Egypt. J. Vet. Sci. **Vol. 56,** No. 8, pp. 1849-1865 (2025)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Extracted Marine Collagen From Nile tilapia (*Oreochromis niloticus* L.) Skin Accelerates Burn Healing: Histopathological, Immunohistochemical and Gene Expression Analysis

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Abstract

HEALTHY SKIN protects our bodies from environmental harm. Burns, however, can lead to infections and delayed healing, often with poor cosmetic results. Therefore, promoting efficient wound healing is crucial to restore normal skin function while minimizing scarring. This study investigated the potential benefits of tilapia skin collagen in accelerating burn repair and explored the underlying molecular mechanisms. Analysis using Fourier-transform infrared spectroscopy (FTIR) confirmed that the extracted tilapia collagen (TC) possessed a triple helical structure characteristic of type I collagen. Histopathological examination revealed that TC treatment promoted collagen deposition, epithelization (skin resurfacing), and complete reconstruction of connective tissue compared to the collagen control group (CG). Masson's trichrome staining further supported these findings. The tilapia collagen group (TCG) displayed dense and organized collagen fibers, indicating a more complete remodeling process compared to CG. Notably, TCG exhibited increased expression of growth factors Basic-fibroblast growth factor (b-FGF) and Vascular Endothelial Growth Factor (VEGF), while showing a decrease in Transforming Growth Factor beta 1 (TGF-β1) and alpha Smooth Muscle Actin (α-SMA), suggesting a potential shift towards wound healing and reduced scarring. Topical application of tilapia collagen (TC) appears to promote burn wound healing through several mechanisms. TC may accelerate the healing process by rapidly reducing wound size and increasing the rate of epithelization (skin resurfacing). It may also stimulate collagen production and deposition, leading to a thicker dermis (skin layer). Additionally, TC may promote new blood vessel formation (neovascularization) in granulation tissue, potentially reducing scar formation. Interestingly, studies suggest that collagen interacts with alpha-SMA, but not TGF-β1, potentially explaining the reduced scarring effect. These findings position tilapia-derived collagen as a promising, safe, and cost-effective alternative to existing wound care products.

Keywords: Tilapia collagen; Burn; Histopathology; Gene expression.

Introduction

Wounds caused by burns are one of the world's most ubiquitous and debilitating medical stresses. Globally, about 300,000 deaths occur yearly due to acute thermal injuries, with the major incidence in both low- and middle-income countries, [1]. Irrespective of the underlying causes of burns, these injuries are complex, challenging to heal, and linked to elevated rates of mortality [2].

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(Received 18/05/2024, accepted 04/07/2024)
DOI: 10.21608/EJVS.2024.290709.2100

Burn injuries occur in all ages and can vary in severity from very minor cases requiring no care to extremely severe cases requiring full intensive care [3]**.** Thick, rigid scars and contractures can lead to about 91% of all burn injuries and nearly 40–70% of surgeries, with debilitating results [4]. More appropriate therapeutic agents for these patients are required during emergency [5]. Despite recent huge progress in wound care products, such as commercial advanced treatments such as stem cells [6], recombinant growth, and cellular tissue-based skin substitution treatments [7], these advanced commercial treatments are considered cost-ineffective for low-income patients. Thus, any new effective burn care agents must be promoted urgently to fulfill the urgent request for low-cost clinically applied burn treatment [8]. Traditional biomaterial therapy, such as collagen, chitosan, and hydrogel mixtures, is thought to be an interesting promising alternative [9]. The most plentiful animal protein is collagen, comprising about 30% of the overall protein [10]. It exists in the skins, bones, sheath of muscles, ligaments, cartilage, and other connective tissues of various animals that contribute to their specific physiological functions [11]. At least 29 collagen forms are known and categorized mainly by composition. About 90% of the body's collagen located in skin, bone, and ligament is type I; meanwhile, types II, III, and IV are considered common collagen. Collagen is formed from three polypeptide α-chains, so they are trimeric molecules characterized by a triple helix-tertiary structure. Moreover, collagen is rich in glycine, proline, and hydroxyproline amino acid residues [12].

Several key characteristics contribute to the widespread use of collagen in many fields. Firstly, collagen exhibits excellent biocompatibility, meaning it integrates well with the body and minimizes rejection [13]. Secondly, its high versatility allows it to be adapted for various applications [14]. Thirdly, collagen has low antigenicity, reducing the risk of allergic reactions [15]. Fourthly, it demonstrates a superior ability to promote cell adhesion, crucial for tissue growth and repair [16]. Finally, collagen boasts a higher degree of biodegradability compared to materials like chitin/chitosan and synthetic polymers [16]. These combined advantages, along with its remarkable ability to influence cell behavior and promote tissue restoration, make collagen a highly valuable tool in healthcare [17]. Also, the biological collagen dressing is bacterial impermeable and provides the optimum physiological interface between the surface of the wound and the environment. Generally, commercial collagen type is traditional and obtained mainly from terrestrial mammals, such as bovine tendon or porcine skin [18]. While animal-derived collagen offers certain benefits, its use is overshadowed by significant safety considerations. The risk of outbreaks from diseases like bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD), and transmissible spongiform encephalopathy (TSE) in animals poses a potential health threat [12]. Furthermore, purifying collagen from animal sources is a complex and expensive process, hindering its widespread use [19]. Also, collagens from bovine and porcine are prohibited in Hinduism and Islamic and Jewish cultures, respectively, due to religious beliefs [20]. So, it is imperative to search for a new alternative source for collagen that compensates for mammalian collagen [21]. Marine and freshwater vertebrates or invertebrates derived from collagen represent an alternative source for collagen obtained from mammals [22]. Marine collagen (MC) is a rich source of structurally innovative and biologically active ingredients. Up to now, the most convenient and secure choice has been to obtain collagen without animal disease risks and pathogens, such as those mentioned above [23]. In addition, Marine collagen (MC) offers a sustainable option as it can be extracted from diverse marine organisms like fish [24], sponges [25], and mollusks [26]. In the case of fish, this is particularly relevant because processing discards a significant amount of material – approximately 30% of the fish consists of skin, bones, and scales [27]. Utilizing this waste stream for collagen production offers a valuable solution for waste management. Therefore, it makes sense to use such by-products as an effective method for protecting the ecological environment and manufacturing valuable products to improve the income from seafood processors and develop products from natural sources that are both low in cost and effective for wound treatment**.**

Nile tilapia is one of the most commonly cultured fish globally, and each year, huge quantities of fish by-products rich in collagen are discarded [28]. [29] and [30] reported that collagen dry weight yields from the skin of tilapia exceed 40%. Nile tilapia skin has been suggested to be an option as a biological material for burn treatments. Tilapia skin has been discovered as a hopeful burn handling since it was first utilized in a clinical test on people in Brazil in 2017 [31]. The skin of tilapia fish represents a promising source of type I collagen, exhibiting a high degree of morphological analogy to human skin collagen [32]. This tilapia-derived collagen possesses high resistance and tensile strength at the point of breakage. Despite ongoing research efforts to develop novel and efficient wound healing interventions, this study aimed to explore the impacts of tilapia skin collagen on burn wound healing and to elucidate its potential modulatory action on the expression patterns of genes associated with the wound healing process. These genes of interest included angiogenic factors, such as b-FGF and VEGF, which play a crucial role in blood vessel

formation, as well as fibrosis-related molecules, such as TGF-β and α-SMA, to investigate the underlying mechanism of action by which tilapia skin collagen may promote wound healing

Material and Methods

Collagen extraction and characterization

Fish skin, obtained from fish averaging 70g ± 5.5g from the Faculty farm, served as the starting material for collagen extraction using the Noitup method , according to Potaros, Raksakulthai [33]. The skin was first separated and cut into small pieces. A treatment with sodium hydroxide (NaOH) removed non-collagenous proteins, followed by extensive washing to achieve a neutral pH. Acid-solubilized collagen was then prepared by exposing the washed skin to acetic acid at a low temperature for 24 hours. Centrifugation separated the collagen-containing supernatant from the residual material. To ensure complete collection, the remaining solids were re-extracted twice. The isolated collagen was further purified using salting out with sodium chloride (NaCl) and subsequent centrifugation. Finally, the purified collagen underwent washing and lyophilization for analysis by Fourier Transform Infrared Spectroscopy (FTIR) (Thermo Scientific Nicolet iS5, MA, USA) [34].

Measurement of the extracted collagen spectrum

To analyze the chemical composition of the lyophilized collagen powder, Fourier-transform infrared spectroscopy (FTIR) was employed. The powder was mixed with potassium bromide (KBr) obtained from Sigma-Aldrich (St. Louis, MO, USA) and then compressed into a pellet for analysis using a standard FTIR instrument [35]**.** The instrument settings were set to a resolution of 4 cm^{-1} and 64 scans, recording the transmission spectrum within a range of 450 to 4000 cm^{-1} (Fig. 1) [34].

Experimental animals

The experiment was conducted in the laboratory animal unit using fourteen male Wistar rats (10 weeks old) and Weight (200 ± 10 g) obtained from a private animal colony (Egypt). All rats received a basal diet (Al Wadi, Egypt) and water ad libitum, housed individually in cages to prevent fighting and wound contamination. The environment was maintained at 25 ± 2˚C with a 12-hour light/dark cycle. Animals were randomly assigned to two groups (7 rats each): a control group (CG) receiving no treatment and a tilapia collagen-treated group (TCG) administered an extract of tilapia skin collagen. Prior to the study, all animals were acclimatized for one week. Fig. 2 illustrates the experimental design, including the timeline for burn area measurement, tilapia collagen administration, and sample collection.

