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Effects of Mackerel Scad Collagen Gel on Superficial Non-Infected Wound in Mice

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ABSTRACT

Marine organisms are rich sources of structurally diverse bioactive compounds with various biological activities, making them increasingly important for biomedical applications. This study aims to evaluate the effects of the topical administration of mackerel scad (*Decapterus macarellus*) fish collagen gel on tissue repair during superficial non-infected wound healing in mice. To make the collagen gel, lyophilized collagen extracted from mackerel skin was incorporated into hydroxypropyl methylcellulose gel at 3% or 5% (w/w) concentrations, and the gel's physical properties were characterized. The collagen gels were applied topically to excision wounds on BALB/c 57 strain mice three times daily at a dose of 300 mg. Assessment of the histological state of the healing wound was done using Hematoxylin-Eosin, Masson's Trichrome, and VEGF immunohistochemistry staining techniques. The collagen gel exhibited favorable characteristics in terms of viscosity, spreadability, and adhesiveness. Histological examination revealed that the topical application of 5% collagen gel reduced wound diameter by 75%, significantly ($p<0.05$) higher than the placebo control treatment (42.5%). By day 14, the wound tissue treated with 5% collagen gel exhibited a well-organized re-epithelialization layer and visible arrangement of new collagen fibers in the extracellular matrix. These tissues entered a proliferation and remodeling phase, resembling normal skin tissue characteristics. Furthermore, the expression of VEGF in the wound healing process treated with topical collagen gel was stronger and more widespread compared to the gel without collagen. Overall, these findings suggest that collagen treatment promotes quicker wound recovery and enhances the overall outcome of wound healing processes.

Keywords: Collagen gel; Mackerel scad; Histology; Wound healing.

Introduction

Skin wounds, characterized by tissue damage caused by punctures, cuts, or friction, can disrupt normal skin function and require immediate and appropriate treatment. The healing process for skin wounds typically ranges from one to ten weeks, depending on factors such as wound type (acute or chronic), trauma severity, hormonal influences, and overall skin condition.[1] This healing process encompasses four overlapping stages: haemostasis, inflammation, proliferation, and remodelling.[2] Effective wound treatment is essential for achieving optimal wound closure and ensuring the restoration of skin integrity. The extracellular matrix (ECM), a skin component composed of collagen fibres, elastin, and glycoproteins, is crucial in regulating the wound-healing process.[3] Most collagen in ECM is collagen type I, the primary component of the skin's dermal layer that functions as a connective tissue. During wound healing, collagen fibres stimulate cell signalling and enhance the mechanical resistance of newly formed tissue.[4] Wound repair also involves the formation of new blood vessels (i.e., neovascularization), mediated by pro-angiogenic factor proteins such as Vascular Endothelial Growth Factor (VEGF). This process supports nutrient distribution in the granulation tissue. VEGF, part of the ECM, recruits chemoattractant to the wound site through cell adhesion and binding interactions.[5]

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Therefore, the optimal level of neovascularization in the wound and the maturity of the ECM in the skin's connective tissue are vital indicators of effective wound healing.
Collagen-based biomaterials are commonly utilized in wound treatment, particularly for healing burns, surgical wounds, and post-transplantation cases.[6] Gel-form wound care products are often preferred due to their superior ability to bind water molecules and penetrate skin layers more efficiently than other topical formulations.[1,7] Incorporating additional collagen in wound care enhances the vascularization process during healing. During angiogenesis, interactions occur between small blood vessels or microvasculature and the extracellular matrix, specifically type I collagen fibres. This interaction leads to increased collagen density through a process known as collagen condensation, where cells recruit collagen to the surface.[8] The exogenous addition of collagen can further stimulate the extracellular matrix remodelling phase by activating growth factors, thereby contributing to effective wound healing.[9]
Collagen used in topical hydrogel wound care is typically sourced from pigs, cows, and chickens.[6] In contrast, marine-derived collagen remains underexplored and underutilized in wound care biomaterials. Marine collagen, primarily obtained from fish and other sea organisms, offers several potential advantages, including a lower risk of zoonotic disease transmission and higher biocompatibility. Additionally, it has unique bioactive properties that can enhance the wound healing process, such as promoting cell proliferation and migration, as well as exhibiting anti-inflammatory effects.[10,11]
Further research and exploration into marine-derived collagen could significantly expand its application in advanced wound care therapies. A previous study[12] demonstrated that oral collagen peptides derived from mackerel scads (*Decapterus macarellus*) fish skin can accelerate wound closure and increase collagen deposition from the first week during the wound healing process in mice models. On the other hand, to our knowledge, no studies have yet reported the application of

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collagen from mackerel scads for topical wound care. Therefore, this study aims to propose a new formulation of topical wound treatment using scads mackerel collagen in a gel form and to investigate its effectiveness on the wound repair process in vivo.

