

Research Paper

Proangiogenic Potential of a Collagen/Bioactive Glass Substrate

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Purpose. Previous attempts to stimulate angiogenesis have focused on the delivery of growth factors and cytokines, genes encoding for specific angiogenic inductive proteins or transcription factors, or participating cells. While high concentrations of bioactive glasses have exhibited osteogenic potential, recent studies have demonstrated that low concentrations of particular bioactive glasses are angiogenic. We hypothesized that a well known bioactive glass (Bioglass[®] 45S5) possesses proangiogenic potential over a limited range of concentrations.

Materials and Methods. Varying amounts of Bioglass were loaded into absorbable collagen sponges. The proangiogenic potential of Bioglass was determined by examining the capacity of the soluble products to induce endothelial cell proliferation, tubule formation in a co-culture, and upregulate vascular endothelial growth factor (VEGF) production.

Results. We determined a range of Bioglass concentrations which exhibit proangiogenic potential. Furthermore, we demonstrated that the proangiogenic capacity of this material is related to the soluble dissolution products of Bioglass and the subsequent production of cell-secreted angiogenic factors by stimulated cells.

Conclusions. These studies suggest that this bioactive glass possesses a robust proangiogenic potential, and this strategy may provide an alternative to recombinant inductive growth factors.

KEY WORDS: angiogenesis; bioactive glass; endothelial cell; osteogenesis; VEGF.

INTRODUCTION

The establishment of a functional vasculature is a critical aspect for numerous applications in wound healing, tissue regeneration and remodeling, and tissue engineering. Recent attempts to stimulate angiogenesis have focused on the delivery of growth factors and cytokines, genes encoding for specific angiogenic inductive proteins or transcription factors, or participating cells (1). Proteins such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), transcription factors such as hypoxia inducible factor-1 α (HIF1- α), and cellular populations including endothelial cells, smooth muscle cells, and mural cells have all demonstrated a vital role in both angiogenesis and vasculogenesis. For this reason, each of these factors has been investigated as a potential tool to promote angiogenesis *in vivo* (2). However, questions remain regarding the identity of the ideal angiogenic factor or cocktail of factors, the appropriate delivery route, duration of delivery, and dosage.

Bioactive glasses, materials formed of certain compositions of Na₂O, CaO, P₂O₅, and SiO₂, react with physiological fluids and form strong bonds with native tissue (3,4). Bioactive glasses have successfully served as skeletal substitutes and to fill bone defects in the oral cavity, largely due

to the osteoconductive properties of the material (5–7). In order to retain these materials in a local defect site, bioactive glasses have been incorporated into composites with synthetic polymers for improved delivery and degradation (8–10). While most osteoconductive biomaterials predominantly serve as a passive site for cellular adhesion, proliferation, and differentiation (11,12), recent reports demonstrate that bioactive glasses may play a more active role in directing cellular behavior. Bioglass[®] 45S5 has exhibited the potential to support the growth of osteoblasts and their precursors *in vitro* and favors osteoblast differentiation by stimulating the synthesis of phenotypic markers such as alkaline phosphatase (ALP), Type I collagen, and osteocalcin (13–17).

In addition to their capacity to promote osteogenesis, bioactive glasses (namely Bioglass[®] 45S5) have also demonstrated the potential to induce angiogenesis. Human colon fibroblasts secreted greater amounts of VEGF and bFGF when cultured in direct contact with Bioglass[®] 45S5, and conditioned media collected from these cells increased endothelial cell proliferation and tubule formation (18). Additional studies by the same investigator report sustained VEGF delivery was attainable by culturing colon fibroblasts in alginate beads loaded with varying amounts of Bioglass[®] 45S5, with a dose–response curve of VEGF production evident based on the concentration of loaded material. However, these studies have focused on the response of cells in direct contact with the bioactive glass, a strategy which does not preclude the possibility that the cellular response could also be related to cell-biomaterial interactions. Fur-

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thermore, the proangiogenic response was characterized using cells which play a major role in producing the extracellular matrix necessary for wound healing and not the endothelial cells which are essential for capillary formation. The migration of distant endothelial cells along a gradient of angiogenic signaling is an early step of angiogenesis, and the direct response of endothelial cells to the soluble products of Bioglass was not examined. Leach *et al.* (19) reported that the ionic dissolution products of Bioglass® 45S5 induced endothelial cell proliferation when cells were in indirect contact with the material, yet only one dose of bioactive glass (approximately 0.5 mg) was examined. In addition, the mass of Bioglass used in this study was capable of enhancing neovascularization in an osseous bone defect.

