

Full length article

Borosilicate bioactive glass 3D fibrous matrices with increased borate content stimulate healing cascades in chronic wounds

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ARTICLE INFO

Keywords:

Bioglass
Borosilicate
Sol-gel
Wound healing
Electrospinning

ABSTRACT

Diabetic wound regeneration is hindered by dysfunctional extracellular matrix (ECM) formation, impaired angiogenesis and prolonged inflammation. We report the first borosilicate bioactive glass (BG) nanofiber wound matrices with borate content exceeding 2 mol %, achieving up to 70 mol % borate, by optimizing modified sol-gel electrospinning parameters. The resultant 3D matrices mimic the morphology of extracellular matrix fibers with homogeneous fiber diameters of 100–300 nm that can biodegrade with the release of therapeutic calcium ions and borate and silicate species. The focus was investigation of the impact of borate content on cellular response in vitro to identify the optimal borosilicate composition; 55SiO₂-30CaO-15B₂O₃ (mol %, 55S30C15B) matrix promoted the greatest expression of vascular endothelial growth factor and basic fibroblast growth factor by fibroblasts and led to the highest stimulation metabolic activity, protein expression and migration of endothelial cells. Our in vivo study (mouse diabetic model) confirmed the efficacy of 55S30C15B wound matrix in improving wound closure, anti-inflammatory response, angiogenesis, tissue granulation (α-SMA), and collagen deposition, while elaborating the distinct roles of borates and nanofiber structure. All evidence suggests that our matrices exhibit great potential for diabetic wound regeneration.

Statement of significance: We developed a new scaffold for wound healing; the first borosilicate BG nanofiber (100–300 nm) mats with borate content in excess of 2 mol %, with boron content of up to 70 mol %. We identified the influence of borate dose delivered by the fibers on both fibroblasts and endothelial cells. Efficacy of the ECM-mimicking scaffold structure was shown across all stages of the wound healing cascade in an in vivo model.

1. Introduction

Diabetic wounds present a formidable challenge as they often fail to regenerate [1,2], which can result in amputations [3,4] and mortality, with a 60 % amputation rate [5,6]. The economic burden of individual diabetic ulcer treatments exceeds \$50k per case, and the regenerated tissue never recovers to the structural and functional integrity of the original skin [7]. Diabetic wounds typically undergo a prolonged inflammatory phase, characterized by the accumulation of pro-inflammatory cytokines and protease in the wound sites and impaired function of cells, growth factors, and the extracellular matrix (ECM) [8–10]. The neuropathy of diabetes also inhibits keratinocyte and fibroblast activities and migration, leading to reduced wound closure, tissue granulation, and ECM formation rate [11,12]. Another major

hindrance to diabetic wound regeneration is local ischemia, which results in compromised angiogenesis that impedes the supply of nutrients and oxygen [13–15].

Grafts remain the most common clinical treatment strategy but are challenged by lack of donor skin, cell death, donor site infection, pain, and loss of wound regeneration factors such as pigments, hair follicles, sweat glands, immune cells, etc. [16,17]. The cost, difficulty, and time consumption for grafting are also unfavorable. The advances in novel biomaterials have enabled the development of a more efficient affordable, and facile approach to treating diabetic wounds using simple wound matrices. Such wound matrices are expected to incorporate biological cues to address excessive inflammation, bacterial infection, ECM degradation, impaired epithelialization, and compromised vascular networks, along with physical cues that promote cell

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<https://doi.org/10.1016/j.actbio.2025.06.033>

Received 17 February 2025; Received in revised form 12 June 2025; Accepted 18 June 2025

Available online 19 June 2025

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attachment, proliferation, and migration, similar to the ECM fibrils in the dermis [18–21].

Bioactive glasses (BGs) have been popular in soft tissue repair due to their unique biodegradability and delivery of various therapeutic elements that can take part in different signaling or stimulation pathways [22–24]. These elements (e.g. Cu, Zn, Co, etc.) stimulate individual steps in the wound healing cascade, including hemostasis [25,26]; antibacterial activities [27,28]; anti-inflammation [29–31]; the up-regulation of vascular endothelial growth factor (VEGF) and basic Fibroblast Growth Factor (bFGF) from fibroblasts [32–34]; epithelialization and wound closure [35,36]; and endothelial tubule formation in vitro and in vivo [37,38]. BGs containing borate showed particularly impressive wound healing, e.g. BGs containing 2–5 mol % of borate accelerated wound closure, tissue granulation, and anti-inflammatory response compared to untreated wounds [39,40]. Compared to silica-based glass, borate-based BGs exhibited much higher glass degradation kinetics, because the B–O bonds break at a much lower pH (pH=7) than Si–O bonds (pH>9) [41,42]. Unfortunately, there has been limited direct comparison between borate-containing BGs and their borate-free counterparts, leaving the role of boron largely unexplored. Nonetheless, silica-free borate-based BG fibers have shown clinical efficacy and are marketed under the name "Mirragen®" (ETS Woundcare, Rolla).

Mirragen fibers have the composition 50 B₂O₃–6.5 Na₂O–8.5 K₂O–24 CaO–8.3 MgO–1.9 P₂O₅–0.34 ZnO–0.82 CuO (wt %). Comparing fibers and particles, the fibers promoted faster wound closure rate and vascularization rate in vivo compared to BG particles [43–47]. Mirragen fibers demonstrated clinical success in treating chronic wounds, such as diabetic foot ulcers and venous ulcers that were not healing with conventional treatments [48]. Within 12 weeks of treatment, 70 % of patients with diabetic foot ulcers were resolved with Mirragen, while those under standard treatment had a 25 % healing rate. However, as Mirragen is made from melt-spinning, its composition necessitates high levels of Na and K (to reduce melting point), which can potentially result in pain and neuropathy [49–51] or, as glass biodegrades rapidly, high local pH that is detrimental to cells [52–57]. In addition, the resultant fibers are also short, thick (>1 µm), and heterogeneous with a high level of beading and crystallization, further limiting their efficacy.

An alternative method of glass making is the sol-gel process, in which the glass network is polymerised from hydrolysed silica and borate precursors, which eliminates the need for Na and K in the glass composition [58]. Electrospinning the sol generates homogeneous BG fibers with diameters similar to natural ECM and reduced likelihood of beading or crystallization [59–61]. However, until now, sol-gel electrospinning of borate or borosilicate fibers was only feasible for glasses with low borate content (2 mol %) [39]. This may be because common thermal stabilization temperatures for BGs exceed 600 °C [62–64], but heating above 450 °C can cause crystallization of calcium borate [65, 66]. Although several studies have explored the sol-gel synthesis of borosilicate glasses using calcium methoxyethoxide as an alternative calcium source, which reduced crystallinity when calcined at 450 °C, the glasses still exhibited crystallinity when calcined at 600 °C [67–69]. Another possible reason is that borates have low solubility in both water and ethanol, and high boron content necessitates excessive amounts of these solvents, resulting in a less viscous solution that cannot be electrospun effectively.

In this paper, we addressed these challenges by developing an improved sol-gel approach that enables the synthesis of 3D ECM-mimic borosilicate BG nanofiber wound matrices with borate content up to 70 mol % (substituting silica at constant Ca content). This was achieved through investigation and optimization of the sol-gel precessing variables, e.g. stirring speed, stirring duration, and time before or after the addition of Ca source. Our resultant matrices exhibited a 3D structure with thickness higher than 1 cm and fiber diameters (100–300 nm) like that of the ECM. In vitro studies revealed that these matrices stimulated the proliferation, migration, and protein expressions of human dermal fibroblasts (HDF) and human umbilical vein endothelial cells (HUVECs).

In vivo evaluations on C57BJ/6 diabetic mice further proved the efficacy of 55S30C15B wound matrix on wound closure, tissue granulation, anti-inflammation, ECM deposition, and angiogenesis, respectively, and explored the synergistic interaction mechanism of borate ions and nanofiber structure.

2. Experimental section

2.1. Preparation and characterization of wound matrices

BG wound matrices with a composition of (70–x) mol % SiO₂–30 mol % CaO–x mol % B₂O₃ (x = 0, 5, 15, and 35, 55, 65, and 70, labeled as (70–x)S30Cx B respectively) were prepared through the sol-gel electrospinning method. All reagents were obtained from Sigma Aldrich (UK). Sol-gel precursors based on the (70–x)SiO₂–30CaO–xB₂O₃ (in mol %) system was prepared through the step-by-step addition of ethanol, water, 1 M nitric acid, tetraethyl orthosilicate (TEOS), triethyl borate (TEB), and calcium nitrate tetrahydrate (Ca(NO₃)₂•4H₂O), as detailed in Table S1 [60,70]. The resultant precursors were stirred at room temperature for 24–48 h, aged for 48–96 h dependent on the composition, and then mixed with a polyvinyl butyral (PVB) as a binder for another 1–2 h. The ratio of PVB and ethanol of the binder was adjusted based on the ethanol content in the sol-gel precursor, in order to achieve a viscosity within the range of 0.05 to 0.6 Pa·s.

The resultant mixture was loaded into a 10 mL syringe with a gauge 22 stainless steel needle with an inner diameter of 0.7 mm. The electrospinning parameters were also dependent on the composition and were generalized as the following: an electric field strength of ~10 kV, a flow rate of 0.4 mLh^{−1}, ~10 cm between the spinneret and the collector, a relative humidity ranging from 23 %–28 %, and room temperature. The retrieved matrices were then heated to 300 °C at a rate of 1 °C/min and held at that temperature for 6 h for a complete removal of the organic compound. Subsequently, the matrices were sintered under 600 °C at a rate of 1 °C/min and dwelled for 3 h before cooling naturally. BG powders were synthesized using the same sample preparation and sintering approach.

X-ray diffraction (XRD, Bruker D2 PHASER) patterns were acquired from the BG wound matrices, using Cu–Kα radiation (λ = 1.542 Å) at 30 kV and 10 mA in the interval of 10° < 2θ < 70° with a step size of 0.035° and count rate of 12 s per step. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR Nicolet iS10) was applied within the range of 500–4000 cm^{−1}. A thin layer of the matrices was sputter-coated with 20 nm chromium and characterized under the Inlens mode of an LEO-1525 scanning electron microscopy (SEM) at 5 kV accelerating voltage and an 8–10 mm working distance. The average diameters of the fibers were determined from the SEM images using ImageJ.

2.2. Dissolution test

The dissolution profiles of the resultant BG wound matrices in Dulbecco's Modified Eagle Medium (DMEM) were acquired according to previously described protocols [60,71], with 150 mg of BG wound matrices immersed in 37.5 mL of 5 mM TRIS buffer or DMEM and cultured in an incubator shaker at 37 °C and 120 rpm. 0.5 mL of the dissolution products from each sample were collected and replaced with fresh DMEM at each time point (4, 24, 48, 72, 96, and 168 h). The extracted products were filtered with a 0.2 µm surfactant-free cellulose acetate filter (Corning, USA) and diluted with 4.5 mL of water before being characterized with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) at wavelengths of 317.9 nm (Ca), 251.6 nm (Si) and 249.7 nm (B).

