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Glycine-Functionalized Polycaprolactone Electrospun Nanofibers as Bioactive Scaffolds for Skin Repair

Leonardo Prieto-Abello , Liliana Lizarazo-Fonseca , Gustavo Salguero  and Ingrid Silva-Cote *

Unidad de Ingeniería Tisular, Instituto Distrital de Ciencia, Biotecnología e Innovación en Salud (IDCBIS), Bogotá D.C. 111611, Colombia; lprieto@idcbis.org.co (L.P.-A.); alizarazo@idcbis.org.co (L.L.-F.); gsalguero@idcbis.org.co (G.S.)

* Correspondence: izsilva@idcbis.org.co

Abstract

Chronic cutaneous ulcers pose a major clinical challenge due to persistent inflammation, impaired angiogenesis, and limited regenerative capacity, underscoring the need to develop bioactive scaffolds that mimic the extracellular matrix (ECM) and promote skin repair. In this study, electrospun Poly(ϵ -caprolactone) (PCL) scaffolds functionalized with 10% (PCLGLI10) and 20% (PCLGLI20) glycine were fabricated, physicochemically and mechanically characterized, and evaluated in combination with human Wharton's jelly mesenchymal stromal cells (hWJ-MSCs). Glycine incorporation reduced fiber diameter to the nanoscale range (140–155 nm) and increased scaffold porosity (~71–72%) while preserving mechanical properties compatible with skin. FTIR and X-ray diffraction analyses confirmed glycine incorporation and a polymorphic transition from α - to γ -glycine during electrospinning. Cell viability remained above 95% in all scaffolds; however, PCLGLI10 significantly enhanced cell proliferation, metabolic activity, and the secretion of VEGF and HGF, mediators associated with angiogenesis and tissue repair. Furthermore, in a guinea pig full-thickness wound model, the PCLGLI10+hWJ-MSCs construct promoted a modulated inflammatory response and more organized collagen deposition. These findings support the potential of glycine-functionalized electrospun scaffolds as a promising strategy for chronic cutaneous ulcer regeneration.

Keywords: electrospun scaffolds; Poly(ϵ -caprolactone) (PCL); glycine; Wharton's jelly mesenchymal stromal cells (hWJ-MSCs); chronic cutaneous ulcers

1. Introduction

The skin is the largest organ of the human body and represents a complex, dynamic structure with critical roles in mechanical protection, thermoregulation, fluid balance, and immune defense [1]. Loss of skin integrity due to trauma, burns, surgery, or chronic conditions such as diabetes mellitus and peripheral vascular disease leads to wounds that often fail to heal properly, generating substantial humanistic and economic burdens [2,3]. Chronic wounds, particularly diabetic foot ulcers, venous leg ulcers, and pressure ulcers, are characterized by persistent inflammation, impaired angiogenesis, recurrent infection, and deficient extracellular matrix remodeling, which severely compromise tissue regeneration and significantly reduce patients' quality of life while increasing healthcare costs worldwide [4–6]. Skin wound healing is a tightly orchestrated process consisting of overlapping phases of hemostasis, inflammation, proliferation, and remodeling, regulated by an intricate network of growth factors, cytokines, immune cells, and ECM components [7].



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When this process is disrupted, fibrotic scarring or chronic non-healing ulcers develop. Therefore, the development of advanced bioactive scaffolds capable of recreating a pro-regenerative microenvironment represents a promising therapeutic approach for treating chronic cutaneous ulcers. To address these limitations, tissue engineering has emerged as a powerful strategy combining three pillars: (i) biomimetic scaffolds that aim to mimic the architecture of the native ECM; (ii) cells with regenerative and immunomodulatory potential; and (iii) bioactive cues capable of guiding cell behavior toward functional repair [4,7,8].

Electrospinning has emerged as a leading technology for fabricating fibrous scaffolds that closely mimic the native ECM at the nano- and microscale while allowing precise control over fiber diameter, porosity, and surface properties [9–11]. In particular, electrospun nanofibers with diameters below 300 nm have been shown to replicate the dimensions of native collagen fibrils, thereby generating a biomimetic topography that promotes cell adhesion, migration, proliferation, and differentiation [12–14]. Among the polymers commonly employed for electrospinning, PCL is widely recognized for its biocompatibility, FDA approval, slow degradation rate, and favorable mechanical and processing properties for biomedical applications. Nevertheless, the intrinsic hydrophobicity and biological inertness of PCL limit its ability to support cellular functions actively, necessitating functionalization strategies to enhance its bioactivity [15,16]. In this context, the incorporation of bioactive molecules into electrospun PCL scaffolds has attracted increasing interest [10,13,17]. Glycine, the smallest amino acid, has emerged as a promising additive due to its cytoprotective properties and its ability to modulate cellular metabolism and inflammatory responses [18–20]. Moreover, glycine has been reported to inhibit Ca^{2+} -dependent proteolysis [21] and protect epithelial cells against oxidative stress-induced damage [22], suggesting its potential to improve cellular stability and regenerative responses under adverse microenvironmental conditions [18,19,23]. Recent evidence has also shown that glycine-containing peptides can promote wound healing processes, including improved vascularization and re-epithelialization, further supporting a potential role for glycine-related systems in tissue repair [24]. Together, these properties support the use of glycine as a promising candidate to functionalize PCL-based scaffolds for skin tissue engineering.

In parallel, mesenchymal stromal cells (MSCs) have emerged as one of the most promising cell sources for regenerative medicine due to their multilineage potential, relatively low immunogenicity, and rich paracrine secretome [25,26]. hWJ-MSCs offer particular advantages, including non-invasive procurement, robust proliferative capacity, and reported immunomodulatory and pro-angiogenic properties [27,28]. Our group has previously demonstrated that hWJ-MSCs can be efficiently isolated, expanded, and combined with various biomaterials, including human acellular dermal matrix and PCL/collagen scaffolds, to support *in vitro* growth factor secretion and regenerative responses in wound healing models [29–32]. Furthermore, when cultured on instructive substrates, hWJ-MSCs have been reported to exhibit features consistent with mesenchymal-to-epithelial transition (MET), including the expression of keratinocyte-associated markers, suggesting their potential contribution to re-epithelialization in skin repair models [33,34].

Despite these advances, the rational integration of an ECM-mimicking architecture with simple, amino acid-based biochemical functionalization strategies and regenerative cell sources remains underexplored, particularly regarding the use of amino acids as bioactive modulators within electrospun systems. Here, we hypothesized that glycine incorporation into electrospun PCL scaffolds would modulate hWJ-MSCs behavior by enhancing their paracrine activity, while supporting cell–material interactions relevant to tissue repair. Secondary outcomes included evaluation of proliferative responses, early epithelial-like phenotypic changes, and modulation of the wound healing microenvironment *in vivo*.

To test this hypothesis, we fabricated electrospun PCL scaffolds containing 10% (PCLGLI10) or 20% (PCLGLI20) glycine, characterized their morphology, crystallinity, chemical composition, porosity, and mechanical properties, and evaluated their interaction with hWJ-MSCs in terms of viability, proliferation, growth factor secretion, and early epithelial differentiation. Finally, we assessed the *in vivo* wound healing performance of the most promising scaffold, alone and in combination with hWJ-MSCs, in a guinea pig full-thickness excisional model. The guinea pig was selected as the *in vivo* model due to its well-documented anatomical and permeability similarities to human skin, making it a translationally relevant surrogate for cutaneous regeneration studies [35,36]. Together, this work presents a glycine-functionalized scaffold that combines an ECM-inspired architecture, glycine-mediated bioactivity, and the regenerative potential of hWJ-MSCs, providing a promising strategy for the development of advanced skin substitutes.

2. Materials and Methods

2.1. Electrospun PCL Scaffolds Functionalized with Glycine

Electrospun scaffolds were fabricated using a modified protocol from Lizarazo-Fonseca et al. [30,32]. Briefly, polymer solutions of PCL (MW $\sim 80,000$ g $\cdot$ mol $^{-1}$; 440744, Sigma-Aldrich, St. Louis, MO, USA) and glycine (50046, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 2,2,2-trifluoroethanol ($\geq 99\%$; 8.08259.1000, Merck, Darmstadt, Germany) to obtain a total solute concentration of 10% (*w/v*). Three solutions were prepared: a PCL-only solution used as a control, and two PCL/glycine solutions at mass ratios of 9:1 and 8:2, designated PCLGLI10 and PCLGLI20, respectively. All solutions were magnetically stirred at 500 rpm for 15 h before electrospinning.

Each solution was loaded into a 5 mL syringe and processed using a single-nozzle electrospinning system (HV 350R Unlimited Company Amherst power supply, NE injector model 4000 Syringe Pump Company, Farmingdale, NY, USA). A 17 kV voltage was applied between the nozzle and a grounded collector positioned 15 cm from the needle. The polymer solution was delivered at a flow rate of 1.3 mL h $^{-1}$, and randomly oriented electrospun fibers were collected. The average temperature and relative humidity during electrospinning were 20 ± 2 °C and 45–50%, respectively. The scaffolds were dried at 37 °C for 120 h.

2.2. Morphology of Electrospun Scaffolds

The morphology of PCL, PCLGLI10, and PCLGLI20 scaffolds was examined by scanning electron microscopy (SEM). Samples were sputter-coated with gold using a sputter coater (Q150R ES, Quorum Technologies, Laughton, UK) operated at 1 kV and 5 mA for 60 s. The coated samples were imaged using a field-emission scanning electron microscope (JSM-7600F, JEOL, Tokyo, Japan) operated at 20 kV. Images were acquired at 1000 $\times$ magnification, and average fiber diameters were determined by measuring at least 50 fibers per scaffold from three independent images using Fiji (ImageJ, NIH, USA). The apparent surface porosity was estimated through two-dimensional (2D) image analysis of SEM micrographs. Images were segmented via manual thresholding to differentiate the fiber network from the void spaces, and the area fraction of voids was calculated using Fiji software. Consistent thresholding parameters were applied across all samples to ensure a standardized comparison of the surface architecture, although these values represent the projected surface porosity rather than the three-dimensional volumetric porosity of the scaffolds.

2.3. Structural and Chemical Analysis

X-ray diffraction (XRD) patterns were recorded using an X'Pert PRO MPD diffractometer (PANalytical, Almelo, The Netherlands) to assess the crystalline structure of glycine and PCL, PCLGLI10, and PCLGLI20 scaffolds. Finely ground samples were placed as a thin layer on a glass sample holder and analyzed in reflection mode using Cu K α radiation at 45 kV and 40 mA. Data were collected over a 2θ range of 3–60° at a scanning rate of 2.5° min^{−1}. Diffraction patterns were analyzed using Match! Version 3 Software (Crystal Impact, Bonn, Germany).

Fourier transform infrared (FTIR) spectroscopy was performed to analyze the functional groups of PCL, glycine, PCLGLI10, and PCLGLI20 using a Prestige-21 spectrometer (Shimadzu, Kyoto, Japan). Spectra of scaffold samples were acquired using the attenuated total reflection (ATR) mode, while glycine powder was analyzed using the KBr pellet method. Spectra were recorded over an appropriate wavenumber range, and peak identification and integration were carried out using OriginPro 9.0 (OriginLab, Northampton, MA, USA).

