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Porcine skin graft for management of cutaneous wounds in dogs

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Porcine skin has the potential to serve as a biocompatible dressing due to its structural similarities. This study standardized the processing of porcine skin grafts for use as biological bandages and evaluated their clinical efficacy in adult dogs with open cutaneous wounds, alongside optimized the preservation techniques to determine their viability. Grafts of varying sizes were prepared and stored under different conditions to assess viability. Clinical evaluation included wound contraction, histopathology, and immunological response, with outcomes compared to conventional bandaging. Preservation in PBS with 15% glycerol at < 18°C extended graft viability up to 24 days. Dogs treated with porcine skin grafts exhibited faster wound healing, with significant improvement observed as early as 12th day post-application, as compared to 48th day in control group. Histopathological findings supported enhanced tissue regeneration with minimal adverse immune reactions. The study concluded that porcine skin grafts are a suitable, therapeutic and economical alternative for open wound management in dogs.

Keywords: Cutaneous wound, Dog, Porcine skin graft

Due to their structural and physiological similarities to human skin, the porcine skin grafts have been used in the treatment of severe burn injuries and chronic wounds in medical and veterinary fields (Seaton *et al.*, 2015, Radzikowska *et al.*, 2023). In addition to their protective role, these grafts have been found to modulate the inflammatory response and accelerate tissue regeneration in various wound models (Wang *et al.*, 2022).

The harvesting, storing and preservation process of such grafts is critical in ensuring its clinical usefulness. Cryopreservation is one of the most widely used techniques to halt biological processes in the harvested skin to minimize tissue degradation (Xu *et al.*, 2021). However, it carries risk of ice crystal formation, which can damage cellular structures (Holzer *et al.*, 2020). Freeze-drying, or lyophilization, although enhances the shelf life and portability of the graft, may compromise tissue elasticity and biomechanical properties (Kim *et al.*, 2020). Chemical preservation, using agents such as glycerol or formaldehyde, may potentially induce undesirable immune responses by the host body (Tognetti *et al.*, 2017). Combined chemical preservation and cryopreservation or freeze-drying methods have also been used to enhance the viability of grafts (Kim *et al.*, 2020).

A bacteriological study found that 40% of skin grafts were contaminated with over 10^5 organisms/g, mainly due to improper storage in home refrigerators with unstable temperature (Kearney *et al.*, 2005). While there is a noted risk of infection with all xenografts, porcine skin has been shown to have a manageable infection rate when used appropriately, making it a viable option for the management of complex cutaneous wounds (Salloum *et al.*, 2023).

The present study was undertaken to standardize porcine skin graft preservation techniques and to evaluate its effectiveness in managing open cutaneous wounds in dogs.

Materials and Methods

The clinical trial was conducted as per Project No. BVC/IAEC/10/2018 approved in the 40th Institutional Animal Ethics Committee (IAEC) meeting held on 17th February 2018.

Collection of porcine skin and preparation of grafts

Porcine skin was aseptically collected from freshly slaughtered pigs from an abattoir. The harvested skin was hygienically placed in a sterile plastic bag containing an antibiotic solution (composed of 1000 mL saline, 1 lakh units/100 mL crystalline penicillin, and 80 mg/100 mL gentamicin (Darvesh and Butcher, 2022) and transported in an icebox to the department. Then, the skin was sanitized with 0.1% benzylkonium chloride solution for 15 min (Pagnoni *et al.*, 2004) and rinsed with normal saline. Thereafter, the grafts were prepared in several sizes using an electric dermatome.

Storage and preservation of grafts

The grafts were preserved and stored using 4 different combinations of temperatures and storage solutions as detailed in table 1.

Table 1: Different preservative solutions and temperature combinations tested for storing porcine skin grafts.

