



Effectiveness of Collagen β -glucan Composite for Wound Healing Treatment

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Abstract

Fish skin-derived collagen and *Saccharomyces cerevisiae*-derived beta-glucan were extracted for potential use in wound healing applications, with a comprehensive investigation into their physicochemical properties such as molecular weight, solubility, and heat stability. Fourier transform infrared spectroscopy (FTIR) was utilized to examine the chemical bonds of fish collagen and compare its resemblance to type 1 collagen. A mouse model was employed to assess the wound-healing impact of β -glucan. The findings revealed that employing a weak acid (CH₃COOH) in combination with a strong base (NaOH) for extraction resulted in high beta-glucan content and an advantageous ratio of polysaccharides, proteins, and lipids. Additionally, rats were utilized to evaluate the healing effects of the chosen β -glucan. The animal model demonstrated that the combination of collagen and β exhibited superior efficacy in wound healing, leading to accelerated wound closure. Based on these results, the isolated fish collagen shows promise for potential utilization in wound treatment.

Keywords: Collagen; β -glucan; Wound healing

1. Introduction

Wound healing progresses through stages such as coagulation, inflammation, proliferation, and maturation, involving complex coordinated biochemical and immunologic actions [1]. Various factors contribute to wound development, including surgical interventions, trauma, extrinsic forces like pressure or shear, and underlying diseases like diabetes or vascular disease. Wounds are broadly categorized as acute (e.g., surgical wounds and

burns) or chronic (e.g., leg ulcers, diabetic foot ulcers, and pressure ulcers) based on their underlying causes [2]. Medications and healthcare products designed for wound healing align with the four stages and include haemostatics, antibiotics, anti-inflammatory drugs, and agents promoting collagen production [3]. Wounds have a significant impact on individuals, caregivers, and the healthcare system, often referred to as the 'Silent Epidemic' [2].

Collagen, constituting approximately 30% of animal protein, is a vital structural protein in mammalian connective tissue [4]. Produced in healing wounds by cells like fibroblasts, collagen undergoes modifications into intricate morphologies [5]. Predominant collagen types in the skin include fibrillar types I, III, and V, as well as fibril-associated types XII, XIV, XVI, and VI [5]. Collagens are widespread in various organs, such as tendons, cornea, ligaments, blood vessels, skin, bones, and cartilage [6].

Traditional methods of collagen extraction from animal sources are challenging, expensive, and time-consuming. Aquaculture waste, particularly fish scales and skin, serves as an alternative collagen source, offering advantages such as being fat-free, antibiotic-free, and prion-free [7]. Fish collagen possesses biodegradability, low toxicity, non-antigenicity, high nutritional value, stability, and unique qualities like strong emulsification abilities, gelation, and water binding capacity [8]. Glucans, complex polysaccharides, play diverse physiological roles with glucose units linked by α - (alpha) and β - (beta) glycosidic linkages [9]. β -Glucans, found in various sources like bacteria, yeast (*Saccharomyces cerevisiae*), fungi, algae,

mushrooms, and cereal grains, exhibit anti-cancer, antibiotic, and immunomodulatory properties, affecting processes like phagocytosis and reducing blood pressure or cholesterol levels [10]. *S. cerevisiae* cell walls are a significant source, containing 55–65% of β -glucan [11]. Extraction methods, such as water, alkaline, acid, or enzymatic extraction, impact β -glucan production and purity [12].

Studies have demonstrated that β -glucan enhances wound healing by stimulating tissue granulation, human dermal fibroblast collagen biosynthesis and deposition, and re-epithelialization [12]. Extracting potent β -glucan from plant and microbial sources involves careful consideration of extraction procedures to ensure usefulness, quality, and structural integrity [13].

Previous research has explored wound dressing materials containing both β -glucan and collagen, demonstrating positive impacts on patient health and wound care [14]. In this study, we developed sheets for potential wound dressings using collagen extracted from fish skin modified by β -glucan. The investigation encompassed the physical, chemical, and biological properties of these sheets.

