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Research paper

Fish skin as a biological dressing for burn injuries

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ABSTRACT

Background: Recently, biological dressings have become popular in treating burn injuries. Fish skin has non-infectious microbiota, high levels of type 1 and type 3 collagen, and a structural property similar to human skin, making it a promising xenograft for managing burn wounds. In this study, we aimed to evaluate the effectiveness of fish skin as a physiological dressing cover in patients with burn injuries.

Method: From June 2017 to June 2021, 18 patients who were admitted to our center within 3 days from a thermal injury, burns with a partial thickness of approximately 20% of the total body surface area (TBSA) or complete partial-thickness burns ranging from 5% to 15% of the TBSA, and treated with the Shaour (*Lethrinus lentjan*) fish skin xenografts were enrolled in this retrospective study. The demographic characteristics of the participants, wound healing process, and final outcome were analyzed.

Results: The mean age of the patients was 31.86 ± 9.14 years (range 19–46) which encompassed 12 (66.7%) men and 6 (33.3%) women. Superficial partial-thickness burns were seen in 13 (72.2%) patients, whereas full-thickness burns were seen in five (27.8%) patients. The mean time to 90% epithelialization and 100% epithelialization were 11.05 ± 2.57 days and 17.27 ± 2.05 days, respectively. The mean pain scores were 6.94 ± 0.72 and 5.22 ± 0.64 on days 7 and 15, respectively. Neither negative consequence nor allergic reaction was associated with using the fish skin grafts. The main reduction in the pain score from the first visit (7 days) to the second visit (15 days) was 1.72 ± 0.95 ($p < 0.001$).

Conclusion: Acellular fish skin is a useful tool for wound healing treatment in complete and partial thickness burns. To validate this result, prospective cohort studies with long-term post-procedural follow-up are needed.

Keywords: burns, biological dressing, *Lethrinus lentjan*, shaour fish skin, wound healing

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INTRODUCTION

Burns kill 180,000 people each year according to the World Health Organization (WHO). Furthermore, nonfatal burns lead to prolonged hospital stays, deformities, as well as disability[1]. Early resection and use of split skin grafting is the line of therapy for superficial, deep dermal, and full-thickness burn injuries to prevent major comorbidities such as sepsis and multi-organ failure[2].

Autologous skin accessibility is a challenge when treating large burns, and burn surgeons frequently rely on allogenic and xenogeneic skin for the momentary covers after surgical debridement. This momentary coverage is primarily derived from human cadavers and pig skin[2]. Using such cadaveric and pig skin grafts involves the risk of an autoimmune response as well as the transmission of bacterial and/or viral infections; and many cultures, such as in Muslim countries, object to using porcine grafts[2,3]. Cadaveric skin is in limited supply and thus incredibly costly[3].

A new xenograft resource based on acellular fish skin has recently become available. It is a safe and reliable skin substitute, which is neither associated with the risk of viral infections nor autoimmune reactions. Moreover, it was successfully used in the healing process of acute and difficult-to-heal wounds such as diabetic foot ulcers and chronic leg wounds of various types[3,4].

Lethrinus lentjan, also known as Shaour or Hegella in Yemeni and Saudi Arabian fisheries, is one of the most important Lethrinus fishes found in the Red Sea and the Arabian Gulf. The Lethrinids, commonly known as the emperor fish family (Emperors), are a group of coastal fishes that dwell mostly on or near reefs and are vital components of numerous coastal fisheries[5,6].

Previous studies focused on the *Aeromonas* load in Tilapia and different fish species[7,8]. *Aeromonas* infections are generally typified by symptoms, including tissue swelling, edema, necrosis, red blisters, ulceration, abnormal protrusion of the eyeball (exophthalmia), and hemorrhagic septicemia. Regarding this infection, Shaour was superior to Tilapia, and a previous study showed no evidence of pathogenicity was found in Shaour species when tested in laboratory-based experiments.[9] In this study, we aimed to evaluate the effectiveness of the skin of Shaour fish as a xenograft dressing to manage burn wounds in patients.

MATERIALS AND METHODS

Study design

This retrospective study was approved by the ethics committees of the Ibb University of Medical Sciences and was performed in accordance with the Declaration of Helsinki. This study was approved by the Yemen Association of plastic and reconstructive surgery and the Scientific Department of Burns Department at Al-Thawra General Hospital in Al-Hodeidah, headed by Dr. Adel Kotkot.

The study participants were recruited from a local burn treatment center, Al-Thora hospital, Al-Hudaydah, Yemen, from January 2017 to January 2021. Eighteen patients having partial or split-thickness skin grafting for burn injuries were treated with the Shaour fish skin xenografts. The following information was collected retrospectively: Sex, age, size of the burn, procedure, pain score, and final outcome.

