

Effects of a 45S5 Bioactive Glass-Containing Ointment on Oxidative Stress, Angiogenesis, and Wound Healing in MRSA-Infected Wounds of Diabetic Rats

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Abstract: Diabetic wounds are chronic wounds associated with a high risk of infection, oxidative stress, and impaired angiogenesis. In recent years, the wound-healing potential of biomaterials has become a significant area of research. One biomaterial that has gained popularity in this context is 45S5 bioactive glass. This study aimed to investigate the effects of a 45S5 bioactive glass ointment on wound healing in a diabetic wound model infected with methicillin-resistant *Staphylococcus aureus* (MRSA). Twenty-four male Wistar Albino rats, rendered diabetic via streptozotocin, were divided into three groups: control (CTR), bioactive glass (BG), and mupirocin (MUP). Full-thickness skin wounds were created on all animals and subsequently infected with MRSA; topical treatments were administered for 21 days. Changes in wound area were evaluated macroscopically, and bacterial load was determined using a quantitative culture method. Additionally, levels of malondialdehyde (MDA), glutathione (GSH), and catalase (CAT) in the wound tissues were analyzed. Histopathological examination was performed alongside an assessment of vascular endothelial growth factor A (VEGFA) immunohistochemical expression. It was determined that bacterial load was significantly reduced in the BG and MUP groups compared to the CTR group ($p<0.05$). Furthermore, the wound area was found to be significantly smaller in both treatment groups compared to the CTR group on days 10, 15, and 21 ($p<0.05$). In the BG group, MDA levels decreased significantly compared to the CTR group, while GSH levels and CAT activities increased significantly ($p<0.05$). Histopathological examinations revealed reduced edema and inflammatory cell infiltration, along with increased granulation tissue formation and angiogenesis, in the BG group. Furthermore, VEGFA immunoreactivity was found to be higher in the BG group compared to the CTR group. These findings suggest that the ointment containing 45S5 bioactive glass may contribute to the reduction of bacterial load and oxidative stress and may be associated with improved angiogenesis and wound healing.

Keywords: 45S5 bioactive glass, infected diabetic wound, MRSA, oxidative stress, streptozotocin.

45S5 Biyoaktif Cam İçeren Merhem MRSA ile Enfekte Diyabetik Sıçan Yaralarında Oksidatif Stres, Anjiyogenez ve Yara İyileşmesi Üzerine Etkileri



Öz: Diyabetik yaralar, yüksek enfeksiyon seviyeleri, oksidatif stres ve zayıf anjiyogenez ile ilişkili kronik bir yara türüdür. Son birkaç yıldır, biyomateryallerin yara iyileştirme potansiyeli önemli bir araştırma alanı olmuştur. Bu bağlamda popülerlik kazanan biyomateryallerden biri de 45S5 biyoaktif camdır. Bu çalışma, metisiline dirençli *Staphylococcus aureus* (MRSA) ile enfekte edilmiş diyabetik bir yara modelinde 45S5 biyoaktif cam merheminin yara iyileşmesi üzerindeki etkilerini araştırmayı amaçlamıştır. Streptozotocin ile diyabet oluşturulan 24 erkek Wistar Albino sıçan, üç gruba ayrılmıştır: kontrol (CTR), biyoaktif cam (BG) ve mupirosin (MUP). Tüm hayvanlara tam kalınlıkta bir deri yarası oluşturulmuş ve daha sonra MRSA ile enfekte edilmiştir; 21 gün boyunca topikal tedaviler uygulanmıştır. Yara alanı değişiklikleri makroskobik olarak değerlendirilmiş; bakteri yükü kantitatif kültür yöntemiyle belirlenmiştir. Ayrıca, yara dokularındaki malondialdehid (MDA), glutatyon (GSH) ve katalaz (CAT) düzeyleri analiz edildi. Histopatolojik inceleme, vascular endothelial growth factor A (VEGFA) immünohistokimyasal ekspresyonunun değerlendirilmesiyle birlikte gerçekleştirildi. BG ve MUP gruplarında bakteriyel yükün kontrol grubuna göre anlamlı düzeyde

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azaldığı belirlendi ($p<0.05$). Ayrıca 10., 15. ve 21. günlerde her iki tedavi grubunda yara alanının CTR grubuna kıyasla anlamlı derecede daha küçük olduğu saptandı ($p<0.05$). BG grubunda MDA düzeyleri CTR grubuna göre anlamlı olarak azalırken, GSH düzeyleri ve CAT aktiviteleri anlamlı şekilde arttı ($p<0.05$). Histopatolojik incelemelerde BG grubunda ödem ve yangısal hücre infiltrasyonunun azaldığı, granülasyon dokusu oluşumu ile anjiyogenezin arttığı gözlemlendi. Ayrıca VEGFA immünoekspresyonunun BG grubunda kontrol grubuna göre daha yüksek olduğu belirlendi. Bu bulgular, merhemın bakteriyel yük ve oksidatif stresin azaltılmasına katkıda bulunabileceğini ve anjiyogenez ile yara iyileşmesinin iyileştirilmesiyle ilişkili olabileceğini düşündürmektedir.

Anahtar kelimeler: 45S5 biyoaktif cam, diyabetik enfekte yara, MRSA, oksidatif stres, streptozotosin.

INTRODUCTION

Wound healing is a complex and dynamic biological process consisting of four fundamental phases that follow one another but partially overlap. These phases are defined, in order, as hemostasis, inflammation, proliferation, and remodeling. The hemostasis phase represents the body's rapid initial response to tissue damage, while the inflammation phase begins immediately following injury and typically lasts approximately 48 hours (Diegelmann & Evans, 2004).