2. Materials and Methods

2.1 The Extraction Process of Collagen from Fish Skin

The process of obtaining collagen from fish skin was conducted according to the following procedures. Fresh marine fish skin, thoroughly cleaned with running and distilled water, was refrigerated at $-20\text{ }^{\circ}\text{C}$ until required. To eliminate non-collagenous proteins and colours, five-gram fish scales underwent a three-day treatment with 0.1 N NaOH. Following this treatment, scales were cleaned with distilled water and left to dry. Subsequently, the samples were subjected to a three-day extraction using 0.5 M acetic acid, followed by centrifugation for one hour at $4\text{ }^{\circ}\text{C}$ and 10,000 rpm. NaCl was added to the supernatants to precipitate them, bringing the final concentration down to 0.9 M. Pellets were collected through centrifugation, washed three times with distilled water to remove residual salts, and, finally, hung in 0.5 M acetic acid. The removal of salts was accomplished through dialysis, and the resulting collagen was lyophilized and ground for further use [8].

2.2 Collagen Sheet and Collagen Dressing Formation.

The creation of collagen films involved casting and lyophilization. First, collagen was dissolved in 0.5 M acetic acid to create a collagen solution. This solution was then cast onto differently-sized containers, followed by lyophilization [15].

2.3 Yeast Strain and Cultivation

Saccharomyces cerevisiae yeasts were cultivated in Yeast Extract-Glucose broth (YG broth), where a yeast suspension was prepared using 500 mL of YPD broth (containing yeast extract 1%, peptone 2%, and glucose 2%). The suspension was agitated at 150 rpm for 48 hours at $30\text{ }^{\circ}\text{C}$. Post-incubation, cells were harvested at $4\text{ }^{\circ}\text{C}$ through centrifugation at 4500 g for 5 minutes. The collected cells underwent three sterile distilled water washes, were weighed, and subsequently freeze-dried, storing them at $20\text{ }^{\circ}\text{C}$ until required for wall preparation [16].

2.4 Extraction of β -glucan

2.4.1 Acid-Base Extraction Method

In this approach, the yeast extract-glucose broth medium served as the medium for cultivating dry active yeast cells over 48 hours. The yeast cell pellets, obtained from the preceding phase, were combined with five times the volume of 1 M NaOH. Subsequently, the mixture underwent centrifugation at $80\text{ }^{\circ}\text{C}$ for two hours. After centrifugation, the pellets were subjected to a rewashing process with distilled water, followed by a final round of centrifugation to achieve the ultimate yield. The pellets containing lysed yeast cells were then collected and used for β -glucan extraction.

Extraction was carried out using a five-fold acetic acid (CH_3COOH) solution, resulting in 20% of the yeast cells being enriched with β -glucan at the conclusion.

To eliminate any residual organic-soluble compounds, additional proteins, or contaminants, the collected β -glucan pellets were dried in an oven at $60\text{ }^{\circ}\text{C}$ and preserved for further analysis. Post-drying, the weight of the pellets was 11.5% higher than that of the initial yeast cells [11].

2.5 SEM Analysis of Scaffolds

SEM was used to examine the microstructure of the fish collagen and β -glucan. The lyophilized samples were cut using punch and fixed to an adhesive carbon stub. Imaging was carried out using a Tabletop SEM (JEOL 6340, Japan) operated at 15 k [12].

2.6 FTIR Analysis of Scaffolds

2.6.1 Spectroscopy of Collagen and β -glucan

Samples that had been freeze-dried were subjected to FTIR spectroscopy using a Shimadzu FTIR 8400S from Japan. A disc with a diameter of 10 mm was created by compressing a mixture of 10 mg of the sample and 100 mg of dried potassium bromide (KBr). Spectra were obtained in the range of 4000 to 500 cm^{-1} .

2.7 Experimental Planimetry Analysis of Wound Closure Rates

Photographs of experimental animals' wound areas were taken from postoperative days 0 to 14 using a digital camera, with a metric ruler serving as the background. The boundaries of clearly visible epithelialization were utilized to determine the measurement of the wound closure area. Each surface area in a two-dimensional plane was measured using the adjacent metric ruler as a reference. Four distinct photometric observations were obtained for each mouse. The percentage of wound healing (% contraction) was calculated using the following formula:

$$(\text{Healed area})/(\text{total area}) = \% \text{ wound contraction}$$

2.8 Preparation of Full Thickness Wound Model

A wound healing model was established using male rats aged 12 to 14 weeks, with body weights ranging from 180 to 220 g. The rats were procured from El-Mowasah University Hospital's Animal House in Alexandria, Egypt. Each group of animals was housed in separate stainless steel cages under controlled conditions of lighting and humidity (25 ± 3 °C; 35–60% humidity) with a 12-hour light-dark cycle [17].

