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Effect of Bioactive Glasses on Angiogenesis: A Review of *In Vitro* and *In Vivo* Evidences

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The incorporation of bioactive glass into bone tissue-engineered scaffolds can be widely beneficial based on emerging evidence in the literature about the angiogenic potential of this material, particularly 45S5 Bioglass®. This article reviews the literature discussing *in vitro* studies which have demonstrated that increases in angiogenic indicators have been achieved through both direct and indirect contact of relevant cells with 45S5 Bioglass® particles or with their dissolution products. A few available *in vivo* studies confirming the ability of bioactive glass, incorporated into scaffolds, to stimulate neovascularization are also discussed. Suggestions for further research are given, highlighting the need for specific investigations designed to assess the effect of particular ion dissolution products from bioactive glasses and their relative concentration on angiogenesis both *in vitro* and *in vivo*.

Introduction

The difficulty to induce rapid vascular ingrowth during new tissue development is a major limitation of tissue engineering (TE) approaches for the replacement of diseased or damaged tissue.[1–5] To fully utilize the number of cells available, these need to be placed into a suitable environment, usually provided by a three-dimensional porous biomaterial scaffold that will promote cell viability and function.[6,7] Transport of oxygen and nutrients to cells in the scaffold is initially dependent on diffusion because cells more than a few hundred micrometers away from blood vessels in the surrounding tissue are destined to die because of lack of oxygen.[8] Thus, enhancement of the angiogenic potential of implantable biomaterial scaffolds is receiving much attention in TE strategies.[9–13] Neovascularization represents a critical contribution to the success of regenerating and growing new tissue because blood vessels provide growing cells with oxygen and nutrients necessary for survival. If tissue-engineered scaffolds have the ability to induce neovascularization, the viability of native or transplanted cells within scaffolds will be increased, which will enhance the possibility of engineering tissues of larger volume.

Because the angiogenic potential of most synthetic and natural materials used to fabricate tissue-engineered scaffolds is limited, insufficient, or even absent, numerous attempts have been made to enhance angiogenesis associated with tissue-engineered constructs, either by changing physicochemical properties or by supplementation with angiogenic factors.[14–16] Neovascularization may be enhanced, for example, through the controlled delivery of certain bioactive molecules, such as specific angiogenic growth factors, including vascular endothelial growth factor (VEGF).[17] Several approaches are being explored to incorporate relevant growth factors into synthetic biomaterials for use in TE.[14–16,18] However, material-processing techniques used to form such bioactive scaffolds typically involve high temperatures (in the case of inorganic biomaterials) or organic solvents, conditions that are expected to denature proteins present during the process. Because of the importance of angiogenesis in new tissue formation, there is a continuous need to develop alternative means to induce angiogenesis in tissue-engineered constructs, particularly for the regeneration of highly vascularized tissues such as bone.

There is emerging evidence in the literature (to be discussed in detail in this review) that the use of bioactive silicate glasses in biomaterial-based TE strategies may improve vascularization and bone regeneration in both healthy or highly compromised experimental models of bone healing.

Bioactive silicate glasses, such as 45S5 Bioglass® (NovaMin, Alachua, FL) (composition [in wt%]: 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5),[19] were the first man-made inorganic materials engineered to bond to bone tissue.[20] These inorganic materials provide an ideal environment for

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colonization, proliferation, and differentiation of osteoblasts to form new bone exhibiting mechanically strong attachment to the implant surface. Moreover, reactions on the bioactive glass surface induce the release of critical concentrations of soluble ions, for example, Si, Ca, and P, which has been shown to lead to favorable intracellular and extracellular responses promoting rapid bone formation.[21,22] This response is genetically controlled, with seven families of genes upregulated within 48 h of exposure of primary human osteoblasts to the ionic dissolution products of bioactive glasses.[21] These significant attributes make bioactive glasses, particularly the 45S5 Bioglass®, as one of the biomaterials of choice for development of scaffold materials for TE.[23] In this context, the chemical composition of bioactive glasses can be varied to tailor their rate of biodegradation. Moreover, the structure and chemistry of glasses, particularly sol-gel–derived glasses,[24] can be tailored at a molecular level by varying either composition or thermal or environmental processing history. It is, in principle, possible to design glasses with degradation properties and bioreactivity specific to a particular TE application.

