cosmetics

MDPI

Article

Therapeutic Potential of Ozonized Glycerin in Skin Inflammation and Repair

John Ivarsson [1,2], Anna Guiotto [1], Giulia Trinchera [3], Alessandra Pecorelli [3], Gotaro Shiota [4] and Giuseppe Valacchi [1,3,5,*]

[1] Department of Food, Bioprocessing and Nutrition Sciences, Plants for Human Health Institute, NC Research Campus, NC State University, Kannapolis, NC 28081, USA; aguiott@ncsu.edu (A.G.)
[2] Department of Animal Sciences, Plants for Human Health Institute, NC Research Campus, NC State University, Kannapolis, NC 28081, USA
[3] Department of Environmental and Prevention Sciences, University of Ferrara, 44121 Ferrara, Italy; apecore@ncsu.edu (A.P.)
[4] Department of Research and Development, Mediplus Pharma Inc., Tokyo 105-0013, Japan
[5] Department of Food and Nutrition, Kyung Hee University, Seoul 02447, Republic of Korea
* Correspondence: gvalacc@ncsu.edu

Abstract

Glycerin is a widely used ingredient in cosmetics due to its cost-effectiveness and safety. While often used to enhance the texture of cosmetics, current research has demonstrated that it improves cutaneous properties such as enhanced skin hydration and moisturization. Due to its widespread use in cosmetics, enhancing the functional capacities of glycerin provides a promising method to improve the effectiveness of numerous cosmetics. Ozonized glycerin has emerged as a novel technology able to enhance glycerin's effectiveness with reported anti-inflammatory, antibacterial, and antiviral effects. This approach leverages ozone stabilization in glycerin for improved stability and prolonged release to the skin. Clinical application of ozonized glycerin has exhibited lightening of aging spots through promoting skin turnover. The objective of this study was to evaluate the enhanced properties of glycerin when ozonized in terms of skin repair and inflammation. Through topical pretreatment of epidermal 3D wound healing models (13 days) and ex vivo human skin biopsies (4 days), ozonized glycerin was able to improve wound closure, enhance skin barrier and extracellular matrix protein expression, and reduce inflammation. Notably, ozonized glycerin enhanced wound closure by 6.8% compared to glycerin, as well as significantly protecting against LPS-induced elastin degradation (67.7% difference from LPS). These data provide evidence for the use of ozonized glycerin as a new technology to prevent and diminish skin inflammation and improve wound repair.

Keywords: oxidative stress; wound healing; skin explants; metalloproteinases; oxinflammation

Check for updates

Academic Editor: Enzo Berardesca

Received: 5 January 2026
Revised: 2 February 2026
Accepted: 10 February 2026
Published: 12 February 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

As our biological external armor, the skin defends against a variety of environmental pollutants and pathogens that can cause inflammation and skin diseases [1]. Although skin inflammation is our body's first response to irritation, injury, infection, or stress. When inflammation becomes chronic or excessive, it can lead to redness, irritation, premature aging, and disease. In response to insults, skin cells can release pro-inflammatory cytokines like interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α), which can upregulate cutaneous matrix metalloproteinases (MMPs) such as MMP-9/MMP-2, which cleave key

dermal proteins like collagen and elastin [2–4]. Tissue inhibitors of metalloproteinases (TIMPs) can influence MMPs by binding directly to the enzyme and blocking its catalytic activity [5]. Prolonged inflammation can drive excessive MMP activity, degrading critical structural proteins like collagen and elastin, impairing wound healing [6]. Outside of extracellular matrix (ECM) proteins, inflammation can compromise epidermal barrier integrity through the disruption of tight junctions and desmosomal proteins, functionally weakening keratinocyte-to-keratinocyte adhesion and making the skin more susceptible to pathogens or external insults [7]. This mechanism is largely mediated through proinflammatory cytokines, which can reduce mRNA and translation of tight junction and desmosomal components [8]. Therefore, protecting the integrity, preventing excessive inflammation, and fortifying the skin are essential in maintaining cutaneous homeostasis and healthy skin.

One promising avenue for cutaneous therapies is ozone-derived compounds, which have demonstrated promising benefits in wound healing and inflammatory skin diseases [9–11]. Ozone therapies are low-cost and non-invasive, making them favorable therapeutic options [12]. However, gaseous therapies face severe challenges due to stability and safety, as high ozone concentrations are toxic to tissues [13]. As an alternative, ozonized oils have been created as carriers of ozone to retain the beneficial properties; however, they provide a much safer, more stable, and more controlled treatment [9,14]. Ozonized oils stabilize ozone in the form of ozonides and contain peroxides, which can exert a variety of effects [15].

Solubilization of ozone in glycerin (G) is an effective method of stabilizing ozone to harness its cutaneous benefits [16]. Current literature has demonstrated a variety of physiological effects with the application of ozonized glycerin (OG) [17–20]. With regard to the skin, in vitro application of OG has been shown to increase skin cell turnover and differentiation by enhancing the levels of involucrin and serine palmitoyltransferase [16]. Furthermore, application of OG has been shown to induce Nrf2-dependent defensive responses, including heme oxygenase-1 (HO-1) and NAD(P)H: quinone oxideoreductase-1 (NQO1) [21]. Additionally, clinical studies have demonstrated the ability of an 8-week application of OG (800 ppm) to improve aging spots through melanin degradation [16]. Importantly, these effects were not detected with regular G application, demonstrating the enhanced abilities of ozonized formulations [16].

While previous work investigated differentiation markers and antioxidant enzymes, there is no evidence regarding the effects of topical OG treatments on modulating structural proteins such as tight junctions and the extracellular matrix. Furthermore, no studies have explored the ability of ozonized compounds to protect against or modulate inflammatory responses in physiologically relevant 3D or ex vivo human skin models.

