Mechanism of Action of Bioactive Glass in Stimulating Angiogenesis

Authors

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Abstract

The objective of this study was to examine the mechanism of action (MOA) of how borate-based bioactive (BBGFM) glass technology, stimulates neovascularization of endothelial cells *in vitro* and *in vivo*. I*n vitro* assays (N=11, *p*-val <0.05) suggest that bioactive glass can stimulate human EPC proliferation but not cell migration or endothelial tube formation on its own. In a diabetic mouse (Db/Db <LepB>) delayed wound healing model system using a ~300 probes custom NanoString CodeSet for wound healing. Five genes were downregulated with statistical significance: *Tnfsf13*, *Faah*, *Itgax*, *Gnas*, and *Dnmt3a* (N=6, adjusted *p*-val < 0.05), while 19 genes were upregulated with statistical significance: *Ctgf*, *Vegfa*, *Jun*, *Itgav*, *Gzmb*, *Rln1*, *Il1rn*, *Shh*, *Gata4*, *Il17a*, *Il1*, *Cxcl1*, *S100a8*, *Csf2*, *Ptgs2*, *Csf3*, *Cxcl2*, *S100a9*, and *Il1a*. Most of the proteins encoded by these genes play a critical role in the extracellular matrix (ECM) by regulating angiogenesis either indirectly through stimulating macrophages (IL10, RLN) to secrete VEGF or by interacting directly with endothelial progenitor cells (EPCs) through multiple signaling pathways (including but not limited to p13k/PKB/Act, p38/MAPk, and cAMP/CREB) to influence EPC behavior and vessel formation. Porcine studies suggest that BBGFM can stimulate blood vessel formation based on CD31 staining quantitatively measured in ImageJ with a > 5-fold increase in vascular density. Our findings suggest that bioactive glass plays a very critical role in directing the angiogenic signaling mediators. This occurs through the indirect activation of macrophages as well as directly acting on EPCs via phosphorylation cascades to activate cell migration, cell stability, and new blood vessel formation.

Cell Proliferation, DB Mouse, & Porcine Model

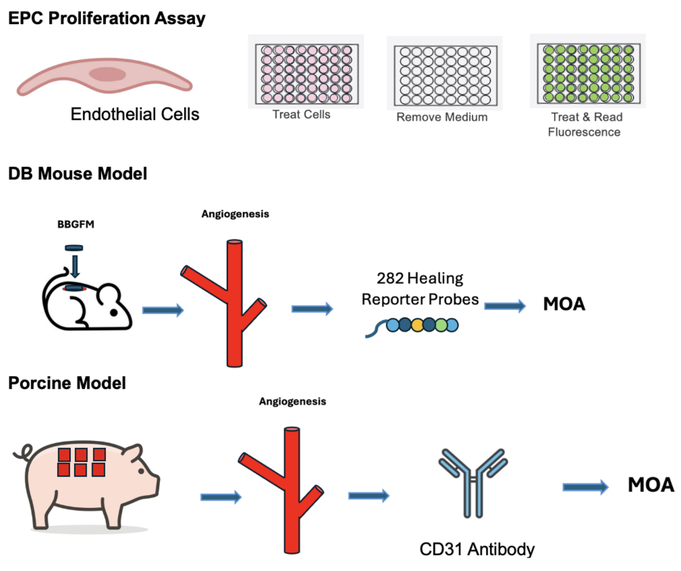


Figure 1. Overview of *in vitro* and *in vivo* cross-species studies to ascertain MOA of BBGFM. We used human EPC to examine cell proliferation, DB mice to study gene expression and a porcine model to measure blood vessel density of defects treated with borate-based bioactive glass fiber matrix (BBGFM) to stimulate angiogenesis. The results suggest that the mechanism of action of BBGFM is conserved among different species from mouse, porcine, and human.

Results: DB Mouse and Cell Based Assays

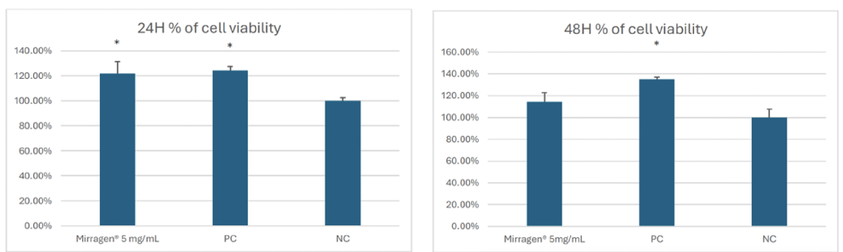


Figure 2. Endothelial Cell Proliferation with BBGFM (n=7 replicates). BBGFM stimulates endothelial cell proliferation at 24 hours with statistical significance (*p* val = 0.014) and trending significance at 48 hours (0.0555).

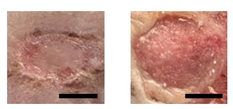


Figure 3. BBGFM-treated wound versus control (untreated) wound in diabetic mice at Day 14. Representative photos of wounds from each treatment group are shown. The BBGFM-treated wound is bright red, reflecting the formation of new blood vessels in the granulation tissue. Scale = 5 mm.

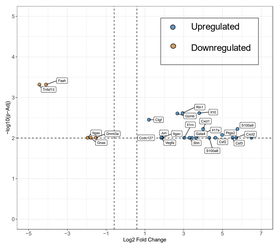


Figure 4. Volcano Plot of upregulated and downregulated genes. This volcano plot was generated on the Rosalind Bioinformatics software package using NanoString raw data. Upregulation and downregulation refer to the gene expression in BBGFM-treated wounds as compared to control wounds.