Upon identification, weighing, and segregation into five groups, each consisting of seven animals ($n=5$), the experimental design was as follows:

- Group I: received saline and underwent the vehicle operation.
- Group II: served as an ointment (dermazine)-treated control group, containing hydrocortisone and iodoquinol.
- Group III: received a collagen sheet.
- Group IV: received collagen with β -glucan.
- Group V: treated with a β -glucan sheet.

Before any surgical procedures, the rats were ensured to have no existing skin lesions at the designated surgical site. Intramuscular injections of 2% xylazine hydrochloride (Calmium®, 0.1 ml/100 g body weight) and 10% ketamine hydrochloride (Dopalen®, 0.1 ml/100 g body weight) were administered to induce anaesthesia. After shaving the back, the area was antisepticated with 4% iodine-based alcohol. A circular incision with a diameter of 2.5 cm was made at the center of the shaved area, extending down to the loose subcutaneous tissue of the upper back [8].

Subsequently, the wounds of each animal group were topically treated daily with a specific dosage of the designated medication for three, five, eight, ten, fourteen, fifteen, sixteen, and eighteen days. Throughout the trial, the wounds were bandaged with sterile gauze and secured with circular adhesive bands [18].

2.9 Standard Histology

After being carefully dissected from the surgical site, tissue samples were fixed in 10% formalin before being processed paraffinically. Day 7 post-operative tissue specimens were used to prepare histology sections. Paraffin sections were serially sectioned, and representative sections were stained with Hematoxylin and Eosin [8].

3. Results

3.1 Scanning Electron Microscopy (SEM) of Scaffolds

Examination of collagen extracted from fish scales, purified through acid treatment, using SEM Microscopy, unveiled a dual-layered structure within each scale: an outer osseous layer and an inner fibrillary plate. SEM analysis of freeze-dried native collagen showcased uniformly distributed multi-layered aggregates with rigorous, orderly, and highly fibrillary structures. (Fig. 1a).

SEM imaging of β -glucan extracted from *Saccharomyces cerevisiae* exhibited particles with irregular geometric shapes, sharp edges, and aggregated formations forming large masses. Additionally, the particle size ranged from 1.56 to 2.36 μm , as depicted in Figure 1b. Moreover, the development of aggregates between β -glucan particles was observed, highlighting the oval to elliptical form of the granular particles.

The examination of collagen with β -glucan, as illustrated in Figure 1c, revealed the presence of collagen bonded with β -glucan. The distinctive structure of collagen is emphasized by the attachment of oval-shaped β -glucan particles.

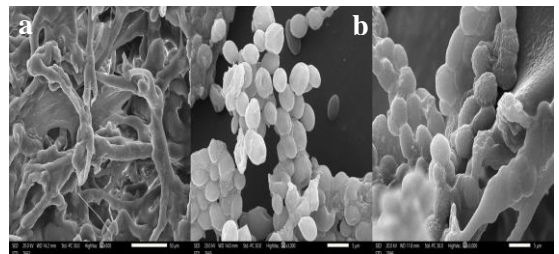


Figure 1. Scanning electron microscope (SEM) of collagen; 1a, β -glucan extracted from *Saccharomyces cerevisiae*; 1b, collagen- β -glucan scaffolds.

3.2 FTIR Analysis of Collagen, β -glucan and Collagen- β -glucan Scaffold

The FTIR spectrum of collagen extracted from collagen skin is depicted in (Fig. 2) as black line. The prominent peaks at (3343 cm^{-1}) and (2864 cm^{-1}) correspond to the amide A and B bands, which are commonly identifiable. The COH stretching vibrations are represented by the band at (1416 cm^{-1}).

¹). The distinctive absorption peaks for the amide I, II, and III bands of polypeptides are observed at (1640 cm^{-1}), (1586 cm^{-1}), and (1258 cm^{-1}), respectively. The resulting spectrum closely resembles collagen isolated from other species.

