Characterization of Tilapia collagen-loaded chitosan nanofibers synthesized by electrospinning method for wound dressing

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Abstract. Burns are traumatic injuries that require intensive therapy. Tilapia skin is known to contain high collagen properties and was developed for wound dressing. This study aimed to investigate the characterization of Tilapia collagen-loaded chitosan nanofibers synthesized by electrospinning for wound dressing. This study evaluated three group materials, i.e. (G1) chitosan, (G2) Tilapia collagen, and (G3) Tilapia collagen-loaded chitosan. The electrospun method was performed by alloy the chitosan nanofibers containing 8% w/v Tilapia collagen. The mix solution was characterized using scanning electron microscopy (SEM), Fourier-transform infrared (FTIR) spectroscopy, pH level, swelling test, water capacity test, and cytotoxicity test. As result, Tilapia collagen-loaded chitosan showed the better swelling test, water capacity test, cytotoxicity test, and FTIR evaluation compared to the collagen or chitosan group. However, all group materials showed similarities in pH level and SEM evaluation. In conclusion, Tilapia collagen-loaded chitosan nanofibers could be considered as a wound dressing based on positive results during characterization.

1. Introduction
The development of wound care has increased rapidly, especially in the last two decades, supported by advances in health technology. In addition, changes in patient profiles associated with metabolic and degenerative diseases are crucial comorbid factors [1]. These conditions usually require proper treatment so that the healing process can be optimal. This issue is very important to emphasize in modern wound care management. This is supported by the increasing number of the latest innovations in wound care products. Basically, the selection of the right product must be based on considerations of cost, comfort, and safety [2].

Wound healing can be done with wound dressings to avoid infection. The ideal wound dressing should support wound healing. According to its characteristics, the ideal wound dressing provides moist conditions for wound healing, controls excess exudate, maintains a stable temperature condition, and prevents microbial infection [3]. Collagen leads a very important role at every stage of the wound
healing process. Collagen has the ability, i.e. homeostasis, interaction with platelets, interaction with fibronectin, increasing fluid exudation, increasing cellular components, increasing growth factors and promoting the process of fibroplasia and epidermal proliferation [4]. The benefits of collagen in the medical field is to accelerate the growth of new tissue. In recent studies, chitosan has been widely developed in various aspects of the medical field. One of the products resulting from chitosan technology is that it has started to be used as a topical drug delivery system in the form of membranes [5]. This study aimed to evaluate the character of Tilapia collagen coated with chitosan nanofiber as a wound dressing.

2. Material and Methods

2.1. Ethical approval

This study was approved by Animal Ethics and Use Committee Universitas Airlangga No 367/HREC.FODM/VII/2021. This ethical letter aimed to explain a clear method during collagen collection from Tilapia.

2.2. Collecting samples

A total of 0.5 g of chitosan from the synthesis of crab shells was dissolved in 25 ml of 0.5 M acetic acid until homogeneous, then stirred for 30 mins. After that, collagen was added with a ratio of (g) 1:1 of collagen with chitosan, then stirred for 30 mins. The membrane can be printed by pouring the collagen-chitosan composite into a glass plate and leveling the surface with a thickness of 1 mm. Then we let stand for 24 h at room temperature. The dried bioplastic was put in a coagulant bath containing 4% NaOH to help the membrane release from the glass. Incubate the membrane for 24 h for easy collection. Then dried on mica to make it easier to take the membrane when it is dry.

The treatment group was divided into three groups of materials, i.e. (G1) chitosan, (G2) Tilapia collagen, and (G3) Tilapia collagen-loaded chitosan. The electrospun method was performed by mixed the chitosan nanofibers containing 8% w/v Tilapia collagen. A total of 18 kV electrical voltage was used with a 12 cm distance from the collector to the tip of the pump syringe [6].

2.3. Interpretation

The characterization test was carried out using scanning electron microscopy (SEM), Fourier-transform infrared (FTIR) spectroscopy, pH level, swelling test, water capacity test, and cytotoxicity test [7].

