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Nile Tilapia Fish Skins (NTFS): Review of a piscine biomaterial for burns in veterinary practice

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Abstract
Burn injuries involve damage to the integument and underlying tissues caused by thermal, chemical, electrical, or mechanical agents and remain a major challenge in both human and veterinary medicine. Conventional burn management relies largely on topical antimicrobials and biosynthetic dressings, which may be costly, require frequent changes, and cause significant pain.

Nile Tilapia Fish Skin (NTFS), a waste by-product of the fish industry, has emerged as a novel, cost-effective biological dressing with promising therapeutic potential. Rich in type I collagen, omega-3 fatty acids, and possessing high tensile strength, NTFS functions as an effective occlusive xenograft that maintains wound moisture, reduces protein and plasma loss, minimizes pain, and accelerates re-epithelialization.

Initially developed in Brazil to address the scarcity of human skin grafts, NTFS has demonstrated significant success in treating partial and full-thickness burns, reducing healing time and frequency of dressing changes compared with conventional silver-based therapies. Its safety and efficacy have been supported by microbiological, histological, and biomechanical studies, showing preservation of collagen structure even after sterilization. Beyond human medicine, NTFS has shown remarkable results in veterinary practice, including treatment of severe burn injuries and chronic wounds in wildlife, companion animals, and equines, often without the need for systemic antibiotics.

The preparation of NTFS involves rigorous cleaning, glycerolization, antimicrobial treatment, and optional gamma irradiation to ensure sterility and long shelf life of up to two years under refrigeration. Given its abundance, low cost, biocompatibility, and therapeutic advantages, NTFS represents a sustainable and effective alternative to conventional burn dressings.

Keywords: Collagen Xenograft, Nile tilapia fish skin, biological dressing, burn wound management, veterinary medicine, wound healing

Introduction

A burn is an injury to the integumentary system involving the skin, subcutaneous tissue, and sometimes underlying muscles, caused by exposure to high temperatures or chemical agents. It can be defined as the tissue damage resulting from excessive absorption of heat by the skin (Nandi *et al.*, 2009) [23]. Based on etiology, burns are classified as Thermal, chemical, mechanical and electrical based on cause of burns i.e. heat, acid or alkali, friction due to rope or leash and electrical cord exposure or lightening respectively. Based on involvement of area involved and severity, Fossum (2013) [8] classified burns as First degree (superficial); Second degree (Deep partial-thickness); Third degree (full thickness) and fourth degree burns.

Traditional burn wound therapy comprised of application of topical silver preparations like silver sulphadiazine or silver nitrate alone or with cerium nitrate or mafenide (Slatter, 2003) [29]. Several biological materials like boiled potato peel dressings (Keswani and Patil, 1985) [14], autoclaved banana leaf dressings (Gore and Akolekar, 2003) [11], honey dressings (Subrahmanyam, 2007) [30] and papaya pulp dressings (Jayarajan *et al.*, 2016) [13] have been used with varied success for treatment of burns in human patients. Registered biosynthetic alternatives like Biobrane (Greenwood *et. al.*, 2010) [12], Aquacel Ag (Lohana and Potokar, 2006) [19], Integra (Alnababtah, 2010) [1] and Matriderm (Shahrokhi *et al.*, 2014) have been prepared using various biomaterials for treatment of burn wounds.

Marine derived biological alternatives are recently becoming popular. Some of the popular ones are Mantle of *Pinctada martensii* (Yang *et al.*, 2019) [31], Chitosan films (Dai *et al.*, 2011) [4], Frog skin and its derivatives (Falcao *et al.* 2002; Liu *et al.* 2014) [6, 18]. But, as phytomedicine impregnated dressings lack some of the properties required in rapid and accelerated wound healing, and biosynthetic dressing materials prove to be an uneconomical option, there was a need of a dressing material which is cost effective, easily available, possessed properties that speed up wound healing and at the same time prevent pain and secondary bacterial infection. That is when Nile Tilapia Fish Skins (NTFS) came into picture. It was invented out of sheer necessity in Brazil because there were more than 30 registered tertiary burn care centres and only 4 Skin banks available. As Brazil is a developing nation, it was not economically feasible for the country to import skins from other developed nations. Also, Brazil is the 4th largest producer of Tilapia Fish and its skin being the inedible part is thrown away as waste product with only about 1% being utilized in leather and baggage making industry.