We hypothesized that bioactive glass has proangiogenic potential over a limited range, and this mass of material could be loaded onto a three-dimensional biomaterial for localized and sustained delivery. In this study, we examined the proangiogenic potential of a bioactive glass using several *in vitro* assays. Furthermore, we sought to elucidate the mechanism of action by examining the production of a well known angiogenic inductive factor by human endothelial cells stimulated by the soluble products of the construct.

MATERIALS AND METHODS

Materials

Bioglass® 45S5 was from NovaBone (Jacksonville, FL, USA) and had a composition of 45.0% SiO₂, 24.6% CaO, 24.4% Na₂O, and 6.0% P₂O₅ by weight. Collagen sponges were from Integra LifeSciences (Plainsboro, NJ, USA), and collagen solutions derived from bovine hide were from Inamed Biomaterials (Fremont, CA, USA). All chemicals were from Sigma Aldrich (St. Louis, MO, USA) unless otherwise noted.

Preparation of Bioglass-Loaded Sponges

Known masses of BG were sterilized by suspending the material in 95% ethanol in microcentrifuge tubes for 30 min, followed by 2 washes in sterile PBS. Biopsy punches (Acuderm, Ft Lauderdale, FL, USA) were used to create disks (5×2.5 mm) from collagen sponges under sterile conditions. BG was then suspended in a solution of bovine hide-derived collagen (2.4 mg/ml) prepared by the manufacturer's instructions. Forty microliters of each BG collagen solution was pipetted onto collagen sponges to incorporate varying amounts of BG (0.06, 0.12, 0.6, 1.2, 6, and 12 mg). Control sponges consisted of 40 µl collagen solution pipetted onto collagen sponges. All collagen sponges were allowed to gel at 37°C for 1 h in a 5% CO₂ incubator, resulting in sponges with final dimensions of 3.5×1.5 mm.

Preparation of Bioglass-Coated Surfaces

Tissue culture dishes were coated with BG by modifying a previously described protocol (20). Briefly, slurries were produced by suspending BG in 95% ethanol with a magnetic stirrer to produce solutions of varying concentrations. A small volume (594 µl) was evenly pipetted into each well of a 24-

well tissue culture plate to deposit known masses of BG (0.06, 0.12, 0.6, 1.2, 6, and 12 mg). Tissue culture plates were left uncovered overnight in the laminar flow hood to allow for ethanol evaporation and resultant BG sterilization.

Endothelial Cell Proliferation

The biological activity of the dissolved ionic constituents of BG released from BG-collagen sponges was determined by testing its ability to stimulate the growth of cultured human microvascular endothelial cells (HMVEC, Cambrex, Walkersville, MD, USA). HMVECs were cultured in endothelial basal media (Cambrex) supplemented with Cambrex's Single-Quot supplement (5% fetal bovine serum, hydrocortisone, gentamycin, human VEGF, human fibroblast growth factor (FGF), human epidermal growth factor (EGF), human insulin-like growth factor (IGF), and ascorbic acid) prior to use. Cells were plated at 6×10⁴ cells/well in 12-well tissue culture dishes. The cells were allowed to attach for 24 h, washed with PBS to remove any nonadherent cells, and the media in each well was then replaced with 1.5 ml of either complete media (for positive control) or growth factor deficient media (GF-deficient: basal media containing 2% serum but lacking VEGF, FGF, and IGF). Transwells (3.0 µm pore size; Corning, Corning, NY, USA) containing BG-loaded collagen sponges covered with 500 µl GF-deficient media were placed over the wells, and dishes were incubated at 37°C/5% CO₂. Wells containing complete media served as the positive control. After 48 h all cells in the experimental and control wells were removed with a solution of 0.25% trypsin/2.21 mM EDTA (Mediatech, Manassas, VA, USA), and counted using a Coulter Z1 Dual Particle Counter (Fullerton, CA, USA).

Rat Aortic Ring Assay

Treatment of all experimental animals was in accordance with UC Davis animal care guidelines and all National Institutes of Health (NIH) animal handling procedures. Aortas were excised from 15-week old male Sprague-Dawley rats and cut into 1 mm sections as previously described (21). Each aortic ring was placed into a well coated with known masses of BG, covered with 25 µl of collagen, and allowed to gel at 37°C for 1 h before adding 1 ml of GF-def media.

The angiogenic potential of BG was characterized by observing the outgrowth of endothelial cells from the aortic ring under brightfield microscopy. Due to difficulties in quantifying cellular outgrowth from the excised aortas (data not shown), we collected 500 µl of conditioned media from BG-treated rat aortas every 72 h for 12 days for use in the tubule formation assay described below.