Group	Storage solution	Temperature
A	Saline	4°C
B	Saline+ 15% glycerol	-18°C
C	Phosphate buffered saline (PBS)	4°C
D	PBS+ 15% glycerol	-18°C

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Viability testing of grafts

The viability of grafts preserved using various techniques was evaluated through the 2,3,5-Triphenyl Tetrazolium Chloride (TTC) assay (Bravo *et al.*, 2000) by testing samples daily from grafts stored at 4°C and at 2-day intervals from grafts stored at -18°C until they were no longer viable. For that, a 2 cm^2 portion was taken from different skin grafts on each day and placed into a clean, dry test tube containing 1.5% TTC and 3% sodium succinate and further incubated at 37°C for one hour under anaerobic conditions. Following incubation, formazan formation was assessed by adding ethylene glycol monomethyl ether to extract the product, and the optical density (OD) was measured at 490 nm using UV-Vis Spectrophotometer. The development of red colour with OD values exceeding 0.20 were considered indicative of graft viability. The mean values were compared between groups by using two-way ANOVA.

Evaluation of porcine skin grafts in the management of open cutaneous wounds in dogs

Twelve adult dogs with a history of open cutaneous wounds of various durations on shoulder, thigh, and back regions were utilized for the study. They were divided into two equal groups of 6 dogs each. Their wounds were treated in a routine manner using povidone-iodine solution and a commercially available herbal antiseptic ointment (Himax, Indian Herbs India Ltd). Inj. streptopenicillin (10,000 IU/kg body weight) and Mmeloxicam (0.2-0.3 mg/kg) were administered intramuscularly for 7 and 5 days, respectively. The wounds were covered with non-adherent dressing in group 1 (control group) and with freshly prepared sterile porcine skin graft in group 2 (test group). The dressing was changed every day in group 1 and at an interval of 4 days in group 2 until complete healing was achieved.

The wound healing was evaluated clinically by observing wound size contraction, reduction in exudate, and the granulation tissue formation at different time intervals during change of graft/ bandages. The wound sizes were measured using a digital Vernier calliper in millimetres, and per cent wound contraction were calculated as per Kodati *et al*. (2011). Punch biopsies were taken from the wound beds at weekly intervals until complete healing of these wounds and subjected to routine histopathological and histochemical examinations. The wound swab was collected aseptically and subjected to microbiological examination before the start of the treatment.

Result and Discussion

Viability testing of grafts preserved with different methods

The skin cells remained viable only for 1 day in saline at 4°C and 2 days in saline + 15% glycerol at -18°C; while in PBS these were viable for 5 days at 4°C and up to 24 days in PBS + 15% glycerol at -18°C (Table 2). Previous studies also indicated that PBS provided an optimal ionic environment that support cellular homeostasis (Martin *et al.*, 2006), while glycerol act as a cryoprotectant, reducing intracellular and extracellular ice formation, thus exhibiting prolonged viability than many other graft-preservation methods (Yoon *et al.*, 2016).

Table 2: Mean Optical Density (OD_{490}) of samples during TTC assay of grafts stored under different conditions.

Groups	Observation days																			
	0	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
Saline (4°C)	0.344 ± 0.027^{ab}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saline + Glycerol (–18°C)	0.344 ± 0.027^{ab}	-	0.262 ± 0.005^{ab}	-	-	-	-	-	-	-	-	-	-	-	-	-
PBS (4°C)	0.313 ± 0.009^{b}	0.278 ± 0.005^{ab}	0.262 ± 0.007^{ab}	0.250 ± 0.006^{a}	0.243 ± 0.005	0.221 ± 0.006^{b}	-	-	-	-	-	-	-	-	-	-
PBS + Glycerol (–18°C)	0.351 ± 0.020^{a}	-	0.335 ± 0.016^{a}	-	0.297 ± 0.009^{a}	-	0.288 ± 0.006	0.281 ± 0.007	0.274 ± 0.003	0.267 ± 0.006	0.264 ± 0.005	0.255 ± 0.003	0.248 ± 0.005	0.237 ± 0.006	0.226 ± 0.004	0.212 ± 0.004

Note: Values with different superscript letters are significantly different ($P < 0.05$)

Table 3: Per cent wound contraction in groups I and II at different observation intervals (Mean±SE).