The increasing interest of bioactive glasses in TE applications is demonstrated in Figure 1, which shows that increased number of scientific publications in the last 15 years have been found using "bioactive glass" and "tissue engineering" as keywords in a search in the Web of Science® (Thomson Reuters, Philadelphia, PA) electronic database. Recently, Hench[25] has summarized the work carried out on designing bioactive glasses for genetic stimulation of cells.

As indicated earlier, there is evidence in recent literature (to be reviewed here, considering both *in vitro* and *in vivo* investigations) that bioactive glasses not only stimulate new bone growth but also (or they dissolution products) can act as an angiogenic factor and induce increased vascularization when incorporated in tissue-engineered scaffolds. The ability of bioactive glasses to stimulate, for example, the release of VEGF from transplanted and/or host fibroblasts that have migrated into the scaffold is extremely beneficial, because the goal in TE is to induce rapid vascular ingrowth sufficient to meet the metabolic requirements of the engineered new tissue.

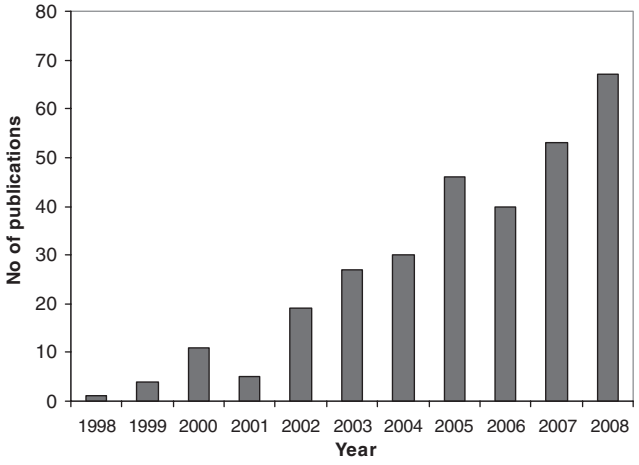

FIG. 1. Number of papers published per year in the fields of "bioactive glass" and "tissue engineering" (according to Web of Science; literature search carried out in March 2009).

The objective of this review is thus to discuss comprehensively the available evidence in the literature pointing to the angiogenic effect of bioactive silicate glasses, focusing on both *in vitro* and *in vivo* studies. Although the specific available literature is still rather limited, we believe that the timely analysis of the results achieved so far under different experimental conditions will contribute to the design of new research strategies conducive to generate further knowledge concerning the angiogenic potential of bioactive glasses. The confirmation of bioactive glasses promoting reliable neovascularization in tissue-engineered constructs will represent a major step in the challenging process of developing improved bioactive scaffolds for bone TE and with ramifications in other areas of regenerative medicine, considering the increasing relevant role that bioactive glasses are finding in TE based on their other attractive properties and versatility in terms of chemistry and surface bioreactivity[25] (Fig. 1).

In Vitro Experiments

Bioactive glass stimulates fibroblasts to secrete angiogenic growth factors

Recent *in vitro* studies have demonstrated that bioactive glasses, particularly the 45S5 Bioglass®, stimulate a significant increase in the secretion of angiogenic growth factors from fibroblasts such as VEGF and basic fibroblast growth factor (bFGF).[26–31] The effect of 45S5 Bioglass® on VEGF secretion was first assessed by Day *et al*.[26] using a rat fibroblast cell line (208F). Enzyme-linked immunosorbent assay of culture medium collected from fibroblasts grown for 24 h on surfaces coated with a colloidal suspension (0.01%, w/v) of 45S5 Bioglass® particles (<5 μm) in distilled and deionized water was found to contain significantly higher concentrations of VEGF. Figure 2 shows representative results of that study, indicating the remarkable VEGF secretion by 208F fibroblasts grown on 45S5 Bioglass®-coated cell culture plates after 24, 48, and 72 h.[26]

Additional studies by the same investigator report that increased secretion of VEGF occurred with human fibroblasts (CCD-18Co) cultured on surfaces coated with 0.01% and 0.1% (w/v) 45S5 Bioglass® compared with control fibroblasts grown on uncoated surfaces.[27] Moreover, fibroblasts grown on surfaces coated with 45S5 Bioglass® also contained increased amounts of VEGF mRNA relative to unstimulated cells as determined by a colorimetric mRNA quantitative assay that detects all known human VEGF mRNA splice variants.[27] In a related investigation, Keshaw *et al*.[31] recently reported that microporous spheres of poly (lactide-*co*-glycolide) (PLGA) containing 10% (w/w) 45S5 Bioglass® particles (mean particle size, 4 μm) stimulated a significant increase in VEGF secretion from CCD-18Co myofibroblasts consistently over a 10-day period compared with the neat PLGA (no Bioglass®) microporous spheres.