In this study, we want not only to understand the beneficial effect of OG topical treatment but also to compare G and OG. To do so, we have used 3D epidermal wound healing models and ex vivo human skin biopsies with and without the presence of a pro-inflammatory stimulus to ascertain the novel effects of OG at both conditions, inflammatory status, and baseline responses. The protection of various key epidermal, extracellular matrix proteins, as well as cutaneous remodeling and inflammatory markers, was assayed to reveal important differences between the compounds. Through these markers, we hoped to identify cutaneous aspects uniquely altered with OG to elucidate its primary mechanisms of action. Understanding these effects could improve the development of topical therapies to protect barrier function and promote ECM integrity in cosmetic and clinical dermatological applications.

2. Materials and Methods

2.1. G/OG Preparation

Preparation of Ozonized Glycerin

Concentrated glycerin was subjected to ozonation using an ozone generator. Ozone gas was introduced into the glycerin phase under continuous mixing until the sample reached a target ozone-equivalent value, which was quantified via standard iodometric titration using potassium iodide. The resulting preparation was designated as ozonized glycerin (OG) and used for all experiments in this study.

Previous literature indicates that ozonized glycerin contains stable ozone-derived reaction products rather than residual ozone, and these compounds are responsible for its oxidative and biological properties. In particular, our patent (JP7575652B2) identified and structurally elucidated a novel cyclic peroxide, 1,2,4-trioxepane, generated during the ozonation of glycerin. This molecule is a newly discovered glycerin-derived compound and represents a key active species contributing to the characteristic oxidative capacity and functional activities of OG. The formation of 1,2,4-trioxepane and other peroxide-containing species is consistent with previously reported analytical observations on ozonated glycerol systems and provides a mechanistic basis for the biological effects evaluated in the present study.

2.2. 3D Skin Wound Closure Model

A wound closure model in 3D reconstructed human epidermis (EpiDerm Mattek™, Ashland, MA, USA) was used to survey the effectiveness of OG in bolstering glycerin effects on wound healing. Three EpiDerm tissues were used per condition and time point (Day 1 and Day 13). Control tissues received only topical water application, whereas G and OG conditions received respective compounds at a 1% concentration topically. The medium of the 3D tissues was replaced daily, and tissues were pretreated topically with 10 µL of G and OG for 13 days. After wound closure, tissues were collected for immunofluorescence analysis. Pictures were taken at baseline and at indicated timepoints, and the closure area was quantified using ImageJ software 1.53K. The closure area was normalized to the baseline area to control for any deviations in wound size. EpiDerm tissues were also collected for paraffin embedding after 1 and 13 days for the purpose of immunofluorescence analysis. This provides insight into the possible biological mechanisms by which ozonized glycerin improves wound closure.

2.3. Ex Vivo Human Skin Explants

Human skin explants were provided from abdominoplasties from 3 different donors, in accordance with the approval of the Institutional Biosafety (IBC) Committee at North Carolina State University [22]. The skin explants were cleaned with phosphate-buffered saline supplemented with Penicillin and Streptomycin to remove excess blood and prevent contamination. Biopsies with a full thickness of 12 mm were extracted from the explant, and the subcutaneous fat was removed. For experiments, biopsies were cultured in 6-well plates with 1 mL of 4.5 g/L high-glucose Dulbecco's Modified Eagle Medium (DMEM). Explants were allowed to recover in an incubator at 37 °C in 5% CO_2 overnight prior to topical pretreatments with 1% OG or 1% G in double-distilled water. For LPS experiments, a concentration of 100 µg/mL was supplemented in fresh medium daily along with new topical G/OG treatments. Skin tissues and culture medium were collected at the indicated timepoints.

2.4. Immunofluorescence Staining and Quantification

After paraffin embedding, tissues were sectioned with a Leica microtome at 5 µM thickness. After allowing slides to dry, ensuring adherence, slides were deparaffinized using a hot plate at 60 °C. Tissues were rehydrated by a series of washes in xylene, 100%, 80%, 70%, and 30% ethanol, and then double-distilled water. Slides were then placed in citrate buffer for antigen retrieval in a water bath at 96 °C; afterwards, slides were allowed to cool and then washed in phosphate-buffered saline (PBS) before blocking with 2% bovine serum albumin (BSA) in PBS. After blocking, slides were incubated with primary antibodies seen in Table 1: MMP-2, TIMP-1, claudin-1, desmocollin-1, collagen type III, collagen type I, and elastin. The next day, slides were washed and incubated with the corresponding secondary antibody for 45 min. After secondary antibody incubation, slides were washed again, incubated with 4',6-diamidino-2-phenylindole (DAPI) (D1306, Invitrogen, ThermoFisher Scientific, Waltham, MA, USA), washed again in PBS, and mounted with Fluoromount. Images were acquired via epifluorescence on a Zeiss Z1 AxioObserver LSM10 confocal microscope at 40× magnification. A total of six images were taken across the paraffin section of either embedded 3D wound closure tissues or ex vivo skin biopsies per replicate. A 100 µM scale bar is provided in the bottom right corner for reference. Quantification of fluorescent intensity was performed using ImageJ software 1.54 g (National Institutes of Health, Bethesda, MD, USA) [23]. The selection tool was used to create an appropriate region specific for the immunofluorescence signal (epidermal/dermal markers) and saved in the ROI manager to ensure equivalent areas were quantified; a new selection was made for each marker and used to take three measurements per photo. Measurements are expressed in graphs as the mean/average pixel intensity within the selection (arbitrary units).

Table 1. Antibody specifications.

