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Nile tilapia fish skin as an alternative biological dressing for canine skin wounds

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Abstract
This study was aimed to assess the effectiveness of Nile tilapia fish skin biological dressings in treating superficial skin wounds in dogs. Six dogs with superficial wounds were selected after thorough physical examinations. The wounds were covered with chlorhexidine and glycerol-processed tilapia fish skin dressings, secured using polyamide sutures, and reapplied as needed. By the 30th day post-surgery, all dogs showed complete wound healing, with full epithelialization, satisfactory functional recovery with no adverse reaction. These findings suggest that tilapia fish skin is an effective biological dressing for managing superficial wounds in dogs.

Keywords: Nile tilapia, fish skin, wound healing, dog, biological dressing

Introduction

A wound is defined as a break in the cellular and anatomical structure of a tissue, which can result from immunological, microbiological, chemical, thermal, or physical trauma (Maver *et al*. 2015) [21]. Current standards for wound care in companion animals focus on cleaning and debriding the wound, maintaining a moist healing environment, preventing infection, and minimizing dead space to enhance the healing process. (Campbell, 2006) [7]. Tilapia fish skin (NTFS) presents a promising alternative source of collagen, which can be utilized as an occlusive biological dressing for the treatment of burns and wounds (Verde *et al*. 2021) [26]. Tilapia fish skin is rich in type I and type III collagen (Lima-Junior *et al.* 2019) [16] which is a biocompatible collagen, suitable for use in clinical regenerative medicine due to its lack of α-Gal antigen, preventing immune responses (Alves *et al*. 2018 [3]). The skin is rich in Omega-3 fatty acids, particularly EPA and DHA, which enhance the skin barrier, possess antimicrobial properties, modulate inflammation, and influence cytokine activity to support wound healing (Seth *et al.* 2022) [23]. Tilapia fish skin contains non-infectious microbiota (Junior *et al*. 2016) [14], making its production process milder, preserving key bioactive components such as collagen structure and omega-3 fatty acids. The present study describes the effectiveness of Nile tilapia fish skin for management of skin wound in dogs.

Materials and Methods

Nile tilapia fishes (*Oreochromis niloticus*) were procured from the Fisheries Research and Information Centre (Inland) located in Hebbal, Bengaluru. The fishes were rinsed and descaled. Following descaling, incisions were made along the three edges of the fish using a sterilized B.P. surgical blade, and the skin was carefully detached from the underlying tissues with the assistance of forceps. Subsequently, the skin was washed with tap water to eliminate residual blood and contaminants. Any remaining muscle and soft tissue were removed from each skin sample using a number 22 B.P. blade under aseptic conditions**.** Extracted skin was subjected to chemical processing in an aseptic environment under vertical laminar airflow at the Department of Veterinary Microbiology. The skins were meticulously cleansed with sterile saline and subjected to sequential treatments comprising 90 minutes in 2% chlorhexidine, 60 minutes in a 3:1 mixture of 99.5% anhydrous glycerol and 2% chlorhexidine and a subsequent 60-minute immersion in 99.5% glycerol, with sterile saline rinses between each step. Following the final lavage with 0.9% sterile saline, the skins were aseptically packaged in sterile transparent packets and stored at 4 °C for subsequent use. Sterile swabs were taken from Nile Tilapia skin pre- and post-processing to assess total viable counts. Skin strips (0.5 × 0.5 cm) were fixed in 10% neutral buffered formalin for histological analysis using H&E and Masson's trichrome staining to evaluate structural features and collagen organization.

