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Original Article

Investigating the Impact of Collagen-Chitosan Derived from *Scomberomorus Guttatus and Shrimp Skin* on Second-Degree Burn in Rats Model

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A B S T R A C T

Background: The present study focused on burning as one of the main causes of mortality with detrimental economic and social effects in the world. The purpose of this study was to investigate the impact of collagen-chitosan gel extracted from ***Scomberomorus guttatus*** and shrimp skin in the treatment of second degree burn healing among rats.
Materials & method: To fulfill the purpose of the study, chitosan and collagen were extracted respectively from shrimp and ***Scomberomorus guttatus*** skin waste by the acid-based method and were evaluated by using Pico Tag, SDS-PAGE. The burn wound healing efficiency of marine collagen-chitosan gel was examined in vivo using rats. Three different ratios of collagen and chitosan blend (Col-CH, 1:3, 1:1 and 3:1) were prepared to obtain the most effective Col-CH gel for burn wound healing and were compared to the animals treated with silver sulfadiazine ointment. Healing burn wound was studied by measuring wound surface area with Image J and histopathologic examination was carried out based on the mean of epithelialization, fibroblastic cells, acute and chronic inflammatory cells, angiogenesis, structure collagen and the amount of collagen on days 15 and 25 post-burn.
Results: The results of SDS-PAGE indicated that the extracted collagen was type I and it was composed of two α (α_1 and α_2) chains. Amino acid analysis showed a much higher glaycin content in extracted collagen which amounted to one-third of the total amino. The wound surface measurement showed a significant reduction in wound size in the group treated with Col-CH (3:1) compared to silver-sulfadiazine treated group on 15th and 25th days. Histopathological findings represented a high score in epithelialization, collagen, collagen structure, fibroblast cell and a decrease in inflammatory cells infiltration in Col-CH (3:1) treated group on 25th day. The most obvious finding of the present study is

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that chitosan-collagen gel (3:1) represented a better efficacy compared to sulfadiazine in burn wound healing on day 25 post-burn.

1. Introduction

Presently burn wounds are becoming increasingly a health problem in the wake of a change of daily routines and progress of technology usage [1,2]. Silver sulfadiazine and Mafenide Acetate solutions are usually used for wound care but poor efficacy, side effects and high costs make them undesirable [3–6]. Therefore, exploring modern treatment mediators for burn wound healing is crucial [7]. Formation and alignment of fibrous structure followed by utilizing natural polymers is increasingly used in burn wound healing [8]. Collagen-chitosan structure wound dressing leads to optimal recovery of burn defect by using generation of fibrous connective tissue around and within the scar zone [9]. Marine collagens are one of the most widely used groups of biologic agents and have been extensively used for decades in wound healing. Collagen is extracted from different marine sources such as fishes, sponges and mollusks [10,11].

Fish collagen is a relatively cheap and easily accessible protein at industrial scale, mainly due the fact that there are large volumes of fish waste tissues containing skin, bone and scale which are nearly 30% of fish volume. Caruso et al. have estimated that more than 50% of fish wastes are discarded, exceeding 20 million tonnes per year [12]. Interestingly enough, these discarded wastes have contained 17–35% lipid-rich compounds and 10–25% high content of valuable protein [13]. Moreover, the utilization of fish waste into collagen production faces less religious and ethical constraints and helps to protect environment and produce value-added products in the fish processing industry. Although previous researchers have shown that the amount of amino acids of fish collagen is less than that of mammalian collagen which causes early denaturizing of fish collagen in lower temperatures, two α_1 and α_2 chains mainly constitute type 1 collagen in the fish skin as well [7,14]. Mammalian sources, however, can pose a potential risk such as foot and mouth disease (FMD), bovine spongiform encephalopathy (BSE), and transmissible spongiform encephalopathy (TSE) for human health [15]. On the other hand, moisture is an important component in wound healing and plays a key role in survive of skin cells in the burn. In addition, absorption capability of fish collagen is more than other collagen sources such as pig and cattle that might be due to the lower weight of peptide molecules. Therefore, recent developments in the field of collagen-based dressing have led to a renewed interest in the use of fish skin collagen for burn wound. However *Scomberomorus guttatus*, which is one of the species of migratory pelagic-neritic fish with its body entirely shielded with small scales, has not been adequately researched yet. *Scomberomorus guttatus* is one of the numerous fishes living in Iranian waters of the Persian Gulf [16].

Chitosan is one of several biopolymers extensively used in a variation of biomedical fields, such as in drug delivery, wound healing dressing, etc. In recent years, the studies have shown that collagen-chitosan combination improve mechanical and biological properties more than the individual polymer scaffold [17].