Methodology

Collagen extraction and gel formulation

Collagen extraction refers to the method described by Herawati and colleagues.[13] In brief, washed fish skin was soaked in 0.1 M NaOH with a ratio of 1:10 (w/v) for 6 hours at 4˚C. The skin was then soaked in 0.5 M acetic acid containing 0.1% pepsin (w/w) with a 1:8 (w/v) ratio for 48 hours at a temperature of 4˚C. The sample was filtered, and the filtrate was centrifuged at 4000 rpm, temperature 4˚C for 60 minutes. The pellets were taken, and then acetic acid 0.5 M was added with a 1:5 ratio (v/v) of pellets to acetic acid. Samples were loaded onto a dialysis membrane (12 kDa cut-off). Stage 1 dialysis was carried out with 0.2 M phosphate buffer for 24 hours at a temperature of 4˚C. Next, the sample was subjected to stage 2 dialysis using distilled water at a temperature of 4˚C for 24 hours. The dialysis samples were dried using the freeze-dry method to obtain collagen. Collagen gel was made by modifying the method described elsewhere.[14] The gel formula (Table 1) comprises collagen, hydroxypropyl methylcellulose (HPMC), glycerine, methylparaben, lemon oil, and distilled water.

The beaker containing HPMC was added with distilled water (w/v), which was previously heated to 70˚C. The mixture was stirred until it expanded and became homogeneous at room temperature. Glycerine, methylparaben, lemon oil, and collagen were homogenized in separate beakers. The two mixed ingredients were homogenized until evenly distributed. A distilled water (aquadest) was then added to the gel until the volume reached 100%. Next, the collagen gel formed was stored at 4˚C.

Gel Characterization

The collagen gel formed was subjected to three tests, i.e., viscosity, spreadability, and adhesiveness. All tests were performed in triplicates unless indicated.

Viscosity Test

The viscosity test was done using a viscometer set consisting of a cone and plate. A sufficient amount of gel was placed on the viscometer plate and squeezed with the cone, giving a little space. A computer was connected to the viscometer, and the Rheosys Micra program was used to read the results.

Spreadability test

The gel was placed in the centre of a round glass and covered with a cover glass. A weight was added, and the glass was left to rest for 1 minute. The diameters of the four sides of the gel spread were recorded.

Adhesiveness test

The gel was placed on Object Glass 1 with a specific area. Object Glass 2 was placed on top of the gel in Object Glass 1. A load of 500 grams was placed on the object glass for 5 minutes. A weight of 80 grams was lifted, and the time gained was recorded.

Excision wound model and collagen gel treatment

The excision wound model was performed by applying different collagen gel treatments on artificially wounded mice (*Mus musculus*). This research obtained ethical clearance from the Faculty of Medicine, Muhammadiyah University of Surakarta (Number 4809/A.1/KEPK-FKUMS/V/2023). Sixteen mice (strain C57BL/6) aged 8 weeks old, weighing 29-32 g, were randomly divided into four groups, i.e., control (placebo) group, commercial gel group (Bioplacenton®), Col-3 group with 3% collagen gel treatment, and Col-5 group with 5% collagen gel treatment.

Mice were anesthetized with isoflurane (20% isoflurane v/v in propylene glycol). The dorsal skin of the mice was shaved. The excision wound was made through a biopsy punch (2 mm in diameter). The wound was left without applying antimicrobials. A topical gel of 300 mg was applied three times a day every 6 hours according to the treatment group for 13 days.

Wound assessment

The diameter and surface area of the wound were measured with a vernier calliper. The wound area measurement was done daily before administering the third dose of the day and repeated three times.

Table 1. Collagen gel formulation

Ingredients	Function	Formulation 1 (gram)	Formulation 2 (gram)	Formulation 3 (gram)
Collagen (w/v)	Active ingredients	0%	3%	5%
HPMC	Gelling agent	2%	2%	2%
Methylparaben	Preservatives	0.2%	0.2%	0.2%
Glycerine	Humectant	10%	10%	10%
Lemon oil	Fragrance	0.5%	0.5%	0.5%
Aquadest	Dilution	Added to 100%	Added to 100%	Added to 100%

Preparation of skin tissue slides

Mice skin tissue with the size of 1.5 × 1.5 cm were fixated with Neutral Buffer Formalin 10% for 18 hours. The microscope slide of skin tissue was prepared using the paraffin embedding technique. All processes involved, i.e., dehydration, clearing, infiltration, and paraffin embedding, were done using an automatic tissue processor.[15] Paraffin blocks were sliced to a thickness of 5 μm and stained with HE or Masson's Trichome.

Immunohistochemical staining of skin tissue was made by placing tissue slices on poly-L-lysine slides and then incubated at 37°C for one night. The slides were deparaffinized by soaking in xylol I, xylol II, xylol III, xylol IV, and absolute alcohol, 95% alcohol, 70% alcohol for minutes sequentially. The slides were washed with running water and distilled water. Then, they were washed with PBS. The antigen was retrieved in a microwave oven with Tris EDTA pH 9 at a temperature of 90°C, then continued at a low temperature.