Establishment of burn injuries

Following a one-week acclimatization period, the rats were anesthetized using an intraperitoneal (IP) injection of a ketamine-xylazine combination (70mg/kg ketamine and 7mg/kg xylazine). Prior to surgery, the hair on their backs was shaved and disinfected with 70% ethanol. To create the burn injuries, a 2 x 2 cm metal bar preheated to 90°C in a water bath was placed on the shaved area of each rat's back for 10 seconds using its own weight (illustrated in Fig. 1). The burn area of each rat was photographed and measured using National Institutes of Health (NIH) ImageJ software (http://rsb.info.nih.gov/ij) on days 0, 3, 6, 9, 12, 15, 18, and 21 post-burn (Fig. 3) until complete skin resurfacing (epithelialization) was observed. Figure 3 depicts the change in wound size throughout the experiment, expressed as a percentage of the initial wound size on day 0.

Treatment of the burn wound with Tilapia Collagen (TC)

To ensure a fair comparison, the control group (CG) received no treatment but underwent regular bandage changes to maintain wound moisture and prevent any influence on wound area measurements (Fig. 3). The tilapia collagen-treated group (TCG) received a topical collagen gel formulation applied directly to the burn site once daily for eight days, starting on day 0 post-burn (days 0, 3, 6, 9, 12, 15, 18, and 21). Following each collagen application, a fresh medical dressing was applied. The wound area in the TCG group was measured at each dressing change to monitor healing progress. Both groups were monitored for wound healing progress, including visual assessment until complete epithelialization (resurfacing) of the wound occurred.

Collection of tissue samples

Upon reaching the experimental endpoint (day 21 post-burn), all rats were euthanized using decapitation following anesthesia with an intraperitoneal injection of pentobarbital (500 mg/kg). The entire burn area, including a 5 mm margin of healthy surrounding skin, was excised for further analysis. These tissue samples were then divided in half. One half was fixed in 10% buffered formalin (pH 7.4) for 48 hours, then embedded in paraffin for subsequent histopathological and immunohistochemical (IHC) staining. The remaining half of the tissue was used for RNA extraction.

Histopathological examination

We processed skin burn tissues for microscopic examination using established histological techniques. The tissues were first fixed in a 10%

buffered formalin solution (pH 7.4) for 48 hours to preserve their structure. After fixation, the tissues were embedded in paraffin wax to allow for sectioning. The obtained sections were then stained with hematoxylin and eosin (H&E) for general morphology and Masson's trichrome stain to specifically identify fibrous connective tissues [36].

Immunohistochemical analysis of VEGF

Vascular Endothelial Growth Factor (VEGF) expression in tissue samples was evaluated using immunohistochemistry (IHC) on 4-micron thick paraffin sections, according to [37]. The established protocol involved deparaffinization, rehydration, and antigen retrieval steps for optimal antibody binding. To minimize background staining, nonspecific protein binding was blocked before incubating the sections with a primary rabbit polyclonal antibody targeting VEGF (dilution: 1:100) overnight at 4°C. Following washes, a secondary goat anti-rabbit antibody linked to horseradish peroxidase was applied. After additional washing, the sections were visualized using a DAB chromogen and counterstained with hematoxylin for light microscopy. To quantify VEGF expression, the percentage of positively stained cells was calculated by analyzing eight high-power fields (×400 magnification) per section.

RNA extraction

Following collection, tissue samples were placed in sterile 2 ml Eppendorf tubes and immediately subjected to a flash-freezing process in liquid nitrogen. The samples were then stored at -80°C until required for RNA extraction. Total RNA was extracted from all samples using a commercially available TriZol reagent kit (iNtRON Biotechnology) following the established protocol provided by the manufacturer. The quality and concentration of the extracted RNA were evaluated using a NanoDrop spectrophotometer (BioDrop) by measuring the absorbance at 260 nm and 280 nm. Extracted RNA samples with a 260/280 nm absorbance ratio of 1.59 and a 280 nm absorbance greater than 1.8 were considered acceptable for downstream experiments. In addition, RNA integrity was verified using agarose gel electrophoresis. The RNA samples were loaded onto a 1.5% agarose gel (Sigma, Germany) stained with ethidium bromide and run in Tris-acetate-EDTA buffer (pH 8.0). The RNA integrity was visualized using a UV transilluminator (azure c200).

cDNA synthesis and rt-qPCR of target genes

Gene expression analysis was performed using real-time quantitative polymerase chain reaction (RT-qPCR). First, complementary DNA (cDNA) was synthesized from 2 µg of total RNA extracted from each sample using an Intron-Power cDNA synthesis kit (iNtRON Biotechnology) following the manufacturer's instructions. This cDNA then served as the template for the RT-qPCR analysis. Rat-specific primers for genes of interest (*VEGF*, *bFGF*, *TGF-β1*, and *αSMA*) and an internal reference gene (GAPDH) were utilized (Table 1) [38]. The RT-qPCR analysis was performed in triplicate for all samples using SYBR green dye on an Mx3005P Real-Time PCR system (Agilent, USA). The data acquisition software (MxPro QPCR Software) calculated the cycle threshold (Ct) values, and the ΔΔCt method was employed to determine the relative expression levels of the target genes compared to the reference gene [39].

Molecular Docking Assessment

The three-dimensional structures of tilapia collagen and rats' α-SMA and TGF-β1 were retrieved from UniProt (https://www.uniprot.org/) database. Molecular docking was done using ClusPro (https://cluspro.org/help.php) server, where tilapia collagen was used as a ligand to rats' α-SMA and TGF-β1. After the docking process, BIOVIA Discovery Studio was used for visualization and interaction recognition between target proteins and ligand.

Statistical analysis

To assess statistical significance between treatment groups, multiple unpaired two-tailed t-tests were employed, incorporating the Holm-Sidak method for correction with multiple comparisons. This analysis was performed using GraphPad Prism software (version 8.00, GraphPad Software, San Diego, CA, USA). For the relative gene expressions of *VEGF, b-FGF, TGF-β1*, and *α-SMA*, a separate unpaired Welch's t-test was used. All data are presented as mean ± SEM. A p-value less than 0.05 was considered statistically significant and indicated with an asterisk (*).

Results

FTIR characterization of the extracted tilapia skin collagen

The FTIR analysis of tilapia skin collagen (Fig. 1) revealed several key peaks. Peaks at 3328 cm^{-1} and 2924 cm^{-1} corresponded to amide A and B bands, characteristic of collagen [40]. We observed an additional peak at 1451 cm^{-1} attributed to C–H stretching vibrations. Furthermore, the spectrum displayed characteristic peaks for amide I, II, and III bands of polypeptides at 1655 cm^{-1}, 1541 cm^{-1}, and 1237 cm^{-1}, respectively, indicating a random coil structure, a common conformation in type I collagen [41]. The presence of the amide A band suggested that the peptide N–H groups participate in hydrogen

bonding. These findings collectively suggest that we successfully extracted type I collagen with its characteristic triple helical structure from tilapia skin.

TC application regarding burn healing

Tilapia collagen (TC) demonstrated promising effects in promoting burn wound repair when applied topically. As shown in (Fig. 3)**,** rats treated with TC displayed a significantly higher percentage of wound closure compared to the untreated control group at all observation time points (days 0, 3, 6, 9, 12, 15, 18, and 21 post-burn). Wound healing was evaluated by measuring the extent of regenerated skin (epidermis) covering the wound area in both groups. Importantly, wounds treated with TC healed considerably faster and showed a visually improved appearance compared to the control group (Fig. 3)**.**

Histopathological examination

Microscopic analysis of control rat skin at day 21 post-burn (Fig. 4) showed delayed healing. This was evident by the presence of a marginal epithelial tongue, indicating incomplete resurfacing of the wounded area. However, in the TC-treated rats, there was marked epithelization with complete connective tissue remodeling. Our findings suggest that tilapia collagen (TC) promotes burn healing by stimulating both blood vessel formation (angiogenesis) and collagen deposition. This is evident in the tilapia collagen-treated group (TCG), which exhibited exceptional skin resurfacing (epithelization) and complete reconstruction of connective tissue. To assess the total collagen deposition within the burn tissues of the rats, Masson's trichrome staining was performed (Figure 5). These results revealed that skin sections of the CG showed immature collagen within the granulation tissue, with a low amount of collagen. The TCG showed marked dense and ordered collagen staining, indicating the rearrangement of the new collagen during the wound healing process with complete remodeling of tissue. This indicates a significant enhancement in burn healing following topical application of TC.

Tilapia collagen treatment significantly enhanced collagen fiber density within the dermis, as shown in Table 2. The percentage of positive area, a measure of collagen fiber density, was markedly higher in the tilapia collagen-treated group (TCG; 75.58 ± 13.53) compared to control rats (36.62 ± 4.58). These findings suggest a potential benefit of tilapia collagen in promoting collagen deposition.

Immunohistochemistry of burn injury

Immunohistochemical analysis revealed a significant increase in *VEGF* expression within the tilapia collagen-treated group (TC) compared to the untreated control group. In the TCG, strong *VEGF* staining was localized to the cytoplasm of endothelial cells in blood capillaries and fibroblastic cells within the mature granulation tissue (62.6%, Fig. 6). The control group exhibited mildly positive cytoplasmic VEGF staining (22.58%, Table 2).

Real-time qPCR of target genes

Application of tilapia collagen (TC) on a topical level showed a significant up-regulation of *VEGF* and *FGF* genes in the TC-treated group compared to the control one (Figure. 7) Genes in this group are classical markers for the process of wound repair. On the contrary, a reduced expression of *TGF-β1* and *α-SMA*, which are implicated with scar formation, was seen in the TC-treated group as compared to the normal untreated group.

Molecular docking assessment

The molecular docking interaction of tilapia collagen against rats' α-SMA is represented in Table 3 and Fig. 8. Tilapia collagen interacted with the binding site of rats' α-SMA by 28 hydrogen bonds, 15 pi interactions, and two salt bridges. Otherwise, tilapia collagen did not interact with the binding site of rats' TGF-β1 at all.