Wound healing is a process that can be influenced by various systemic and local factors, such as diabetes, infection, malnutrition, and aging (Kırgız et al., 2024). The World Health Organization has reported that there are 422 million people with diabetes worldwide. In individuals with diabetes, foot wounds and associated infections are a major cause of morbidity and pose a serious public health problem (Del Core et al., 2018). The ulceration of these wounds increases the risk of infection and, in severe cases, can result in limb loss or mortality (Jiang et al., 2015). Numerous factors influence diabetic wound healing, including recurrent microbial infections, excessive accumulation of reactive oxygen species (ROS), cellular dysfunction, and impaired angiogenesis and vasculogenesis associated with aging (Xu et al., 2024). One of the main challenges complicating the treatment of these wounds in clinical practice is the colonization of the wound site by resistant microorganisms (Ali et al., 2022; Saltoglu et al., 2018). In severe wound infections, *Staphylococcus aureus* and *Escherichia coli* are among the most commonly encountered colonizing microorganisms (Kalan et al., 2019). The biofilms formed by these bacteria and the virulence factors they secrete not only contribute to bacterial resistance but also negatively affect the angiogenesis process and the functions of immune cells, thereby exacerbating the inflammatory response (Hall & Mah, 2017).

In recent years, biomaterials have been used in wound healing, yielding effective results as alternatives to standard treatments (Ahmad et al., 2026; Li et al., 2022; Raina et al., 2022). 45S5 bioactive glass has long been recognized as a biomaterial that effectively supports osteogenic cell activity in bone regeneration (Özarslan, 2023). More recent studies, however, show that 45S5 bioactive glass containing 45% SiO₂, 24.5% CaO, 24.5% Na₂O, and 6.0% P₂O₅ can interact not only with bone tissue

but also with soft tissues (Kırgız et al., 2024; Temel-Soylu et al., 2024). It has been reported that this material supports angiogenesis and accelerates the wound healing process by increasing the expression of vascular endothelial growth factor (VEGF) (Kırgız et al., 2024; Temel-Soylu et al., 2024). Furthermore, it is stated that 45S5 bioactive glass nanoparticles accelerate hemostasis and contribute positively to overall biocompatibility (Kırgız et al., 2024; Temel-Soylu et al., 2024). On the other hand, thanks to the ion release from 45S5 bioactive glass, it not only supports tissue regeneration but can also exert an antibacterial effect by increasing the local pH (Kırgız et al., 2024; Temel-Soylu et al., 2024). There are some studies indicating that bioactive glass yields positive results on diabetic wounds (Tang et al., 2021; Vargas Guerrero et al., 2024; Xie et al., 2021). However, most existing studies address diabetic wound healing in general, and no data have been found regarding the effect of bioactive glass on infected diabetic wounds. Yet in cases accompanied by infection particularly due to resistant microorganisms that form biofilms wound healing becomes even more challenging. Therefore, there is a need for new treatment approaches that can both combat infection and support tissue healing.

The aim of this study was to evaluate the effects of 45S5 bioactive glass on wound healing in an infected diabetic wound model and to investigate its effects on oxidative stress, angiogenesis, and microbial load.

MATERIAL AND METHOD

Preparation of a Bioactive Glass Ointment: In this study, 45S5 bioactive glass (BONE-G ACTIVE; REF: BGA025.0.5/2, Turkey) was used to prepare a composite wound ointment. Vaseline, a commercially available ointment (Krauterhof Vaseline, Germany), served as the carrier matrix due to its history of clinical use. This vaseline was chosen because it has no known positive or negative effect on wound healing (Penven et al., 2005). The bioactive glass-vaseline formulation was prepared at the Chemistry and Metallurgy Laboratory of Yıldız Technical University based on previously described methods (Mao et al., 2014; Özarslan et al., 2023). The vaseline was first liquefied by heating it to approximately 65 °C. Next, bioactive glass powder (average particle size <50 µm) was slowly added to the molten vaseline under continuous stirring to ensure a homogeneous distribution. The final composition consisted of 16% (by weight) bioactive glass and 84% (by weight)

vaseline. The mixing process continued until the formulation began to cool; afterward, the ointment was allowed to reach room temperature. The prepared composite was stored under appropriate conditions until it was used in in vivo experiments.

Ethics Approval and Laboratory Animals: The ethical approval required for this study was obtained from the Local Animal Experimentation Ethics Committee of Hatay Mustafa Kemal University (Decision No. 2026/05-11). All experimental procedures were conducted in accordance with national legislation regarding the welfare and protection of laboratory animals and with European Union Directive 2010/63/EU on the protection of animals used for scientific purposes.

A total of 24 adult male Wistar Albino rats weighing between 250 and 300 g were used in the study. The animals were housed individually in polycarbonate cages in a controlled environment maintained at 23 ± 2 °C and 55% humidity, with a 12-hour light–12-hour dark cycle. All rats were provided with water and standard chow ad libitum. The MRSA strains used in the experimental procedures were obtained from the Microbial Bank of the Microbiology Laboratory at the Faculty of Medicine Hospital of Hatay Mustafa Kemal University.