This paper aimed to find the best ratio of collagen-chitosan combination which was respectively extracted from *Scomberomorus guttatus* Skin and Persian Gulf's shrimp for healing of second-degree burn in rats. In addition, this study critically discussed wound size and histopathological results on day 15 and 30 of post burn.

2. Materials and methods

2.1. Sample preparation

For the purpose of this study, *Scomberomorus guttatus* fish were collected from the fisher market. The fish were sacrificed and their skins were removed. They were cleaned manually in order to take out fat and other adhering tissues. Then, they were washed with cold deionized water. The skins were cut into small pieces (0.5×0.5 cm^2) and were packed in polyethylene bags and they were stored at 20 °C until used.

2.2. Chemicals

B- Mercaptoethanol (BME), Pepsin from porcine gastric mucosa, powdered; 0.7 FIP/mg dry matter (Ec 3.4.23.1), Acetic acid, Bothyle alcohol (Butanol), Tris (Hydroxymethyl) aminomethane, Sodium dodecyl sulfate (SDS), and Coomassie Brilliant blue R-250. The companies of these materials are Sigma–Aldrich Corporation.

2.3. Collagen extraction

All procedures were performed at 4 °C. Acid–soluble collagen extraction was done as described by Naderi Gharagheshlagh et al. (2019), which involved an initial extraction with 0.1 M NaOH (pH 12) for 24 h at 4 °C to remove non-collagenous proteins. Then, they were washed with DW and they were added 10% (w/v) butyl alcohol for 24 h for removing the fat tissue samples. Next, three volumes of 0.5 M acetic acid were added to the samples, stirred for 3 days and centrifuged at 10,000 g for 20 min. After that for collagen precipitating, 2.6 M NaCl was added and centrifuged at 10,000 g for 30 min. Finally, it was dialyzed by dialysis bags for 3 days and dried in −40 °C for 2 days [18].

2.4. Extraction of chitin and chitosan preparation

To date various methods have been developed and introduced to Chitin and chitosan preparation. The Chemical method was used for the deproteinization of shrimp shells. 5% (w/v) NaOH solution was applied for 4 h at room temperature. After that, they were washed with DW and their PH was notarized. The demineralization of shells pieces was done by adding 1 L 4% (v/v) HCl at 30 C for 12 h. Then they were washed and PH notarizing was accomplished. For the deacetylation, samples were treated by 100% (w/v) NaOH at 105 °C for 6 h using a mechanical stirrer and followed by centrifugation 4000 rpm for 15 min. Finally, they were stored in −40 °C overnight and dried for storing in room temperature [19].

2.5. SDS-PAGE test

SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed based on the method of laemmli [20], using 7.5% gel containing 10% SDS at pH 8.8. The protein samples, containing 50 μl

dialysis collagen, 10 μl SDS10%, and 3 μl 2-mercaptoethanol were heated in boiling water for 5–10 min. Then, they were added to the solution of 50 μl glycerol 20% and bromophenol blue 0.005%. The proteins of the gel were stained by Coomassie brilliant blue R-250.

2.6. Amino acid analysis

In our previous report [21], Amino acid composition in collagen of ***Scomberomorus guttatus*** was analyzed by using waters–Pico Tag high performance liquid chromatography amino acid analyzer, Waters Model 88,131 WISP™ (Millipore Corp, Milford, MA, USA) according to the method of Bidlingmeyer et al., (1984) [22].

2.7. Preparation of collagen-chitosan hydrogel

We made four ratios of hydrogel based on various percentages of collagen–chitosan containing Col-CH (1:3, w/w) or (SGC0.25), Col-CH (1:1) or (SGC0.5), Col-CH (3:1) or (SGC0.75) and Col 1% –CH 1% in order to specify the optimal mix of collagen–chitosan (Col-CH). Collagen-Chitosan hydrogel combination was prepared by adapting the procedure used by Chen et al., (2006) [23]. Finally, PH mixture reached 7.2 by adding NaOH (1 N). Samples were held at 4 C for future experiments.

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2.8. Burn model in rats

Male Sprague–Dawley rats (n = 64) weighing 300–350 g (prepared from Razi Vaccine and Serum Research Institute) were used for this experiment. All rats were given a standard diet several days before the study for 12 h, observing light/dark cycle and at environment temperature of 22–24 °C. Accommodation and maintenance of the animals were in accordance with the National Research Council guidelines. In the present study, the thermal injury model was used for experimental burns model creation. The rats were anesthetized by intramuscular injection of ketamine 10% (Alfasan Inc., Woerden, Netherland) (70 mg/kg) and Xylazin 2% (Alfasan Inc., Woerden, Netherland) (9 mg/kg). After that, the dorsum of each rat was shaved with electric clippers. Next, a second degree of burn injury (2 × 4 cm) was created by stainless steel rod (2 × 4 × 1 cm). The rod was preheated in boiling water (100 °C) for 15 min and vertically applied to the skin surface for 6 s without pressure. After the model creation, Ibuprofen 1.5 mg/100 g was orally given for analgesia.