Antibody	Company/Code	Dilution
MMP-2	Invitrogen 436000	1:250
TIMP-1	Invitrogen MA1-733	1:250
CLAUDIN-1	Santa Cruz (Santa Cruz, CA, USA) sc-166338	1:50
DESMOCOLLIN-1	Santa Cruz sc-398590	1:50
COLLAGEN III	Santa Cruz sc-514601	1:50
COLLAGEN I	Abcam (Waltham, MA, USA) ab138492	1:2000
ELASTIN	Abcam ab9519	1:500

2.5. Cytokine and Growth Factor Analysis

IL-1α/TGF-1β measurements were conducted in cell culture medium via ELISA assays following the manufacturer's protocol.

2.6. Statistical Analysis

Appropriate statistical analyses were performed using GraphPad Prism version 10.5.0 (673) for macOS, GraphPad Software, Boston, MA, USA, www.graphpad.com. An ordinary one-way ANOVA with subsequent Tukey multiple comparison was conducted for each variable/condition tested. Data are expressed as mean with standard error of the mean (SEM) obtained from three biopsies. Statistical significance is considered at * $p <$ or equal to 0.05.

3. Results

3.1. Comparisons of G and OG in a Wound Closure Model

To survey the ability of OG to modulate wound closure mechanisms, 3D epidermal tissues were pretreated daily for a 13-day period. Models treated with OG demonstrated significantly accelerated wound closure over the period compared to G and control groups, as depicted in Figure 1a. Immunofluorescent analysis of the tissues exhibited upregulation of MMP-2 expression with G and OG treatments compared to control (Figure 1b,c). No significant changes were seen at Day 1 in the MMPs inhibitor TIMP-1 across the different treatments; however, at Day 13, significant decreases were observed in G- and OG-treated tissues (Figure 1d,e). In the early phase of wound closure (Day 1), OG-treated models exhibited a significant decrease in IL-1α release compared to controls and G-treated models (Figure 1f). Conversely, OG increased TGF-1β (Figure 1g) secretion, suggesting the promotion of wound closure.

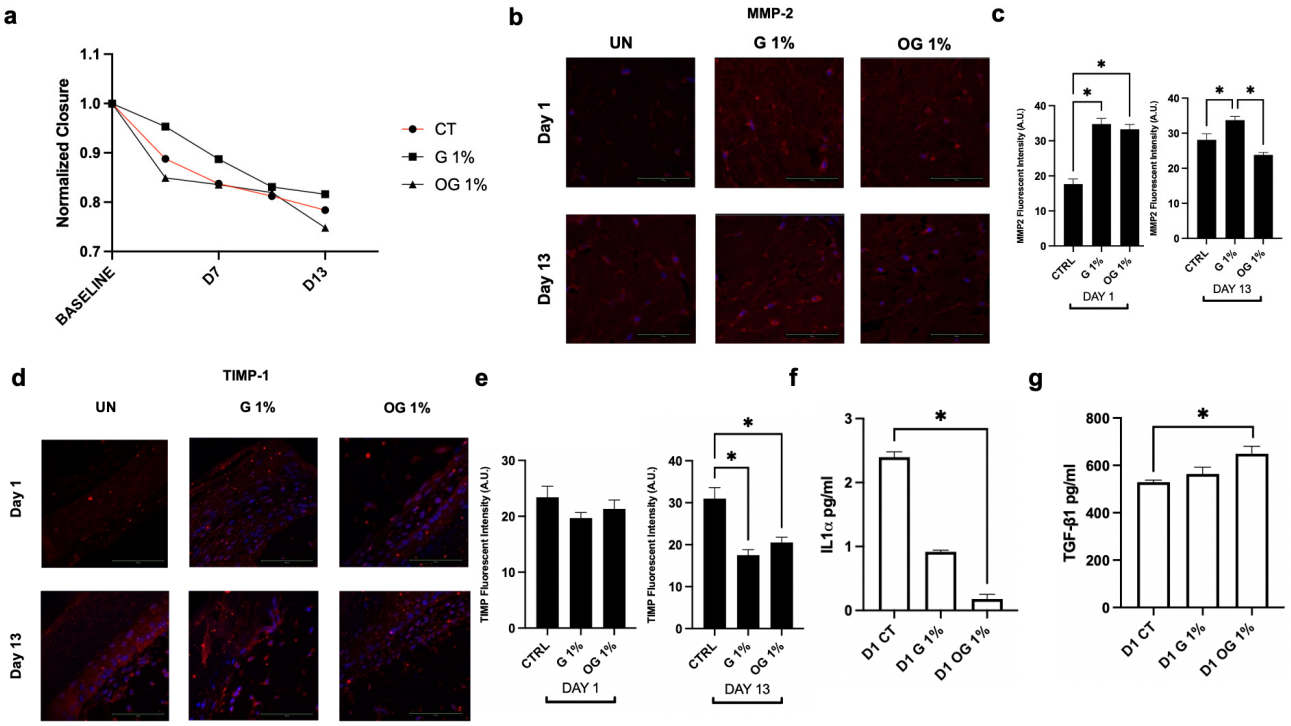

Figure 1. OG promotes wound closure and modulates remodeling and inflammatory markers in 3D skin tissues. (**a**) Wound closure quantified over a 13-day period. Representative immunofluorescent images of (**b**) MMP-2 and (**c**) quantification of protein expression levels at Day 1 and Day 13. Representative immunofluorescent images of (**d**) TIMP-1 and (**e**) quantification of protein expression levels at Day 1 and Day 13. (**f**) IL-1α and (**g**) TGF-1β release in culture medium after pretreatment with OG and G in the wound closure model at Day 1. Nuclei are counterstained with DAPI (blue). Data are presented as mean ± standard error of the mean. Statistical significance was determined using one-way analysis of variance with Tukey's post hoc test for multiple comparisons (n = 3). A p-value < 0.05 was considered statistically significant and denoted by an asterisk (*).