In Vitro Tubule Formation

The capacity of soluble BG degradation products to induce the formation of endothelial tubules was determined using a commercially available angiogenesis tubule formation assay (AngioKit, TCS Cellworks, Buckinghamshire, UK) following the manufacturer's instructions and as previously described (22). Media on the cells was aspirated and replaced with a 1:1 mixture of conditioned medium (500 µl of medium collected from the aortic ring assay) and commercially

provided optimized medium (500 μ l) in triplicate. Media was replaced every 3 days with fresh mixtures of conditioned and optimized medium for 12 days. On day 13, samples underwent immunohistochemistry for CD31, and tubules were immediately imaged using a Nikon Eclipse TE2000-U microscope and SpotRT digital camera (Diagnostic Instruments, Sterling Heights, MI, USA). Five images were taken per well and tubules were manually counted and averaged over the five images. The longest smooth and continuous tubules were identified as the primary tubules and counted, while tubules branching from the primary tubules were also counted. Further branching was also noted and counted. Care was taken to ensure that no tubules were counted twice.

VEGF mRNA Quantitation

HMVECs seeded on 12-well tissue culture plates were treated with varying amounts of BG delivered from a collagen sponge (0, 0.12, 1.2, and 12 mg) placed in 3 μ m pore size cell culture inserts. After exposure to BG for 72 h, HMVECs were collected and analyzed using a VEGF mRNA Quantitation Kit (R&D Systems, Minneapolis, MN, USA) per the manufacturer's instructions. A calibrator curve produced with known quantities of RNA calibrator was used to determine the concentration of mRNA in each sample.

Inhibition of HMVEC-Produced VEGF

A standard curve of endothelial cell proliferation in response to known concentrations of recombinant human VEGF was generated. HMVECs were seeded in complete media in 24-well tissue culture dishes at 10,000 cells/well and cultured for 24 h. Media was aspirated and replaced with 500 μ l GF-deficient media supplemented with known concentrations of rhVEGF (PeproTech, Rocky Hill, NJ, USA). Proliferation of HMVECs was quantified after 48 h with a Coulter Z1 Particle Counter. We determined an equivalent concentration of rhVEGF (25 ng/ml) which induced endothelial cell proliferation comparable to 1.2 mg of BG (data not shown). Per the manufacturer's instructions, 1 μ g/ml VEGF antibody (R&D Systems, Minneapolis, MN, USA) is needed to neutralize every 10 ng/ml of VEGF.

HMVECs were seeded as described above. Media was aspirated and replaced with 1.5 ml GF-deficient media, and transwells containing empty or BG-loaded collagen sponges (1.2 mg) covered with 500 μ l GF-deficient media were placed over the wells. The impact of the following experimental groups on 48 h HMVEC proliferation was quantified: (1) collagen-soaked collagen sponge, (2) 1.2 mg BG collagen sponge, and (3) 1.2 mg BG collagen sponge + VEGF antibody. Endothelial cell proliferation was quantified as described above.

Osteogenic Differentiation

Mouse preosteoblasts (MC3T3-E1) were kindly provided by Clare Yellowley (University of California, Davis) and expanded in α -MEM (Invitrogen, Plainsboro, NJ, USA) supplemented with 10% FBS (JRS Scientific, Woodland, CA, USA) containing 100 units/ml of penicillin and streptomycin (Invitrogen). Collagen sponges were fabricated containing 0, 0.12, 1.2, and 12 mg of BG as described above. $5 \times$

10^5 MC3T3s in 25 μ l α -MEM were statically seeded onto each sponge and allowed to adhere for 3 h. The cell-seeded constructs were then transferred to 12-well plates with 2 ml of α -MEM containing osteogenic supplements (10 mM β -glycerophosphate, 50 μ g/ml ascorbate-2-phosphate, and 10 nM dexamethasone) and cultured on an XYZ shaker at 25 rpm. Media was changed three times per week.

For analysis, sponges were minced with a razor blade, incubated in 500 μ l of $1 \times$ passive lysis buffer (Promega, Madison, WI, USA) at room temperature for 10 min, sonicated briefly, and centrifuged at 10,000 rpm for 5 min. The supernatant was assayed for alkaline phosphatase (ALP) activity by incubating with 50 mM *p*-nitrophenyl phosphate (PNPP) in an assay buffer (100 mM glycine, 1 mM $MgCl_2$, pH=10.5) at 37°C. Absorbance was measured at 405 nm and converted to ALP activity using the extinction coefficient for PNPP ($1.85 \times 10^4 M^{-1} cm^{-1}$). To determine the amount of total DNA in each sample, double-stranded DNA was quantified using a commercially available DNA Assay (FluoReporter[®] Blue Fluorometric dsDNA Quantitation Kit, Invitrogen) following the manufacturer's instructions, and the data was used to generate results in the form of ALP units per milligram DNA.