The slides were cooled with PBS. Then, they were dripped with 3% hydrogen peroxide (H_2O_2) and washed with running water. Slides were stained with blocking serum, drained, then dripped with the anti-VEGF antibody (Santa Cruz Biotechnology®), and incubated at 4°C. The slides were rewashed with PBS. Slides were blotted with biotin and washed with PBS. Slides were blotted with streptavidin and washed with PBS. The slides were treated with the peroxidase enzyme substrate diaminobenzidine (DAB) and washed with water. The slides were stained with haematoxylin and washed again with water. Finally, the next slide was mounted with entellan and covered with a deck glass cover glass. The histology of the skin was observed with a light microscope, and images were taken with a magnifying lens at 200× magnification.

Data analysis

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The percentage reduction in wound diameter data obtained was analyzed using one-way ANOVA. When ANOVA results showed a significant difference between groups at $p<0.05$, Duncan's post-hoc test was done. Analysis of the histological structure of the skin was presented in a comparative descriptive manner.

Result and Discussion

Physical characteristics of collagen gel

The collagen extraction from mackerel scad (*D. macarellus*) fish skin yielded an average yield of 8.1973 ± 1.25 grams, corresponding to approximately 11.66 ± 2.5% of wet weight. The collagen exhibited a white colour and had a fibrous texture (Figure 1A), with a pH of 6.9. This collagen was subsequently incorporated as an active ingredient in a base gel, which was then topically applied to excision wounds on the dorsal skin of mice. The resulting collagen gels exhibited a semi-solid form, a slightly yellow and translucent colour, and a citrus aroma (Figure 1B). Our previous organoleptic studies indicated that panellists positively perceived the collagen gel's texture and colour.[14] The pH of the gel base ranged from 5 to 6, whereas the pH of the gel formulated with collagen ranged from 6 to 7 (Table 2), which suggests that the collagen gel tends to be slightly alkaline. The pH of normal skin typically fluctuates between 5.7 to 6.5, while infected wounds consistently maintain an alkaline environment.

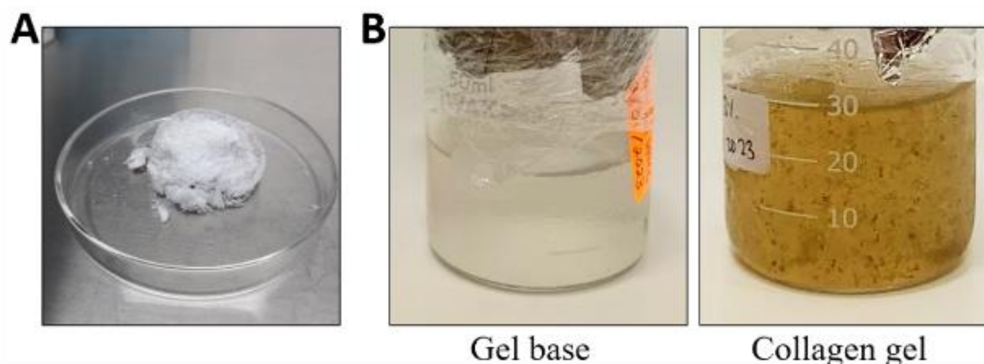

Figure 1. Collagen extracted from the skin of mackerel scad fish (A) and its gel form (B). The gel base was incorporated with 3% of collagen.

Table 2. The pH, viscosity, spreadability, and adhesiveness value of collagen gel

Gel formulation	pH	Viscosity (cp) ±SD	Spreadability (g.cm/s)	Adhesiveness (s)
Base	5-6	17,655.31 ± 58	16.25	3.2
Collagen-3%	6-7	9,620.91 ± 15	17.69	15.2
Collagen-5%	6-7	9,261.79 ± 62	16.25	18

In contrast, the pH during non-infected wound healing initially shifts from alkaline (7.4) to acidic (6.7) and then returns to an alkaline state (7.9). To summarize, a dynamic pH environment, transitioning from alkaline to acidic and back to alkaline, is conducive in wound healing process.[16]

The viscosity test was done to measure the thickness of the gel formulations. In this study, the collagen gel displayed a lower viscosity than the gel base (Table 2), indicating a faster flow rate of collagen gel. According to the Indonesian standard guide (SNI 16-4399-1996), the viscosity for topical cosmetic products needs to be within 2,000 to 50,000 centipoise (cp). All gel formulations in this study have met the SNI viscosity standards. Ensuring the viscosity level of the topical product meets the standard is essential since a high viscosity ensures good skin adhesion and efficient delivery of active ingredients; meanwhile, an excessively high viscosity can make the gel too dense, reducing its adhesiveness.