2.3. Biocompatibility and protein expression on fibroblasts

DMEM culture media was prepared by supplementing DMEM

(Gibco) with 10 % (v/v) fetal bovine serum and 1 % penicillin/streptomycin (FBS, Thermo Trace Ltd., Melbourne, Australia). BG-conditioned media were prepared by dissolving 200, 100, and 50 mg of BG wound matrices into 50 mL of basal media, followed by incubation in a shaker at 37 °C and 120 rpm for 24 h before filtering using a 0.2 µm sterile membrane filter. Human dermal fibroblasts (HDFs, Sciencell, USA, passage number 6) seeded in 96-well plates at a density of 1×10^3 cells per cm^2 were first cultured with fresh media for 24 h before being cultured with BG-conditioned media for 7 days. HDFs in the control group were cultured in the DMEM culture media only. The metabolic activity (MA) of fibroblasts was assessed on days 1, 3, and 7 using a Cell Counting Kit (CCK)-8 assay (Beyotime) following the manufacturer's instructions. MA was quantified as units of optical density (OD) absorbance at 450 nm using an enzyme-linked immunosorbent assay microplate reader (Synergy 2, Bio-TEK). Supernatants from the HDF cultures were collected for the characterization of Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), Platelet-Derived Growth Factor (PDGF), HIF-1 α (Hypoxia Induced Factor 1 α), and Transforming Growth Factor-beta (TGF- β) using an ELISA assay (Beyotime).

Total DNA (tDNA) was quantified using the Hoechst H33258 fluorescent dye following the manufacturer's instructions (Total DNA, Sigma-Aldrich, UK). HDFs were washed with PBS and water, lysed through 5 freeze-thaw cycles and the resultant lysates were diluted by 1:1000 with 10 % assay buffer and water. A standard curve was prepared using calf thymus DNA, with concentrations ranging from 0 to 10 µg mL^{-1} . For the measurement, 100 µL of cell lysates and standard solutions were added to a black 96-well plate, followed by the addition of 100 µL of Hoechst dye. Fluorescence was detected using a microplate reader (Fluoroskan Ascent FL, Thermo Labsystems, UK) with excitation and emission wavelengths set at 360 nm and 460 nm, respectively.

2.4. Metabolic activity, migration, and protein expressions on endothelial cells

Endothelial cell culture medium (Sciencell, USA) was supplemented with 5 % (v/v) fetal bovine serum (FBS, Thermo Trace Ltd., Melbourne, Australia) and 1 % (v/v) endothelial cell growth supplement/heparin kit (ECGS/H, Promocell). Media conditioned with HDFs was prepared by culturing HDFs with DMEM alone or with BG-conditioned media (4 mg mL^{-1}) for 3 days, followed by filtering and mixing with supplemented endothelial cell culture medium at 1:1 (labeled as DMEM/BG-HDF media). Human umbilical vein endothelial cells (HUVECs, Sciencell, USA, passage number 6) were seeded in 96-well plates at a density of 3×10^3 cells per cm^2 and initially cultured with fresh endothelial cell culture medium for 24 h before being cultured with the DMEM/BG-HDF media for 7 days. The MA of HUVECs was assessed using the CCK-8 assay, and the expressions of VEGF, bFGF, and eNOS were determined through ELISA assays on day 3.

Endothelial cell migration was assessed using the Transwell assay. HUVECs were cultured in a 24-well Transwell plate with fresh endothelial cell culture medium until they reached confluence. 250 µL of DMEM/BG-HDF media were added to the upper layer of the well, while 300 µL of supplemented endothelial cell culture medium was added to the bottom layer. After incubating for 16 h at 37 °C in a 5 % CO_2 atmosphere, non-migrated cells on the upper layer were removed using a cotton swab. Cells that migrated through the filter were fixed with 4 % Paraformaldehyde and subsequently stained with crystal violet for 30 min. Migrated cells were visualized using an inverted microscope (Leica Microsystems, Germany) and the cell population was counted using ImageJ.

2.5. In vivo diabetic wound healing assay

All animal procedures were conducted in accordance with ethical guidelines and were approved by the Ethical Committee at Northeastern

University. C57BL/6 J mice (5–6 weeks old, male) were purchased from the Huafukang Animal Center (Beijing, China) and fed for another 2 weeks. The type II diabetic model was created by intraperitoneal injections of streptozocin (Sigma-Aldrich, 50 mg kg^{-1}) to the mice for 7 days. Following established protocols, the glucose level of the mice was checked 3 times a day, in the morning, at noon and in the evening. Mice were considered diabetic when their blood glucose level stabilized at 12–15 mmol/L for one week [72]. A total of 48 diabetic mice were randomly divided into four groups, each consisting of 12 mice: the control group, the 70S30C powder group, the 55S30C15B powder group, and the 55S30C15B matrix group.

The mice were anesthetized, and the dorsal hair was shaved. A circular full-thickness skin wound with a diameter of 1 cm was cast on each mouse. The wound sites were then implanted with 100 mg of BG devices accordingly and covered with a transparent dressing (Tegaderm, 3 M) (the control group was only covered with Tegaderm). Wounds of different groups were photographed on days 0, 3, 7, 10, and 13 with a ruler included for size reference. The relative wound area was then quantified using ImageJ and expressed as a percentage of the wound area at each time point relative to the original wound area.

2.6. Histology and immunohistochemistry assay

The mice were sacrificed on days 7 and 13 respectively (6 mice per time point). Wound tissues were carefully excised and photographed from the underside to visually assess tissue granulation and the degree of blood vessel infiltration within the newly formed dermal layer. Then, removed tissues were fixed in 4 % paraformaldehyde for 48 h, followed by embedding in paraffin and sectioning into 7 µm-thick sections. The sections were then dried, dewaxed, washed, and stained using Hematoxylin and eosin (H&E) for histology analysis according to the manufacturer's instructions. The cell numbers were quantified using ImageJ at three randomly selected areas, while the wound gaps were calculated by the distances between two peripheries of wounds.

The collagen deposition of the wounds was analyzed through the Masson Trichrome staining. The sections were fixed with Bouin's solution overnight and went through Masson Trichrome staining according to the manufacturer's instructions. The collagen deposition was quantified using ImageJ based on previously reported research [73]. The blue color was segregated through 'Color deconvolution' and changed into 'black and white' figures using a threshold of 170 (Figure S1 (A)). The collagen deposition was represented by an area of staining relative to the overall area.

Immunohistochemical analysis was performed with CD31 (Abcam), VEGF (Abcam), α -SMA (Abcam), IL-1 β (wanleibio), and IL-10 (wanleibio) in order to analyze the angiogenesis, ECM deposition, and anti-inflammation effect of the wound matrix to diabetic ulcers. All antibodies were monoclonal antibodies and diluted by 1:100 with PBS. The sections were dewaxed, washed, fixed using citrate buffer solution (pH = 6), and then cultured using 3 % H_2O_2 for protein retrieval. 10 % BSA in PBS was used as a blocking solution to quench endogenous biotins or enzymes. The sections were then subjected to primary antibody staining under 4 °C overnight following the manufacturer's instruction, followed by secondary antibody staining using goat anti-rabbit antibodies and labeling using Horseradish Peroxidase. The sections were further visualized using DAB staining and counterstained with hematoxylin. Images were captured using an optical microscope equipped with a digital camera (DMI 4000, Leica). Angiogenesis was assessed by counting the number of blood vessels from the CD31 staining images. The remaining proteins were quantified using ImageJ by relative stained area to the overall area of the wound (area for protein quantifications were taken at higher magnifications). The staining area was quantified using the same approach as that used for collagen deposition. High-magnification images of the staining were processed using the 'Color Deconvolution' program in ImageJ (Figure S1 (A)), and the results were reported as the stained area relative to the total image area. All analyses were taken

from three randomly selected areas.

2.7. Statistical analysis

Data distributions were checked for normality with the Shapiro–Wilk test and for homogeneity of variance with Levene's test. A one-way analysis of variance (ANOVA) was then applied to detect overall differences among groups, followed by Dunn's multiple-comparison post-hoc procedure to define whether certain groups are significant from the others. A p value < 0.05 was considered statistically significant.

For *in vitro* assays, symbols indicate: * $p < 0.05$ vs DMEM; @ $p < 0.05$ vs 70S30C; # $p < 0.05$ vs 65S30C5B; & $p < 0.05$ vs 55S30C15B. For *in vivo* evaluations, symbols denote: * $p < 0.05$ vs control; @ $p < 0.05$ vs 70S30C powder; # $p < 0.05$ vs 55S30C15B powder. All statistical analyses were performed in GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Sample sizes for each experiment are provided in the corresponding figure legends.

3. Results

3.1. 3D ECM-mimicking borate-based bioactive glass wound matrices

Seven formulations of BG wound matrices, with B_2O_3 concentration ranging from 0 to 70 mol %, were successfully produced through this improved approach. Sol-gel process variables optimized included stirring speed, stirring duration, and time before or after the addition of Ca

source. Electrospinning variables optimized to promote stronger whipping and bending of the sol included operating voltage and spinneret-to-collector distance. All matrices had total fiber matrix thickness greater than 1 cm (Fig. 1(a)), which was defined as being a 3D structure from previous research [60,74], with long and homogeneous fibers whose diameters fell within 96–320 nm (Fig. 1(b), Table S2), mimicking the collagen and fibronectin fibrils in the ECM. The incorporation of B_2O_3 up to 70 mol % did not interrupt fiber morphology, but fibers with >35 mol % B_2O_3 were much thinner. This was attributed to that change in BG: PVB ratio as more PVB was needed in the binder to balance the increase of ethanol in the sol.

XRD patterns (Fig. 1(C)) indicated that BGs with <35 mol % borate or less displayed an amorphous halo around 20° – 34° (2θ) and no obvious crystal peaks. Fibers with 55 mol % borate or greater exhibited XRD peak around 26.4° which corresponded to calcium borate [66]. BGs with >65 mol % borate exhibited more peaks of calcium borate at 28.75° and 32.8° . They also exhibited a significant peak around 29° , which can be a combination of calcium carbonate (where carbon comes from PVB) and calcium silicate [75]. The minor peaks 31 – 33° were likely due to polymorphs of calcium carbonates (e.g. aragonite), calcium borate, or boron carbide [66].