3.2. Effect of OG on Epidermal Barrier Proteins

Ex vivo human skin biopsies pretreated with either G or OG demonstrated significantly different impacts on epidermal barrier proteins and extracellular matrix integrity. As seen in Figure 2a,b, Claudin-1 was significantly upregulated at Day 4 in OG-treated explants; no increases were detected in explants treated with G. Similarly, desmocollin-1 expression was elevated with OG treatments across both time points compared to G and controls (Figure 2c,d). Outside of epidermal proteins, OG also promoted structural enhancement of

the dermis. Furthermore, in Figure 2e,f, significant upregulation of collagen type III was exhibited in tissues pretreated with OG at both time points compared to control. These data aligned with significantly upregulated TIMP-1 protein expression (Figure 2g,h) with OG treatments in human skin explants compared to control, suggesting enhanced deposition of ECM components.

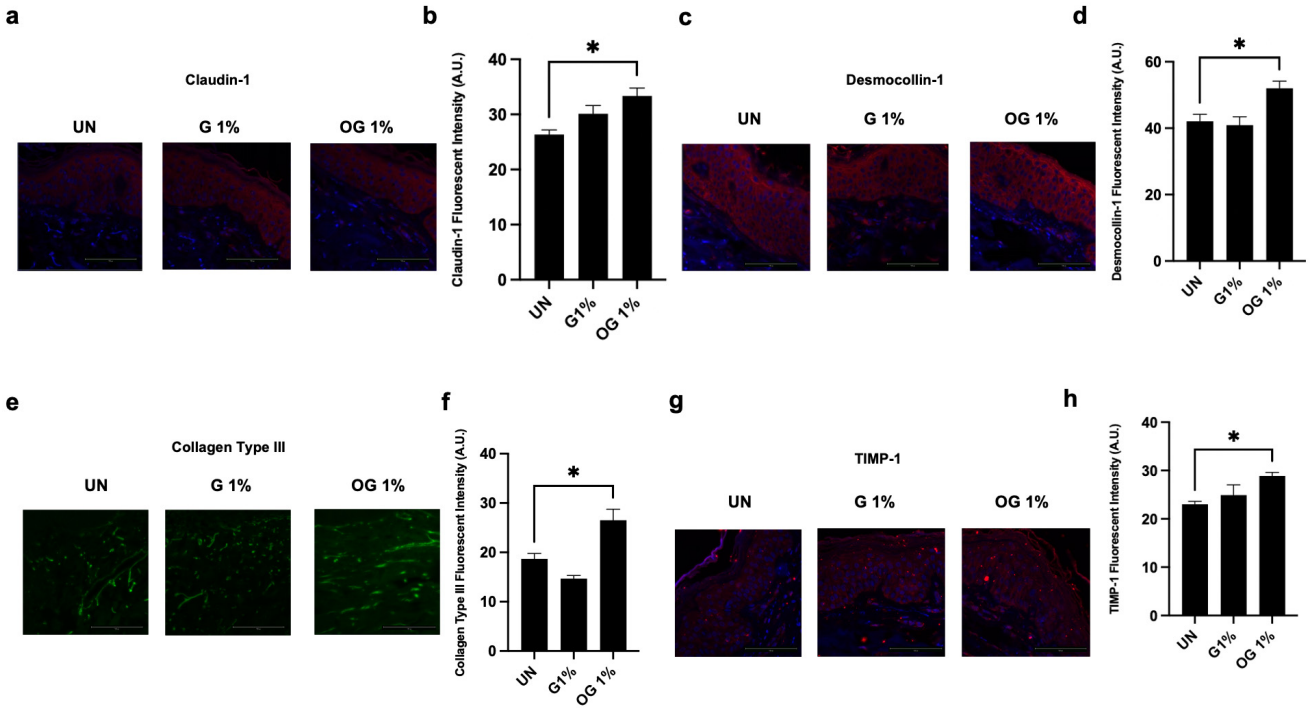

Figure 2. Ex vivo human skin biopsies treated with OG enhance barrier and matrix-related proteins. Representative immunofluorescent images of (**a**) claudin-1 and (**b**) quantification of protein expression levels. Representative immunofluorescent images of (**c**) desmocollin-1 and (**d**) quantification of protein expression levels. Representative immunofluorescent images of (**e**) collagen type III and (**f**) quantification of protein expression levels. Representative immunofluorescent images of (**g**) TIMP-1 and (**h**) quantification of protein expression levels in human skin biopsies pretreated daily with OG at Day 4. Nuclei are counterstained with DAPI (blue). Data are presented as mean ± standard error of the mean. Statistical significance was determined using one-way analysis of variance with Tukey's post hoc test for multiple comparisons ($n = 3$). A p-value < 0.05 was considered statistically significant and denoted by an asterisk (*).

3.3. Cutaneous Anti-Inflammatory Properties of OG

In LPS-challenged ex vivo skin biopsies, OG treatment attenuated inflammatory damage, which preserved the levels of key extracellular matrix components. As shown in Figure 3a,b, skin explants treated with LPS demonstrated significant increases in MMP-9 levels; this upregulation was substantially decreased at Day 4 in explants treated with both G and OG. As expected, LPS treatments degraded the extracellular matrix with marked decreases in key dermal proteins, elastin, and collagen type I (Figure 3a–f). Degradation of elastin was significantly prevented in OG-treated samples. TIMP-1 levels were significantly reduced in LPS-treated tissues; both OG and G treatments were effective in increasing TIMP-1 expression in the ECM compared to LPS-treated tissues (Figure 3g,h).