2.4. Mechanical Testing

The tensile properties of the scaffolds were evaluated using a universal testing machine (AG-IS, Shimadzu, Kyoto, Japan) equipped with a 50 N load cell and rubber-coated grips to prevent slippage. The gauge length was set to 20 mm, and tests were performed at a crosshead speed of 10 mm min^{−1} under ambient conditions (24 ± 3 °C and 45–50% relative humidity).

Three rectangular specimens (10 × 35 mm) were prepared from each scaffold type, and their thickness was measured before testing. Young's modulus was determined from the initial linear region of the stress–strain curve using TRAPEZIUM 2 software (Shimadzu, Kyoto, Japan). Results are presented as mean ± standard deviation.

2.5. Scaffolds Sterilization

The PCL, PCLGLI10, and PCLGLI20 scaffolds were cut using a 1 cm diameter punch. For *in vitro* assays, the scaffolds were placed in 48-well culture plates and then sterilized using γ -irradiation equipment (Gamma-Medical with Cesium-137 BIOBEAM 2000 radiation source (Eckert & Ziegler BEBIG GmbH, Berlin, Germany)) at 50 Gy at room temperature under dark conditions, following a previously reported protocol [32]. For *in vivo* test, sterilization was performed in a γ -irradiation equipment Gammabeam 651 PT irradiator (Nordion/Atomic Energy of Canada Ltd., Ottawa, Canada) equipped with a Cobalt-60 (⁶⁰Co) source located at the Instituto de Ciencias Nucleares (UNAM, México D.F, México) with a total dose of 25 kGy at room temperature.

2.6. Biofabrication of Tissue-Engineered Constructs

hWJ-MSCs were isolated from umbilical cords obtained from healthy pregnant women after informed consent, in accordance with protocols approved by the Ethics Committee of the Secretaría Distrital de Salud de Bogotá, Colombia. Umbilical cord blood was screened for infectious diseases, including hepatitis B and C, syphilis, Chagas disease, HIV, and HTLV I and II. The immunophenotype and multilineage potential of the hWJ-MSCs were evaluated as previously reported [37].

To assess construct formation, hWJ-MSCs viability and cytocompatibility were evaluated by seeding 3 × 10⁴ hWJ-MSCs (passage 5), onto PCL, PCLGLI10, and PCLGLI20 scaffolds. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; 11885-084, Gibco, Life Technologies, Carlsbad, CA, USA) supplemented with 10% human platelet lysate (hPL; GMP Group, Advance Therapy Unit, IDC BIS, Bogotá, Colombia), 1% antibiotic–antimycotic

(15240-062, Gibco, Grand Island, NY, USA), and 0.16% heparin (103/AP/RR/97/F/R, Gland Pharma Ltd., Hyderabad, Telangana, India), and maintained at 37 °C in a humidified atmosphere with 5% CO₂.

Cell viability was assessed at 24, 72, and 120 h using a LIVE/DEAD™ assay (L3224, Invitrogen, Waltham, MA, USA). At each time point, constructs were washed with PBS (1×) and incubated with 200 µL of staining solution for 10 min at 37 °C. Fluorescence images were acquired using an inverted microscope (DMI8, Leica Microsystems, Wetzlar, Germany). Viability was quantified using Fiji (ImageJ, NIH, USA) by calculating the ratio of calcein-positive (live) to total stained area.

Cell morphology was evaluated by SEM. Constructs were fixed in 4% paraformaldehyde for 1 h and washed twice with deionized water. Samples were then dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 96% for 15 min each, followed by 100% ethanol twice for 15 min each) and treated with 1,1,3,3,3-hexamethyldisilazane (HMDS; 804324, Sigma-Aldrich, St. Louis, MO, USA) for chemical drying. Samples were sputter-coated with gold (Q150R ES, Quorum Technologies, Laughton, UK) and imaged using a field-emission SEM (JSM-7600F, JEOL, Tokyo, Japan) at 20 kV and 5000× magnification.

Metabolic activity was assessed using a resazurin assay (resazurin sodium salt powder, Sigma-Aldrich, St. Louis, MO, USA). At each time point, the culture medium was replaced with fresh medium containing 1% resazurin, and the cultures were incubated for 3 h. Supernatants (100 µL) were collected, and absorbance at 570 and 600 nm was measured using a Synergy MX spectrophotometer microplate reader (Biotek Synergy, Winooski, VT, USA). hWJ-MSCs cultured on standard tissue culture plates were used as controls. Metabolic activity was calculated from resazurin reduction using a previously reported method [34], and expressed as a percentage relative to control cells. This approach was used as an indirect indicator of cell metabolic activity and proliferation, as described in prior studies employing hWJ-MSCs-based skin constructs.

All experiments were performed in triplicate using hWJ-MSCs from three independent donors, and results are presented as mean ± standard deviation.

2.7. Growth Factors Quantification Involved in Skin Tissue Repair

To evaluate the effect of PCL, PCLGLI10, and PCLGLI20 scaffolds on growth factor production by hWJ-MSCs, constructs were maintained at 37 °C in a humidified atmosphere with 5% CO₂. Supernatants were collected at 24, 48, and 72 h, centrifuged at 1200 rpm for 6 min to remove cell debris, and stored at −20 °C until analysis. A cell-free DMEM supplemented with hPL at the same concentration used in each experimental group was included as a baseline control to account for growth factors present in the culture medium. Additionally, hWJ-MSCs cultured on tissue culture plates (TCP) were used as cell controls.

The production of six growth factors associated with wound healing—Angiopoietin-1 (Ang-1), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), platelet-derived growth factor-BB (PDGF-BB), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF)—was quantified using a ProcartaPlex™ multiplex immunoassay (Cat. No. 358901579, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, 50 µL of conditioned medium was incubated with 50 µL of magnetic beads for 2 h, then incubated with a biotinylated detection antibody cocktail for 1 h at room temperature. After washing, streptavidin-phycoerythrin was added and incubated for 30 min. Beads were washed, resuspended, and analyzed using a Luminex 200 instrument (Luminex Corp., Austin, TX, USA).

Concentrations were calculated by correcting for the sample dilution factor (1:100) and are reported as absolute values. No normalization to cell number or protein content was performed; therefore, results reflect total factor production per construct.

2.8. Epithelial-like Differentiation of hWJ-MSCs on PCL and Glycine Scaffolds

For epithelial-like induction, 1.5×10^4 hWJ-MSCs were seeded onto PCL and PCLGLI10 scaffolds and cultured in keratinocyte basal medium (KGM Bullet Kit w/o Ca^{2+} , Lonza Bioscience, Basel, Switzerland) supplemented with bovine pituitary extract (0.4%), epidermal growth factor (EGF, 0.01%), insulin (0.01%), hydrocortisone (0.01%), and antibiotic–antimycotic (0.01%). In parallel, cells were cultured in DMEM supplemented as a bioactive non-epithelial inductive control condition. All cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2 , with medium changes every 3 days. Samples were collected at days 7 and 10, fixed with 4% paraformaldehyde (PFA), and processed for immunocytochemical and SEM analyses.

Immunocytochemical analysis was performed to evaluate mesenchymal and epithelial-associated protein expression in hWJ-MSCs cultured on PCL and PCLGLI10 scaffolds. Samples were washed three times with PBS (1×) and permeabilized with 0.5% Triton X-100 for 10 min. After washing, samples were incubated in PBS/0.5% bovine serum albumin (BSA) and subsequently blocked in PBS/5% fetal bovine serum (FBS) for 1 h at room temperature. Samples were incubated overnight at 4 °C with an Alexa Fluor® 594-conjugated anti-vimentin antibody (2.5 µg/mL, BioLegend, San Diego, CA, USA) and an anti-involucrin primary antibody (5 µg/mL, BioLegend, San Diego, CA, USA). After washing, involucrin was detected using an Alexa Fluor® 488-conjugated goat anti-rabbit secondary antibody (1:1000, Abcam, Cambridge, UK) for 1 h at 4 °C. Nuclei were counterstained with DAPI (0.1 µg/mL). Constructs were imaged using a confocal laser scanning microscope (LSM 900 with Airyscan 2, ZEISS, Oberkochen, Germany). All analyses were performed on at least three independent scaffolds per condition, with representative fields acquired per sample.

For SEM analysis, PCL and PCLGLI10 scaffolds, cultured under DMEM and KGM conditions, were prepared following graded ethanol dehydration (30%, 50%, 70%, 90%, and 96% for 15 min each, followed by two incubations in 100% ethanol for 15 min). Samples were then chemically dried using 1,1,1,3,3,3-hexamethyldisilazane (HMDS; Sigma-Aldrich, St. Louis, MO, USA) and sputter-coated with gold (Q150R ES, Quorum Technologies, Laughton, UK). Imaging was performed using a field-emission scanning electron microscope (JSM-7600F, JEOL, Tokyo, Japan) at 20 kV. Multiple regions per scaffold were analyzed to ensure representative surface evaluation.

2.9. In Vivo Healing Assays

Among the formulations evaluated, PCLGLI10 demonstrated the most favorable *in vitro* performance and was therefore selected for subsequent *in vivo* assessment, while PCL scaffolds were used as the material control. Ten (10) Hartley guinea pigs (*Cavia porcellus*; 8–10 weeks old, 600–800 g) were obtained from the Instituto Nacional de Salud (Bogotá, Colombia) and randomly allocated into two experimental groups ($n = 5$ animals per group). All animal procedures were reviewed and approved by the Institutional Committee for the Care and Use of Laboratory Animals of the Instituto Nacional de Salud (CICUAL-INS; approval No. R-23-2023) and were conducted in accordance with Colombian regulations governing animal research, including Resolution No. 008430 of 1993 issued by the Ministry of Health and Law 84 of 27 December 1989, ensuring compliance with established ethical and animal welfare standards.

In both experimental groups, each animal received three standardized full-thickness excisional wounds (1×1 cm) on the dorsal region. In the scaffold group, wounds were

randomly assigned to receive a PCL scaffold, a PCLGLI10 scaffold, or to be left untreated as an internal negative control. In the construct group, wounds were randomly assigned to treatment with a PCL scaffold seeded with mesenchymal stromal cells (PCL+hWJ-MSCs), a PCLGLI10 scaffold seeded with mesenchymal stromal cells (PCLGLI10+hWJ-MSCs), or left untreated as an internal negative control. This within-animal experimental design enabled direct comparison of the different treatments while minimizing inter-animal variability and reducing the total number of animals required.

This experimental design resulted in a total of 30 wounds: PCL ($n = 5$), PCLGLI10 ($n = 5$), PCL+hWJ-MSCs ($n = 5$), PCLGLI10+hWJ-MSCs ($n = 5$), and untreated controls ($n = 10$). Constructs were prepared by seeding 5.0×10^4 hWJ-MSCs onto 1×1 cm PCL or PCLGLI10 scaffolds and cultured for 24 h prior to implantation. Animals were anesthetized with isoflurane (5% for induction and 3% for maintenance) and placed in a prone position. The dorsal surgical site was shaved and disinfected with 70% ethanol and 4% chlorhexidine solution. Scaffolds and constructs were secured to the wound beds using Prolene® 6-0 sutures and covered with Mepitel® One dressing.

