Published in final edited form as:

Biomaterials. 2010 August ; 31(24): 6131–6141. doi:10.1016/j.biomaterials.2010.04.033.

The Dependence of MG63 Osteoblast Responses to (Meth) Acrylate-based Networks on Chemical Structure and Stiffness

Kathryn E. Smith¹, Sharon L. Hyzy², MoonHae Sunwoo³, Ken Gall^{1,4}, Zvi Schwartz², and Barbara D. Boyan^{2,4,*}

¹George Woodruff School of Mechanical Engineering, Georgia Institute of Technology, 771 Ferst Drive, Atlanta, Georgia, 30332, USA

²Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, 315 Ferst Drive NW, Atlanta, Georgia, 30332, USA

³Musculoskeletal Transplant Foundation, 125 May Street, Edison, NJ, 08837, USA

⁴School of Materials Science and Engineering, Georgia Institute of Technology, 771 Ferst Drive, Atlanta, Georgia, 30332, USA

Abstract

The cell response to an implant is regulated by the implant's surface properties including topography and chemistry, but less is known about how the mechanical properties affect cell behavior. The objective of this study was to evaluate how the surface stiffness and chemistry of acrylate-based copolymer networks affect the *in vitro* response of human MG63 pre-osteoblast cells. Networks comprised of poly(ethylene glycol) dimethacrylate (PEGDMA; Mn~750) and diethylene glycol dimethacrylate (DEGDMA) were photopolymerized at different concentrations to produce three compositions with moduli ranging from 850 to 60MPa. To further decouple chemistry and stiffness, three networks comprised of 2-hydroxyethyl methacrylate (2HEMA) and PEGDMA or DEGDMA were also designed that exhibited a range of moduli similar to the PEGDMA-DEGDMA networks. MG63 cells were cultured on each surface and tissue culture polystyrene (TCPS), and the effect of copolymer composition on cell number, osteogenic markers (alkaline phosphatase specific activity and osteocalcin), and local growth factor production (OPG, TGF- β 1, and VEGF-A) was assessed. Cells exhibited a more differentiated phenotype on the PEGDMA-DEGDMA copolymers compared to the 2HEMA-PEGDMA copolymers. On the PEGDMA-DEGDMA system, cells exhibited a more differentiated phenotype on the stiffest surface indicated by elevated osteocalcin compared with TCPS. Conversely, cells on 2HEMA-PEGDMA copolymers became more differentiated on the less stiff 2HEMA surface. Growth factors were regulated in a differential manner. These results indicate that copolymer chemistry is the primary regulator of osteoblast differentiation, and the effect of stiffness is secondary to the surface chemistry.

© 2010 Elsevier Ltd. All rights reserved.

*Corresponding Author: Barbara D. Boyan, Ph.D., Department of Biomedical Engineering, Georgia Institute of Technology, 315 Ferst Drive NW, Atlanta, Georgia 30332-0363, Phone: 404-385-4108, FAX: 404-894-2291, barbara.boyan@bme.gatech.edu .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Surface Stiffness; Osteoblasts; Hydroxyethyl methacrylate; Polyethylene glycol dimethacrylate; *In vitro*; mineralized and demineralized bone

INTRODUCTION

Understanding the interaction between cells and an implant material is of great importance in ensuring the implant's effectiveness. Many studies have examined how various material surfaces affect the osteoblast response and peri-implant bone formation [1–11]. These studies indicate that implants are more likely to osseointegrate with surrounding bone when cells cultured *in vitro* on the material surface form a cuboidal morphology, exhibit elevated levels of differentiation markers including alkaline phosphatase specific activity and osteocalcin, and produce more osteogenic factors. Materials with these properties have improved bone to implant contact and increased pullout strength when tested *in vivo* [11–13]. Therefore studying the *in vitro* response of various cell types to the material surface can provide insight regarding the *in vivo* performance of the implant material.

In order to understand how materials affect bone formation, it is important to determine how specific surface characteristics including the surface wettability, topography, and stiffness mediate the osteoblast response. For example, wettability, as measured by the contact angle between water droplets and the surface, affects both cell attachment and proliferation [14] by changing the surface presentation of extracellular matrix proteins to which cells bind. In particular, it has been shown that a contact angle above 45 degrees is necessary for adequate cell adhesion and proliferation on polymer films [1]. On hydrophilic metal oxide surfaces, human osteoblast-like MG63 cells exhibit a differentiated phenotype and also secrete factors that create an osteogenic microenvironment [9]. Increasing surface roughness also promotes osteoblast attachment and differentiation in a manner dependent on the size scale of the topographical features [2,7,10–11,15]. For instance, MG63 cells become more differentiated on titanium with micron scale roughness in comparison to smooth or sub-micron scale rough surfaces [16]. Similar responses have also been observed on polycarbonate surfaces containing micropores, where cells begin to exhibit a more differentiated phenotype above a 3mm pore size [6].

More recently, much interest has been generated on whether cells, including osteoblasts, can mechano-sense their extracellular environment. Several studies have reported that cell attachment, proliferation, and differentiation are all modulated by the substrate rigidity to a degree dependent upon the substrate stiffness in relation to the stiffness of the native tissue [3,5,8,17–19]. In other words, cells will mimic their *in vivo* behavior more when they are cultured on substrates whose rigidity matches that of that cell type's native tissue matrix. Thus, considering the relatively stiff nature of bone, it is expected that osteoblasts will attach more, increase their proliferation rate, and exhibit a more mature phenotype on stiffer surfaces, greater than 100kPa [5,18]. Kong et al. have proposed that this modulated osteoblast response relates to the cells' ability to more efficiently generate force through their cytoskeleton in response to the larger resistance induced by the rigid substrate [17], and that these generated traction forces will alter the assembly of intracellular machinery that drives its ability to enter the cell cycle or differentiate [18]. These findings suggest that the mechanical microenvironment can have implications on developing therapies to promote bone formation and osseointegration.

Despite these advancements, it has proven challenging to independently vary the stiffness of the substrate without drastically changing chemistry or functionalizing the surface with bioactive molecules [3,5,8]. For most studies, polyacrylamide substrates crosslinked with

different concentrations of bis-acrylamide were produced to attain surfaces with various moduli [3,8], but it has been shown that osteoblasts can alter their morphology in response to changes in network structure, suggesting that network chemistry, independent of wettability, could be a potential regulator in the overall cell response [20]. To overcome the chemistry effect, polymer substrates are coated with extracellular matrix ligands, usually either collagen or arginine-glycine-aspartic acid (RGD) peptides [3,5], but this approach does not represent how actual polymer implant surfaces affect the osteoblast response.

The relationship between matrix elasticity and cell response has mostly been reported on substrates with moduli less than 1MPa; however most orthopedic polymer implants possess moduli in the megapascal to gigapascal range. In one instance, the effect of moduli ranging between 100–500MPa was indirectly studied using bone marrow stromal cells on a biodegradable polymer network, but no significant difference in cell number, alkaline phosphatase activity, or osteopontin expression was observed, potentially due to the complex trade-offs of chemistry, degradation and substrate mechanics [4]. Additionally, variations in stiffness have been shown to exist in bone depending upon the component of bone and degree of mineralization, representing different stages of remodeling. Thus, a copolymer system with a modulus that is tunable over several orders of magnitude with little change in chemistry would be useful to study the relationship between polymer stiffness and osteoblast response. Photopolymerizable acrylate-based networks serve as promising orthopaedic implant materials due to their tunable mechanical properties and ability to be formed *in situ* into complex geometries rendering them useful in minimally invasive procedures [21–22]. For example, these materials have already been demonstrated for use in fixation devices [23] and dental resins [24].