The compressive moduli of BG-collagen composites were determined using a Bose Enduratec ELF 3200 (Eden Prairie, MN, USA). Samples were compressed between platens with a constant deformation rate of 1 mm/min. A small preload was applied to each sample to ensure that the entire construct surface was in contact with the compression plates prior to testing. Experimental moduli of sponges ($n=3$) loaded with 0.12, 1.2, and 12 mg BG were compared to moduli of sponges without BG via a Student's *t* test to determine significant differences.

Statistical Analysis

In each experiment, three samples were analyzed per condition unless otherwise specified. The values on the graphs represent means and standard error unless otherwise stated. Statistical analysis was performed using a paired Student's *t* test. *P* values less than 0.05 were considered statistically significant.

RESULTS

Mitogenic Response of HMVECs to Bioglass

HMVECs cultured in indirect contact with BG exhibited a dose-related proliferative response to the soluble products of the biomaterial (Fig. 1). We did not observe increases in HMVEC proliferation compared to GF-deficient media at dosages below 0.6 mg. BG dosages of 0.6, 1.2, and 6 mg resulted in enhanced proliferation, with the greatest proliferative response achieved with collagen sponges loaded with 1.2 mg BG. HMVEC proliferation was effectively negated at the highest BG mass (12 mg), generating cell counts comparable to GF-deficient media.

Tubule Formation in Response to Bioglass

In order to further explore the proangiogenic potential of BG, we examined the capacity of conditioned media

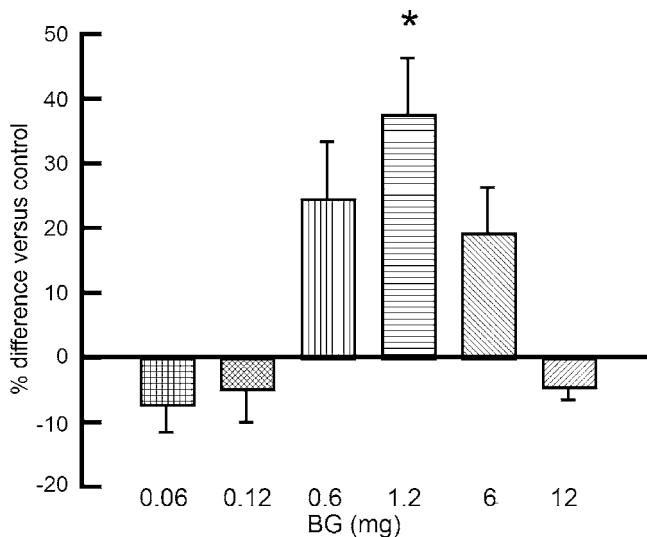


Fig. 1. Mitogenic stimulation of HMVECs achieved through indirect contact with BG. The extent of HMVEC proliferation was dependent upon the mass of BG in the collagen sponge. * $P < 0.05$ vs collagen sponge without BG (control).

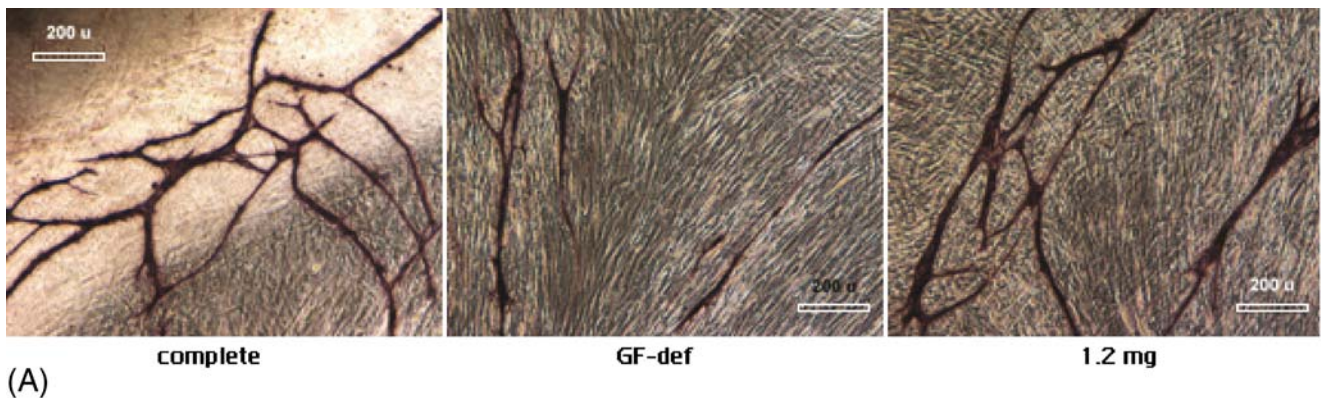
containing solubilized BG to induce tubule formation within a co-culture of fibroblasts and endothelial cells. Following immunohistochemistry for CD31, tubules were clearly visible under brightfield microscopy (Fig. 2a). Similar to the results

obtained in the endothelial proliferation assay, we observed a dose-related response of tubule formation to BG. The greatest average number of tubules was generated using 1.2 mg BG, while other masses (0.12, 0.6, and 6 mg) failed to produce enhanced tubule formation over collagen sponges lacking BG (Fig. 2b).

