

Investigating the expression level of VEGF and FLT 1 genes in wound healing using electrospun multilayer scaffold in animal model

Asefeh Kamrani ¹, Mitra Heydari Nasrabadi ^{1*}, Raheleh Halabian ², Masoud Ghorbani ³

1. Department of Biology, Parand Branch, Islamic Azad University, Tehran, Iran

2. Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

3. Tissue Engineering and Regenerative Medicine Research Center ,Baqiatallah university of medical sciences



Asefeh Kamrani, PhD

Abstract

Background: Treating skin lesions with bioscaffolds has been a new step in biotechnology and medical studies in recent years. Due to the multi-layered structure of skin tissue, the use of a three-layer scaffold made using an electrospinning technique can lead to planned and targeted skin regeneration. The purpose of this research is to investigate the role of effective VEGF expression and collagen production in healing full-thickness wounds using a three-layer scaffold that has a structure imitating skin tissue and can reduce the duration of wound healing.

Materials and methods: Here, the physical properties of the scaffold were investigated using XRD. Also, after L929 fibroblasts were seeded, cellular scaffolds were implanted in full thickness wounds of rats, and the degree of wound closure was evaluated using histological and genetic techniques.

Result: The evaluations performed on the scaffold confirmed the physical characteristics. In vivo studies showed better wound closure with scaffolds containing zinc ions. This scaffold also showed better observations with histological evaluations than nontreated and control groups. Real-time PCR confirmed the higher expression of VEGF and FIT1 genes in mice treated with scaffolds containing zinc ions.

Conclusion: Scaffolds with bio-nano glass, whether 45s5 type or type containing zinc ions, showed a better ability to heal skin injuries.

Received: 13 Feb. 2026

Revised: 15 Apr. 2026

Accepted: 14 Jul. 2026

Introduction

The skin is the body's largest organ, which is the interface between the outside and the inside of the body and protects the body against pathogenic factors. Wound healing naturally follows various molecular and biological events that depend on the type and size of the wound and occurs when the patient has adequate blood circulation, nutrition, and an appropriate immune response [1]. Nowadays, tissue engineering tries to reduce the duration of wound

healing. [2]. Recent studies have shown that skin bio-substitutes have made major progress in tissue engineering, which has wide applications in wound healing [3]. Using biomaterials and cells to help the body heal itself is the most critical tissue engineering strategy [4]. To improve the efficiency of cell and tissue culture, three-dimensional conditions similar to skin tissue must be created. Therefore, the construction of biological scaffolds should be done with an acceptable similarity to this tissue [5]. Bio scaffolds provide a surface that encourages the desired

** Corresponding author: , Mitra Heydari Nasrabadi, E-mail address: heydarimitra45@iau.ac.ir*

Collagen is one of the main proteins of the extracellular matrix and contributes to the mechanical strength and elasticity of the skin. It also provides a suitable substrate for cell adhesion, proliferation, and differentiation [8,9]. This characteristic of collagen was confirmed by a study that used collagen as electrospun nanofibers to increase the attachment and proliferation of L-929 fibroblasts [10]. Also, in previous studies, collagen nanofibers were cross-linked by glutaraldehyde to increase its mechanical properties [11]. However, this toxic solvent can be replaced by more biocompatible crosslinkers named Ethyl-dimethylaminopropyl carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) [12].

For this reason, in a study in which collagen scaffolds with elastin were made using the electrospinning method, cross-linking was done by EDC. The prepared scaffold demonstrated desired cell adhesion and proliferation after culture by fibroblasts and human keratinocytes without toxicity [13]. Research has shown that the use of collagen in wound dressings shows extreme changes within 10 to 14 days [14] and causes the absorption of wound fluids and secretions. On the other hand, it provides a moist environment around the wound [15]. Combining collagen with skin cells in the wound bed increases the speed of epithelialization [16]. It has been indicated that wound dressings containing collagen can effectively release drugs, growth factors, antibiotics, and natural antimicrobial compounds such as silver in the wound bed [17]. In most cases, to regenerate damaged skin tissue, especially when the scale of damage is large, it should be strengthened with the help of other materials, such as bioactive silicate materials. Among various nanoparticles, it has been shown that 45S5 bioglass can stimulate fibroblasts to develop angiogenesis in the presence of endothelial cells. This phenomenon is required to regenerate large skin injuries [18,19].

Bioglass, as a bioceramic, has the ability to connect to the living tissues of the body by forming a layer at the interface with the living tissues. [20]. Bioglass nanoparticles can accelerate rats' skin damage due to increased cell adhesion, migration, and proliferation [21]. The ability of bioglass to regenerate tissue can be enhanced by adding some ions. Zn ions are other nanoparticles that combine with bioglass due to their antibacterial

ability [22]. Also, it has been observed that zinc ions can induce keratinocyte migration and promote re-epithelialization after being released into the environment [23]. One of the growth factors involved in wound healing is vascular endothelial growth factor (VEGF), a hemodermal glycoprotein, and it also stimulates endothelial cell proliferation, enhances cell migration, and inhibits apoptosis. The use of these ions causes problems related to factors. It dissolves biologicals such as vascular endothelial growth factor (VEGF). Among these problems, we can mention the induction of the immune system. Therefore, besides helping to repair skin wounds successfully, there is a necessity to find other substances to replace them [24].