Euthanasia was performed on day 18 by CO₂ displacement. Skin samples (approximately 1.5×1.5 cm), including the repaired wound and surrounding healthy tissue, were collected for histological processing. Collected tissues were first preserved in 4% paraformaldehyde and then progressively dehydrated in increasing concentrations of ethanol. Samples were subsequently cleared with xylene and embedded in paraffin blocks for histological analysis. Thin sections (3–4 μ m thick) were cut and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT). A histopathologist blinded to the sample identity quantified inflammatory cells (macrophages, lymphocytes, and polymorphonuclear cells) on H&E-stained slides and blood vessels on MT-stained slides. Cells were counted in three equally spaced regions within the central area of the repaired tissue. Histological images were acquired using a motorized DMi8 microscope equipped with a K5 monochrome camera and a DMC4500 color camera (Leica Microsystems, Wetzlar, Germany) at $5\times$ and $63\times$ magnifications.

2.10. Statistical Analysis

Statistical analyses were carried out using GraphPad Prism (version 8.0.1) and R Studio version 2023.06.1+524 software. Data are expressed as mean $\pm$ standard deviation (SD), and all experiments were performed in triplicate. Comparisons between experimental groups were conducted using two-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Repeated-measures ANOVA was used to analyze temporal changes within groups. A p -value < 0.05 was considered indicative of statistical significance.

3. Results

3.1. Morphological Analysis of Electrospun Scaffolds

SEM analysis showed randomly oriented fibers with submicron diameters across all scaffold formulations. Glycine incorporation resulted in a significant reduction in fiber diameter and was associated with the appearance of bead-like defects, suggesting changes in fiber formation during electrospinning (Figure 1a–c). PCL fibers exhibited an average diameter of 510 ± 279 nm (Figure 1d), whereas PCLGLI10 and PCLGLI20 fibers showed significantly smaller diameters of 140 ± 58 nm and 155 ± 55 nm, respectively (Figure 1e,f). PCL scaffolds displayed the lowest estimated porosity (51%). In contrast, PCLGLI10 and PCLGLI20 scaffolds exhibited comparable estimated porosity values (71–72%), both significantly higher than that of pure PCL ($p < 0.0001$).

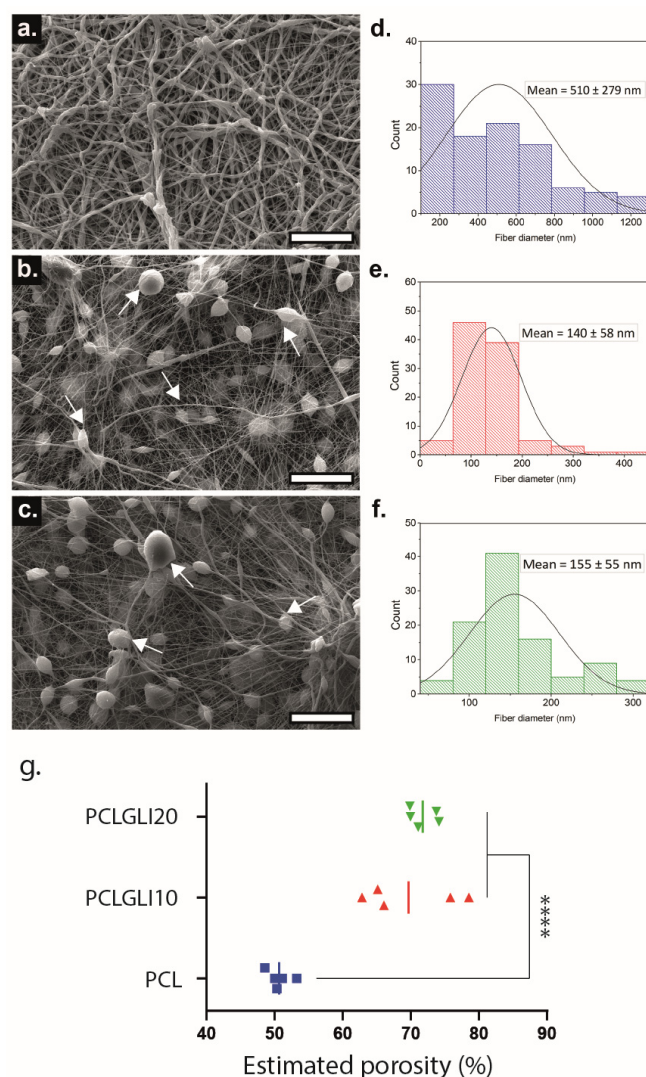


Figure 1. Representative scanning electron microscopy (SEM) micrographs and fiber diameter distributions of (a,d) PCL, (b,e) PCLGLI10, and (c,f) PCLGLI20 electrospun scaffolds. White arrows indicate bead-like structures observed in PCLGLI10 and PCLGLI20 scaffolds. Scale bar = 20 μ m. (g) Estimated porosity of PCL, PCLGLI10, and PCLGLI20 scaffolds. PCL scaffolds exhibited the lowest porosity, whereas no significant differences were observed between glycine-containing scaffolds (PCLGLI10 and PCLGLI20). Statistical significance was determined using two-way ANOVA followed by Tukey's post hoc test (**** $p < 0.0001$). White arrows indicate beads on electrospun scaffolds.

3.2. Crystallinity and Functional Group Analysis

XRD analysis revealed that glycine presented a highly crystalline pattern, whereas PCL exhibited a broad amorphous halo along with two characteristic crystalline peaks, as expected from its semi-crystalline nature. In the PCLGLI10 and PCLGLI20 scaffolds, additional diffraction peaks appeared at $2\theta = 15^\circ$ and $2\theta = 30^\circ$ (Figure 2a,b).

FTIR spectroscopy showed the characteristic bands of pure PCL at 2945 cm^{-1} , 2865 cm^{-1} , and 1725 cm^{-1} . The two main peaks of pure glycine at 3180 cm^{-1} and 2100 cm^{-1} were also clearly visible in both PCLGLI10 and PCLGLI20 spectra. Furthermore, a new band emerged at 3436 cm^{-1} in the glycine-containing fibers (Figure 2c). The detailed peak positions and assignments are summarized in Table 1.

Table 1. Characteristic peaks observed in FTIR spectra of PCL, Glycine, PCLGLI10, and PCLGLI20.

Wavenumber (cm ⁻¹)	PCL	Glycine	PCLGLI10	PCLGLI20	Description
3436			X	X	Symmetrical stretching NH ₂
3180		X	X	X	Asymmetrical stretching NH ₂
2945	X		X	X	Vibration CH ₂
2865	X		X	X	Vibration CH ₂
2100		X	X	X	Torsion NH ₃
1725	X		X	X	Vibration C=O

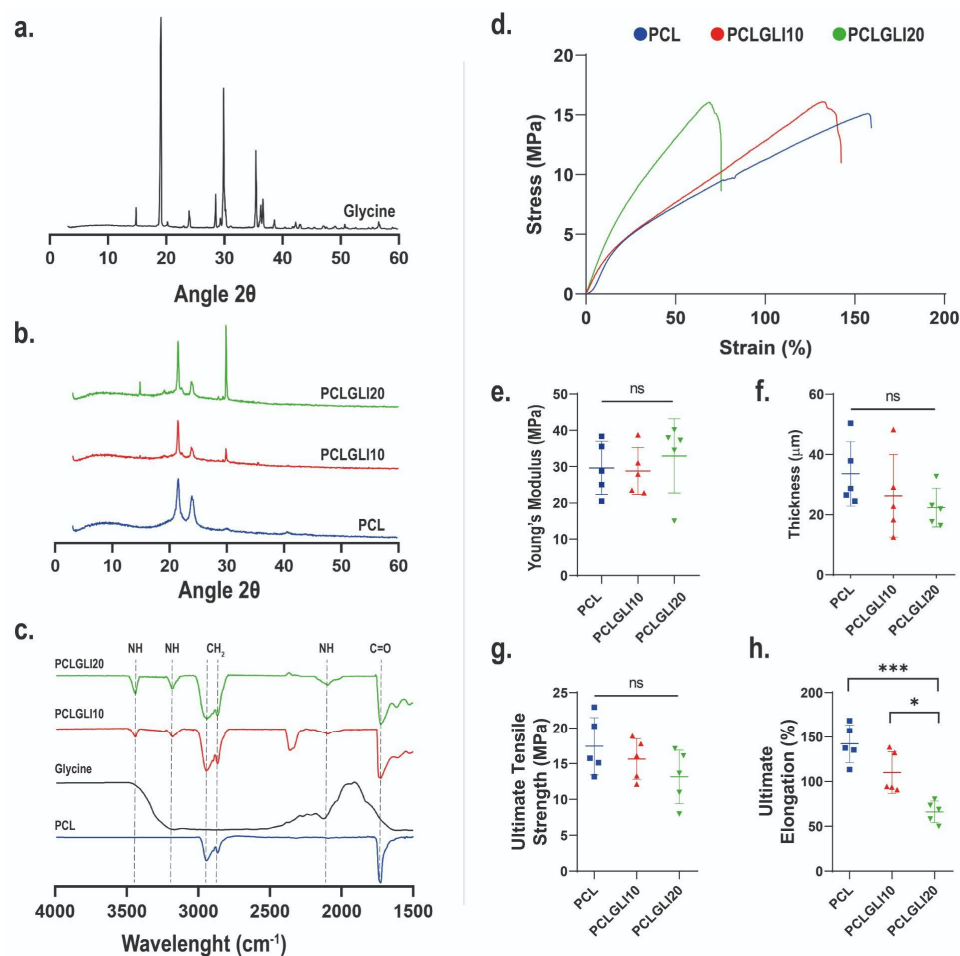


Figure 2. Structural, chemical, and mechanical characterization of PCL, PCLGLI10, and PCLGLI20 scaffolds. (a) X-ray diffraction (XRD) pattern of highly crystalline glycine. (b) XRD diffractograms, showing the characteristic semi-crystalline structure of PCL and the emergence of glycine-associated crystalline peaks in glycine-containing scaffolds. (c) Fourier-transform infrared (FTIR) spectra of glycine, PCL, PCLGLI10, and PCLGLI20. The presence of N–H vibrational bands in PCLGLI10 and PCLGLI20 confirmed the successful incorporation of glycine into the electrospun matrices. (d) Representative stress–strain curves. Quantification of (e) Young’s modulus, (f) specimen thickness, (g) ultimate tensile strength, and (h) ultimate elongation. Data are shown as individual values with mean $\pm$ SD; $n = 5$ independent specimens per group. Statistical comparisons were performed using one-way ANOVA followed by Tukey’s multiple-comparisons test. * $p < 0.05$; *** $p < 0.001$; ns, not significant. Although scaffold thickness, Young’s modulus, and ultimate tensile strength did not differ significantly among groups, ultimate elongation decreased significantly with glycine incorporation. These findings indicate that glycine incorporation did not substantially affect scaffold stiffness or tensile strength but reduced scaffold ductility at higher glycine concentrations.