FTIR spectra of the fibers (Fig. 1(d)) showed presence of Si-O bonding, with bands at 1040 cm^{-1} (vibration of Si-O bond), 790 cm^{-1} (vibration of Si-OH bond), and 470 cm^{-1} (Si-O-Si rocking) [76–78]. FTIR spectra also indicated successful incorporation of boron into the BG microstructure, consistent with previous literature [67–69,79], with all

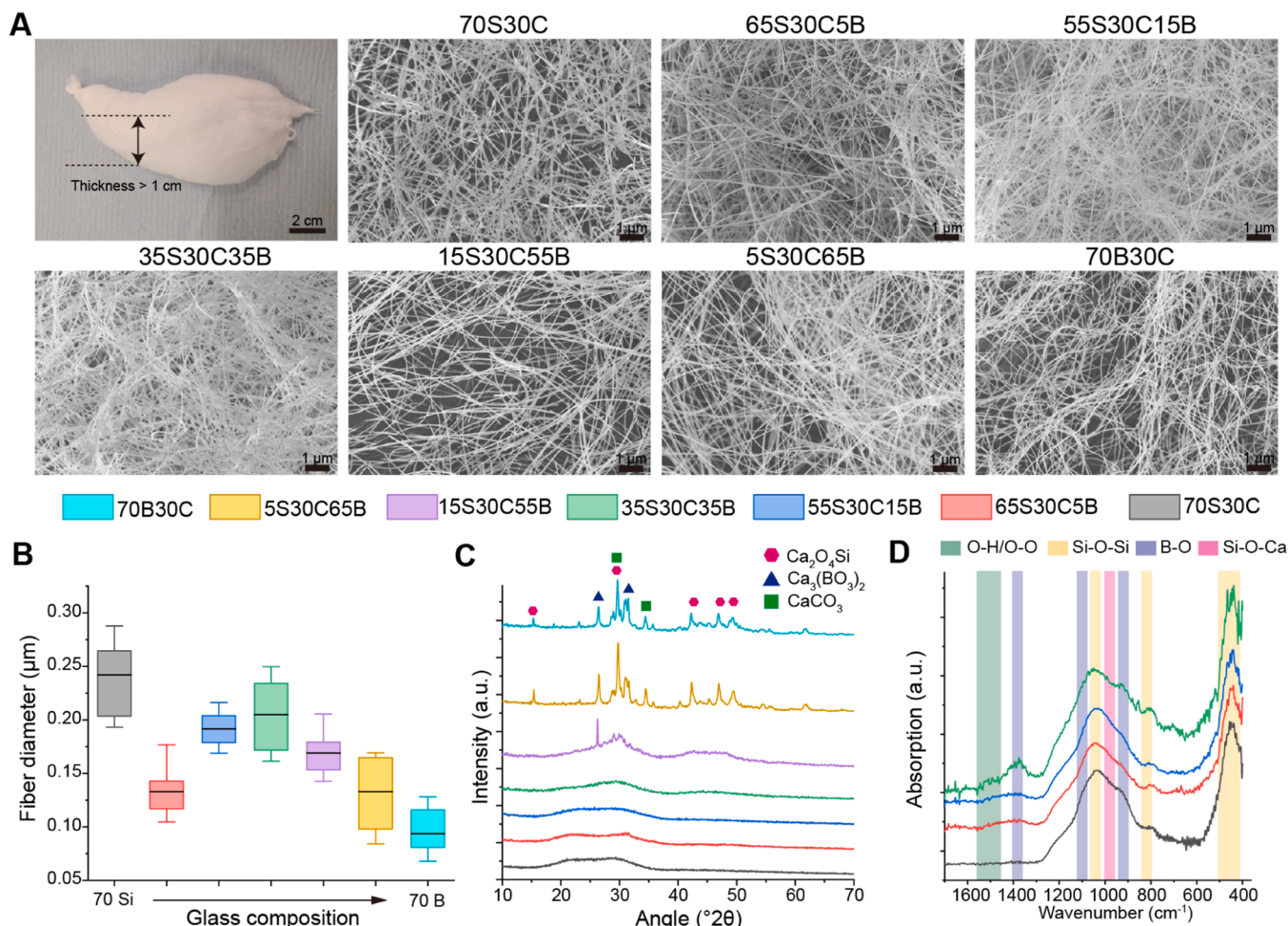


Fig. 1. Characteristics of bioactive glass nanofiber wound matrices: (A) Photograph and SEM images of spun matrices with glass compositions of $(70-x)\text{SiO}_2\text{-}30\text{CaO-xB}_2\text{O}_3$ in mol % ($x = 0, 5, 15$, and $35, 55, 65$, and 70), labeled as $(70-x)\text{S}30\text{C}x\text{B}$; (B) Fiber diameters; (C) XRD patterns and (D) FTIR spectra of $(70-x)\text{SiO}_2\text{-}30\text{CaO-xB}_2\text{O}_3$ (mol %, $x = 0\text{-}70$) matrices.

spectra having bands at 925 cm^{-1} (Si-O-B vibration) and 1095 cm^{-1} (B-O vibration), but only 35S30C35B had a small band around 1400 cm^{-1} (B-O-B stretching) [80,81]. This indicated that for the glasses with borate content $<35\%$, most borate groups were locked within silicate groups (Si-O-B bonding only).

3.2. Biodegradability of wound matrices

BG wound matrices exhibited a sustained release of therapeutic ions including Si (expected to be in the form of SiO_4^{4-}), Ca (Ca^{2+}), and B (expected to be in the form of BO_3^{3-}) in DMEM (Fig. 2(a)). Large structures of the BG matrices were not seen 2 days after the immersion, but some BG still remained even on day 7. SEM images revealed the presence of micro-scale BG fiber clusters that retained the intertwining structure of the nanofibers (Figure S2 (A–D)). Although the XRD pattern (Figure S2 (E)) revealed signs of mineral deposition, the SEM images revealed no obvious signs of large mineral formation, indicating that minerals may deposit due to the static environment during the dissolution studies and are less likely to form in real-life dynamic situations.

The release of Si and Ca from BG matrices remained relatively steady, while B release increased over time. Si release from B-BGs was higher than that from Si-BGs for the first 48 h, even though the Si content in the glass was lower, while 35S30C35B had the highest Si release on day 7 (Fig. 2(a1)). Similarly, Ca release from 35S30C35B was also the highest for the first 96 h (Fig. 2(a2)). B release at all time points seemed proportional to the B content in BGs. The pH release followed a trend consistent with the release of Ca and B, where the highest B content in the BG stimulates the highest pH (Fig. 2(a4)). While there has not been a unified consensus regarding the therapeutic levels of these elements, we selected 3 BG doses with relatively high, medium, and low ion concentration levels and further explored their impact on the viability and protein expression of fibroblasts in vitro.

3.3. In vitro metabolic activity assessment on fibroblasts

Human dermal fibroblasts (HDFs) were cultured with media conditioned with doses of 4, 2, and 1 mg mL^{-1} of BG wound matrices. On day 1, 70S30C media of all different doses induced higher MA of HDFs (Fig. 2 (b1, c1, d1)). 65S30C5B medium slightly reduced the MA, while the other B-BGs showed no significant difference compared to the control. On day 3 and day 7, all media conditioned with 4 and 2 mg mL^{-1} BGs reduced the MA of HDFs (Fig. 2 (b1, c1)), with 65S30C5B prompting the lowest MA, followed by 35S30C35B, 55S30C15B and finally 70S30C. A lower dose (1 mg mL^{-1}) of BG, except for 35S30C35B, induced a similar MA compared to the control (Fig. 2(d1)). Although HDF proliferation was negatively impacted by the BG media, the tDNA assay (Fig. 2(e)) revealed that the reduction in cell proliferation was not as pronounced as the 10-fold decrease indicated by the MA assay.

3.4. Protein expression from fibroblasts

The expression of 5 proteins associated with wound healing and angiogenesis were assessed, Platelet-derived growth factor (PDGF), Transforming growth factor-beta ($\text{TGF-}\beta$), HIF-1 α , VEGF, and bFGF, to study to role of BG matrices on HDFs in vitro. All 4 mg mL^{-1} BG media stimulated the expression of PDGF compared to the control (Fig. 2(b2)). The PDGF expression levels among all B-BGs were similar and lower than those of 70S30C. In addition, 2 and 1 mg mL^{-1} BG media had little impact on the PDGF expressions from HDFs, some even exhibited adverse effects (Fig. 2 (c2, d2)).

Dose of 4 mg mL^{-1} 70S30C and 65S30C5B significantly reduced $\text{TGF-}\beta$ expression from HDFs (Fig. 2(b2)), while that of 55S30C15B and 35S30C35B were similar to the control. In contrast, 2 mg mL^{-1} 70S30C and 65S30C5B media induced higher $\text{TGF-}\beta$ levels than the control, while 2 mg mL^{-1} 55S30C15B and 35S30C35B media resulted in lower

levels of $\text{TGF-}\beta$ (Fig. 2(c2)). 1 mg mL^{-1} of BG did not promote $\text{TGF-}\beta$ expression compared to the control.

All doses of BG stimulated the expression of HIF-1 α (Fig. 2 (b2, c2, d2)). Media conditioned with 4 mg mL^{-1} BG stimulated less overall HIF-1 α compared to other doses but given the slight reduction in cell population (Fig. 2(e)), the average HIF-1 α per cell for 4 mg mL^{-1} BG might be the highest. 4 mg mL^{-1} of 70S30C up-regulated the expressions of VEGF and bFGF from fibroblasts (Fig. 2(b2)), while the borate containing BGs further stimulated the expressions of VEGF and bFGF compared to DMEM and 70S30C. 55S30C15B stimulated the highest expression of both VEGF and bFGF from HDFs, while glass with higher B content (35S30C35B) presented an adverse effect on VEGF and bFGF expression. All 2 and 1 mg mL^{-1} media had little impact on VEGF and bFGF expressions, likely due to reduced ion levels. In general, although 4 mg mL^{-1} BG reduced the metabolic activity of HDFs, it did not significantly impact the cell population but stimulated the highest VEGF and bFGF expressions. Combined with cellular studies on boric acid (Figure S3), it can be deduced that $\sim 250\text{ }\mu\text{g mL}^{-1}\text{BO}_3^{3-}$ level was optimal in promoting highest growth factor expressions with little cell death, whereas higher BO_3^{3-} ($>300\text{ }\mu\text{g mL}^{-1}$) led to cytotoxicity and lower BO_3^{3-} ($<150\text{ }\mu\text{g mL}^{-1}$) did not significantly up-regulated protein expression compared to the control. 4 mg mL^{-1} was deemed the most suitable dose and was further applied to in vitro studies on endothelial cell activities.

3.5. In vitro endothelial cell activities

BG wound matrices are hypothesized to stimulate angiogenesis through a combined effect of (1) the angiogenic factors released from fibroblasts and (2) the role of released ions directly on the proliferation, migration, and protein expressions of endothelial cells. To investigate this combined effect, supplemented BG-HDF conditioned media, that were pre-cultured with HDFs for two days, were applied to endothelial cells to assess their impact on HUVEC viability and protein expressions. On day 1 and day 3, MA increased with B content in BG at 5 mol % and 15 mol % but decreased when B content reached 35 % (Fig. 3(A)). This agrees with the trend on the VEGF and bFGF expressions from HUVECs (Fig. 3(B)). Endothelial Nitric Oxide Synthase (eNOS) expression from 70S30C–HDF was significantly higher than the control but that from B-BG-HDF media was not significantly different. On day 7, MA for all media was similar, with only 55S30C15B-HDF media being distinctly higher. It can thus be deduced that BO_3^{3-} stimulated MA and protein expressions of HUVECs until reaching $125\text{ }\mu\text{g mL}^{-1}$, at which point it started exhibiting adverse effects.

HUVEC migration was assessed using ELISA assay. Within 16 h, all BG-HDF media stimulated more migration of HUVECs than the DMEM-HDF control media (Figure (C)). 55S30C15B stimulated the greatest HUVEC migration. In general, the 55S30C15B wound matrix exhibited little inhibition on the fibroblast MA, while promoting protein expression from fibroblast that further stimulated the HUVECs proliferation and migration. Therefore, this composition was further analyzed for its efficacies and functional mechanisms in vivo.