The spreadability test measures how well a gel formulation spreads on the skin. According to Indonesian standard SNI 16-4399-1996, the ideal spreadability range for topical cosmetic gels is approximately 5-7 cm/g·s. In this study, the base gel had a spreadability of about 16.25 g·cm/s. Adding collagen at 3% or 5% did not significantly change the spreadability, with values of 17.69 g·cm/s and 16.25 g·cm/s, respectively. However, these values exceed the SNI standards. Adding gelling agents to reduce the spreadability value will solve this problem.

The adhesiveness test measures how long a topical formulation stays on the skin after application. The results of this study showed that the collagen gel exhibits excellent adhesion. Specifically, the gel with 5% collagen adhered for approximately 18 seconds, and the 3% collagen gel adhered for about 15.2 seconds. These findings indicate that the gel formulation can adhere to the skin for an extended period, enhancing the efficient delivery of active substances to the skin's epidermal layers. The overall physical characteristics of the collagen gel indicate it has favourable attributes as a topical gel.

The healing effect of collagen gel on skin wound

The contraction of wound edges and the formation of new epithelial cells facilitate the closure of the wound defect. Mice treated with 5% collagen gel showed accelerated wound closure starting from day 6 compared to the other groups. Ultimately, they exhibited the fastest wound closure, with a reduction in wound diameter of up to 75% by day 13. In contrast, the control group experienced a diameter reduction of only 42.5% over the same period (Figure 2). Significant differences (p-value < 0.05) were observed among the four treatment groups.

Haematoxylin-Eosin (HE) staining vividly depicts the histological stages of wound regeneration. Key elements such as re-epithelialization (E), granulation tissue (G), and hair follicles (HF) were clearly visible. The wound area initially shows a noticeable gap (Figure 3, two-way arrow) at day 0. Within 2 days, the wound area initially fills with a blood clot to prevent further blood loss and in the inflammatory phase.[1,17]

By the 4th day, the wound area showed nearly complete closure (Figure 3). All treatment groups exhibited cell proliferation and differentiation in the wound area. Re-epithelialization was notably thickened, and underlying granulation tissue began forming.

However, the wound healing process in group Col-5 appeared slightly delayed, as shown by an uneven thickness in neo-epidermis formation (arrowhead; Figure 3). This observation led us to investigate whether the formation of new blood vessels (neo-angiogenesis), which typically occurs early in wound healing,[18] was also impeded. Angiogenesis is considered crucial for proper wound repair. During angiogenesis, the fibrin clot is replaced by granulation tissue rich in blood vessels, which

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is then replaced by collagenous scar tissue containing fewer mature blood vessels.

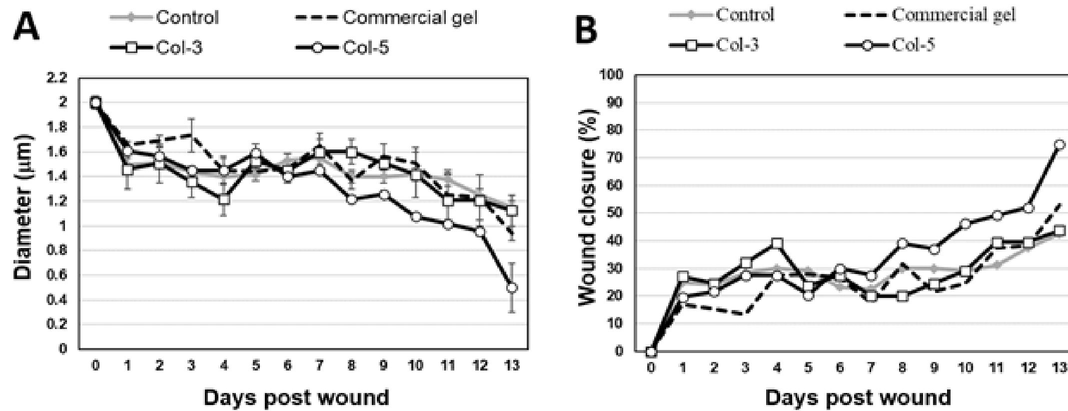

Figure 2. Gross wound healing measures. Wound size quantification (A) indicates Col-5 gel-treated wounds showed accelerated wound closure (B) relative to control that uses base gel (n = 8 per condition)