Related studies have been carried out on PLGA disks containing different concentrations of Bioglass® particles (<5 μm).[29] It was found that fibroblasts (L929) cultured on the surface of PLGA disks with 0.01%, 0.1%, and 1% (w/v) 45S5 Bioglass® particles secreted increased amounts of VEGF compared with cells cultured on PLGA alone. The most

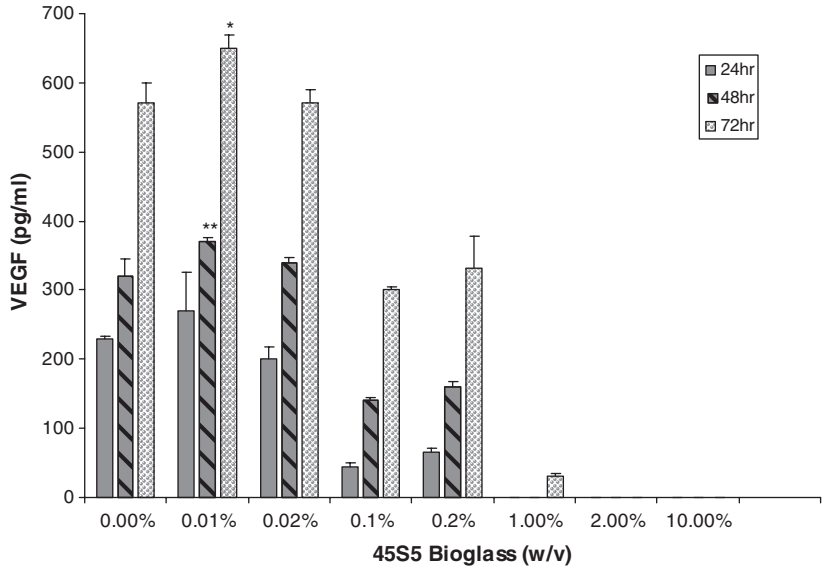

FIG. 2. VEGF secretion by 208F fibroblasts grown on 45S5 Bioglass®-coated cell culture plates. Assessment in conditioned culture medium collected after 24, 48, and 72 h. Data are the mean values of triplicate experiments. The vertical bars are the standard deviations. Reproduced from Day *et al.*,[26] with permission from Elsevier Ltd. VEGF, vascular endothelial growth factor.

consistent significant increase in VEGF secretion occurred with addition of 0.1% (w/v) Bioglass® after culturing for 48 and 72 h.[29] The key results of that investigation are presented in Figure 3, which shows VEGF secretion from L929 fibroblasts cultured on PLGA-Bioglass® composite disks for 24, 48, or 72 h.[29] Several studies have shown that concentrations of 45S5 Bioglass® greater than 0.1% (w/v) inhibited secretion of VEGF from fibroblasts possibly because of cytotoxic effects related to either increased concentration of ion dissolution products or increased pH of the culture medium associated with higher concentrations of Bioglass®.[26–28]

On the basis of the above results, Keshaw *et al*.[28] assessed the feasibility of incorporating cells and bioactive glass particles into a three-dimensional system that could be used as a bioreactor to deliver angiogenic growth factors for therapeutic angiogenesis. Human fibroblasts encapsulated in alginate beads containing 0.01% and 0.1% (w/v) 45S5 Bioglass® powder with a mean particle size of 4 μm secreted increased quantities of VEGF compared with cells encapsulated in alginate alone or with 1% (w/v) 45S5 Bioglass® particles.[28]

In addition to their capacity to promote the gene expression and protein secretion of VEGF, 45S5 Bioglass® has also demonstrated the potential to stimulate the bFGF production from human fibroblasts. bFGF secretion was significantly increased when the cells were grown on surfaces coated with 0.1–2% (w/v) 45S5 Bioglass® for periods of 24 and 48 h.[27,30]