In this present study, the first objective was to measure the local surface modulus of cortical and trabecular human bone both in mineralized and demineralized states to determine the mechanical environment osteoblasts sense during different stages of remodeling. Based upon the determined moduli, several (meth)acrylate networks were designed with different moduli that corresponding to the measured moduli of different bone regions and states of mineralization. These selected copolymer networks were then used to evaluate the *in vitro* response of human MG63 osteoblast-like cells to changes in surface chemical structure and stiffness. Using a copolymer system comprised of two PEG-based crosslinkers, poly(ethylene glycol) dimethacrylate (PEGDMA) and diethylene glycol dimethacrylate (DEGDMA), the modulus of the network was adjusted by varying the ratio of the two crosslinkers while still presenting the same surface chemistry. The PEG-based networks created in this study were formulated from low molecular weight PEGDMA chains such that when polymerized with DEGDMA, they will form highly crosslinked networks resulting in decreased wettability [25]. To further decouple the effects of chemistry and stiffness, a second copolymer network comprised of 2-hydroxyethyl methacrylate (2HEMA) and PEGDMA with various molecular weights was also used to test different chemical compositions while maintaining a range of moduli similar to the PEGDMA-co-DEGDMA networks. The response of MG63 cells on each surface was evaluated by assessing morphology, cell number, and the production of certain osteogenic markers. These synthesized networks are potential materials for tendon or spinal disc repair where it is important that the material osseointegrate with surrounding bone to ensure implant stability.

METHODS

Materials

Diethylene glycol dimethacrylate (DEGDMA), poly(ethylene glycol) dimethacrylate (PEGDMA; Mn~550 or 750), and 2-hydroxyethyl methacrylate (2HEMA) monomers were

obtained from Sigma-Aldrich (St. Louis, MO) and used as received. 2,2-dimethoxy-2-phenylacetophenone (DMPA, Sigma Aldrich) was used as the photoinitiator.

Nanoindentation of Bone

Human trabecular and cortical (both periosteal and endosteal) bone were taken from donor femurs and tibias, courtesy of Musculoskeletal Transplant Foundation (Edison, NJ). The condyles were cut into 3mm thick trabecular sheets, then after all the surrounding cortical tissue was removed, cut to 2cm × 2cm trabecular wafers. The shafts were cleaned and cut to 2cm rings, and the rings with flat sides were used to make the 2cm × 2cm × 1cm cortical wafers. The wafers were cleaned with a combination of antibiotics, surfactant, and ethanol and then washed with deionized (DI) water. To demineralize the bone matrix, the wafers were soaked in 0.6N hydrochloric acid for 5 minutes. Both mineralized and demineralized wafers were rinsed in DI water, soaked in ethanol, dried, and stored at −70°C prior to shipping. Upon receipt, the wafers were stored at 5°C prior to use. The surface elastic moduli were determined by performing nanoindentation using a 130μm diameter spherical ruby tip on a NanoIndenter XP (MTS Systems Corporation, Eden Prairie, MN). The displacement of the tip into the surface was recorded as a function of tip load. Using Hertz' theory for the contact of a sphere into an elastic half space [26], model load vs. displacement curves were created from the following equation:

$$\delta = \left(\frac{3}{4E^*} \right)^{2/3} \frac{P^2}{R}$$

where P is the applied tip load, δ is the displacement into the surface, R is the tip radius, and E^* is the reduced modulus as determined by:

$$\frac{1}{E^*} = \frac{(1 - \nu^2)}{E} + \frac{(1 - \nu'^2)}{E'}$$

where ν and ν' are the Poisson's ratios and E and E' are the elastic moduli of the specimen surface and indenter tip, respectively. To determine the elastic modulus from the experimental load versus displacement curves, E values of the predicted curve were adjusted until the experimental data fit the predicted curve in the unloading region. Two wafers from each bone region were indented with the average modulus value calculated from at least 10 indents for each bone specimen.

Network Synthesis and Disc Preparation

Monomer solutions were formulated by combining various weight ratios of PEGDMA (Mn~750) and DEGDMA with 0.2 wt.% DMPA. The elastic modulus of the copolymer system was adjusted by varying the weight ratio of PEGDMA to DEGDMA. Three compositions (by weight %) were selected based on their determined moduli values: (1) 10%PEGDMA:90% DEGDMA (10PEG), (2) 50%PEGDMA:50%DEGDMA (50PEG), and (3) 90%PEGDMA: 10%DEGDMA (90PEG). Each solution was mixed manually in a glass vial, filtered through 5μm filter paper under vacuum, and injected using a glass pipette between two glass slides separated with two glass spacers (thickness = 1mm). The sheets were placed in a UV chamber (UVP, Model CL-1000L Ultraviolet Crosslinker, Upland, CA; $\lambda=365\text{nm}$; energy=2000×100μJ/cm²) for 10 minutes. Discs were laser-cut from the polymerized sheets to a diameter such that the disc of each composition would swell to fill the bottom of a well in a 24-well cell culture plate when incubated in cell culture media. All discs were post-cured in

an oven at 90°C for 90 minutes and boiled in distilled water for 30 minutes to remove excess monomer. Discs were then sterilized by autoclaving at 121°C for 15 minutes.

To prepare the 2HEMA-co-PEGDMA surfaces, sheets were photopolymerized using the same procedure as the PEGDMA-co-DEGDMA copolymers using glass slides coated with poly (dimethylsiloxane) (PDMS; Dow Corning Sylgard 184; Midland, MI) to enhance release. Three compositions with the appropriate moduli were selected for the *in vitro* cell experiments (by weight %): (1) 65%HEMA:35%DEGDMA (65HEMA), (2) 80%2HEMA:20%PEGDMA (Mn~550) (80HEMA), and (3) 98%2HEMA:2%PEGDMA (Mn~750) (98HEMA). After laser cutting and sanding the edges, the discs were post-cured for 90 minutes at 90 °C and then soaked in distilled water for 3 days to remove excess monomer. Because these materials were unable to maintain their structural integrity during autoclaving, discs for these compositions were sterilized by UV light ($\lambda=254\text{nm}$) for 90 minutes.

Surface Characterization

Surface wettability was determined by performing contact angle measurements using the sessile drop method on a Ramé-hart goniometer (Mountain Lakes, NJ). A 3 μL drop of deionized water was dropped onto the disc surface, and the contact angle between the side of the drop and polymer surface was measured using a camera and DROPimage CA software (Mountain Lakes, NJ). Three measurements of the left and right contact angle were taken on three separate discs of each composition.

Elemental analysis was performed by x-ray photoelectron spectroscopy (XPS) on a SSX-100 spectrometer (Surface Science Laboratories, Mountain View, CA) under high vacuum (3×10^{-8} Torr) using Al-K α radiation (1486.6eV) and a pass energy of 50eV. Charge buildup on the polymer surfaces was neutralized by an electron flood gun at 3mV. To further correct for charging effects, the binding energies were offset such that the C1 peak was assigned to the binding energy of 284.6eV. Survey scans were obtained in the 0–1000eV range using 1 eV/step and 10 scans/time. Peak assignments and their corresponding area were determined using ESCA software (Service Physics; Bend, OR). Three scans were performed on three separate discs for each composition.