Surgical Procedure: Laboratory animals were randomly assigned to three experimental groups (CTR, MUP, and BG; n = 8 per group) using a computer-generated random number sequence. To induce diabetes in the rats, each rat was fasted for 12 hours and then administered a single intraperitoneal dose of streptozotocin (65 mg/kg), freshly prepared in 0.1 M sodium citrate buffer (pH 4.5; CAS No. 6132-04-3, ISOLAB Laborgeräte GmbH, Eschau, Germany). After 72 hours, blood was collected from the tail vein, and blood glucose levels were measured using a glucose meter (Accu-Check Instant, Germany). Rats with blood glucose levels ≥ 250 mg/dL were considered diabetic (Table 1). Following the onset of diabetes, all rats included in the study were administered general anesthesia via intramuscular injection of 10 mg/kg xylazine HCl (Sanazalin, Santavet, Turkey) and 100 mg/kg ketamine HCl (Keta-kontrol, Doga Ilac, Turkey) prior to wound creation in the dorsal interscapular region. The area where the wound was to be created was shaved with an electric razor and then disinfected with an antiseptic solution. Subsequently, a full-thickness skin wound measuring 1.5×1.5 cm was created in each animal. In all groups, freshly prepared MRSA strains at a concentration of 10^8 CFU/30 μ L were inoculated into the wound site immediately after surgery. To prevent contamination of the wound, it was quickly covered with a hydrocolloid wound dressing (SafWound, Turkey). After three days, the dressing was removed, and infection was confirmed following an assessment of the presence of secretions in the area and bacterial culture counts.

Control Group (CTR):

No treatment was administered to the animals in this group; wound care was provided solely by applying saline solution to the wound surface once a day.

Bioactive Glass Group (BG):

The bioactive glass-containing ointment, which formed the basis of the study, was applied topically to the wound surfaces of the animals in this group once every 24 hours for 21 days.

Mupirocin Group (MUP):

The 2% mupirocin ointment (Dextrocin, Pharmaactive, Turkey) was applied topically once daily for 21 days to the wounds of the rats in this group.

In the experimental applications, 45S5 bioactive glass ointment and mupirocin-containing ointment were applied once daily to each wound, ensuring complete coverage of the wound surface. Since a fixed weight of ointment was not weighed and used during application, the amount of ointment applied was not determined in milligrams.

Wound Area Measurement: For macroscopic evaluation, photographs of the wound site were taken on days 1, 5, 10, 15, and 21, and measurements were obtained using the ImageJ 2X software.

Biochemical Analysis of Wound Tissues: Each wound tissue sample was diluted 1:10 (weight/volume) ratio with phosphate-buffered saline (0.01 M, pH = 7.4), then homogenized at +4 °C at 5,000 g for 5 minutes using a homogenizer (IKA T18 Digital Ultra-Turrax, Germany), and the supernatants were collected. Malondialdehyde (MDA) levels (MDA ELISA Kit, Catalog No.: ER1878, FineTest, China), glutathione (GSH) levels (GSH ELISA Kit, Catalog No.: E-EL-0026, Elabscience, USA), and CAT activities (CAT ELISA Kit, Catalog No.: QT-ER0264, FineTest, China) were measured according to the methods specified in the respective rat-specific commercial kits. MDA levels in wound tissue were reported in ng/mL, GSH levels in μ g/mL, and CAT activities in mIU/mL.

Histopathological and immunohistochemical examination: Skin samples collected following necropsy were fixed in a 10% neutral formaldehyde solution for 72 hours, then trimmed and washed overnight in tap water. They were subsequently processed through routine tissue preparation procedures and embedded in paraffin. Sections cut from the paraffin blocks onto slides were stained with hematoxylin and eosin and examined under a light microscope. The histopathological evaluation was performed semi-quantitatively according to a previously reported study (Erol et al., 2025; Kirgiz Akçakavak, 2025; Kirgiz et al., 2026). In this context, edema, inflammatory cell infiltration, and the absence of angiogenesis were scored as 0 (none), mild (+), moderate (++), severe (+++) and very severe (++++). In addition, scores ranging from (+) to

(++++)) were assigned based on the presence of fibroblasts, fibrocytes, and collagen in the region during granulation tissue formation.

Sections cut from paraffin blocks onto slides were subjected to paraffin removal procedures. Immunohistochemical staining was performed using a commercial kit according to a previously reported method. The VEGFA antibody (Affinity Biosciences, AF5131, USA) was used as the primary antibody, and DAB was used as the chromogen. Immunohistochemical evaluation was scored semi-quantitatively as absent (0), mild (+), moderate (++), severe (+++) and very severe (++++)) (Kirgiz et al., 2026).

Wound area measurements, histopathological evaluations, and immunohistochemical VEGFA scoring were performed under blinded conditions, in which the evaluators were unaware of the information regarding the experimental groups. Histopathological and immunohistochemical scores were evaluated observationally.

Statistical Analysis: The data obtained from the present study were analyzed using SPSS 25 (Inc., Chicago, USA). The Shapiro-Wilk test was used to assess the normality of the data distribution, and the Levene test was used to assess the homogeneity of variances. Bacterial load, wound area, and biochemical results were analyzed using ANOVA and post-hoc Duncan tests. The accepted level of significance was $p < 0.05$.

RESULTS

Blood Glucose, Quantitative Microbiological Cultures, and Wound Site: Blood glucose levels increased significantly 72 hours after streptozotocin administration compared with pre-STZ levels in all experimental groups ($p < 0.001$; Table 1). Quantitative culture results obtained from wound tissue revealed that the bacterial load was significantly reduced in both the BG and MUP groups compared with the CTR group ($p < 0.05$; Figure 1A). The lowest bacterial count was observed in the bioactive glass (BG) group, while a statistically significant reduction was also observed in the mupirocin (MUP) group compared to the CTR group (Figure 1A) ($p < 0.05$). In the macroscopic wound area assessment, a reduction in wound area was observed over time in all groups. However, wound contraction and closure were found to be more pronounced in the BG group compared to the other groups. Specifically, on days 10, 15, and 21, the wound areas in the BG and MUP groups were found to be significantly smaller than those in the CTR group (Figure 1B) ($p < 0.05$).

