SHORT COMMUNICATION

Effectiveness of Collagen Extracted From the Skin of Nile Tilapia Fish (*Oreochromis niloticus*) to Accelerate Wound Healing *in vivo*: A Narrative Review

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ABSTRACT

Developing wound dressings with good properties to accelerate wound healing has always been challenging. Mammal collagen is well known as the desired choice among naturally produced dressings despite experiencing major drawbacks. Therefore, researchers have explored the by products of *Oreochromis niloticus* fish or red Nile tilapia, such as the scales and skin as alternative source of collagen. This narrative review analyzes the effectiveness of collagen extracted from *Oreochromis niloticus* skin and its beneficial properties to accelerate wound healing *in vivo*. Recent studies were compiled to provide a compact review on the effectiveness of the collagen in hastening wound healing process. Faster wound closure with upregulation of multiple growth factors, and high hydroxyproline content are some of the findings. The collagen extract could also promote the formation of epidermal layers, fibroblasts proliferation, and dermal reconstitution. Thus, *Oreochromis niloticus* skin collagen extract has the potential to be developed as an effective wound dressing.
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INTRODUCTION

Wounds impose a huge burden on patients and healthcare facilities. Wound care accounts for 1.6-1.8% of the total yearly healthcare expenditure (1). Since the impact of chronic wound is growing over time, there is a need for economical, safe, and reliable wound care products. Land-based animals are typically the major source of commercialized collagen. This is because collagen is the main structural protein in mammalian connective tissue, accounting for around 30% of total protein in mammals. Collagen is crucial for the formation of extracellular matrix (ECM), and cell and tissue migration. Collagen production from terrestrial animals has, however, increased the danger of infections in recent years as the frequency and incidences of diseases such as mad cow disease, bird flu, and other infectious illnesses have escalated (2). In addition, porcine collagen is outlawed in some countries and by various religious organizations, including Muslims and Jews, owing to the religious and consumer concerns (3). Therefore, the need for a new source of collagen for wound dressings is crucial.

Most recently, researchers have found that the by products of *Oreochromis niloticus* (*O. niloticus*) fish, or Nile Tilapia such as the scales and skin can be used as an alternative source (4). The extraction of scale and skin-derived collagen from *O. niloticus* would not just increase the value of the fish, but also reduce environmental pollution (5). The utilization of *O. niloticus* collagen as biomaterial for wound healing has also been proposed in multiple studies in laboratory animal model. In addition, the commercialized traditional dressings for wound healing currently used are also experiencing a major drawback for being less effective and having prolonged treatment time (6). Thus, this narrative review analyzed the effectiveness of *O. niloticus* collagen extracted from its skin to accelerate wound healing through *in vivo* studies.

MATERIALS AND METHODS

Study design and method

The study design for this narrative review was selected based on a broad perspective and explore the general debates on the topic. The information about the effectiveness of *O. niloticus* skin collagen extracts to accelerate wound healing *in vivo* were obtained from published papers via online databases. The online databases used were PubMed, Google Scholar, and ScienceDirect from 2015 to 2021. A total of 187 papers were retrieved from the online databases to be reviewed, rephrased, compiled, and discussed.

Literature search and selection criteria

The literature search was conducted in English. The following keywords were used in various combinations: "wound healing", "Nile Tilapia", "collagen extract", "*in vivo*". The number of articles retrieved from this broad search was 187. Articles published in non-scientific journals and those published before 2015 were excluded. Duplicates, non-free full text, and articles written in a language other than English were also excluded. The articles which do not match the criteria of the review were also excluded (Example: *In vivo* studies using Nile Tilapia fish skin as xenograft, disease-induced animals). The selected articles were analyzed qualitatively after being screened for important details and data. Different categories were created for the findings (Example: "Histological analysis" and "Immunohistochemistry analysis").

RESULTS AND DISCUSSION

Several significant studies which previously investigates the effectiveness of *O. niloticus* skin collagen extracts to accelerate wound healing *in vivo* are presented in Table I.

Comparison of wound area over time

All studies reported that *O. niloticus* collagen extract produced positive outcomes in expediting wound healing process at different time intervals. The findings on the wound area over time indicates a significant acceleration in wound healing using *O. niloticus* skin collagen extracts as compared to the control group and the commercial product treated groups. This is due to the healing characteristic of collagen which could be absorbed and eventually degraded by the wound. Consequently, this will help increase collagen fibers and fibroblasts formation in the epidermal tissue, and thus replacing the wounds with newly regenerated dermis (6).