Fibroblasts are the main cells of the connective tissue and play a central role in the stages of proliferation and wound healing. These cells can secrete different cytokines, growth factors, and collagen [25]. Vascular endothelial growth factor (VEGF) is a unique factor in initiating the migration process of fibroblast cells by inducing components of the wound healing cascade, such as angiogenesis, collagen deposition, and epithelialization. Human VEGF binds to flt1-(VEGFR) and KDR(VEGFR-2) receptors. Angiogenesis plays an important role in forming new vessels to remove cellular debris and provide oxygen and nutrients to the wound bed [26]. During the inflammatory process, many growth factors and cytokines (proangiogenic molecules) are secreted by lymphocytes, macrophages, and epithelial cells to recruit fibroblast cells to the injured area. In this way, the wound bed becomes an angiogenic site, which increases the migration of endothelial cells, proliferation, and creation of neovascularization of the wound bed. Newly formed vessels from endothelial progenitor cells (EPC) are transferred to the wound area and differentiated. Angiogenesis and neovascularization in the wound bed (granulation stage) will progress during the days of wound healing. The electrospun scaffold by bioglass solution and zinc ion showed that these nanoparticles have a significant antibacterial effect and also increase fibroblast proliferation [27]. In addition, PU polyurethane polymer can create mechanical properties similar to skin tissue [28].

The mechanism of zinc ions on cell proliferation and migration is through the activation of MAPK/ERK and Akt pathways [29]. It is clear that divalent zinc ions must be released from the scaffold to the skin tissue to exert

their antiseptic and regenerative roles. Therefore, a suitable design for the scaffold should be such that it provides a platform for creating a stable release pattern of growth factors and various ions, including zinc. In this regard, scaffolds with multiple layers have been introduced for the stable release of zinc ions. On the other hand, the skin tissue consists of 3 layers: epidermis, dermis, and hypodermis, which have protective and regenerative functions [30]. However, these layers have other roles and work together to repair skin damage. If the skin scaffolds are made in multilayers, they will not only be able to control growth factors but may also show better mechanical behavior. The purpose of this study is to investigate the expression level of effective genes in the wound healing process and to examine angiogenesis using fabricated scaffolds: PU/PCL-Collagen/PCL-Collagen, PU/PCL-Collagen-bioglass 45s5/PCL-Collagen and PU/PCL-Collagen-bioglass Zn/PCL-Collagen.

Materials and methods

The desired scaffolds have been examined and evaluated in a separate project as the first phase

of this study.

X-ray diffraction (XRD) analysis

To investigate the crystal structure of standard bio glass nano (45s5) with Zn +2 ion used in the scaffold, the powder sample is subjected to X-ray diffraction analysis using an XRD device with wavelength $\lambda = 1.5406$ Å. The dwell time in each step is one second, the step size is 0.058 degrees, and the range of 2θ is chosen between 10 and 80 degrees. The results will be compared in both control and control groups

In vitro studies

Cell culture

In this study, fibroblast cell line (Pasture Institute, Iran) L-929 was used to analyze the efficiency of the fabricated scaffolds. Cells were detached using trypsin (Gibco, cat. no 15090046) and then counted. After that, about 15×10^3 were planted on scaffold samples measuring 1×1 cm². Cell plates with Dulbecco's modified Eagle's medium (DMEM, Thermofisher, cat. no 11965092) They were incubated with 10% fetal bovine serum (FBS, Thermofisher, cat. no 10082147) at 37°C and 5% CO₂.

In vivo studies

Full thickness wound design

Animal studies were performed on 20 adult male Wister rats weighing 200 to 220 grams, obtained from the Clinical Research Development Center of Baqiyatullah Hospital in Tehran. All animal studies were conducted according to the regulations approved by the Islamic Azad University of Parand, and this project was allowed to perform the animal phase based on the contents of the contract with the ethical code IR.IAU.PIAU REC.1399.003.

The animals were randomly divided into four groups of five, which are:

- No treatment group
- PCL/Collagen-PU scaffold (without bio glass nanoparticles)
- PCL/Collagen-PCL/Collagen/45S5-PU scaffold
- PCL/Collagen-PCL/Collagen/ZN-PU scaffold

All rats were kept under standard conditions with free access to food and water. The animals were first anesthetized with ketamine (80 mg/kg, Sigma) and xylazine (6 mg/kg, Sigma), and then, the hair on the animal's back was shaved to remove the hair completely. Then, the wound area is disinfected, and using an 8 mm punch, we make a full-thickness wound on the back of the animal. Four wounds are made on the back of each mouse for all four groups, and then one wound will remain untreated; one wound with The control scaffold is coated with Collagen-PCL/PU. The other wound is covered with a scaffold containing bioglass 45s5, PCL/Collagen-PCL-bioglass45s5/PU, and the last wound is covered with a scaffold containing zinc bioglass, PCL/Collagen-PCL-zinc/PU. Mice were observed for two weeks, and gene expression evaluations were performed.

Histological analysis

Collagen staining

In this section, on days 7 and 14 after surgery, approximately 5 mm of wound tissue was removed for histological analysis. The prepared tissues were fixed in paraformaldehyde (4% Sigma) for 24 hours and embedded in paraffin (Thermofisher). The paraffin-embedded tissues were subsequently cut at a thickness of 6 μ m using a microtome blade (Leica 2135, Germany) and then dehydrated by serial ethanol dilution. The dried samples were stained

using Masson's trichrome method and studied using a light microscope.