Table 1. Blood glucose levels of the experimental groups before and 72 hours after streptozotocin administration.

Group	Before STZ (mg/dL)	72 h after STZ (mg/dL)	p
CTR	96.13 $\pm$ 4.64	401.75 $\pm$ 18.17	<0.001
MUP	107.00 $\pm$ 5.24	422.13 $\pm$ 15.25	<0.001

BG	106.00 $\pm$ 4.08	401.25 $\pm$ 22.66	<0.001
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Values are presented as mean $\pm$ standard error of the mean (SEM) ($n = 8$ per group). Statistical analysis was performed by comparing the pre- and post-STZ blood glucose levels of the same animals using a paired t-test.

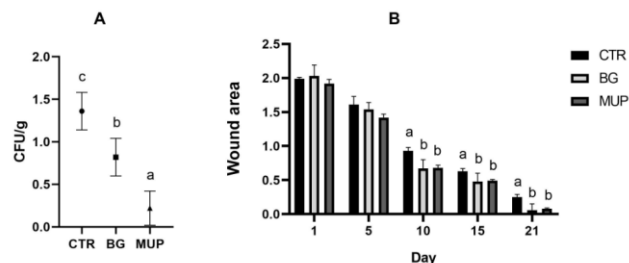


Figure 1. Quantitative microbiological culture results and changes in wound area in the experimental groups. (A) Quantitative MRSA culture results obtained from wound tissue (CFU/g tissue). (B) Changes in wound area measured on days 1, 5, 10, 15, and 21 in the experimental groups. Data are presented as mean $\pm$ standard error of the mean (SEM) ($n = 8$ /group). Different letters (a–c) at each time point indicate statistically significant differences between experimental groups ($p < 0.05$). Individual data points represent the measurement values for each animal.

Biochemical Findings: The MDA levels, GSH levels, and CAT activities in the wound tissues of the groups are presented in Figure 2. The CTR group was found to have higher MDA levels than the BG and MUP groups, and the lowest MDA level was observed in the BG group ($p < 0.05$). No statistically significant difference was found in GSH levels between the BG and MUP groups ($p > 0.05$); however, it was determined that the GSH levels in these groups were higher than those in the CTR group ($p < 0.05$). It was found that the BG group had the highest CAT activity among the groups, and that the MUP group's CAT activity was also higher than that of the CTR group ($p < 0.05$).

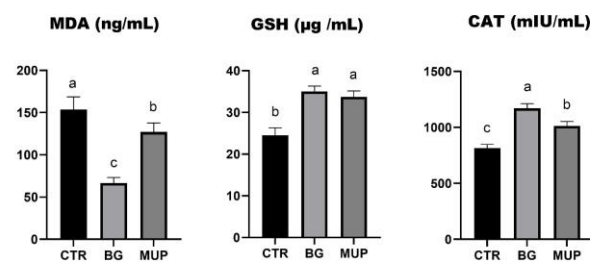


Figure 2. Levels of MDA (A), GSH (B), and CAT (C) in wound tissues of the experimental groups. Data are presented as mean $\pm$ standard error (SEM). Differences between groups denoted by different letters are statistically significant ($p < 0.05$, one-way ANOVA and Duncan's multiple range test). CTR: Control group, BG: Bioactive glass group, MUP: Mupirocin group, MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase.

Histopathological and Immunohistochemical Findings: The histopathological and immunohistochemical scores across groups are presented in Table 2. The highest scores for edema and inflammatory cell infiltration were observed in the CTR group, whereas the BG group showed

lower scores for these parameters. The lowest scores for granulation tissue formation and angiogenesis were observed in the CTR group, whereas the BG group showed higher scores for these parameters. In the immunohistochemical evaluation of VEGFA, the CTR group had the lowest scores, whereas the BG group resulted in an increase in immunoreactivity. VEGFA immunoreactivity showed cytoplasmic localization in

keratinocytes, fibroblasts, macrophages, and vascular endothelial cells (Figure 3).

Table 2. Appearance of histopathological and immunohistochemical scores among experimental groups.

Parameter	CTR	BG	MUP
Edema	+++	+	++
Inflammatory cell infiltration	+++	++	+++
Granulation tissue formation	+	++	++
Angiogenesis	+	+++	++
VEGFA	+	++	++