The inclusion criteria were dermal wounds caused by burns with a partial thickness of approximately 20% of the total body surface area (TBSA) or complete partial-thickness burns ranging from 5% to 15% of the TBSA referred to our center within 72 hours of the thermal injury and age >16 years.

The exclusion criteria were burns $>20\%$ TBSA, previous treatment for the existing burn, chemical or electrical burns, use of medications which may have an effect on wound healing, such as steroids, hypersensitivity to materials used in the study or related compounds, pregnancy, and miscarriage in the 12 weeks before the scheduled start of treatment. Furthermore due to the difficulty of our material to adhere to these areas, patients with burns on the face, neck, genitalia, perineum, axillae, groin, and buttocks were excluded.

Preparation of Shaour fish skin xenografts

After removing the fish scales and washing with water, the Shaour fish skin was chemically sterilized with 4% chlorhexidine gluconate (Hibiclens Mölnlycke Health Care, Norcross, GA) and irradiated with an ultraviolet lamp (wavelength 400 nm to 315 nm). It was then tested for microbes, that is, bacteria and fungi and stored in aseptic, refrigerated packaging[10,11].

Procedure

First, the fish skin was soaked in saline. It was then applied and secured with a supplementary covering of dry gauze. Each participant's skin graft donor sites were thus treated. After seven days of surgery, the dressing was changed at the wound site, followed by another at 15 days (surgery did not exceed two procedures) until fully recovered (Figures 1–4).

Outcome assessment

The symptoms and signs of infection were evaluated at each dressing change, and the pain was evaluated using a verbal rating score of 0–10[2]. The number of days until 90% and 100% epithelialization were documented as visually determined by the surgeon[2].

Statistical analysis

The mean ± standard deviation (SD), median, and inter-quartile range (IQR) described the quantitative variables; and frequency (percentage) was used for qualitative variables. The analysis of variance (ANOVA) was applied to compare the difference of the means among various different levels. A p value of <0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) software (IBM SPSS, version 20, Armonk, New York: IBM Corp).

RESULT

The mean age of patients was 31.86 ± 9.14 years (range 19–46) and comprised 12 (66.7%) men and 6 (33.3%) women. The mean body mass index (BMI) was 22.94 ± 2.22 kg/m^2 (range 19–27). Superficial partial thickness burns were seen in 13 (72.2%) patients, whereas 5 (27.8%) of them had full-thickness burns. The size of the donor sites ranged (50(750 cm^2). The mean time to 90% epithelialization was 11.05 ± 2.57 days (range 7–15). The mean time to 100% epithelialization was17.27 ± 2.05 days (range 13–21). The mean pain scores were 6.94 ± 0.72 (range 6–8) and 5.22 ± 0.64 (range 4–6) at days 7 and 15, respectively (Table 1). None of the patients showed noticeable symptoms of infection, neither negative consequences nor allergic reactions associated with using fish skin grafts. The carrying of Shaour fish skin was extremely good, strong, and pliable xenograft and was easy to apply. The efficiency of donor site recovery was assessed to be satisfactory in all the participants (Figure 1–4).

The main reduction in pain score from the first visit (seven days) to the second visit (15 days) was 1.72 ± 0.95 ($p < 0.001$). There was no association between pain scores on the first visit and full and partial thickness burns, 6.80 ± 0.44 vs. 7.00 ± 0.81 ($p = 0.20$). There was no association between pain scores on the second visit and full and partial thickness burns, 5.40 ± 0.54 vs. 5.15 ± 0.68 ($p = 0.83$).

Figure 1. Shaour fish skin graft.

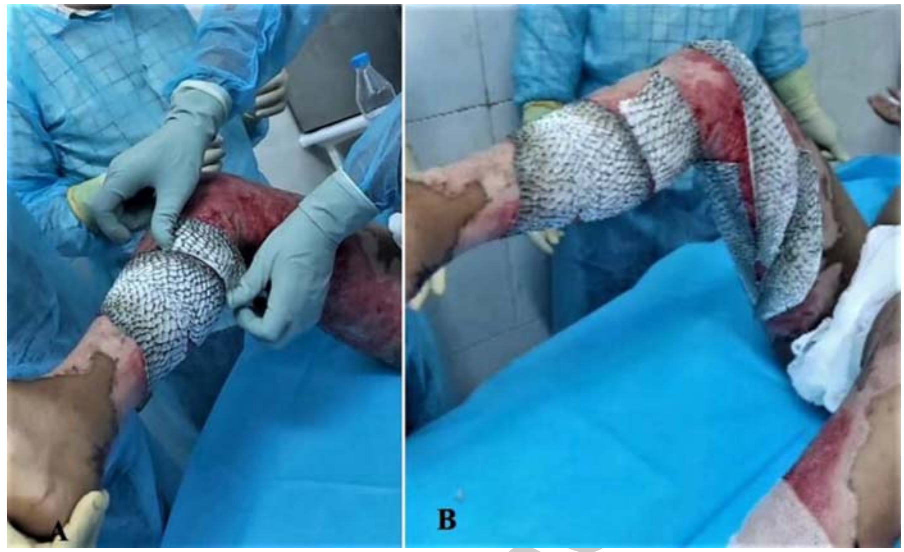

Figure 2. **(A and B). The appearance of the left lower limb during Shaour fish skin graft application, after cleaning the lesion (removing necrotic and fibrinous tissue from the lesion).**