Discussion

Skin is the first barrier to protection against environmental risks [42], such as microbial infection, chemical, thermal, and mechanical factors. One of the most important causes of skin damage is burn wound [43]. Burn wounds result in different pathophysiological processes in the body, either local or systemic. Like all sorts of wounds, burn wounds initiate the ongoing process of inflammation, which is accompanied by various cytokines release [44]. Wound healing is a complex process orchestrated by a variety of growth factors. Key players include fibroblast growth factor (FGF), transforming growth factor alpha (TGF-α) and beta (TGF-β), and vascular endothelial growth factor (VEGF) [45]**.** These growth factors are produced by various cell types within the wound microenvironment, including endothelial cells, fibroblasts, and inflammatory cells. Research suggests that growth factors and cytokines may offer a promising therapeutic approach for accelerating wound closure in burn injuries [46]. However, in spite of the huge numbers of research concerned with handling choices for shallow and incomplete thickness burns, the ideal treatment protocol has not yet been recognized [47]. Effective treatment of burn injuries remains a critical challenge in clinical practice. Developing new therapeutic options is crucial to restore patients' skin function and integrity. So, research efforts are needed to determine a way to limit complications in healing processes such as scarring and infection [48].

In this study, FTIR was used for determining proteins and polypeptide's secondary structure regarding molecular chemical structures [49]. In this trial, we characterized marine tilapia skin collagen using FTIR spectrum analysis (Fig. 1); the obtained figure revealed that the extracted collagen was

collagen type I, which is characterized by its triple-helical configuration. This paralleled [12] findings, which revealed that collagen is a trimeric molecule comprising three polypeptide α-chains, characterized by a triple helix-tertiary structure, and rich in the residue of glycine, proline, and hydroxyproline amino acids. According to [49], at a range of 3292–3315 cm^{-1} bands, amide A appeared which indicates that the peptides N–H groups are included in the bonding of hydrogen. Meanwhile, amides I, II, and III bands appeared at 1656, 1538–1548, and 1232–1238 cm^{-1}, respectively. Moreover**,** [50] analyzed the collagen of tilapia skin using the FTIR spectra. In particular, FTIR reveals that collagen type I can be isolated and has a triple-helical structure [51].

Throughout the course of this study, we demonstrated the effect of the obtained collagen on the process of burn healing relying on histopathological, IHC assessment alongside the pattern of gene expression of several wound healing-associated genes, such as *b-FGF* and *VEGF*, as well as fibrosis-related molecules, such as *TGF- β1* and *α-SMA* in a rat model. Our results affirmed a rapid diminish in the measured wound size ascertained by the wound area percentage. The average size differed between the untreated CG and TCG groups from day 6 and continued until day 21 post-burning. [52] found that TC nano-fibers improve the speed of skin wound healing in rats. This may be a result of promoting cell adhesion, proliferation, and differentiation**.** Similarly, [53] detected that tilapia skin enhances the wound healing process. Likewise, [54] provided promising results by applying marine collagen peptides (MCPs) from tilapia skin to deep partial-thickness scalds in rabbits. In the existing study, histopathological findings revealed a noticeable reduction in the dermal tissue thickness with an increasing rate of epithelization, which explained that the rapid and healthy wound closure in TCG outperformed those in the CG. Also, TC can accelerate degree of burn healing with elevating collagen production and deposition, as revealed by the elevated percentage of positive area within the dermis stained with Masson's trichrome. The TCG expressed higher level of *VEGF* than in the control group within the epithelial covering and the fibroblasts underlining the epidermis. These findings strongly suggest that TC has ability to boost wound healing via enhancing the deposition of collagen. [55] detected an accelerated phase of epithelization and identified the promotion and differentiation of keratinocytes after treatment with TC. [56] performed a study on 75 diabetic foot ulcer patients using a dressing of alginate containing collagen (90%) and achieved complete healing, although the patient treated with gauze moistened with normal saline revealed only 36%. Additionally, [57] recorded that Chum Salmon skin collagen improves the healing of cutaneous wounds and angiogenesis when administered orally in rats**.** Furthermore, [52] proved that electrospun TC nanofibers could rapidly and effectively accelerate skin wounds healing in rat model.

The phase of proliferation includes epithelialization and angiogenesis, where the epidermal growth factor (EGF) and the transforming growth factors (*TGF*) are essential factors to stimulate fibroblast and keratinocyte proliferation, migration, as well as differentiation [58]. Angiogenesis process ensures that nutrients are supplied to the newly generated granulation tissue [59]. The formation of new blood vessels, known as angiogenesis, within the temporary wound healing matrix (provisional matrix) is stimulated by various factors. Two key factors include basic fibroblast growth factors (*b-FGF*) [60] and vascular endothelial growth factor (*VEGF*) [61]**.**

In this study, TC topical application strikingly enhanced the expression levels of *VEGF* and *b-FGF* genes compared to the control group, indicating that TC is able to promote neovascularization and increase fibroblasts in granulation tissue. Paralleling our findings, [55] findings revealed that TC markedly enhances the expression of *EGF* and *FGF*, promoting fibroblast and keratinocyte proliferation and differentiation. Similarly, [62] revealed that collagen plays a vital role in extracellular matrix (ECM) formation and in cells and tissue development/migration.

Furthermore, after the end of the proliferation phase, the remodeling phase starts as the wound is re-epithelized. Also, during this phase, the skin/scar acquires the last morphological appearance that mostly depends on the collagen fibers final organization [58]. Regarding abnormal pathologic skin scarring, hypertrophic scarring appears after burn injury, which negatively impacts the quality of life and impairs function [63]. Fibroblasts are considered the principle cell type in the dermis that are critical for skin remodeling and wound healing [64]. Fibroblast secretes different growth factors (*TGF-β*), cytokines (*TNF-α*), along with matrix metalloproteinases (MMPs), and generates ECM (collagen, elastin, and proteoglycans). The high levels of the profibrotic growth factor *TGF-β1* generated by inflammatory cells activate the fibroblasts in the wound region during normal healing. After activation, such fibroblasts become myofibroblasts, and extremely contractile at the end of the granulation stage [65]. In pathologic dermal scarring, the main effector cells are myofibroblasts, which can produce ECM, reduce its turnover, and supply the contractile forces inside the matrix to provide scar contractures [66]. *TGF-β1* induces quieting fibroblasts to express *α-SMA*, an essential material basis for scar contraction, and a signature protein for myofibroblasts [67]**.** Also, myofibroblasts often create much more collagen than their normal counterparts [58] and reduce the metalloproteinase

matrix (MMP), normally leading to ECM turnover [68]. After resolving the healing, these myofibroblasts either experience apoptosis or become senescence because of the loss of their profibrotic characteristics [69]. However, the conditions for either de-activation or de-differentiation of myofibroblast remain unclear, and the exact mechanisms remain unestablished [69]. In addition, *TGF-β* plays a vital role in the remodeling process of the collagen matrix and inhibiting the production of MMP to control such a convenient healing environment [70]**.** However, in the abnormal healing process, myofibroblasts remain in the wound area and sustain the production of more ECM, inhibit the turnover of the ECM, and contribute to scar contractures [71].

We investigated the potential role of tilapia collagen (TC) in promoting burn healing. Our study suggests that TC may influence the TGF-β1 signaling pathway. Animals treated topically with TC (tilapia collagen group, TCG) displayed a marked decrease in the expression of both TGF-β1 and α-SMA compared to the control group (CG). These results suggest that TC may interact with the TGF-β1 pathway, potentially leading to reduced scar formation. Our findings of the suppressive effect of TC application on the expression levels of *α-SMA* are greatly supported by a recent study that greatly considered how to advance apoptosis of myofibroblast or inhibit fibroblasts transformation process into myofibroblasts [72]**.** The antifibrotic drug pirfenidone can be used to avoid the translation of fibroblasts into myofibroblasts [67]. Pirfenidone eliminates hypertrophic scarring after burn, bites of the animal and diabetic ulcer wounds [73]. This occurred via the inhibition of differentiation of fibroblast cells into myofibroblasts and the reduction of *αSMA* expression while enhancing MMP-1 mRNA expression [72]**.**

Conclusions

Applying tilapia collagen (TC) topically offers significant advantages for burn wound healing. TC demonstrably reduces wound size and accelerates healing, with a noticeable thinning of the dermal layer and a faster rate of skin resurfacing. Furthermore, TC stimulates collagen production and deposition within the wound. Importantly, the marked upregulation of genes for growth factors *bFGF* and *VEGF* suggests that TC promotes the formation of new blood vessels in granulation tissue, contributing to a more robust healing environment. However, the observed downregulation of *TGF-β1* as well as *α-SMA* expressions was attributed to the TC inhibitory effect on scar formation. Therefore, tilapia skin-derived collagen can be provided as a new promising safe alternative cost-effective option compared to other commercial wound care products.

Availability of data and materials

All the data generated or analysed during this study are included in this article.

Acknowledgement

The authors wish to acknowledge the support of The Biotechnology Lab., Faculty of Aquatic and Fisheries Science, Kafrelsheikh University, Egypt, where the laboratory investigations were carried out.

Funding statement

This work did not receive any external fund.

Conflict of interest

The authors declare no conflict of interest

List of abbreviations

TC: tilapia collagen

MC: marine collagen

FTIR: Fourier transform infrared spectroscopy

IHC: immune-histochemichal staining

TABLE 1. Primer sequence of selected genes used in RT-PCR analysis.