3.3. Mechanical Properties by Uniaxial Tension Testing

Representative stress–strain curves for PCL, PCLGLI10, and PCLGLI20 scaffolds are shown in Figure 2d. Mechanical characterization revealed that PCL scaffolds exhibited a Young's modulus of 29.65 ± 7.35 MPa (Figure 2e). Glycine incorporation influenced this parameter in a concentration-dependent manner; specifically, PCLGLI10 scaffolds displayed a slightly lower Young's modulus (28.81 ± 6.51 MPa), whereas PCLGLI20 scaffolds exhibited the highest value (32.97 ± 10.24 MPa). However, no statistically significant differences were observed among groups. In a similar manner, no significant differences were found in scaffold thickness (33.98 ± 10.52 μ m for PCL, 26.22 ± 13.77 μ m for PCLGLI10, and 22.34 ± 6.45 μ m for PCLGLI20; Figure 2f) or in ultimate tensile strength (17.44 ± 4.03 MPa for PCL, 15.63 ± 2.87 MPa for PCLGLI10, and 13.15 ± 3.74 MPa for PCLGLI20; Figure 2g). In contrast, ultimate elongation was significantly reduced with increasing glycine concentration, decreasing from $142.28 \pm 21.32\%$ in pure PCL to $109.96 \pm 23.41\%$ in PCLGLI10 and $66.11 \pm 12.13\%$ in PCLGLI20 (Figure 2h). Overall, while the addition of glycine led to a significant decrease in ultimate elongation at the highest concentration, it did not significantly affect the thickness, stiffness, or tensile strength of the electrospun scaffolds, indicating that their structural integrity was largely preserved despite compositional modifications.

3.4. Biological Response of Human Wharton's Jelly Mesenchymal Stromal Cells on Glycine-Functionalized Electrospun PCL Scaffolds

Live/Dead staining revealed high hWJ-MSCs viability on PCL, PCLGLI10, and PCLGLI20 scaffolds throughout the culture period (24, 72, and 120 h), as evidenced by the predominance of green-fluorescent live cells and the scarce presence of red-fluorescent dead cells (Figure 3a). Quantitative image analysis using Fiji confirmed that cell viability remained above 95% on all scaffold formulations over the 5-day culture period. Notably, PCLGLI10 exhibited the highest viability ($\sim 99.6\%$) and showed a statistically significant increase compared with PCL at 120 h. In contrast, no significant differences were detected between PCLGLI10 and PCLGLI20 at any of the evaluated time points (Figure 3c). These findings demonstrate that glycine incorporation did not compromise cell viability and may enhance the cytocompatibility of the electrospun scaffolds, particularly at the 10% concentration.

As shown in Figure 3b, hWJ-MSCs exhibited their characteristic fibroblast-like morphology on all scaffold formulations. Cells adhered efficiently to the nanofibrous architecture and displayed extensive spreading along the fibers, as well as over the bead-like structures present in the glycine-containing scaffolds. Consistent with these observations, the resazurin reduction assay demonstrated that incorporating 10% glycine (PCLGLI10) significantly enhanced cell metabolic activity at 72 h compared with PCL (Figure 3d). Given the established correlation between metabolic activity and cell number under these experimental conditions, these findings indicate an increased proliferative response on PCLGLI10. Collectively, the results demonstrate that PCLGLI10 provides a favorable microenvironment for hWJ-MSCs growth, supporting high cell viability, robust adhesion, and enhanced proliferation. Based on its superior biological performance, PCLGLI10 was selected for subsequent differentiation studies and *in vivo* evaluation.

3.5. Growth Factor Production of hWJ-MSCs Modulated by the Scaffolds

hWJ-MSCs seeded onto tissue culture plates (TCP) as well as on PCL, PCLGLI10, and PCLGLI20 scaffolds produced or consumed several growth factors. Regarding angiopoietin-1 (Ang-1), cells cultured on TCP showed clear production, while all electrospun scaffolds tended toward its consumption (Figure 4a). For FGF-2 (Figure 4b), no production was detected in TCP or PCLGLI20. In contrast, both PCL and PCLGLI10 exhibited produc-

tion at 48 h, with PCL showing higher levels at 72 h, although the difference was not statistically significant.

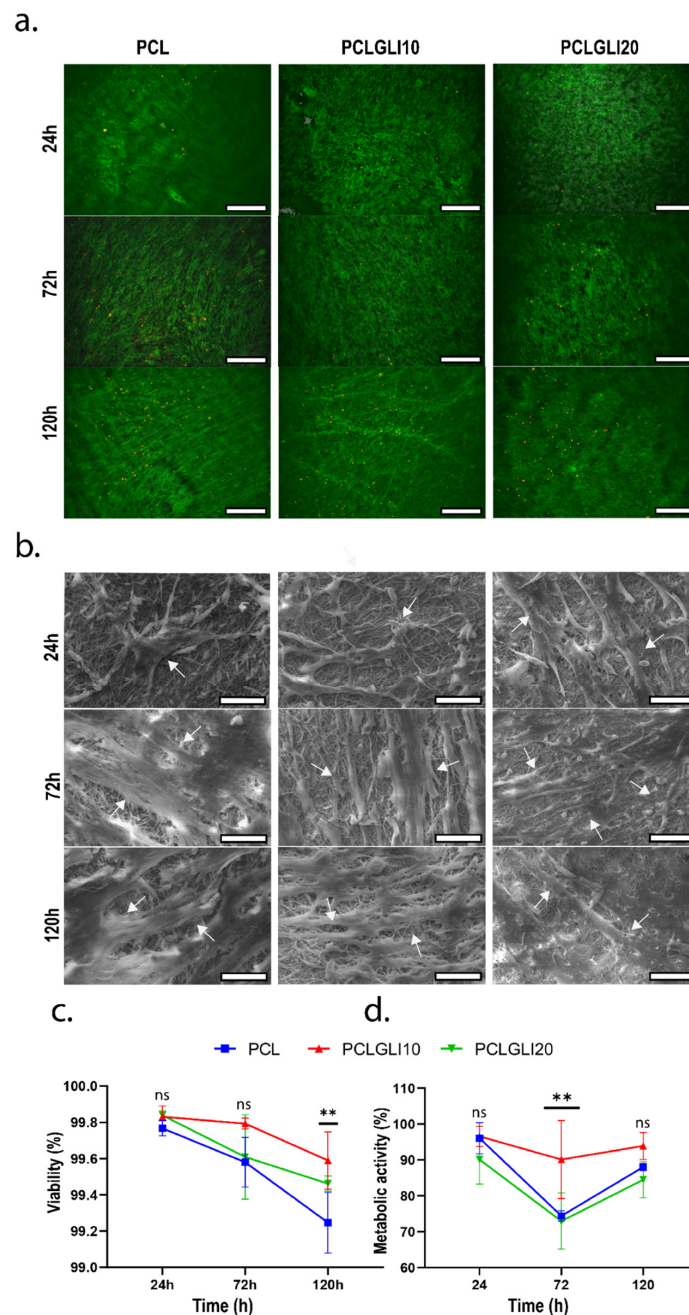


Figure 3. *In vitro* biological characterization of hWJ-MSCs cultured on electrospun scaffolds. (a) Representative Live/Dead fluorescence images showing the viability of hWJ-MSCs cultured on PCL, PCLGLI10, and PCLGLI20 scaffolds after 24, 72, and 120 h. Live cells are shown in green and dead cells in red. Scale bar = 500 μ m. (b) SEM micrographs of hWJ-MSCs cultured on PCL, PCLGLI10, and PCLGLI20 scaffolds at 24, 72, and 120 h. Cells exhibited a characteristic fibroblast-like morphology and extensive spreading along the scaffold surface; elongated cells are indicated by white arrows. Scale bar = 50 μ m. (c) Quantification of hWJ-MSCs viability on PCL, PCLGLI10, and PCLGLI20 scaffolds. A statistically significant increase in viability was observed for PCLGLI10 compared with PCL after 120 h of culture. (d) Metabolic activity of hWJ-MSCs cultured on PCL, PCLGLI10, and PCLGLI20 scaffolds, determined by the resazurin reduction assay. PCLGLI10 exhibited significantly higher metabolic activity than PCL at 72 h. Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance was determined using two-way ANOVA followed by Tukey's post hoc test (** $p < 0.01$, ns indicates no statistical significance).

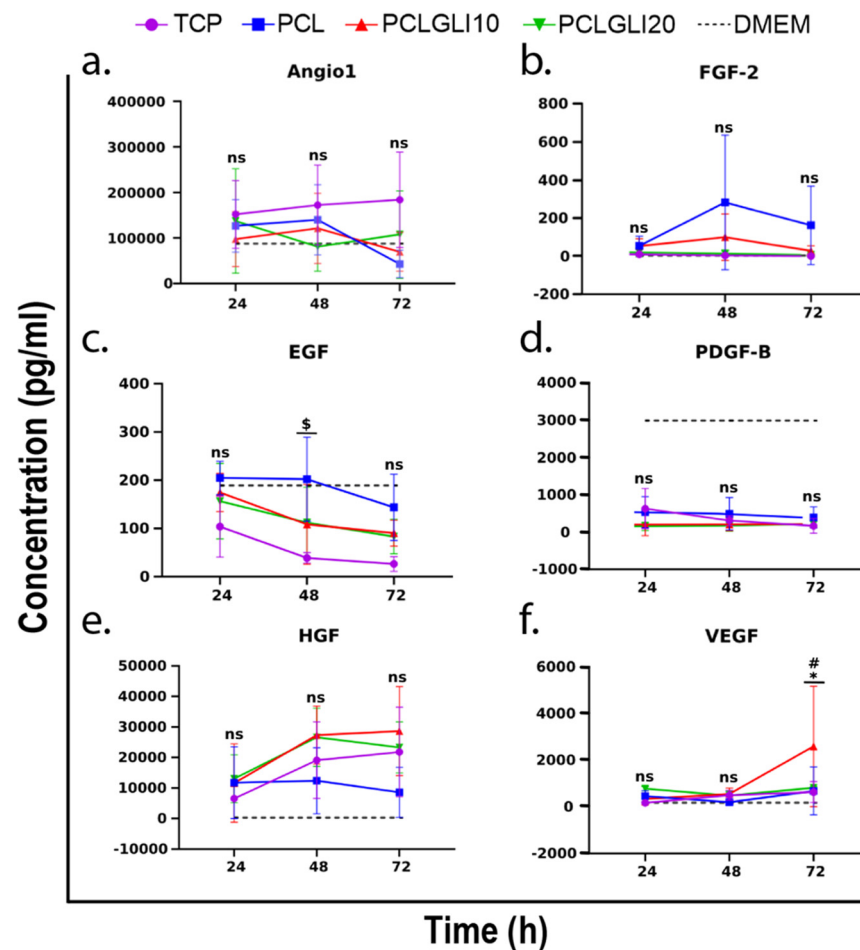


Figure 4. Growth factor secretion profile of hWJ-MSCs cultured on tissue culture plates (TCP), PCL, PCLGLI10, and PCLGLI20 scaffolds. Concentrations of (a) angiopoietin-1 (Ang-1), (b) fibroblast growth factor-2 (FGF-2), (c) epidermal growth factor (EGF), (d) platelet-derived growth factor-B (PDGF-B), (e) hepatocyte growth factor (HGF), and (f) vascular endothelial growth factor (VEGF) were quantified at 24, 48, and 72 h. The dotted line represents the concentration of each growth factor in a cell-free culture medium and serves as a reference to distinguish factor production from consumption. Positive values indicate net growth factor production, whereas negative values indicate net consumption by the cells. PCLGLI10 promoted increased secretion of HGF and VEGF compared with the other conditions, with VEGF levels at 72 h being significantly higher than those observed for TCP and PCL. Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance was determined using two-way ANOVA followed by Tukey's post hoc test ($* p < 0.05$), where * indicates comparison between PCL and PCLGLI10, # indicates comparison between TCP and PCLGLI10, and \$ indicates comparison between TCP and PCL, ns indicates no statistical significance.