Real-time PCR

In this method, wound tissue was cut from all animals and all four wound areas on days 3 and 7 after surgery, and the samples were immersed in cold PBS. The tissues were ground in liquid nitrogen, and the total RNA of the samples was extracted using a Trizol reagent (Sigma). Possible protein contamination was detected using 2 μ l of extracted RNA and diluted by adding 98 μ l. Then, its OD was measured using NanoDrop USA (Thermo Fisher and Scientific) at 260 and 280 nm. cDNA synthesis kit (Sigma) was used for cDNA synthesis. RT-PCR was performed on Rotor-gene Q Corbett software, and all reactions were repeated three times. After the completion of the PCR reactions, the Mellat program was run on all samples, and Excel file of threshold cycle was extracted. Data analysis was done through Rotor-gene Q software (Corbett). Specific primers for VEGF and FLT1 genes are listed in Table 1. Target genes were normalized against the GAPDH gene as an endogenous reference using the $2^{-\Delta\Delta C_t}$ method [32], and the values of all groups were calibrated using TCPS.

Table 1 primer list

Oligo name	Seq(5-3)
Flt1-F	CGGCAGACCAATACAATCCT
Flt1-R	TGTATTGAGGTCCGTGGTGA
VEGF-F	GCAATGATGAAGCCCTGGAG
VEGF-R	CCTATGTGCTGGCTTTGGTG

Results

XRD

The crystal structure of bioglass nanocomposites is of considerable importance and significantly impacts their structure and biological behavior. With the help of the XRD technique, it is possible to evaluate the crystal structure of biological nano-glasses. It can be seen in Figure 1 that the pure sample S1 has two crystal structures at angles of 21.4 and 23.73 degrees. In sample S2, where BG nanoparticles are placed, the amount of crystalline plates has increased dramatically, possibly due to the better interaction of this nanoparticle with the polymers used. The crystalline structures are significantly

reduced in sample S3, where zinc nanoparticles are located. It seems that in this sample, these nanoparticles have been able to reduce the regular parts in this sample by penetrating the crystalline structures and making the sample move toward becoming amorphous.

Wound morphological analysis

The effect of fabricated scaffolds on wound closure in rats was investigated. This review was done on days 7 and 14. Untreated wounds were considered as a control group.

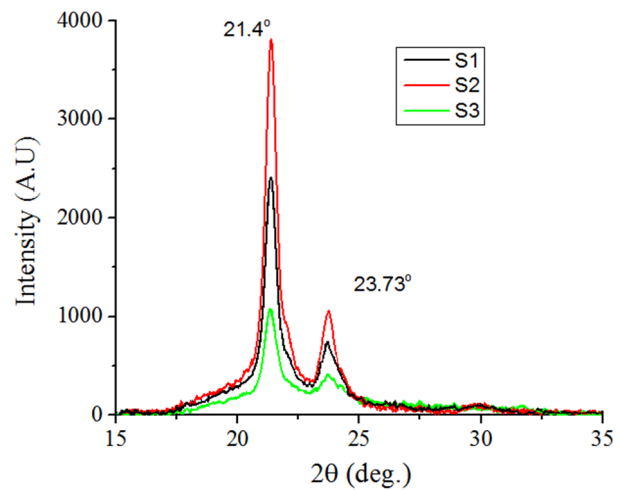


Figure 1- XRD analysis. S1 control group scaffold, S2 scaffold with 45s5 bio-nanoglass, S3 scaffold with Zinc bio-nanoglass

Wounds covered with PCL/Collagen-PU scaffold (without bioglass nanoparticles), PCL/Collagen-PCL/Collagen/45S5-PU PCL/Collagen-PCL/Collagen/ZN-PU scaffold, different effects on wound healing at time points showed different through macroscopic analysis. Wounds treated with scaffolds containing zinc nanoparticles, as shown in Figure 2, showed epithelialization healing much faster than other groups in the early days. After 14 days, they were almost covered with epidermis, healed faster, and provided complete wound closure with a value of $98.5 \pm 3\%$.

Histological analysis

Collagen evaluation

Collagen formation during the wound healing process was studied using Masson's trichrome staining. All groups formed Collagen fibers during wound healing, as seen in Figure 3. However, in the group treated by Zinc scaffold, the amount of collagen fibers is much higher compared to other groups. Figure 3 shows hair follicles

on the 14th day, which shows the regeneration of ECM by collagen. As can be seen in Figure 3, on both the seventh and fourteenth days after surgery in the groups without treatment and treated with control scaffolds, the border between the epidermis and the dermis is hardly visible, but in the groups treated with scaffolds With bio-nanoglass with ECM regeneration as well as neovascularization that occurred beyond the thickness of the dermis in scaffolds containing bio-nanoglass, this border is well visible.

Figure 3: Micrographs of Masson's trichrome and skin wounds on days seven and fourteen. A) group without treatment. B) group treated with control scaffold. C) 45s5 scaffold-treated group. D) Zinc scaffold treated group. Abbreviations: epithelial layer: EL, collagen fibers: CF, hair follicle: HF.