Fourier transform infrared (FTIR) spectroscopy in attenuated total reflectance (ATR) mode was performed to assess the chemical bonding present on the surface and compare the differences in the amounts of key chemical groups among the different copolymer systems. Spectra were obtained on discs using a Bruker Optics Tensor Spectrometer (Billerica, MA) with a KBr crystal. Ten scans were obtained on each sample at a 1Hz frequency and peak wavenumbers were determined using OMNIC software (Thermo Electron Corporation, Madison, WI). Three spectra were obtained for three separate discs for each composition.

To evaluate the surface roughness, atomic force microscopy (AFM) was performed using a Pacific Nanotechnology Nano-R AFM (Irvine, CA) in close-contact mode. Each sample was scanned using a scan size of 25 $\times$ 25 μm at 0.5Hz frequency and a resolution of 512 scan lines. Average roughness (R_a) was calculated for each scan using the Pacific Nano Software (Irvine, CA). Three scans were performed on three separate discs for each copolymer system.

Surface elastic modulus was determined by performing nanoindentation using the same loading regime described above for bone. To simulate cell culture conditions, samples were soaked in phosphate buffered saline (PBS) prior to performing nanoindentation and placed on a sample mount immersed in PBS-soaked absorbent foam to maintain hydration during indentation [27]. Previous testing (data not shown) suggested that the elastic modulus of a polymer changed with time when immersed in water. Thus, the elastic modulus was determined for all compositions after immersion in PBS for 24h or 7 days. Moduli values were determined from

the load versus displacement curves using the method described above for the bone and the average elastic moduli was determined from at least 10 indents measured on at least three discs.

Cell Culture

Human MG63 osteoblast-like cells (ATCC, Manassas, VA) were cultured in Dulbecco's modification of Eagle's medium (DMEM, Cellgro, Manassas, VA), 10% fetal bovine serum (Hyclone, Waltham, MA), and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA) at 37°C, 5% CO₂, and 100% humidity. Polymer discs were soaked in media for 24h prior to plating in order to allow the discs to swell to fit the bottom of the culture well. To overcome the buoyancy of the polymer discs, CellCrown polymer inserts (Scaffdex, Finland) were used to anchor the disks. Cells were plated either on tissue culture polystyrene (TCPS) or polymer discs at a density of 20,000 cells/cm² and cultured until cells reached confluence on TCPS, about 5–7 days.

Analysis of Cell Response

At confluence, fresh media were added to the cultures and cells were incubated for 24h prior to harvesting. At harvest, the conditioned media were collected from each well. Cells were released from the surfaces using two sequential trypsinizations to ensure all cells were collected. Cell number was determined using a Beckman Coulter Z1 particle counter (Fullerton, CA). Cells were lysed and alkaline phosphatase specific activity was measured as the release of *p*-nitrophenol from *p*-nitrophenylphosphate at pH 10.2 as previously described [28] and normalized to total protein (Pierce BCA Protein Assay, Thermo Scientific, Waltham, MA). Osteocalcin was measured in the conditioned media using a commercially available radioimmunoassay kit (Human Osteocalcin RIA Kit, Biomedical Technologies, Inc., Stoughton, MA) as previously described [7]. The amount of osteoprotegerin (OPG), vascular endothelial growth factor (VEGF), and total transforming growth factor- β 1 (TGF- β 1) released by the cells was determined by performing an enzyme-linked immunosorbent assay (ELISA) of the conditioned media (DuoSet ELISA kits, R&D Systems, Inc., Minneapolis, MN) as previously described [29–30].

Cell Morphology

To determine if cell morphology was affected by copolymer composition, cells cultured on the copolymer surfaces were examined by scanning electron microscopy (SEM). Cells were plated as described and cultured for 24h or to confluency. At each time point, the media were removed and the samples rinsed three times with PBS to remove the excess media. Samples were fixed with Karnovsky's fixative (Electron Microscopy Sciences, Hatfield, PA) for 24h at room temperature. After fixation, the samples were freeze-dried for 48h. Samples were coated with a thin layer of gold and imaged using a Zeiss Ultra60 SEM with an SE2 detector (Carl Zeiss SMT Inc., Peabody, MA).

Statistical Analysis

Six experiments were performed using the PEGDMA-co-DEGDMA surfaces. For each individual experiment, there were six independent cultures per variable normalized by the average of the TCPS control for that experiment resulting in a treatment/control ratio for each variable in a given experiment. The data shown represent the mean $\pm$ SEM of the treatment/control ratios for the six separate experiments (cell number and alkaline phosphatase specific activity) or four separate experiments (osteocalcin, OPG, VEGF-A, TGF- β 1). Data were compared by the Wilcoxon rank sum test. Data presented from the 2HEMA-based surfaces are from one of two independent experiments, each with comparable results. Data are the mean $\pm$ SEM of six independent cultures and show absolute values rather than treatment/control ratios. Data were analyzed by performing an analysis of variance, and, when statistical differences

were found, a Student's t-test for multiple comparisons using Bonferroni's modification was used. P values < 0.05 were considered significant.

RESULTS

Nanoindentation of Human Bone

The average elastic moduli values of all bone specimens are listed in Table 1. As would be expected, the moduli of demineralized cortical and trabecular bone decreased relative to fully mineralized bone by almost an order of magnitude, and mineralized cortical bone (periosteal or endosteal) had higher moduli compared with trabecular bone. In order to identify any similarities in the nanomechanical loading behavior of bone compared with the copolymers, representative load vs. displacement curves from the unloading regime are illustrated for each type of bone and copolymer in Figure 2. The load versus displacement behavior of 10PEG fell in between the measured moduli of the demineralized cortical periosteum and trabecular bone while the behaviors of 65HEMA and 50PEG were similar to the demineralized and mineralized trabecular bone. The other three copolymers had smaller moduli compared to the measured values of any of the bone types tested.

Polymer Surface Characterization

The average surface roughness, contact angle, and elastic modulus values for all six copolymer surfaces are shown in Table 2. Average roughness (R_a) was below 70nm for all copolymer surfaces. There was no significant difference in contact angle between each PEGDMA-DEGDMA or 2HEMA-PEGDMA surface with TCPS ($82.0 \pm 6.4^\circ$). 50PEG was moderately more hydrophilic compared with the other surfaces having a significantly lower contact angle compared with 90PEG, 65HEMA, 80HEMA, and 98HEMA. The elastic moduli of the PEGDMA-DEGDMA copolymers varied over several orders of magnitude and decreased as the weight fraction of PEGDMA increased. Similarly, moduli values for the three 2HEMA-PEGDMA compositions varied over several orders of magnitude, but fell within the same range as the PEGDMA-DEGDMA copolymers. When nanoindentation was performed after one day or one week immersion in PBS, slight decreases in moduli were observed between the two time points; however, there was no significant difference in the moduli of each copolymer composition after one day or one week soaking.

The percent elemental compositions obtained by XPS are listed in Table 3. Peaks corresponding to C and O were detected on all copolymer surfaces at a elemental ratio (C:O) of around 7:3, as would be expected given the chemical structures of the incorporated monomers. There was no significant difference in composition amongst the six copolymer surfaces.

