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Comparison between acellular ovine skin and acellular bovine pericardium on the induced skin loss healing in local breed cats

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Abstract

This study aimed to compare the effects of acellular ovine skin and acellular bovine pericardium on healing of large skin loss in cats. Eighteen local breed adult cats were used and divided into two equal groups. A full-thickness square of 4x4 cm dimensions wounds were created on the backs of all cats. In the first group, acellular ovine skin of a 3x3 cm patch was used to close these full-thickness back wounds and sutured with wound edges and bandaged, while acellular bovine pericardium of the same size was used in the second group. Wounds were observed macroscopically and assessed physically and clinically daily, while histopathological examination was achieved on days 7, 14, and 21 post-operations. The clinical results showed a significant difference ($P<0.05$) between both groups in the rate of wound healing. The physical evaluation using Doppler ultrasound exhibited the presence of good cutaneous blood perfusion since day 12 in the first group and day 14 in the second experimental group. The histopathological manifestations revealed significant differences in most of the observed histopathological parameters, especially interwoven with the wound beds, angiogenesis, well-developed granulation tissue, and good tissue architecture associated with acellular ovine skin group. The immunohistochemistry results of vascular endothelium growth factor expression detected that 1st group exhibited more staining intensity than 2nd group. In conclusion, acellular ovine skin is much better than acellular bovine pericardium as dressing for extensive wound healing in cats.

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Introduction

Cutaneous wounds are still among the most common daily cases in small animal practice (1,2). Skin and its associated appendages achieve a lot of dynamic roles, especially protection against various invaders and factors, thermoregulation, and prevention of water and electrolyte losses (3). Its position and huge proportion predisposing it to be the first tissue that faces any invasion or injury, especially large extensive wounds, which are still representing an economical and physiological challenge for both the human and veterinary world due to their extended healing time, need for a continues complex management and being more vulnerable for various complications (4), particularly in the feline family because cats are one of the most problematic behavioral animals that are more curious and cautious about investigating things (5). This curious nature makes cats more vulnerable to being injured with many kinds of wounds, particularly those with extensive skin loss that need to adapt successful wound management to prevent complications (6), especially in cats because their cutaneous healing process is not easy and fast as in other animals besides it is entirely different from what happens with other domestic animals because of the deficiencies in many wound healing features especially poor inflammatory response, weakened granulation tissue formation, deprived blood vascularization and diminish contraction (7,8), that leading to poor quality healing response (9). Besides, many studies revealed

significant differences in the macroscopic appearance of wounds, skin perfusion, and even the microscopic cell structure of feline wounds that make extended wound healing and pseudo-healing phenomena much more happening in cats comparable with other species, especially dogs (2,6). Furthermore, the normal histology and anatomy of a cat's skin are relatively different from that of other species, which, in turn, influences and deeply influences its healing process. Moreover, the blood circulation of the skin, especially within the trunk region, is much less than that of other mammals due to their relatively low mass of tertiary blood vessels, leading to a low degree of cutaneous perfusion (10,11). All these variations of feline skin make healing their extensive skin loss wounds challenging, requiring special alternative therapeutic options to deal efficiently and successfully with such wounds (6). Although dealing with and treating various wounds is a common daily practice in veterinary clinics, extensive wounds are more challenging cases that necessitate suitable open-wound therapy to guarantee effective management (8,12). Surgical rebuilding after the excision of large volumes of tissue, as it happens in association with the treatment of severe trauma, infection, or even neoplastic lesions, represents a huge, challenging, tricky task for the surgeon because accomplishing quick, uncomplicated wound healing is always required for large volume wounds because failure of wound repair is potentially disastrous (13). The purpose of extensive wound care and dressing is to generate a suitable situation for healing, initiate the beginning of healing, adjust the exudate, contract pain, provide mechanical support, and avoid contamination (14,15). Therefore, there is a significant need to find and supply new revolutionary materials and products to speed up healing (4). Various biodegradable, biocompatible natural and synthetic materials are continuously being driven into the biomedical field, especially for wound dressing and treatment (16). Biological viable scaffolds that derived from the pericardium, skin, small intestine, and submucosa that can be harvested from human and nonhuman sources like porcine, equine, or even bovine is nowadays constitute the primary sources for the biomaterial industry (17,18). These derived biological materials, which could be either degradable or permanent, are usually subjected to a series of procedures to achieve complete decellularization and preserve their extracellular matrix components only without any antigenic or inflammatory properties (19,20). The use of natural biomaterials to promote wound healing has become a very trendy trial due to their highly positive influence on the stages of wound healing. They supply the appropriate healing atmosphere and act as a scaffold that facilitates bridging the healing aspects, including cell movement, propagation, and differentiation, thus progressing tissue regeneration and repair. Furthermore, most of these materials display multifunctional roles that participate during different phases of the wound-healing process (21,22). Acellular bovine pericardium is widely accepted for synthesizing numerous bio prostheses, such as vascular stents and heart valves associated with lung and cardiovascular surgeries. Cardiac patches are also used to repair several soft tissue deficiencies, such as cerebral dura mater or even diaphragmatic hernia (23-25). The ovine acellular matrix is becoming a new trend that could be applied extensively, especially in wound healing or treating burn injuries (26,27).