Real time

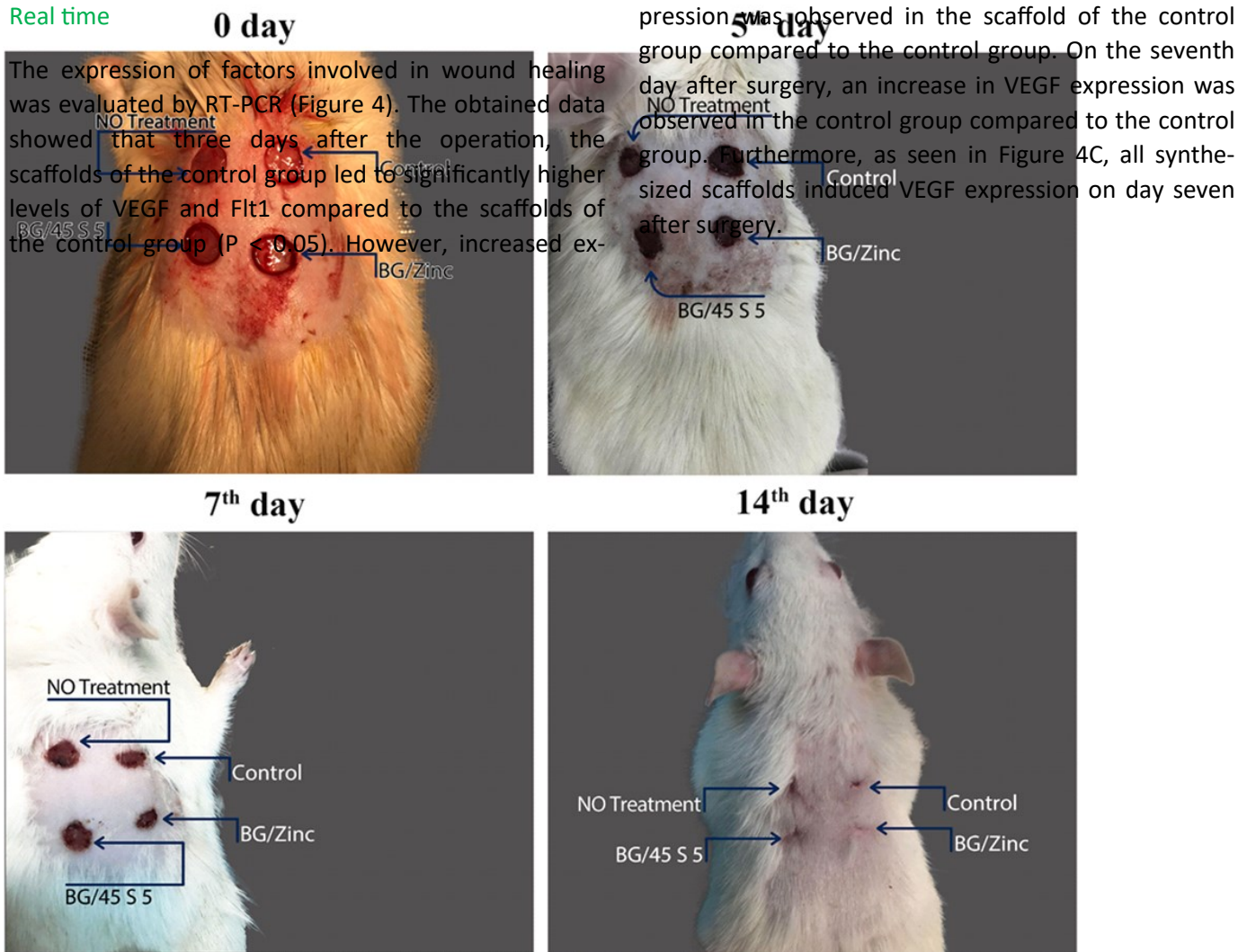
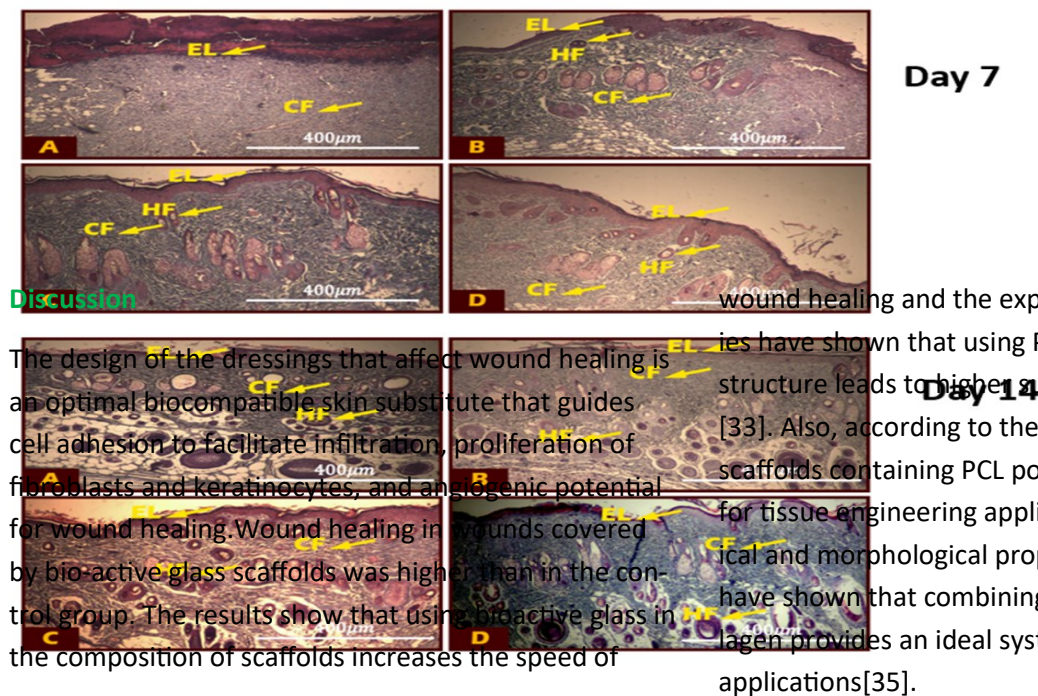