Representative FTIR-ATR spectra for the PEGDMA-DEGDMA and 2HEMA-PEGDMA copolymers are shown in Figure 2A and 2B, respectively. For the PEGDMA-DEGDMA copolymers, key chemical groups including the $-\text{CH}_3$ or $-\text{CH}_2$, $\text{O}-\text{C}=\text{O}$, and $\text{C}-\text{O}-\text{C}$ can be identified by peaks at $2800\text{--}3000\text{cm}^{-1}$, 1750cm^{-1} , and 1140cm^{-1} wavenumbers, respectively. As more PEGDMA was added, the intensity of the peak at 2800cm^{-1} increased due to the increased surface presentation of $-\text{CH}_2$ in the ethylene glycol groups. An increased surface presentation of PEG was also evident by a right shift in frequency of the $\text{C}-\text{O}-\text{C}$ peak. Looking at the spectra for 2HEMA-PEGDMA copolymers, a different chemical profile was observed, specifically with a peak appearing around $3200\text{--}3450\text{cm}^{-1}$ due to the addition of $-\text{OH}$ side groups presented on the surface. The intensity of this peak increased as more 2HEMA was added to the system. A biphasic peak around $1000\text{--}1150\text{cm}^{-1}$ became more distinct as the 2HEMA concentration increased due to the dual presentation of $\text{C}-\text{O}$ in both the 2HEMA and PEGDMA monomers.

Cellular Morphology

Substrate-dependent differences in cell morphology were observed on the PEGDMA-DEGDMA copolymers using scanning electron microscopy. Cells were able to attach on all surfaces by 24h in culture. In general, MG63 cells on 10PEG were elongated and exhibited spindle-like shapes about 5–10 μ m diameter and over 40 μ m long (Figure 3a). Conversely, cells on 50PEG were more flat and spread on the surface with multiple extensions stretching over 10 μ m (Figure 3b). The fewest number of cells were observed on 90PEG with the majority of cells on the surface forming a polygonal shape around 10–20 μ m in diameter (Figure 3c).

MG63s also attached by 24h in culture on the 2HEMA-PEGDMA copolymers, exhibiting variable morphologies depending on the copolymer composition. Cells on 65HEMA exhibited mostly an elongated, spindle-like shape (Figure 3d) similar to that observed on the 10PEG surfaces (Figure 3a). A range of morphologies from flat and spread to round and cuboidal was observed on both the 80HEMA and 98HEMA compositions (Figure 3e and 3f, respectively), but more cells were seen attached on 98HEMA and 65HEMA than 80HEMA.

Cellular Response

Cell number for the PEGDMA-DEGDMA copolymers was sensitive to copolymer composition and stiffness (Figure 4a). Compared to TCPS, cell number was reduced 3-fold on the PEGDMA-DEGDMA surfaces. Cell numbers were at least two-fold higher on 50PEG and 90PEG surfaces than on 10PEG surfaces, the stiffest surface with the highest cell number observed on 50PEG, the medium stiffness surface.

Osteoblast differentiation was also affected by the surface chemistry and stiffness. Compared with TCPS, the PEGDMA-DEGDMA copolymers had a variable effect on alkaline phosphatase specific activity with activities increasing slightly on the less stiff 50PEG and 90PEG surfaces, but decreasing by 30% on the most stiff 10PEG (Figure 4b). Like cell number, the alkaline phosphatase specific activity increased at least 2-fold on 50PEG and 90PEG with respect to 10PEG. A three-fold increase in osteocalcin levels in the conditioned media of cells grown on the copolymer surfaces was observed compared to TCPS (Figure 4c). Osteocalcin levels were also affected by polymer stiffness, decreasing by at least 60% on 50PEG and 90PEG compared with 10PEG. The osteocalcin levels of cells on 50PEG, the moderately stiff surface, were significantly reduced compared with both 10PEG and 90PEG. The production of local regulatory factors was also sensitive to copolymer composition. OPG levels increased 4-fold on the 10PEG and 50PEG surfaces and at least 6-fold on 90PEG (Figure 4d). In contrast, both VEGF-A and TGF- β 1 levels were higher on 10PEG surfaces than on 50PEG and 90PEG (Figure 4e and 4f).

The MG63 cell response on the 2HEMA-PEGDMA surfaces was affected by copolymer composition in a different manner compared with the PEGDMA-DEGDMA copolymers. To effectively compare the changes in cell response between the two copolymer systems, 90PEG was included in these experiments because its surface modulus was within the same order of magnitude of 80HEMA. Cell number was affected by both the copolymer composition and stiffness (Figure 5a), reducing by 50% on the 65HEMA and 98HEMA surfaces and 75% on the 80HEMA in comparison with TCPS. Compared with 90PEG, cell number was increased 2-fold on 65HEMA and 98HEMA surfaces, but there was no significant difference in cell number between 80HEMA and 90PEG.

Osteoblast differentiation was affected by copolymer composition. Similar to the results described above for the PEGDMA-DEGDMA surfaces, alkaline phosphatase specific activity and osteocalcin levels increased on 90PEG compared with TCPS (Figure 5b and 5c). Comparing the 2HEMA-based surfaces with TCPS, alkaline phosphatase specific activities

were reduced 50% on the moderately stiff and least stiff surfaces (80HEMA and 98HEMA, respectively). Activities also decreased on the three 2HEMA-PEGDMA copolymers compared with 90PEG. In contrast, osteocalcin levels on 80HEMA and 98HEMA surfaces were elevated at least 2-fold from the levels detected on TCPS, but there was no difference between 65HEMA and TCPS. The levels on the 2HEMA-based surfaces were reduced by at least 50% compared with the levels on 90PEG. Surface stiffness did not affect osteocalcin production using the 2HEMA compositions.

The production of local regulatory factors was also regulated by copolymer composition. Both OPG and VEGF-A levels were again significantly increased on the 90PEG in comparison to TCPS (Figure 5d and 5e). OPG levels increased at least three fold on the three 2HEMA copolymers compared with TCPS, but there was no difference amongst the three compositions. Compared with 90PEG, OPG levels were reduced by at least 50% on 65HEMA and 98HEMA, but were not significantly different on 80HEMA. There was no significant change in VEGF levels between the 2HEMA copolymers, but levels were 60% lower on all three 2HEMA-PEGDMA compositions in comparison to 90PEG. TGF- β 1 was also evaluated on the 2HEMA-PEGDMA compositions, but no detectable levels were observed.

DISCUSSION

The results of this study demonstrate that bone stiffness on the micro-scale varies amongst the different structural components and mineralized states. The surface modulus of different bone components and mineralized state has been measured previously [31–32], but to the best of our knowledge, this is the first study comparing the elastic surface moduli of the cortical periosteal, endosteal surfaces of cortical and trabecular bone, both in mineralized and demineralized states. As would be expected, the elastic modulus of cortical bone was significantly higher than trabecular bone. This result can be attributed to the structural differences between the components in that cortical bone is a dense, compact tissue, whereas trabecular bone is more porous consisting of mostly strut-like trabeculae. The decrease in modulus for demineralized bone compared with mineralized bone for all regions occurs because the demineralized tissue is deplete of the mineral content known to give bone its rigidity. Interestingly, there was no difference in modulus between the periosteal and endosteal bone surfaces when mineralized, but the average modulus of the periosteal bone was significantly lower than the endosteal bone after demineralization.