3.6. In vivo diabetic wound closure

In vivo wound healing assessments were conducted on the STZ-induced diabetic C57BL/6 mouse model to evaluate the healing efficacy of the wound matrix and understand the impact of BGs on individual healing steps. As shown in (Fig. 4 (a, b)), the 55S30C15B matrix induced faster wound healing compared to the 55S30C15B powder, followed by the 70S30C powder and finally the control. Exudates started appearing on day 3, with their levels following the same trend as the wound healing rate. On day 7, both the control and 70S30C powder groups continued to suffer from exudates, whereas wounds treated with 55S30C15B powder or matrix exhibited a thin layer of epithelialization,

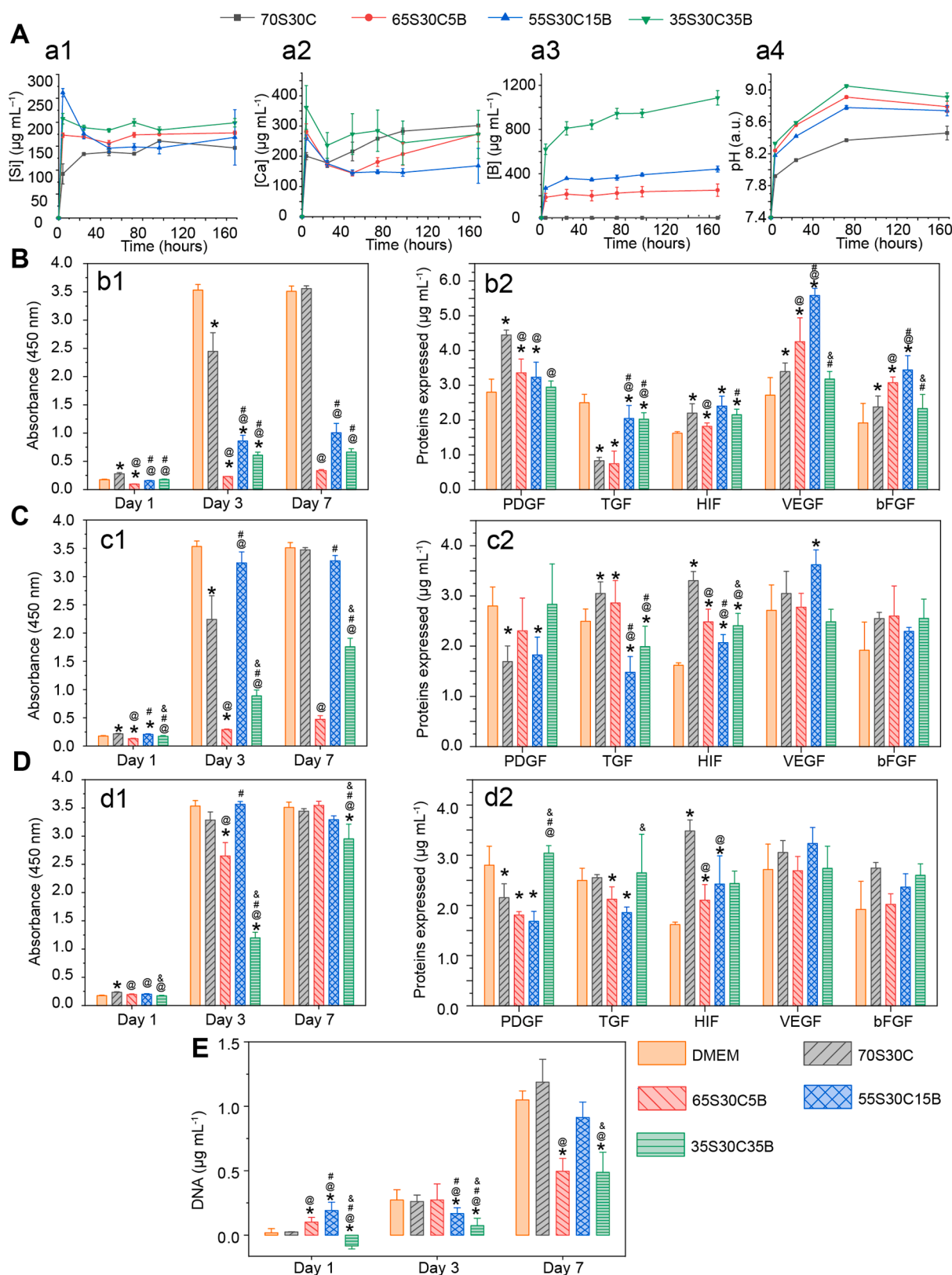


Fig. 2. Ion release from wound matrices and their impact on HDF viability and protein expression. (A) elemental concentration profiles (ICP) in DMEM following immersion of 70S30C, 65S30C5B, 55S30C15B, and 35S30C35B wound matrices over 7 days; (B–D) effect of dissolution products of BG wound matrices on HDF metabolic activity (MA) at days 1, 3, and 7, and protein expressions on day 3 at doses of (B) 4 mg mL⁻¹, (C) 2 mg mL⁻¹, and (D) 1 mg mL⁻¹. (E) Proliferation (in total DNA $\mu\text{g mL}^{-1}$) of HDFs exposed to 4 mg mL⁻¹ BG dissolution products at days 1, 3, and 7. (* $p < 0.05$ compared to DMEM group; @ $p < 0.05$ compared to 70S30C group; # $p < 0.05$ compared to 65S30C5B group; & $p < 0.05$ compared to 55S30C15B group).

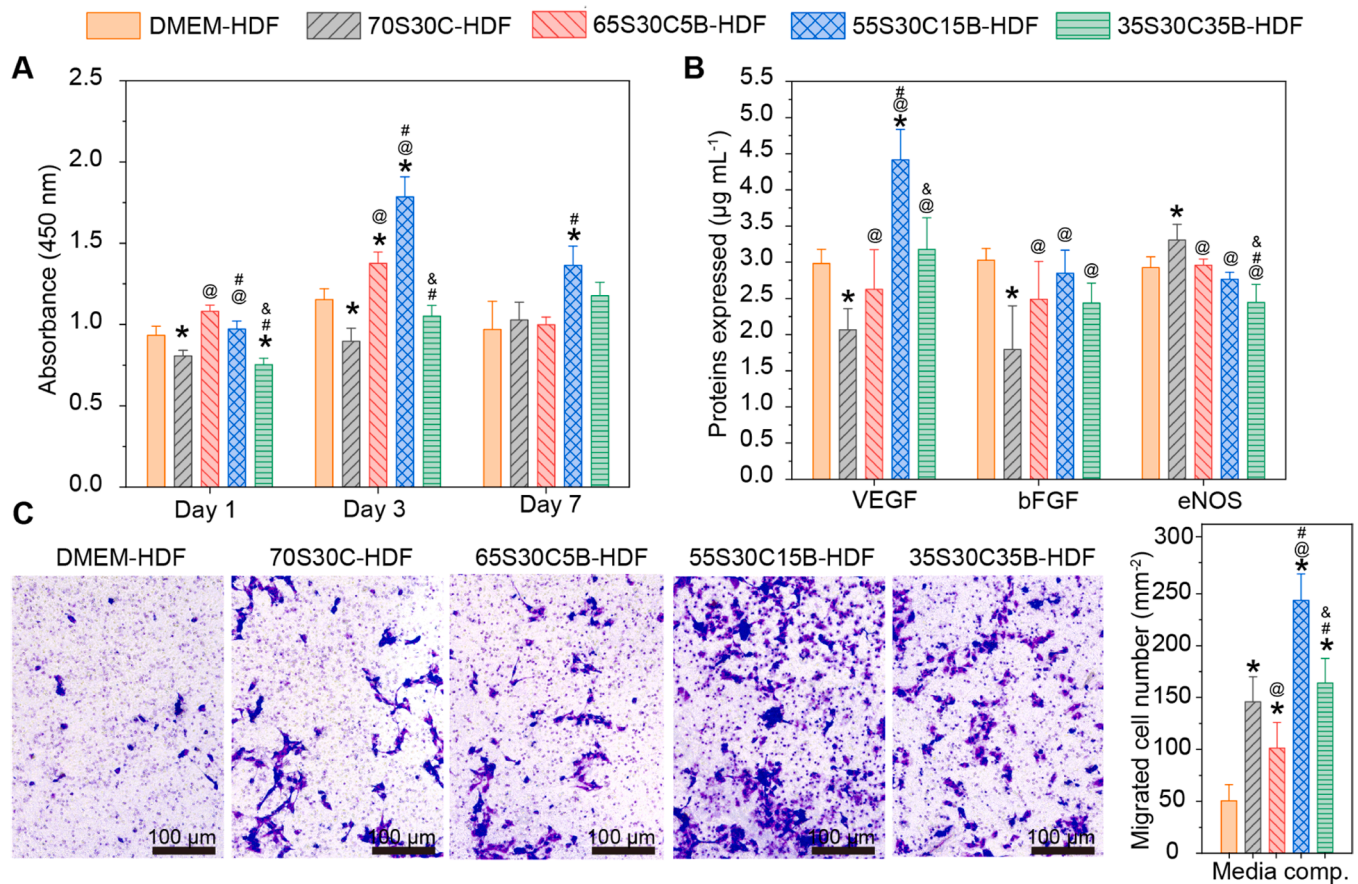


Fig. 3. In vitro culture of HUVECs in media conditioned with HDFs and BG wound matrices/DMEM (labeled as BG/DMEM-HDF media). (A) Metabolic activity of HUVECs at days 1, 3, and 7; (B) Protein expression of VEGF, bFGF (FGF-2), and eNOS expression at day 3; (C) Images and cell number of migrated HUVECs (Transwell assay) after being cultured for 16 h with BG/DMEM-HDF media. (* $p < 0.05$ compared to DMEM group; @ $p < 0.05$ compared to 70S30C group; # $p < 0.05$ compared to 65S30C5B group; & $p < 0.05$ compared to 55S30C15B group).

which may indicate the anti-inflammatory effects of B-BGs. All BG-treated groups reached the wound contraction stage on day 10, although high levels of keratinocytes were observed at the edge of the wound. The changing color of the skin also indicated that the mice went into the hair follicle regeneration phase, which may lead to even faster wound healing. The control group healed much slower and merely reached the final stage on day 13.

Half of the animals were sacrificed on day 7 and the other half on 13, and the dermis layers of the removed tissue were characterized under an optical microscope to assess the blood vessel formation/infiltration (Fig. 4(c)). On day 7, the wounded dermis of the control group appeared significantly larger and thicker than other groups, with minimal signs of blood vessel infiltration. The wound dermis for BG-treated groups were thinner with exudates visible from the back of the skin, but there were obvious signs of blood vessel infiltration. This may indicate the transition from the 'inflammation' to the 'proliferation' phase in the healing cascade, where capillary networks start growing in the wound area to replace infiltrated arteriole (larger blood vessels with a thin smooth muscle layer). By day 13, BG-treated wounds exhibited thickness similar to that of intact tissues and a well-developed capillary network. In comparison, wounds of the control group remained thicker and poorly vascularized.