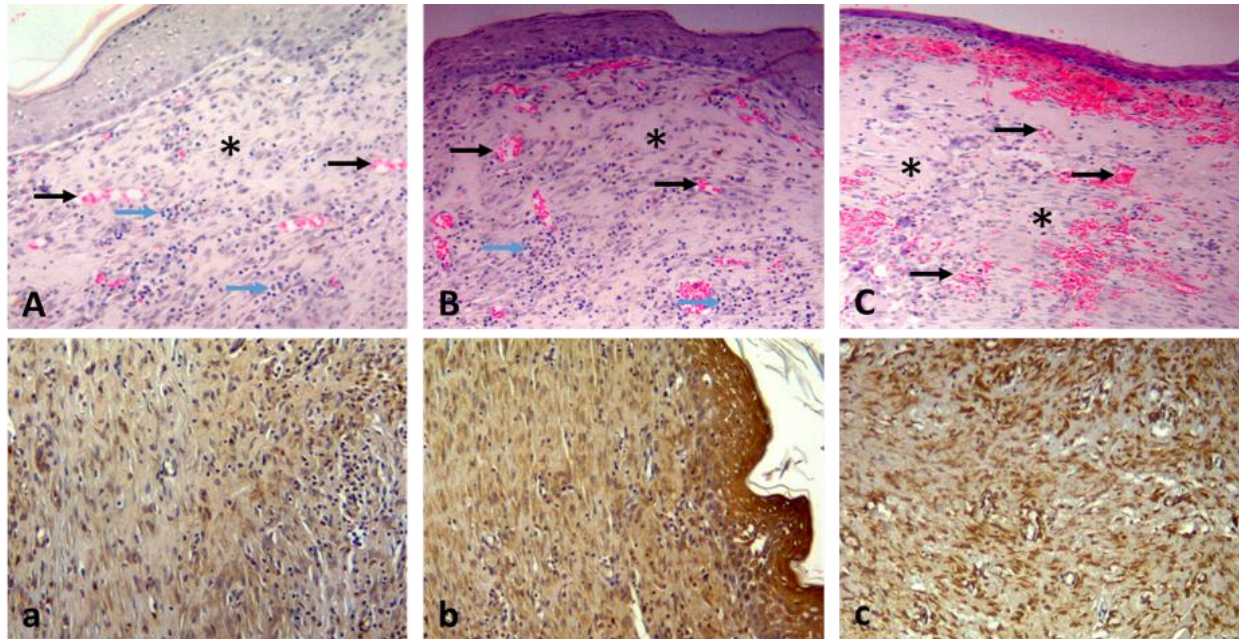


Figure 3. Appearance of histopathological and immunohistochemical photomicrographs across experimental groups; A,a represent the CTR group; B,b, the MUP group; and C,c, the BG group. Black arrows indicate angiogenesis; blue arrows, inflammatory cell infiltration; and asterisks, granulation tissue.

DISCUSSION

Wound healing is defined as a biological process consisting of four phases hemostasis, inflammation, proliferation, and remodeling that occurs to restore tissue integrity. It is generally known that in the normal wound healing process, the proliferation phase is largely completed within 21 days, and the remodeling phase begins (Gonzalez et al., 2016; Landén et al., 2016; Sorg et al., 2017). On the other hand, diabetic wounds follow a different course from the normal wound healing process due to the development of various pathophysiological events particularly subacute or chronic inflammation, increased oxidative stress, reduced angiogenesis, and inadequate granulation tissue formation which prolong the wound healing process (Deng et al., 2021; Okonkwo & DiPietro, 2017). In the study presented in this context, a 21-day experimental protocol was established, and edema, angiogenesis, inflammatory cell infiltration, and granulation tissue formation were evaluated histopathologically. The findings indicated that the BG group had positive effects on edema, inflammatory cell infiltration, granulation tissue, and angiogenesis compared to the CTR group. Based on this result, it is thought that

BG may reduce the inflammatory response and exert effects that support regenerative processes (Lin et al., 2019; Rahaman et al., 2011).

In the present study, when the effects of mupirocin and the ointment containing 4SS5 bioactive glass were evaluated together, it was observed that both treatments yielded positive results compared to the CTR group in terms of reducing bacterial load and decreasing wound area. While the lowest bacterial load was observed in the BG group, a statistically significant reduction was also detected in the MUP group compared to the CTR group ($p < 0.05$). However, although wound contraction and closure appeared more pronounced in the BG group, no statistically significant difference was observed. While mupirocin is thought to contribute to the control of wound infection primarily through its antibacterial activity, it is believed that 4SS5 bioactive glass may exert its effects not only through antibacterial activity but also via multiple biological mechanisms that could influence the different stages of wound healing. In the present study, the BG group showed not only a reduction in bacterial load but also lower MDA levels, higher GSH levels and CAT activity, and greater granulation tissue formation, angiogenesis, and VEGFA immunoexpression. The ointment containing

45S5 bioactive glass may therefore help control infection while also contributing to wound repair through reduced oxidative stress and support for processes such as angiogenesis. VEGF has important functions in wound healing, especially in the formation of new blood vessels. It stimulates endothelial-cell proliferation, migration, and new-vessel formation, thereby facilitating the delivery of oxygen and nutrients to the wound site (Byrne et al., 2005; Shi et al., 2023). In diabetic wounds, by contrast, VEGF signaling is reduced by oxidative stress, hyperglycemia, and chronic inflammation, which leads to inadequate angiogenesis and delayed healing (Bao et al., 2009; Kolluru et al., 2012; Okonkwo & DiPietro, 2017). The present study likewise found the lowest VEGFA expression in the CTR group, a result interpreted as reflecting insufficient granulation tissue formation, inadequate angiogenesis, and irregular tissue perfusion. VEGFA levels were significantly higher in the BG group than in the CTR group. This may be because BG stimulated angiogenesis, fibroblast activity, and granulation tissue development more effectively. The rise in VEGFA expression may also have supported the maturation of new blood vessels, improving the tissue's supply of oxygen and nutrients (Dudley & Griffioen, 2023; Pérez-Gutiérrez & Ferrara, 2023).