In this study, it was hypothesized that the osteoblast response was affected by copolymer stiffness in a manner related to the rigidity the cells experience within the native tissue. The results of this study demonstrate that MG63 cells respond in a differential manner to both polymer stiffness and chemistry. The two copolymer systems, PEGDMA-DEGDMA and 2HEMA-PEGDMA, were designed to exhibit specific mechanical properties and chemical features. In the PEGDMA-DEGDMA copolymer system, the same chemical features (the PEG and acrylate groups) were presented on the surface regardless of composition, but in varying concentrations. For example because PEGDMA ($M_n \sim 750$) has more PEG repeating units compared with DEGDMA, the addition of PEGDMA to the material allowed for more surface presentation of ethylene glycol units while keeping the concentration of oxygen and carbon atoms constant and a lower crosslinking density compared with compositions with higher DEGDMA content (i.e., 90PEG vs. 10PEG). By systematically varying the PEGDMA to DEGDMA ratio, the surface modulus was effectively tailored over several orders of magnitude. With the 2HEMA-PEGDMA system, the hydroxyl groups in the 2HEMA structure created a different interfacial chemistry compared with the PEGDMA-DEGDMA copolymers. Adding more PEGDMA to the 2HEMA network resulted in less hydroxyl groups on the surface, lower crosslink density, and subsequently lower surface modulus. The range of surface moduli attained by changing the monomer ratio (PEGDMA:DEGDMA or PEGDMA:2HEMA) were

within the same orders of magnitude for the two copolymer systems, thus allowing for distinct comparisons between the effects of copolymer chemistry on the cell response when stiffness is controlled. As observed in the results and to be discussed below, the effect of surface stiffness on the cell response was not comparable between the two copolymer systems indicating that the base chemical structure serves as the primary mediator of the osteoblast response while the effect of surface modulus differs with surface chemistry.

On both copolymer systems, the low cell numbers suggest that cell attachment or proliferation was not well supported on these surfaces. This result agrees well with previous findings showing that cell number is decreased on PEG and HEMA-presenting surfaces compared with TCPS [33–34]. Cell attachment and adhesion are usually dependent upon the surface wettability, which is controlled by hydrophilicity and roughness. Despite having a significantly lower contact angle, 50PEG surfaces promoted the highest cell number amongst the three PEGDMA-DEGDMA copolymers suggesting that cell number was affected by surface features other than wettability. These findings are consistent with previous studies reporting that contact angle is not always a suitable measure of wettability on copolymer surfaces or an accurate way to predict degree of cell attachment due to the inherent heterogeneity of the network structure [35]. Considering that the contact angles were similar for all 2HEMA-PEGDMA surfaces, the increased cell numbers on 65HEMA and 98HEMA compared with 80HEMA further suggests that other surface effects possibly including stiffness could affect cell attachment and/or proliferation. Because proliferation and cell viability were not directly measured, it is difficult to ascertain whether the increased cell number on 50PEG and 80HEMA is the result of increased cell attachment or proliferation or decreased viability. From the SEM images, it is apparent that cells adhered to all copolymer surfaces after 24h in culture, but the number of cells attaching is variable on composition. Although surface wettability did not differ, it is also possible that protein adsorption was altered in a manner dependent on copolymer composition that resulted in key osteoblast binding proteins to be present in active conformations. The effect of polymer chemistry on protein adsorption has been detailed elsewhere [36].

On the PEGDMA-DEGDMA surfaces, MG63 cells grown on the stiffest surface (10PEG) exhibited characteristics corresponding to a more mature osteoblast phenotype. Alkaline phosphatase specific activity and osteocalcin production are both well-studied differentiation markers for osteoblasts [37]. In general, alkaline phosphatase specific activity increases during the early stages of osteoblast differentiation, but then decreases over time as differentiation progresses and mineral deposition begins to occur. In contrast, osteocalcin levels are low during the early stages of differentiation and then increase and plateau as the cell reaches a mature osteoblast phenotype. Thus in this present study, the reduced alkaline phosphatase specific activity in combination with elevated osteocalcin levels on the PEGDMA-DEGDMA surfaces indicate that MG63 cells are more differentiated on these surfaces compared with cells grown on TCPS. Considering that differentiated osteoblasts will cease to proliferate, the reduced cell numbers observed on these surfaces further supports this hypothesis and agrees with previous studies showing that PLL-g-PEG coating on rough titanium can promote an osteogenic response from MG63 cells [33].

By comparing the cell numbers and osteocalcin levels produced on the 2HEMA-PEGDMA surfaces with 90PEG, it can be suggested that cells differentiate at a faster rate on the PEGDMA-DEGDMA surfaces than on the 2HEMA-PEGDMA surfaces. Interestingly, changing the 2HEMA concentration did not statistically affect osteoblast differentiation within the context of this copolymer system. This result contrasts with what is observed on the PEGDMA-DEGDMA surfaces where cell differentiation was increased the most on 10PEG, the stiffest surface, indicating that cells were preferentially responding to changes in copolymer chemistry versus changes in surface stiffness. Considering that contact angle and average

roughness measurements were essentially similar amongst all copolymer compositions, this differential response signifies that osteoblast differentiation is primarily affected by copolymer chemistry in a manner that is not dependent on surface wettability and roughness.

Copolymer chemistry and stiffness may affect osseointegration through mechanisms involving changes in the production of local osteogenic factors. The results of this study indicate that both OPG and TGF- β 1 production are modulated by the copolymer chemistry. OPG is produced by osteoblasts and acts as a decoy receptor by binding with RANKL to suppress osteoclastogenesis and regulate the bone remodeling cycle [38]. TGF- β 1 is a growth factor that stimulates extracellular matrix formation and is believed to mediate OPG production [15]. It is produced in a latent form by osteoblasts and then stored in the extracellular matrix until it becomes activated [39]. In previous studies, fully differentiated osteoblasts exhibited elevated levels of OPG and TGF- β 1 when cultured on microrough titanium surfaces [29,40]. The elevated OPG and TGF- β 1 production from MG63 cells cultured on the PEGDMA-DEGDMA surfaces further support the contention that MG63 cells are in a more differentiated state on these copolymer surfaces compared with TCPS by synthesizing mediators that enhance the osteogenic microenvironment. However, MG63 cells appear to produce more OPG on the less stiff surface (90PEG) while TGF- β 1 levels are elevated more on the stiffest surface suggesting that the effects of copolymer stiffness mediate OPG and TGF- β 1 production through different pathways. The reduced OPG levels on the 2HEMA-PEGDMA surfaces compared with 90PEG further indicates that the copolymer chemistry regulates osteoblast differentiation in a manner that affects osteogenic factor production.

Interestingly, the increased OPG production on 80HEMA compared with 65HEMA and 98HEMA also mirrors the change in OPG production with copolymer stiffness observed on the PEGDMA-DEGDMA copolymer (90PEG vs. 10PEG and 50PEG). It has been suggested that cells mimic their *in vivo* behavior on synthetic matrices when the matrix stiffness matches the rigidity of the native tissue [3,18]. In bone, the stiffness of the tissue is changing as bone is continuously remodeling itself. During the initial stages of bone formation, osteoblasts are beginning to lay down osteoid, which consists mostly of collagen and other matrix proteins and has a reported modulus of 27kPa [3]. As the matrix begins to mineralize during latter stages of bone formation, the stiffness increases to between 1 and 30GPa depending on the bone type, cortical or trabecular and degree of mineralization [31–32,41]. In the present study, the moduli of 90PEG and 80HEMA represent the mechanical environment during early stages of bone development when the extracellular matrix is beginning to mineralize. During early osteogenesis, it is important that osteoclast activity is reduced so that bone resorption is repressed and enough of the matrix is deposited. The increased OPG production on the 90PEG and 80HEMA suggest that softer copolymer substrates, within the 10–70MPa range, will inhibit bone resorption by preventing osteoclast activation. Conversely on stiffer substrates such as 10PEG that mechanically mimic mature, mineralized bone, OPG production is reduced, potentially allowing osteoclasts to become activated and initiate bone resorption.