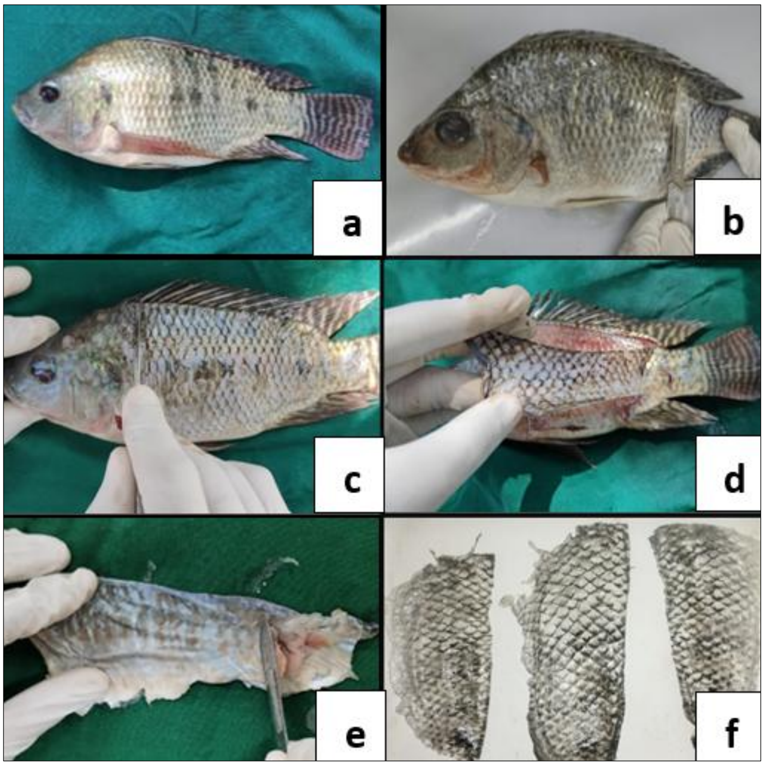

Fig 1: a. Nile Tilapia fish b. Descaling c. Skin incisions made with BP blade No - 22 d. Separation of skin from underlying structures e. Removal of excessive soft tissue using B. P. blade f. Extracted and cleaned NTFS

The present study was carried out in six clinical cases of dogs with superficial skin wounds (Dog 1 to Dog 6) presented to the Department of Veterinary Surgery and Radiology, Veterinary College Hospital, Hebbal, Bengaluru. Detailed anamnesis, thorough physical examination was conducted and blood samples were collected for hematobiochemical examination.

Table 1: Anamnesis of the six cats under study

Dog No.	Breed	Age	Gender	Aetiology	Location
1.	Mudhol	4 months	female	Automobile accident	Right hock
2.	Pomeranian	5 years	Female	Automobile accident	Right radius & ulna
3.	Non-descript	5 months	Female	Unknown	Left metacarpal
4.	Non-descript	2 months	Female	Automobile accident	Left tarsal
5.	Non-descript	1 year	Male	Automobile accident	Right tarsal
6.	Labrador retriever	2 years	male	Dehiscence	Right tibia

All dogs were subjected to fasting for 12 hours preoperatively. Ceftriaxone (25 mg/kg, IV) and Meloxicam (0.2 mg/kg, SC) were administered 30 minutes before surgery as prophylactic antibiotic and analgesic, respectively. Sedation was achieved with Atropine sulphate (0.04 mg/kg, IM) and Xylazine hydrochloride (1 mg/kg, IM). Anaesthesia was induced and maintained with Thiopentone sodium (12.5 mg/kg, IV) to effect.

Dogs were positioned with the wound site facing upward to facilitate the surgical intervention. The hair surrounding the wound area was clipped. wound bed was debrided and wound edges were freshened with a sterile B. P. blade. The preserved Tilapia skin was thoroughly washed with normal saline for 5 minutes. The fish skin was then trimmed to match the dimensions of the recipient site, ensuring that the wound's edges were covered by a minimum of one centimetre of the processed fish skin to prevent any exposure of the wound from movement. The fish skin was positioned over the wound with the scale side facing upward and held in place using 3-0 monofilament polyamide sutures in a simple interrupted pattern. After application, the tilapia dressing was covered with paraffin gauze to prevent it from sticking to the bandage. This was followed by the placement of a soft padded bandage to manage exudate, and a self-adhesive cohesive bandage (Fig 2).

Postoperatively, Cephalexin (25 mg/kg, PO, BID) was administered for five days, and Carprofen (2 mg/kg, PO, SID) for three days for analgesia. An Elizabethan collar was recommended to prevent self-trauma. Wounds were evaluated sequentially at regular intervals to monitor healing.

Results and Discussion

The extraction of NTFS was straightforward following similar methods used by Arun *et al*. (2022) [4] and Alisha (2023) [2]. Nile tilapia fish skin was chemically processed and sterilized as a biological dressing following the protocol of Choi *et al.* (2021) [8], modified from Costa *et al.* (2019) [9]. Costa *et al.* (2019) [9] recommended cold sterilization as an effective method for Tilapia skin sterilization while preserving collagen, omega-3 fatty acids, and its three-dimensional matrix.