3.7. Histology analysis

The histology of the wound areas was analyzed through H&E and Masson Trichrome staining. H&E staining was conducted to study the levels of epithelialization, wound gaps, and cell number. On day 7,

neoepidermis formed in wounds treated with 55S30C15B powder or matrix, whereas not in the control or those treated with 70S30C powder (Fig. 5(A)). On day 13, the epidermis layer from the control group remained detached from the dermis layer, separated by a clot of keratin and fibrin. The wound gaps on both day 7 and day 13 (Fig. 5(C)) were derived by calculating the distance between the two peripheries of the wound. Untreated and 70S30C treated wounds were larger than the maximum field of view of the microscope and were defined by stitching adjacent images. The trend in wound gaps at both time points agrees with the trend on wound sizes (Fig. 4 (A, B)).

Mass infiltration of cells with the formation of a fibrin clot was observed in BG-treated wounds on day 7 (Fig. 5(A)), while fibrin clots were barely formed in untreated wounds. Wounds treated with 70S30C powder had the strongest cell infiltration probably because of the mass macrophage infiltration that may indicate an ongoing 'inflammation' phase. For wounds treated with 55S30C15B powder or matrix, cells were highly concentrated at the center of the wound, while less concentrated and more uniformly distributed at the wound edges. The morphology of the cells at wound edges is also more similar to that of the fibroblast (elongated rather than round in macrophages). These findings may indicate a transition from the 'inflammation' phase to the 'proliferation' phase at the wound edges. On day 13, the cell population in all groups was indistinguishable and the cell distribution was uniform, seemed to be primarily fibroblasts.

The Masson Trichrome staining was applied to analyze the collagen deposition (light blue) and alignment in the dermis layer. Fig. 5(b) revealed that the control group at day 7 had the least amount of collagen, as the wound area was highly occupied with macrophages

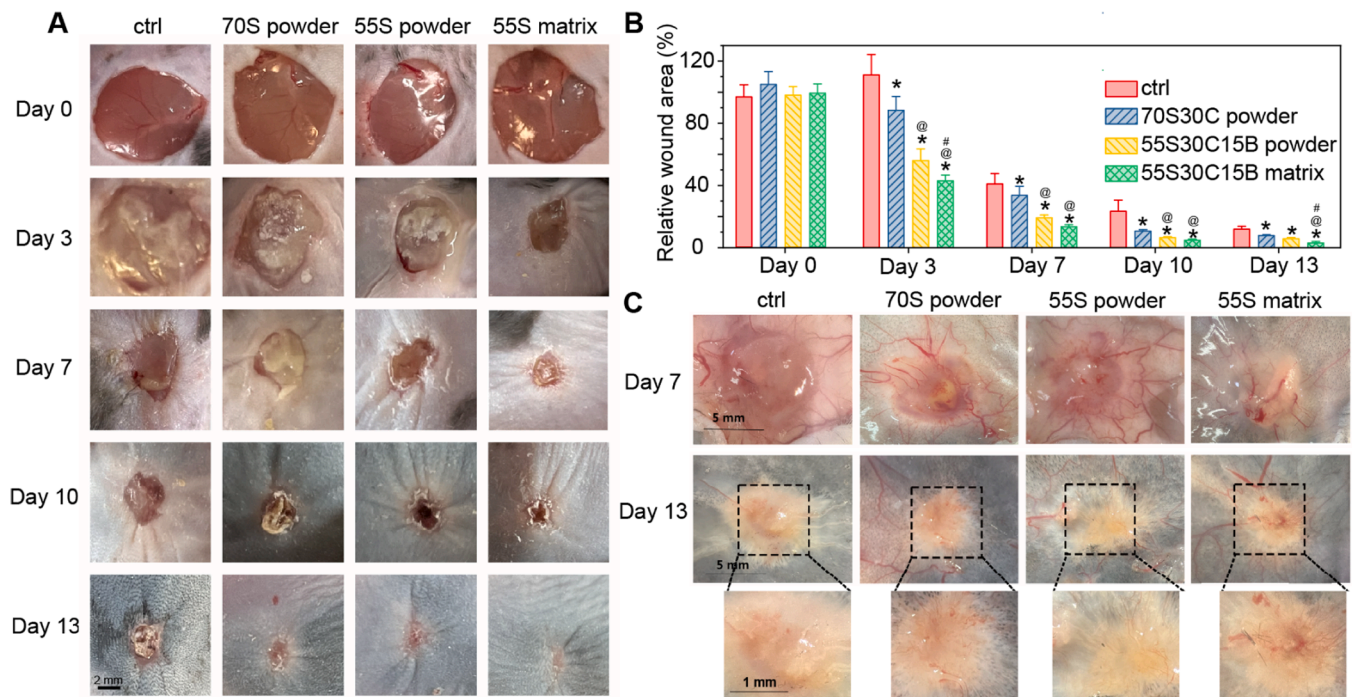


Fig. 4. In vivo wound closure on diabetic C57BJ/6 mice treated with 70S30C powder, 55S30C15B powder, and 55S30C15B wound matrix. (A) Images of regenerated wound area and (B) Quantification of wound area on days 0, 3, 7, 10, and 13 post-surgeries compared to original wound area taken; (C) Optical images of relative wound size and angiogenesis of the sacrificed skin tissue of different treatment groups after 7 and 13 days post-surgery. (* $p < 0.05$ compared to ctrl group; @ $p < 0.05$ compared to 70S30C powder group; # $p < 0.05$ compared to 55S30C15B powder group).

(purple dots) and infiltrated adipose tissue (large white space within frames of collagen fibrils) from the hypodermis. Quantification of collagen deposition was conducted on images with higher magnification that display only uniform tissues using the color deconvolution method on ImageJ. BG-treated wounds exhibited more collagen deposition than the control (Fig. 5(d)), with 55S30C15B matrix stimulated the highest collagen deposition. On day 13, all wounds exhibited collagen fibrils that horizontally aligned across the dermis layer (Fig. 5(B)), but their densities were much less than wounds treated with the 55S30C15B matrix, indicating that 55S30C15B matrix had the most effective stimulation of collagen deposition in diabetic ulcers.

3.8. In vivo anti-inflammation evaluation

The anti-inflammatory effects of the BGs were characterized through the immunochemical staining of IL-1 β (inflammatory cytokines) and IL-10 (anti-inflammatory cytokines). On day 7, wounds in the control group exhibited the highest IL-1 β and lowest IL-10 (Fig. 6 (a, b)) expressions compared to the wounds treated with BGs, consistent with the level of chronic exudates observed in wound images (Fig. 4(a)). There were no significant differences in IL-1 β expressions between wounds treated with 70S30C powder, 55S30C15B powder, and 55S30C15B matrix, but both 55S30C15B powder and matrix induced higher IL-10 expressions than 70S30C powder (Fig. 6 (a, b)). On day 13, both IL-1 β and IL-10 expressions were similar among all wounds, with most of these expressions located at the peripheral blood vessels and follicles. This study may indicate that BO_3^{3-} ions contribute to the up-regulate expression of anti-inflammation cytokine in vivo during the inflammatory phase, while the nanofiber structure showed little anti-inflammatory impact.

3.9. In vivo angiogenesis evaluation

The angiogenic effect of the BG samples was analyzed through in vivo VEGF expressions and the number of blood vessels per unit area

(highlighted through CD31 immunohistochemistry). On day 7, high levels of VEGF were expressed from fibroblasts in the BG-treated wounds (Fig. 7(a)) while wounds in the control group only exhibited VEGF surrounding the infiltrated adipose tissue (adipose stem cells can also express VEGF, but not at high level). The incorporation of boron further stimulates the VEGF expressions, with 55S30C15B matrix promoted less VEGF expressions than 55S30C15B powder. 55S30C15B matrix promoted less VEGF expressions than 55S30C15B powder. On day 13, the control group induced the highest level of VEGF expression, followed by the 70S30C powder group, 55S30C15B powder group, and 55S30C15B matrix group (Fig. 7(c)). This may indicate that the angiogenesis in the BG-treated wounds was almost complete, while that in the untreated wounds was still ongoing. 55S30C15B matrix merely induced half of the VEGF expressions than 55S30C15B powder, which suggests that the vessel formation for the 55S30C15B matrix may be fully complete.

There were few blood vessels in untreated wounds, except for those that infiltrated from the hypodermis (Fig. 7(b)). Significant vascularization was observed in wounds treated with BGs (red arrows), with B-BGs exhibited an even higher angiogenic effect (Fig. 7(d)). It can be deduced from the in vitro studies that B stimulated the angiogenic factor expressions from both fibroblasts (Fig. 2) and endothelial cells (Fig. 3). The discrepancy between the 55S30C15B powder and 55S30C15B matrix groups was not significant. On day 13, BG stimulated an almost 2-fold increase in blood vessel formation compared to the control. The discrepancy among BG-treated wounds was not significant probably because these wounds reached the maximum level of angiogenesis.

3.10. In vivo tissue granulation evaluation

On day 7, minimal α -SMA expressions were observed in the control group (Fig. 8(a)). 70S30C powder stimulated higher myofibroblast formation, predominantly at the bottom half of the dermis layer. 55S30C15B powder stimulated higher α -SMA expressions than 70S30C powder with a more uniform distribution across the dermal layer, with