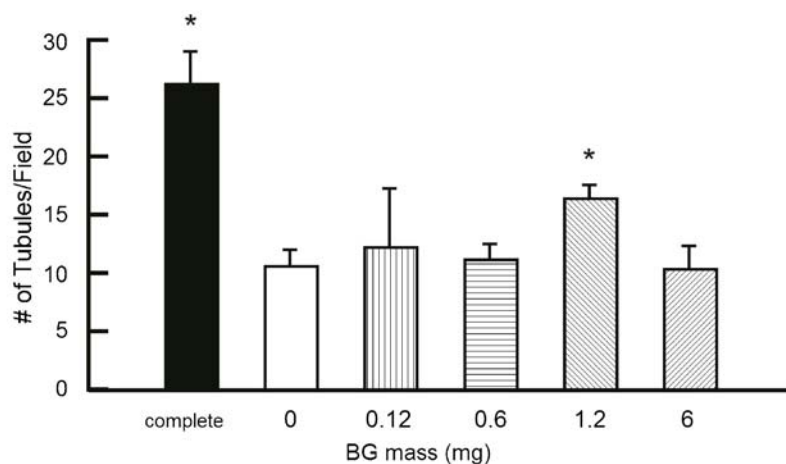
Production of VEGF in Response to Bioglass

In order to examine the mechanism of endothelial cell mitogenicity in response to the soluble ionic products of Bioglass, we measured the production of VEGF mRNA in cultured HMVECs using a commercial mRNA assay. This assay measures the production of all known VEGF splice variants and is not specific for VEGF₁₆₅. Compared to 0 and 12 mg, HMVECs exposed to 0.12 and 1.2 mg of BG demonstrated greater VEGF mRNA production after 72 h of BG exposure (Fig. 3a). HMVECs treated with 12 mg of BG exhibited comparable VEGF mRNA production to the negative control (0 mg).

To determine the presence of VEGF₁₆₅ in the conditioned media of BG-stimulated HMVECs, we measured endothelial cell proliferation induced by 1.2 mg of BG in the presence of a soluble antibody to VEGF₁₆₅ (Fig. 3b). Compared to collagen sponges containing BG, we observed a substantial inhibition of endothelial cell proliferation in the presence of BG and the antibody to near control levels ($P = 0.059$).



(A)



(B)

Fig. 2. Tubule formation assay with BG-conditioned media. **A** Extent of tubule formation was dependent upon the mass of BG in the conditioned media. **B** Quantification of tubule formation. * $P < 0.05$ vs collagen sponge without BG (0 mg BG).

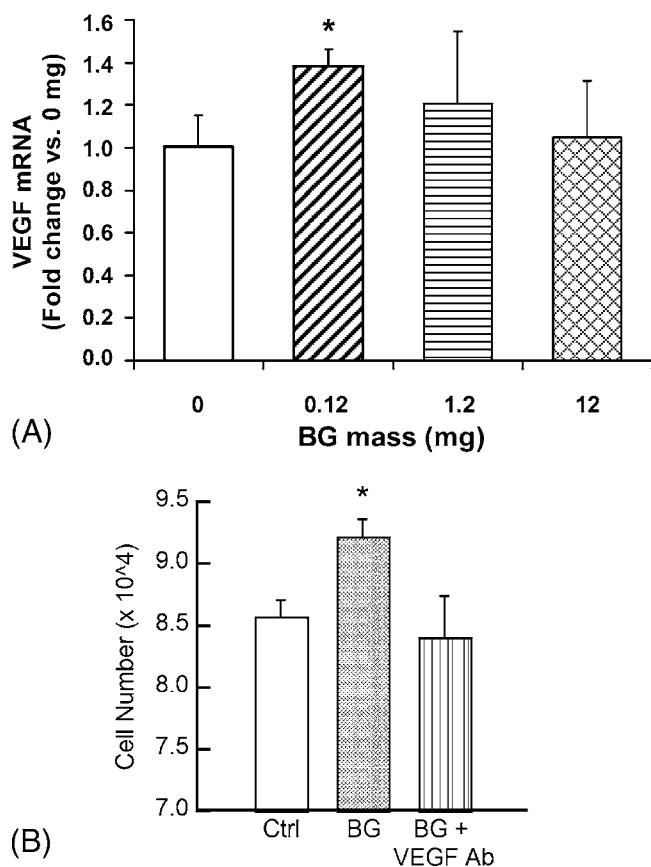


Fig. 3. BG induces the production of VEGF by HMVECs cultured in indirect contact with the substrate. **A** Quantification of VEGF gene-specific mRNA by HMVECs exposed to varying amounts of BG. **B** HMVEC proliferation stimulated by BG-collagen sponges was inhibited by a soluble VEGF antibody. * $P < 0.05$ vs control.

