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Xenografting of tilapia fish skin for snare wound management in wild sloth bear (*Melursus ursinus*): A novel approach

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ABSTRACT

In a rescue operation, Wildlife SOS Bannerghatta Bear Rescue Centre and the Karnataka Forest Department rescued a wild female sloth bear injured by a poacher's snare. Approximately 2-3 years of age, the bear had suffered a lethal injury on her right foreleg just above the metacarpal region due to the snare. So, the bear was missing the first four digits and claws with gangrenous and septic wounds along the entire paw region. It was estimated that the bear had been injured for 6-10 days prior to her rescue. After a thorough medical examination and stabilization period that lasted for five days, a procedure of skin grafting using fresh tilapia fish skin xenograft was performed to facilitate the healing of the wound. A bandage was applied to cover the graft, that remained intact for two weeks. Fourteen days after the procedure, a close re-examination of the wound bed showed partial absorption of the tilapia skin graft. It diffused a microcapillary network and a thin whitish layer formed over the wound's surface, indicating granulation and formation of the collagen layer. The wild sloth bear was made to undergo training which utilized the principles of positive conditioning wherein the bear was rewarded with a treat every time she performed a desired action such as extending her paw. This allowed wound dressing to happen regularly and smoothly. After six weeks, the wounds completely healed.

Key words: Leg injury, poachers' snare, sloth bear, tilapia fish skin, xenograft

INTRODUCTION

Skin grafting is an ancient technique dating back to 1500 BC once practiced by the Egyptian empire. It is mentioned in Egyptian papyrus role called 'Elbert Papyrus' and one of the Ayurveda's earliest pieces of literature 'Sushruta Samhita' which depicts that this technique was even 3000 years ago (Kohlhauser et al., 2021). The skin grafting procedure involves harvesting skin from the donor and incorporating it into the recipient. It is widely used in human medicine. Recent advancements in skin graft procedure in veterinary practice are helpful techniques for rapid healing of wounds that are delayed naturally or wounds that cannot be healed by the movement of local skin around the wound (Erwin et al., 2016).

The grafts are generally classified into three categories. The first being autografts (donor site and the recipient site is on the same animal), second being allografts (a tissue graft from a donor of the same species as the recipient, but not genetically identical), and third being xenograft (donor and recipient species are different). Autografts are the most successful grafts as the host and recipient of the graft are immunologically identical. Allografts and xenografts are temporary and used in burns and large denuded wounds (Swaim, 1990). In the case of wild animals, practicing autograft is difficult due to their aggressive behavior and added stress to the animal by harvesting extra tissue from the same individual, which will result in a double wound, and managing both would be an additional challenge for

the wildlife veterinarian. On top of it, the expected behavior of any wild animal is self-mutilation, and there are bleak possibilities of applying e-collar or restraining them frequently in a crush cage (Arun et al., 2020, 2022).

Tilapias are freshwater fish and typically have laterally compressed, deep bodies like other cichlids. Tilapia fish skin has non-infectious microbiota even after sterilization of the skin, and it has a high amount of type I collagen and type III collagen (Lima-Junior et al., 2019). Tilapia skin is readily available. It is a qualitative, safe and an inexpensive material that can be easily applied in surgical procedures. Nile tilapia skin also has a high level of biocompatibility in nature. The collagen extract is a biocompatible type I collagen with potential biomedical material for use in clinical regenerative medicine (Alves et al., 2018; Ibrahim et al., 2020). The objective of the experiment was to use fish graft to use in wound healing of bear using standard protocols.

Fig. 1. Usable fish skin measurement

Fig. 3. Skin removal and debridement of underlying tissues

MATERIALS AND METHODS

Preparation of fish skin

Freshly caught tilapia fish from the local fishermen was procured and euthanized as per the standard protocol. The fish was examined and checked for any skin discontinuations. Usable fish skin was measured (Fig. 1) with the measuring tape and later on it was matched with the dimensions of the wound taken (Fig. 2) to avoid a mismatch of graft to the wound. Scales were brushed and obliterated from the skin. With the help of a sterile surgical blade, incisions were made across the three borders as per the wound dimensions, with one edge attached to the spine of the fish to provide grip and avoid tearing of the skin while debriding. The skin was separated from the underlying muscles, and debridement of underlying tissues was done (Fig. 3).