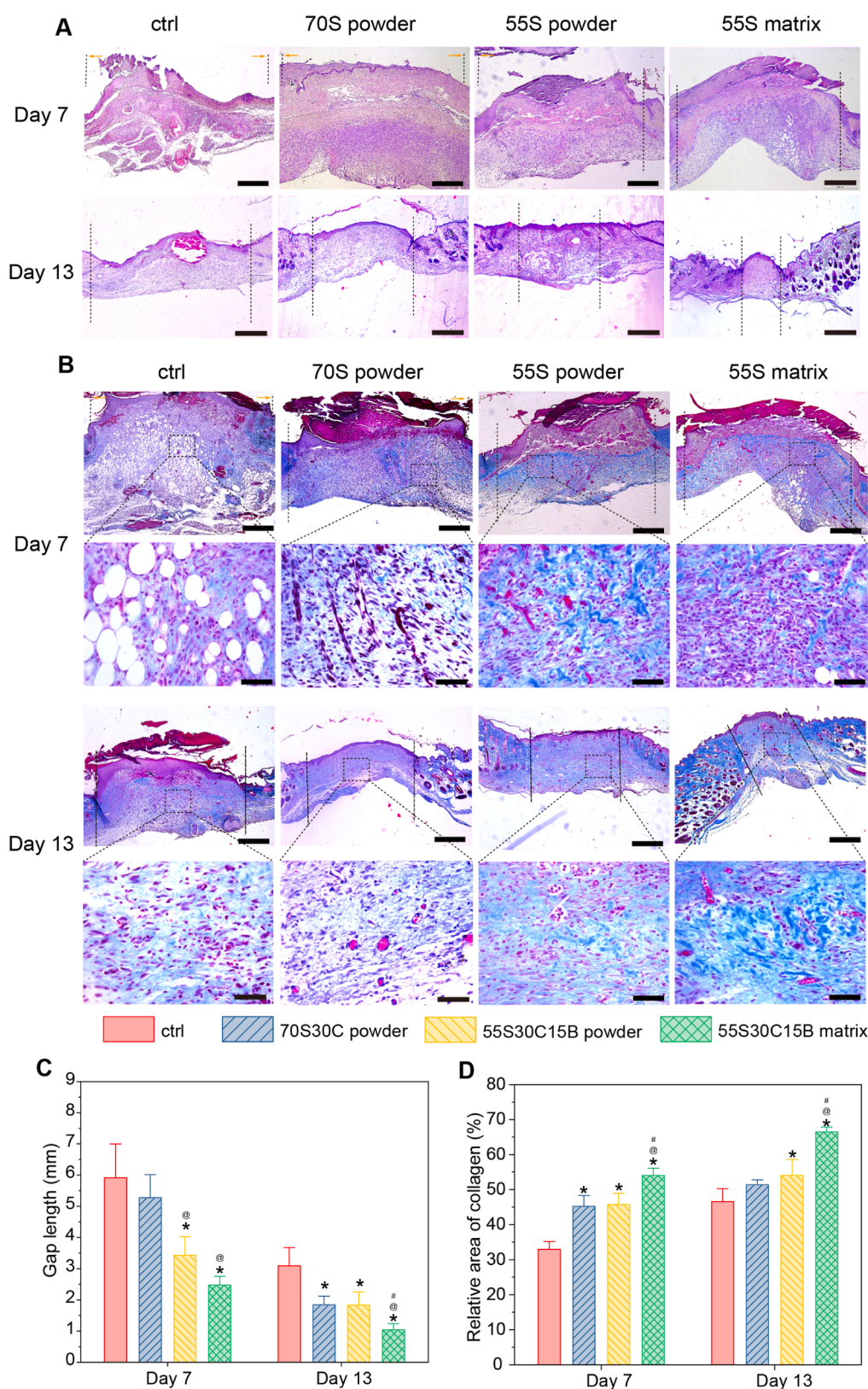


Fig. 5. Histology analysis of wounds treated with 70S30C powder, 55S30C15B powder, and 55S30C15B wound matrix. (A–B) Images of (A) H&E staining and (B) Masson Trichrome staining on days 7 and 13 post-surgery; (C–D) Quantification of (C) The length of the wound gap derived from H&E staining and (D) The relative area of collagen deposition derived from Masson Trichrome staining. Bar size = 100 μ m for (A) and 1st and 3rd row of (B); Bar size = 25 μ m for 2nd and 4th row of (B). (dashed lines indicate the location of the wound edges, orange arrows indicate that the wound edge was out of the image, * $p < 0.05$ compared to ctrl group; @ $p < 0.05$ compared to 70S30C powder group; # $p < 0.05$ compared to 55S30C15B powder group).

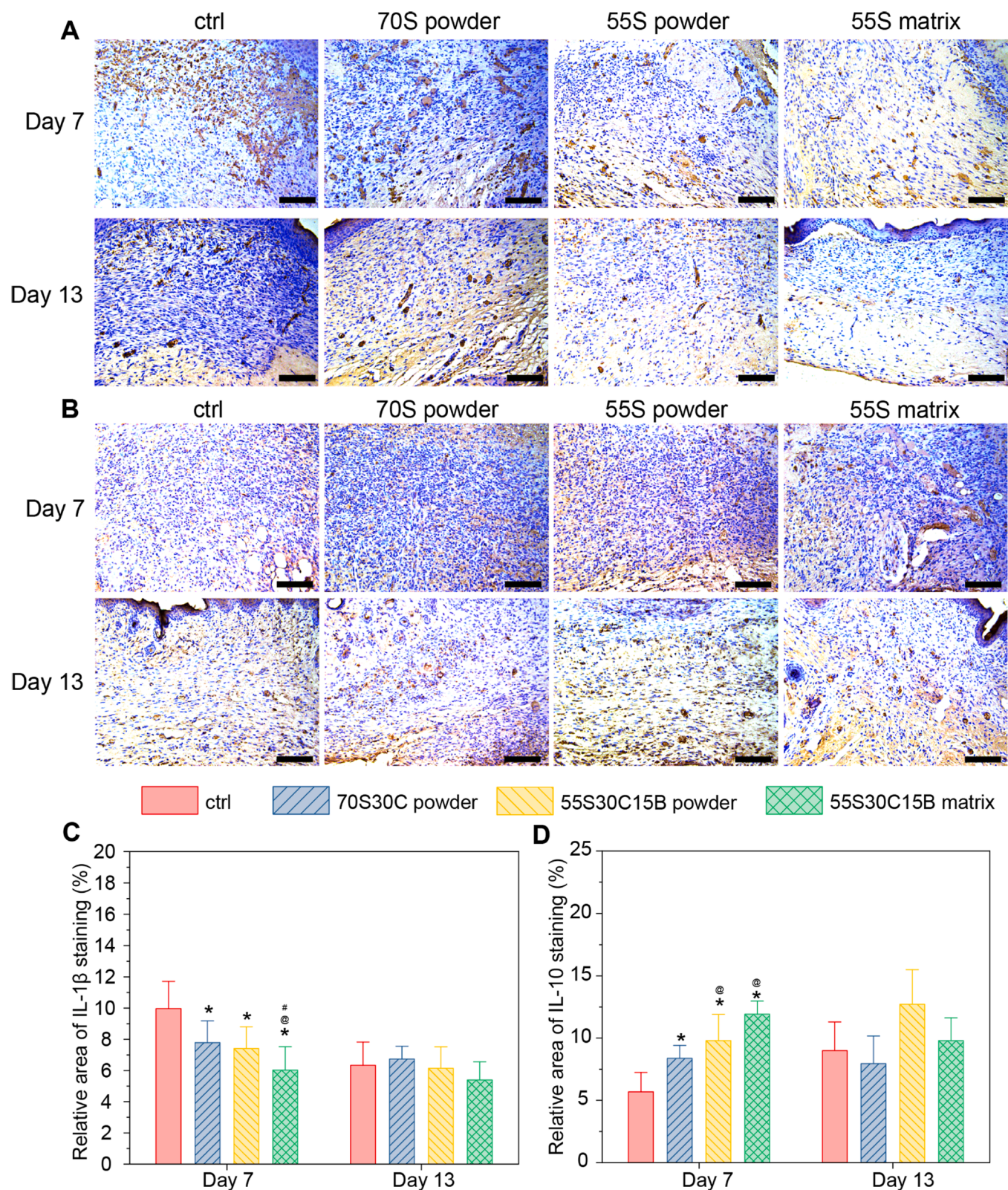


Fig. 6. Anti-inflammatory effect of wounds treated with 70S30C powder, 55S30C15B powder, and 55S30C15B wound matrix. (A-B) Images of (A) IL-1 β immunohistochemistry staining and (B) IL-10 immunohistochemistry staining on days 7 and 13 post-surgery; (C-D) Quantification of (C) IL-1 β and (D) IL-10 relative area from immunohistochemistry images. Bar size = 50 μ m for (A) and (B). (* p < 0.05 compared to ctrl group; @ p < 0.05 compared to 70S30C powder group; # p < 0.05 compared to 55S30C15B powder group).

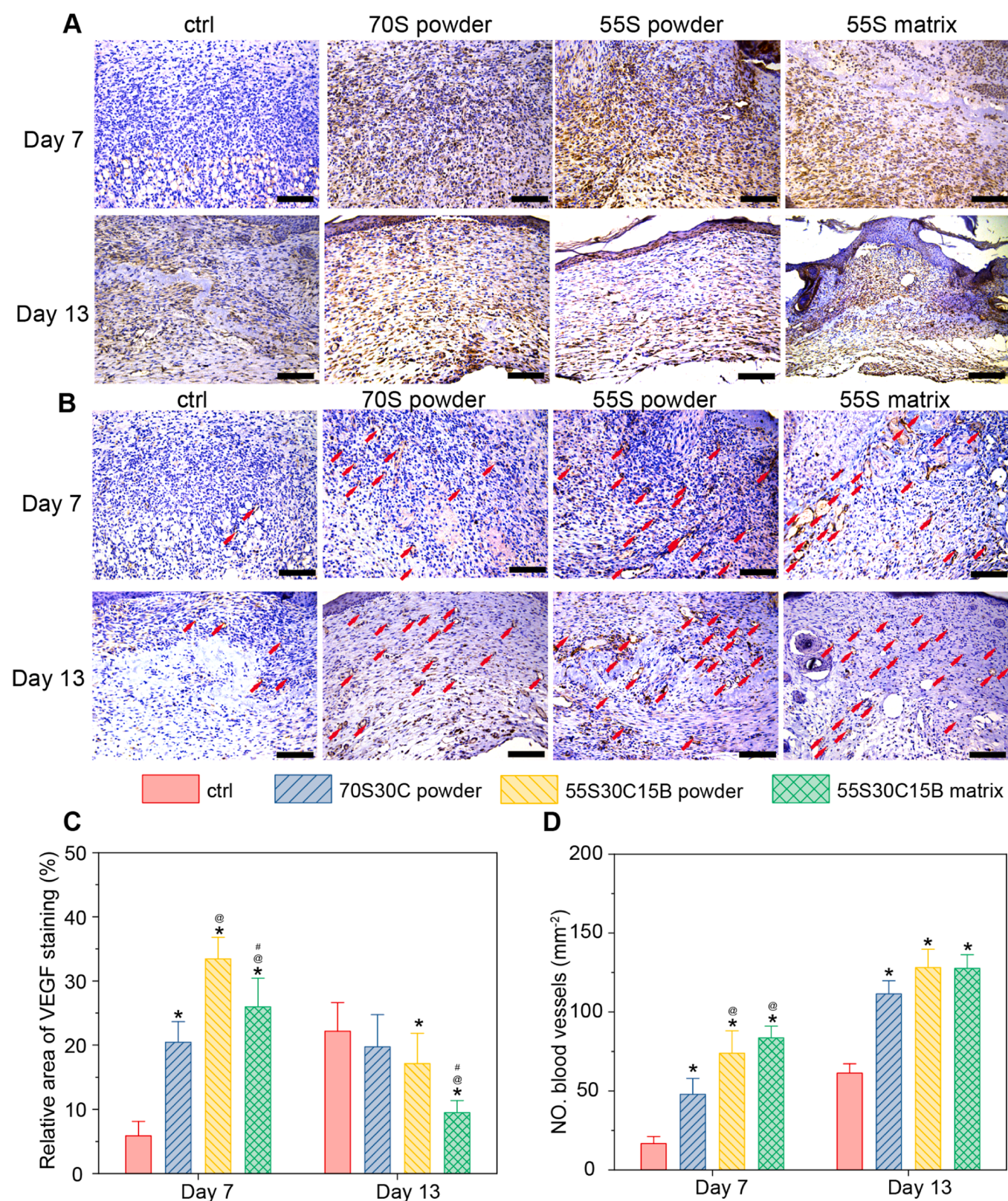


Fig. 7. Angiogenesis of wounds treated with 70S30C powder, 55S30C15B powder, and 55S30C15B wound matrix. (A-B) Images of (A) VEGF and (B) CD31 immunohistochemistry staining on days 7 and 13 post-surgery. (C-D) Quantification of (C) VEGF per mm² and (D) Number of blood vessels per mm² from immunohistochemistry images. Bar size = 50 µm for (A) and (B). (**p* < 0.05 compared to ctrl group; @*p* < 0.05 compared to 70S30C powder group; #*p* < 0.05 compared to 55S30C15B powder group).