Osteogenic Differentiation in Response to Bioglass

Based on the reduced proangiogenic potential detected with higher masses of BG in the angiogenesis assays, we aimed to confirm that these greater concentrations were osteogenic. To determine the osteogenic potential of various BG concentrations in 3D, we seeded MC3T3 preosteoblasts on collagen sponges containing various masses of BG and quantified the production of intracellular alkaline phosphatase, an early marker for osteogenic differentiation (Fig. 4a). After one week of culture in osteogenic media, we did not detect significant differences in alkaline phosphatase production between the four groups. However, alkaline phosphatase was significantly upregulated in constructs containing 12 mg BG after 2 weeks of culture and steadily increased throughout the experiment, continually exhibiting the greatest alkaline phosphatase values. In contrast, sponges containing 0, 0.12, and 1.2 mg BG did not exhibit significant differences in the differentiation marker at any time point. When examining the gross morphology of MC3T3-seeded collagen sponges in osteogenic media, we did not observe appreciable changes in shape and size after one week of culture. However, substantial differences were clearly visible after 4 weeks of culture between sponges containing 12 mg of BG and the other sponges (Fig. 4b).

To explore the potential contribution of substrate rigidity related to the presence of BG on osteogenic differentiation, we compared the compressive moduli of BG-loaded sponges in the absence of cells. Compared to collagen sponges lacking BG (2.5 ± 0.2 kPa), compressive moduli increased upon the addition of 0.12 mg BG (2.6 ± 0.3 kPa), 1.2 mg BG (3.5 ± 0.1 kPa, $P < 0.03$ vs control), and 12 mg BG (8.3 ± 0.8 kPa, $P < 0.005$ vs each group).

DISCUSSION

Contemporary approaches to promote angiogenesis and neovascularization are primarily focused on the localized delivery of angiogenic growth factors. However, these strategies suffer from limitations including identifying the appropriate factor(s), the necessary concentration, and the duration of presentation to induce the formation of robust vessels. These drawbacks continue to motivate the exploration of alternative methods to facilitate neovascularization and perfusion of regenerating tissues. The results of these studies confirm that BG has a biphasic nature in that it possesses proangiogenic potential over a limited range of concentrations and greater osteogenic potential at higher concentrations.

Using two indicators of angiogenic potential (proliferation and tubule formation), we observed a robust proangiogenic response with collagen sponges loaded with 1.2 mg of BG (Figs. 1 and 2). Our results demonstrate that the soluble dissolution products of BG possess proangiogenic potential when delivered to endothelial cells. Previous studies have reported the proangiogenic potential of Bioglass when cultured in direct contact with fibroblasts, which subsequently produced quantifiable levels of VEGF and bFGF capable of inducing endothelial cell proliferation (18,20). However, the potential use of Bioglass as a strategy to enhance neovascularization necessitates exploring the interaction between Bioglass and vessel-forming endothelial cells. Moreover, treatment strategies such as Guided Tissue Regeneration may seek to exclude the presence of fibroblasts or other connective tissue cells from the wound bed (23), potentially inhibiting the production of angiogenic factors by these cells.

Importantly, increases in these angiogenic indicators were achieved through indirect contact with BG following exposure to its soluble ionic products and not in direct contact with this material. We pursued this delivery route to more accurately mimic the *in vivo* condition of angiogenic therapies which employ the sustained delivery of angiogenic growth factors to a localized region (24,25). Furthermore, we observed significant reductions in cell survival (data not shown) when endothelial cells were cultured in direct contact with BG, suggesting that the high concentrations of these ionic constituents initially presented to HMVECs when in direct contact could be detrimental to angiogenic processes.