DISCUSSION

Complete healing can be anticipated in two weeks for superficial partial thickness burns (SPTB) and in more than three weeks for deep partial thickness burns (DPTB)[12]. In a study by Alam, the time to 90% epithelialization was an average of 8.5 days, and the time to 100% epithelialization was an average of 11.5 days[2]. According to previous studies, the average time for re-epithelialization in patients with SPTB and DPTB was 11.70 and 21.30 days, respectively[1]. Our study was in some agreement with previous reports, and we reported that the mean time for 90% re-epithelialization was 11 days, and 100% re-epithelialization was seen in 17 days.

The mean age of the patients in our study was 31.86 $\pm$ 9.14 years, which was similar to the study by Júnior et al.[1], where the mean age of the participants was 32.50 $\pm$ 8.15 years.

Chlorhexidine and povidone iodine are commonly used for sterilization and disinfection during a grafting procedure because they are effective against Gram-negative and Gram-positive bacteria and fungi and kill by disrupting the cell membrane[13–15].

An effective sterilization method must eliminate or reduce microbial populations while preserving the structure, bioactive composition, and biocompatibility of the skin graft[10]. Regarding the standard sterilization technique of fish skin, Ibrahim et al. compared the efficiency of three sterilizing agents; chlorhexidine gluconate 4%, povidone iodine 10%, and silver nanoparticles (25 μg/mL) at three different times (5, 10, and 15 min) on Tilapia fish skin based on the microbial count, histology, and collagen properties. They concluded that sterilization of fish skin using silver nanoparticles was the most effective with 100% reduction in microbial growth and no change in collagen content at three different mentioned times[10]. In our study, after removing the fish scales and washing with water, sterilization was performed with chlorhexidine and irradiation with an ultraviolet lamp.

Previous studies have mentioned that chlorhexidine was effective in reducing the microbial load on Tilapia fish skin to varying degrees, and the bactericidal effect increased with increasing contact time[10, 16]. Chlorhexidine is a bisbiguanide compound with broad antibacterial and antifungal activity against both Gram-positive and Gram-negative bacteria and fungi, even in the presence of interfering material such as blood or serum. It dissociates and releases the chlorhexidine cation, which is positively charged. This cation then binds to the negatively charged bacterial cell walls, resulting in cellular content leakage, intracellular compound precipitation, and adenosine triphosphate inhibition, all of which eventually inactivate or kill the bacteria[13].

In our study, the main pain score reduction from the first visit (seven days) to the second visit (15 days) was statistically significant ($p < 0.001$). However, there was no association among pain scores on the first and second visits with full and partial thickness burns. Similarly, Junior et al. postulated that the existence of minimal to no pain when wounds are nearing full re-epithelialization resulted in no difference in the pain intensity on the final visits for patients managed with Tilapia fish

Figure 3. The appearance of the left lower limb during Shaour fish skin graft removal.

skin or silver sulfadiazine, regardless of treatment in use[1]. Although the pain associated with a burn injury can be considered a part of tissue protection and regeneration processes, long-lasting acute pain can result in pain centralization, a rise in the occurrence of postoperative pain, the development of depression, and a loss of trust in the medical team, all of which have a negative impact on therapeutic efficacy and compliance with the healthcare team. As a result, the medical team must provide suitable burn pain management, including dressing options which can reduce related pain in conjunction with analgesic pharmacological interventions[1,17,18].

Animal-derived biological tissues, such as dog, pig, and frog skin, are used as biological grafts in burn wounds. However, to approve their routine clinical use, these materials should meet strict testing to determine their true donation, safeness, effectiveness, and biocompatibility[19]. Cadaver skin allografts are an alternative choice with promising results; however, there is a dearth of the required human skin banks.

The acellular fish skin is an economical and novel biomaterial choice for treating burns in developing countries. The recent randomized clinical trial (RCT) by Júnior et al. and the study by Allam, both

Figure 4. The appearance of the left lower limb after Shaour fish skin graft application.

Table 1. Patient characteristics.