The FTIR spectrum of β -glucan isolated from *Saccharomyces cerevisiae* is illustrated as green line in figure 2. The absorption bands of β -glucan samples at 3319 were attributed to the existence of a free hydroxyl group (O-H stretching). Furthermore, the presence of absorption in the 900–1200 cm^{-1} range supported the peaks of the C–C and C–O stretching vibrations, indicating that polysaccharides constitute a significant component. Additionally, the identification of β -glucan was confirmed by the prominent absorption peak at 1632.87 cm^{-1} , signifying the presence of β -glycosidic linkages and the (C1–H) deformation mode.

The results obtained for collagen β -glucan scaffold is illustrated as purple color in figure 2. The primary collagen linkages that have been documented match wavenumbers: 3433, 2917, 2847, 1739, and 1650 cm^{-1} respectively, and they are typical of samples that contain collagen. Amide A's absorption properties, which are frequently linked to N–H stretching vibration, happen in the wavenumber range of 3300–3440 cm^{-1} . Our collagen's maximal absorption peak was discovered to be 3400 cm^{-1} . In the β -glucan spectrum that was examined, the distinctive peak was noted at 1015 cm^{-1} . Polysaccharides are present in the area of 871–1240 cm^{-1} , as indicated by the FTIR peak (mostly caused by C–C and C=O stretching vibrations).

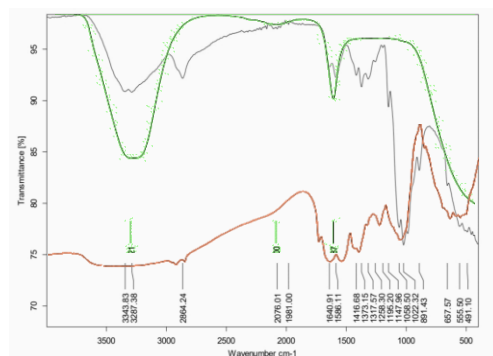


Figure 2. Showing FTIR spectrum of extracted collagen represented as black line, the isolated β glucan represented as green line and β -glucan+collagen scaffold represented as purple line.

3.3 Assessment of In-vivo Wound Healing

Compared to the saline and dermazine groups (collagen & β -glucan with collagen), it was evident that effective wound dressing was demonstrated, promoting a faster and improved re-epithelialization during the 7–14 days post-injury

period. Furthermore, it enhanced adhesion and had no adverse effects on the healing process (Fig. 3). Macroscopic analysis (Fig. 3) focused on observing potential erythema in the vicinity of the lesion, with profuse exudate being present, keeping the wound wet. The dressing comprising collagen and glucan proved effective for cleaning dry wounds and encouraging autolytic debridement. It exhibited permeability and did not elicit a response from injured biological tissue. The dressing further facilitated wound re-epithelialization and left no residue on the wound. In the early stages of the healing process, there was no evidence of exudate in the wounds. By Day 3, a reduction in the size of all wounds relative to the injury day was observed. However, deviations from the saline group were noticeable. The collagen sheet resulted in a crust formation over the wound, indicating dryness. Granulated tissue was observed only in groups receiving collagen-based dressings, but the wounds remained clean, devoid of exudate, inflammation, or microbiological contamination (pus or edema). By Day 7, a thin tissue layer had grown over the entire surface of the wounds, primarily in those dressed with collagen. All abrasions at this point had reduced in size. By Day 14, a further reduction in the size of the wounds was noted, with the collagen-based groups exhibiting full re-epithelialization.

The results showed that the collagen sheet and glucan accelerated wound healing compared to the other collagen-control group (saline & dermazine). The glucan with collagen scaffold dressing proved instrumental in faster wound healing, presenting itself as a valuable addition to clinical applications for wounds.