3. Results and Discussion

Evaluation using the FTIRs method aimed to determine the functional groups formed from the collagen-chitosan composite. Collagen-chitosan has a characteristic peak at the absorption wave number of 3452.64 cm\(^{-1}\) which is a hydroxyl group (-OH) (Figure 1). At absorption wave number 1657.57 cm\(^{-1}\) is amide I. Amide I is an important factor in understanding the secondary structure of protein [8]. The presence of amide II was shown in the absorption wave number 1560.47 cm\(^{-1}\). Amide II shows the presence of a helical structure [9]. The results showed that the most dominant interaction between collagen molecules and chitosan molecules was physical interaction [10]. Compounds –OH, C=O, and –NH\(_2\) which are formed from collagen composites are derived from the combination of compounds contained from collagen and chitosan.
Figure 1. Evaluation of collagen-loaded chitosan using (a) SEM and (b) FTIR

Table 1. Evaluation of pH level, swelling, water capacity, and cytotoxicity test

| Group | pH      | Swelling test (%) | Water capacity (%) | Cytotoxicity test (%) |
|-------|---------|-------------------|--------------------|-----------------------|
| G1    | 6.0 ± 0.06 | 265.3 ± 6.03       | 31.7 ± 4.16        | 17.7 ± 3.06          |
| G2    | 6.2 ± 0.10 | 301.7 ± 27.68      | 45.7 ± 3.79        | 14.0 ± 1.00          |
| G3    | 6.1 ± 0.12 | 282.3 ± 15.50      | 38.0 ± 3.61        | 8.7 ± 2.08           |

Values are expressed in mean ± standard deviation. One-way Anova test was performed followed by Tukey multiple comparison test. a,b,c Different superscript in the same column indicated significantly difference (p<0.05).

The SEM findings showed soft fibers on the surface and smear layer (Figure 1). Collagen-chitosan cushion structure can provide a greater water absorption capacity so that it is more suitable during wound healing (Table 1). The percentage of water retention is highly dependent on the hydrophilic and micro properties of the membrane, because collagen and chitosan are both hydrophilic materials, the ability to maintain a porous membrane structure is the main explanation for the difference in the percentage of water retention results. The ideal water retention reaches 200-500% to increase the chances of wound healing [11].

Primary wound dressings are generally composite products covered by a thin layer that serves as a wound protector so that it is easily removed, so as not to damage new tissue [12]. Products that meet these requirements include collagen coated with chitosan, because it has high regeneration and absorption power [13]. Previous studies have proven that collagen coated with chitosan can close the wound and maintain a moist balance around the wound, easy to use and remove, elastic, antibacterial and non-toxic, does not cause allergies, is non-carcinogenic, biodegradable and biocompatible, because it can be catabolized into monosaccharides and can be absorbed by the body [14]. It is also known that wound healing is 30% - 50% faster when wound dressings made of collagen are used [15].

Primary dressings must absorb wound fluid, maintain temperature and humidity around the wound, regulate the oxidation of gases coming out of the wound, so that the wound becomes moist and heals faster [16]. The main requirements for primary dressings are non-toxic, non-allergenic, easy to sterilize, strong, elastic, biodegradable and biocompatibility [17]. The low mechanical properties of collagen allow the porous structure to not be maintained when removed from the distilled water [18]. In contrast, chitosan has a higher elasticity which can help maintain the porous membrane structure. Therefore, the percent water absorption will decrease along with the increase in the addition of collagen [19].

4. Conclusion
It can be concluded from the swelling test, water capacity test, cytotoxicity test, and FTIR evaluation, Tilapia collagen-loaded chitosan nanofibers revealed slightly significant compared to the collagen or
chitosan group, respectively. Tilapia collagen-loaded chitosan nanofibers may be developed as a candidate for wound dressing based on their characterization.

5. References
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6. Acknowledgments
The study was funded by the Faculty of Veterinary Medicine, Universitas Airlangga (No. 212/UN3/2021), Surabaya, Indonesia. We would like to thank the anatomy laboratory staff for their help in the current study.