Intervention was carried out with hydrogels that had different ratios of Col-CH on the wounds. Routine treatment was completed using Silver sulfadiazine (SS group). As it is mentioned in Table 1, first, the ratio of Col-CH was compared in the five groups for evaluation of scaffold efficacy on burn wound healing. Then, the highly effective scaffold was compared to Control group or WD% and SS. The wound dressing was done every day and the rats were taken care of over time.

2.9. Burn wound healing evaluation

2.9.1. Measurement of wound surface area

After the wound dressing in each group, wound surface area was measured on days 15 and 25. Wound sizes were measured using a ruler and digital photographs taken by the Canon IXUS Digital Camera. Also the percentage of epithelialization was calculated by Image J software, version 1.45, (National Institutes of Health, Bethesda, Maryland, USA).

2.9.2. Histopathological assessment

Fifteen and twenty-five days after the surgery, animals were killed by an overdose of ether and the wound area was removed. Wound tissue samples were fixed in a 10% formaldehyde solution at 4 °C for 48 h, dehydrated through a graded series of ethanol solutions in an automatic tissue processor, embedded in paraffin and sectioned in 5 μm thickness slices.

Sections were then placed on a glass slide and stained with hematoxylin-eosin (HE). As shown in Table 2, histological evaluation was done by our pathologist using the Semi-quantitative method to score fibroblast cells, angiogenesis, epithelialization, acute and chronic inflammatory cells, and the deposition and arrangement of collagen in the wound area.

2.10. Statistical analysis

Statistical analysis was performed by Anova and post-hocTukey's tests for multiple comparisons. P-values were calculated using GraphPad Prism (version 6.1, GraphPad software, USA). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ were considered as statistically significant different.

3. Results

3.1. Chitosan

The amount of 20.7% chitosan was extracted from shrimp skin by Tahvildari method.

3.2. Collagen

14.5% collagen (based on dry/weight ratio) was gotten from the skin of *Scomberomorus guttatus* fish through acid-soluble method.

Table 1
Experimental design.

No	Group	Treatment
1	Control	Burn with no treatment (n = 6)
2	Col 1%	Burn with treatment (hydrogel containing 1% Col, n = 6)
3	Col-CH (3:1)	Burn treated with hydrogel containing Col-CH (3:1) (n = 6)
4	Col-CH (1:1)	Burn treated with hydrogel containing Col-CH (1:1) (n = 6)
5	Col-CH (1:3)	Burn treated with hydrogel containing Col-CH (1:3) (n = 6)
6	CH1%	Burn treated with hydrogel containing CH 1% (n = 6)
7	SS	Burn treated with silver sulfadiazine (n = 6)

Table 2
Pathological scores based on the severity of the parameters.

Re-epithelialization: migration of keratinocytes, bridging of cells and keratinization	<25% 1	25%–50% 2	50%–75% 3	>75% 4
Inflammatory cells	++ and + 1	± 2	±± 3	− 4
Fibroblastic cells	− 1	± 2	+ 3	++ 4
Collagen	− 1	± 2	+ 3	
Collagen Structure	− 1	± 2	+/± 3	+ 4
Angiogenesis	3 > 1	3–5 2	5–9 3	10≥ 4

3.2.1. Collagen profiling in the skin by SDS-PAGE

The results obtained from the analysis of SDS-PAGE test by 7.5% resolving gel are presented in Fig. 1. According to the data, *Scomberomorus guttatus* fish collagen contains two distinct chains of α (α_1, α_2) and β.

It seems that the molecular weight of α_2 was smaller than that of α_1. This was the result of different mobility positions in α region. Therefore, there were various α_1 and α_2 chains. From the data of electrophoretic mobility, it is apparent that the type I of *Scomberomorus guttatus* collagen was composed of two α_1 and α_2 chains.

3.2.2. Amino acid compositions of collagen

As Table 3 shows, there was the amino acid composition that consisted *of Scomberomorus guttatus* collagen based on amino acid residues per 1000 total amino acid residues. In Table 3, as it is clearly indicated the amount of glaycin is high in the *Scomberomorus guttatus* skin collagen which is about one-third of its amino acids. In addition, the amount of proline was 86.8 residues per 706.1 residues in place of a unique amino acid in of acid-soluble collagen from ***Scomberomorus guttatus*** (ASC). Even though the count of proline was different based on the species[26,45], other amino acids including Alanine, Glutamate and Arginine were in the highest levels. There were no amino acids such as tryptophan and cysteine in the extracted collagen and some amino acids including Methionine, Tyrosine, and Histidine were in the lowest levels of collagen.