Variables	Mean ± standard deviation; *N* (%)
Age (years)	31.86 ± 9.14
Sex	
Male	12 (66.7%)
Female	6 (33.3%)
BMI (kg/m^2)	22.94 ± 2.22 kg/m^2
Type of burn	
Superficial partial-thickness burns	13 (72.2%)
Full-thickness burns	5 (27.8%)
Time to 90% epithelialization (days)	11.05 ± 2.57
Time to 100% epithelialization (days)	17.27 ± 2.05
Mean pain scores on first visit	6.94 ± 0.72
Mean pain scores on second visit	5.22 ± 0.64

BMI: body mass index.

confirmed that in humans, the Tilapia skin xenograft was effective as an occlusive organic dressing for treating burn wounds[1,2]. In *in vitro* experiments, the structure of fish skin xenograft resembled human skin, with a deep dermis created by thick organized collagen fibers. Compared to the human skin, there was a higher concentration of type 1 collagen and high resistance and tensile strength. When subjected to chemical sterilization and supplementary irradiation processes, the Tilapia skin xenograft did not show differences in its microscopic structure and retrieved its natural consistency after rehydration[20].

There is no need for dressing changes as the biomaterial adheres well to the wound bed when the Tilapia skin xenograft is used, suggesting that it may reduce patient suffering and healthcare expenses[1]. Another benefit of this xenograft is the anti-inflammatory action of omega-3, which promotes quicker skin burn recovery[16]. To differentiate the impact of omega-3 from the effect of the fish dermal matrix in wound treatment, a separate RCT is needed[3].

Every week, the fish skin dressings were changed in our study. This was in agreement with Lima-Junior et al. and Ibrahim et al.[21,22], who discovered that fish skin dressings did not need to be changed as frequently as gauze dressings and could stay for up to 10 days. Minimizing wound dressing periods may relieve the pain and anxiety arising from changing dressings regularly, particularly in chronic wounds.

In a prior RCT study, acellular fish skin grafts were compared to pig grafts. The findings revealed that 77.5% of wounds treated with the fish graft completely healed after 3.5 weeks, whereas only 65% of wounds treated with a pig graft fully recovered. Fish grafts cure wounds quicker than pig grafts, according to these results[3]. The acellular fish skin graft structure is absorbent rather than dense compared to amnion/chorion membrane products. This highly permeable structure promotes wound cell ingrowth in three-dimensional structures[23].

Kirsner et al.[24] compared fish skin and human amniotic membrane allografts for acute wound healing and reported that the cost of preparation and treatment with human amniotic membrane allografts was 76% more expensive than treatment with fish skin grafts. They stated that unlike mammal tissue production processes, the gentle processing of cod skin did not include viral deactivation, preserving the natural structure and molecular components of the cod skin. Furthermore, the total fatty acid content of cod skin contains approximately 30% omega-3 polyunsaturated fatty acid, whereas human skin, amnion membrane, and collagen matrix contain less than 1% omega-3 of total fatty acid content[24,25]. Both these products have similar application profiles in that chronic wounds frequently require five or more applications to be closed. As a result, if one has a significantly higher or lower cost than the other, the difference will grow quickly with various application strategies[24].

In another paper, Baldursson et al. compared the effects of fish skin graft and porcine small intestine submucosa extracellular matrix on the healing of 162 full-thickness 4-mm wounds on the forearms of 81 volunteers. Wounds treated with fish skin acellular matrix healed much faster without any superiority. The authors performed the autoimmune reactivity tests on the fish skin, revealing no reactivity. They proved the claims of safety and efficacy of fish skin graft for wound care[3].

A recently published study by Badois et al. used acellular fish skin matrix on thin-skin graft donor sites in 21 patients. This study showed that the healing time was halved when using the acellular fish skin matrix, from 68 to 32 days on average. The visual analog pain scale was ≥ 3 at five days ($p = 0.0034$), and the infection rate reduced from 60% to 0% ($p = 0.0039$)[26].

Our study had a few limitations. It was a retrospective series in a single center with no comparison, and the sample size was admittedly too small to run any powerful statistical analyses. Nevertheless, at this stage of scientific development, our results provide a preliminary promising outcome. It is suggested that future studies with a large sample size with strict inclusion criteria and randomized criteria be conducted. Furthermore, we have only focused on the short-term prognosis of Shaour fish skin grafting and have failed to provide long-term results. Finally, a subjective pain evaluation method was used. In future studies, quantitative sensory testing modalities to perform the objective pain assessments may be used.

CONCLUSIONS

Acellular fish skin is a potent resource to manage full and partial thickness burns. This finding needs to be confirmed in a prospective cohort study with long-term post-procedural follow-up.

Funding

None.

Conflicts of Interest

The authors have nothing to disclose.

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Informed Consent

Written informed consent was obtained from the patients for treatment and participation in our study.

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