It should be noted that the increase in secretion of VEGF and bFGF from cells cultured on 45S5 Bioglass®-coated surfaces was not associated with a relative increase in the number of metabolically active cells.[27,28] However, the inclusion of Bioglass® particles into PLGA composite disks produced the opposite effect, with an increase in fibroblast cell proliferation observed with increasing quantity of Bioglass® added.[29] The authors stated that one explanation might be attributed to better local conditions resulting from Bioglass® particles being embedded in the polymer, preventing cells from being directly exposed to the highly alkaline ionic dissolution products of Bioglass®.[29]

Bioactive glass stimulates endothelial cell proliferation

Several studies have reported the mitogenic response of human endothelial cells to 45S5 Bioglass®.[27,28,32–34] It has been shown that the presence of conditioned medium from human fibroblasts (CCD-18Co) grown for 72 h on surfaces coated with a slurry of 0.1% (w/v) 45S5 Bioglass® particles (size <5 μm) produced a 61.5% increase in the number of adult human dermal microvascular endothelial cells after 24 h of stimulation compared with cells cultured in endothelial basal medium alone. This result indicates a predominant presence of fibroblast-secreted angiogenic factors (e.g., VEGF, bFGF) in the conditioned medium which are capable of inducing endothelial cell proliferation.[27] This result is in agreement with another study in which endothelial cell proliferation was also significantly increased by conditioned medium collected from human fibroblasts encapsulated in alginate beads containing 0.1% (w/v) 45S5 Bioglass® particles.[28]

In contrast, when bovine aortic endothelial cells were plated directly on different slabs of zinc-doped 45S5 bioactive glasses,[32] a significantly higher number of bovine aortic endothelial cells were observed on samples containing 5% ZnO (wt%) compared with control (45S5 Bioglass®) and bioactive glass containing 20% ZnO. The authors stated that as endothelial cells are very sensitive to pH changes, the large and rapid pH increase caused by 45S5 bioactive glass dissolution, as well as the high Zn release from bioactive glass containing 20% ZnO (wt%), can heavily affect cell proliferation over a 6-day period.[32]

More recently, the response of human microvascular endothelial cells to the soluble ionic dissolution products of 45S5 Bioglass® released from tissue-engineered scaffolds was evaluated by Leach *et al*.[33] They reported significantly enhanced mitogenic stimulation of endothelial cells in the presence of a three-dimensional porous scaffolds made from PLGA with approximately 0.5 mg 45S5 Bioglass® coating (1.8–4.1% [w/w] of the construct mass). In related experiments, Leu and Leach[34] showed that endothelial cells