Fig. 2. Wound dimensions measurement

Sterilization of the fish skin

Sterilization of skin was carried out using chlorhexidine gluconate, povidone-iodine, and metronidazole. The tilapia skin was subjected to successive treatments of each disinfectant for different timing. The skin was dipped in chlorhexidine gluconate (4%) for 5 minutes, followed by 10% povidone-iodine for 10 minutes and metronidazole for 15 minutes, respectively (Fig. 4 - 5). After the treatment with the disinfectants, the skin was placed on a sterile surgical cloth over a sterile sponge, and skin was spread over it. The

edges of the skin were held with the help of sterile needles to avoid rolling and shrinking of the skin (Alves et al., 2018; Ibrahim et al., 2020) (Fig. 6).

Fig. 4. Process of sterilization of fish skin

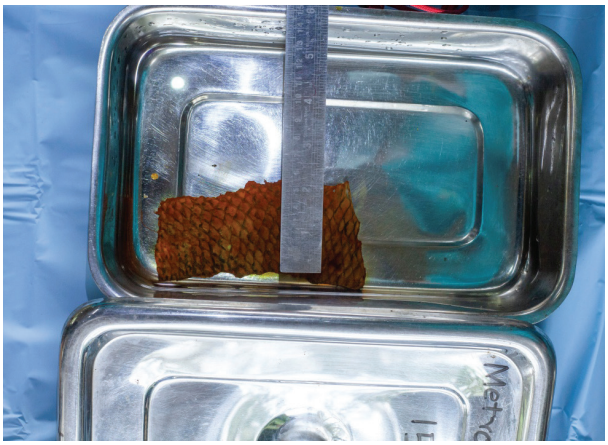

Fig. 5. Sterilization of fish skin

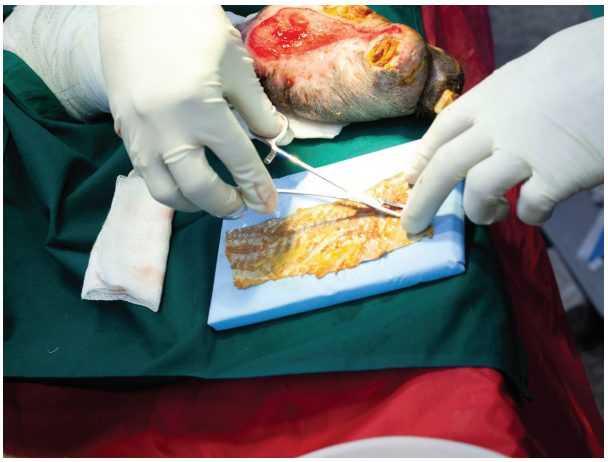

Fig. 6. Holding of fish skin

Patient preparation

The sloth bear was chemically immobilized by the combination of drug ketamine hydrochloride and xylazine hydrochloride with a dose rate of 5 mg kg^{-1} body weight and 2 mg kg^{-1} body weight, respectively with the aid of Dart gun (Fig. 7). Before the immobilization, the animal was made to fast for 12 hours. During the procedure, the utmost caution was maintained and all the vitals were monitored (Fig. 8). The surrounding area of the wound and the leg fur was shaved up to the elbow and later cleaned with 4% chlorhexidine gluconate, 10% povidone-iodine, and metronidazole. The wound was washed with normal saline including cleaning of visible dirt and debris, and disinfection of the wound was undertaken by the same agents as described above (Fig. 9).

Fig. 7. Chemical immobilization using dart gun

Fig. 8. Monitoring of the vital signs

Fig. 9. Wound cleaning and grafting site preparation

Wound preparation

For successful grafting, the vascularity of wound tissue is an essential factor. The excessive fat and unwanted tissues which can hinder the adhesion of the graft were scrapped with the surgical blade, and the wound was freshened across edges along the entire surface (Fig. 10).

Fig. 10. Wound preparation

Graft preparation

The dimensions of the graft were decided as per the dimensions of the wound. With the help of the tip of the 22 number bp blade, tiny 7-8 pores were made on the graft for aeration of the wound (Fig. 11). A thin layer of collagen ointment (silver sulphadiazine USP 1% w w-1 and collagen base) was applied over the surface in contact with the wound (Fig. 12). The graft was placed over the wound and sutured from the edges of the wound with absorbable suture (4-0) by deep, simple interrupted suture pattern. Initially, one border of the wound was sutured to ensure that while suturing opposite edge proper tension is available, and the graft stays in close contact with the underlying injury (Fig. 13). Four sutures in the central part, its adjacent areas were made to hold the graft in contact with the same place (Fig. 14).

Fig. 11: Graft skin on the wound with pores for the aeration of the wound

Fig. 12. Application of collagen ointment on the contact surface of the graft

Fig. 13. Suturing of the graft

Fig. 14. Central sutures for holding of the graft

Bandaging of the wound

Aerated gauze bandages were applied over the graft site to facilitate easy airflow to the wound. Apart from the wound bandaging, additional bandages were extended till the shoulder joint as a safety measure to ensure that even if the bear tries to remove the dressing, the bandage will not tear from the main site of the operation (Fig. 15).