Besides modulating the ability of MG63 cells to promote bone formation versus resorption, copolymer chemistry and stiffness can also potentially regulate other processes that accompany bone formation. VEGF-A is an important angiogenic factor that osteoblasts produce to promote blood vessel formation within newly formed bone matrix [42]. Previous studies have suggested that VEGF-A production in pre-osteoblasts signifies the latter stages of osteoblast differentiation [43]. Within this current study, the elevated VEGF-A levels on the PEGDMA-DEGDMA surfaces compared with TCPS further indicate that cells cultured on these surfaces exhibit characteristics of mature osteoblasts, and the extent of VEGF-A production by MG63 cells on these surfaces is mediated by stiffness. However, there was no significant difference in VEGF-A production on the 2HEMA-PEGDMA copolymers compared with TCPS. Considering the reduced osteocalcin and OPG levels observed on the 2HEMA-PEGDMA

copolymers in comparison with 90PEG, it can be suggested that cells on these surfaces have not fully differentiated and thus are not readily producing key functional proteins characteristic of mature osteoblasts.

This study illustrates the importance of surface chemistry in evaluating the cell response on polymer surfaces. Comparing the two copolymer systems investigated, the PEGDMA-DEGDMA copolymer overall better promoted osteoblast differentiation compared with 2HEMA-PEGDMA. This result is significant as PEGDMA and 2HEMA are both common biocompatible polymers used in many implants and tissue engineering applications, but their osteogenic potential has never been compared. Previous studies have shown that cell attachment and spreading does not differ when osteoblasts are cultured on both PEG and HEMA surfaces [34]. These findings are supported by the SEM images shown in this study indicating that the copolymer chemistry affects osteoblast behavior through mechanisms other than attachment and adhesion, possibly by changing the conformation of adsorbed matrix proteins and causing certain extracellular membrane receptors including integrins to be differentially expressed. Several studies have documented how protein adsorption and subsequently cell attachment are altered by the addition of 2HEMA [36], but this effect has not been fully correlated to changes in osteoblast differentiation. Looking at the structure of each network, the PEGDMA-DEGDMA copolymers were more highly crosslinked than the 2HEMA-PEGDMA compositions, a structural characteristic that could affect protein adsorption and cell binding on the surface leading to changes in intracellular signaling. This hypothesis is supported by a previous study showing that osteoblast attachment is affected by changes in the molecular weight between crosslinks in a degradable acrylate-based network [20], although there is no prior evidence that this leads to altered cell differentiation potential.

Previous studies have shown that the extracellular matrix rigidity within the kPa range can influence the cell morphology, proliferation, and differentiation of MC3T3-E1 cells [3,5,18]. To the best of our knowledge, this is the first study examining the effect of stiffness within the MPa range on the MG63 cell response. For the PEGDMA-DEGDMA system, it is apparent that MG63 cells differentiate and begin to create an osteogenic microenvironment at a faster rate on the stiffest surface (in the 100 MPa range), but the response of MG63s on the 2HEMA-PEGDMA surfaces suggests that a lower stiffness material around 10MPa will better promote osteogenesis. These results challenge whether MG63 cells can sense their mechanical environment at this rigidity. If cells do alter their function in response to the surface stiffness, then this effect is dependent on the copolymer chemistry presented. Thus when selecting a copolymer surface to interface with bone, osseointegration can be potentially optimized by first selecting the appropriate surface chemistry, PEG in this present study, and then the appropriate stiffness of the copolymer. Given their easy tailorability of chemical composition and mechanical properties, acrylate-based networks serve as excellent candidate materials for implementation in bone-interfacing orthopedic applications.

CONCLUSIONS

This study indicates that osteoblast differentiation is mediated by the copolymer chemistry and that PEG-based acrylate chemistries support osteoblast differentiation better than 2HEMA-based networks. Specifically, cell numbers were decreased and osteocalcin, OPG, and VEGF-A levels were elevated on the PEGDMA-DEGDMA surfaces versus 2HEMA-PEGDMA surfaces. In order to study the effect of various surface properties on the cell response, polymer networks are typically used because their surface properties can be easily tailored by varying the chemical structure. These results emphasize the importance of minimizing changes in surface chemistry when evaluating the role of surface stiffness. Further studies will examine potential cellular mechanisms creating the differential response to copolymer composition and stiffness, particularly the role of integrin expression and cytoskeleton organization. A better

understanding of these cell-polymer interactions will facilitate the development of orthopedic polymer materials that will better promote osseointegration.

Acknowledgments

This work was funded by the NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases (USPS 5R21AR054339). The authors would like to thank Dr. Robert Braga and the School of Chemistry for the use of their FTIR, the Microelectronics Research Center for the use of their XPS and SEM, and Rolando Gittens from the Tannenbaum Lab for his assistance with AFM.

REFERENCES

1. Adden N, Hoffmann A, Gross G, Windhagen H, Thorey F, Menzel H. Screening of photochemically grafted polymer films for compatibility with osteogenic precursor cells. *J Biomater Sci, Polym Ed* 2007;18(3):303–316. [PubMed: 17471767]
2. Boyan BD, Lohmann CH, Dean DD, Sylvia VL, Cochran DL, Schwartz Z. Mechanisms involved in osteoblast response to implant surface morphology. *Annu Rev Mater Res* 2001;31:357–371.
3. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;126(4):677–689. [PubMed: 16923388]
4. Kavlock KD, Pechar TW, Hollinger JO, Guelcher SA, Goldstein AS. Synthesis and characterization of segmented poly(esterurethane urea) elastomers for bone tissue engineering. *Acta Biomater* 2007;3(4):475–484. [PubMed: 17418651]
5. Khatiwala CB, Peyton SR, Metzke M, Putnam AJ. The regulation of osteogenesis by ECM rigidity in MC3T3-E1 cells requires MAPK activation. *J Cell Physiol* 2007;211(3):661–672. [PubMed: 17348033]
6. Lee SJ, Choi JS, Park KS, Khang G, Lee YM, Lee HB. Response of MG63 osteoblast-like cells onto polycarbonate membrane surfaces with different micropore sizes. *Biomaterials* 2004;25(19):4699–4707. [PubMed: 15120516]
7. Lincks J, Boyan BD, Blanchard CR, Lohmann CH, Liu Y, Cochran DL, et al. Response of MG63 osteoblast-like cells to titanium and titanium alloy is dependent on surface roughness and composition. *Biomaterials* 1998;19(23):2219–2232. [PubMed: 9884063]
8. Pelham RJ, Wang YL. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci USA* 1997;94(25):13661–13665. [PubMed: 9391082]
9. Zhao G, Schwartz Z, Wieland M, Rupp F, Geis-Gerstorfer J, Cochran DL, et al. High surface energy enhances cell response to titanium substrate microstructure. *J Biomed Mater Res A* 2005;74A(1):49–58. [PubMed: 15924300]
10. Zinger O, Zhao G, Schwartz Z, Simpson J, Wieland M, Landolt D, et al. Differential regulation of osteoblasts by substrate microstructural features. *Biomaterials* 2005;26(14):1837–1847. [PubMed: 15576158]
11. Schwartz Z, Raz P, Zhao G, Barak Y, Tauber M, Yao H, et al. Effect of Micrometer-Scale Roughness of the Surface of Ti6Al4V Pedicle Screws in Vitro and in Vivo. *J Bone Joint Surg-Am Vol* 2008;90A(11):2485–2498.
12. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants—a histomorphometric study in miniature pigs. *J Biomed Mater Res* 1991;25(7):889–902. [PubMed: 1918105]
13. Li DH, Ferguson SJ, Beutler T, Cochran DL, Sittig C, Hirt HP, et al. Biomechanical comparison of the sandblasted and acid-etched and the machined and acid-etched titanium surface for dental implants. *J Biomed Mater Res* 2002;60(2):325–332. [PubMed: 11857440]
14. Redey SA, Nardin M, Bernache-Assolant D, Rey C, Delannoy P, Sedel L, et al. Behavior of human osteoblastic cells on stoichiometric hydroxyapatite and type A carbonate apatite: Role of surface energy. *J Biomed Mater Res* 2000;50(3):353–364. [PubMed: 10737877]
15. Schwartz Z, Olivares-Navarrete R, Wieland M, Cochran DL, Boyan BD. Mechanisms regulating increased production of osteoprotegerin by osteoblasts cultured on microstructured titanium surfaces. *Biomaterials* 2009;30(20):3390–3396. [PubMed: 19395022]