This article evaluates the effectiveness of using biomaterial xenografts obtained from ovine skin and bovine pericardium to dress extensive cat wounds.

Materials and methods

Ethical approve

The surgical and other associated procedures in this experimental work were achieved according to the ethics and rules of the Committee of Animal Care and Use of Mosul University/Faculty of Veterinary Medicine. U.M.VET.2023. 067.

Experimental animals

Eighteen (N=18) local breed male cats, one to two years old and with a usual weight of 2-3± 0.3 kg, were acclimatized one week before the start of this research. All the surgical operatives and accompanying handling manipulative actions were done following the ethical standards line and rules for animal utilization protocols. To ascertain the health of our experimental cat, all animals were inspected to ensure they were free from any infectious disease and pathological conditions.

Preparation of acellular ovine skin

Ovine skin was gained from the neighboring abattoir and instantly saved within an ice-cold box with sterile phosphate-buffered saline (PBS, pH 7.4) supplemented with wide-spectrum antibiotic (Amikacin-1 mg/ml) and a proteolytic inhibitor Ethylenediaminetetraacetic acid (0.02% EDTA). In the preparation room, the skin was shaven to remove all skin hair, while all the adhesive debris, tissues, and blood were rinsed thoroughly using a PBS sterile solution. Skin de-epithelization was completed using trypsin 0.25% and 2M sodium chloride solution for eight hours, followed by 2% sodium deoxycholate for 48 hours to decellularize the dermis. During both de-epithelization and decellularization processes, to ensure the best contact of skin with chemical solutions and obtain the ideal result, the specimens were exposed to nonstop agitation using a horizontal orbital shaker at the rate of 220 rpm. To ascertain the complete removal of all cells from the processed skin samples, a microscopic examination after staining with Hematoxylin and Eosin (H&E) was done (Figure 1). Then, the treated acellular ovine skin was rinsed with sterile PBS solution six times (2 hours each) to eliminate all the remaining chemicals and lastly kept at -20oC in PBS solution comprising 0.1%

amikacin solution (26,28). Lastly, the treated acellular ovine skin specimen was kept at -20 °C in PBS holding 1% Gentamycin (29).

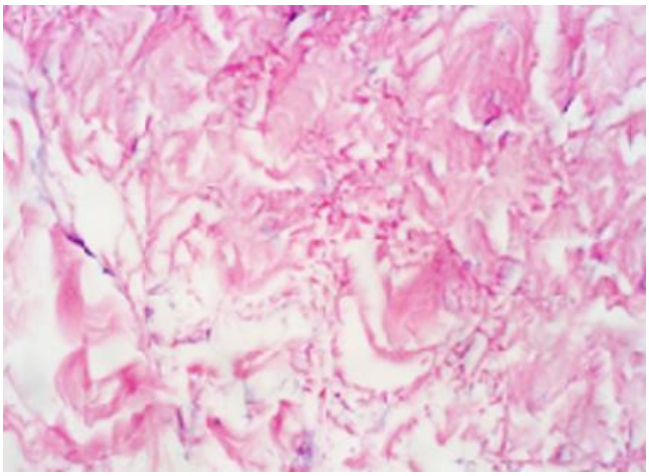

Figure 1: Histological section of acellular ovine skin patch. Shows efficiently eliminates the cellular part of the tissue, absent of the nucleus. H&E, 400x.

Preparation of a cellular bovine pericardium

To prepare a cellular bovine pericardium, the pericardium membrane was obtained from the patriot abattoir and rapidly saved inside an ice-cold solution of sterile buffered saline of sodium (PBS, pH 7.4) enhanced with wide-spectrum antibiotic (Amikacin-1 mg/ml), and (0.02% EDTA) as a proteolytic inhibitor. Next, inside the preparation room, all the associated debris, fats, and blood were removed and cleaned out carefully using sterile gauze and PBS. Decellularization of the bovine pericardium was accomplished using a new modified approach through complete immersion of pericardium membrane inside 2% of sodium deoxycholate SDS solution for 9 hours with unceasing agitation of the horizontal orbital shaker at the rate of 220 rpm to ensure optimal contact of the membrane with a chemical solution. To ascertain the de-cellularity of the processed pericardium specimens, a microscopic inspection after Hematoxylin and Eosin (H&E) staining was completed (Figure 2). Then, the ready acellular bovine pericardium was rinsed for 120 minutes with sterile PBS solution for six periods to remove any outstanding substances and finally stored at 4ºC in PBS solution containing 0.1% amikacin solution (26,28). Finally, the ready cellular bovine skin was kept at 4ºC in a PBS solution saturated with 1% Gentamycin (29).