Gene	Primer sequence 5'-3'	Gene bank accession number	Annealing temperature	Reference
GAPDH	5'-CAGCAATGCATCCTGCAC-3' 5'-GAGTTGCTGTTGAAGTCACAGG-3'	XM_017592435.1	60ºC	[74]
VEGF	5'-AGGCTGCACCCACGACAGAA-3' 5'-CTTTGGTCTGCATTCACATC-3'	NM_001110333.2 NM_019305.2	55ºC	[74]
bFGF	5'-GTCAAACTACAGCTCCAAGC-3' 5'-TTTATACTGCCCAGTTCGTT-3'	NM_031004.2	50 ºC	[74]
αSMA	5’-CGATAGAACACGGCATCATC-3’ 5’-CATCAGGCAGTTCGTAGCTC-3’	X52498.1	58 ºC	[74]
TGF1β	5'-CCAGATCCTGTCCAAACTAA-3' 5'-TTTTGTCATAGATTGCGTTG-3'		53 ºC	[74]

Vascular endothelial growth factor: (*VEGF);* Basic fibroblast growth factor *:(bFGF);* transforming growth factors beta: (*TGF-β*) and α-smooth muscle actine: *(α-SMA).*

TABLE 2. Density of collagen fibers in control and Tilapia collagen treated groups

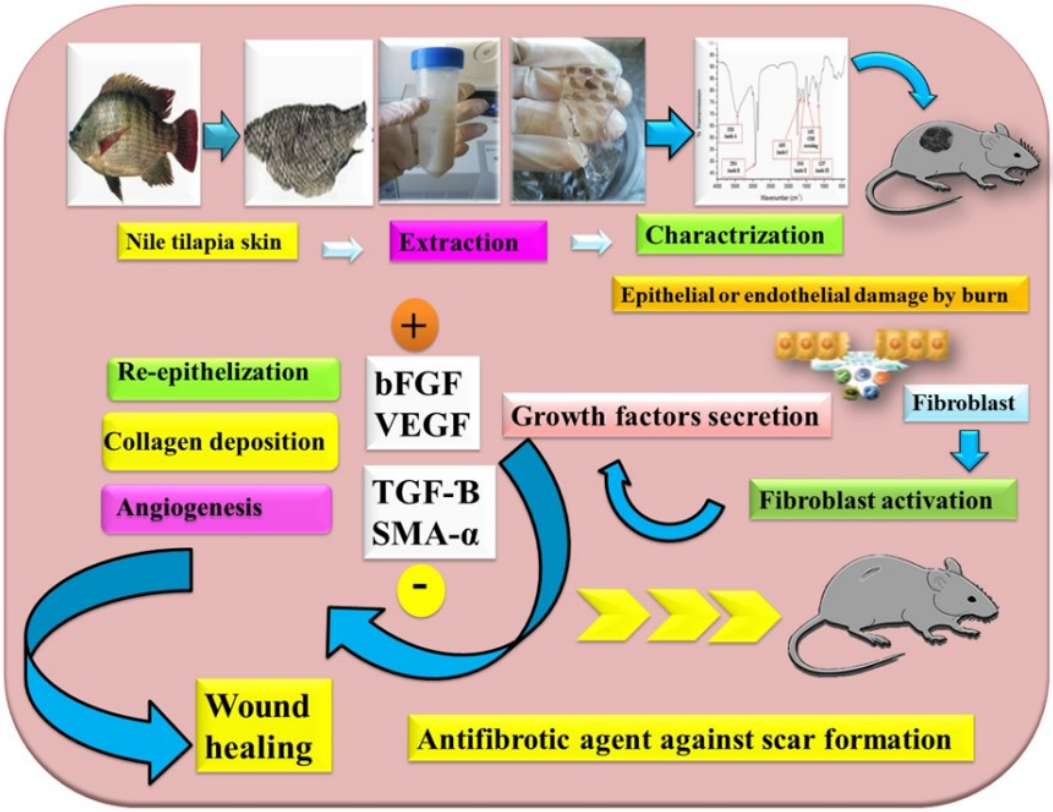
	Control	Tilapia Collagen
(A) Positive area of MTS	36.62± 4.58	75.58± 13.53
(B) Positive area of VEGF	22.58± 2.80	62.6± 5.08

Density of collagen fibers indicated by (A) percent of positive area of Masson Trichrome Staining (MTS) within the dermis. (B) The percent of positive area of Vascular endothelial growth factor (*VEGF*) immunostaining within the dermis using ImageJ analysis software (mean±SE). N=5

TABLE 3. Molecular docking interaction of tilapia collagen against rats' α-SMA

α-SMA residue	Collagen residue	Interaction Constituents	Distance	Type
Hydrogen bonds				
A:HIS75	A:GLN152	A:HIS75:HD1 - A:GLN152:O	1.8648	Conventional
A:HIS75	A:GLY153	A:HIS75:HD1 - A:GLY153:O	2.0113	Conventional
A:LYS115	A:ASP158	A:LYS115:HZ3 - A:ASP158:O	2.094	Conventional
A:TYR135	A:ASP162	A:TYR135:HH - A:ASP162:OD1	1.9629	Conventional
A:ARG149	A:LEU169	A:ARG149:HH22 - A:LEU169:O	1.8697	Conventional
A:THR150	A:SER167	A:THR150:HG1 - A:SER167:OG	1.8454	Conventional
A:ARG185	A:GLU151	A:ARG185:HH12 - A:GLU151:O	1.7647	Conventional
A:ARG198	A:ASN769	A:ARG198:HE - A:ASN769:OD1	2.3034	Conventional
A:ARG198	A:LEU770	A:ARG198:HE - A:LEU770:O	2.7945	Conventional
A:ARG198	A:LEU770	A:ARG198:HH22 - A:LEU770:O	1.811	Conventional
A:TYR200	A:LEU770	A:TYR200:HH - A:LEU770:O	2.0296	Conventional
A:THR205	A:SER147	A:THR205:H - A:SER147:O	1.9826	Conventional
A:THR205	A:SER147	A:THR205:HG1 - A:SER147:O	1.8972	Conventional
A:VAL249	A:ALA773	A:VAL249:H - A:ALA773:O	2.878	Conventional
A:ARG256	A:GLY212	A:ARG256:HH21 - A:GLY212:O	1.8611	Conventional
A:LYS338	A:GLN208	A:LYS338:HZ3 - A:GLN208:OE1	1.6314	Conventional
A:TYR145	A:SER164	A:SER164:H - A:TYR145:OH	1.9667	Conventional
A:THR353	A:HIS165	A:HIS165:HD1 - A:THR353:OG1	1.9301	Conventional
A:ALA297	A:ARG170	A:ARG170:HH12 - A:ALA297:O	2.9007	Conventional
A:ASN298	A:ARG170	A:ARG170:HH12 - A:ASN298:OD1	1.7551	Conventional
A:ALA297	A:ARG170	A:ARG170:HH21 - A:ALA297:O	1.6912	Conventional
A:LYS217	A:ARG211	A:ARG211:H - A:LYS217:O	2.5399	Conventional
A:CYS219	A:ARG211	A:ARG211:HE - A:CYS219:O	2.0072	Conventional
A:LYS215	A:ARG211	A:ARG211:HH21 - A:LYS215:O	2.0151	Conventional
A:ARG198	A:ASN769	A:ASN769:HD22 - A:ARG198:O	1.9173	Conventional
A:VAL249	A:ALA773	A:ALA773:H - A:VAL249:O	1.9044	Conventional
A:GLY148	A:MET168	A:GLY148:CA - A:MET168:O	3.1027	Carbon
A:ARG185	A:GLU151	A:ARG185:CD - A:GLU151:O	3.4386	Carbon
Pi interactions				
A:PHE377	A:ARG161	A:ARG161:NH2 - A:PHE377	3.2615	Pi-Cation
A:TYR308	A:ARG211	A:ARG211:NH2 - A:TYR308	3.4197	Pi-Cation
A:TYR171	A:ASP162	A:ASP162:H - A:TYR171	3.0859	Pi-Donor
A:LEU112	A:PHE156	A:LEU112:CD1 - A:PHE156	3.882	Pi-Sigma
A:ILE347	A:PHE166	A:ILE347:CG2 - A:PHE166	3.9561	Pi-Sigma
A:GLU197	A:PHE768	A:GLU197:C,O;ARG198:N - A:PHE768	4.4496	Pi-Amide Stacked

α-SMA residue	Collagen residue	Interaction Constituents	Distance	Type
A:TYR145	A:SER164	A:SER164:C,O;HIS165:N - A:TYR145	4.3751	Pi-Amide Stacked
A:TYR171	A:ARG161	A:TYR171 - A:ARG161	3.9665	Pi-Alkyl
A:TYR171	A:PRO163	A:TYR171 - A:PRO163	4.8447	Pi-Alkyl
A:TYR220	A:PRO214	A:TYR220 - A:PRO214	4.8224	Pi-Alkyl
A:PHE377	A:ARG161	A:PHE377 - A:ARG161	5.4766	Pi-Alkyl
A:MET192	A:HIS149	A:HIS149 - A:MET192	5.1178	Pi-Alkyl
A:LEU351	A:HIS165	A:HIS165 - A:LEU351	4.9891	Pi-Alkyl
A:LEU238	A:PHE213	A:PHE213 - A:LEU238	5.4402	Pi-Alkyl
A:ARG198	A:PHE768	A:PHE768 - A:ARG198	4.7415	Pi-Alkyl
Salt bridges				
A:LYS70	A:GLU151	A:LYS70:HZ3 - A:GLU151:OE2	1.7954	Salt bridge
A:LYS115	A:ASP159	A:LYS115:HZ2 - A:ASP159:OD2	1.7271	Salt bridge