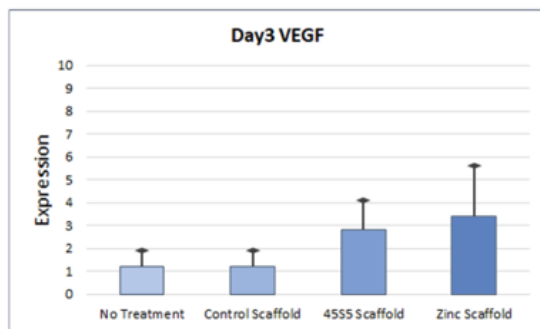
Figure 2: An overview of the wound healing process during 14 days of treatment



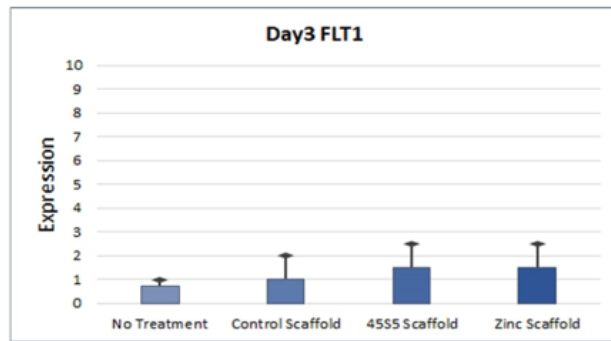
gen is widely used as a significant component in wound dressings due to its hemostatic and proliferative effects in fibroblasts and keratinocytes [41]. Collagen is electrospun with other natural and artificial polymers and cross-links to form bonds with various materials [42]. The results of the studies have shown that the combination of two natural and synthetic polymers, PLGA and collagen nanofibers, did not show any evidence of cytotoxicity

natural
ts indi-
on and

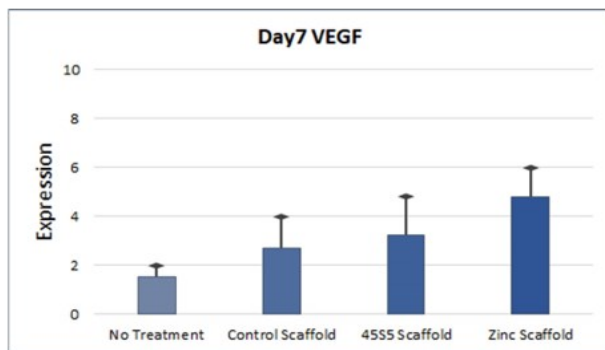
is, com-
nd 6%
ported
active
ptable
found
studies
ne pro-
l fibro-
blasts to wound sites increase collagen deposition and
y accel-
, bioac-
ssential
II com-
endo-
eagues
e disso-
roduce
effects



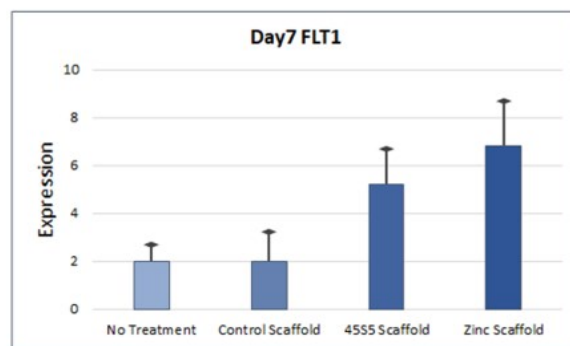
A



B



C



D

Figure 4: Expression level of VEGF and FLT1 genes. A,B three days after surgery, C,D seven days after surgery.

maintain cell differentiation, direct cell growth, and support ECM function [36]. Collagen is the main component of connective tissues and is characterized by the presence of a proline-rich peptide-binding domain[37]. Type I, II, and III collagens are the most common collagens and are called classic fibril forming collagens [38]. Collagen has a relatively stable structure due to the formation of covalent bonds among fibers[39]. However, its proteinaceous nature determines its biodegradability [40]. Colla-

Id con-
taining 45s5 biological nanoglass as well as Zn can observe the antibacterial effects of Zn against *E. coli* [48]. Because the growth of fibroblasts is necessary for skin repair, this study's beneficial result shows better skin regeneration due to the presence of zinc ions. The mechanism of this method includes Zn^{2+} ions reaching the wound area and then fibroblast proliferation[49].The higher expression of FLT1 and VEGF with the fabricated scaffold in the presence of bioactive zinc glass nanoparticles indicates the vital role of these ions in stimulating the dermal molecular signaling pathway. This result is consistent with previous studies in which the level of

VEGF expression was investigated on the same scaffold [50]. In this study, the amount of collagen fibers, followed by re-epithelialization, has increased in the scaffolds containing bioactive nanoglass, consistent with the results reported in other studies conducted on a full thickness mouse wound model [51].