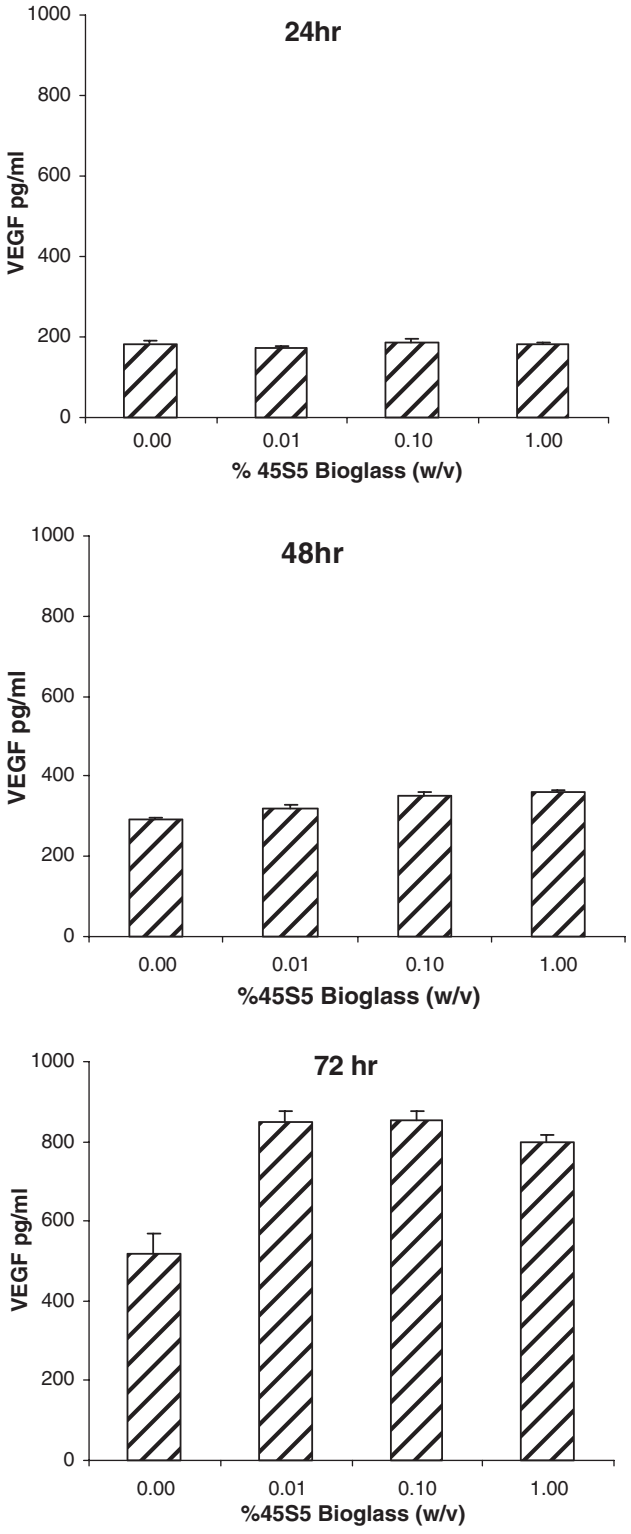

FIG. 3. VEGF secretion from L929 fibroblasts cultured on disks of PLGA containing different concentrations (%, w/v) of 45S5 Bioglass® particles for 24, 48, or 72 h. Significantly increased secretion of VEGF was observed after 48 h at 0.1% and 1% (w/v) Bioglass® and after 72 h at 0.01%, 0.1%, and 1% (w/v) Bioglass® compared with control cells cultured on PLGA alone (no Bioglass®). Adapted from Day *et al.*[29] PLGA, poly(lactide-*co*-glycolide).

cultured in indirect contact with Bioglass®-loaded collagen sponges exhibited a dose-related proliferative response to the soluble products of the constructs. 45S5 Bioglass® 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 of Bioglass® particles. In contrast, a substantial inhibition of endothelial cell proliferation was observed with sponges containing the highest Bioglass® mass (12 mg).[34] To examine the mechanism of endothelial cell mitogenicity in response to the soluble ionic products of 45S5 Bioglass®, Leu and Leach[34] studied the production of VEGF at the genetic level using a commercial mRNA assay. Compared with negative control (0 mg) and with the highest Bioglass® content (12 mg), the endothelial cells exposed to 0.12 and 1.2 mg of Bioglass® demonstrated greater VEGF mRNA production after 72 h of Bioglass® exposure. However, the aforementioned authors did not analyze which specific bioactive glass ionic dissolution product(s) causes upregulated production of VEGF. Future studies are warranted to study the response of endothelial cells to bioactive glasses at a cellular, molecular, and proteomic levels. It can be anticipated that the correct design of bioactive glass compositions will follow from understanding the direct effect of dissolution products on the upregulation mechanisms of VEGF.

Little has been done to investigate the effects of the ionic dissolution products released from other inorganic biomaterial systems on *in vitro* endothelial cell proliferation. A recent study[35] demonstrated that endothelial cell proliferation was significantly improved when treated with degradation fluid from porous strontium-doped calcium polyphosphate (CPP) scaffolds compared with the treatment by CPP degradation fluid. It was suggested that strontium, whose concentration (1.2–1.8 mg/L) was far higher in strontium-doped CPP degradation fluid than that in CPP fluid, could significantly promote the proliferation of endothelial cells human umbilical vein endothelial cells (HUVECs) although the mechanism was not clearly identified.[35] In this context, new strontium-delivering bioactive glasses[36–38] could further represent a promising biomaterial-based strategy to initiate angiogenesis and to induce desired neovascularization.

Bioactive glass stimulates formation of endothelial tubules

It has been reported that conditioned medium from human fibroblasts (CCD-18Co) grown for 72 h on surfaces coated with a slurry of 0.1% (w/v) 45S5 Bioglass® particles (<5 μm) induced a significant increase in the formation of anastomosing networks of newly formed endothelial tubules as evidenced by immunocytochemistry staining for CD31, a specific glycoprotein expressed by vascular endothelial cells.[27]

Leu and Leach[34] explored the proangiogenic potential of 45S5 Bioglass® by examining its capacity to promote the generation of tubules within a coculture of endothelial cells and fibroblasts. Cocultures were stimulated with conditioned medium from 45S5 Bioglass®-treated rat aortic rings. Similar to the results obtained in the endothelial proliferation assay, the authors observed a dose-related response of tubule formation to 45S5 Bioglass®. The greatest average number of tubules was generated using 1.2 mg 45S5 Bioglass®, whereas other masses (0.12, 0.6, and 6 mg) failed to produce enhanced tubule formation over collagen sponges without 45S5 Bioglass®.[34]