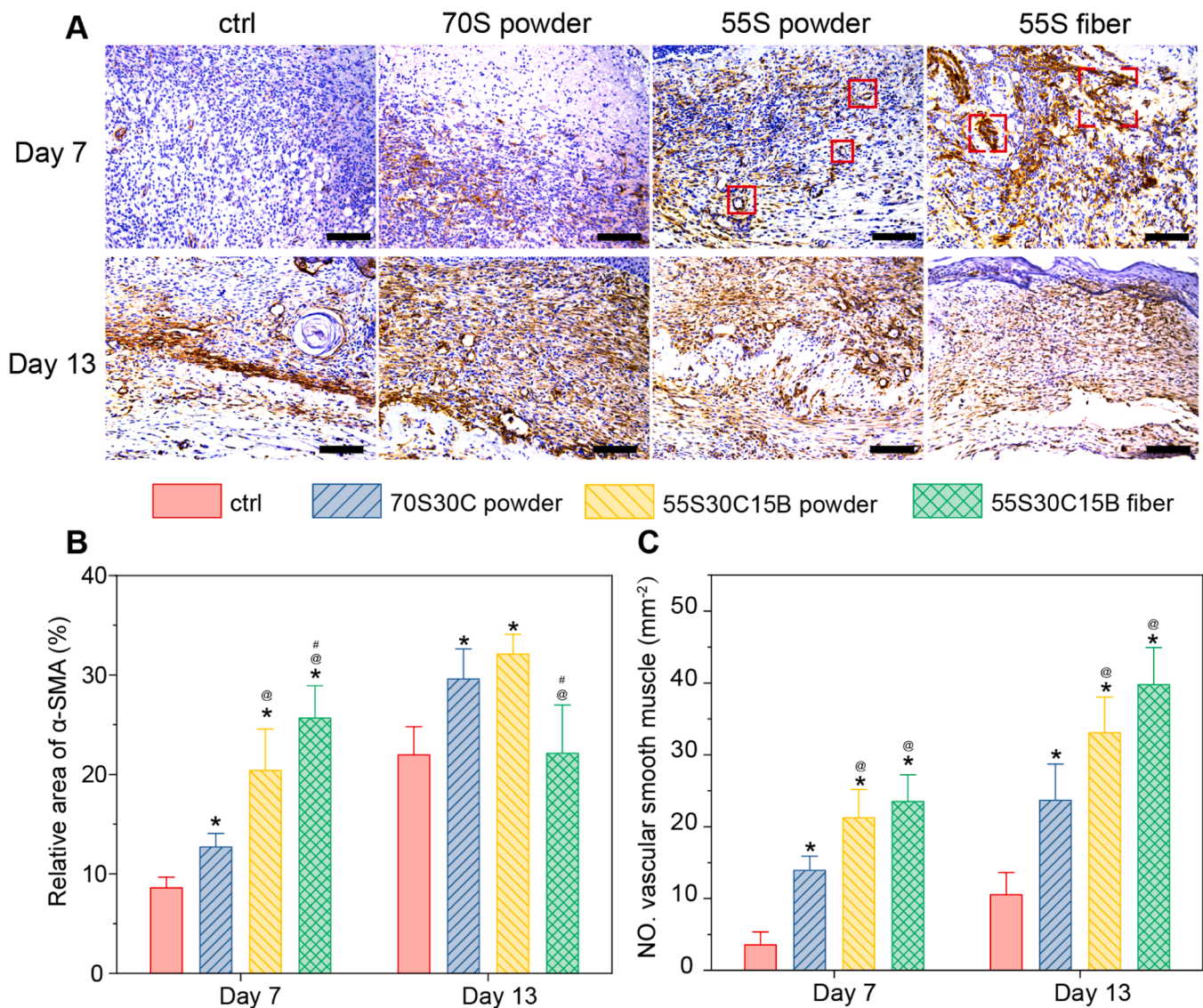


Fig. 8. Tissue granulation and vascular infiltration of wounds treated with 70S30C powder, 55S30C15B powder, and 55S30C15B wound matrix. (A) Images of α -SMA immunohistochemistry staining on days 7 and 13 post-surgery; (B) Quantification of α -SMA relative area in percentage and (C) Number of vascular smooth muscle per mm² derived from α -SMA immunohistochemistry. Bar size = 50 μ m for (A). (* p < 0.05 compared to ctrl group; @ p < 0.05 compared to 70S30C powder group; # p < 0.05 compared to 55S30C15B powder group).

55S30C15B matrix stimulated the highest α -SMA expression (Fig. 8(b)). On day 13, wounds treated with 55S30C15B matrix seemed fully closed so that the α -SMA levels were lower compared to that in wounds treated with 70S30C and 55S30C15B powder. Wounds in the control group had much slower wound contraction and therefore exhibited the lowest level of α -SMA.

α -SMA is also commonly expressed in the vascular smooth muscle of arterioles or larger blood vessels [82,83]. In the context of vascular smooth muscle, α -SMA forms a closed loop, while in myofibroblasts, it is scattered within the pericellular matrix of the cells. The numbers of large blood vessels in different animal groups were derived from the immunohistochemistry analysis (Fig. 8(c)). The control group had the least vascular infiltration. Wounds treated with 70S30C powder had much more vessels, but all failed to infiltrate the upper dermis layer. Both 55S30C15B powder and 55S30C15B matrix induced a thorough blood vessel infiltration, characterized by α -SMA loops parallel to the wound surface, which indicates that these arterioles were formed inside the wounds rather than infiltrated from the hypodermis. The same trend was found on day 13, with 55S30C15B powder or matrix stimulating the

highest arterioles formation and the control group the least.

4. Discussion

Previous research focused on borosilicate BG fibers with thicknesses over 1 μ m, including melt-derived fibers with high Na/K content or sol-gel-derived fibers with low boron content [39,48]. Several obstacles lie ahead in the effort to prepare BG nanofibers with high boron content, which we have overcome to produce BG wound matrices. First, to prevent crystallization of calcium borate during thermal stabilization, sol parameters such as the stirring speed, stirring duration, and time before or after the addition of Ca source were carefully adjusted to ensure that individual borate groups were effectively integrated among multiple silicate groups, as evident from the FTIR spectrum (Fig. 1); a schematic is included in Figure S4. Viscosity and electrospinning parameters, such as operating voltage and spinneret-to-collector distance, were optimized to promote stronger whipping and bending of the sol, further enhancing the fiber separation (SEM, Figure S5). Secondly, increased solvent volume with an increase in B content due to the low solubility of borate

groups has been overcome. An ideal viscosity value, in contrast to a wide viscosity range reported in previous literature [60], has been proposed and used as a standard to balance solvent volume in the binder and sol-gel crosslinking time with the change of solvent volume in sol (SEM, Figure S5). The impact of electrospinning parameters, such as the voltage and working distance, was also studied (Figure S6). Thirdly, humidity has been a long-standing parameter affecting fiber thickness and the 3D structure. While previous literature suggests a humidity > 35 % [60], we found that a humidity level within 23–28 % stimulated the formation of long, thin, and homogeneous with little beading (Figure S7). The finalized sol and electrospinning parameters are displayed in Table S1 and the Experimental section.

All BG wound matrices exhibited a 3D structure with fiber thicknesses of 100–300 nm, resembling collagen fibrils in the dermis and facilitating cell attachment and migration. These matrices are biodegradable with a controlled release of B, Ca, and Si at levels comparable to those reported in previous studies [84–86]. Si and Ca release from borosilicate BGs was higher than that from silicate BGs on the first day, probably due to the fast degradation kinetics of borosilicate glasses (Figure S8) [52–57,87]. Borosilicate glass has lower network connectivity because B-O-B has fewer bridging bonds [67–69]. In addition, B-O-B bonding is weaker and can be broken at pH=7, while Si-O-Si bonding only breaks at pH >9 [88]. Simultaneously, breaking the glass network resulted in the removal of H⁺ ions from the media due to cation exchange, which may explain the increase in pH level [89]. B level in the media increases linearly with the dissolution time and generally agrees with B content in glass.

Therapeutic effects of BGs are mainly attributed to the release of ions, although the therapeutic level has not been defined. Our data showed a reduced MA at high doses for borate-free BG matrices. Since Ca [90–92] and Si [93–95] ions were previously reported to be non-toxic but stimulate the MA of fibroblasts, we hypothesize that this is due to a static culture environment, which results in a cumulation of local pH (pH > 8.5), or precipitation. Brown et al. claimed that a dynamic environment, such as the *in vivo* condition, would not exhibit the same reduction effect as seen in static *in vitro* culture [96]. Following *in vivo* implantation, borate fibers were fully biodegraded, with no sign of precipitation or obvious cell apoptosis because of local pH.

Here, the introduction of borate ions further reduced the MA of fibroblasts but only exhibited a slight reduction in cell population. This is likely due to a pseudo-hypoxic environment where cells were stimulated by growth factors without being harmed, as further supported by the up-regulation of HIF-1 α expression (Fig. 2) [97]. The role of B in inducing a pseudo-hypoxia environment has been previously documented in borate containing BGs [98], as well as other borate nanomaterials such as boric acids [99]. Its mechanism is explained as boron elevates intercellular reactive oxidative species (ROS), which is a known oxygen-independent route to inhibit prolyl-hydroxylases and thereby stabilize HIF-1 α . Pseudo-hypoxia is commonly observed in pro-angiogenic environment, where a low MA is usually connected with an increase in angiogenic factor expressions. Borate ions in BG significantly up-regulated HIF-1 α as well as the expressions of VEGF and bFGF, which may further evident the presence of this scenario.

MA rose steadily as the BG dose fell. At 2 mg mL⁻¹ 55S30C15B overtook 70S30C in MA, and at 1 mg mL⁻¹ all four BGs yielded similarly high MA as the control group. This is most likely because lower particle concentrations generated a milder, less-alkaline microenvironment that better supports proliferation. A reduced flux of therapeutic ions—especially borate—may also have lessened the pseudo-hypoxic stress reported for high-borate systems, further enhancing MA. Cytokine profiles shifted in parallel. Lowering the dose suppressed PDGF, VEGF, and bFGF but elevated TGF- β , suggesting a transition from a proliferative to a remodeling phenotype as ionic strength declined. Within a given dose, borate content modulated the profile in a non-monotonic fashion. At 4 mg mL⁻¹, VEGF and bFGF peaked at 55S30C15B, whereas PDGF was highest for the boron-free control. At 2 mg mL⁻¹, 35S30C35B boosted

PDGF and TGF- β , while VEGF again peaked with 55S30C15B. These data show that all therapeutic ions played an important role in tuning protein expressions and will be interpreted in concert with the *in vivo* findings in the following sections.