Histological examination of unprocessed Nile Tilapia skin revealed densely packed dermal collagen fibres and superficial melanophores. Processed skin showed mild collagen disaggregation, occasional focal disruption, and reduced staining intensity, likely due to the low chlorhexidine concentration used, as levels between 0.5% and 2.0% are not associated with significant collagen fibril dissociation. These findings concur with those of Alves *et al.* (2018) [3] and Verde *et al*. (2021) [26].

Microbiological analysis of fish skin showed a pre-processing Total Viable Count (TVC) of 8.5-9.3 cfu(colony forming unit)/swab, with negligible growth post-processing, confirming effective sterilization. This outcome is attributed to chlorhexidine's broad-spectrum bactericidal activity and aligns with findings by Alves *et al*. (2018) [3], Choi *et al.* (2021) [8] and Alisha (2023) [2].

Gross wound evaluation

A gross wound assessment was conducted before (on day 0) and after (3rd, 7th, 15th, 21st, and 30th) the application of fish skin following the Bates-Jenson Wound Assessment Tool as recommended by MacEwan *et al.* (2017) [17]. By 7th postoperative day, necrotic tissue was absent in all cases except Case 4; by 15th day, all wounds exhibited complete absence of necrosis. All wounds were free from exudates by day 15. This could be attributed to Tilapia skin's antimicrobial properties, which help prevent infection and promote a moist healing environment. These findings align with studies by Choi *et al.* (2021) [8], Costa *et al.* (2020) [10], Alisha (2023) [2] and Tozetto *et al.* (2023) [25]. The application of Tilapia skins significantly promoted granulation tissue formation, with all cases showing healthy, bright red tissue by third day. Tilapia skin, enriched with collagen and amino acids such as proline and alanine, promoted fibroblast proliferation and extracellular matrix deposition, facilitating granulation tissue formation. These observations align with Sastri *et al.* (2022) [22], Esmaeili *et al.* (2023) [12], and Alisha (2023) [2]. Application of Nile Tilapia Fish Skin enhanced epithelialization. By day 15, Case 3 was fully epithelialized, while others exceeded 75% coverage. By day 21, all cases except Case 6 achieved complete epithelialization, with Case 6 exceeding 75% coverage. By day 30, all wounds reached 100% epithelial coverage. Tilapia skin provides a porous, robust extracellular matrix that facilitates keratinocyte migration and ingrowth, accelerating epithelial tissue formation. These findings are consistent with Magnusson *et al.* (2017) [18], Mauer *et al.* (2022) [20] and Manzoor *et al.* (2023) [19]. Tilapia skin was well tolerated, with no discomfort or allergic reactions observed, and wound healing progressed with minimal scar formation (Fig. 2).