Graphical abstract

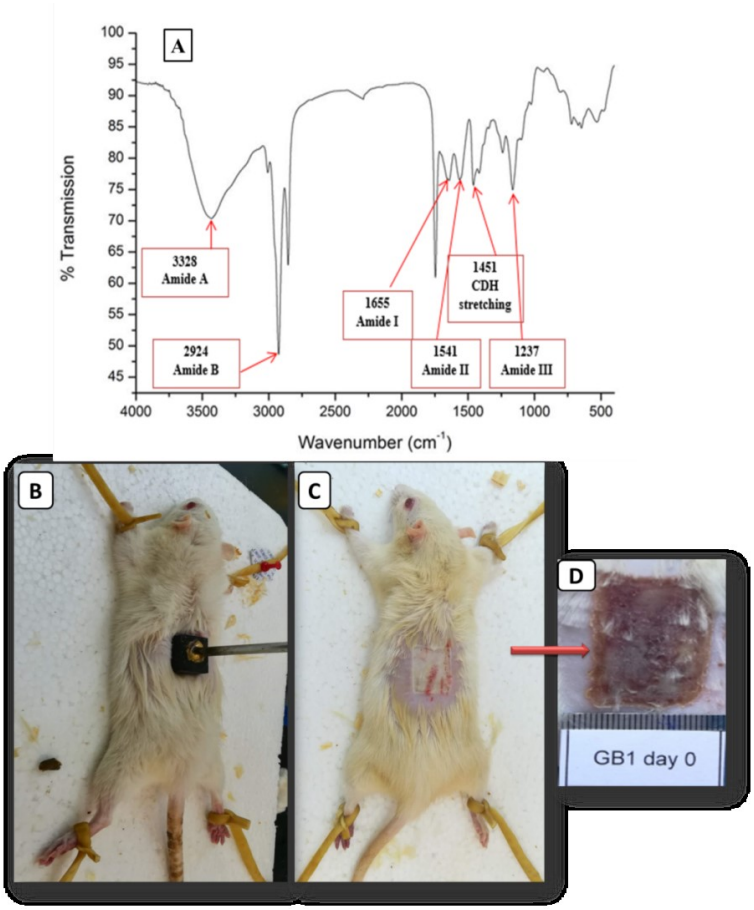


Fig. 1. (A) Characterization of collagens from tilapia skin using FTIR spectrum analysis [41]. (B) Preparation of rat skin by back hair clipping and disinfection by ethyl-alcohol. (C) Marking definite burn area. (D) Photos represent day of burn injury (GB1= group B animal number1).

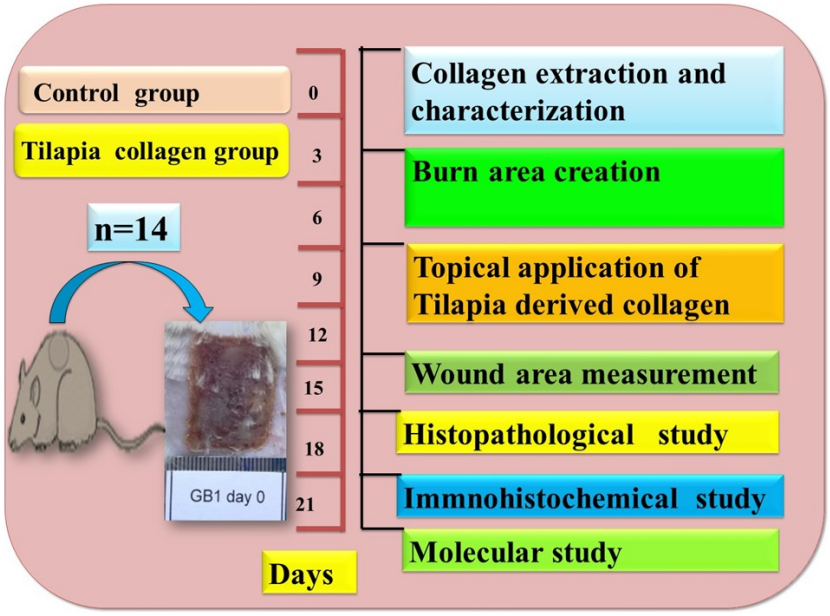


Fig. 2. Experimental scheme, schedule time of burn area measurement, TC extract administration and sampling.

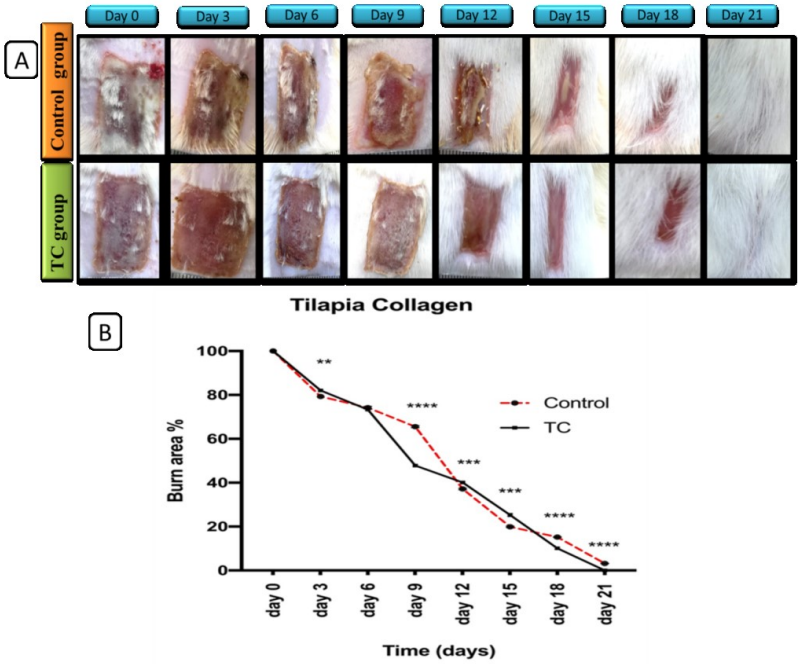


Fig. 3. (A) Effect of topical application of TC extract on burn area contraction in TCG: Tilapia collagen group compared to the CG: Control group. (B) The percentage of wound area observed on days 0,3,6,9,12,15,18 and 21 post wounding as compared with burn wound closure in CG. The wound closure rate was expressed as the percentile of wound area compared with that on post wounding day 0 (100%). Values are represented as mean ±SE.

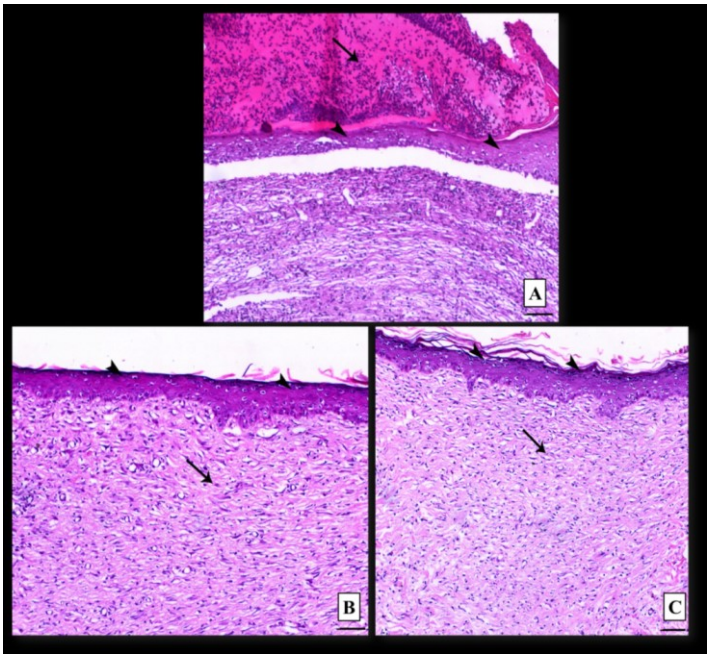


Fig. 4. Skin of control rat group (A) showing epithelial tongue (arrowheads) under the protective (arrow)(control group images are adopted from Elbialy et al. [63]), (B) Skin of Tilapia collagen treated animal showing marked epithelization (arrowheads) and complete remodeling of connective tissue (arrow),(C) Skin of Tilapia collagen treated animal showing marked epithelization (arrowheads) and complete remodeling of connective tissue (arrow), H&E,X100, bar= 200 µm.

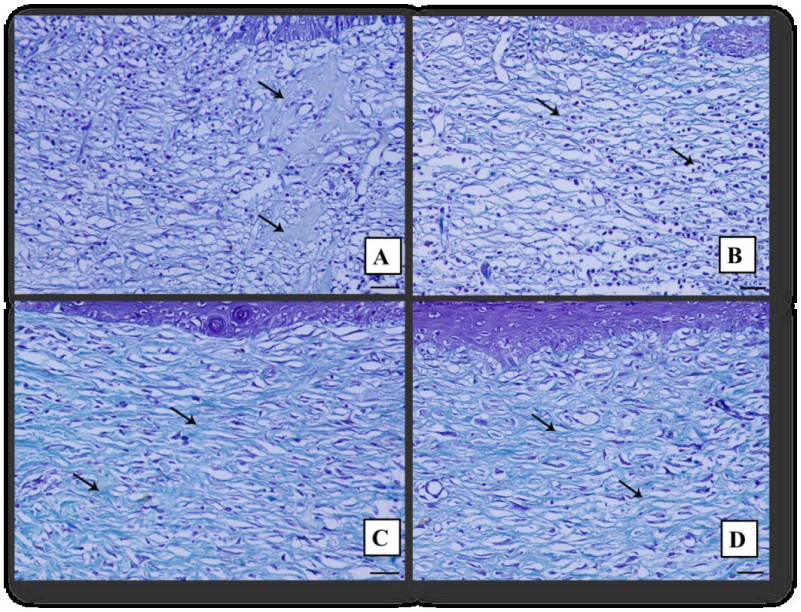


Fig. 5. Masson's Trichrome stain (A) Skin section of control rat group showing immature collagen within the granulation tissues (arrows). (B) Skin section of control rat group showing low amount of the collagen within the granulation tissues (arrows) (control group images are adopted from Elbialy et al. [63]). (C) Skin section of rat group with topical tilapia derived collagen application showing marked collagen staining with complete remodeling of collagen tissue (arrows). (D) Skin section of rat group with topical tilapia derived collagen application showing remodeling of collagen tissue (arrows) X200, bar= 50 µm.