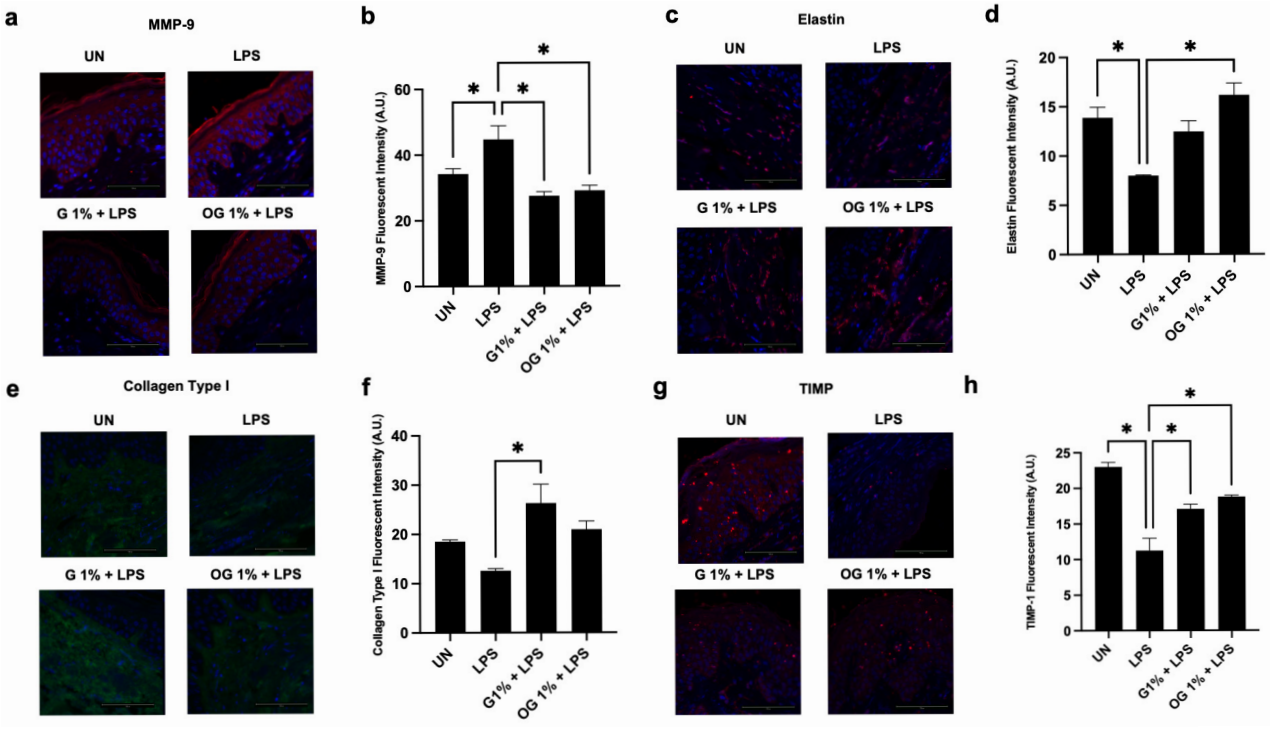

Figure 3. OG attenuates inflammation and preserves ECM components in LPS-challenged ex vivo human skin biopsies. Representative immunofluorescent images of (**a**) MMP-9 and (**b**) quantification of protein expression levels. Representative immunofluorescent images of (**c**) elastin and (**d**) quantification of protein expression levels. Representative immunofluorescent images of (**e**) collagen type I and (**f**) quantification of protein expression levels. Representative immunofluorescent images of (**g**) TIMP-1 and (**h**) quantification of protein expression levels in human skin biopsies pretreated daily with OG and challenged with LPS at Day 4. Nuclei are counterstained with DAPI (blue). Data are presented as mean ± standard error of the mean. Statistical significance was determined using one-way analysis of variance with Tukey's post hoc test for multiple comparisons ($n = 3$). A p-value < 0.05 was considered statistically significant and denoted by an asterisk (*).

4. Discussion

This study highlights the potential of OG to modulate critical aspects of cutaneous physiology under both normal and inflammatory conditions in 3D tissues and human skin biopsies. Compared to G and untreated controls, OG demonstrated consistent benefits toward skin tissue repair and barrier structure maintenance in both 3D epidermal skin models and human ex vivo skin explants. However, in an inflammatory model, G and OG demonstrated comparable anti-inflammatory effects, exhibiting similar modulations of MMPs and TIMPs expressions, with some advantages for OG in ECM preservation. These findings help rationalize the use of OG in cosmetics to target and enhance skin regeneration and resilience.

In the 3D wound closure model, accelerated healing was exhibited with OG treatments compared to both untreated control and G-treated tissues. OG's effect on wound closure is associated with the modulation of MMPs and TIMP-1 protein expression. Our results demonstrate that OG increased MMP-2 expression compared to control; at the same time, TIMP-1, an endogenous MMP-2 inhibitor, was decreased, suggesting the management and balance of matrix remodeling mechanisms, which likely contributed to accelerated matrix turnover [24]. The idea that OG forms and supports an enhanced regenerative environment was supported by cytokine analysis. IL-1α, a key inflammatory molecule that drives cutaneous inflammation and stress responses, was reduced in OG-treated tissues [25]. Conversely, the secretion of TGF-1β, a central growth factor, was significantly promoted

in OG-treated tissues compared to control [26]. Together, the simultaneous reduction in pro-inflammatory signaling and the enhancement of regenerative pathways support the accelerated wound closure demonstrated in OG treatments. Compared to G, OG formed a favorable environment for cutaneous repair, suggesting the role of ozonation in enhancing glycerin functionality.