Tables

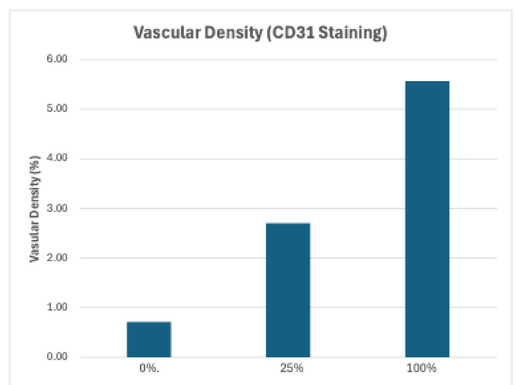
Table 1. Genes downregulated

Name	Description	Fold Change	p-Value	p-Adj
Tnfsf13	tumor necrosis factor (ligand) superfamily, member 13	-21.7263	2.34E-06	0.000481
Faah	fatty acid amide hydrolase	-17.1396	3.67E-06	0.000481
Itgax	integrin alpha X	-3.87012	0.000935	0.009846
Gnas	GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus	-3.46604	0.000441	0.009846
Dnmt3a	DNA methyltransferase 3A	-2.88154	0.000651	0.009846

Table 2. Genes upregulated

Name	Description	Fold Change	p-Value	p-Adj
Ctgf	connective tissue growth factor	2.31235	8.10E-05	0.003539
Vegfa	vascular endothelial growth factor A	3.58929	0.000858	0.009846
Jun	jun proto-oncogene	3.64094	0.000884	0.009846
Itgav	integrin alpha V	3.71974	0.000939	0.009846
Gzmb	granzyme B	6.3964	4.72E-05	0.002476
Rln1	relaxin 1	7.7023	2.87E-05	0.002426
Il1rn	interleukin 1 receptor antagonist	8.18017	0.000607	0.009846
Shh	sonic hedgehog	9.83333	0.000689	0.009846
Gata4	GATA binding protein 4	10.7585	0.000903	0.009846
Il17a	interleukin 17A	12.4627	0.000875	0.009846
Il10	interleukin 10	13.9706	3.70E-05	0.002426
Cxcl1	chemokine (C-X-C motif) ligand 1	16.0551	0.000177	0.005969
S100a8	S100 calcium binding protein A8 (calgranulin A)	19.977	0.000823	0.009846
Csf2	colony stimulating factor 2 (granulocyte-macrophage)	24.8696	0.000655	0.009846
Ptgs2	prostaglandin-endoperoxide synthase 2	31.664	0.000289	0.008412
Csf3	colony stimulating factor 3 (granulocyte)	45.9582	0.000908	0.009846
Cxcl2	chemokine (C-X-C motif) ligand 2	52.5865	0.000729	0.009846
S100a9	S100 calcium binding protein A9 (calgranulin B)	54.8945	0.000182	0.005969
Il1a	interleukin 1 alpha	90.6076	0.00051	0.009846

Figure 5. Blood Vessel Density %



Figures 5. We treated Pig Defects with 0, 25, and 100 % BBFG to determine whether we could stimulate angiogenesis based CD31 staining while using imageJ to quantify blood vessel density. 100% BBGFM had a >5 fold increase in vascular density.

Discussion and Conclusions

In vitro cell based assays with human endothelial cells suggest that BBGFM stimulates endothelial cell proliferation at the concentrations tested (1, 5, and 20 mg/ml).

Our NanoString analysis in a diabetic mouse delayed healing model suggest that BBGFM stimulated multiple pathways in macrophages and endothelial cells to promote angiogenesis. Five genes were downregulated with statistical significance: *Tnfsf13*, *Faah*, *Itgax*, *Gnas*, and *Dnmt3a* while nineteen genes were upregulated with statistical significance: *Ctgf*, *Vegfa*, *Jun*, *Itgav*, *Gzmb*, *Rln1*, *Il1rn*, *Shh*, *Gata4*, *Il17a*, *Il10*, *Cxcl1*, *S100a8*, *Csf2*, *Ptgs2*, *Csf3*, *Cxcl2*, *S100a9*, and *Il1a* (adjusted *p*-value < 0.05).

In a porcine incision model, BBGFM exhibited a concentration-dependent induction of angiogenesis based on CD31 staining utilizing quantitative analysis blood vessel density using ImageJ. The negative control had limited CD31 vasculature 0.71% while the highest concentration of the BBGFM had the most pronounced CD31 staining and a vascular density 5.57% (>5 fold) after 6 weeks. This result was consistent within the three replicates of each group.

We hypothesize that BBGFM promotes angiogenesis via a series of indirect and direct signaling pathways (**Figure 7**). Some of the effector molecules act indirectly on macrophages while others act directly on endothelial cells to stimulate cell proliferation.

It also should be noted that there are limitations with cell-based assays. The effect of boron on endothelial cells and VEGF is dose- and context-dependent. High concentrations tend to be anti-angiogenic, while lower concentrations or specific boron-containing materials can have pro-angiogenic effects, often by working with VEGF or stimulating its release. This dual effect has implications for its use both in cell-based assays and the treatment of diseases that require anti-angiogenesis and in applications like wound healing and tissue engineering that require pro-angiogenesis.

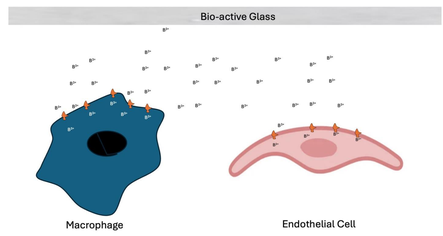


Figure 7. Indirect and Direct activation of Endothelial Cells with BBGFM.

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