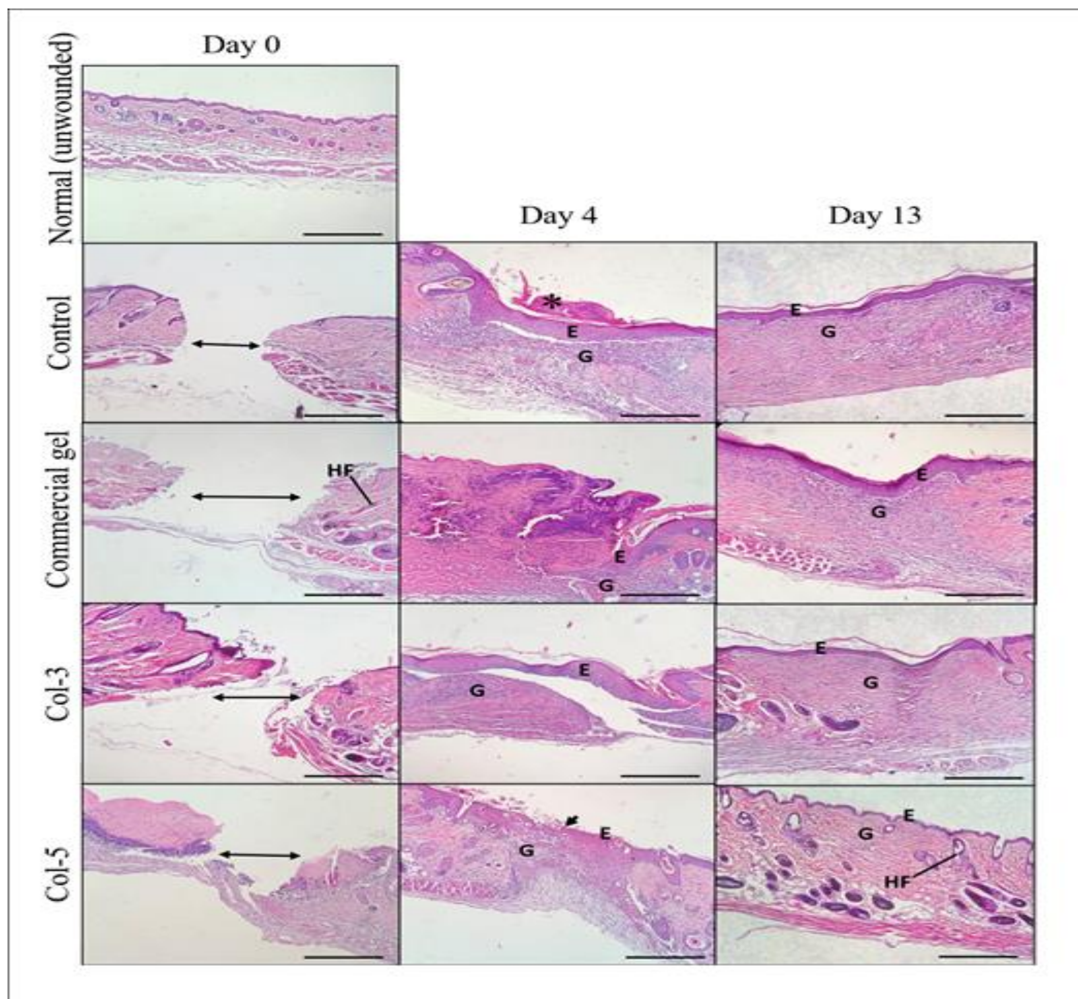

Figure 3. Effect of topical application of collagen gel on tissue repair in the non-infected wound area. Experimental group skin photomicrographs: Control (2nd row) using gel base, commercial gel (3rd row), 3% collagen gel (4th row), and 5% collagen gel (5th row) on Days 0, 4, and 13 post-treatments. E: newly formed epidermal cells; G: granulation tissue; HF: hair follicle; two-way arrow: the area of the wound. An asterisk (*) shows the presence of a scab. Haematoxylin-Eosin (HE) stain was used to prepare the slides. A representative picture of the normal group showed intact skin without wounds. Scale bars 300 µm.

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During angiogenesis, the fibrin clot is replaced by granulation tissue rich in blood vessels, which is then replaced by collagenous scar tissue containing fewer mature blood vessels. In our study, we assessed the expression of the pro-angiogenic factor VEGF-A. Interestingly, VEGF-A expression in group Col-5 was remarkably higher than in the control group and covered the entire granulation tissue. Thus, collagen appears to promote neo-angiogenesis, which is essential for nutrient supply, oxygenation, and immune response in wound healing.[18,19] By day 13, the Col-5 group exhibited the most pronounced epithelial cell differentiation. The epidermal layer had become thinner, indicating advanced differentiation. Additionally, mature extracellular matrix components and the initial formation of hair follicles and glands were observed, resulting in skin architecture resembling normal skin (Figure 3). The histological appearance of the wound tissue on day 13 correlates with the reduction in wound diameter (Figure 2), demonstrating that using 5% collagen gel effectively accelerates wound healing and promotes the remodelling stage of wound tissue.

To further clarify, we examined the tissue using Masson's Trichrome staining to visualize the synthesis of a new collagen matrix as an indicator of maturation in the remodelling phase (Figure 5).[20, 21] Four days after the intervention, all the animals displayed extensive granulation tissue with numerous blood vessels and clear signs of inflammatory and proliferative phases of the reparative process, as observed in histological images (Figure 3; Figure 5).