EGF was consumed under all conditions (Figure 4c), but the rate of consumption was slowest on PCL, followed by the glycine-containing scaffolds, and fastest on TCP. A similar pattern was observed for PDGF-BB (Figure 4d), with comparable consumption across all groups. Interestingly, PCL scaffolds showed sustained production of HGF (Figure 4e), while PCLGLI10 induced even higher levels of this factor. Although no statistically significant differences were detected among scaffold groups, both glycine-containing formulations showed a clear trend toward increased HGF secretion relative to PCL, particularly at 48 and 72 h. Given the established role of HGF in tissue repair, angiogenesis, and modulation of inflammation, this response suggests that glycine incorporation may promote a more pro-regenerative secretory profile in hWJ-MSCs.

Notably, VEGF production was only observed in the PCLGLI10 condition (Figure 4f). VEGF levels increased progressively over time in PCLGLI10 cultures, reaching their highest

values at 72 h. At this time point, VEGF secretion was significantly higher than that observed in TCP and PCL cultures, whereas PCLGLI20 induced only a slight increase. The selective enhancement of VEGF production by PCLGLI10 that suggests this formulation may provide specific physicochemical cues capable of stimulating the pro-angiogenic activity of hWJ-MSCs.

Overall, the growth factor secretion profile demonstrated that scaffold composition influenced the paracrine behavior of hWJ-MSCs. Among the evaluated formulations, PCLGLI10 elicited the most favorable response, characterized by increased production of HGF and VEGF, two key mediators of angiogenesis and wound healing. These findings support the selection of PCLGLI10 as the lead scaffold for all subsequent *in vitro* studies.

3.6. hWJ-MSCs Differentiation on the Constructs

At days 7 and 10, hWJ-MSCs cultured on both PCL and PCLGLI10 scaffolds displayed a flattened morphology when maintained in KGM, in clear contrast to the typical fibroblast-like shape observed in cells grown in DMEM (Figure 5a). SEM analysis revealed extensive cell spreading and the formation of a continuous cellular layer across the scaffold surface, particularly after 14 days of culture. This effect was more evident under KGM, where cells exhibited a polygonal morphology and increased cell–cell contacts, consistent with epithelial differentiation.

Vimentin expression decreased in hWJ-MSCs seeded on both scaffolds compared to cells cultured on tissue culture plates (TCP) (Figure 5b). This reduction was most pronounced in cells cultured in KGM for 10 days on either PCL or PCLGLI10. Furthermore, a decrease in vimentin expression was also noted at day 10 in cells cultured on PCLGLI10 scaffolds in DMEM. Since vimentin is a mesenchymal marker, its downregulation suggests a progressive loss of the mesenchymal phenotype during culture on the electrospun scaffolds, particularly under differentiation-inducing conditions.

Involucrin expression was detected under all conditions evaluated. However, its expression appeared more intense in cells cultured in KGM, especially after 10 days of culture on both PCL and PCLGLI10 scaffolds. The merged fluorescence images showed a progressive decrease in the mesenchymal marker vimentin and an increase in involucrin expression, indicating advancement of the differentiation process toward an epithelial-like phenotype. Notably, hWJ-MSCs cultured on PCLGLI10 exhibited a more homogeneous involucrin distribution and reduced vimentin expression than cells cultured on PCL, suggesting that glycine incorporation may promote epithelial differentiation.

Overall, these findings demonstrate that both electrospun scaffolds support hWJ-MSCs differentiation toward a keratinocyte-like phenotype, while PCLGLI10 appears to provide a microenvironment more conducive to this process. Based on its superior biological performance, including enhanced cytocompatibility, growth factor secretion, and differentiation-associated marker expression, PCLGLI10 was selected for *in vivo* evaluation.

3.7. In Vivo Wound Healing Evaluation

Histological evaluation was performed on wound tissues treated with PCL and PCLGLI10 scaffolds, as well as with the corresponding cell-seeded constructs (PCL+hWJ-MSCs and PCLGLI10+hWJ-MSCs). Complete wound closure was observed in all experimental groups, including the untreated control, by day 18 post-injury, corresponding to the study endpoint (Figure 6a). Although none of the acellular scaffolds achieved permanent integration within the wound bed, they provided transient structural support throughout the healing process and detached progressively as tissue repair advanced. In contrast, the cell-seeded constructs (PCL+hWJ-MSCs and PCLGLI10+hWJ-MSCs), which were secured to the wound margins with sutures, remained in place throughout the study period.

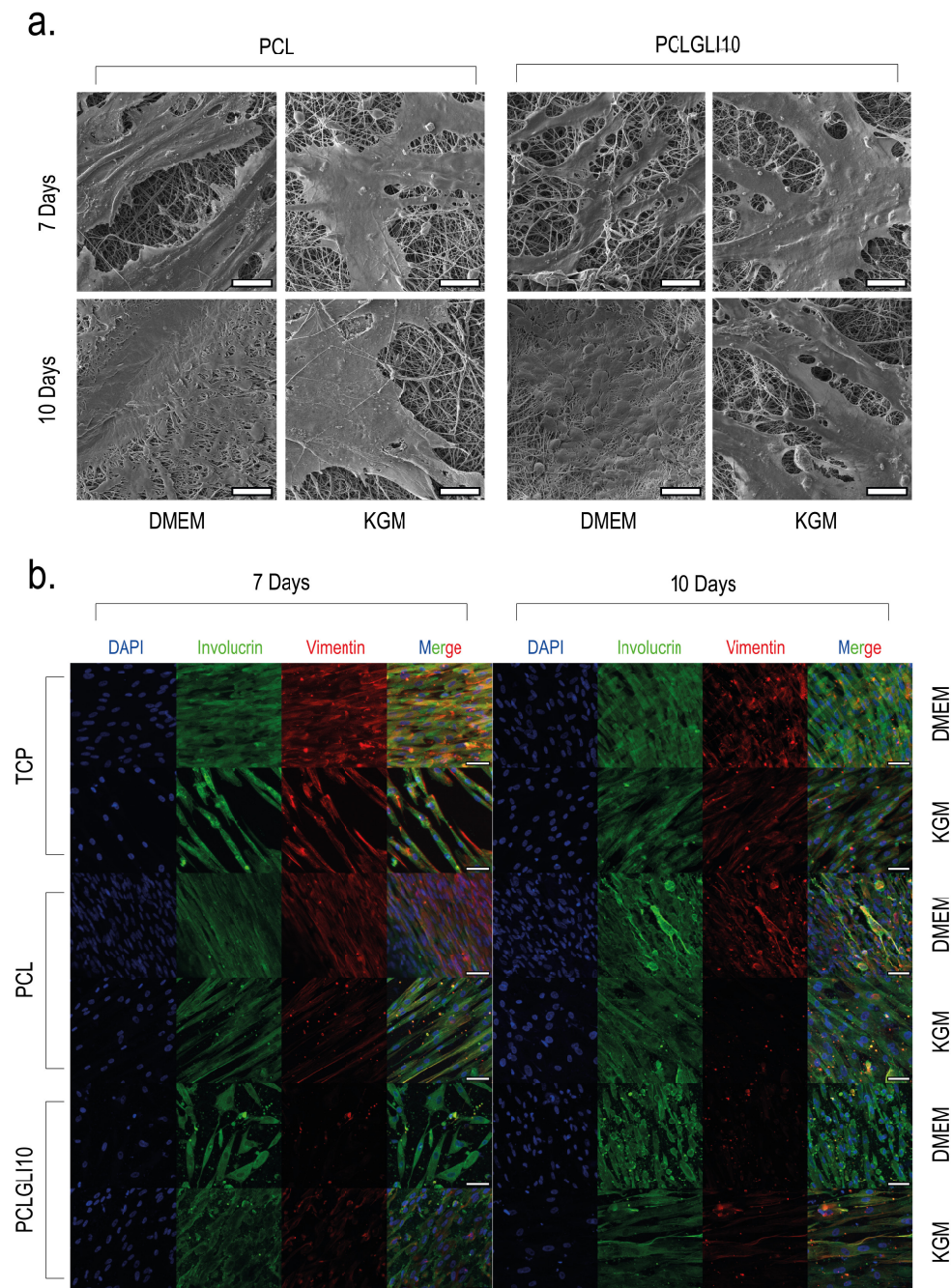


Figure 5. *In vitro* epithelial differentiation of hWJ-MSCs cultured on PCL and PCLGLI10 scaffolds. (a) Representative SEM micrographs of hWJ-MSCs cultured on PCL and PCLGLI10 scaffolds in DMEM supplemented with human platelet lysate (hPL) or keratinocyte growth medium (KGM) for 7 and 10 days. Cells cultured in KGM exhibited a transition from the characteristic fibroblast-like morphology to a flattened, polygonal morphology associated with epithelial differentiation. Scale bar = 20 μ m. (b) Immunofluorescence staining of involucrin (epithelial marker) and vimentin (mesenchymal marker) in hWJ-MSCs cultured on tissue culture plates (TCP), PCL, and PCLGLI10 scaffolds under DMEM supplemented with hPL or KGM for 7 and 10 days. A reduction in vimentin expression was observed in cells cultured on PCLGLI10 scaffolds at both time points and under both culture conditions. In PCL scaffolds, vimentin downregulation was primarily observed in cells cultured in KGM. Concurrently, involucrin expression was maintained or increased, supporting the progression toward an epithelial-like phenotype. Scale bar = 50 μ m.