The beneficial effects of OG were also seen in ex vivo human skin explants, supporting its capacity to strengthen structural and barrier components of the skin. Pretreatments of OG increased the expression of both claudin-1 and desmocollin-1, two critical proteins involved in maintaining epidermal integrity [27]. Claudin-1 is a major component of tight junctions, which are necessary for barrier function [28], while desmocollin-1 is involved in cell-to-cell adhesion in the upper epidermal layers [29]. Together, their upregulation indicates that OG enhances epidermal cohesion and reinforces the cutaneous barrier. In addition to the epidermal effects, OG pretreatment also increased collagen type III and TIMP-1 expression, indicating impacts on ECM remodeling. Collagen type III co-assembles with collagen type I and is important for supporting and promoting ECM deposition and stabilization [30]. TIMP-1 is a natural inhibitor of MMPs, which are critical for maintaining ECM stability through balancing proteolytic activity [5]. The harmonized increase in collagen type III and TIMP-1 under basal conditions suggests that OG, compared to G alone, stimulates an environment that strengthens ECM deposition and reinforcement. The mechanism of action could be due to the generation of peroxides or other species through the ozonation of glycerin, although we have not measured these biomarkers. Based on published literature, OG contains small amounts of a trioxepane derivative, which can have mild oxidative activity through physicochemical analyses [13]. Therefore, pretreatment with OG may generate a mild oxidative environment, which drives its beneficial biological effects, similar to a hormetic effect [31,32]. This hypothesis is corroborated by previous research that has demonstrated the ability of OG to induce Nrf2, normally bound to Keap1 and inactive, and upregulate antioxidant enzymes such as HO-1 and NQO1 [21]. In the skin, HO-1 and NQO1 help control and reduce oxidative stress and inflammation through antioxidant response (carbon monoxide/bilirubin) and detoxification of reactive quinones [33,34]. Considering that Nrf2 activation is a very early response of the cells, due to the lack of early time points in our experimental procedure, we plan to investigate Nrf2 activation in the next steps of this project by focusing on early time tissue responses. Together, the upregulation of these enzymes has been shown to inhibit LPS-induced inflammatory mediators like TNF-α [35,36]. These proinflammatory mediators can drive and perpetuate cutaneous inflammatory responses [37]. For example, TNF-α and the upregulation of MMP-9 expression contribute to significant degradation of collagen and elastin [38].

Indeed, in our proinflammatory model, LPS induced significant upregulation of MMP-9, degrading both collagen type I and elastin. Treatment with either OG or G effectively reduced MMP-9 expression and prevented the LPS-induced degradation of key ECM proteins, elastin, and collagen-1, respectively. This indicates that both formulations have anti-proteolytic and anti-inflammatory benefits. Notably, no significant differences were observed between glycerin (G) and ozonized glycerin (OG) with respect to MMP-9 and TIMP-1 levels in LPS-treated skin explants. These findings may suggest that, under inflammatory conditions, OG preferentially modulates pathways related to elastin remodeling. Alternatively, it is possible that the LPS stimulation was not sufficiently robust to reveal differential effects between OG and G.

Overall, when considering the results obtained in non-inflammatory models, our data indicate that OG is able to improve several baseline markers associated with skin aging.

In contrast, the anti-inflammatory effects of OG warrant further investigation using more mechanistic and optimized inflammatory models.

Given previous research, it is likely that the OG protective effects are mediated through the upregulation of antioxidant enzymes, thwarting the accumulation of oxidants, which drive the activation of proinflammatory pathways, and cytokines are responsible for the protective benefit. The increases and protection of ECM components by OG or G are supported by the upregulation of TIMP-1, which confirms ECM stabilization through inhibition of matrix metalloproteinases.

Interestingly, despite the clear benefits of OG compared to G in other models, both formulations demonstrated similar anti-inflammatory effects with specific differences in collagen/elastin preservation. While both G and OG attenuated ECM degradation, OG appeared to be more effective in preserving elastin, whereas its effects on collagen type I were less pronounced. Although the basis for these differential effects is unclear, it may reflect unique susceptibilities of these ECM proteins in response to inflammatory stimuli.

Taken together, OG and G appear to exert complementary protective effects in an inflammatory environment through modulation of MMP/TIMP balance. In a proinflammatory environment, OG, plausibly through its mild oxidative signaling, may preferentially enhance antioxidant defenses and stabilize elastic fibers; alternatively, G may contribute to ECM protection through its known humectant properties, enhancing cutaneous hydration through attraction of moisture into the skin [39].

One shortcoming of our study is the absence of an untreated and unwounded control; therefore, it is difficult to understand whether the effects of OG represent a restoration or overcompensation to baseline values of the proteins investigated. Future studies should investigate the use of OG in in vivo models to capture the full complexities of wound healing and improve translatability. Additionally, further investigation is needed to understand the absorption of OG and quantify the release of compounds (such as ozone-derived peroxides) in the skin.

5. Conclusions

Overall, these results demonstrate that ozonation enhances the biological activity of glycerin as a cosmetic ingredient. In a wound-healing model, ozonized glycerin (OG) promoted wound closure through increased growth factor production and modulation of IL-1α release. When topically applied to human skin explants, OG significantly upregulated epidermal and extracellular matrix (ECM) proteins compared with glycerin alone. In inflammatory models, OG reduced MMP-9 expression and preserved key ECM components. Taken together, these findings reveal the novel multifunctional properties of OG, supporting its potential as a cosmetic ingredient to improve skin integrity and protect against cutaneous inflammation.

Author Contributions: Conceptualization, G.V., J.I. and G.S.; Methodology, J.I., A.G. and G.T.; Software, J.I. and A.P.; Validation, G.T., A.G. and A.P.; Formal Analysis, J.I. and A.P.; Investigation, J.I. and A.G.; Resources, G.V.; Data Curation, J.I., A.G. and A.P.; Writing—Original Draft Preparation, J.I. and G.V.; Writing—Review and Editing, G.V. and A.P.; Supervision, G.V. and G.S.; Project Administration, G.V.; Funding Acquisition, G.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Mediplus Pharma to NCSU, Grant.