Table 1. Angiogenic Indicators Stimulated in Response to Bioactive Glass

Angiogenic indicator	*Cell line*	*Material*	*Reference*
Secretion of growth factors			
VEGF	Rat fibroblasts (208F)	BG-coated cell culture plates	26
	Mouse fibroblasts (L929)	PLGA/BG composite disks	29
	Human fibroblasts (CCD-18Co)	BG-coated cell culture plates	27
		BG/alginate beds	28
		BG/PLGA porous microspheres	31
	Human endothelial cells, human microvascular endothelial cells (HMVEC)	Collagen/BG scaffolds	34
bFGF	Human fibroblasts (CCD-18Co)	BG-coated cell culture plates	27,30
Endothelial cell proliferation	Human endothelial cells (HMVEC)	PLGA/BG scaffolds	33
		Collagen/BG scaffolds	34
	Human endothelial cells, human dermal microvascular endothelial cells (HDMEC)	Conditioned medium from human fibroblasts (CCD-18Co) and/or encapsulated in BG/alginate beds	27,28
	Bovine aortic endothelial (BAE-1)	Slabs of zinc-doped BG	32
Endothelial tubule formation	Endothelial cells/fibroblasts coculture	Conditioned medium from human fibroblasts (CCD-18Co)	27
		Conditioned medium from BG-treated aortic ring assay	34

VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; BG, bioactive glass; and PLGA, poly(lactide-*co*-glycolide).

Table 1 shows a summary of the analyzed results, providing a compact overview of the available *in vitro* evidence of bioactive glass as an angiogenic factor. The table also includes the specific material or device, incorporating bioactive glass in particulate form, which has been analyzed in each case.

In Vivo Experiments: Bioactive Glass Stimulates Neovascularization of Tissue-Engineered Scaffolds

To our knowledge, the first demonstration of the relationship between a bioactive glass porous matrix and *in vivo* vascular development was reported by Mahmood *et al*.[39] Vascularization of two geometrically different constructs, porous-ball and bundle-shaped structures, based on a fiber-form bioactive glass (composition [in mol%]: 32.24% CaO, 9.26% P_2O_5, 41% SiO_2, 17.5% Al_2O_3) combined with recombinant human bone morphogenetic protein-2 (rhBMP-2), was evaluated by mRNA expression of Flt-1 and KDR, two receptors for VEGF. The receptors Flt-1 and KDR were expressed in the porous-ball but not in the bundle-shaped scaffolds at both 2 and 4 weeks after subcutaneous implantation in rats. Illustrating the key results of the study, Figure 4 shows photographs of agarose electrophoresis gels showing the reverse transcription–polymerase chain reaction products of mRNA extracted from ball-shaped and bundle-shaped constructs at 2 and 4 weeks after implantation.[39] Importantly, the histology showed remarkably higher bone formation in the porous-ball constructs at 2 and 4 weeks than in the bundle-shaped scaffolds. However, rhBMP-2 is known to induce bone formation and previous reports have documented that rhBMP-2 also promotes vascularization.[40] On the basis of these studies, the potential contribution to vascularization induced by the localized release of rhBMP-2 from the scaffolds developed by Mahmood *et al*.[39] cannot be excluded.

Andrade *et al*.[41] conducted *in vivo* tests in which sol-gel bioactive glass-coated collagen scaffolds were placed subcutaneously in mice to evaluate angiogenic and inflammatory responses. Vascularization, as determined by hemoglobin (Hb) content extracted from implants, was higher in the glass-coated collagen implants at 14 days after