For endothelial cells, a clear borate-dependent optimum for cell viability and migration emerged. When HUVECs were cultured in fibroblast-conditioned media, the 55S30C15B extract produced the strongest pro-angiogenic response. Metabolic activity peaked on day 3, rising roughly 50 % above the DMEM-HDF control and surpassing both the 70S30C-HDF and the high-borate 35S30C35B-HDF groups. This enhancement correlated with the highest VEGF concentration and a concomitant surge in bFGF, while eNOS levels remained comparably elevated across all BG-treated conditions. Functional assays confirmed that the 55S30C15B-HDF medium accelerated endothelial migration, a nearly 4-fold increase from the control. These data indicate that a moderate borate content maximizes the paracrine angiogenic signals from fibroblasts, translating into superior endothelial proliferation and chemotaxis; excessive or absent borate, by contrast, attenuates this synergy.

Diabetic ulcers lack the signals needed for epithelial cells to migrate and completely cover the wound [12,100,101]. Both wound area images and histology analysis revealed an accumulation of epithelial cells at wound edges. Our BG wound matrices promoted faster wound closure than the control. Although this is the first investigation of the effects of BG nanofibers on wounds *in vivo*, evidence from previous literature on polymer nanofibers containing BGs supports these findings [102,103]. Wounds treated with 55S30C15B powder epithelialized faster than those treated with 70S30C powder and those treated with 55S30C15B wound matrices were the highest. Given the limited knowledge of the effect of boron on epithelialization, this result may provide compelling evidence of its impact.

Inflammation is the second phase in the wound-healing cascade. In chronic wounds, substantial macrophage infiltration results in the expression of cytokines and protease which leads to chronic inflammation, healthy cell apoptosis, and ECM degradation [104]. The amount of exudates upon implantation as well as the immunohistochemistry evidence indicated that all BGs induced anti-inflammatory effects compared to the control. This aligns with previous literature where SiO₂-CaO based BGs such as 45S5 [29], 60SiO₂-36CaO-4P₂O₅ (58S) [105] and 75SiO₂-20CaO-5P₂O₅ [106] have been reported to down-regulate inflammatory cytokines and up-regulate anti-inflammatory cytokines. B-BG stimulated higher even higher expressions of anti-inflammatory cytokines, indicating the anti-inflammatory response of B ions. While the mechanism remains unclear, B ion may inhibit iNOS and COX-2 [99,107], two important inflammatory mediators, and possess anti-oxidant properties [108]. 100-mmol supplemental boron down-regulated IL-1 β , TNF- α , macrophage inflammatory protein-1 alpha (MIP-1a), and inducible nitric oxide synthase (iNOS) [109], while 200 μ g boric acid or sodium pentaborate reduced both cyclooxygenase-2 (COX-2) and iNOS [107].

Angiogenesis is one of the most important steps in the chronic wound healing cascade as it ensures better delivery of nutrients, oxygen, and proteins to the wound site [13]. The interplay of HIF-1 α and fibroblasts up-regulates the expressions of VEGF and bFGF, two major proteins in wound angiogenesis [110–112]. *In vitro* results revealed that all BG wound matrices significantly upregulated VEGF and bFGF expressions from both HDFs and HUVECs. For the highest BG dose (4 mg mL⁻¹), incremental increases in borate from 0, 5, to 15 mol % progressively increased VEGF and bFGF expressions, while a further increase to 35 % reduced their expressions. The stimulatory effect was greatly mitigated at 2 mg mL⁻¹ dose and completely disappeared at 1 mg mL⁻¹. Since VEGF and bFGF bind to their receptors to stimulate the proliferation and migration of endothelial cells to form tubules [113–115], 55S30C15B further resulted in the highest HUVEC viability and migration. The same tendency was maintained *in vivo*, where 70S30C stimulated a 2-fold increase in blood vessel/capillaries formation and VEGF expressions

compared to the control, with 55S30C15B inducing an even greater effect. This aligns with previous literature on silicate BGs [32,33,116,117], in which the release of SiO_4^{4-} [118–120] and Ca^{2+} ions [121,122] stimulate angiogenesis. The incorporation of B further enhances the angiogenic effect, for example, 45S5 BG with 2–5 wt % borates stimulated higher VEGF and bFGF expressions compared to 45S5 and control [37,123]. Finally, the 3D structure and ECM-mimicking environment did not induce significant improvement in the angiogenesis effect.

Histology further revealed the impact of BG wound matrices in tissue granulation from multiple aspects such as fibroblast proliferation, fibroblast maturation, and ECM deposition. Alpha-smooth muscle actins (α -SMA) are microfilaments in myofibroblasts that produce tension to the surrounding ECM for wound contraction [124,125] and are a crucial marker of fibroblast differentiation into myofibroblasts [126,127]. Elevated levels of α -SMA typically indicate faster tissue granulation and wound closure kinetics. This is the first assessment of α -SMA expression in BG-treated wounds *in vivo*, although such upregulation was observed in other cells *in vitro*, such as urine-derived stem cells [128]. Little was known about its mechanism, but it may be because BGs stimulated higher ambient growth factor expressions, such as PDGF and TGF- β , two major growth factors on fibroblast differentiation [129] and ECM deposition [130–132]. Both proteins were up regulated by BG wound matrices at different doses, probably due to the combined effect of Ca and B ions. Ca ions are essential for the activation of both PDGF [133,134], and TGF- β [135,136], but excessive Ca may display an adverse effect. Borates tend to balance the adverse effect of high Ca^{2+} content, but they seem not to have a direct stimulatory effect on both PDGF and TGF- β expressions. BGs also significantly promoted collagen deposition in wounds, which is consistent with previous literature [102,103]. The incorporation of borates within BG presented little impact, which contradicts previous literature that reported borates stimulated more collagen deposition in wounds *in vitro* [40]. 55S30C15B matrix stimulated the highest collagen deposition, which is likely because the ECM-mimic structure exhibited better physical cues for ECM protein attachment than the powders.

Collectively, our *in vitro* and *in vivo* findings delineate the mechanism underlying the therapeutic efficacy of the 3D ECM-mimicking, high-borate wound matrix (Fig. 9). Upon implantation, the matrix integrates seamlessly with chronic-wound tissue, rendering an ECM-like structure that supports cell adhesion and accelerates closure. Sustained release of B, Ca, and Si ions then operates synergistically to (1) stimulate fibroblast proliferation and collagen deposition, enabling tissue granulation; (2) enhance secretion of angiogenic factors by fibroblasts and endothelial cells, driving endothelial proliferation and migration for angiogenesis;

and (3) up-regulate anti-inflammatory while down-regulate pro-inflammatory cytokines, guiding macrophages toward a reparative phenotype for anti-inflammation. Together, these coordinated effects highlight the matrix's strong potential for chronic wound healing.

5. Conclusion

In this paper, an improved sol-gel electrospinning approach was proposed to synthesize borosilicate BG wound matrices, with enhanced borate content, for diabetic wound regeneration. The wound matrices exhibited a 3D structure composed of entwined fibers (diameters 100–300 nm), which closely resembled the ECM environment, and biodegradability with the release of therapeutic ions such as BO_3^{3-} , Ca^{2+} , and SiO_4^{4-} . *In vitro* analysis revealed that the 55S30C15B fibers stimulated the highest VEGF and bFGF expressions from HDFs compared to other BG formulations. The impact of BG wound matrices on endothelial cell viability, migration, and protein expression was assessed, where 55S30C15B facilitated the highest MA and migration of HUVECs. The ideal BO_3^{3-} levels for HDFs and HUVECs are approximately 250 and 125 $\mu\text{g mL}^{-1}$ respectively, with higher concentrations resulting in cytotoxicity and lower concentrations leading to insignificant protein expression. *In vivo* studies on C57BJ/6 mice confirmed that BGs accelerated wound closure and vascularization with reduced chronic inflammation compared to untreated wounds. Histology and immunohistochemistry further proved that BGs improve anti-inflammatory response (IL-1 β and IL-10), angiogenesis (VEGF and CD31), and tissue granulation (Masson Trichrome, α -SMA). Fiber morphology and ion release had an impact. The incorporation of a certain level of borate ions bolstered the angiogenic and anti-inflammatory response, while the 3D nanofiber structure accelerated tissue granulation, epithelialization, and collagen deposition. In summary, this paper establishes the efficacy of the proposed borosilicate BG wound matrices in diabetic wound healing and provides insights into the potential of BGs to release therapeutic ions for soft tissue engineering.

CRedit authorship contribution statement

Xingchen Zhao: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Dong Zhai:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Gavin Jell:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Chengtie Wu:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Huiling Gao:** Writing – review & editing, Validation, Supervision, Resources, Methodology,

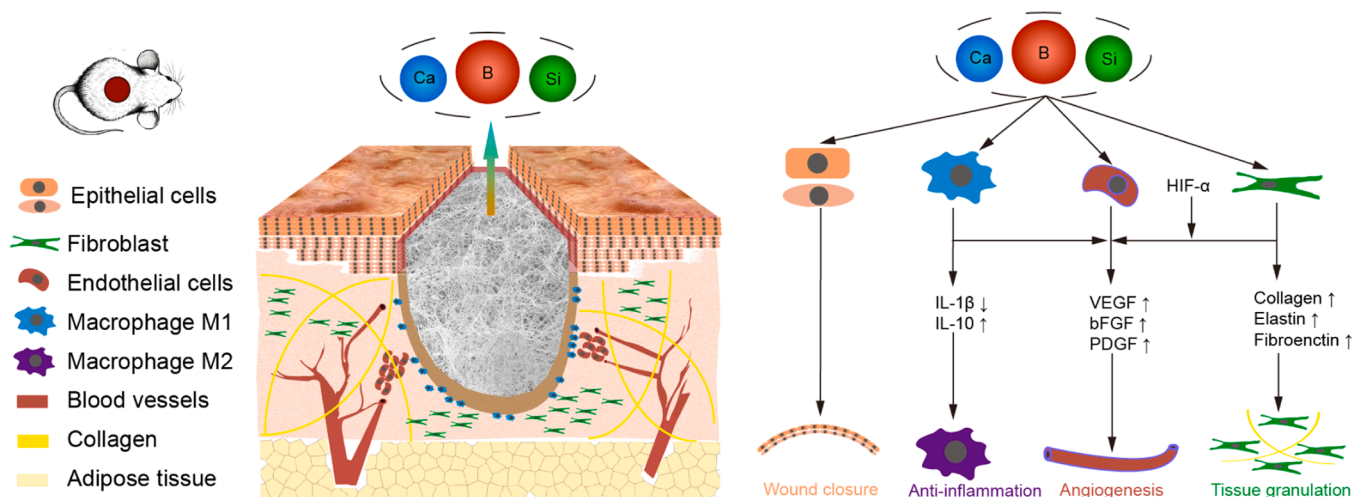


Fig. 9. Schematics of the mechanism of BG wound matrices in promoting individual healing cascades, including wound closure, anti-inflammation, angiogenesis, and tissue granulation in chronic wounds.

Investigation. **Julian R. Jones:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors acknowledge the EPSRC (EP/N025059/1). Raw data can be obtained by reasonable request from rdm-enquiries@imperial.ac.uk.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.actbio.2025.06.033](https://doi.org/10.1016/j.actbio.2025.06.033).

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