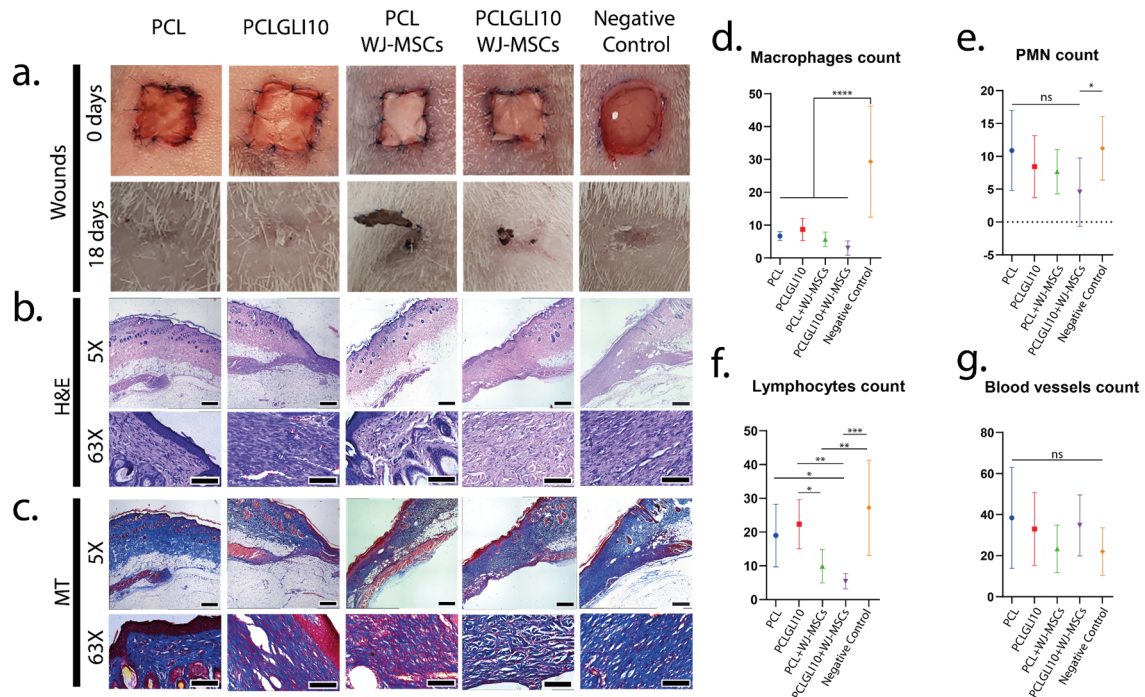


Figure 6. *In vivo* evaluation of PCL and PCLGLI10 with and without hWJ-MSCs. (a) Wounds in guinea pigs at 0 and 18 days, the time of euthanasia. (b) Hematoxylin and eosin staining of the repair area and (c) Masson's trichrome staining were performed at 5× and 63× magnifications of the repaired skin area. At 5×, the transition from healthy to repaired tissue was evident, characterized by a thinner repaired layer and absence of subcutaneous muscle. At 63×, we quantified cells characteristic of an inflammatory environment, including (d) macrophages, (e) polymorphonuclear cells (PMN), and (f) lymphocytes, as well as (g) blood vessels. Scale bar at 5×: 2 mm; scale bar at 63×: 250 μm. Statistical significance was determined using two-way ANOVA followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns indicates no statistical significance).

H&E staining at low magnification (5×) revealed a clear transition zone between healthy and repaired skin, marked by reduced tissue thickness and the absence of the subcutaneous muscle layer in the repaired area (Figure 6b). At higher magnification (63×), various inflammatory cells, including lymphocytes, macrophages, and polymorphonuclear cells, were observed within the newly formed tissue, consistent with an active wound healing response. Masson's trichrome (MT) staining confirmed these observations, showing a distinct boundary between healthy and repaired tissue, along with the presence of immature collagen (darker blue staining with densely packed fibers) in the repaired region (Figure 6c). Higher magnification images also enabled the quantification of newly formed blood vessels.

Quantitative assessment of inflammatory cells (Figure 6d–f) showed a significant reduction in macrophage numbers in all scaffold- and construct-treated groups compared to the untreated control (Figure 6d). Notably, the PCLGLI10+WJ-MSCs construct resulted in a significantly lower number of polymorphonuclear cells relative to the negative control (Figure 6e). Both constructs demonstrated reduced lymphocyte counts relative to other treatments, with the PCLGLI10+WJ-MSCs construct showing significant differences compared to both scaffolds and the negative control, whereas the PCL+WJ-MSCs construct did not differ significantly from the PCL scaffold (Figure 6f). Assessment of blood vessel density (Figure 6g), a key indicator of neovascularization and tissue perfusion, revealed no significant differences between the treatment groups.

4. Discussion

The development of bioactive electrospun scaffolds capable of supporting MSCs function is a cornerstone of skin tissue engineering [5,7,30]. In this study, we fabricated and characterized PCLGLI10 and PCLGLI20 scaffolds, evaluated their interaction with hWJ-MSCs, and assessed the wound-healing performance of the most promising construct in a guinea pig excisional wound model. Our findings demonstrate that incorporating 10% glycine into PCL produces a scaffold with favorable physicochemical properties, enhanced hWJ-MSCs proliferation and growth factor secretion, an instructive niche for epithelial differentiation, and improved modulation of the inflammatory microenvironment *in vivo*.

Several strategies have been used to improve the intrinsic hydrophobicity and limited biological activity of PCL scaffolds, including blending with natural polymers such as gelatin or collagen, plasma-based surface treatment, laser-mediated surface modification, and coating with platelet-rich plasma. These approaches may improve cell–material interactions and regenerative responses; however, they can also introduce limitations related to biological-source variability, the need for crosslinking, surface-limited effects, specialized equipment, or challenges in standardization. To position glycine incorporation within this context, Table 2 compares the principal advantages and limitations of these approaches with the glycine-functionalized PCL scaffolds evaluated in the present study.

Table 2. Comparative evidence-based rating of PCL functionalization strategies for skin repair. Rating scale: ++ = Limited evidence; +++ = moderate evidence; ++++ = Substantial evidence; +++++ = strong, multi-level evidence.

Functionalization Strategy	Mechanical Properties	Biocompatibility	Evaluation in Biomodels	Wound-Repair Performance	Scalability Potential	Reference
Gelatin blending	+++	++++	++++	++++	++	[38,39]
Collagen blending	+++	+++++	+++++	+++++	+++	[30,40]
Plasma surface treatment	+++	++++	++	++	++	[41]
PRP coating or immobilization	+++	++++	+++	+++	++	[42]
Glycine incorporation PCLGLI10	+++++	+++++	+++++	+++++	+++++	Present work

Overall, the comparative scoring indicates that gelatin- and collagen-based blends provide strong biological performance and have been evaluated as dermal substitutes and in wound-repair biomodels; however, their prospective scale-up may be limited by water sensitivity, crosslinking requirements, concentration-dependent changes in mechanical behavior, biological-source variability, and cost [30,38–40]. Plasma treatment can improve surface bioactivity while preserving the fibrous architecture, but its action is mainly restricted to the scaffold surface and requires specialized equipment, whereas PRP-based approaches provide pro-regenerative factors and enhance cell adhesion and proliferation but remain affected by donor variability, release kinetics, and standardization challenges [41,42]. In this context, PCLGLI10 obtained the highest overall rating because glycine is a chemically defined, low-molecular-weight additive that is incorporated directly during electrospinning without a post-fabrication coating, crosslinking step, or donor-derived component. In the present study, 10% glycine increased porosity and reduced fiber diameter to the nanoscale range while preserving Young’s modulus and ultimate tensile strength. PCLGLI10 also maintained hWJ-MSCs viability above 95%, enhanced metabolic and proliferative activity, increased HGF secretion and selectively induced VEGF produc-

tion, supported epithelial-like differentiation, and promoted re-epithelialization, organized collagen deposition, neovascularization, and modulation of the inflammatory response in a full-thickness wound model. Taken together, these findings identify PCLGLI10 as the most balanced strategy in this comparison in terms of mechanical performance, biocompatibility, biomodel validation, wound-repair potential, and prospective scalability inferred from manufacturing simplicity.

To further substantiate the comparative positioning of the glycine-functionalized scaffolds, the following sections examine in detail how glycine incorporation influenced the physicochemical, structural, mechanical, and biological performance of electrospun PCL. Particular attention is given to the concentration-dependent responses observed for PCLGLI10 and PCLGLI20, beginning with changes in fiber morphology and porosity and subsequently addressing mechanical behavior, cell-scaffold interactions, paracrine activity, epithelial differentiation, and *in vivo* wound-repair outcomes. This sequential analysis provides the experimental basis for identifying PCLGLI10 as the formulation that achieved the most favorable balance between structural integrity, bioactivity, and translational potential.

Fibers with diameters below 300 nm are highly desirable for skin tissue engineering, as they closely mimic the size scale of the native ECM, which typically ranges from tens to hundreds of nanometers [12,13,32]. Both PCLGLI10 and PCLGLI20 met this criterion, with mean diameters of 140 ± 58 nm and 155 ± 55 nm, respectively, and SEM analysis also revealed the formation of beads upon glycine incorporation. The appearance of beads is mainly attributed to a reduction in the effective polymer concentration in the spinning solution, which decreases viscoelastic forces relative to surface tension during jet whipping, thereby promoting bead-on-string morphologies and the concomitant decrease in fiber diameter [9,10,17,43]. Importantly, beaded electrospun architectures are not necessarily detrimental: as demonstrated by Santillán et al. (2019), PCL beads-on-string membranes provide preferential anchoring sites that favor cell adhesion, spreading, and proliferation [44]. Our SEM observations of hWJ-MSCs spanning both fibers and beads support this interpretation and reinforce that the mixed nano/micro topography of PCLGLI10 and PCLGLI20 actively contributes to the cell-scaffold interaction.

In addition to fiber size, PCLGLI10 and PCLGLI20 exhibited markedly higher porosity (~71–72%) than pure PCL (51%, $p < 0.0001$). Increased porosity is highly desirable, as it facilitates nutrient diffusion, gas exchange, cell infiltration, and waste removal, parameters critical for sustained MSC viability and tissue regeneration [45,46]. Loh and Choong (2013) demonstrated that interconnected porous architectures with appropriate pore size significantly enhance cell attachment, proliferation, and migration [46], while Murphy et al. (2010) further showed that pore size critically modulates cell adhesion and proliferation in fibrous scaffolds [47]. The combination of nanoscale fibers with high porosity in PCLGLI10 thus generates a biomimetic microenvironment closely resembling native ECM, providing topographical cues that support stem cell function [48,49].

These improved morphological properties were accompanied by distinct molecular-level changes in the crystalline structure of the incorporated glycine. XRD analysis revealed an interesting polymorphic transition during the electrospinning process. The diffractogram of pure glycine corresponded to the α -glycine conformation [50]. In contrast, the peaks observed at $2\theta \approx 15^\circ$ and 30° in PCLGLI10 and PCLGLI20 are characteristic of the γ -glycine conformation [51], indicating a clear change in glycine crystalline structure during the fabrication of the composite scaffolds. The semi-crystalline nature of PCL, evidenced by its two hallmark diffraction peaks, was preserved in the composite scaffolds, demonstrating that the polymer backbone organization was unaffected by the addition of glycine [50–53].

The FTIR spectra further support this $\alpha \rightarrow \gamma$ transition. The signals at 3180 cm^{-1} and 2100 cm^{-1} , corresponding to the asymmetric stretching of NH_2 and the torsion of NH_3^+ bonds, respectively, are characteristic of α -glycine [52]. In the PCL spectrum, peaks at 2945 cm^{-1} , 2865 cm^{-1} , and 1725 cm^{-1} stand out, corresponding to CH_2 vibrations and C=O stretching [54]. All five signals were preserved in PCLGLI10 and PCLGLI20, and notably, a new peak appeared at 3436 cm^{-1} , which is associated with the symmetric stretching of NH_2 bonds and is characteristic of the γ -glycine polymorph [53]. These spectroscopic fingerprints are in excellent agreement with the detailed PCL characterization reported by Elzein et al. (2011) [54] and confirm that glycine was successfully embedded into the scaffolds in a metastable γ -form, without disrupting the polymeric backbone, supporting the use of electrospinning as a versatile platform for incorporating bioactive small molecules and tuning their solid-state behavior [15].

In addition to favorable morphological and structural characteristics, the mechanical properties of the scaffolds are also well-suited for skin tissue engineering, the Young's modulus of the scaffolds should be similar to or higher than that of native skin [55]. In this work, all scaffolds exhibited moduli above 25 MPa (PCL: 29.65 ± 7.35 MPa; PCLGLI10: 28.81 ± 6.51 MPa; PCLGLI20: 32.97 ± 10.35 MPa), values that surpass those reported for back skin (~ 14.87 MPa), abdominal skin (~ 14.96 MPa), and scalp skin (~ 18.8 MPa) [56,57], confirming their mechanical adequacy for cutaneous applications.

