Copolymer Stiffness Regulates the Differentiation of MG63 Osteoblast-like Cells

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Statement of Purpose: The cellular microenvironment, including biochemical, topographical, and mechanical cues, can greatly affect a cell's ability to differentiate and perform its physiological functions. The objective of this study was to examine the role of substrate stiffness in regulating cell differentiation. It has proven challenging to independently vary the stiffness of the substrate without changing chemistry or functionalizing the surface with bioactive molecules (1). Thus, we took advantage of a ternary (meth)acrylate network (MA-co-MMA-co-PEGDMA) whose surface modulus can be tuned by varying the ratio of two structurally-similar linear monomers (MA & MMA). This copolymer system is an advantageous material platform because it has tailorable material properties and demonstrates enhanced mechanical properties for implementation as an orthopedic implant material (2). For these experiments, we used a well characterized cell culture model for studying osteoblast differentiation in response to substrate microstructure and chemistry. MG63 pre-osteoblast-like cells were grown on substrates in which copolymer stiffness was varied and we assessed the effects on cell response.

Methods: Copolymer solutions consisting of methyl acrylate (MA), methyl methacrylate (MMA), and poly(ethylene glycol) dimethacrylate (PEGDMA MW~750) were photopolymerized under 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. Elastic modulus was determined by performing tensile strain-to-failure testing at 37°C in PBS (n=4). Contact angle measurements were performed to determine the wettability of each material (n=3).

MG63 cells were plated at a density of 20,000 cells/cm^2 on tissue culture polystyrene (TCPS) and each copolymer surface. At confluence on TCPS, cells were harvested and the total cell number was determined using a Coulter counter. Osteocalcin and osteoprotegerin (OPG) production were measured in the conditioned media using commercially available RIA and ELISA kits, respectively. Data were calculated as mean±SEM (n=6). Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test. Results shown are one of two different experiments, both with comparable results.

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. Cells cultured on the copolymer surfaces had significantly reduced cell numbers when compared to cells grown on TCPS (18MA > 29MA > 72MA>40MA) (Figure 1A). Osteocalcin levels increased as the stiffness of the copolymer network increased with 18MA (310MPa) exhibiting 50% higher levels compared with TCPS and at least 20% more osteocalcin than the other copolymer compositions (Figure 1B). While OPG production was at least 50% higher on the copolymer surfaces compared with TCPS, there was no correlation between OPG production and stiffness for the materials used in this study (Figure 1C).

Conclusions: Osteoblast number and differentiation are sensitive to substrate stiffness. Stiffer surfaces (18MA and 29MA) exhibited decreased cell numbers compared with TCPS, but supported greater differentiation, as determined by osteocalcin production. However, this did not correlate to increased production of other osteogenic factors such as OPG. These results indicate that osteoblast differentiation can be controlled by tailoring copolymer stiffness. Further studies will examine possible intracellular mechanisms, including integrin signaling and cytoskeletal organization that may govern this response.

References: (1) Engler A et al., Cell Mech 83:521-545, 2007; (2) Smith K et al., Polymer 50(21): 5112-5123.
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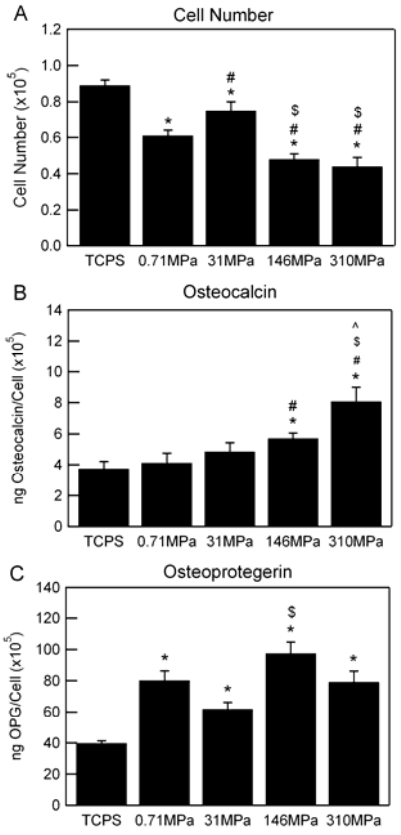

Fig 1. Effect of copolymer stiffness on (A) cell number, (B) osteocalcin and (C) osteoprotegerin production. Values represent mean±SEM. *p<0.05 vs. TCPS; #p<0.05 vs. 0.11MPa; $p<0.05 vs. 4.7MPa; ^p<0.05 vs. 153MPa.