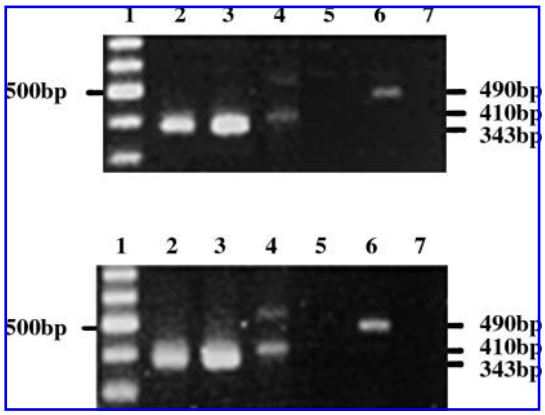

FIG. 4. Photographs of 2% agarose electrophoresis gels showing the reverse transcription–polymerase chain reaction products of mRNA extracted from ball- and bundle-shaped constructs at (a) 2 weeks and (b) 4 weeks after implantation and stained with ethidium bromide. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) bands of ball- and bundle-shaped scaffolds appearing at the 343 bp sites are observed in lanes 2 and 3, respectively. Flt-1 and KDR bands of the ball-shaped scaffold appear at the 410 and 490 bp sites in lanes 4 and 6, respectively. No detectable Flt-1 or KRD bands are seen for bundle-shaped scaffolds (lanes 5 and 7, respectively). The first lane on the left is a 100-bp marker ladder. Reproduced from Mahmood *et al*.,[39] with permission from Oxford Journals, Oxford University Press.

implantation (μg Hb per mg wet tissue: 6.0 ± 0.3) compared with the glass-free (control) group (μg Hb per mg wet tissue: 1.6 ± 0.1). One important finding of this study was that the glass-coated collagen samples did not present inflammatory cells associated with the presence of Hb as observed for the control group.[41]

Ghosh *et al*.[42] evaluated the biological response to porous (35–40% by volume) bioactive glass blocks prepared from a melt-derived glass powder of composition (in wt%) 43.70% SiO_2, 19.20% CaO, 5.46% P_2O_5, 9.40% B_2O_3, 22.24% Na_2O. Three months after implantation in a bone defect performed in the lateral aspect of diaphysis of radius bone of black Bengal goats, the bioactive glass porous blocks were seen to allow well-formed vascularization toward the implant block and bone tissue ingrowth making direct integration with a neighboring bone possible.

Using the same experimental model, Nandi *et al*.[43] described by angiography that there was well-organized trans-implant angiogenesis and establishment of vascular supply across the bone defects treated with bioactive glass porous struts of 35–40 vol% porosity, prepared from a bioactive glass powder of composition (in wt%) 58.60% SiO_2, 23.66% CaO, 3.38% P_2O_5, 3.78% B_2O_3, 1.26% TiO_2, 9.32% Na_2O.

Related results were obtained by Ross *et al*.,[44] who showed that microvessel density was significantly greater in the tissue surrounding silicone tubes coated with 45S5 Bioglass® powder (90–125 μm), compared with the tissue around uncoated tubes implanted subcutaneously in a rat model.

Previous and recent *in vivo* studies have evaluated the effect of incorporation of relatively small quantities of 45S5 Bioglass® (in particulate form) into tissue-engineered scaffolds on the angiogenic response in both soft connective and bone tissues.[26,29,31,33,45,46] In these investigations, a biodegradable polyglycolic acid mesh coated with Bioglass® particles was developed and implanted subcutaneously into rats.[26] It was shown that the composite scaffolds became infiltrated by a significantly increased number of blood vessels compared with uncoated control scaffolds. This response probably was due in part to a significant increase in the gene expression and protein secretion of VEGF from fibroblasts, as observed by Day *et al*.[26] and as previously described in the *In Vitro* Experiments section. However, unlike these original studies that demonstrated increased vascularization associated with scaffolds containing Bioglass®, polymer (PLGA) foam composites containing 0.1% (w/v) Bioglass® particles implanted subcutaneously into mice did not produce a significant increase in the number of blood vessels counted in the granulation tissue surrounding the scaffolds.[29] Similarly, a quantitative assessment of the number of blood vessels infiltrating the voids inside microporous spheres of PLGA containing 10% (w/w) 45S5 Bioglass®, placed into a subcutaneous wound model in rats, revealed no significant difference between the neat PLGA microporous spheres and those containing Bioglass® after 2 weeks of implantation.[31] Similarly, Choi *et al*.[46] observed that inclusion of 45S5 bioactive glass particulate (comprising 30% of the weight of the implant) did not result in any significant increase in the rates of fibrovascular ingrowth in porous polyethylene orbital implants in rabbits.