16. Zhao G, Zinger O, Schwartz Z, Wieland M, Landolt D, Boyan BD. Osteoblast-like cells are sensitive to submicron-scale surface structure. *Clin Oral Implants Res* 2006;17(3):258–264. [PubMed: 16672020]
17. Bischofs IB, Schwarz US. Cell organization in soft media due to active mechanosensing. *Proc Natl Acad Sci USA* 2003;100(16):9274–9279. [PubMed: 12883003]
18. Kong HJ, Polte TR, Alsberg E, Mooney DJ. FRET measurements of cell-traction forces and nano-scale clustering of adhesion ligands varied by substrate stiffness. *Proc Natl Acad Sci USA* 2005;102(12):4300–4305. [PubMed: 15767572]
19. Peyton SR, Ghajar CM, Khatiwala CB, Putnam AJ. The emergence of ECM mechanics and cytoskeletal tension as important regulators of cell function. *Cell Biochem Biophys* 2007;47(2):300–320. [PubMed: 17652777]
20. Brey DM, Erickson I, Burdick JA. Influence of macromer molecular weight and chemistry on poly (beta-amino ester) network properties and initial cell interactions. *J Biomed Mater Res A* 2008;85A(3):731–741. [PubMed: 17896761]
21. Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Langer R. Transdermal photopolymerization for minimally invasive implantation. *Proc Natl Acad Sci USA* 1999;96(6):3104–3107. [PubMed: 10077644]
22. Yakacki CM, Shandas R, Lanning C, Rech B, Eckstein A, Gall K. Unconstrained recovery characterization of shape-memory polymer networks for cardiovascular applications. *Biomaterials* 2007;28(14):2255–2263. [PubMed: 17296222]
23. Yakacki CM, Shandas R, Safranski D, Ortega AM, Sassaman K, Gall K. Strong, tailored, biocompatible shape-memory polymer networks. *Adv Funct Mater* 2008;18(16):2428–2435. [PubMed: 19633727]
24. Lovell LG, Lu H, Elliott JE, Stansbury JW, Bowman CN. The effect of cure rate on the mechanical properties of dental resins. *Dental Materials* 2001;17(6):504–511. [PubMed: 11567688]
25. Jin H, Yoon SS, Kim SC. Synthesis and characterization of interpenetrating polymer networks from polyurethane and poly(ethylene glycol) diacrylate. *J Appl Polym Sci* 2008;109(2):805–812.
26. Johnson, KL. *Contact Mechanics*. Cambridge, U.K: Cambridge University Press; 1985.
27. Ebenstein DM, Pruitt LA. Nanoindentation of soft hydrated materials for application to vascular tissues. *J Biomed Mater Res A* 2004;69A(2):222–232. [PubMed: 15057995]
28. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J, et al. Effect of titanium surface-roughness on proliferation, differentiation, and protein-synthesis of human osteoblast-like cells (MG63s). *J Biomed Mater Res* 1995;29(3):389–401. [PubMed: 7542245]
29. Lossdorfer S, Schwartz Z, Wang L, Lohmann CH, Turner JD, Wieland M, et al. Microrough implant surface topographies increase osteogenesis by reducing osteoclast formation and activity. *J Biomed Mater Res, Part A* 2004;70A(3):361–369.
30. Kieswetter K, Schwartz Z, Hummert TW, Cochran DL, Simpson J, Dean DD, et al. Surface roughness modulates the local production of growth factors and cytokines by osteoblast-like MG-63 cells. *J Biomed Mater Res* 1996;32(1):55–63. [PubMed: 8864873]
31. Rho JY, Ziopos P, Currey JD, Pharr GM. Microstructural elasticity and regional heterogeneity in human femoral bone of various ages examined by nano-indentation. *J Biomech* 2002;35:189–198. [PubMed: 11784537]
32. Zysset PK, Guo E, Hoffler CE, Moore KE. Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nanoindentation in the human femur. *J Biomech* 1999;32:1005–1012. [PubMed: 10476838]
33. Tosatti S, Schwartz Z, Campbell C, Cochran DL, VandeVondele S, Hubbell JA, et al. RGD-containing peptide GCRGYGRGDSPG reduces enhancement of osteoblast differentiation by poly(L-lysine)-graft-poly(ethylene glycol)-coated titanium surfaces. *J Biomed Mater Res A* 2004;68A(3):458–472. [PubMed: 14762925]
34. Schneider GB, English A, Abraham M, Zaharias R, Stanford C, Keller J. The effect of hydrogel charge density on cell attachment. *Biomaterials* 2004;25(15):3023–3028. [PubMed: 14967535]
35. Hutcheon GA, Messiou C, Wyre RM, Davies MC, Downes S. Water absorption and surface properties of novel poly(ethylmethacrylate) polymer systems for use in bone and cartilage repair. *Biomaterials* 2001;22(7):667–676. [PubMed: 11246960]