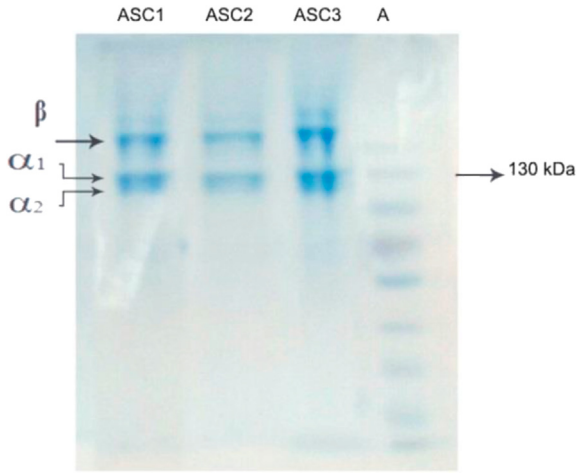

Fig. 1. SDS-PAGE patterns of acid-soluble collagen from ***Scomberomorus guttatus***. (*ASC1*, ASC2, ASC3) and protein marker (Lane A).

3.3. Burn surface area

3.3.1. Comparison of the various ratios of collagen-chitosan combination on the wound surface area

After the creation of burn model in male rats, for determination of effective ratio of collagen-chitosan, rats were divided into four groups as shown in Table 1. It can be seen from the data in Fig. 2 that in measuring the wound surface area on day 15 and 25 of post-burn, Col-CH (3:1) group had a significant reduction compared to other groups on two time points (**P < 0.003 and **P < 0.005). As indicated by the, the treated group with Col-CH (3:1) gel had the smallest wound area, whereas control groups showed the largest burn wound area on those days.

3.3.2. Comparison of collagen-chitosan gel with chitosan and silver sulfadiazine

As shown in Fig. 3A, wound size changes in collagen-chitosan gel and chitosan and silver sulfadiazine groups were compared; the wound treated with Col-CH (3:1) showed an impressive decrease in wound size on day 15 and 25. Also from the quantified results using Image j software in Fig. 3B, it is apparent that wound sizes in animals treated with Col-CH (3:1) had more significant decrease than others on day 15 and 25 (***P: 0.001).

3.3.3. Histopathological assessment

For tissue sampling on 15th and 25th days of post-burn, punching and taking whole wound surface were done respectively. Histological evaluation was done by our pathologist using Semi-quantitative method based on Table 2 to score fibroblast cells, angiogenesis, epithelialization, acute and chronic inflammatory cells, and the deposition and arrangement of collagen in the wound area.

The amount of wound healing among groups was evaluated by scoring based on Table 2, the highest score indicating maximum healing.

3.3.3.1. Histopathological assessment on day 15. In terms of the histopathologic parameters, there was a significant difference in wound healing scores in the five groups (P < 0.05).

The results shown in Table 4 and Fig. 4 indicate that the group treated with Col-CH (3:1) had the highest score among collagen Arrangement (2.5), collagen (3), Acute Inflammatory Cell (2.66) and fibroblast cells accumulation (2.57). Totally, the highest wound healing average score belonged to Col-CH (3:1) group.

3.3.3.2. Histopathological assessment on day 25. From the data in Table 5 and Fig. 5, the mean score of histopathologic parameters on 25th day of post-burn for Col 1%, Col-CH (3:1) and CH 1% was the same and it was a high score in epithelialization. Whereas high mean scores in the number of structure collagen (2.66),

Table 3
Amino acids composition of extracted Collagen

Amino acids composition of extracted Collagen[a]			
Alanine	84.3	Leucine	22.1
Arginine	71.8	Lysine	33.3
Aspartate	41.4	Methionine	14.1
Cysteine	0	Phenylalanine	20.7
Glutamate	87.8	Proline	86.8
Glycine	188.6	Serine	26.3
Histidine	9.1	Threonine	20.7
Isoleucine	10.8	Tyrosine	3.9

[a] Values were given as mean ± standard deviation of triplicate.