Oxidative stress an imbalance between free radicals and antioxidants affects diabetic wound healing and the development of wound complications. Excessive production of reactive oxygen species (ROS) damages tissue and delays healing (Kumar et al., 2019, Deng et al., 2021). MDA, a byproduct of lipid peroxidation and a biomarker of oxidative stress in an organism, forms when ROS break down polyunsaturated lipids; its levels rise under diabetic conditions (Ghaisas et al., 2014). GSH and CAT, meanwhile, are antioxidants abundant in the skin. They provide a primary defense against free-radical damage and help counter the harmful effects of excessive ROS, particularly during diabetic wound healing (Kumar et al., 2019; Tombulturk and Demirci 2025). It is known that reducing oxidative stress and strengthening antioxidant defense are necessary for faster healing of diabetic wounds (Tombulturk and Demirci 2025, Kirgiz et al., 2026). To accelerate the wound healing process, ferulic acid (Ghaisas et al., 2014), curcumin (Kant et al., 2014), quercetin (Ahmed et al., 2018), hemin (Kumar et al., 2019), syringic acid (Ren et al., 2019), gallic acid-silver nanoparticles (Vendidandala et al., 2021), metformin (Tombulturk and Demirci, 2025), and eucalyptol (Kirgiz et al., 2026), it has been reported that oxidative stress markers such as MDA increase in diabetic rats, while endogenous antioxidants such as GSH and CAT decrease. Furthermore, these studies have demonstrated that increased oxidative stress leads to impaired wound healing in diabetic rats and

that the bioactive compounds used have the ability to accelerate wound healing in diabetic rats by alleviating oxidative stress, thereby revealing their therapeutic effects. Consistent with the literature, the present study observed significant increases in CAT and GSH antioxidant levels, along with a concurrent significant decrease in MDA levels an oxidant in rats treated topically with a bioactive glass-containing ointment every 24 hours for 21 days, compared to diabetic control rats. In short, it is believed that the 45S5 bioactive glass-containing ointment contributes to diabetic wound healing by increasing antioxidant capacity and simultaneously reducing oxidants that is, by alleviating oxidative stress.

A limitation of the present study is that, although vaseline was used as a carrier in the ointment formulation containing 45S5 bioactive glass, the experimental design did not include a separate vaseline carrier control group. Although our previous study reported that vaseline had neither a positive nor a negative effect on wound healing (Kirgiz et al., 2024), the current study lacks a vehicle control group that would allow for the direct differentiation of the effects of vaseline and 45S5 bioactive glass. In future studies, the use of an appropriate vehicle control group containing vaseline will allow for a clearer demonstration of the independent effect of 45S5 bioactive glass.

CONCLUSION

In conclusion, the topical application of the 45S5 bioactive glass-containing ointment appears to be associated with a reduction in bacterial load and inflammation; furthermore, the decrease in MDA levels and the increase in GSH levels and CAT activity may be related to the alleviation of oxidative stress, while the ointment may also contribute to wound healing by promoting VEGFA expression and angiogenesis. These findings suggest that the 45S5 bioactive glass-containing ointment may be a promising alternative treatment option for diabetic infected wounds; however, further studies investigating its molecular mechanisms, long-term safety, and clinical efficacy are needed before it can be implemented in clinical practice.

REFERENCES

- Ahmad, U., Hanaffi, W.N.W., Islam, A., Salman, A., Khan, M.M., Shakeel, F., Cai, Q., Cai, X., & Shubhra, Q.T. (2026). Cutting edge strategies for diabetic wound care: nanotechnology, bioengineering, and beyond. *BMEMat*, 4(1), e70033. <https://doi.org/10.1002/bmm2.70033>
- Ahmed, O.M., Mohamed, T., Moustafa, H., Hamdy, H., Ahmed, R.R., & Aboud, E. (2018). Quercetin and low level laser therapy promote wound healing process in diabetic rats via structural reorganization and modulatory effects on inflammation and oxidative stress. *Biomedicine & Pharmacotherapy*, 101, 58-73. <https://doi.org/10.1016/j.biopha.2018.02.040>