References

1. D. He, H. Li, Biomaterials affect cell-cell interactions in vitro in tissue engineering, *J. Mater. Sci. Technol.* 63 (2021) 62–72. <https://doi.org/10.1016/j.jmst.2020.03.054>
2. P. Ma, Y. Wang, B. Li, H. Hou, Cross-linking effects of carbodiimide, oxidized chitosan oligosaccharide and glutaraldehyde on acellular dermal matrix of basa fish (*Pangasius bocourti*), *Int. J. Biol. Macromol.* 164 (2020) 677–686. <https://doi.org/10.1016/j.ijbiomac.2020.07.147>
3. J.J. Vázquez, E.S.M. Martínez, Collagen and elastin scaffold by electrospinning for skin tissue engineering applications, *J. Mater. Res.* 34 (16) (2019) 2819–2827. <https://doi.org/10.1557/jmr.2019.231>
4. E. Norris, C. Ramos-Rivera, G. Poologasundarampillai, J.P. Clark, Q. Ju, A. Obata, J.V. Hanna, T. Kasuga, C.A. Mitchell, G. Jell, Electrospinning 3D bioactive glasses for wound healing, *Biomed. Mater.* 15 (1) (2020) 015014. <https://doi.org/10.1088/1748-605X/ab55fa>
5. J. Zhu, G. Jiang, G. Song, T. Liu, C. Cao, Y. Yang, Y. Zhang, W. Hong, Incorporation of ZnO/bioactive glass nanoparticles into alginate/chitosan composite hydrogels for wound closure, *ACS Appl. Bio Mater.* 2 (11) (2019) 5042–5052. <https://doi.org/10.1021/acsabm.9b00756>
6. R. Sergi, D. Bellucci, R. Salvatori, V. Cannillo, Chitosan-based bioactive glass gauze: microstructural properties, in vitro bioactivity, and biological tests, *Materials* 13 (12) (2020) 2819. <https://doi.org/10.3390/ma13122819>
7. P. Kumar, V. Kumar, R. Kumar, R. Kumar, C.I. Pruncu, Fabrication and characterization of ZrO₂ incorporated SiO₂–CaO–P₂O₅ bioactive glass scaffolds, *J. Mech. Behav. Biomed. Mater.* 109 (2020) 103854. <https://doi.org/10.1016/j.jmbbm.2020.103854>
8. F. Rezaie, M. Momeni-Moghaddam, H. Naderi-Meshkin, Regeneration and repair of skin wounds: various strategies for treatment, *Int. J. Low Extrem. Wounds* 18 (3) (2019) 247–261. <https://doi.org/10.1177/1534734619859062>
9. Z.P. Rad, J. Mokhtari, M. Abbasi, Calendula officinalis extract/PCL/Zein/Gum arabic nanofibrous bio-composite scaffolds via suspension, two-nozzle and multilayer electrospinning for skin tissue engineering, *Int. J. Biol. Macromol.* 135 (2019) 530–543. <https://doi.org/10.1016/j.ijbiomac.2019.05.181>
10. C. Kimna, S. Tamburaci, F. Tihminlioglu, Novel zein-based multilayer wound dressing membranes with controlled release of gentamicin, *J. Biomed. Mater. Res. B Appl. Biomater.* 107 (6) (2019) 2057–2070. <https://doi.org/10.1002/jbm.b.34298>
11. M. Nair, S.M. Best, R.E. Cameron, Crosslinking collagen constructs: achieving cellular selectivity through modifications of physical and chemical properties, *Appl. Sci.* 10 (19) (2020) 6911. <https://doi.org/10.3390/app10196911>
12. A.B.D. Nandiyanto, R. Oktiani, R. Ragadhita, How to read and interpret FTIR spectroscopy of organic material, *Indones. J. Sci. Technol.* 4 (1) (2019) 97–118. <https://doi.org/10.17509/ijost.v4i1.15806>
13. Z. Neščáková, K. Zheng, L. Liverani, Q. Nawaz, D. Galusková, H. Kaňková, M. Michálek, D. Galusek, A.R. Boccaccini, Multifunctional zinc ion doped sol–gel derived mesoporous bioactive glass nanoparticles for biomedical applications, *Bioact. Mater.* 4 (2019) 312–321. <https://doi.org/10.1016/j.bioactmat.2019.10.002>
14. R. Dwivedi, S. Kumar, R. Pandey, A. Mahajan, D. Nandana, D.S. Katti, D. Mehrotra, Polycaprolactone as biomaterial for bone scaffolds: review of literature, *J. Oral Biol. Craniofac. Res.* 10 (1) (2020) 381–388. <https://doi.org/10.1016/j.jobcr.2019.10.003>
15. H. Wang, X. Liu, D.E. Christiansen, S. Fattahpour, K. Wang, H. Song, S. Mehraeen, G. Cheng, Thermoplastic polyurethane with controllable degradation and critical anti-fouling properties, *Biomater. Sci.* 9 (4) (2021) 1381–1396. <https://doi.org/10.1039/D0BM01503C>
16. M. Bartnikowski, T.R. Dargaville, S. Ivanovski, D.W. Huttmacher, Degradation mechanisms of polycaprolactone in the context of chemistry, geometry and environment, *Prog. Polym. Sci.* 96 (2019) 1–20. <https://doi.org/10.1016/j.progpolymsci.2019.05.004>
17. J.R. Yu, J. Navarro, J.C. Coburn, B. Mahadik, J. Molnar, J.H. Holmes IV, A.J. Nam, J.P. Fisher, Current and future perspectives on skin tissue engineering: key features of bio-