Day	Group I	Group II
0	0.00±0.00a (n=6)	0.00±0.00a (n=6)
4	12.00±4.34a (n=6)	31.07±4.12b (n=6)
8	25.69±7.32a (n=6)	50.06±8.39b (n=6)
12	45.22±9.84a (n=6)	66.84±9.26b (n=6)
16	59.83±11.72 (n=6)	74.69±12.15 (n=6)
20	52.95±4.58 (n=4)	54.94±1.71 (n=4)
24	63.50±3.10 (n=4)	66.79±0.23 (n=4)
28	71.67±0.79 (n=4)	73.38±0.82 (n=4)
32	76.99±1.77 (n=4)	80.62±1.32 (n=4)
36	80.84±0.87 (n=4)	88.96±0.34 (n=4)
40	85.06±1.21 (n=4)	98.14±0.79 (n=4)
44	91.26±0.98 (n=4)	-
48	96.86±0.84 (n=4)	-

Evaluation of porcine skin graft vis-a-vis regular bandage in management of open cutaneous wounds in dogs

The mean size of cutaneous wounds at day-0 was 17.28±0.41 cm² (range: 16.5–18.1 cm²) in group I and 20.12±0.38 cm² (range: 19.40–20.80 cm²) in group II. Both groups showed progressive wound contraction following the start of treatment with significant intra-group variation.

The mean per cent wound contraction was 12.00±4.34, 25.69±7.32, 45.22±9.84 on 4th, 8th and 12th day, respectively in group I. At the same intervals, those values were 31.07±4.12, 50.06±8.39 and 66.84±9.26 in group II. The recording of parameters could be done for all dogs for 16 days in group 1 and 12 days in group II as the wounds healed satisfactorily in some dogs by this period. Hence, intergroup statistical comparison of results was done only up to the 12th day; however, the results of the remaining dogs are presented in the table for the subsequent observation periods as well. Inter-group comparison revealed significantly greater wound contraction in group II in this initial follow-up period. The median healing time of wounds was 20 days in group I and 12 days in group II.