In this context, recent results from our laboratory have shown that 45S5 Bioglass®-derived glass-ceramic scaffolds did not produce an angiogenic response when they were placed on the chorioallantoic membrane of chick embryos.[47] It remains to be determined whether this effect is the result of suboptimal concentration of Bioglass® or it is related to the physical properties of the scaffold, such as pore dimension, pore-size distribution, interconnectivity, and pore orientation. It should be highlighted that the dose-dependent effect of bioactive glass on cell behavior (adhesion, growth), including MG-63 (human osteosarcoma cell line), A549 cells (human lung carcinoma cell line) and human bone marrow stromal cells, has been discussed in the literature[48,49] and similar effects could be operative in the mechanisms of angiogenesis. For example, Leach *et al*.[33] demonstrated that approximately 0.5 mg of 45S5 Bioglass® particles coated on a VEGF-releasing PLGA porous scaffold were capable of enhancing neovascularization in a critical-sized cranial bone defect in rats. It is also noteworthy that a significant angiogenic capacity was found for 45S5 Bioglass®-coated scaffolds lacking VEGF. Importantly, this result indicates that the mass of 45S5 Bioglass® used provides a comparable, and potentially additive, response to localized VEGF delivery.

More recently, Leu *et al*.[45] have reported greater neovascularization and bone regeneration in irradiated critical-sized calvarial defects filled with collagen sponges loaded with 1.2 mg of Bioglass® than in controls at 2 weeks post-implantation in rats.

Clearly, the evidence from *in vivo* investigations to support the effect of bioactive glasses to stimulate neovascularization of tissue-engineered scaffolds is still limited and further dedicated research is needed. In this regard, the success of the model based on the chorioallantoic membrane of chick embryos to assess angiogenesis[50,51] could see the broader application of this model in TE to investigate neovascularization induced by bioactive glass-containing scaffolds, offering a convenient platform to screen different bioactive glass compositions, particle sizes, and scaffold structures in terms of their angiogenic potential.

To our knowledge, few reports are available in the literature on the capacity of tissue-engineered scaffolds loaded with inorganic angiogenic factors, such as copper ions, to guide a vascularized wound tissue.[52–55] It has been shown that hyaluronan–copper composite hydrogels had an angiogenic potential upon implantation in rats.[52–54] More recently, Barralet *et al*.[55] compared the tissue responses with macroporous calcium phosphate scaffolds implanted in the peritoneal cavity of mice that had been loaded with either VEGF or copper sulfate. Controls without angiogenic factors exhibited only poor tissue growth within the scaffolds; in contrast, low doses (in the order of ng) of copper sulfate led to the formation of microvessels oriented along the macropore axis and enhanced wound tissue ingrowth. Surprisingly, the formation of microvessels within the pores loaded with copper sulfate did reach the same extent as that obtained by VEGF.

Conclusions and Outlook

In vitro studies have demonstrated increases in angiogenic indicators through both direct and indirect contact of cells with bioactive glass particles or with their dissolution prod-

ucts. Moreover, *in vivo* studies have confirmed the ability of certain bioactive glasses to stimulate neovascularization. The incorporation of Bioglass® into bone tissue-engineered scaffolds is thus perceived to be widely beneficial in biomaterial-based regenerative medicine strategies. Further research should concentrate on assessing the specific effect of particular ion dissolution products from bioactive glasses and their relative concentration on angiogenesis in standard *in vitro* or *in vivo* models. The results from such investigations will enable the formulation of optimal bioactive glass compositions to stimulate angiogenesis. In addition, the possible effect of scaffold morphology on neovascularization must be investigated, including porosity, pore size, interconnectivity, and pore orientation. In this context, also the morphology of bioactive glass in particulate form, for example, as addition to biopolymers to form composite scaffolds,[56] will need to be considered to design new bioactive glasses with angiogenic potential. The recent availability of nanosized bioactive glass particles[57,58] and the successful incorporation of such nanoparticles in biodegradable polymer matrices for tissue-engineered scaffolds[59,60] provide an opportunity to assess for the first time the effect of enhanced bioreactivity and degradation rate, exhibited by the nanoparticles, on neovascularization. Finally, the success of the model based on the chorioallantoic membrane of chick embryos to assess angiogenesis[50,51] could see the broader application of this model in TE to investigate neovascularization induced by bioactive glass-containing scaffolds. Overall, improved understanding of the angiogenic effect of bioactive glasses, also used as carriers of angiogenic ions, as demonstrated in the studies reviewed here, will increase the attractiveness of this material for applications in TE, which should encompass not only bone regeneration but also selected areas of soft TE.

Disclosure Statement

No competing financial interests exist.

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