- medical research, translational assessment, and clinical application, *Adv. Healthc. Mater.* 8 (5) (2019) 1801471. <https://doi.org/10.1002/adhm.201801471>
18. S. Hosseinzadeh, S. Hamed, E. Esmaili, M. Kabiri, A. Babae, M. Soleimani, A. Ardeshirylajimi, Mucoadhesive nanofibrous membrane with anti-inflammatory activity, *Polym. Bull.* 76 (9) (2019) 4827–4840. <https://doi.org/10.1007/s00289-018-2641-0>
19. Z. Lu, W. Wang, J. Zhang, P. Bártolo, H. Gong, J. Li, Electrospun highly porous poly (L-lactic acid)-dopamine-SiO₂ fibrous membrane for bone regeneration, *Mater. Sci. Eng. C* 117 (2020) 111359. <https://doi.org/10.1016/j.msec.2020.111359>
20. K. Shirani, M.S. Nourbakhsh, M. Rafienia, Electrospun polycaprolactone/gelatin/bioactive glass nanoscaffold for bone tissue engineering, *Int. J. Polym. Mater. Polym. Biomater.* 68 (10) (2019) 607–615. <https://doi.org/10.1080/00914037.2018.1473268> (Corrected standard domain prefix to 10.1080)
21. N. Dehnavi, K. Parivar, V. Goodarzi, A. Salimi, M.R. Nourani, Systematically engineered electrospun conduit based on PGA/collagen/bioglass nanocomposites: The evaluation of morphological, mechanical, and bio-properties, *Polym. Adv. Technol.* 30 (9) (2019) 2192–2206. <https://doi.org/10.1002/pat.4653>
22. H. Elkhoully, W. Mamdouh, D.I. El-Korashy, Electrospun nano-fibrous bilayer scaffold prepared from polycaprolactone/gelatin and bioactive glass for bone tissue engineering, *J. Mater. Sci. Mater. Med.* 32 (9) (2021) 111. <https://doi.org/10.1007/s10856-021-06584-0>
23. Rajzer, A. Kurowska, J. Frankova, R. Sklenářová, A. Nikodem, M. Dziadek, A. Jabłoński, J. Janusz, P. Szczygieł, M. Ziąbka, 3D-printed polycaprolactone implants modified with bioglass and Zn-doped bioglass, *Materials* 16 (3) (2023) 1061. <https://doi.org/10.3390/ma16031061>
24. J. Bejarano, A.R. Boccaccini, C. Covarrubias, H. Palza, Effect of Cu- and Zn-doped bioactive glasses on the in vitro bioactivity, mechanical and degradation behavior of biodegradable PDLLA scaffolds, *Materials* 13 (13) (2020) 2908. <https://doi.org/10.3390/ma13132908>
25. R. Wetzel, O. Bartzok, D. Brauer, Influence of low amounts of zinc or magnesium substitution on ion release and apatite formation of Bioglass 45S5, *J. Mater. Sci. Mater. Med.* 31 (10) (2020) 89. <https://doi.org/10.1007/s10856-020-06429-2>
26. R. Sergi, V. Cannillo, A.R. Boccaccini, L. Liverani, Incorporation of bioactive glasses containing Mg, Sr, and Zn in electrospun PCL fibers by using benign solvents, *Appl. Sci.* 10 (16) (2020) 5530. <https://doi.org/10.3390/app10165530>
27. P. Zhou, B.L. Garcia, G.A. Kotsakis, Comparison of antibacterial and antibiofilm activity of bioactive glass compounds S53P4 and 45S5, *BMC Microbiol.* 22 (1) (2022) 112. <https://doi.org/10.1186/s12866-022-02528-0>
28. K. Schuhladen, L. Stich, J. Schmidt, A. Steinkasserer, A.R. Boccaccini, E. Zinser, Cu, Zn doped borate bioactive glasses: Antibacterial efficacy and dose-dependent in vitro modulation of murine dendritic cells, *Biomater. Sci.* 8 (8) (2020) 2143–2155. <https://doi.org/10.1039/D0BM00142C>
29. Ali, B.U. Amin, W. Yu, T. Gui, W. Cong, K. Zhang, Z. Tong, J. Hu, X. Zhan, Q. Zhang, Eco-friendly biodegradable polyurethane based coating for antibacterial and antifouling performance, *Chin. J. Chem. Eng.* 54 (2023) 80–88. <https://doi.org/10.1016/j.cjche.2022.03.023>
30. J.A. Claudio-Rizo, I.A. González-Lara, T.E. Flores-Guía, L.F. Cano-Salazar, D.A. Cabrera-Munguía, J.J. Becerra-Rodríguez, Study of the polyacrylate interpenetration in a collagen-polyurethane matrix to prepare novel hydrogels for biomedical applications, *Int. J. Biol. Macromol.* 156 (2020) 27–39. <https://doi.org/10.1016/j.ijbiomac.2020.04.032>
31. Kamrani, M.H. Nasrabadi, R. Halabian, M. Ghorbani, A biomimetic multi-layer scaffold with collagen and zinc doped bioglass as a skin-regeneration agent in full-thickness injuries and its effects in vitro and in vivo, *Int. J. Biol. Macromol.* 253 (2023) 127163. <https://doi.org/10.1016/j.ijbiomac.2023.127163>

