Osteoblast Proliferation and Differentiation are Sensitive to Substrate Stiffness

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Statement of Purpose: Osteoblasts are anchorage dependent cells that produce osteoid and calcify their extracellular matrix on pre-existing surfaces, including their normal bone surface and on biomaterials used clinically. It is not known how the mechanical stiffness of the substrate affects this process, although it is known that matrix elasticity influences differentiation of stem cells (1) and the behavior of cells in their native tissue (2). To examine this, we took advantage of acrylate-based networks, because of the ability to tune their stiffness without dramatic changes in chemistry. Poly(ethylene glycol) (PEG) can be used to assess effects of stiffness in the kPa range (3), but more clinically relevant stiffnesses ranging from MPa to GPa have not been examined. We designed polymeric substrates in this range to assess the effects of surface stiffness on the osteoblast phenotype.

Methods: Sheets of poly(ethylene glycol) dimethacrylate (PEGDMA MW=750) mixed with diethylene glycol dimethacrylate (DEGDMA) were photopolymerized under 365nm UV light using 2,2 dimethoxy 2-phenylacetophenone as a photoinitiator. Stiffness in the rubbery, water absorbed state was adjusted by varying the weight ratio of PEGDMA to DEGDMA, producing three copolymer systems: 90%PEGDMA-co-10%DEGDMA (90% PEG), 50%PEGDMA-co-50%DEGDMA (50% PEG), and 10%PEGDMA-co-90%DEGDMA (10% PEG). 1mm thick discs were laser cut and sanded to a diameter that allowed each disk to swell in cell culture media to a final diameter of 15mm. All disks were baked and subsequently boiled in water to remove any excess monomer. Surface modulus was determined using an atomic force microscope (AFM) and nanonindentation. In addition, contact angle measurements were performed to determine the wettability of each material.

MG63 human osteoblast-like cells derived from a male osteosarcoma cell line were plated at a density of 20,000 cells/cm^2 on both tissue culture polystyrene (TCPS: DEG 0%/PEG 0%) and polymer surfaces. At confluence, total cell number and alkaline phosphatase activity were measured. Data were calculated as means$\pm$SEM for N=6 independent cultures for each variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

Results: The moduli of substrates containing 90% PEG, 50% PEG, and 10% PEG were determined to be in the range of 40MPa, 900 MPa, and 2GPa, respectively. Average contact angles (N=5) for 90% PEG, 50%PEG, and 10% PEG were 73.17$\pm$8.18°, 83.52$\pm$11.46°, and 65.51$\pm$4.87°, respectively. Cells cultured on the PEG surfaces had decreased cell numbers when compared to cells on TCPS (% decrease: 10%PEG > 90%PEG > 50%PEG) (Figure 1A). Cellular alkaline phosphatase specific activity increased on the 90% PEG surfaces in comparison to TCPS, but decreased on 10% PEG (Fig 1B). Alkaline phosphatase activity on 50% PEG was comparable to TCPS.

Conclusions: Osteoblast number and differentiation are sensitive to substrate stiffness. Softer surfaces (10% PEG) supported greater differentiation than TCPS and the stiffest surface supported the least differentiation, as determined by alkaline phosphatase activity. The moderately stiff hydrogel, 50% PEG, had the greatest number of cells on the polymer surface, although this was still reduced in comparison to TCPS. Statistically significant differences in contact angle were not found. These results demonstrate that stiffness is an important variable but they do not indicate which stiffness is optimal for osteoblast differentiation. Alkaline phosphatase varies with time and it is not known whether the cells were assayed during the early phase when activity is increasing or at the later stage when activity is decreasing as osteocalcin production and terminal differentiation are underway.

References: (1) Engler A et al., Cell Mech 83:521-545, 2007; (2) Discher D et al., Science 310:1139-1143, 2005; (3) Peyton S et al., Biomat 27 :4881-4893, 2006

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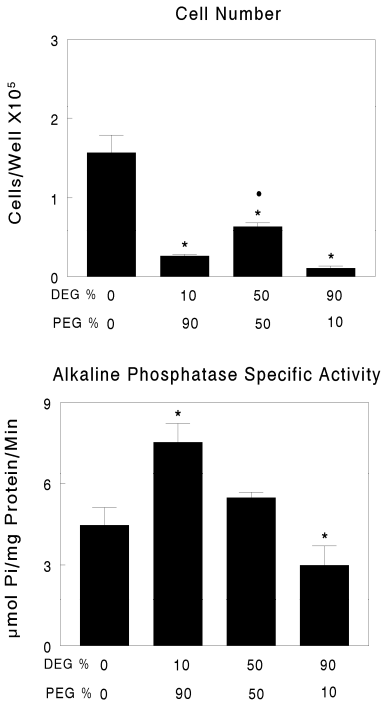

Figure 1: The effect of different concentrations of DEG and PEG on osteoblast cell number (top) and differentiation alkaline phosphatase (bottom) activity Data are means $\pm$ SEM, N=6 independent cultures, and are from one of two separate experiments. *P<0.05.