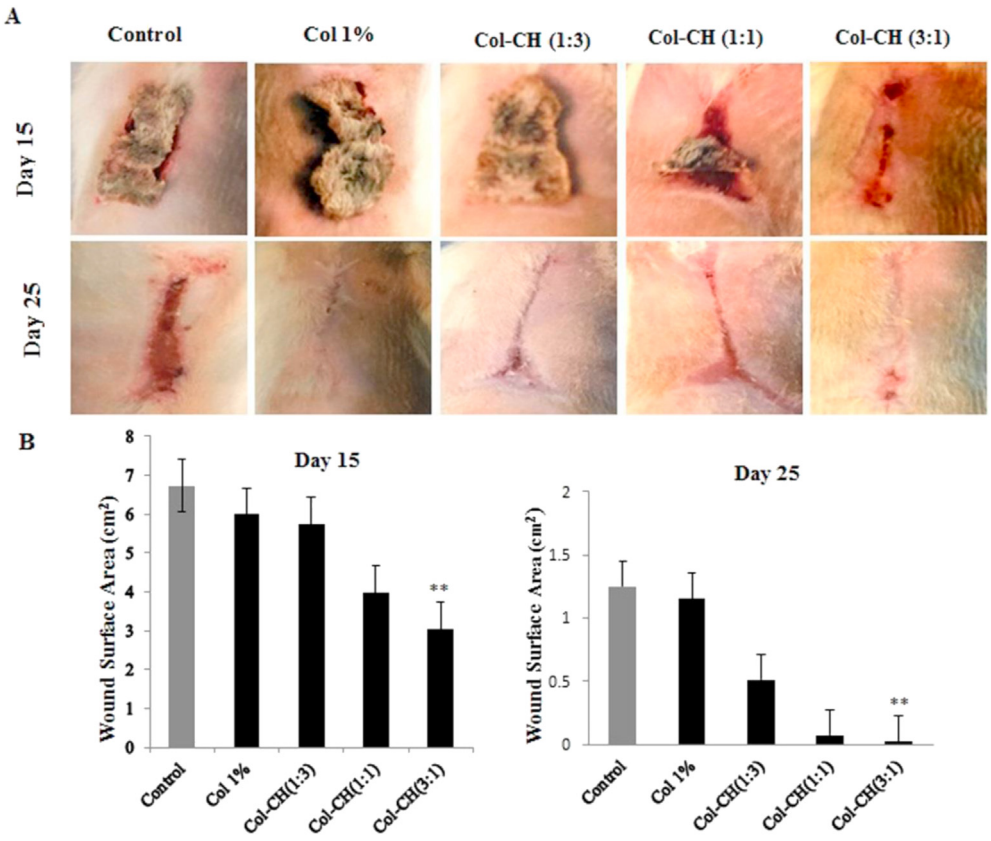

Fig. 2. (**A**) Representative images of wound healing on 15th and 25th days of post-burn in groups treated with different ratios of collagen Col 1%, Col-CH (1:3), Col-CH (1:1) and Col-CH (3:1). (**B**) Comparison of wound surface area on 15th and 25th days among above-mentioned groups.

collagen accumulation (3), acute Inflammatory cell (3.83) and fibroblast cells accumulation (3) were seen in Col-CH (3:1) group ($P < 0.05$), the group treated with CH 1% indicated the high mean score for chronic inflammatory cell infiltration (3). In addition, the lowest average scores (2.11 and 2.354) were seen in silver sulfadiazine treated and control groups respectively along with incomplete epithelialization.

4. Discussion

This study set out with the aim of accessing the amino acid composition of *Scomberomorus guttatus* skin collagen and finding an effective ratio of collage–chitosan combination for burn wound healing in rats. The results of this study indicated that Glycine was the most abundant amino acid and Col-CH (3:1) combination had the highest efficacy in burn wound healing. Histopathological evaluation on day 15 indicated a high score in collagen arrangement, collagen, acute inflammatory cells and fibroblast cells accumulation in Col-CH (3:1) treated group. After 25 days, our results indicated that Col 1%, Col-CH (1:1) and CH 1% groups had the equivalent and high score in epithelialization.

From the data of electrophoretic mobility, type I of *Scomberomorus guttatus* collagen was composed of two α_1 and α_2 chains. The results of electrophoretic mobility of the specimens indicated that *Scomberomorus gtatusut* extracted from collagen consisted of collagen type I with two $\alpha 1$ and α_2 chains. The present findings seem to be consistent with other studies such as Tylingo (2016) [24], Alizadeh Node (2014) [25], Naderi Gharehgheshlagh [26], Senaratne et al. (2006) [27], Zhang et al. (2009) [28], Yan et al. (2008) [29], Wang et al. (2007) [30], Duan et al. (2009) [31], Jongjareonrak et al. (2005) [32], Ogawa et al. (2004) [33], and Singh et al. (2011) [34].