Institutional Review Board Statement: Human tissues were obtained via elective abdominoplasty with donor consent under Pearl IRB approval in accordance with FDA 45 CFR 46.102 and 21 CFR 56.102 regulations (Pearl Pathways, Exemption Determination Submission, IRB Study Number: 21-TENB-101, Study Title: Collection, culture, and distribution of human abdominoplasty skin tissue) on 4 March 2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: Dataset available on request from the authors.

Acknowledgments: Mediplus Pharma for research support.

Conflicts of Interest: Gotaro Shiota is the employee of Mediplus Pharma Inc. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Haykal, D.; Lim, H.W.; Calzavara-Pinton, P.; Fluhr, J.; Cartier, H.; Berardesca, E. The impact of pollution and climate change on skin health: Mechanisms, protective strategies, and future directions. *JAAD Rev.* **2025**, *6*, 1–11. [CrossRef]
2. Xiao, T.; Yan, Z.; Xiao, S.; Xia, Y. Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. *Stem Cell Res. Ther.* **2020**, *11*, 232. [CrossRef]
3. Han, Y.P.; Downey, S.; Garner, W.L. Interleukin-1α–induced proteolytic activation of metalloproteinase-9 by human skin. *Surgery* **2005**, *138*, 932–939.
4. Van Doren, S.R. Matrix metalloproteinase interactions with collagen and elastin. *Matrix Biol.* **2015**, *44–46*, 224–231. [CrossRef]
5. Brew, K.; Nagase, H. The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity. *Biochim. Biophys. Acta* **2010**, *1803*, 55–71.
6. Cabral-Pacheco, G.A.; Garza-Veloz, I.; Castruita-De la Rosa, C.; Ramirez-Acuña, J.M.; Perez-Romero, B.A.; Guerrero-Rodriguez, J.F.; Martinez-Avila, N.; Martinez-Fierro, M.L. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. *Int. J. Mol. Sci.* **2020**, *21*, 9739. [CrossRef]
7. Simpson, C.L.; Patel, D.M.; Green, K.J. Deconstructing the skin: Cytoarchitectural determinants of epidermal morphogenesis. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 565–580. [CrossRef]
8. Naydenov, N.G.; Baranwal, S.; Khan, S.; Feygin, A.; Gupta, P.; Ivanov, A.I. Novel mechanism of cytokine-induced disruption of epithelial barriers. *Tissue Barriers* **2013**, *1*, e25231. [CrossRef]
9. Liu, L.; Zeng, L.; Gao, L.; Zeng, J.; Lu, J. Ozone therapy for skin diseases: Cellular and molecular mechanisms. *Int. Wound J.* **2022**, *20*, 2376–2385. [CrossRef]
10. Valacchi, G.; Lim, Y.; Belmonte, G.; Miracco, C.; Zanardi, I.; Bocci, V.; Travagli, V. Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice. *Wound Repair Regen.* **2011**, *19*, 107–115.
11. Travagli, V.; Zanardi, I.; Valacchi, G.; Bocci, V. Ozone and ozonated oils in skin diseases: A review. *Mediat. Inflamm.* **2010**, *2010*, 610418. [CrossRef] [PubMed]
12. Serra, M.E.G.; Baeza-Noci, J.; Mendes Abdala, C.V.; Luvisotto, M.M.; Bertol, C.D.; Anzolin, A.P. The role of ozone treatment as integrative medicine. An evidence and gap map. *Front. Public Health* **2023**, *10*, 1112296.
13. Bocci, V. The Potential Toxicity of Ozone: Side Effects and Contraindications of Ozonetherapy. *OZONE* **2010**, *24*, 75–84. [CrossRef]
14. Valacchi, G.; Zanardi, I.; Lim, Y.; Belmonte, G.; Miracco, C.; Sticozzi, C.; Bocci, V.; Travagli, V. Ozonated oils as functional dermatological matrices: Effects on the wound healing process using SKH1 mice. *Int. J. Pharm.* **2013**, *458*, 65–73. [CrossRef] [PubMed]
15. Repciuc, C.C.; Vișan, G.A.M.; Teleky, B.E.; Pintea, A.; Novac, C.Ș.; Oros, N.V. Physico-Chemical and Antimicrobial Evaluation of Ozonated Olive Oil Produced with a Medical-Grade Generator for Veterinary Purposes. *Microorganisms* **2025**, *13*, 1932.
16. Hanada, K.; Okuda, D.; Ogi, R.; Kojima, S.; Tsuruoka, R.; Shiota, G. Ozonized glycerin (OG)-based cosmetic products lighten age spots on human facial skin. *J. Cosmet. Dermatol.* **2022**, *21*, 3133–3139.
17. Niinomi: The Bactericidal Effects of Ozone Gel. Available online: https://scholar.google.com/scholar_lookup?journal=J%20Showa%20Univ%20Dent%20Soc&title=The%20bactericidal%20effects%20of%20ozone%20gel&author=K%20Niinomi&author=A%20Kanaishi&author=A%20Shiba&volume=24&issue=2&publication_year=2004&pages=103-109& (accessed on 5 October 2025).