In addition to HMVEC proliferation, we explored the proangiogenic potential of BG by examining its capacity to promote the generation of tubules within a co-culture of endothelial cells and fibroblasts. Co-cultures were stimulated with conditioned media from aortic rings in direct contact with varying masses of BG, and we observed a correlation between tubule formation and the mass of BG used to stimulate the aortic rings (Fig. 2) which was in good agreement with HMVEC proliferation. However, previous reports have documented the secretion of

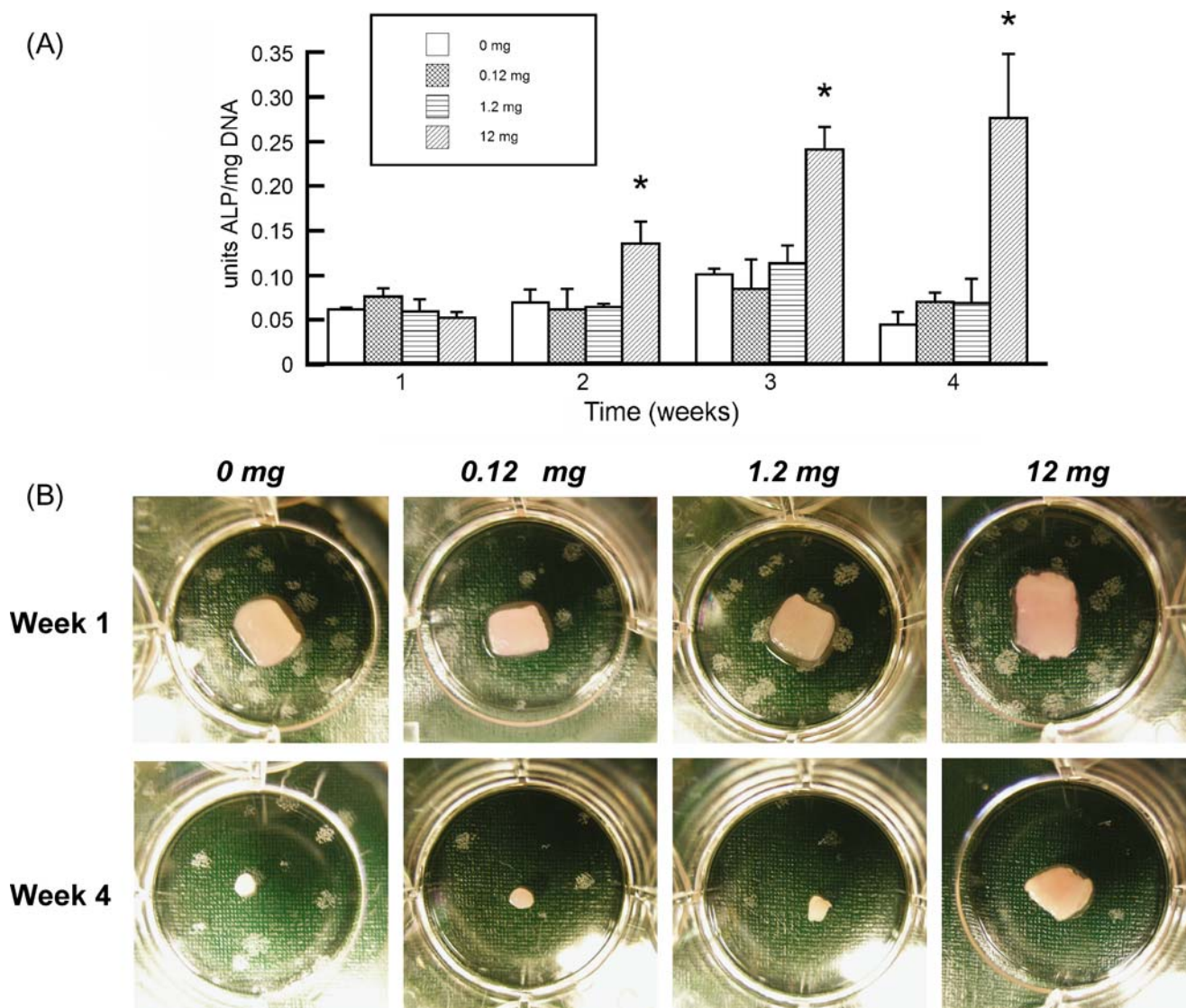


Fig. 4. Culture of MC3T3 preosteoblasts on BG-collagen sponges. **A** Alkaline phosphatase expression by MC3T3s seeded on BG-collagen sponges. **B** Differences in construct morphology were observed between sponges containing 12 mg BG and the other formulations after 4 weeks in culture. * $P < 0.05$ vs collagen sponge without BG (0 mg).

angiogenic factors (e.g. VEGF, bFGF) by fibroblasts in direct contact with BG (18,20). Based on these studies, we cannot exclude the potential contribution of fibroblast-secreted angiogenic factors in tubule formation.