As mentioned in the Results section, Glycine was the most abundant amino acid, which included one third of the total amino acids found in extracted collagen. The findings in this study indicated that the amount of essential amino acids in *Scomberomorus*

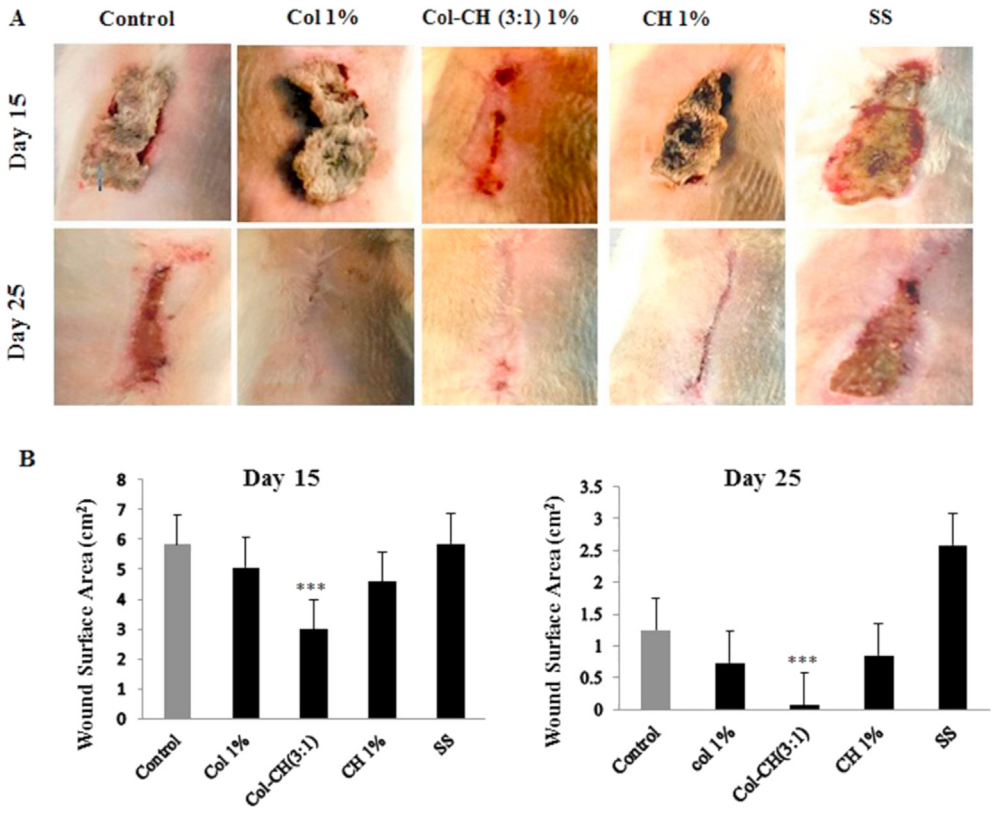

Fig. 3. (**A**) Representative images of wound healing on 15th and 25th days of post-burn in groups treated with Col 1%, Col-CH (1:1), CH 1% and SS; Control. (**B**) Quantification of wound surface area (cm^2) on 15th and 25th days.

Table 4
The average scores of the histopathologic parameters in the five groups on the 15th day after burn.

Day 15								
Groups	Vessel	Collagen arrangement	Collagen	Chronic inflammatory cell	Acute inflammatory cell	Fibroblast cell	Epithelialization	Average
Col 1%	2.71	2.14	3	2.42	2.57	2.42	2.71	2.56
Col-CH (3:1)1%	2.71	2.5	3	2	2.66	2.57	2.66	2.58
CH 1%	2.85	1.71	2.142	2.42	2.42	2.14	2.28	2.281
SS	1	1	1	1	1	1	1	1
Control	2.33	2.1	2.83	1.16	1.16	2.16	1	1.82
P	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	