Fig. 15. Wound bandaging

Postoperative measures

Before the shifting of bear, one extra room was made available to allow the bear to rest while existing room is washed and cleaned. It was ensured that both the chambers were free from visible dirt and any infective materials. Both rooms were checked for any sharp protruding elevations. Daily examination of the bandages was carried out to avoid infections at the site through soiling with water or urine.

Postoperative treatment regime

Daily supplementation included two capsules of cod liver oil and NASID drug carprofen @4 mg kg^{-1} body weight for seven days twice a day. No antibiotics were supplemented. Diet Supplementation included 6-7 liters of nutritiously standardized porridge twice a daily, along with ten boiled eggs a day with 2 kg seasonal fruits like watermelon.

RESULTS AND DISCUSSION

Fourteen days after the procedure, the bandages were still intact as the bear had no complications like irritation and did not try to remove the bandages. After 14 days, the bandages were removed, and the grafting site was examined. The graft was absorbed partially, and the wound showed a white layer over it, depicting that the collagen had absorbed. The injury was evident in the presence of microcapillaries at the contact surface. No secondary infections and inflammation were observed over the surface (Fig. 16). Both the pre operative injury and the post operative successful skin grafting have been presented here in Fig. 17 & 18 respectively.

In the recent past, skin grafting has been a promising development in the veterinary field and can be widely practiced for wound management. Several studies have recorded successful results in cats, dogs, and bears (Aisa and Vernon, 2016). Tilapia skin as a xenograft can be utilized as a biological dressing in the veterinary field. Under wildlife veterinary field, the skin grafting has rarely been practiced and there are least available literatures. Present study with a complete stepwise procedure along with clear documentation will no doubt make the wildlife veterinarians job much easier in future. This will be also tremendously beneficial to the wildlife especially in captivity.

Fig. 16. Post procedure wound healing and changes after 14 days

Fig. 17. Pre-operative wound

Fig. 18. Post-operative wound changes

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REFERENCES

Aisa, J. and Vernon, J. 2016. The use of skin grafts in small animal reconstructive surgery. *Companion Animal* **21**(11): 642-648.

Alves, A.P.N.N., Lima Júnior, E.M., Piccolo, N.S., de Miranda, M.J.B., Lima Verde, M.E.Q., Ferreira Júnior, A.E.C., de Barros Silva, P.G., Feitosa, V.P., de Bandeira, T.J.P.G., Mathor, M.B. and de Moraes, M.O. 2018. Study of tensiometric properties, microbiological and collagen content in nile tilapia skin submitted to different sterilization methods. *Cell Tissue Bank.* **19**(3): 373-382.

Arun, A. Sha., Virk, A.S. and Patil, S.P. 2022. Surgical treatment of baculum fracture in sloth bear (*Melursus ursinus*): A case report. *e-planet* **20**(1): 74-78.

Arun, A.S., Bakde, R. and Adhithyan, N.K. 2020. Successful rescue and rehabilitation of an injured wild sloth bear trapped in a poacher's snare. *e-planet* **18** (2): 158-163.

Erwin, G., Handharyani, E. and Noviana, D. 2016. Subjective and objective observation of skin graft recovery on Indonesian local cat with different periods of transplantation time. *Vet. World* **9**(5): 481-486.

http://www.ucdavis.edu/health/news/healing-animals-fish-skin

http://www.vice.com/en/article/us_/article/ne4x3k/burned-bear-paws-fish-skin

Ibrahim, A., Hassan, D., Kelany, N., Kotb, S. and Soliman, M. 2020. Validation of three different sterilization methods of Tilapia skin dressing: impact on microbiological enumeration and collagen content. *Front. Vet. Sci*. **7:** 597751.

Kohlhauser, M., Luze, H., Nischwitz, S.P. and Kamolz, L.P. 2021. Historical evolution of skin grafting—a journey through time. *Medicina* **57**(4): 348.

Lima-Junior, E.M., de Moraes Filho, M.O., Costa, B.A., Fechine, F.V., de Moraes, M.E.A., Silva-Junior, F.R., Soares, M.F.A.D.N., Rocha, M.B.S. and Leontsinis, C.M.P., 2019. Innovative treatment using tilapia skin as a xenograft for partial thickness burns after a gunpowder explosion. *J. Surg. Case Rep*. **2019**(6): rjz181.

Swaim, S.F. 1990. Skin grafts. *Vet. Clin. North America: Small Anim. Pract.* **20**(1): 147-175.