Stiffness Modulates Chondrogenic and Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Statement of Purpose: Current regenerative medicine strategies aim to incorporate stem cells and biomaterials to restore structure and function to injured or diseased tissues. Stem cell differentiation can be affected by chemical, mechanical, and topographical cues provided by the biomaterial. It has been shown that physical cues such as microroughness and stiffness can influence stem cell fate. However, it has been difficult to determine the effect of material stiffness on stem cell differentiation since decoupling substrate stiffness and chemistry presents a challenge. Here, we designed a photopolymerizable ternary (meth)acrylate network (MA-co-MMA-co-PEGDMA) with a tunable surface modulus over several orders of magnitude. This is achieved by varying the ratio of methyl acrylate (MA) and methyl methacrylate (MMA), two structurally similar linear monomers, thus changing substrate stiffness while maintaining chemistry as close as possible. The aim of this study was to determine the effect of copolymer stiffness on osteogenic and chondrogenic differentiation of human mesenchymal stem cells (HMSCs), in the absence of exogenous media supplements that enhance differentiation.

Methods: Copolymer solutions consisting of methyl acrylate (MA), methyl methacrylate (MMA), and poly(ethylene glycol) dimethacrylate (PEGDMA MW~750) were photopolymerized with 365nm UV light using 2,2-dimethoxy-2-phenylacetophenone as a photoinitiator. The weight ratio of MA to MMA was varied while the crosslinking concentration of PEGDMA was held constant at 10 wt% to produce 4 copolymer networks (by wt. % of MA): 18MA, 29MA, 40MA, and 72MA. Materials were photopolymerized between 1mm thick glass sheets under 365nm UV light for 30 minutes. Discs were laser cut from the polymer sheets to fit into a well of a 24-well culture plate and UV sterilized for 90 minutes. Elastic modulus was determined by performing tensile strain-to-failure testing at 37°C in PBS and calculating the slope of the linear portion of the stress-strain curve (n=4). Contact angle measurements were performed to determine the wettability of each material (n=3).

HMSCs were plated at a density of 10,000 cells/cm^2 on copolymer surfaces and cultured in basal media. Cells were harvested and total cell number, alkaline phosphatase specific activity in the cell lysate, and secreted osteocalcin (OCN), osteoprotegerin (OPG), active TGF-β1, and latent TGF-β1 measured to determine osteogenic differentiation. To determine chondrogenic differentiation, sulfated proteoglycans were stained using Alcian blue. In a second experiment, RNA extraction performed and expression of integrins (α1, α2, α5, αv, α6, β1, β3), osteogenic markers (OCN, COL1), chondrogenic markers (SOX9, COMP, ACAN), and transcription factors important for commitment to the osteochondroprogenitor lineage (RUNX2, MSX2) measured by real-time qPCR. Data are mean±SEM for n=6 cultures/ variable. Statistical significance was determined by ANOVA and Bonferroni's Student's t-test.

Results: The average elastic moduli of 18MA, 29MA, 40MA, and 72MA were 310±6.5MPa, 146±45MPa, 31±7.7MPa, and 0.71±0.11MPa, respectively. Average contact angles for 18MA, 29MA, 40MA, and 72MA were 75.9±2.6°, 74.5±3.6°, 89.4±2.0°, and 86.9±1.7°, respectively. Cell number increased with decreasing stiffness; the highest number of cells was on the 72MA surfaces. Alkaline phosphatase specific activity increased at 40MA and 72MA with the highest peak at 40MA. Interestingly, OCN (Fig. 1) and OPG increased with decreased stiffness, peaking at 40MA, but decreasing at the lowest on level in 72MA. Proteoglycan staining was lowest on 18MA and 29MA surfaces, but increased as stiffness decreased, with highest staining on 72MA substrates (Fig. 1). Expression of ITGA1, ITGA5, and ITGB1 increased as stiffness decreased. ITGA2 increased with decreasing stiffness, reaching a peak at 40MA and decreasing at 72MA. ITGA5 was similar on 18MA and 29MA, but increased 2-fold on 40MA and an additional 50% on 72MA in comparison to the stiffer surfaces. ITGB3 was highest on 18MA, but decreased as stiffness decreased. Expression of ITGA6 was lowest on 18MA, but 300% higher on 29MA and 42MA in comparison. Expression on 72MA was 100% higher than cells on softer substrates. RUNX2 increased as stiffness decreased. Chondrogenic markers SOX9, ACAN, and COMP had highest expression on the 72MA substrates. Osteogenic genes MSX2, OCN, and COL1 had highest expression on 40MA.

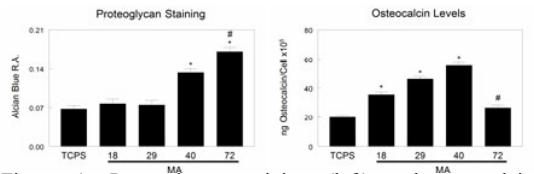

Figure 1. Proteoglycan staining (left) and osteocalcin production (right) of HMSCs cultured on polymer substrates. *p<0.05, vs. TCPS, #p<0.05, vs. 40MA.

Conclusions: Gene expression and protein production of HMSCs can be modulated by changing substrate stiffness while maintaining similar chemistry and surface energy. It is important to note that HMSC differentiation was achieved in absence of exogenous molecules of proteins that enhance stem cell differentiation. Osteogenic markers were highest on 40MA substrates, while chondrogenic differentiation was greatest on the softest surface (72MA). The results demonstrate that HMSC differentiation and integrin expression are sensitive to substrate stiffness. The MA-MMA-PEGDMA system is an advantageous material platform due to the ease with which it can be formed into complex geometries, as well as exhibit unique mechanical properties for possible implementation as an orthopaedic implant material.

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