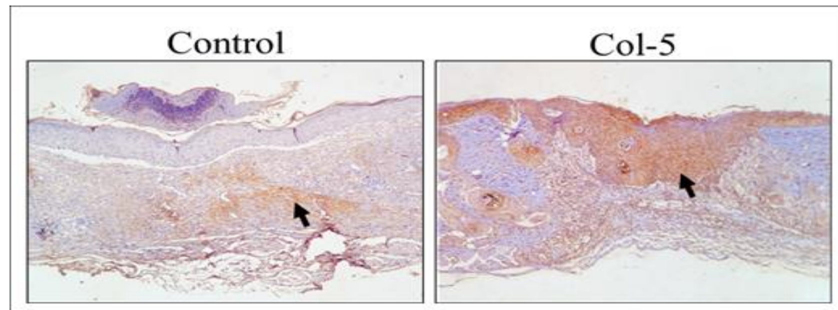

Figure 4. VEGF protein production in the healing wound area. Photomicrographs of histological sections represent control and Col-5 groups on day 4. VEGF (indicated by arrows) was stained brown.

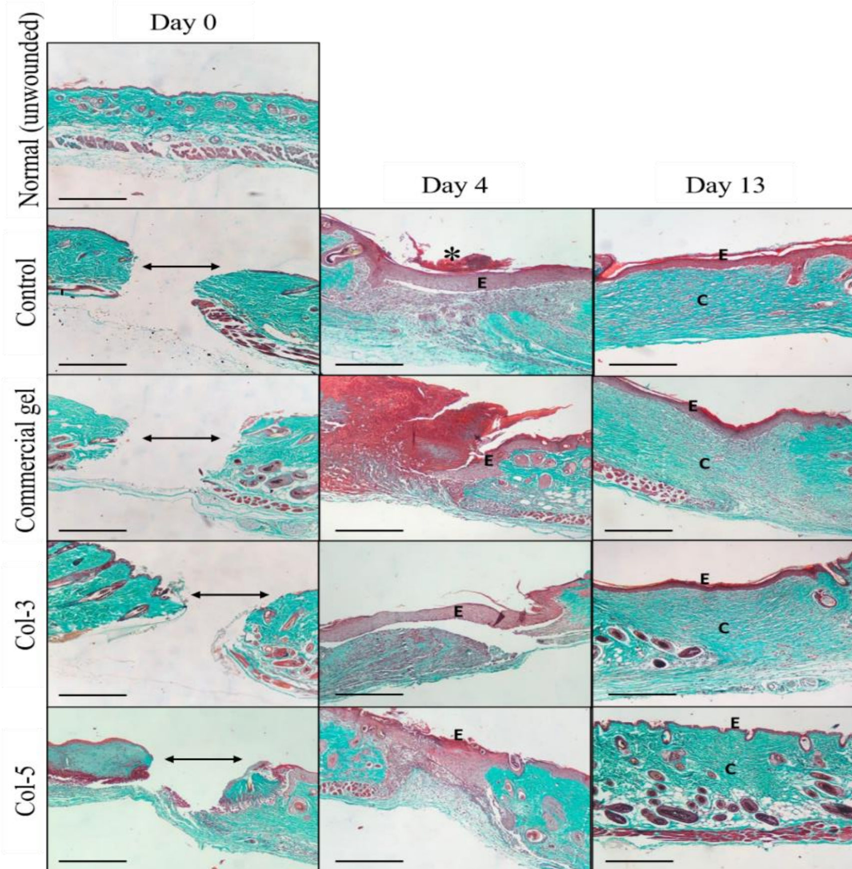

Figure 5. Effect of topical application of collagen gel on collagen formation in the non-infected wound area. Experimental group skin photomicrographs: Control (2nd row) using gel base, commercial gel (3rd row), collagen 3% (4th row), and collagen 3% (5th row) on Days 0, 4, and 13 post-treatments. Collagen fibers are stained green and cell cytoplasm is stained redish-brown. The area of the wound, marked by the disruption of normal architecture and skin structure, is indicated with a double arrowhead. E: newly formed epidermal cells; G: granulation tissue; C: collagen fibers. An asterisk (*) shows the presence of a scab. Masson's Trichrome stain was used. A representative picture of normal group showed the intact skin without skin wound. Scale bars 300 µm.