Use in Human medicine

The first patient to receive Nile tilapia fish skin for treatment burn wounds was 36-year-old Maria Ines Candido da Silva in October 2016. She suffered 2nd degree wounds on her arms, face and neck as a result of explosion of a gas cooker canister in a terrible workplace accident. She was treated as a part of the pilot project. This novel marine derived biological dressing was developed over a period of more than two years by a research team led by Dr. Odorico Moraes, Prof. Elisabete Moraes, and Dr. Ana Paula Negreiros at the Nucleus of Research and Development of Medicines (NPDM), Federal University of Ceará (UFC). The project is coordinated by plastic surgeons Dr. Edmar Maciel from the Burns Unit of Dr. José Frota Institute (IJF), Fortaleza, and Dr. Marcelo Borges from the SOS Burns and Wounds Unit at São Marcos Hospital, Recife, in northeastern Brazil.

Until January 2018, 129 patients were successfully treated using this treatment, according to Dr. Odorico de Moraes. Due to its huge success in healing wounds, it was also used as a biological graft for neovaginoplasty in 41-year-old lady suffering from vaginal stenosis as a result of radiotherapy for vaginal cancer (Dias *et al.*, 2019) [5] and in Male to female Gender-affirming surgery as a biocompatible graft (Rodriguez *et al.*, 2020) [26].

Lima Junior *et al.* (2020) [26] described several advantages of use of NTFS as occlusive biological dressing. Due to its adherence to skin, it prevents external contamination and limits loss of protein and plasma that can generate dehydration and cause death. It was reported that intensity of pain was significantly lower in patients treated with NTFS than in those treated with silver sulphadiazine cream (SSDC) along with reduced need for dressing changes in NTFS compared to SSDC. They also noted a more clinically relevant healing time reduction for Deep Partial Thickness Burns (DPTB) whereas, the number of days for complete reepithelialization was significantly lower in the groups treated with NTFS than in the groups treated with SSDC. Franco *et al.* (2013) [9] compared the skins of tilapia (*Oreochromis niloticus*), pacu and tambaqui regarding histology, centesimal composition, physical-chemical and physical-mechanical characteristics. It was found out that the thicker the collagen fibres the more resistant the skin is. Alves *et al.* (2018) [2] studied Nile Tilapia skin and concluded that the tensiometric properties did not change even after undergoing chemical sterilization and radiation.

Tilapia and its farming

There are about 70 species of tilapias fish, of which nine species are used in aquaculture world-wide. The Mozambique tilapia (*Oreochromis mossambicus*), blue tilapia (*O. aureus*), Nile tilapia (*O. niloticus*), Zanzibar tilapia (*O. hornorum*), and the red belly tilapia (*O. zilli*) are important commercial species of tilapia. About 75% of tilapia raised worldwide are of the Nile Tilapia/Nile Perch breed (Mitchell, 2015) [22].

Fig 1: Nile Tilapia (*Oreochromis niloticus*)

Characteristics of Nile tilapia

Tilapia may be raised in saline water, as well as in pond or cage systems, because it is tolerant of a wide range of aquaculture environments. It can only live and reproduce in warmer water, making it a seasonal species. A tilapia farm should have water that is between 27 to 30 °C. India is therefore a good place to grow tilapia. "Monosex tilapia fish farming" is the practice of raising male tilapia fish by isolating females since male tilapia grow more quickly than female tilapia. Tilapia uses small combs in its gills to filter algae from water, eats plants, and adores duckweed, which is as high in protein as commercial fish feed. It is an omnivorous grazer that consumes benthic fauna, phytoplankton, periphyton, aquatic plants, small invertebrates, detritus, and bacterial films connected to detritus.