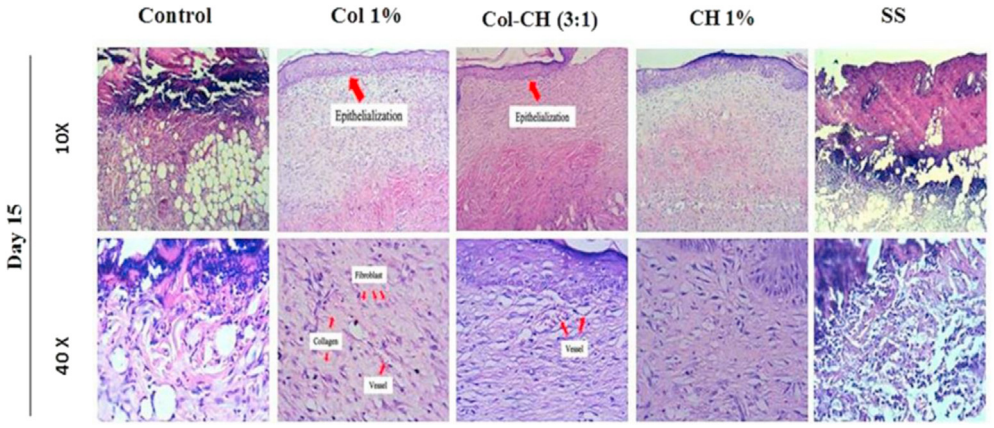

Fig. 4. H&E staining of samples on 15th day of post-burn for all groups (Col 1%, Col-CH (1:1), CH 1% and SS); Control. All images were represented in two different magnifications (10x and 40x).

Table 5
Average scores of the histopathologic parameters in the five groups on the 25th day after burn.

Day 25								
Groups	Vessel	Structure collagen	Collagen	Chronic Inflammatory Cell	Acute Inflammatory Cell	Fibroblast Cell	Epithelialization	Average
Col 1%	3.66	2.5	3	2.33	3.5	3	4	3.14
Col-CH (3:1)1%	3.33	2.66	3	2.66	3.83	3	4	3.21
CH1%	3.12	2.37	3	3	3.71	2.14	4	3.04
SS	2.33	2	2.8	1.16	2.16	1	3.33	2.11
Control	3	2.33	3	1.33	1.33	2.83	2.66	2.354
P	**<0.05**	**>0.05**	**>0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	

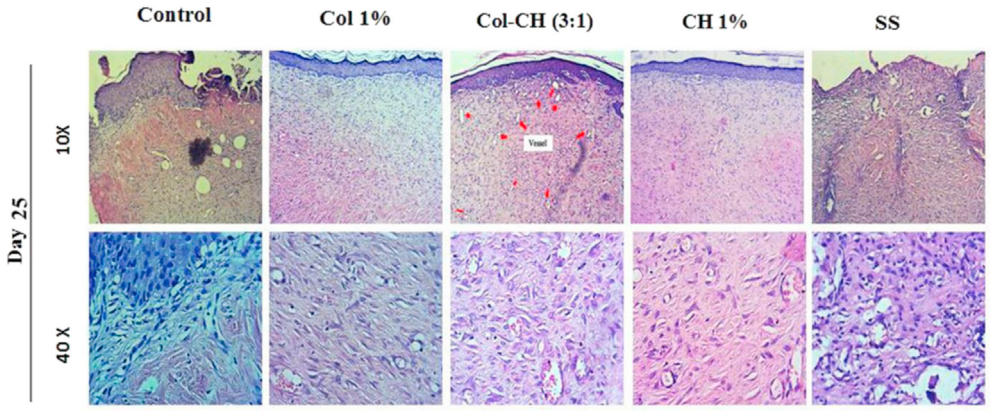

Fig. 5. H & E staining of samples on 25th day of post-burn for all groups (Col 1%, Col-CH (3:1), CH 1% and SS); Control. All images were represented in two different magnifications (10x and 40x).

guttatus was lower than those of Salmon fish reported by Tylingo et al., 2016 [24], but it is in agreement with Nile Perch as reported by Muyonga et al. (2004) [35], and channel catfish as reported by Liu et al. (2007) [36]. A possible explanation for the lower content of glycine in the *Scomberomorus guttatus* compared to salmon fish might be the contamination by other proteins.

This study supported the presence of high levels of Alanine, −Glutamate, − Arginine, and Proline and very low levels of Tyrosine, Histidine, Methionine, and Isoleucine in the extracted collagen. The present findings seem to be consistent with the reported results for the skin of albacore, Rohu, and lungfish [37,38]. These distribution patterns of the amino acid composition in the *Scomberomorus guttatus* skin collagen further supported the idea of similarity to that of channel catfish [36]. It was proved that the *Scomberomorus guttatus* ASC could be considered as type I collagen. The results of this study indicated that the group treated with collagen-chitosan gel (3:1) represented the smallest wound surface on the 15th, 25th days and the wounds became almost completely closed on 25th day of post-burn. The current study found out that IIIb skin burns got treated with collagen-chitosan dressing through increasing the granulation and fibrous connective tissues [8,39]. The findings of the current study are consistent with those of Basha et al. (2011) [40] who found fish scale-extracted collagen reduced the healing time (15 ± 0.82 days) compared to untreated group (23 ± 0.99 days). In addition, in the current study Col-CH (3:1) caused wound size reduction more than others.