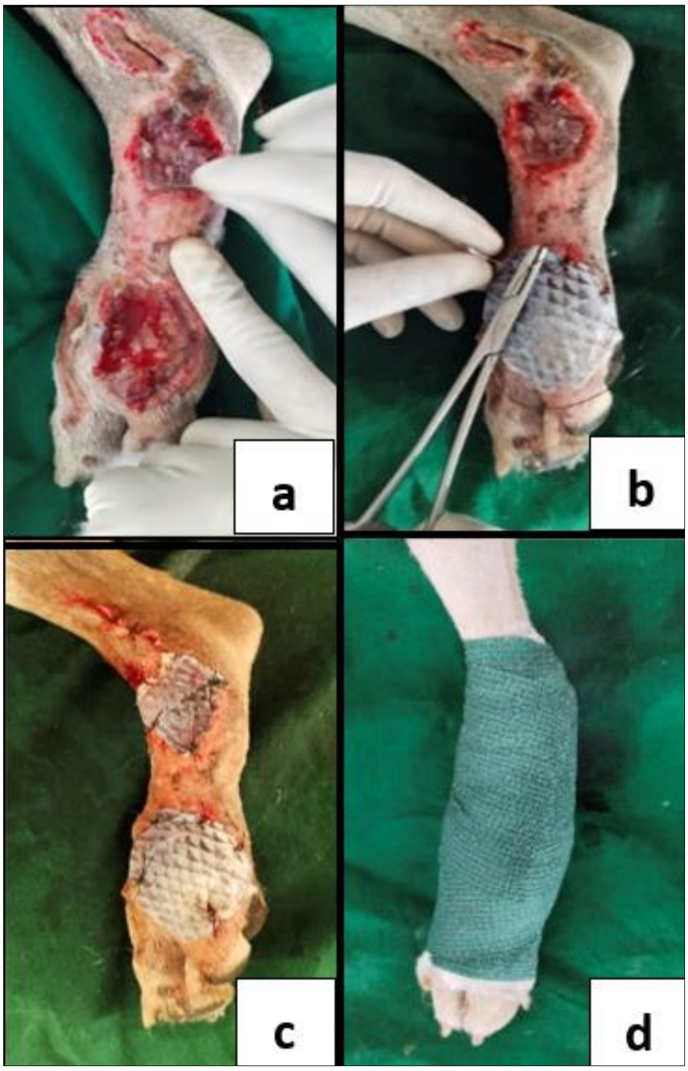

Fig 2: a. Debridement of wound bed b. Suturing of NTFS to the wound bed c. Tilapia skin fixed to the wound bed d. Post operative dressing

The wound dimensions were measured using the ImitoMeasure, smartphone allowing application digital quantification of wound size, as outlined by Bodea *et al.* (2021) [6]. This non-contact digital planimetry method proved to be an efficient, non-invasive, and cost-effective approach for wound measurement. Regular use of the same smartphone for assessments ensured consistent results. This method aligns with findings from Biagioni *et al.* (2021) [5], Sastri *et al.* (2022) [22], Aarts *et al.* (2023) [1] and Alisha (2023) [2].

Rate of advance of the wound margin per day

This formula accurately represented the healing rate, independent of wound area according to Cukjati *et al.* 2001[11] given by the formula, Rate of Advance of the wound margin per day in cm = 2 S0 [cm/day] / P0 T Where, S0 = initial wound area, P0= initial wound perimeter, T= time needed to complete wound closure in days. In this study, advancement rate was 0.104 ± 0.005 cm/day. Tobias and Johnston (2017) [24] reported a typical epithelialization rate of 0.1 cm/day for primarily closed wounds. The tilapia skin application led to a epithelialization rate of 0.104 cm/day for secondary wound healing, indicating faster healing. This may be due to the highly porous extracellular matrix of Nile tilapia fish skin, which supports keratinocyte ingrowth and cell migration. Additionally, the collagen content in tilapia skin likely aids fibroblast proliferation, further accelerating wound healing. These findings are consistent with studies by Sastri *et al.* (2022) [22], Choi *et al.* (2021) [8] and Alisha (2023) [2].

Histopathological analysis (Fig. 3) revealed progressive and accelerated granulation tissue formation with increasing maturity over time. This is likely due to collagenase mediated degradation of Tilapia skin, providing collagen-rich fragments that create a favourable wound microenvironment, stimulating TGF-β (Transforming Growth Factor beta) expression, and promote granulation tissue growth, consistent with Li *et al.* (2021) [15] and similar reports by Hu *et al.* (2017) [13], Choi *et al.* (2021) [8], Zhang *et al.* (2022) [27] and Manzoor *et al.* (2023) [19]. Histology also indicated accelerated epithelialization, likely mediated by tilapia skin induced vascular endothelial growth factor (VEGF) and transforming growth factor β1 expression, as demonstrated by Zhang *et al.* (2022) [27].

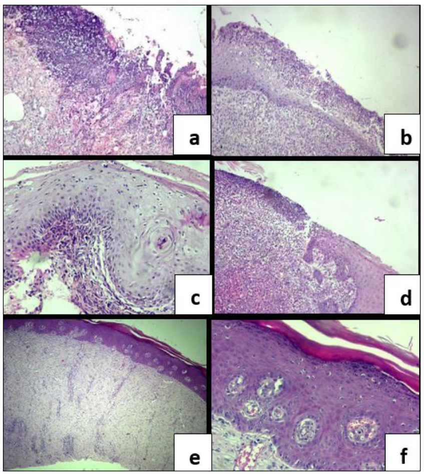

Fig 3: a. Section showing no epithelialization at day 0(10x) b. Day 7 showing no epithelialization(10x) c. Day 15 with thin partial epithelization layer(10x) d. Day 21 with complete epithelization layer(10x) e. Day 30 showing complete & mature re-epithelization layer(10x) f. Day 30 (40x).

Conclusion

In this study, the superficial skin wound in dogs were treated effectively with the use of tilapia skin as a biological dressing. Tilapia was well tolerated, facilitating complete re-epithelialization of the wound. Nile Tilapia fish skin has proven to be an effective, cost-efficient, and safe biological dressing for managing skin wounds in dogs, owing to its unique properties such as abundant collagen, Omega-3 polyunsaturated fatty acids, a porous extracellular matrix, and high biocompatibility.

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