Breeding characteristics of Nile tilapia

In ponds or cages, sexual maturity occurs between 5 to 6 months of age. When the water temperature hits 24 °C, spawning starts. Three months before tilapia reach a size suitable for consumption, they begin reproducing. Adult female tilapia fish can produce up to 100 fries per week due to their exceptionally rapid breeding rate. After hatching, the fry are brooded until the yolk sac is absorbed by the female, who incubates the eggs in her mouth. A female weighing 600-1000 grammes can generate 1,000-1,500 eggs every spawn, but a female weighing 100 grammes will produce roughly 100 eggs.

Nile tilapia have a lifespan of over ten years and can weigh more than five kilogrammes. While many native animals are careless parents, tilapia, an omnivore, is a superb example of parental care. Both males and females of tilapia dig burrows to construct nests, and they protect their offspring until they are juveniles. They retain the juveniles in their mouths when predators get close.

Tilapia farming in India

Tilapia species (*Oreochromis mossambicus*) was introduced in India in 1952. It is an aggressive invasive species of in India, now occupying most of water bodies in the state of Karnataka, elbowing out many native species. Tilapia, commonly called as Jilepi in Tamil is a fish native to Africa and parts of west Asia. Tilapia can survive in waters with extremely low oxygen level and its eggs are remaining viable even under dry conditions. It has destroyed the habitat of native fauna, which are already destroyed by pollution. Hence it was banned in 1959. It was banned because it wreaked havoc and proved a threat to the native biodiversity because, As a signatory of the convention on Biological Diversity, India has a duty to protect its wild germplasm before it is totally eradicated by exotic species (Kolappan, 2016) [15].

After being introduced in 1970, Nile tilapia became a significant part of aquaculture in India and become famous as "Aquatic chicken". In 2015, the National Fisheries Development Board released guidelines for the fish's responsible production. GIFT (genetically enhanced tilapia) farming was authorised under certain conditions (Mitchell, 2015) [22].

In 2005, the Yamuna River contained only a negligible presence of Nile tilapia; however, within a span of two years, its share increased to approximately 3.5% of the total fish population in the river. At present, tilapia constitute around 7% of the total fish species in the Ganga river system. For tilapia farming in India, the optimum temperature for best growth is 15 to 35 °C. However, tilapia can survive in 10 to 40 °C temperature.

Histology of fish skin

The external (outside medium) and internal (body) environments must be physically balanced by the skin. It is the first line of defence against mechanical damage and pathogens. The epidermis (1) and dermis are the two normal layers of teleost skin. This layer typically consists of two strata: a deeper compactum (3) and an upper spongiosum (2) directly below the epidermis. Collagen has a blue stain. Cross (4) and longitudinal (5) slices of the left striated muscles (Genten *et al*., 2009) [10]. Trichrome Stain perprtuates visualization of collagen (blue), muscle (red) and nuclei purple. Reddish purple colour is the top layer of skin composed of keratin.

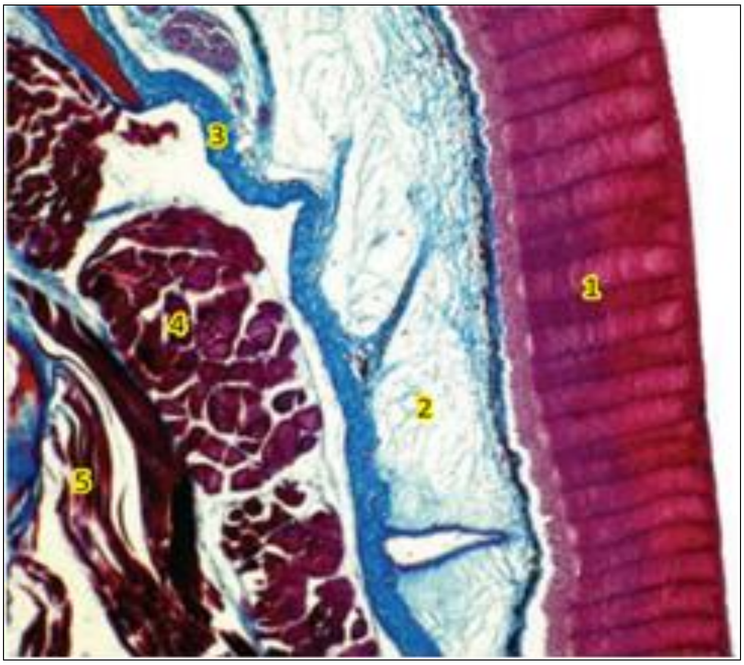

Fig 2: Histology of fish skin; 1: Epidermis, 2: Upper spongiosum, 3: Depper compactum, 4 and 5: Cross and longitudinal sections of left straited muscles