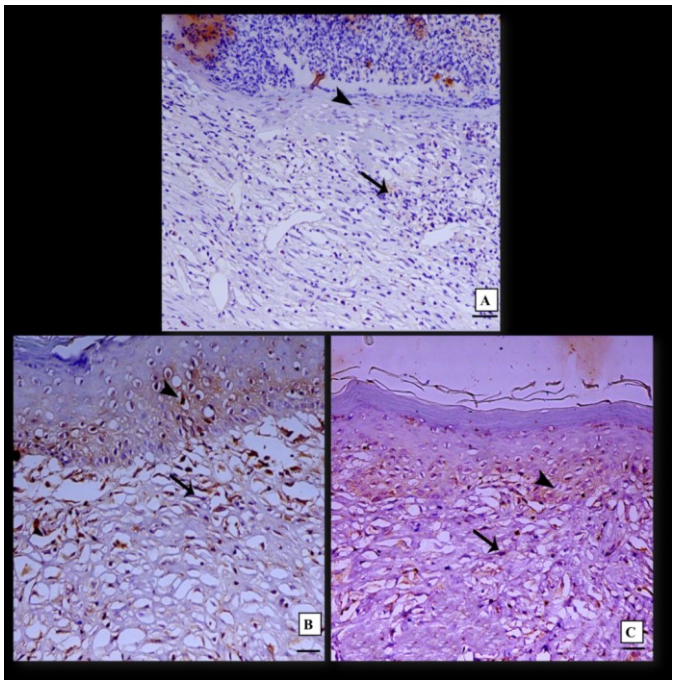


Fig. 6. Immunostaining of skin of group control rat group (A) showing marked decrease of Vascular endothelial growth factor*: VEGF* in both the epithelial covering (arrowhead) and fibroblasts of the granulation tissues (arrow) (control group images are adopted from Elbialy et al. [63]). (B) Skin of rat group with topical tilapia derived collagen application showing marked expression of *VEGF* within the epithelial layer (arrowhead) and fibroblasts underlining the epidermis (arrow), (C) Skin of rat group with tilapia derived collagen topical application showing marked *VEGF* expression within the epithelial covering (arrowhead) and fibroblasts underlining the epidermis (arrow), X200, bar= 50 µm.

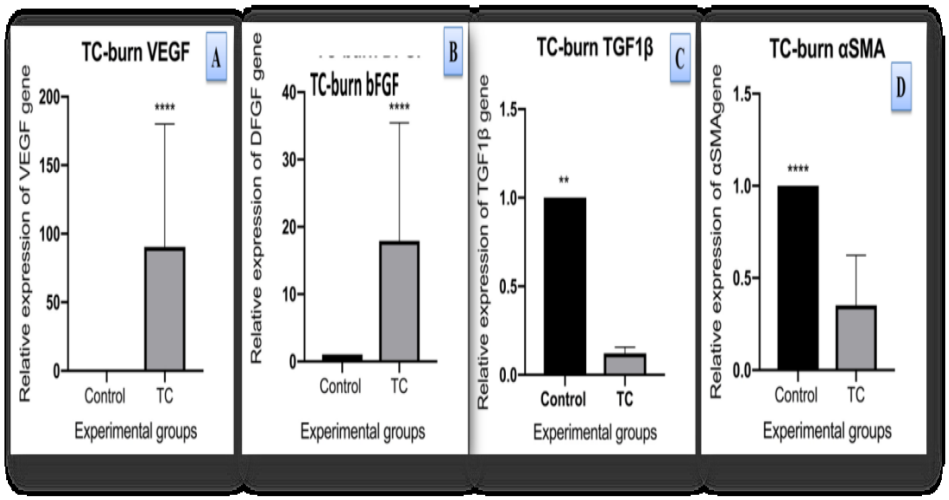


Fig.7. Relative gene expression of (A) *VEGF:* Vascular endothelial growth factor, (B) *bFGF:* basic fibroblast growth factor, (C) *TGF-B1*: Transforming growth factor and (D) *α-SMA*: Alpha smooth muscle actin genes in wounded rats of both CG: control group and TCG: tilapia collagen-treated group. All values are expressed as mean±SE (*n*=5). Bars with an asterisk are considered significantly different from those of CG ($P < 0.05$).

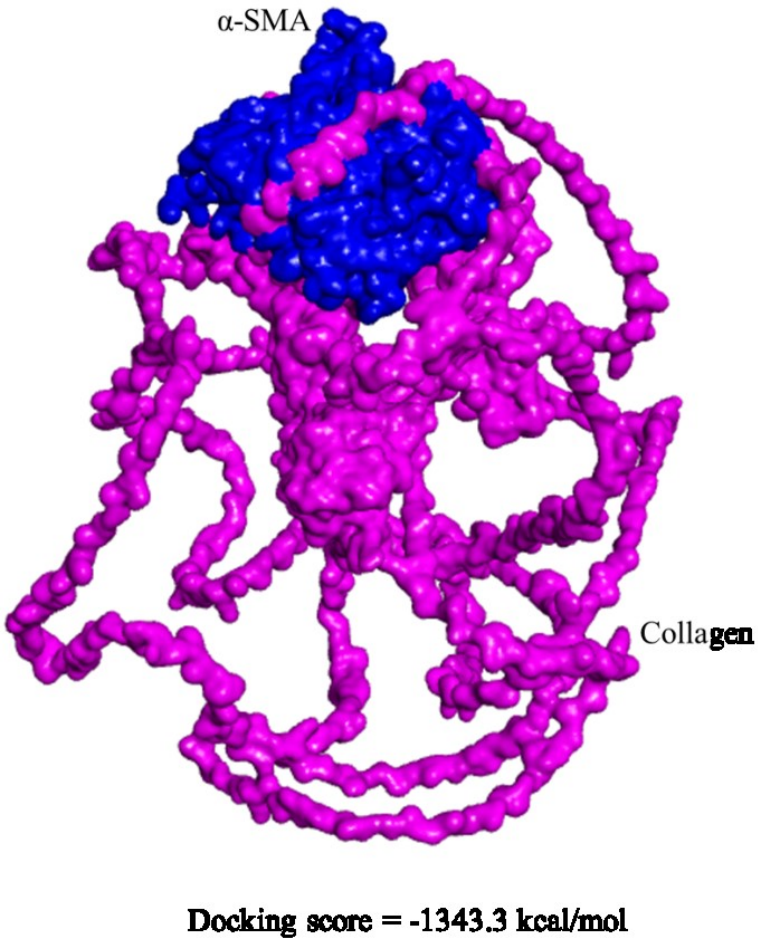


Fig. 8. Molecular docking interaction of tilapia collagen against rats' α-SMA.