Table I : Selected significant *in vivo* studies describing the effectiveness of *O. niloticus* skin collagen extract to accelerate wound healing

No.	Author, year, species	Type of collagen extraction used	Form of collagen used in the study	Findings
1	Hu, Z., et al., 2017, rabbit	Not specified	Peptide powder	Faster wound closure and healing rate. Effective to accelerate wound healing.
2	Zhou, T., et al., 2017, rat	Pepsin-solubilized	Nanofibers	Faster wound closure with accelerated COL-1 protein secretion. Upregulated expression of CD31 marker. Effective to accelerate wound healing.
3	Chen, J., et al., 2019, rat	Acid-solubilized and pepsin-solubilized	Collagen sponge	Faster wound closure with higher total protein secretion. Increased Hyp content, FGF and EGF expression. Upregulated CD31 marker and elevated expression of granulation tissues and fibroblasts cells. Effective to accelerate wound healing.
4	Sitje, T.S., et al., 2018, rat	Not specified	Collagen sponge	Faster wound closure. Increased keratinocyte proliferation and epidermal differentiation. Effective to accelerate wound healing.
5	Ge, B., et al., 2020, rat	Pepsin-solubilized	Hydrogel	Faster wound closure with increased Hyp content. Accelerated formation of new collagen and skin appendages. Effective to accelerate wound healing.
6	Elbialy, Z.I., et al., 2020, rat	Acid-solubilized	Gel	Faster wound closure. Upregulated expression of VEGF & bFGF, and TGF-β1. Effective to accelerate wound healing

Protein content at wound site

A sufficient supply of proteins is important for effective wound healing process. Thus, protein content is used in the study to evaluate the progress of wound healing. According to previous finding, the secretion of COL-1 protein was accelerated after *O. niloticus* collagen extract treatment (7). *O. niloticus* Type 1 collagen derived from fish skin has been proven of having low toxicity level through *in vivo* study on its biocompatibility for application in biomedicine (8). This is due to its properties and capability to promote tissue repair, regeneration, and accelerating recovery that progressively completes wound healing. In addition, other beneficial properties of Type 1 collagen include promoting cellular adhesion and proliferation, collagen synthesis, and increasing the expression of various growth factors which enables the collagen extract to accelerate wound healing process (6). All of these characteristics made the collagen derived from *O. niloticus* skin a very promising substitute for the traditional wound dressings.

Hydroxyproline (Hyp) content at wound site

Collagen is a component of growing cells which is synthesized by healing tissues. The presence of collagen is measured by the concentration of hydroxyproline, and the high amount of hydroxyproline signifies a more rapid wound healing process (7). Enhanced synthesis of hydroxyproline on *O. niloticus* collagen treated wound site was demonstrated previously (6), and this was also reflected with its increased concentration during cellular proliferation (9). Therefore, increasing the synthesis of collagen will expedite the wound healing process.

Histological examination

Various cells on the wound site were evaluated through histological examination after the treatment with *O. niloticus* collagen extract. For instance, in collagen-treated groups, the granulation tissue and fibroblasts cells which are important for wound healing were elevated as compared to the control group (6). It is also reported that the healed wound site was covered with epithelial tissues paired with substantial collagen deposition, fibroblast proliferation, and granulation tissue formation. The study described that the *O. niloticus* collagen treated groups developed higher vascularization and collagen deposition than the control group. The hematoxylin and eosin (H&E) staining further revealed that in the collagen treated wound, skin tissues and skin appendages were steadily formed with less inflammatory cells seen at the wound site as compared to the other groups. These positive findings indicate that *O. niloticus* collagen extract was effective in enhancing wound healing *in vivo*.

Immunohistochemistry examination

Immunohistochemistry examination was conducted in order to examine the effect of *O. niloticus* skin collagen on angiogenesis, increase in fibroblast formation and collagen deposition. As a result, multiple growth factors were found to be upregulated after the treatment with *O. niloticus* skin collagen. The growth factors including transforming growth factor beta 1 (TGF-β1), fibroblast growth factor (FGF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) are upregulated in the process of wound healing through cell proliferation involving activation of processes including formation of extracellular matrix. re-epithelialization, and angiogenesis (10, 11). TGF-β1 plays an important role during wound healing process and is involved in every stage of wound healing, particularly to suppress inflammatory reactions and promoting granulation tissue formation (10). In addition, TGF-β1 is an important component for the synthesis of ECM by the activated fibroblast and differentiated myofibroblast (12). The higher production of EGF by *O. niloticus* collagen extract can trigger keratinocytes proliferation in order to induce re-epithelialization of the wound (6). Through its role as mitogenic agent and chemotactic agent of fibroblasts, EGF can stimulate the proliferation and differentiation process of fibroblasts, and synthesize collagen for faster wound healing.