Normal microbiota of fish skin and mouth comprises of

- *Pseudomonas* spp.
- *Aeromonas* spp.
- *Shewanella putrefaciens*
- *Moraxella* spp.
- *Acinetobacter* spp.

A quantitative culture shows in all samples studied, a Colony Forming Unit (CFU) load of less than 1,00,000 CFU/ gram of tissue. This value is a cutoff standardized by the American society of Microbiology to differentiate the causal agent from colonizer isolates in human wounds. In light of these results and considering the absence of infectious signs in the fish samples, we conclude that CFU values found in this study indicate presence of normal, non-infectious microbiota (Lima Junior *et al.,* 2016) [16].

Preparation of sterilized Nile Tilapia fish skins

Preliminary skin retrieval

Fishes are obtained from fish farms that use culture systems (tanks-net), passing through a sterilization process, as described below. After slaughtering, Using a turquoise tool, tilapia skins weighing between 800 and 1000 grammes are removed, rinsed under running water to remove any remaining blood or impurities, and then placed in sterile saline (0.9% NaCl solution) that has been cooled to 4 °C for a final washing. After that, the excess muscle that is still connected to the skin is cut into 10.0 cm by 5.0 cm pieces, cleansed with saline, and sterilised.

Fig 3: Harvesting and slaughter of NTF

Figu 4: Deskinning of fish

Fig 5: Washing and cleaning

Fig 6: Clean room environment facility at the Laboratory workstation

Processing of sterilized Nile tilapia fish skins

- **Step 1:** The skins are placed in a sterile container containing 2% chlorhexidine gluconate and left for 30 min.
- **Step 2:** Following step 1, the skins will be washed with sterile saline solution and placed in another container containing 2% chlorhexidine gluconate solution and left for another 30 minutes.
- **Step 3:** After rinsing the skins in sterile saline solution, they are placed in a container with 50% glycerol, 49% sterile saline solution, and 1% penicillin, streptomycin, and fungisol solution. They are then packed for transportation in an isothermal box with ice. The following procedures will be carried out in a sterile setting with either horizontal or vertical laminar flow in the NPDM of UFC.
- **Step 4:** Before 24 hours, the skins should be taken out of the previous solution, cleaned with sterile saline, and put in a sterile, hermetically sealed container with 75% glycerol, 24% saline, and a 1% solution containing penicillin, streptomycin, and fungisol. The skins are then massaged in this solution for 5 minutes and kept in a water bath (centrifuge) for 3 hours at a constant speed of 15 revolutions per minute.
- **Step 5:** The skins will be taken out, cleaned with sterile saline again and placed in another sterile and hermetic container containing 99% glycerol, 1% solution with penicillin, streptomycin and fungisol, with massage of the skins for 5 minutes in this solution and and they will be kept in a water bath in a centrifuge set at 37 °C and 15 revolutions per minute for an additional three hours.
- **Step 6:** Following the final step of the glycerol, the skins will be sealed in sterile double plastic envelopes and stored at 4 °C for up to two years.

Starting on the natural skin, seven microbiological tests for gram-positive and gram-negative bacteria and fungus will be carried out, both before and after the first of the six previously mentioned stages. The skin will be usable if the Bioburden levels, which are used for bacterial counts (i.e., Bioburden Microbiological Analysis or Microbial Limit Test, are within the acceptable limits.

When the skins are used, they will be taken out of the envelope and cleaned three times for five minutes each in various sterile saline solutions. Following this procedures, the skins will be trimmed to fit the wound and used as an occlusive dressing. A further supplementary radiation sterilization sterilisation using Gamma rays from radioisotope Cobalt 60 should be added if the bacterial count is more than 10/3.