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The extracellular matrix was loose and poorly organized, with noticeable immature collagen deposition revealed by the green colour of Masson's Trichrome stain (Figure 5). Thirteen days after the wound was made, the skin exhibited features of the initial stages of maturation and remodelling (Figure 5). Almost all the animals showed completely epithelialized wound areas; however, the control groups displayed a thicker epidermal area, suggesting that differentiation was still ongoing. The repaired tissue appeared more organized in the Col-5 group than that of the other group, showing a remarkable collagen deposition with a more homogeneous distribution in the newly formed tissue. The collagen architecture in this group was indistinguishable in the tissue edges adjacent to the uninjured area. Furthermore, the collagen fibres in the control group were arranged parallelly, indicating the early stages of new collagen reorganization. In contrast, the Col-5 treatment group exhibited a reticular arrangement of collagen fibres. This reticular pattern allows for flexibility and elasticity, characteristics of collagen fibre organization that closely resemble normal skin tissue or tissue fully healed from injury.[22]
Based on the observed collagen deposition and organization (Figure 5), applying topical collagen gel may accelerate the remodeling phase. Indeed, faster wound closure was observed in the mice treated with the collagen gel (Figure 2). These results align with a previous study[12] showing that orally administered collagen from mackerel scad promotes wound healing in mice. It is evident that collagen, regardless of the route of administration, is beneficial for wound tissue regeneration.
Marine collagen application for wound recovery has been demonstrated in several studies,[23,24,25] although further research is needed to elucidate its mechanisms of action. Collagen derived from mackerel scad offers several advantages, including easy extraction, abundant availability, well-characterized properties, and proven bioactivity, as shown in previous studies.[12,13,14,26] While this study emphasizes collagen's role in the later phases of wound healing, other studies have shown its involvement in earlier phases, such as the inflammatory and proliferative stages, indicating that collagen plays a complex and multifaceted role in wound healing. An in vivo study using marine-derived collagen incorporated into a hydrogel demonstrated that collagen application reduced inflammatory cell infiltration and promoted neovascularization in the new tissue. Additionally, the porous structure of collagen-based scaffolds is well-regarded for its ability to attract and support cell migration, further enhancing the wound-healing process.[27]

Conclusion

The present study demonstrated that incorporating mackerel scad fish-derived collagen into a topical gel formulation enhances the superficial non-infected wound healing process. The findings showed that the collagen gel promoted faster wound closure, enhanced the formation of new epithelial layers, and improved collagen tissue regeneration compared to the control group. Additionally, the collagen gel increased the expression of proangiogenic factor, VEGF, indicating a positive effect on neovascularization. These results underscore the potential of exogenous marine biomaterials to advance wound care practices and expedite the healing process.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. Pharm. 2020;12(8):735. DOI: 10.3390/pharmaceutics12080735
2. Gaspar-Pintiliescu A, Stanciuc AM, Craciunescu O. Natural composite dressings based on collagen, gelatin and plant bioactive compounds for wound healing: A review. Int J Biol Macromol. 2019;138:854–865. DOI: 10.1016/j.ijbiomac.2019.07.155
3. Mathew-Steiner SS, Roy S, Sen CK. Collagen in wound healing. Bioeng. 2021;8(5):63. DOI: 10.3390/bioengineering8050063
4. Naomi R, Ratanavaraporn J, Fauzi MB. Comprehensive review of hybrid collagen and silk fibroin for cutaneous wound healing. Mater. 2020;13(14):3097. DOI: 10.3390/ma13143097
5. Bao P, Kodra A, Tomic-Canic M, Golinko MS, Ehrlich HP, Brem H. The role of vascular endothelial growth factor in wound healing. J Surg Res. 2009;153(2):347–358. DOI: 10.1016/j.jss.2008.04.023
6. Cascone S, Lamberti G. Hydrogel-based commercial products for biomedical applications: A review. Int J Pharm. 2020;573:118803. DOI: 10.1016/j.ijpharm.2019.118803
7. Abdelrahman T, Newton H. Wound dressings: principles and practice. Surg (Oxford). 2011;29(10):491–495. DOI:https://doi.org/10.1016/j.mpsur.2011.06.007
8. Minor AJ, Coulombe KLK. Engineering a collagen matrix for cell-instructive regenerative angiogenesis. J Biomed Mater Res. 2020;108(6):2407–2416. DOI: 10.1002/jbm.b.34573
9. Kumar M, Banerjee P, Das A, Singh K, Guith T, Kacar S, Gourishetti K, Sen CK, Roy S, Khanna S. Hydrolyzed collagen powder dressing improves wound inflammation, perfusion, and breaking strength of repaired tissue. Adv Wound Care. 2024;13(2):70–82. DOI: 10.1089/wound.2023.0065
10. Lu Y, Zhu X, Hu C, Li P, Zhao M, Lu J, Xia G. A fucoidan-gelatin wound dressing accelerates wound healing by enhancing antibacterial and anti-inflammatory activities. Int J Biol Macromol. 2022;223(Pt A):36–48. DOI: 10.1016/j.ijbiomac.2022.10.255
11. Gaspar-Pintiliescu A, Anton ED, Iosageanu A, Berger D, Matei C, Mitran RA, Negreanu-Pirjol T, Craciunescu O, Moldovan L. Enhanced wound healing activity of undenatured type I collagen isolated from discarded skin of black sea gilthead bream (*Sparus aurata*) conditioned as 3D porous dressing. Chem Biodiver. 2021;18(8):e2100293. DOI: 10.1002/cbdv.202100293
12. Herawati E, Setyawan VA, Listyawati S. Collagen peptides of mackerel scad (*Decapterus macarellus*) fish accelerate wound healing in mice. J Pharm Sci Res. 2023;8(2):278–290. DOI: 10.20961/jpscr.v8i2.74260
13. Herawati E, Titisari RS, Astirin OP. Characterization of collagen hydrolysate gel from mackerel scad skin (*Decapterus macarellus*). JPPIPA. 2023;9(7):5568–5573. DOI: 10.29303/jppipa.v9i7.3983
14. Herawati E, Akhsanitaqwim Y, Agnesia P, Listyawati S, Pangastuti A, Ratriyanto A. In vitro antioxidant and antiaging activities of collagen and its hydrolysate from mackerel scad skin (*Decapterus macarellus*). Mar Drugs. 2022;20(8):516. DOI: 10.3390/md20080516
15. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. (8th ed.). Amsterdam: Elsevier; 2019. 557 p.
16. Cui T, Yu J, Wang C, Chen S, Li Q, Guo K, Qing R, Wang G, Ren J.. Micro-gel ensembles for accelerated healing of chronic wound via pH regulation. Adv Sci. 2022;9(22):2201254. DOI: 10.1002/advs.202201254
17. Masson-Meyers DS, Andrade TAM, Caetano GF, Guimares FR, Leite MN, Leite SN, Frade MAC. Experimental models and methods for cutaneous wound healing assessment. Int J Exp Pathol. 2020;101(1-2):21–37. DOI: 10.1111/iep.12346
18. Shams F, Moravvej H, Hosseinzadeh S, Mostafavi E, Bayat H, Kazemi B, Bandehpour M, Rostami E, Rahimpour A, Moosavian H. Overexpression of VEGF in dermal fibroblast cells accelerates the angiogenesis and wound healing function: in vitro and in vivo