The biphasic nature of BG was further explored by culturing MC3T3 preosteoblasts on collagen sponges loaded with various masses of BG. Over the course of 4 weeks, we observed increased alkaline phosphatase production only for cells cultured on sponges containing the highest BG dose. Furthermore, we did not observe a steady increase in alkaline phosphatase, a hallmark of these cells in osteogenic culture conditions, for MC3T3s cultured on sponges with lower BG amounts. Bergeron *et al.* have reported increased alkaline phosphatase production by MC3T3s cultured in indirect contact with Bioglass microspheres suspended in a collagen matrix (17). However, our findings do not necessarily demonstrate that low levels of BG are anti-osteogenic, and

we cannot attribute the differences in alkaline phosphatase production solely to the presence of BG.

Previous studies have shown that the mechanical characteristics of the extracellular matrix (i.e., its stiffness or compliance) provide vital instructional cues to numerous cells including smooth muscle cells (26), neural cells (27), endothelial cells (28,29), fibroblasts (29), and osteoblastic precursors and progenitor cells (30–33). Collagen sponges loaded with 12 mg of BG possessed a compressive modulus two- to three-fold higher than other substrates due to the presence of the bioceramics, and the inability of MC3T3s to deform sponges with high BG amounts was evident (Fig. 4b). Recent studies have demonstrated the role of substrate rigidity on MC3T3 differentiation using tunable PEG hydrogels by detecting higher alkaline phosphatase levels on more rigid substrates (32). Therefore, the role of substrate rigidity may be a contributing factor to the osteogenic response of MC3T3s

cultured on BG-loaded collagen sponges, and additional studies are necessary to clearly delineate these contributions.

Based on previous studies which have demonstrated the angiogenic potential of BG following exposure of cells through both direct (18,20) and indirect contact (19), we hypothesized that BG was upregulating the production of multiple angiogenic factors which were acting in a paracrine fashion. We examined the production of VEGF, a potent mitogen of endothelial cells, at the genetic level. We observed an increase in VEGF mRNA production for HMVECs stimulated with 0.12 and 1.2 mg BG (Fig. 3a), while 12 mg BG resulted in VEGF mRNA production comparable to control values. This is in good agreement with earlier studies which report increased VEGF production by human fibroblasts co-cultured with BG at 0.01 and 0.1% (w/v) but not at 0 or 1% (w/v) (18).

Although mRNA provides the blueprint for all proteins, it is not the last chemical step in protein formation, and gene expression does not necessarily correlate to protein produced due to various post-translational mechanisms. Our attempts to measure VEGF production using a protein-specific ELISA were unsuccessful, most likely due to the low levels of VEGF produced in our cultures. As an alternative, we examined the potential to inhibit endothelial proliferation using an antibody to VEGF₁₆₅, a logical candidate for inducing proliferation as it is one of the most prevalent isoforms of VEGF (34). We observed a profound inhibition of endothelial cell proliferation in the presence of 1.2 mg of BG and the VEGF antibody (Fig. 3b). Colon fibroblasts cultured in direct contact with BG exhibit increased bFGF production, another potent angiogenic factor (20). These data suggest that multiple angiogenic factors are likely produced by neighboring cells exposed to the dissolution products of BG. For this reason, the production of angiogenic factors by BG-stimulated cells may be advantageous compared to the local delivery of a single angiogenic protein. The benefit of locally presenting multiple angiogenic factors to enhance vessel density and maturity has been clearly observed (35). In addition, we determined that the proangiogenic response of HMVECs to 1.2 mg BG is comparable to a low rhVEGF concentration (25 ng/ml). The production of low levels of VEGF may be beneficial, as previous studies have shown that the local concentration of VEGF, and not the total dosage, modulates normal or aberrant angiogenesis (36). Moreover, these studies do not exclude the possibility that other factors which play a critical role in VEGF signal transduction cascades may be responsible for their results. For example, certain extracellular matrix molecules enhance the effectiveness of VEGF signaling, and recent studies suggest that BG mediates differential expression of ECM components such as glycosaminoglycan and collagen (37).

These studies confirm that a biomaterial-based approach can be effective to initiate angiogenesis. In addition to BG, other materials are under investigation with regards to their potential to promote angiogenesis. A direct role of copper to facilitate angiogenesis has been known for more than two decades (38,39), and the capacity of copper sulfate to promote wound healing has been linked to the upregulation of VEGF by stimulated cells, thereby increasing angiogenesis and enhancing the local oxygen microenvironment (40,41). These findings suggest promising new strategies to induce desired vascularization that could potentially be used in concert with other biomaterials.

CONCLUSIONS

The current study demonstrates the proangiogenic potential of BG and its effect on endothelial cells. Furthermore, we show that the proangiogenic response results from the secretion of at least one angiogenic inductive factor (VEGF) by cultured cells. These results suggest that a robust angiogenic response may be achievable exclusively through a biomaterials-based strategy.

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