Besides, angiogenesis is a crucial step for the maintenance of granulation tissues and hastening wound healing process by ensuring adequate supply of oxygen, nutrients, and immune cells to the stroma (13), and it is also induced by several angiogenic factors such as FGF and VEGF (14). The proliferation and migration of fibroblasts, re-epithelialization, angiogenesis, and production of ECM in wound healing are activated by FGF (15). Meanwhile, VEGF stimulates wound healing through angiogenesis but likely promotes collagen deposition and re-epithelialization as well (16). Another finding is the higher expression of CD31, or also known as the marker for angiogenesis that is important for vessel proliferation in *O. niloticus* collagen extract. The findings illustrate that CD31 marker, FGF and VEGF gene expression were significantly higher in *O. niloticus* treated wounds than the other groups, thus, implying that collagen derived from *O. niloticus* has the ability to increase the production of these angiogenic factors (16). A summary of the outcomes from the reviewed immunohistochemistry studies is illustrated in Fig. 1.

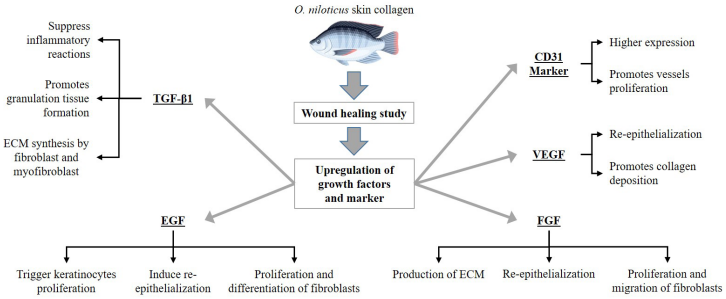

Figure 1 : A summary illustrating immunohistochemistry studies of *O. niloticus* skin collagen for wound healing and the upregulation of growth factors and marker.

Incorporation of additive ingredient in fish skin collagen

To compensate the lack of antibacterial activity in fish collagen, some bioactive components could be added to make it more suitable for clinical use. This may help in preventing possible infection to the exposed wound and also effectively induce regeneration of the skin. Therefore, it is vital to find an effective and safe additive ingredient to be incorporated with *O. niloticus* collagen. Previous study proved that *O. niloticus* collagen has high biocompatibility and was successfully prepared as a composite material with bioactive glass (BG) which significantly improved its mechanical and biological properties (17). Therefore, BG has been considered to have angiogenic potential. Nevertheless, although adding the second component may significantly improve the collagen mechanical strength, it may however compromise the function of collagen overall (18).

Wet wound healing theory

Another notable finding from the previous work is the "wet wound healing theory", which means the wound dressing of the *O. niloticus* collagen extract should be kept in a moist environment (9). To ensure a moist environment, hydrogel form of *O. niloticus* collagen was used. It is reported that the wet healing condition contributed towards the growth of granulation tissues and facilitate the division of skin cells which consequently promotes the completion of wound healing process (7). It was also reported that collagen hydrogels possess the advantage to prevent bacterial infections, moisturizes the skin, and as a result accelerate wound healing progress and re-epithelialization (20). Thus, this suggests that hydrogel form of collagen from *O. niloticus* skin can be used as wound dressing for wound treatment.

CONCLUSION

To conclude, *in vivo* studies showed that *O. niloticus* skin collagen extract is effective in accelerating wound healing by enhancing the expression of multiple growth factors such as VEGF, FGF, EGF, TGF- β1, CD31 marker and producing higher hydroxyproline content. Compared with commercial products and the control groups, the *O. niloticus* collagen extract has the ability to promote epidermal layer formation and the maturation of skin appendages, collagen synthesis, fibroblasts proliferation, re-epithelialization, and dermal reconstitution *in vivo*. All of these beneficial properties of *O. niloticus* skin collagen extracts suggest that it has the potential to be developed as a novel and effective dressing material for wound treatment, thus substituting the traditional land animal based wound dressing. Nevertheless, further studies are still required in order to fully understand the unclear mechanisms and answer any remaining questions on faster wound healing process using *O. niloticus* skin collagen extract.

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