The thickness of the scaffolds did not differ significantly among formulations, with values of $33.98 \pm 10.52\text{ }\mu\text{m}$ for PCL, $26.22 \pm 13.77\text{ }\mu\text{m}$ for PCLGLI10, and $22.34 \pm 6.45\text{ }\mu\text{m}$ for PCLGLI20. This finding indicates that glycine incorporation did not produce a statistically significant change in the overall thickness of the electrospun scaffolds under the fabrication conditions used. Maintaining comparable thickness among groups is important because thickness contributes directly to the calculation of cross-sectional area and, consequently, to the determination of Young's modulus. It also indicates that the observed biological differences among the scaffolds are unlikely to be explained simply by differences in scaffold thickness.

Similarly, the ultimate tensile strength remained statistically comparable among PCL, PCLGLI10, and PCLGLI20 scaffolds, with values of 17.44 ± 4.03 MPa, 15.63 ± 2.87 MPa, and 13.15 ± 3.74 MPa, respectively. These results suggest that incorporation of glycine did not significantly compromise the resistance of the PCL fiber network to failure under unidirectional tensile loading. Therefore, under the conditions evaluated, glycine incorporation did not significantly reduce the capacity of the scaffolds to withstand the maximum applied tensile stress before failure. This result is relevant because the glycine-containing scaffolds presented morphological differences, including reduced fiber diameter, increased porosity, and bead formation. However, these morphological changes were not accompanied by a significant reduction in ultimate tensile strength. In electrospun PCL scaffolds, tensile strength is determined not only by fiber diameter, but also by factors such as fiber arrangement, fiber junctions, porosity, and polymer-chain organization. Accordingly, changes in fiber morphology do not necessarily produce a proportional decrease in the tensile strength of the complete scaffold [58].

In contrast, ultimate elongation was significantly reduced as glycine concentration increased. PCL scaffolds exhibited an ultimate elongation of $142.28 \pm 21.32\%$, while PCLGLI10 and PCLGLI20 showed values of $109.96 \pm 23.41\%$ and $66.11 \pm 12.13\%$, respectively. Post-hoc analysis showed that PCLGLI20 had significantly lower ultimate elongation than PCL and PCLGLI10, whereas the reduction observed for PCLGLI10 compared with PCL was not statistically significant. This result indicates that the higher glycine concentration reduced scaffold ductility, despite preserving Young's modulus and ultimate tensile strength. The lower deformability of PCLGLI20 may be associated with the morphological

changes observed at this concentration, including bead formation and alterations in fiber architecture, which may modify stress distribution and interfiber sliding. Consistent with this interpretation, recent studies of electrospun PCL have reported that processing-driven changes in fiber morphology and pore structure can affect tensile strength, elastic modulus, and elongation simultaneously, although the direction and magnitude of those effects depend on the specific scaffold architecture and formulation [58,59].

Although no statistically significant differences were detected among PCL, PCLGLI10, and PCLGLI20 in terms of elastic modulus and ultimate tensile strength, morphological features such as the presence of beads and the reduction in fiber diameter would, in principle, be expected to influence mechanical behavior [56,60]. Croisier et al. (2012) previously demonstrated that the elastic modulus of electrospun PCL fibers strongly depends on fiber diameter and processing parameters [55], and Wong et al. (2008) showed that thinner fibers can confer enhanced tensile properties due to greater molecular orientation along the fiber axis [61]. Interestingly, despite these changes, our PCLGLI10 and PCLGLI20 scaffolds maintained stiffness and strength comparable to PCL. This suggests that the higher porosity and altered fiber architecture are partially offset by efficient stress transfer through the fibrous network. However, the significant reduction in elongation for PCLGLI20 indicates that the threshold for glycine incorporation should be carefully considered, as excessive concentrations may limit the flexibility required for scaffolds to accommodate the natural movements of skin tissue.

In addition to suitable mechanical properties, the scaffolds exhibited strong cytocompatibility with hWJ-MSCs. Evaluation of cell viability revealed robust cytocompatibility of the electrospun scaffolds with hWJ-MSCs over a 5-day culture period. High viability (>95%), as indicated by minimal red fluorescence and predominant green fluorescence across all scaffolds (PCL, PCLGLI10, PCLGLI20), demonstrates a favorable microenvironment for cell survival, comparable to viability reported in biologic [29] and other biosynthetic scaffolds [30,31]. This highlights the capacity of these scaffolds to maintain cellular viability while causing negligible cytotoxicity, which is essential for efficient skin repair.

Furthermore, hWJ-MSCs cultured on PCL, PCLGLI10, and PCLGLI20 scaffolds adopted a fibroblast-like morphology consistent with the native phenotype of umbilical cord-derived MSCs [28], adhering to and spanning across the nanofibers. The presence of beads within the scaffold structure did not impair cell attachment; rather, it facilitated the formation of a complex network that supported cell–scaffold interactions [44]. These findings highlight the ability of the scaffold to mimic the structure of the ECM, thereby promoting cell adhesion and spreading processes actively modulated by nanotopographical features and biochemical cues [48,49,62].

Notably, the enhancement of cell proliferation at 72 h on PCLGLI10 and the increased viability at 120 h compared with PCL suggest that glycine loading creates a particularly conducive environment for metabolic activity and cellular proliferation. This effect can be rationalized by the multiple cytoprotective and pro-proliferative roles of glycine: as the simplest amino acid, glycine modulates mTORC1-mediated protein synthesis [23], inhibits Ca^{2+} -dependent proteolysis [21], and protects epithelial cells against oxidative damage via the GLYT1 transporter [22]. In addition, glycine acts as an anti-inflammatory micronutrient through NF- κ B modulation [18] and directs macrophage polarization via specific glycinergic signaling pathways [19], properties that may collaboratively enhance the favorable microenvironment observed in PCLGLI10 cultures.

hWJ-MSCs are particularly attractive for skin regeneration applications due to their easy isolation, robust expansion potential, low immunogenicity, and rich paracrine secretome [27,28,63]. Our results align with previous work by our group demonstrating that hWJ-MSCs can be efficiently isolated, cryopreserved, and combined with biomaterials for

in vivo tissue regeneration [29–32], and they extend this body of evidence by showing that scaffold composition can be fine-tuned to enhance hWJ-MSCs proliferative behavior, with PCLGLI10 emerging as the most permissive substrate.

To further characterize the secretory profile of hWJ-MSCs within the scaffolds, the observed patterns in growth factor production provide valuable insights into the interaction between hWJ-MSCs and the electrospun scaffolds. The significant enhancement of cell proliferation on PCLGLI10, accompanied by its unique production of VEGF and elevated levels of HGF, suggests a strong correlation between scaffold composition and the activation of pro-regenerative paracrine signaling. Specifically, the sustained production of HGF on PCLGLI10, the appearance of VEGF, and the consumption of Angio-1, EGF, and PDGF-B may collectively promote angiogenesis, cell proliferation, and re-epithelialization—processes essential for tissue repair [64–67]. Importantly, Xin et al. demonstrated that HGF and VEGF act synergistically to promote endothelial cell survival, tubulogenesis, and *in vivo* neovascularization, an effect not observed with either factor alone [65]. The unique ability of PCLGLI10 to elicit simultaneous VEGF secretion and elevated HGF production therefore suggests that this scaffold creates a stimulatory microenvironment particularly aligned with the angiogenic and re-epithelializing demands of cutaneous repair [65,68,69].

The production of FGF-2 in PCL and PCLGLI10, but not in PCLGLI20 or TCP, further supports a scaffold-mediated activation of mitogenic and angiogenic pathways relevant to skin regeneration, as comprehensively reviewed by Werner and Grose, and Barrientos et al. [66]. EGF and PDGF-B were consumed across all conditions, with slower kinetics on PCL and PCLGLI10, suggesting that the scaffolds may modulate the bioavailability of these factors, possibly by providing a reservoir-like microenvironment that prolongs their effective concentration [64–66]. Conversely, the consumption of Angio-1 across all scaffolds (versus production on TCP) likely reflects active engagement of hWJ-MSCs in vascular stabilization processes induced by the 3D fibrous environment [67–70]. The relevance of VEGF in particular has been thoroughly documented by Bao et al., who established it as a master regulator of neovascularization during cutaneous repair [67]. HGF additionally facilitates interactions between mesenchymal cells and epithelial or endothelial cells, contributing to integrated tissue repair [65–68].

These results are consistent with the current secretome-centered understanding of MSC-based therapies, according to which MSCs exert much of their regenerative activity through the paracrine release of growth factors, cytokines, chemokines, and extracellular matrix components [63,71]. Recent studies have further demonstrated that MSC-derived secretomes and conditioned media can accelerate wound healing by promoting cell proliferation, angiogenesis, and extracellular matrix deposition [72–74]. Our findings extend this concept by demonstrating that a suitably designed glycine-functionalized PCL scaffold can modulate and enhance the secretory profile of hWJ-MSCs in a manner aligned with the temporal requirements of cutaneous wound repair [64,66,71]. These results are also consistent with previous studies from our group using PCL/collagen and PLA/ACP electrospun scaffolds and hADM-based constructs, in which scaffold composition influenced the secretion of bFGF, PDGF, HGF, and Angiopoietin-I [29–32].

These findings are further complemented by the observed morphological and phenotypic changes in hWJ-MSCs seeded on PCL and PCLGLI10 scaffolds, which indicate a strong potential for epithelial regeneration. Cells cultured in keratinocyte growth medium (KGM) exhibited a clear transition from a spindle-shaped, fibroblast-like morphology to a flattened, polygonal phenotype at 7 and 10 days. This morphological adaptation is widely recognized as an early hallmark of keratinocyte differentiation and epithelial commitment [75,76].

These observations were supported by immunocytochemical findings, which revealed a decrease in the expression of vimentin, a canonical mesenchymal intermediate filament, alongside sustained expression of involucrin, an early epithelial differentiation marker. The downregulation of vimentin coupled with the maintenance of epithelial cytoskeletal proteins is indicative of a mesenchymal-to-epithelial transition (MET), a reversible biological process in which mesenchymal cells lose migratory characteristics and acquire epithelial polarity and cell–cell adhesion properties [77,78]. This transition underscores the intrinsic plasticity of mesenchymal stem cells and aligns with previous reports demonstrating that hWJ-MSCs can differentiate into ectodermal lineages and form organized epithelial-like layers under appropriate microenvironmental cues [33,34,79].

Scanning electron microscopy further confirmed that hWJ-MSCs cultured on both scaffolds in KGM adopted a flattened morphology by days 7 and 10, suggesting that biochemical cues present in KGM, such as epidermal growth factor and elevated calcium concentrations, are sufficient to override the native mesenchymal phenotype. This is consistent with evidence showing that keratinocyte-specific media can effectively induce epidermal marker expression and initiate early lineage commitment in MSCs populations [33,34]. In parallel, microenvironmental signals, including substrate composition, nanotopography, and stiffness, play a decisive role in directing stem cell fate toward epithelial phenotypes [48,49,62,80].