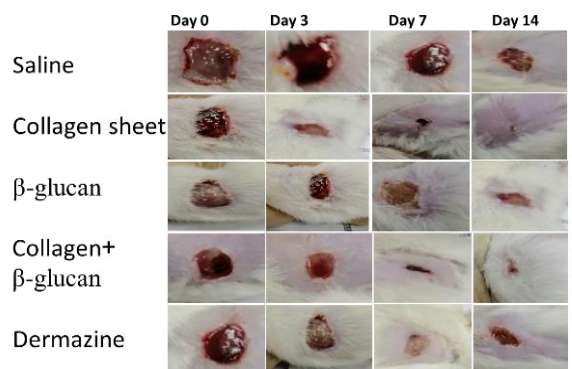


Figure 3. Digital photos showing macroscopic wound subjected to different treatments.

3.4 Histological Evaluation of Healed Wounds

Histological evaluation of wounded skin showing the development of an ulcer with early healing as seen by a thin layer of squamous epithelial cells

infiltrating the dermis and epidermis, accompanied by a high number of polymorphneutrophils and lymphocytes, which are inflammatory cells. (Fig. 4) a skin slice from an animal given dermazine plus saline treatment demonstrating the development of an ulcer that healed quickly to generate a thin layer of squamous epithelium, lack of sebaceous glands and extensive polymorphneutrophil and lymphocyte infiltration in the epidermis and dermis. By day 7, In all collagen, β -glucan, collagen with β -glucan groups, the damaged areas were seen to be fully sealed by re-epithelialization, whereas in the control groups saline and cream, the epidermal layer at the wound's center had not yet fully grown. These findings suggested that collagen and β -glucan had the ability to mend wounds. This could be explained by collagen's extensive antimicrobial action, which lowers infection and speeds up the healing process.

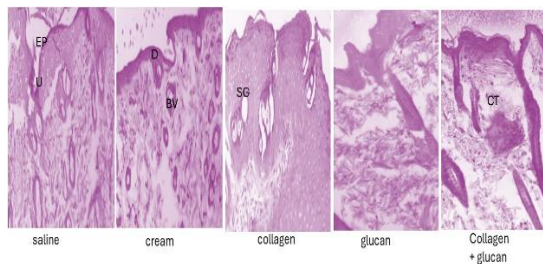


Figure 4. Histological analysis of wound treatments (10X). Ep: Epidermis; BV: Blood vessels; SG: Sebaceous glands; U: Ulcer; C: Collagen; CT: Connective tissue (CT).

5. Discussion

In this study, collagen sourced from marine organisms was utilized, offering the advantage of versatility in molding into various shapes. This biomaterial holds potential as a scaffold for tissue regeneration, providing multiple benefits not achievable with conventional dressings.

Collagen sheets were produced incorporating β -glucan, which were then applied to cover wounds in clinical trials involving rats, yielding positive results. β -glucan was extracted from *Saccharomyces cerevisiae* yeast using the acid-base extraction method. This extraction method offers advantages in terms of simplicity, cost-effectiveness, and time efficiency. The study demonstrated that a high-quality β -glucan could be obtained through a combination of weak acid (CH₃COOH) and strong base (NaOH) extraction.

Apart from its therapeutic advantages, utilizing β -glucan-collagen for treating chronic wounds has the potential to reduce healing times and overall treatment expenses [19]. However, further clinical investigations are necessary to validate

the efficacy of β -glucan-collagen composite-based wound dressings in treating a broad spectrum of burns and chronic wounds.

6. Conclusion

In this study, collagen extracted from marine organisms was used, one benefit determined by the separated collagen is that it may be molded into a variety of shapes. It is suggested that this biomaterial be used as a scaffold for tissue regeneration. Multiple benefits that are not possible from traditional dressings are supplied by collagen sheets.

Collagen sheets were made with beta-glucan, the wound was covered with them, and they were applied in clinical trials in rats, results were good, and beta-glucan was extracted from *Saccharomyces cerevisiae* yeast using the acid-base extraction method. The advantages of this method are that it is not difficult, less expensive, and not time-consuming. The current study also proved that high-quality of beta-glucan could be produced by combining an extraction with a weak acid (CH₃COOH) and a strong base (NaOH).

In addition to its therapeutic benefits, using beta-glucan-collagen to treat chronic wounds can reduce healing times and overall treatment costs. However, further clinical studies are required to confirm the effectiveness of beta-glucan-collagen composite-based wound dressings in treating a wide range of burns and chronic wounds.

7. References

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