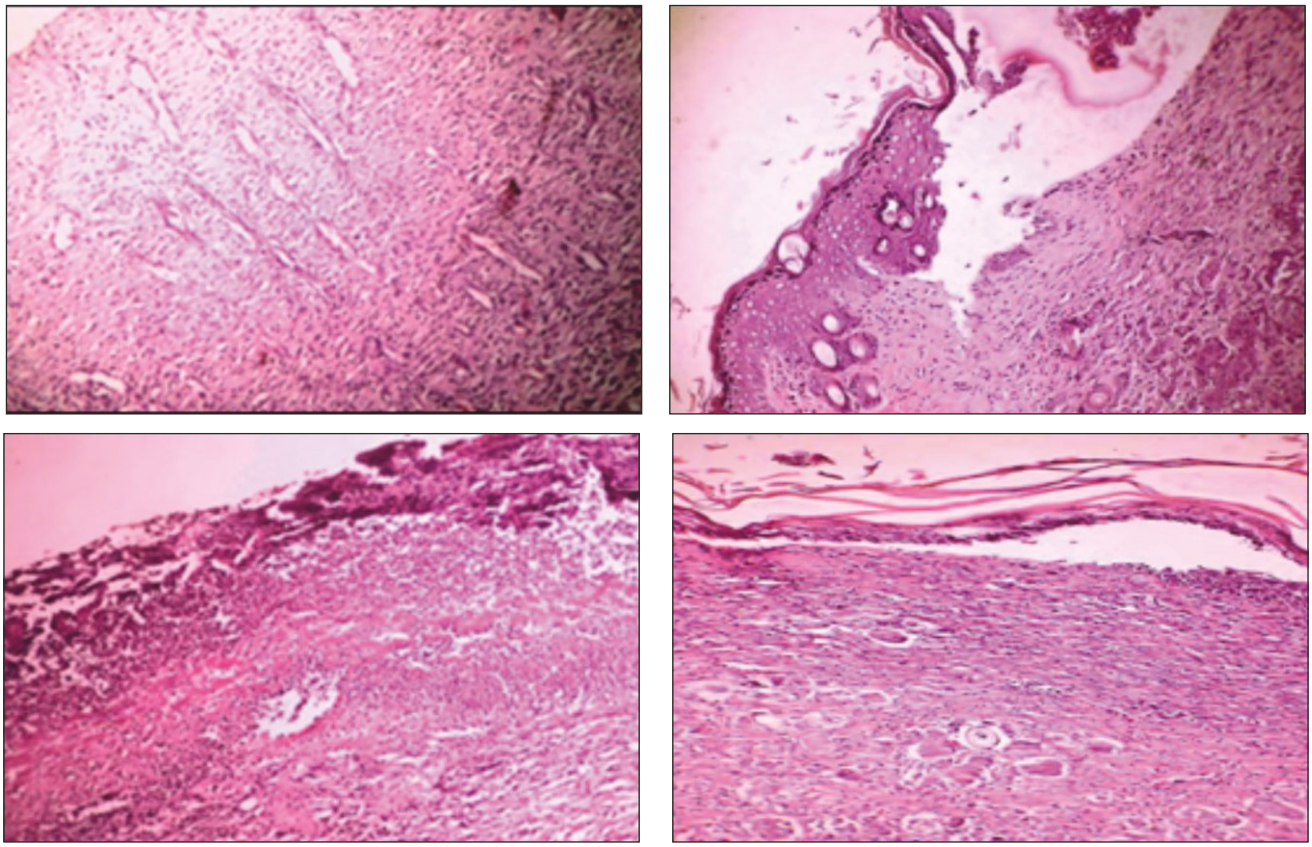

Fig. 1: Histopathological observations of wound biopsies of dogs at different time intervals (5x): (A) Group I, 8th day: Few inflammatory cells at oedema-tous granulation tissue. Presence of some blood clots mild leukocytic infiltration, minimal fibroplasia, mild neovascularization; (B) Group I, 16th day: Incomplete regeneration process of epidermis, some amounts of polymorphoneuclear lymphocytes seen; (C) Group II, 8th day: Minimal to mild leukocytic infiltrat-ion, mild to moderate fibroplasia, minimal laid down collagen and moderate neovascularization. Slight growth of the epithelial cells; (D) Group II, 16th day: Regeneration process of epidermis completed. Moderate fibroplasia and randomly dispersed polymorphoneuclear lymphocytes seen.

Case 1 (group I) and g4 (group II) showed malodorous discharge during the follow-up period, which was addressed successfully with a course of antibiotics. The physiological, haematological and biochemical parameters of the dogs remained within the normal ranges throughout the study in both groups. This suggests the absence of systemic involvement in any of the groups and indirectly validates the safety of porcine skin grafts.

The histopathological examinations of the wound bed biopsies revealed a faster and greater degree of tissue regeneration in group II. On day 8, group I showed fewer inflammatory cells, milder neovascularization and a lesser degree of fibroplasia as compared to group II. On the 16th day, group II showed complete regeneration of the epidermis, neovascularization, moderate fibroplasia, and some amounts of polymorphonuclear lymphocytes, indicative of early wound healing, whereas the healing process was still incomplete in group I (Fig. 1).

In group I, *Pseudomonas aeruginosa* and *Proteus mirabilis* were found in one case, whereas Klebsiella pneumonia and Staphylococcus aureus in two cases. In group II, similar organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were isolated prior to treatment; however, the overall healing progression in these animals remained as per the general trend in the group, suggesting that the treatment conditions favoured recovery despite initial microbial presence.

Many studies have used porcine skin grafts for wound dressing purposes in the past. It was reported that its structure closely mimics canine and human skin, providing an environment conducive to healing and acting as a barrier to microbial invasion. It was also reported that such dressings promote re-epithelialization, reduce fluid loss, minimize pain, and accelerate granulation tissue formation. Clinical veterinary applications have shown that porcine-derived scaffolds significantly improved wound healing parameters, such as less oedema, necrosis, and faster wound contraction compared to commercial bovine collagen matrices (Karthika *et al*., 2018, Jo *et al*., 2024). A wound-healing time range of 9-14 days was reported in yet another study utilizing porcine skin scaffolds indicating faster overall recovery (Irilouzadian *et al*., 2023). The present study did not result in any adverse effects, while Yang *et al*. (2016) observed adverse effects like bleeding in some cases.

Though the group II dogs showed overall faster healing, significant intra-group differences of the rate of healing were evident in both groups, which may be attributed to wide variations in local factors like wound size, chronicity, presence of infection or the systemic factors like the age and condition of the patient etc.

It was concluded that the use of porcine skin grafts resulted in faster healing of open cutaneous wounds compared to routine dressing methods in a limited clinical study on dogs; more studies involving larger data base are needed to validate the repeatability of such results.

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