Notably, the reduction in vimentin expression was more pronounced at day 10, indicating a time-dependent maturation of the epithelial-like phenotype. Interestingly, the PCLGLI10 scaffold induced partial downregulation of vimentin even in basal conditions (DMEM), an effect not observed on PCL, suggesting that its physicochemical properties may provide instructive cues that promote MET independently of specialized differentiation media. This phenomenon highlights the relevance of scaffold-driven differentiation, where biomaterial properties such as surface chemistry, fiber architecture, mechanical signaling, and nanotopography contribute to lineage specification [24,62,81].

Involucrin expression across all experimental conditions further supports the notion that hWJ-MSCs possess a primed or inherently plastic state toward ectodermal differentiation [76]. However, the coexistence of epithelial (involucrin) and mesenchymal (vimentin) markers suggests the presence of an intermediate or hybrid phenotype, which is commonly reported during *in vitro* MSC-to-keratinocyte differentiation [82]. Such transitional states reflect incomplete or partial MET and are often observed in the absence of additional maturation signals such as an air–liquid interface or *in vivo*-like environmental conditions [78,82].

From a translational perspective, the ability of hWJ-MSCs to undergo MET and adopt epithelial-like characteristics on PCLGLI10 scaffolds has important implications for skin tissue engineering. Effective wound healing, particularly in full-thickness injuries, requires rapid re-epithelialization and controlled modulation of mesenchymal activity to prevent fibrosis [5,7]. In this context, constructs capable of downregulating mesenchymal markers such as vimentin while promoting epithelial organization are highly desirable. The observed synergy between KGM and the PCLGLI10 scaffold suggests a promising platform for the development of advanced, off-the-shelf skin substitutes that exploit the regenerative and immunomodulatory properties of hWJ-MSCs to enhance epithelial repair [29,34].

Furthermore, the promising *in vitro* results were translated into an *in vivo* setting using a guinea pig full-thickness wound model. The *in vivo* evaluation demonstrated complete full-thickness wound closure across all experimental treatments, including the PCLGLI10 scaffolds and the PCLGLI10+hWJ-MSCs constructs. The selection of the guinea pig model lends strong translational value to these findings, as its skin acts as an excellent surrogate for human skin and shares close structural, immunological, and permeability

characteristics [35,36]. Furthermore, guinea pig skin possesses interfollicular melanocytes and melanocyte stem cells that actively migrate to the injured epidermis to facilitate repigmentation, making it a highly relevant model for studying skin repair [83]. The electrospun fibers with diameters below 300 nm successfully mimicked the architecture and size scale of the native ECM. Although the scaffolds did not permanently integrate, they functioned effectively as temporary structural supports, providing a provisional framework that facilitated endogenous cell migration, attachment, and protection of the wound bed [5,7].

While macroscopic wound closure alone is not sufficient to assess the quality of tissue repair [5,7,84], the histological analyses provided important mechanistic insights into the quality of the repaired tissue. Hematoxylin and eosin (H&E) staining revealed an active inflammatory infiltrate within the repaired tissue. While prolonged or excessive inflammation is a recognized hallmark of non-healing chronic wounds, a controlled inflammatory phase is a crucial driver of physiological healing [5,84,85]. Specifically, macrophages play an indispensable role in transitioning the wound from the inflammatory to the proliferative phase by clearing apoptotic neutrophils and releasing vital growth factors [83,86–88]. Through robust paracrine signaling, these immune populations secrete factors such as TGF- β , PDGF, and VEGF, which collectively stimulate angiogenesis, fibroplasia, and matrix synthesis [66,67,89]. Quantitative analysis confirmed a significant reduction in macrophage counts in all scaffold-treated groups compared to the untreated control, and the PCLGLI10+hWJ-MSCs construct produced the lowest counts of polymorphonuclear cells and lymphocytes, consistent with the well-established immunomodulatory role of hWJ-MSCs and their capacity to polarize macrophages toward a pro-healing M2 phenotype while attenuating excessive infiltration of neutrophils and lymphocytes [68,72,86–88].

Importantly, the modulation of inflammation observed in PCLGLI10+hWJ-MSCs constructs may also reflect a contribution from glycine itself. Aguayo-Cerón et al. described glycine as the smallest anti-inflammatory micronutrient, exerting its effects via NF- κ B suppression and downregulation of pro-inflammatory cytokines [18], and Gan et al. demonstrated that glycinergic signaling can modulate macrophage polarization through cellular signaling pathways and microRNAs [19]. Consistently, Feng et al. recently reported that a glycine-rich peptide accelerates cutaneous wound healing by enhancing macrophage M1-to-M2 polarization, vascularization, and re-epithelialization via the TLR4/MAPK/NF- κ B pathway [24]. Together, these data provide strong mechanistic support for the hypothesis that glycine, in synergy with hWJ-MSCs paracrine activity, contributes to the favorable inflammatory milieu of PCLGLI10-based constructs.

Progression toward the tissue remodeling phase was further confirmed by Masson's trichrome staining, which highlighted the robust deposition of newly synthesized collagen fibers in the repaired region [83]. In standard physiological healing, migrating fibroblasts differentiate into myofibroblasts under the influence of mechanical tension and TGF- β [90], generating contractile forces that align collagen fibers into dense, parallel bundles, ultimately leading to scar formation. However, highly porous engineered scaffolds can mechanically disrupt this process by physically limiting excessive wound contraction. In addition, the scaffold disperses myofibroblast assemblies and promotes a more random, dermis-like organization of collagen fibers. The presence of organized, newly formed connective tissue with immature collagen in our study indicates that the PCLGLI10 scaffold provided a highly biocompatible microenvironment that suppressed severe foreign body reactions and limited restrictive scarring by controlling wound contraction [90].

Furthermore, the successful integration of skin substitutes depends heavily on rapid vascularization, given that efficient oxygen and nutrient diffusion is restricted to short distances from the nearest capillary, a known physiological limit of approximately 200 μ m,

which requires the rapid formation of capillary-like networks to prevent cell death within thicker constructs [89]. The inclusion of hWJ-MSCs provided a significant advantage in promoting robust angiogenesis. MSCs are well documented to accelerate wound healing via their potent paracrine effects and immunomodulatory capabilities [63,68,71]. Specifically, hWJ-MSCs seeded on scaffolds secrete high levels of pro-angiogenic factors, including VEGF and HGF, as confirmed by our *in vitro* analysis, that actively recruit host endothelial cells and stimulate capillary sprouting, thereby helping to overcome the critical challenge of ischemia in full-thickness defects [65,67,70,89]. Although blood vessel density did not differ significantly between groups at day 18, this likely reflects the late time-point of evaluation, when neovascular pruning and vessel maturation typically occur [89,91]. As discussed by DiPietro, the angiogenic response during wound healing is highly dynamic, and excessive or persistent vascularization can be as detrimental as insufficient angiogenesis [91]. The consistent VEGF and HGF secretion observed *in vitro* on PCLGLI10 supports the hypothesis that earlier time points might reveal an enhanced angiogenic response, an aspect that warrants further investigation [65,67,70,89].

An important limitation of this study is that glycine-release kinetics and long-term changes in scaffold properties were not evaluated. Although PCL degrades slowly under physiological conditions [92], glycine may be released from the electrospun fibrous network because of its water solubility [93]. However, considering the bead-like structures observed in the glycine-containing scaffolds, it is possible that a significant portion of the glycine was entrapped within these dense regions rather than being fully exposed at the fiber surface [94–96]. This localization could reduce immediate exposure to the aqueous medium and potentially delay diffusion, resulting in a slower and more sustained release profile [95,96]. Nevertheless, glycine distribution within the fibers and beads, as well as the specific release kinetics, were not measured in this study; therefore, this mechanism remains hypothetical. Over time, both glycine release and PCL degradation may influence the biological activity and potentially the mechanical behavior and apparent surface porosity of the scaffolds. Previous studies have shown that PCL-based scaffolds can undergo time-dependent changes in morphology, porosity, molecular characteristics, and mechanical properties during degradation under aqueous or *in vivo* conditions [59,97,98]. Therefore, future studies should evaluate glycine distribution, release kinetics, and longitudinal changes in scaffold architecture and tensile properties under physiologically relevant conditions.

5. Conclusions

The synergistic combination of the structural properties of the PCL/glycine scaffold, designed to mimic key features of the native extracellular matrix, with the pro-angiogenic and immunomodulatory secretome of hWJ-MSCs promoted rapid re-epithelialization, organized collagen deposition, and neovascularization. The PCLGLI10+hWJ-MSCs construct also contributed to the modulation of the inflammatory response and the reduction in factors associated with excessive scar formation. Furthermore, glycine incorporation enhanced hWJ-MSCs proliferation, increased the secretion of regenerative growth factors, particularly HGF and VEGF, and supported epithelial differentiation. Collectively, these findings position PCLGLI10+hWJ-MSCs as a promising bioactive construct for the treatment of complex skin defects and support its further development as a potentially ready-to-use regenerative therapy.

Several limitations should be acknowledged. First, the *in vivo* evaluation was performed at a single endpoint, on day 18, which limited the assessment of the early inflammatory, proliferative, and angiogenic phases of wound healing. Studies incorporating multiple time points would provide a more comprehensive understanding of the healing

dynamics. Second, epithelial differentiation was evaluated for only 10 days; although early phenotypic changes were observed, longer induction periods and additional maturation strategies, such as air–liquid interface culture, may be required to achieve a more advanced and stable keratinocyte-like phenotype. Third, although the results support the bioactive role of glycine, the molecular mechanisms through which it modulates hWJ-MSCs behavior on PCL-based scaffolds remain unclear. Future studies should include transcriptomic and proteomic analyses, evaluate the construct in chronic and diabetic wound models, and assess long-term tissue remodeling, scaffold degradation, and scar quality.

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Abbreviations

The following abbreviations are used in this manuscript:

Ang-1	Angiopoietin-1
DMEM	Dulbecco's Modified Eagle Medium
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
FBS	Fetal Bovine Serum
FGF-2	Fibroblast Growth Factor-2
FTIR	Fourier Transform Infrared Spectroscopy
H&E	Hematoxylin and Eosin
HGF	Hepatocyte Growth Factor
hPL	Human Platelet Lysate
hWJ-MSCs	Human Wharton's Jelly Mesenchymal Stromal Cells
KGM	Keratinocyte Growth Medium
MET	Mesenchymal-to-Epithelial Transition

MSC	Mesenchymal Stromal Cell
MT	Masson's Trichrome
PBS	Phosphate-Buffered Saline
PCL	Poly(ϵ -caprolactone)
PCLGLI10	Poly(ϵ -caprolactone) with 10% Glycine
PCL+hWJ-MSCs	Human Wharton's Jelly Mesenchymal Stromal Cells cultured on Poly(ϵ -caprolactone)
PCLGLI10+hWJ-MSCs	Human Wharton's Jelly Mesenchymal Stromal Cells cultured on Poly(ϵ -caprolactone) with 10% Glycine
PCLGLI20	Poly(ϵ -caprolactone) with 20% Glycine
PDGF-BB	Platelet-Derived Growth Factor-BB
SEM	Scanning Electron Microscopy
TCP	Tissue Culture Plate
VEGF	Vascular Endothelial Growth Factor
XRD	X-ray Diffraction

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