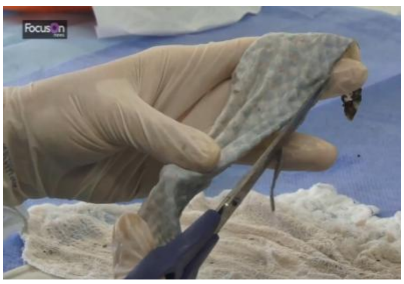

Fig 7: Cutting of NTFS bandages in 5 X 10 cms pieces

Fig 8: Sterilizing in 2% Chlorhexidine gluconate

Fig 9: Placing in Isothermal box for transporting at NPDM of UFC

Fig 10: Hermetically sealed container

Fig 11: Centrifuge water bath machine

Fig 12: Sealing of NTFS in plastic envelope

Use in veterinary medicine

Dr. Jamie peyton, chief of integrative medicine and critical care veterinarian at UC Davis Veterinary Teaching Hospital, first started this treatment during the California's Thomas wildfire. She used Nile tilapia fish skins bandages to cover the burn wounds of rescued wild animals which included a Mountain Lion and two Brown Bears. Since the burn wounds of these rescued animals were severe and it was not possible to follow the routine burn wound care, these biological bandages were used along with supplementary pain relief therapy including acupuncture and laser therapy.

The mountain lion treated using NFTS consumed its bandage and hence when a bear came in with burn wounds, it was decided to cover the fish skin on burn wounds using rice paper and corn husk, so as to delay eating of bandages for few more days.

Because these bandages gave good results like improved healing, good pain relief and fast recovery, its use was also done in other species.

Fig 13: Burn wounds of Mountain lion and Brown bear

Fig 14: Burn wounds after application of NTFS in Mountain lion and Brown bear

Fig 15: Bandages covered with rice paper and corn in brown bear

An 18 months old pony suffering from a malicious acid attack had severe second and third degree burns on the face. NTFS bandages were used to cover her wounds and it showed an excellent accelerated healing of wound within 2 weeks.

Fig 16: Pony with chemical burns before and 14 days after application of biological

Nebraska Humane Society (NHS), an animal shelter in Nebraska, USA, treated a cat rescued from a terrible house-fire. The male cat, King suffered burns on more than 50% of total body surface area (TBSA). Fresh Nile Tilapia fish was bought from the frozen market, the skin removed and sterilized in an antiseptic solution and it was sutured on the wounds.

Fig 17: Retrieval, sterilization and application of NTFS bandage on burn wounds of King male cat

Fig 18: Preoperative day 1 image (immediate after application)

Fig 19: Healing of the wounds after 10 days of application of NTFS bandages

Silva *et al.* (2019) [28] used NTFS bandages in chronic wounds in equines and reported complete recovery of wound without use of any topical or systemic antibiotics in two horses, whereas Costa *et al.* (2020) [26] reported complete recovery of traumatic wound within 42 days. The NTFS bandages in both the cases were placed over the wound after thorough cleaning and bandages were changed every 7 days after washing the wound with chlorhexidine solution.

Fig 20: Chronic wounds after 42 days of application of NTFS bandages (Horse 1)

Fig 21: Chronic wounds after 35 days of application of NTFS bandages (Horse 2)

Fig 22: Complete healing of traumatic wound after 42 days of application of NTFS bandages

Comparative evaluation of Nile tilapia fish skin

Nile tilapia fish skin xenograft and Silver based hydrofiber dressing were compared in treatment of second degree burns in adult and it was found out that NTFS xenograft is an effective occlusive biological dressing with treatment time similar to Silver dressing. In addition, neither the degree of pain following dressing nor the necessity for replacement during therapy showed any discernible intergroup differences (Miranda and Brandt, 2019) [21].