Another important finding was the significant difference of epithelialization scores among the groups on the 25th day. Additionally, the collagen-chitosan gel (3:1) group had the highest score of epithelialization on the 15th day. But, silver sulfadiazine and control groups showed the lowest epithelialization scores. Therefore, it seems possible that acceleration of epidermis and dermis formation and thickness of epidermis were in compliance with the high score of collagen-chitosan gel.

It is also interesting to note that all the three groups treated with Col, Col-CH and CH gel on the 25th day exhibited a faster recovery process in comparison with silver sulfadiazine and control groups. The point to be made here is that wound contraction and epithelialization rate in rats' skin is quicker in comparison with humans. Hence upon the recommendation of Dorsett-Martin and Montandon et al. [41,42], a greater would size and square shaped wounds were applied in the present study in order to address the less relevance of using rats in human clinical settings.

The most interesting finding was that Col 1% group revealed the high score of epithelialization on 25th day that was possibly due to the non-toxicity and similar antigenic determinants of the collagen in marine animals [7].This finding is in agreement with Shen et al. (2017) which showed the wounds were entirely healed in the group treated with the shark collagen on day 12 [43]. These results are consistent with those of other studies that revealed the wound healing process by the dressings comprising chitosan-collagen with mammalian extracted collagen (pig, cow, and buffalo) [44] alongside with high fibroblast proliferation [45]. Furthermore, previous studies confirmed that lessening the inflammation phase was due to the use of chitosan-collagen dressings and, this caused acceleration of collagen generation in the burn wounds [8].

The findings of the current study are consistent with those of Cui et al. who found that the chitosan-collagen hydrogel combined with lysostaphin which was developed for MRSA (Methicillin-resistant Staphylococcus aureus) infected burn wounds caused wound healing through bacterial growth inhibition [39]. These results also accord with our earlier observations, which showed that combination of type I collagen and chitosan along with polyethylene oxide had high efficacy in wound healing [23] even as a supportive scaffold for growth factor producing cells [45,46].

Another important finding was that Col-CH (3:1) gel indicated a decrease in both acute and chronic inflammatory cells which contributes to healing outcome through reducing inflammation,

promoting granulation tissue formation, and assisting rapid proliferation of epithelial, endothelial and fibroblasts cells [7]. In addition, inflammatory cells were more than other groups in the sulfadiazine group. A possible explanation for this result may be due to the earlier starting of proliferative phase in the Col-CH(3:1) group compared to the sulfadiazine group that corroborates Kirichenko et al.'s (2011) finding [8].

In the current study, comparing the average collagen level of the groups on 15th and 25th days after burn revealed that the mean score of collagen formation was high in all of the groups except sulfadiazine group. This result is in agreement with Basha et al.'s (2011) finding [40].

As mentioned in the previous studies, one of the cells that plays a main role in the wound healing are fibroblast cells [47]. Being consistent with the findings of the previous studies, our histological results indicated that Col-CH (3:1) samples had most active fibroblasts around the wound on days 15 and 25.

The observed increase of fibroblast cells in the wound treated by Col-CH (3:1) could be the factor behind strengthening the new tissue via generation of different substances, such as collagen, proteoglycan, and elastin [47,48]. This finding was in agreement with Yates et al. (2007) which showed that collagen and chitosan provided the fibroblasts migration and proliferation by increasing angiogenesis and elevating the release of oxygen from hemoglobin [49].

Rane and Mengi (2003) found out that the high content of hydroxyproline in granulation tissue used collagen film in the wound [32], thereby leading to fast healing of wounds [50]. Findings of the present study seem to be consistent with Hu et al.'s (2017) results which found collagen peptide extracted from the skin of Nile Tilapia to be effective in repairing a full-thickness burn wounds. They indicated that marine collagen peptides in the new epidermis and active hair follicle proliferation created new capillaries and a complete muscular layer structure [7].

5. Conclusion

The present study has purported to give an account of as well as the reasons for the widespread use of chitosan-collagen gel in burn wound healing. The most outstanding finding to emerge from this study is that chitosan-collagen gel (3:1) represented a better efficacy compared to sulfadiazine in burn wound healing on day 25 post-burn. The fast wound closure, increase of angiogenesis and fibroblast proliferation, decrease of inflammation phase, and boosting self-renewal capability of the tissue were observed as a result of using Col-CH (3:1) in burn wound. Taken together, these results suggest that the fish-extracted collagen-chitosan gel, as a new type of wound dressing, can accelerate burn wound healing. These findings provide the new insights for using the biological scaffolds in the human wound treatment.

Declaration of competing interest

The authors declare that we have no conflict of interest.

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