- Ali, R., Khamis, T., Enan, G., El-Didamony, G., Sitohy, B., & Abdel-Fattah, G. (2022). The healing capability of clove flower extract (CFE) in streptozotocin-induced (STZ-induced) diabetic rat wounds infected with multidrug resistant bacteria. *Molecules*, 27(7), 2270. <https://doi.org/10.3390/molecules27072270>
- Bao, P., Kodra, A., Tomic-Canic, M., Golinko, M.S., Ehrlich, H.P., & Brem, H. (2009). The role of vascular endothelial growth factor in wound healing. *Journal of Surgical Research*, 153(2), 347-358. <https://doi.org/10.1016/j.jss.2008.04.023>
- Byrne, A. M., Bouchier-Hayes, D. J., & Harme, J. H. (2005). Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *Journal of cellular and molecular medicine*, 9(4), 777-794. <https://doi.org/10.1111/j.1582-4934.2005.tb00379.x>
- Del Core, M.A., Ahn, J., Lewis III, R.B., Raspovic, K.M., Lalli, T.A., & Wukich, D.K. (2018). The evaluation and treatment of diabetic foot ulcers and diabetic foot infections. *Foot & Ankle Orthopaedics*, 3(3), 2473011418788864. <https://doi.org/10.1177/2473011418788864>
- Deng, L., Du, C., Song, P., Chen, T., Rui, S., Armstrong, D.G., & Deng, W. (2021). The role of oxidative stress and antioxidants in diabetic wound healing. *Oxidative Medicine and Cellular Longevity*, 2021(1), 8852759. <https://doi.org/10.1155/2021/8852759>
- Diegelmann, R.F., & Evans, M.C. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. *Front biosci*, 9(1), 283-289.
- Dudley, A.C., & Griffioen, A.W. (2023). Pathological angiogenesis: mechanisms and therapeutic strategies. *Angiogenesis*, 26(3), 313-347. <https://doi.org/10.1007/s10456-023-09876-7>
- Erol, H., Karakas, E., Ermis, M., Akcakavak, G., Aslan, N. E., & Karatas, O. (2025). Comparison of the Effects of Autologous Cytokine-Rich Serum (ACRS) and Platelet-Rich Plasma (PRP) on Skin Wound Healing. *Acta Veterinaria*, 75(2), 151-165. <https://doi.org/10.2478/acv-2025-0013>
- Ghaisas, M.M., Kshirsagar, S.B., & Sahane, R.S. (2014). Evaluation of wound healing activity of ferulic acid in diabetic rats. *International wound journal*, 11(5), 523-532. <https://doi.org/10.1111/j.1742-481X.2012.01119.x>
- Gonzalez, A.C.d.O., Costa, T.F., Andrade, Z.d.A., & Medrado, A.R.A.P. (2016). Wound healing-A literature review. *Anais brasileiros de dermatologia*, 91, 614-620. <https://doi.org/10.1590/abd1806-4841.20164741>
- Hall, C. W., & Mah, T.-F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, 41(3), 276-301. <https://doi.org/10.1093/femsre/fux010>
- Jiang, Y., Ran, X., Jia, L., Yang, C., Wang, P., Ma, J., Chen, B., Yu, Y., Feng, B., & Chen, L. (2015). Epidemiology of type 2 diabetic foot problems and predictive factors for amputation in China. *The International Journal of Lower Extremity Wounds*, 14(1), 19-27. <https://doi.org/10.1177/1534734614564867>
- Kalan, L.R., Meisel, J.S., Loesche, M.A., Horwinski, J., Soaita, I., Chen, X., Uberoi, A., Gardner, S. E., & Grice, E.A. (2019). Strain-and species-level variation in the microbiome of diabetic wounds is associated with clinical outcomes and therapeutic efficacy. *Cell host & Microbe*, 25(5), 641-655. <https://doi.org/10.1016/j.chom.2019.03.006>
- Kant, V., Gopal, A., Pathak, N.N., Kumar, P., Tandan, S.K., & Kumar, D. (2014). Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats. *International Immunopharmacology*, 20(2), 322-330. <https://doi.org/10.1016/j.intimp.2014.03.009>
- Kirgiz, Ö., & Akçakavak, G. (2025). Clinical, Biochemical and Histopathological Evaluation of Medetomidine–Butorphanol–Ketamine Combination (Kitty Magic Protocol) in Japanese Quails. *J. Anatolian Env. and Anim. Sciences*, 10(5), 580-587. <https://doi.org/10.35229/jaes.1709040>
- Kirgiz, Ö., Altuğ, M.E., Özkan, H., Han, M.C., Akçakavak, G., Özarslan, A.C., & Yücel, S. (2024). 45S5 Bioactive Glass-Ointment Positively Effects on Wound Healing in Rats by Regulating TNF α , IL-10, VEGF, and TGF β . *Journal of Clinical Laboratory Analysis*, 38(17-18), e25094. <https://doi.org/10.1002/jcla.25094>
- Kirgiz, Ö., Kirgiz, F.C., Akçakavak, G., Alakuş, İ., Karataş, Ö., Alakuş, H., Gökçek, İ., Bayraktar, H. S., Ceylan, E., & Akçakavak, F.K. (2026). Topical eucalyptol accelerates the healing of MRSA-infected diabetic wounds in rats by regulating TLR4, NF- κ B, VEGFA and MMP-9 pathways. *Tissue and Cell*, 103602. <https://doi.org/10.1016/j.tice.2026.103602>
- Kolluru, G. K., Bir, S. C., & Kevil, C. G. (2012). Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *International Journal of Vascular Medicine*, 2012(1), 918267. <https://doi.org/10.1155/2012/918267>
- Kumar, D., Jena, G.R., Ram, M., Lingaraju, M.C., Singh, V., Prasad, R., ..., & Kumar, D. (2019). Hemin attenuated oxidative stress and inflammation to improve wound healing in diabetic rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 392(11), 1435-1445. <https://doi.org/10.1007/s00210-019-01682-7>
- Landén, N.X., Li, D., & Stähle, M. (2016). Transition from inflammation to proliferation: a critical step during wound healing. *Cellular and Molecular Life Sciences*, 73(20), 3861-3885. <https://doi.org/10.1007/s00018-016-2268-0>
- Li, R., Liu, K., Huang, X., Li, D., Ding, J., Liu, B., & Chen, X. (2022). Bioactive materials promote wound healing through modulation of cell behaviors. *Advanced Science*, 9(10), 2105152. <https://doi.org/10.1002/advs.202105152>
- Lin, R., Deng, C., Li, X., Liu, Y., Zhang, M., Qin, C., Yao, Q., Wang, L., & Wu, C. (2019). Copper-incorporated bioactive glass-ceramics inducing anti-inflammatory phenotype and regeneration of cartilage/bone interface. *Theranostics*, 9(21), 6300. <https://doi.org/10.7150/thno.36120>
- Mao, C., Lin, C., & Chen, X. (2014). Enhanced healing of full-thickness diabetic wounds using bioactive glass and Yunnan baiyao ointments. *Journal of Wuhan University of Technology-Mater. Sci. Ed.*, 29(5), 1063-1070. <https://doi.org/10.1007/s11595-014-1044-y>
- Okonkwo, U.A., & DiPietro, L.A. (2017). Diabetes and wound angiogenesis. *International Journal of Molecular Sciences*, 18(7), 1419. <https://doi.org/10.3390/ijms18071419>
- Özarslan, A.C. (2023). Preparation of biosilica glass-based porous scaffolds using various elements (Mg–Zn, Mg–Cu, and Sr–Cu) as bioactive glass co-dopants: In vitro bioactivity and biocompatibility behaviors. *Journal of Materials Research*, 38(16), 3874-3889. <https://doi.org/10.1557/s43578-023-01105-7>
- Özarslan, A.C., Özel, C., Okumuş, M.D., Doğan, D., & Yücel, S. (2023). Development, structural and rheological characterization, and in vitro evaluation of the zinc-