References

1. Smolle, C., Cambiaso-Daniel, J., Forbes, A.A., Wurzer, P., Hundeshagen, G., Branski, L.K., Huss, F. and Kamolz, L.-P. Recent trends in burn epidemiology worldwide: A systematic review. *Burns*, **43**(2), 249-257(2017).
2. Markiewicz-Gospodarek, A., Kozioł, M., Tobiasz, M., Baj, J., Radzikowska-Büchner, E. and Przekora, A. Burn Wound Healing: Clinical Complications, Medical Care, Treatment, and Dressing Types: The Current State of Knowledge for Clinical Practice. *International Journal of Environmental Research and Public Health,* **19**, 19031338(2022) DOI: 10.3390/ijerph19031338..
3. Tripathee, S. and Basnet, S.J. Epidemiology of burn injuries in Nepal: a systemic review. *Burns & Trauma*. **5**, (2017).
4. Gauglitz, G.G., Korting, H.C., Pavicic, T., Ruzicka, T., and Jeschke, M.G., Hypertrophic Scarring and Keloids: Pathomechanisms and Current and Emerging Treatment Strategies. *Molecular Medicine*. **17**(1), 113-125(2011).
5. Finnerty, C.C., Jeschke, M.G., Branski, L.K., Barret, J.P., Dziewulski, P. and Herndon, D.N. Hypertrophic scarring: the greatest unmet challenge after burn injury. *The Lancet*, **388**(10052), 1427-1436 (2016).
6. Hirsch, T., Rothoeft, T., Teig, N., Bauer, J.W., Pellegrini, G., De Rosa, L., Scaglione, D., Reichelt, J., Klausegger, A. and Kneisz, D. Regeneration of the entire human epidermis using transgenic stem cells. *Nature*, **551**(7680), 327-332 (2017).
7. Rahmanian-Schwarz, A., Beiderwieden, A., Willkomm, L.-M., Amr, A., Schaller, H.-E. and Lotter, O. A clinical evaluation of Biobrane® and Suprathel® in acute burns and reconstructive surgery. *Burns*, **37**(8),1343-1348(2011).
8. Rahman, M.S., Akhtar, N., Hasan, M.Z. and Asaduzzaman, S.M. Human tissue banking in Bangladesh: hope for the patients of massive burns, surgical wound and bone associated complications. *International Journal of Burns and Trauma*. **9**(2), 23(2019).
9. Iacob, A.-T., Drăgan, M., Ghețu, N., Pieptu, D., Vasile, C., Buron, F., Routier, S., Giusca, S.E., Caruntu, I.-D. and Profire, L., Preparation, characterization and wound healing effects of new membranes based on chitosan, hyaluronic acid and arginine derivatives. *Polymers*, **10**(6), 607(2018).
10. Silvipriya, K., Kumar, K.K., Bhat, A., Kumar, B.D. and John, A. Collagen: Animal sources and biomedical application. *Journal of Applied Pharmaceutical Science*, **5**(3), 123-127 (2015).
11. Tan, Y. and Chang, S.K. Isolation and characterization of collagen extracted from channel catfish (Ictalurus punctatus) skin. *Food Chemistry*, **242**, 147-155 (2018).
12. Lim, Y.-S., Ok, Y.-J., Hwang, S.-Y., Kwak, J.-Y. and Yoon, S. Marine collagen as a promising biomaterial for biomedical applications. *Marine Drugs*, **17**(8), 467 (2019).
13. Hernández-Rangel, A. and Martin-Martinez, E.S. Collagen based electrospun materials for skin wounds treatment. *Journal of Biomedical Materials Research Part A*. **109**(9), 1751-1764 (2021).
14. Parenteau-Bareil, R., Gauvin, R., Cliche, S., Gariépy, C., Germain, L. and Berthod, F. Comparative study of bovine, porcine and avian collagens for the production of a tissue engineered dermis. *Acta Biomaterialia*, **7**(10), 3757-3765 (2011).
15. Pati, F., Datta, P., Adhikari, B., Dhara, S., Ghosh, K. and Mohapatra, P.K.D. Collagen scaffolds derived from fresh water fish origin and their biocompatibility. *Journal of Biomedical Materials Research Part A*. **100**(4), 1068-1079, (2012).
16. Lee, C.H., Singla, A. and Lee, Y. Biomedical applications of collagen. *International Journal of Pharmaceutics*, **221**(1-2), 1-22 (2001).
17. Yeung, D.A. and Kelly, N.H. The role of collagen-based biomaterials in chronic wound healing and sports medicine applications. *Bioengineering*, **8**(1), 8(2021).
18. Avila Rodríguez, M.I., Rodríguez Barroso, L.G. and Sánchez, M.L. Collagen: A review on its sources and potential cosmetic applications. *Journal of Cosmetic Dermatology*, **17**(1), 20-26(2018).
19. Leary, D., Vierros, M., Hamon, G., Arico, S. and Monagle, C. Marine genetic resources: a review of scientific and commercial interest. *Marine Policy*, **33**(2), 183-194 (2009).
20. Easterbrook, C. and Maddern, G. Porcine and bovine surgical products: Jewish, Muslim, and Hindu perspectives. *Archives of Surgery*, **143**(4), 366-370 (2008).
21. Sadowska, M., Kołodziejska, I. and Niecikowska, C. Isolation of collagen from the skins of Baltic cod (Gadus morhua). *Food Chemistry*, **81**(2), 257-262 (2003).
22. Singh, O., Gupta, S.S., Soni, M., Moses, S., Shukla, S. and Mathur, R.K. Collagen dressing versus conventional dressings in burn and chronic wounds: a retrospective study. *Journal of Cutaneous and Aesthetic Surgery*, **4**(1), 12-16 (2011).
23. Jafari, H., Lista, A., Siekapen, M.M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H. and Shavandi, A. Fish collagen: Extraction, characterization, and applications for biomaterials engineering. *Polymers*, **12**(10), 2230(2020).
24. Muthumari, K., Anand, M. and Maruthupandy, M. Collagen extract from marine finfish scales as a potential mosquito larvicide. *The Protein Journal*, **35**, 391-400(2016).

25. Silva, J.M., Silva, T.H., Prata, M.B., Cerqueira, M., Pirraco, R., Giovine, M., Marques, A. and Reis, R. Potential of marine sponge collagen coatings for skin regeneration strategies. Wiley-Blackwell, *Journal of Tissue Engineering and Regenerative Medicine*, **7**(S1),33pages (2013).

26. Su, X.-R., Sun, B., Li, Y.-Y. and Hu, Q.-H. Characterization of acid-soluble collagen from the coelomic wall of Sipunculida. *Food hydrocolloids*, **23**(8),2190-2194(2009).

27. Wang, L., An, X., Yang, F., Xin, Z., Zhao, L. and Hu, Q. Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (Sebastes mentella). *Food Chemistry*, **108**(2), 616-623(2008).

28. Li, J., Wang, M., Qiao, Y., Tian, Y., Liu, J., Qin, S. and Wu, W. Extraction and characterization of type I collagen from skin of tilapia (Oreochromis niloticus) and its potential application in biomedical scaffold material for tissue engineering. *Process Biochemistry*, **74**, 156-163(2018).

29. Zeng, S.-k., Zhang, C.-h., Lin, H., Yang, P., Hong, P.-Z. and Jiang, Z. Isolation and characterisation of acid-solubilised collagen from the skin of Nile tilapia (Oreochromis niloticus). *Food Chemistry*, **116**(4), 879-883(2009).

30. Potaros, T., Raksakulthai, N., Runglerdkreangkrai, J. and Worawattanamateekul, W. Characteristics of collagen from nile tilapia (Oreochromis niloticus) skin isolated by two different methods. *Agriculture and Natural Resources*, **43**(3), 584-593 (2009).

31. Lima Júnior, E.M., De Moraes Filho, M.O., Costa, B.A., Rohleder, A.V.P., Sales Rocha, M.B., Fechine, F.V., Forte, A.J., Alves, A.P.N.N., Silva Júnior, F.R. and Martins, C.B. Innovative burn treatment using tilapia skin as a xenograft: a phase II randomized controlled trial. *Journal of Burn Care & Research*, **41**(3), 585-592(2020).

32. Alves, A.P.N.N., Lima Júnior, E.M., Piccolo, N.S., de Miranda, M.J.B., Lima Verde, M.E.Q., Ferreira Júnior, A.E.C., de Barros Silva, P.G., Feitosa, V.P., de Bandeira, T.J.P.G. and Mathor, M.B. Study of tensiometric properties, microbiological and collagen content in nile tilapia skin submitted to different sterilization methods. *Cell and Tissue Banking*, **19**, 373-382(2018).

33. Potaros, T., Raksakulthai, N., Runglerdkreangkrai, J. and Worawattanamateekul, W. Characteristics of collagen from nile tilapia (*Oreochromis niloticus*) skin isolated by two different methods. *Nat. Sci*., **43**,584-593(2009).

34. Elbialy, Z.I., Atiba, A., Abdelnaby, A., Al-Hawary, I.I., Elsheshtawy, A., El-Serehy, H.A., Abdel-Daim, M.M., Fadl, S.E. and Assar, D.H. 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 Veterinary Research*, **16**, 1-11 (2020).

35. Cheng, X., Shao, Z., Li, C., Yu, L., Raja, M.A. and Liu, C. Isolation, characterization and evaluation of collagen from jellyfish Rhopilema esculentum Kishinouye for use in hemostatic applications. *PloS one*, **12**(1), e0169731(2017).

36. Ezzat, N., Al-Hawary, I., Elshafey, A., Althobaiti, N. and Elbialy, Z.I. Effect of Seasonal Changes in Heavy Metals on the Histomorphology of the Liver and Gills of Nile Tilapia (Oreochromis niloticus L.) in Burullus Lake, Egypt. *Egyptian Journal of Veterinary Sciences*, **55**(6), 1705-1716 (2024).

37. Saber, S., Khalil, R.M., Abdo, W.S., Nassif, D. and El-Ahwany, E. Olmesartan ameliorates chemically-induced ulcerative colitis in rats via modulating NFκB and Nrf-2/HO-1 signaling crosstalk. *Toxicology and Applied Pharmacology*, **364**, 120-132 (2019).

38. Hu, W., Criswell, M.H., Fong, S.-L., Temm, C.J., Rajashekhar, G., Cornell, T.L. and Clauss, M.A. Differences in the temporal expression of regulatory growth factors during choroidal neovascular development. *Experimental Eye Research*, **88**(1), 79-91 (2009).

39. Pfaffl, M.W. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*. **29**(9), e45-e45(2001).

40. Yang, H., Yang, S., Kong, J., Dong, A. and Yu, S. Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy. *Nature Protocols*, **10**(3), 382-396 (2015).

41. Yang, G., Wu, M., Yi, H. and Wang, J. Biosynthesis and characterization of a non-repetitive polypeptide derived from silk fibroin heavy chain. *Materials Science and Engineering: C*, **59**, 278-285 (2016).

42. Chen, H., Cheng, J., Ran, L., Yu, K., Lu, B., Lan, G., Dai, F. and Lu, F. An injectable self-healing hydrogel with adhesive and antibacterial properties effectively promotes wound healing. *Carbohydrate Polymers*, **201**, 522-531 (2018).

43. Hettiaratchy, S. and Dziewulski, P. Pathophysiology and types of burns. *BMJ*., **328**(7453), 1427-1429(2004).

44. Guo, S.a. and DiPietro, L.A., Factors affecting wound healing. *Journal of Dental Research*, **89**(3), 219-229 (2010).

45. Ucuzian, A.A., Gassman, A.A., East, A.T. and Greisler, H.P. Molecular mediators of angiogenesis. *Journal of Burn Care & Research*, **31**(1), 158-175(2010).

46. Behm, B., Babilas, P., Landthaler, M. and Schreml, S. Cytokines, chemokines and growth factors in wound healing. *Journal of the European Academy of Dermatology and Venereology*, **26**(7), 812-820(2012).

47. Pipelzadeh, M., Siahpoosh, A., Sheikhi, A.R. and Jafarzadeh, E. Effectiveness of green tea cream in comparison with silver sulfadiazine cream in the treatment of second degree burn in human subjects. *Journal of Herbal Medicine*, **32**, 100533(2022).

48. Sedighi, A., Mehrabani, D. and Shirazi, R. Histopathological evaluation of the healing effects of human amniotic membrane transplantation in third-degree burn wound injuries. *Comparative Clinical Pathology*, **25**, 381-385(2016).