36. McFarland CD, Mayer S, Scotchford C, Dalton BA, Steele JG, Downes S. Attachment of cultured human bone cells to novel polymers. *J Biomed Mater Res* 1999;44(1):1–11. [PubMed: 10397899]
37. Lian JB, Stein GS. Concepts of osteoblast growth and differentiation - Basis for modulation of bone cell-development and tissue formation. *Crit Rev Oral Biol Med* 1992;3(3):269–305. [PubMed: 1571474]
38. Khosla S. Minireview: The OPG/RANKL/RANK system. *Endocrinology* 2001;142(12):5050–5055. [PubMed: 11713196]
39. Bonewald LF. Regulation and regulatory activities of transforming growth factor beta. *Crit Rev Eukaryot Gene Expr* 1999;9(1):33–44. [PubMed: 10200910]
40. Bonewald LF, Schwartz Z, Swain LD, Ramirez V, Poser J, Boyan BD. Stimulation of plasma-membrane and matrix vesicle enzyme-activity by transforming growth-factor-beta in osteosarcoma cell-cultures. *J Cell Physiol* 1990;145(2):200–206. [PubMed: 2246323]
41. Balooch M, Habelitz S, Kinney JH, Marshall SJ, Marshall GW. Mechanical properties of mineralized collagen fibrils as influenced by demineralization. *J Struct Biol* 2008;162:404–410. [PubMed: 18467127]
42. Clarkin CE, Emery RJ, Pitsillides AA, Wheeler-Jones CPD. Evaluation of VEGF-Mediated signaling in primary human cells reveals a paracrine action for VEGF in osteoblast-mediated crosstalk to endothelial cells. *J Cell Physiol* 2008;214(2):537–544. [PubMed: 17685428]
43. Rausch-fan XH, Qu Z, Wieland M, Matejka M, Schedle A. Differentiation and cytokine synthesis of human alveolar osteoblasts compared to osteoblast-like cells (MG63) in response to titanium surfaces. *Dental Mater* 2008;24(1):102–110.

Figure 1.

Representative load versus displacement curves of bone wafers using nanoindentation. The copolymer networks tested in this study are included for comparative purposes. Calculated elastic moduli for each bone type, region, and degree of mineralization are listed in Table 3

Figure 2. FTIR-ATR spectra of (A) PEGDMA-co-DEGDMA and (B) 2HEMA-PEGDMA/DEGDMA surfaces. Peaks corresponding to key chemical components of the networks are indicated. Dotted lines represent the wavenumber corresponding to the $\text{C}=\text{O}$ and $\text{C}-\text{O}-\text{C}$ peaks for 90PEG. It can be observed that these peak wavenumbers shift for the other copolymer systems relative to the 90PEG.

Figure 3. Scanning electron micrographs of MG63 cells after 24h in culture on 10PEG, 50PEG, and 90PEG (A–F) and 65HEMA, 80HEMA, and 98HEMA (G–L) at 500× (A–C and G–I) and 2kX (D–F and J–L) magnifications. Scale bar represents 40µm and 10µm for 500× and 2kX images, respectively.

Figure 4.

Effect of composition and stiffness on differentiation of MG63 cells. Cells were cultured on tissue culture polystyrene (TCPS) or the copolymer surfaces with various weight ratios of PEGDMA to DEGDMA: 90% PEGDMA (90PEG), 50% PEGDMA (50PEG), and 10% PEGDMA (10PEG). At confluence (A) cell number, (B) alkaline phosphatase specific activity, (C) osteocalcin, (D) OPG, (E) VEGF-A, and (F) total TGF- β 1 levels were measured. Data is the ratio of polymer surfaces to TCPS control for at least four independent experiments. * $p < 0.05$, polymer surface vs. TCPS; # $p < 0.05$, polymer surface vs. 10PEG; \$ $p < 0.05$, 90PEG vs. 50PEG.

Figure 5.

Effect of composition and stiffness on differentiation of MG63 cells. Cells were cultured on either tissue culture polystyrene (TCPS) or the copolymer surfaces (65HEMA, 80HEMA, and 98HEMA) with similar stiffness values to PEGDMA-DEGDMA materials. 90PEG was included as a positive control for the PEGDMA-DEGDMA surfaces. At confluence, (A) cell number, (B) alkaline phosphatase specific activity, (C) osteocalcin, (D) OPG, (E) VEGF-A, and (F) total TGF- β 1 levels were measured. * $p < 0.05$, HEMA copolymer surface vs. TCPS; # $p < 0.05$, HEMA copolymer surface vs. 90PEG; ‡ $p < 0.05$, 80HEMA or 98HEMA vs. 65HEMA.

Table 1

Average elastic moduli values for human bone measured by nanoindentation. Values represent mean $\pm$ standard deviation. The corresponding load versus displacement behaviors are displayed in Figure 2

Bone Region	E (MPa)	
	Mineralized	Demineralized
Cortical Periosteum	9905 $\pm$ 1763	871 $\pm$ 218 [*]
Cortical Endosteum	11500 $\pm$ 1656	2364 $\pm$ 588 ^{*#}
Trabecular	365 $\pm$ 223 ^{#^}	92.7 $\pm$ 74.9 ^{*# ^}

^{*} p<0.05 vs. mineralized for that region;

[#] p<0.05 vs. cortical periosteum;

[^] p<0.05 vs. cortical endosteum

PEGDMA-DEGDMA and 2HEMA-PEGDMA/DEGDMA compositions and their corresponding contact angle, average roughness (R_a), and surface elastic modulus (E_s) after soaking for 24h and 7 days in PBS. Values are mean $\pm$ SEM.

Table 2

Substrate	Composition Wt. %	Contact Angle (°)	R_a (nm)	E_s : 24h (MPa)**	E_s : 1 week (MPa)**
TCPS	Tissue culture polystyrene	82.0 $\pm$ 6.5*		2150 $\pm$ 100	2150 $\pm$ 100
10PEG	10% PEGDMA750 90% DEGDMA	73.1 $\pm$ 4.6	12.8 $\pm$ 9.7	851 $\pm$ 223	507 $\pm$ 120
50PEG	50% PEGDMA750 50% DEGDMA	63.1 $\pm$ 7.5	5.4 $\pm$ 2.3	355 $\pm$ 63	311 $\pm$ 26
90PEG	90% PEGDMA750 10% DEGDMA	81.9 $\pm$ 5.8*	26.2 $\pm$ 8.7	67.9 $\pm$ 5.5	63.6 $\pm$ 2.0
65HEMA	65% 2HEMA 35% DEGDMA	78.4 $\pm$ 5.4*	60.3 $\pm$ 22.8	609 $\pm$ 70	582 $\pm$ 103
80HEMA	80% 2HEMA 20% PEGDMA550	80.2 $\pm$ 4.9*	18.7 $\pm$ 2.5*#	8.74 $\pm$ 1.2	8.80 $\pm$ 1.8
98HEMA	98% 2HEMA 2% PEGDMA750	84.5 $\pm$ 2.7*	7.5 $\pm$ 1.8	5.55 $\pm$ 1.9	5.6 $\pm$ 2.0

* p<0.05 vs. 50PEG;

p<0.05 vs. 65HEMA;

** E_s values for different compositions are statistically significant (p<0.05) except 10PEG vs. 65HEMA.

Table 3

Percent elemental composition of PEGDMA-DEGDMA and 2HEMA-PEGDMA/DEGDMA copolymers using XPS. Values represent mean $\pm$ SEM.

Copolymer Composition	%C	%O
10PEG	69.2 $\pm$ 1.7	30.9 $\pm$ 1.7
50PEG	73.1 $\pm$ 0.1	27.0 $\pm$ 0.1
90PEG	73.3 $\pm$ 0.9	26.8 $\pm$ 0.9
65HEMA	72.8 $\pm$ 5.0	27.2 $\pm$ 5.0
80HEMA	70.3 $\pm$ 5.1	29.8 $\pm$ 5.1
98HEMA	74.8 $\pm$ 1.0	25.2 $\pm$ 1.0