- doped 45S5 bioactive glass-vaseline ointment for potential wound healing applications. *Journal of Materials Research*, 38(6), 1557-1572. <https://doi.org/10.1557/s43578-023-00908-y>
- Penven, K., Leroy, D., Verneuil, L., Faguer, K., & Domp Martin, A. (2005). Evaluation of vaseline oil applied prior to UVB TL01 phototherapy in the treatment of psoriasis. *Photodermatology, Photoimmunology & Photomedicine*, 21(3), 138-141. <https://doi.org/10.1111/j.1600-0781.2005.00157.x>
- Pérez-Gutiérrez, L., & Ferrara, N. (2023). Biology and therapeutic targeting of vascular endothelial growth factor A. Nature reviews *Molecular cell biology*, 24(11), 816-834. <https://doi.org/10.1038/s41580-023-00631-w>
- Rahaman, M. N., Day, D. E., Bal, B. S., Fu, Q., Jung, S. B., Bonewald, L. F., & Tomsia, A. P. (2011). Bioactive glass in tissue engineering. *Acta Biomaterialia*, 7(6), 2355-2373. <https://doi.org/10.1016/j.actbio.2011.03.016>
- Raina, N., Rani, R., Pahwa, R., & Gupta, M. (2022). Biopolymers and treatment strategies for wound healing: an insight view. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 71(5), 359-375. <https://doi.org/10.1080/00914037.2020.1838518>
- Ren, J., Yang, M., Xu, F., Chen, J., & Ma, S. (2019). Acceleration of wound healing activity with syringic acid in streptozotocin induced diabetic rats. *Life Sciences*, 233, 116728. <https://doi.org/10.1016/j.lfs.2019.116728>
- Saltoglu, N., Ergonul, O., Tulek, N., Yemisen, M., Kadanali, A., Karagoz, G., Batirel, A., Ak, O., Sonmezer, C., & Eraksoy, H. (2018). Influence of multidrug resistant organisms on the outcome of diabetic foot infection. *International Journal of Infectious Diseases*, 70, 10-14. <https://doi.org/10.1016/j.ijid.2018.02.013>
- Shi, Z., Yao, C., Shui, Y., Li, S., & Yan, H. (2023). Research progress on the mechanism of angiogenesis in wound repair and regeneration. *Frontiers in Physiology*, 14, 1284981. <https://doi.org/10.3389/fphys.2023.1284981>
- Sorg, H., Tilkorn, D.J., Hager, S., Hauser, J., & Mirastschijski, U. (2017). Skin wound healing: an update on the current knowledge and concepts. *European Surgical Research*, 58(1-2), 81-94. <https://doi.org/10.1159/000454919>
- Tang, F., Li, J., Xie, W., Mo, Y., Ouyang, L., Zhao, F., Fu, X., & Chen, X. (2021). Bioactive glass promotes the barrier functional behaviors of keratinocytes and improves the Re-epithelialization in wound healing in diabetic rats. *Bioactive Materials*, 6(10), 3496-3506. <https://doi.org/10.1016/j.bioactmat.2021.02.041>
- Temel-Soylu, T.M., Kececiler-Emir, C., Rababah, T., Ozel, C., Yucel, S., Basaran-Elalmis, Y., Altan, D., Kirgiz, O., Secinti, I.E., & Kaya, U. (2024). Green electrospun poly (vinyl alcohol)/gelatin-based nanofibrous membrane by incorporating 45S5 bioglass nanoparticles and urea for wound dressing applications: Characterization and in vitro and in vivo evaluations. *ACS Omega*, 9(19), 21187-21203. <https://doi.org/10.1021/acsomega.4c01102>
- Tombulturk, F.K., & Demirci, H.D. (2025). Reducing oxidative stress and enhancing antioxidant defenses for faster healing in diabetic wounds: The role of topical metformin. *International Journal of Medical Biochemistry*, 8(2), 89. <https://doi.org/10.14744/ijmb.2025.15428>
- Vargas Guerrero, M., Aendekerk, F.M., de Boer, C., Geurts, J., Lucchesi, J., & Arts, J.J. (2024). Bioactive-glass-based materials with possible application in diabetic wound healing: a systematic review. *International Journal of Molecular Sciences*, 25(2), 1152. <https://doi.org/10.3390/ijms25021152>
- Vendidadala, N.R., Yin, T.P., Nelli, G., Pasupuleti, V.R., Nyamathulla, S., & Mokhtar, S.I. (2021). Gallocatechin-silver nanoparticle impregnated cotton gauze patches enhance wound healing in diabetic rats by suppressing oxidative stress and inflammation via modulating the Nrf2/HO-1 and TLR4/NF-κB pathways. *Life Sciences*, 286, 120019. <https://doi.org/10.1016/j.lfs.2021.120019>
- Xie, X., Zhong, R., Luo, L., Lin, X., Huang, L., Huang, S., Ni, L., Chen, B., Shen, R., & Yan, L. (2021). The infection characteristics and autophagy defect of dermal macrophages in STZ-induced diabetic rats skin wound Staphylococcus aureus infection model. *Immunity, Inflammation and Disease*, 9(4), 1428-1438. <https://doi.org/10.1002/iid3.492>
- Xu, J., Lin, Y., Wang, Y., Gao, H., Li, Y., Zhang, C., Chen, Q., Chen, S., & Peng, Q. (2024). Multifunctional Regeneration Silicon-Loaded Chitosan Hydrogels for MRSA-Infected Diabetic Wound Healing. *Advanced Healthcare Materials*, 13(10), 2303501. <https://doi.org/10.1002/adhm.202303501>