Fig 23: NTFS xenograft Vs. Silver based hydrofiber dressing

Studies conducted on comparison of Acellular fish skin (AFS) graft and Foetal bovine collagen (FBC) graft for treatment of chronic wounds in porcine models suggests that the histologic features of AFS make it amenable to cellular modulation, and its ability to provide omega-3 fatty acids to the local tissue makes it an unprecedented anti-inflammatory, antibacterial, antiviral, and analgesic component, thus causing recovery of wounds within 40-45 days as opposed to 60 days required for wound healing with FBC (Patel and Lantis, 2019) [25].

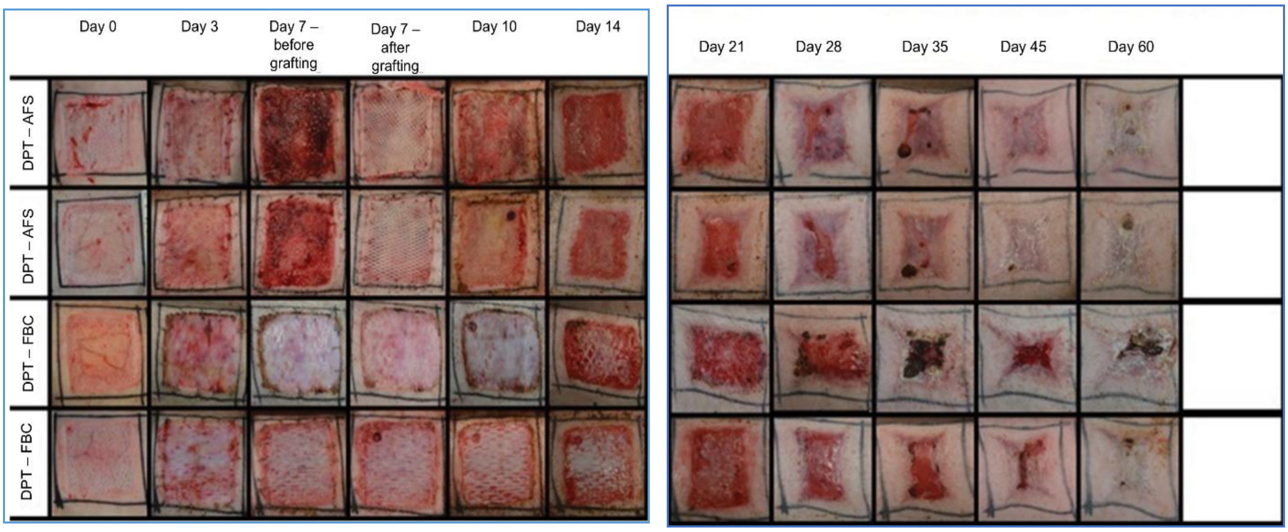

Fig 24: AFS graft Vs. FBC graft for treatment of chronic wounds

Conclusions

Tilapia skin are waste by-product of fish industry, thus economically feasible and a more effective option. It has long shelf-life and hence it can be used up to 2 years once prepared, packed and stored at refrigeration temperature.

NTFS has large composition of Type I collagen and its Nano fibers speed up the skin wound healing. Tilapia skin has good tensile strength and provides high resistance. It protects the wound like a barrier, keeping it moist and healthy as well as preventing loss of moisture and thus dehydration. The Omega-3 fatty acids in the skin have anti-inflammatory effect which provides pain relief. Nile tilapia fish has antioxidant and proteolytic enzymes which cause release of amino-acids and peptides that have anti-microbial activity.

NTFS Bandages yield good results compared to the traditional therapy and available biological and biosynthetic alternatives. As it is a naturally derived biomaterial, there are less chances of complications like intestinal blockage following accidental consumption of these biological bandages. NTFS causes no relevant changes in hematological or biological parameters and no side effects have been observed making it a good alternative.

Future prospects

Nile Tilapia Fish Skin Bandages provide adequate amount of Type I collagen, eliminate pain and accelerate wound healing seemingly becoming a promising agent in burn care and must be incorporated in Veterinary Burn care in India.

Use of NTFS bandages can be explored as an alternative to skin grafting, as a protective in surgical procedures involving resection of large amount of tissue. Its use can also be studied in field of Veterinary dermatology for affections not responding to routine therapies.

Conflict of Interest

Not available

Financial Support

Not available

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