18. Fukui, T.; Masuno, K.; Makita, Y.; Fujiwara , S.; Shiota, G.; Imamura, Y.; Shiba, A.; Wang, P.-L. Antimicrobial Effects of Ozone Gel Against Periodontal Bacteria. *J. Hard Tissue Biol.* **2014**, *23*, 445–448. [CrossRef]
19. Oda: Effect of Ozone Gel on Oral Pathogens and Human Gingiva and Periodontal Ligament Fibroblasts. Available online: https://scholar.google.com/scholar_lookup?journal=Jpn%20J%20Conserv%20Den&title=Effect%20of%20ozone%20gel%20on%20oral%20pathogens%20and%20human%20gingiva%20and%20periodontal%20ligament%20fibroblasts&author=H%20Oda&author=K%20Maruyama&author=M%20Tsubokawa&volume=57&publication_year=2014&pages=369-376& (accessed on 5 October 2025).
20. Takeda, Y.; Jamsransuren, D.; Makita, Y.; Kaneko, A.; Matsuda, S.; Ogawa, H.; Oh, H. Inactivation of SARS-CoV-2 by Ozonated Glycerol. *Food Environ. Virol.* **2021**, *13*, 316–321. [CrossRef]
21. Shiota, G.; Tsuruoka, R.; Hanada, K. 404 Novel glycerin compounds improve skin health. *J. Investig. Dermatol.* **2022**, *142*, S69. [CrossRef]
22. Ferrara, F.; Bondi, A.; Pula, W.; Contado, C.; Baldisserotto, A.; Manfredini, S.; Boldrini, P.; Sguizzato, M.; Montesi, L.; Benedusi, M.; et al. Ethosomes for Curcumin and Piperine Cutaneous Delivery to Prevent Environmental-Stressor-Induced Skin Damage. *Antioxidants* **2024**, *13*, 91.
23. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [CrossRef] [PubMed]
24. Cui, N.; Hu, M.; Khalil, R.A. Biochemical and Biological Attributes of Matrix Metalloproteinases. *Prog. Mol. Biol. Transl. Sci.* **2017**, *147*, 1–73. [PubMed]
25. Macleod, T.; Berekmeri, A.; Bridgewood, C.; Stacey, M.; McGonagle, D.; Wittmann, M. The Immunological Impact of IL-1 Family Cytokines on the Epidermal Barrier. *Front. Immunol.* **2021**, *12*, 808012. [CrossRef] [PubMed]
26. Liarte, S.; Bernabé-García, Á.; Nicolás, F.J. Role of TGF-β in Skin Chronic Wounds: A Keratinocyte Perspective. *Cells* **2020**, *9*, 306. [CrossRef]
27. Brandner, J.; Zorn-Kruppa, M.; Yoshida, T.; Moll, I.; Beck, L.; De Benedetto, A. Epidermal tight junctions in health and disease. *Tissue Barriers* **2014**, *3*, e974451. [CrossRef]
28. Furuse, M.; Hata, M.; Furuse, K.; Yoshida, Y.; Haratake, A.; Sugitani, Y.; Noda, T.; Kubo, A.; Tsukita, S. Claudin-based tight junctions are crucial for the mammalian epidermal barrier. *J. Cell Biol.* **2002**, *156*, 1099–1111. [CrossRef]
29. Kowalczyk, A.P.; Green, K.J. Structure, Function and Regulation of Desmosomes. *Prog. Mol. Biol. Transl. Sci.* **2013**, *116*, 95–118.
30. Singh, D.; Rai, V.; Agrawal, D.K. Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration. *Cardiol. Cardiovasc. Med.* **2023**, *7*, 5–16. [CrossRef]
31. Xiao, W.; Tang, H.; Wu, M.; Liao, Y.; Li, K.; Li, L.; Xu, X. Ozone oil promotes wound healing by increasing the migration of fibroblasts via PI3K/Akt/mTOR signaling pathway. *Biosci. Rep.* **2017**, *37*, BSR20170658.
32. Xiao, W.R.; Wu, M.; Bi, X.R. Ozone oil promotes wound healing via increasing miR-21-5p-mediated inhibition of RASA1. *Wound Repair Regen.* **2021**, *29*, 406–416.
33. Pae, H.O.; Chung, H.T. Heme Oxygenase-1: Its Therapeutic Roles in Inflammatory Diseases. *Immune Netw.* **2009**, *9*, 12–19. [CrossRef]
34. Ross, D.; Siegel, D. Functions of NQO1 in Cellular Protection and CoQ10 Metabolism and Its Potential Role as a Redox Sensitive Molecular Switch. 24 August 2017. Available online: https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2017.00595/full (accessed on 5 October 2025).
35. Lee, I.T.; Luo, S.F.; Lee, C.W.; Wang, S.W.; Lin, C.C.; Chang, C.C.; Chen, Y.-L.; Chau, L.-Y.; Yang, C.-M. Overexpression of HO-1 protects against TNF-alpha-mediated airway inflammation by down-regulation of TNFR1-dependent oxidative stress. *Am. J. Pathol.* **2009**, *175*, 519–532. [CrossRef]
36. Rushworth, S.A.; MacEwan, D.J.; O'Connell, M.A. Lipopolysaccharide-induced expression of NAD(P)H:quinone oxidoreductase 1 and heme oxygenase-1 protects against excessive inflammatory responses in human monocytes. *J. Immunol.* **2008**, *181*, 6730–6737.
37. van Loo, G.; Bertrand, M.J.M. Death by TNF: A road to inflammation. *Nat. Rev. Immunol.* **2023**, *23*, 289–303.
38. Bahar-Shany, K.; Ravid, A.; Koren, R. Upregulation of MMP-9 production by TNFalpha in keratinocytes and its attenuation by vitamin D. *J. Cell. Physiol.* **2010**, *222*, 729–737.
39. Chen, H.J.; Lee, P.Y.; Chen, C.Y.; Huang, S.L.; Huang, B.W.; Dai, F.J.; Chau, C.F.; Chen, C.S.; Lin, Y.S. Moisture retention of glycerin solutions with various concentrations: A comparative study. *Sci. Rep.* **2022**, *12*, 10232. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.