49. Zhang, Q., Wang, Q., Lv, S., Lu, J., Jiang, S., Regenstein, J.M. and Lin, L. Comparison of collagen and gelatin extracted from the skins of Nile tilapia (Oreochromis niloticus) and channel catfish (Ictalurus punctatus). *Food Bioscience*, **13**, 41-48(2016).

50. Chen, J., Li, L., Yi, R., Xu, N., Gao, R. and Hong, B. Extraction and characterization of acid-soluble collagen from scales and skin of tilapia (Oreochromis niloticus). *LWT-Food Science and Technology*, **66**, 453-459(2016).

51. Riaz, T., Zeeshan, R., Zarif, F., Ilyas, K., Muhammad, N., Safi, S.Z., Rahim, A., Rizvi, S.A. and Rehman, I.U. FTIR analysis of natural and synthetic collagen. *Applied Spectroscopy Reviews*, **53**(9), 703-746(2018).

52. Zhou, T., Wang, N., Xue, Y., Ding, T., Liu, X., Mo, X. and Sun, J. Electrospun tilapia collagen nanofibers accelerating wound healing via inducing keratinocytes proliferation and differentiation. *Colloids and Surfaces B: Biointerfaces*, **143**, 415-422(2016).

53. Lima Júnior, E.M., de Moraes Filho, M.O., Costa, B.A., Fechine, F.V., Rocha, M.B.S., Vale, M.L., Diógenes, A.K.d.L., Uchôa, A.M.d.N., Silva Júnior, F.R. and Martins, C.B. A randomized comparison study of lyophilized Nile tilapia skin and silver-impregnated sodium carboxymethylcellulose for the treatment of superficial partial-thickness burns. *Journal of Burn Care & Research*, **42**(1), 41-48(2021).

54. Hu, Z., Yang, P., Zhou, C., Li, S. and Hong, P. Marine collagen peptides from the skin of Nile Tilapia (Oreochromis niloticus): Characterization and wound healing evaluation. *Marine Drugs*, **15**(4), 102 (2017).

55. Chen, J., Gao, K., Liu, S., Wang, S., Elango, J., Bao, B., Dong, J., Liu, N. and Wu, W. Fish collagen surgical compress repairing characteristics on wound healing process in vivo. *Marine Drugs*, **17**(1), 33 (2019).

56. Donaghue, V.M., Chrzan, J.S., Rosenblum, B.I., Giurini, J.M., Habershaw, G.M. and Veves, A. Evaluation of a Collagen-Alginate Wound Dressing in the Management. *Adv. Wound Care*, **11**, 114-118 (1998).

57. Zhang, Z., Wang, J., Ding, Y., Dai, X. and Li, Y. Oral administration of marine collagen peptides from Chum Salmon skin enhances cutaneous wound healing and angiogenesis in rats. *Journal of the Science of Food and Agriculture*, **91**(12), 2173-2179 (2011).

58. Hardwicke, J., Schmaljohann, D., Boyce, D. and Thomas, D. Epidermal growth factor therapy and wound healing—past, present and future perspectives. *The Surgeon*, **6**(3), 172-177 (2008).

59. El Ayadi, A., Jay, J.W. and Prasai, A. Current approaches targeting the wound healing phases to attenuate fibrosis and scarring. *InternationalJournal of Molecular Sciences*, **21**(3), 1105 (2020).

60. Li, D., Yuan, Q., Yu, K., Xiao, T., Liu, L., Dai, Y., Xiong, L., Zhang, B. and Li, A. Mg–Zn–Mn alloy extract induces the angiogenesis of human umbilical vein endothelial cells via FGF/FGFR signaling pathway. *Biochemical and Biophysical Research Communications*, **514**(3), 618-624 (2019).

61. Tang, E., Wang, Y., Liu, T. and Yan, B. Gastrin promotes angiogenesis by activating HIF-1α/β-catenin/VEGF signaling in gastric cancer. *Gene*, **704**, 42-48(2019).

62. Ge, B., Wang, H., Li, J., Liu, H., Yin, Y., Zhang, N. and Qin, S. Comprehensive assessment of Nile tilapia skin (Oreochromis niloticus) collagen hydrogels for wound dressings. *Marine Drugs*, **18**(4), 178 (2020).

63. Elbialy, Z.I., Assar, D.H., Abdelnaby, A., Asa, S.A., Abdelhiee, E.Y., Ibrahim, S.S., Abdel-Daim, M.M., Almeer, R. and Atiba, A. Healing potential of Spirulina platensis for skin wounds by modulating bFGF, VEGF, TGF-ß1 and α-SMA genes expression targeting angiogenesis and scar tissue formation in the rat model. *Biomed Pharmacother*., **137**(111349), 10.21037(2021).

64. Cerqueira, M.T., Pirraco, R.P. and Marques, A.P. Stem cells in skin wound healing: are we there yet? *Advances in Wound Care*, **5**(4), 164-175(2016).

65. Xue, M. and Jackson, C.J. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. *Advances in Wound Care*, **4**(3),119-136 (2015).

66. Sarrazy, V., Billet, F., Micallef, L., Coulomb, B. and Desmoulière, A. Mechanisms of pathological scarring: role of myofibroblasts and current developments. *Wound Repair and Regeneration*, **19**, s10-s15 (2011).

67. Hoerst, K., van den Broek, L., Sachse, C., Klein, O., von Fritschen, U., Gibbs, S. and Hedtrich, S. Regenerative potential of adipocytes in hypertrophic scars is mediated by myofibroblast reprogramming. *Journal of Molecular Medicine*, **97**, 761-775 (2019).

68. Klingberg, F., Hinz, B. and White, E.S. The myofibroblast matrix: implications for tissue repair and fibrosis. *The Journal of Pathology*, **229**(2), 298-309(2013).

69. Kim, K.-H., Chen, C.-C., Monzon, R.I. and Lau, L.F. Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. *Molecular and Cellular Biology*, **33**(10), 2078-2090 (2013).

70. Jun, J.-I. and Lau, L.F. Resolution of organ fibrosis. *The Journal of Clinical Investigation*, **128**(1), 97-107(2018).

71. Kis, K., Liu, X. and Hagood, J.S. Myofibroblast differentiation and survival in fibrotic disease. *Expert Reviews in Molecular Medicine*, **13**, e27 (2011).

72. Wells, A.R. and Leung, K.P. Pirfenidone attenuates the profibrotic contractile phenotype of differentiated human dermal myofibroblasts. *Biochemical and Biophysical Research Communications*, **521**(3), 646-651(2020).

73. Janka-Zires, M., Almeda-Valdes, P., Uribe-Wiechers, A.C., Juárez-Comboni, S.C., López-Gutiérrez, J., Escobar-Jiménez, J.J. and Gómez-Pérez, F.J. Clinical Study Topical Administration of Pirfenidone Increases Healing of Chronic Diabetic Foot Ulcers: A Randomized Crossover Study. *J. Diabetes Res.*, **2016,** 7340641 (2016).

74. Nakahara, T., Hashimoto, K., Hirano, M., Koll, M., Martin, C.R. and Preedy, V.R. Acute and chronic effects of alcohol exposure on skeletal muscle c-myc, p53, and Bcl-2 mRNA expression. *American Journal of Physiology-Endocrinology and Metabolism*, **285**(6), E1273-E1281 (2003).

الكولاجين البحرى المستخلص من جلد البلطى النيلى يحفز إلتئام الحروق: دراسة هستوباثولوجية، مناعية وجينية

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المستخلص

يحمي الجلد الجسم من الأضرار البيئية. تسبب حروق الجلد التهابات ميكروبية، مما يؤخر الشفاء ويؤدي إلى نتائج تجميلية غير مقبولة. لذلك، يعد التئام الجروح أمرًا بالغ الأهمية لاستعادة الوظيفة الطبيعية لأنسجة الجلد مع الحد الأدنى من تكوين الندبات. في هذه الدراسة، قمنا بفحص التأثير المعزز للكولاجين المستخرج من جلد البلطي على إصلاح الحروق وأوضحنا الآلية الجزيئية الكامنة وراءه.

كشف تحليل طيف الكولاجين FTIR أن TC الذي تم الحصول عليه كان من النوع الأول من الكولاجين ذو بنية حلزونية ثلاثية. أظهرت النتائج التشريحية المرضية أن TC يسبب ترسب الكولاجين مع الظهارة وإعادة بناء النسيج الضام بالكامل بالمقارنة مع CG. أظهرت صبغة ماسون ثلاثية الألوان لأجزاء الجلد من TCG تلطيخ كولاجين كثيف ومرتب بشكل ملحوظ، مما يشير إلى إعادة ترتيب الكولاجين الجديد أثناء عملية شفاء الحروق مع إعادة تشكيل كاملة مقارنة بـ CG. ومن المثير للاهتمام أن TCG أظهر b-FGF و VEGF محسّنًا لكنه انخفض مستويات التعبير TGF-β1 و α-SMA مقارنة بـ CG.

من المحتمل أن يؤثر التطبيق الموضعي لـ TC على شفاء حروق الجلد عن طريق تقليل حجم الجرح بسرعة، وتسريع الشفاء مع انخفاض ملحوظ في عرض أنسجة الجلد، وزيادة معدل الظهارة، وزيادة إنتاج الكولاجين بالإضافة إلى ترسب الكولاجين لاحقًا. كما أنه يعزز الأوعية الدموية في الأنسجة الحبيبية عن طريق تثبيط تكوين الندبات. وبالتالي، يمكن توفير الكولاجين المشتق من البلطي كخيار جديد واعد وآمن وفعال من حيث التكلفة مقارنة بمنتجات العناية بالجروح التجارية الأخرى.

الكلمات الدالة: الكولاجين البحرى- الحروق- الهستوباثولوجى- التعبير الجينى.