32. S. Zare, M. Mohammadpour, Z. Izadi, S. Ghanzafari, S. Nadri, H. Samadian, Nanofibrous hydrogel nanocomposite based on strontium-doped bioglass nanofibers for bone tissue engineering applications, *Biology* 11 (10) (2022) 1472. <https://doi.org/10.3390/biology11101472>
33. Y. Lin, L. Zhang, Y. Yang, M. Yang, Q. Hong, K. Chang, J. Dai, L. Chen, C. Pan, Y. Hu, Loading gentamicin and Zn²⁺ on TiO₂ nanotubes to improve anticoagulation, endothelial cell growth, and antibacterial activities, *Stem Cells Int.* 2021 (2021) 1–13. <https://doi.org/10.1155/2021/5598150>
34. S. Yadav, A. Ali, S. Krishnamurthy, P. Singh, R. Pyare, In-vitro analysis of bioactivity, hemolysis, and mechanical properties of Zn substituted calcium zirconium silicate (baghdadite), *Ceram. Int.* 47 (11) (2021) 16037–16053. <https://doi.org/10.1016/j.ceramint.2021.02.183>
35. J. Rashed, Combined surgical treatments in cutaneous malignant melanoma, in: *MedEspera: The 9th International Medical Congress for Students and Young Doctors*, May 12–14, 2022, Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova, 2022, p. 251.
36. S. Yao, Y. Zhao, Y. Xu, B. Jin, M. Wang, C. Yu, Z. Guo, S. Jiang, R. Tang, X. Fang, Injectable dual-dynamic-bond cross-linked hydrogel for highly efficient infected diabetic wound healing, *Adv. Healthc. Mater.* 11 (14) (2022) 2200516. <https://doi.org/10.1002/adhm.202200516>
37. M.M. Malara, B.N. Blackstone, M.E. Baumann, J.K. Bailey, D.M. Supp, H.M. Powell, Cultured epithelial autograft combined with micropatterned dermal template forms rete ridges in vivo, *Tissue Eng. Part A* 26 (21–22) (2020) 1138–1146. <https://doi.org/10.1089/ten.tea.2020.0051>
38. M. Vinayak, S. Jana, P. Datta, H. Das, B. Chakraborty, P. Mukherjee, S. Mondal, B. Kundu, S.K. Nandi, Accelerating full-thickness skin wound healing using zinc and cobalt doped-bioactive glass-coated eggshell membrane, *J. Drug Deliv. Sci. Technol.* 81 (2023) 104273. <https://doi.org/10.1016/j.jddst.2023.104273>
39. N. Mutlu, L. Liverani, F. Kurtuldu, D. Galusek, A.R. Boccaccini, Zinc improves antibacterial, anti-inflammatory and cell motility activity of chitosan for wound healing applications, *Int. J. Biol. Macromol.* 213 (2022) 845–857. <https://doi.org/10.1016/j.ijbiomac.2022.06.012>
40. D.K. Chang, M.R. Louis, A. Gimenez, E.M. Reece, The basics of integra dermal regeneration template and its expanding clinical applications, *Semin. Plast. Surg.* 33 (3) (2019) 185–189. <https://doi.org/10.1055/s-0039-1693436>
41. D. Singh, V. Rai, D.K. Agrawal, Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration, *Cardiol. Cardiovasc. Med.* 7 (1) (2023) 5–16. <https://doi.org/10.26502/fccm.92920301>
42. S.S. Mathew-Steiner, S. Roy, C.K. Sen, Collagen in Wound Healing, *Bioengineering (Basel)* 8 (5) (2021) 63. <https://doi.org/10.3390/bioengineering8050063>
43. Kisling, R.M. Lust, L.C. Katwa, What is the role of peptide fragments of collagen I and IV in health and disease?, *Life Sci.* 228 (2019) 30–34. <https://doi.org/10.1016/j.lfs.2019.04.042>
44. S. Holm Nielsen, L. Jonasson, K. Kalogeropoulos, et al., Exploring the role of extracellular matrix proteins to develop biomarkers of plaque vulnerability and outcome, *J. Intern. Med.* 287 (5) (2020) 493–513. <https://doi.org/10.1111/joim.13025>
45. R. Mohindra, D.K. Agrawal, F.G. Thankam, Altered vascular extracellular matrix in the pathogenesis of atherosclerosis, *J. Cardiovasc. Transl. Res.* 14 (4) (2021) 647–660. <https://doi.org/10.1007/s12265-020-10091-8>
46. F.G. Thankam, D.K. Agrawal, Hypoxia-driven secretion of extracellular matrix proteins in the exosomes reflects the asymptomatic pathology of rotator cuff tendinopathies, *Can. J. Physiol. Pharmacol.* 99 (2) (2021) 224–230. <https://doi.org/10.1139/cjpp-2020-0252>