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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

studies. Sci Rep. 2022;12(1):18529. DOI: 10.1038/s41598-022-23304-8

19. Johnson KE, Wilgus TA. Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair. Adv Wound Care. 2014;3(10):647–661. DOI: 10.1089/wound.2013.0517
20. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37(5):1528–1542. DOI: 10.1177/147323000903700531
21. Tobat SR, Yenny SW, Wahyuni FS, Etriyel MYH, Sartika D, Supriwardi E, Azyenella L, Wahyudi R, Annisa A, Akbar PD, Fauziah, F. Wound healing activity of phyllanthin-rich sub-fractions ointment: isolated from Meniran (Phyllanthus niruri L.) leaf in experimental rats using hydroxyproline as biochemical marker. Trop J Nat Prod Res. 2024;8(7):7722–7733. DOI: https://doi.org/10.26538/tjnpr/v8i7.15
22. Verhaegen PD, van Zuijlen PP, Pennings NM, van Marle J, Niessen FB, van der Horst CM, Middelkoop E. Differences in collagen architecture between keloid, hypertrophic scar, normotrophic scar, and normal skin: An objective histopathological analysis. Wound Repair Regen. 2009;17(5):649–656. DOI: 10.1111/j.1524-475X.2009.00533.x
23. Zhang Z, Wang J, Ding Y, Dai X, Li Y. Oral administration of marine collagen peptides from Chum Salmon skin enhances cutaneous wound healing and angiogenesis in rats. J Sci Food Agric. 2011;91(12):2173–2179. DOI: 10.1002/jsfa.4435
24. Felician FF, Yu RH, Li MZ, Li CJ, Chen HQ, Jiang Y, Tang T, Qi WY, Xu HM. The wound healing potential of collagen peptides derived from the jellyfish *Rhopilema esculentum*. Chin J Traumatol. 2019;22(1):12–20. DOI: 10.1016/j.cjtee.2018.10.004
25. Elbialy ZI, Atiba A, Abdelnaby A, Al-Hawary II, Elsheshtawy A, El-Serehy HA, Abdel-Daim MM, Fadl SE, Assar DH. Collagen extract obtained from Nile tilapia (*Oreochromis niloticus* L.) skin accelerates wound healing in rat model via up regulating VEGF, bFGF, and α-SMA genes expression. BMC Vet Res. 2020;16(1):352. DOI: 10.1186/s12917-020-02566-2
26. Titisari RS, Herawati E, Astirin OP. Oral intake of collagen hydrolysate from mackerel scad (*Decapterus macarellus*) attenuates skin photoaging by suppressing the UVB-induced expression of MMP-1 and IL-6. J Complement Integr Med. 2023;21(1):71–79. DOI: 10.1515/jcim-2023-0209
27. McGrath M, Zimkowska K, Genoud KJ, Maughan J, Gutierrez GJ, Browne S, O'Brien FJ. A biomimetic, bilayered antimicrobial collagen-based scaffold for enhanced healing of complex wound conditions. ACS Appl Mater Interfaces. 2023;15(14):17444–17458. DOI: 10.1021/acsami.2c18837

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