osteogenic cells. Since different types of cells live in the extracellular environments with different stiffness or modulus, we hypothesize that stem cell may behave differently, in terms of proliferation and differentiation, on the substrates with different stiffness or modulus if the chemistry of the substrates is kept the same. A series of extracellular matrix based highly biocompatible hydrogels are used to test this hypothesis. The model hydrogel is based on thiol functionalized carboxymethyl hyaluronic acid (CMHA-DTPH) and thiol functionalized carboxymethy gelatin (Gtn-DTPH), which may be crosslinked by poly(ethylene glycol) diacrylate(PEGDA)(3, 4). By controlling crosslink density, and therefore controlled stiffness, can be obtained. Human adult bone marrow derived mesenchymal stem cells (MSCs) are seeded on the surface of the hydrogels of different stiffness and cultured under osteogenic environment.

Methods: CMHA-DTPH solution and Gtn-DTPH solution (Glycosan BioSystems Inc. Salt Lake City, UT, USA) were prepared in sterile deionized distilled water under aseptic conditions. A 4.5% (w/v) PEGDA (MW 750, Sigma) stock solution was prepared by dissolving PEGDA powder in 1x PBS. Two volumes of CMHA-DTPH solution and two volumes Gtn-DTPH solution were then mixed with one volume of PEGDA solution of varying concentrations (4.5%, 2.25%, 1.5%, 0.75% and 0%) to obtain hydrogels of different stiffness. An AR1000 rheometer (TA Instruments Inc.) with standard steel parallel-plate geometry of 40 mm diameter was used for the rheological characterization of all hydrogel samples. Hydrogel solution with different crosslink densities was added to cell culture wells. 30 min later, 11mM iodoacetamide (MW 185, Sigma) solution in 1x PBS was added to the hydrogel surface to block residual free thiols and thereby prevent any additional crosslinking. Hydrogels was washed with 1x PBS 5 times. Then 4,000 hMSCs was added to each well. The cells were cultured under osteogenic media (PT-3002, Lonza, USA) for 3 and 7 days in a 37 ^{o}C, 5% CO_2, 95% humidity incubator. RT-PCR analysis, flow cytometry, and immunocytochemistry were used to characterize the stem cells and their osteogenic differentiation.

Results: Oscillatory time sweeps were performed to record the temporal evolution of shear storage moduli (G') of hydrogels. Figure 1 shows the time sweep profiles of G' for the 4.5%, 2.25%, 1.5%, 0.75% and 0% hydrogel networks within the small time frame. The development of G', appears to be solely governed by the cross-linking density (or PEGDA concentration). Self -S-S- cross-links contribute insignificantly to the network structure properties, confirming a strong correlation between the measured G' and the number of effective intermolecular cross-links formed in a hydrogel network. hMSCs cultured under osteogenic environment for 7 days results in a decrease in ration of the undifferentiated cells (STRO-1, CD44, CD29, or CD105 expressing cells) in flow cytometry studies, and an increase in ALP activity based on immunocytochemistry experiments (Fig. 2), reflecting their degree of progression into the osteogenic lineage. The extent of increase in ALP activity is much more in hMSCs grown on hydrogels of higher stiffness (Fig. 2B and Fig. 2C). Gene expression tested with RT-PCR (Fig. 3) further confirm that cells on hydrogels of higher stiffness express more mRNA of osteogenic lineage gene ALP. All of data demonstrate that MSCs osteogenic differentiation is inclined to occur on substrate of relatively higher stiffness.

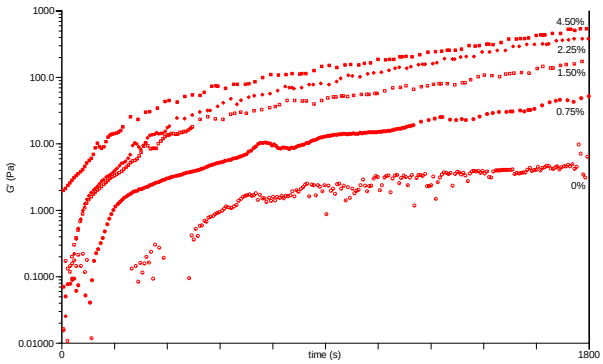


Figure1: Evolution of G' as a function of curing time.

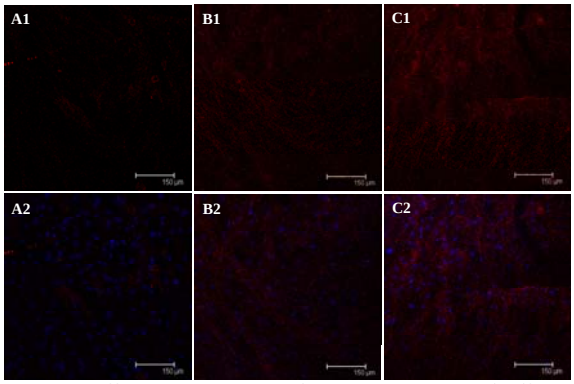
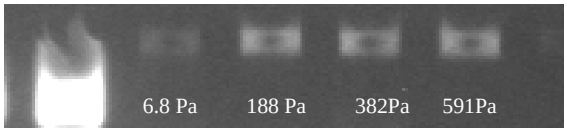


Figure 2: Differentiation potential of MSCs on hydrogels of different stiffness in osteogenic media (A:6.8 Pa, B. 382 Pa, and C 591 Pa).



Beta Actin (House-keeping gene)

Osteogenic gene (Alkaline Phosphatase ALP)

Figure 3: RT-PCR analysis of lineage specific marker (ALP) expressed in MSCs on hydrogels of different stiffness.

Conclusions: CMHA-DTPH, Gtn-DTPH and PEGDA are a valid system for making substrate with tunable mechanical properties. MSCs osteogenic differentiation is inclined to occur on substrate of relatively higher stiffness.

Acknoledgement: This work is supported by NSF (EPSCoR EPS-0447660) and NIH/NCRR P20 RR-016461.

References:

1. Discher D. E., etc. (2005) Science 310, 1139-43.
2. Engler, A. J., etc. (2006) Cell 126, 677-689.
3. Ghosh, K., etc. (2007) Biomaterials 28, 671-9.
4. Ghosh, K., etc. (2005) Biomacromolecules 6, 2857-2865.