Experimental design

Eighteen (N=18) cats were divided randomly into equal groups, 9 for each. A 4x4 cm square of full-thickness skin defect was created in the back region of all cats. In the 1st experiment group, a 3x3 cm segment of fenestrated acellular ovine skin (0.25cm diameter of pores and 0.5 cm spacing between them) was implanted onto the skin defect and sutured with 2.0 Nylon using simple interrupted. Meanwhile, fenestrated acellular bovine pericardium was used to repair the induced back defect in the second group. The total thickness of the dressing area was examined clinically and physically and subjected to histological examinations on days 7, 14, and 21 following dressing.

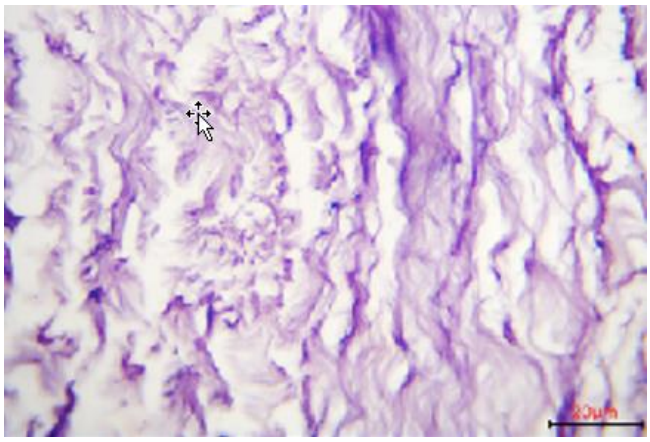

Figure 2: Histological section of acellular bovine pericardium. Shows efficiently eliminates the cellular part of the tissue, absent of the nucleus. H&E, 200x.

Preoperative preparation

All cats were deprived of feed for 12 hours before the surgical operation. The site of the procedure on the back of each animal was prepared strictly for aseptic intervention, first by clipping and shaving hair and then applying an antiseptic solution containing 5% povidone iodine and 70% ethyl alcohol.

Anesthetic Protocol

All surgical procedures were accomplished under general anesthesia using an intramuscular combination of 10 mg/kg of Ketamine Hydrochloride (Narketan®-10, Troy Laboratories PTY Limited, Australia) and 1 mg/kg of Xylazine (ILium Xylazil-100, Troy Laboratories PTY Limited, Australia) (30).

Surgical procedure

The cat was positioned in sternal recumbency, and the experimental wound outline was determined using a skin marker and caliper. Then, a 4x4 cm square full-thickness skin wound without removing the panniculus muscle was excised with a #15 scalpel blade on the center of the trunk, beneath the caudal border of the scapula (Figure 3).

Following defect creation, a 3x3cm segment of acellular ovine skin was used to cover the induced skin defects and sutured into the edges of that wound by 2.0 Nylon (Ethilon*, Ethilon. LLC.) using simple interrupted in the 1st group.

Cellular bovine pericardium pieces were applied for the 2nd group. Lastly, the wounds were bandaged. All wounds were protected with a four-layer padded bandage as follows: the contact layer was covered with a sterile, semi-occlusive, low-adherent pad then, the second layer consisted of a cotton pad, covered with a third layer using cotton roll gauze, applied in several layers over the body to prevent the bandages from sliding, and the fourth of a self-adhesive bandage.

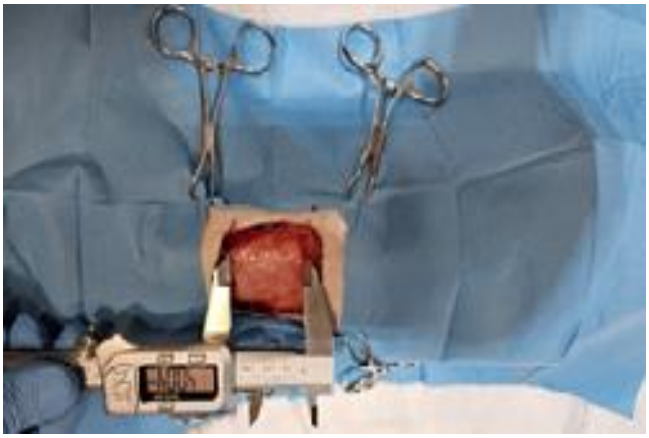

Figure 3: Photographic image shows the determination of the size of the induced wound on the back of each cat.

Postoperative care

Daily, the cutaneous wounds were checked for any apparent gross signs of wound dehiscence or complications. The cats were given ketoprofen (2.2 mg/kg for three days) and Penicillin (40000 IU/kg for five days).

Wound healing assessment

Daily gross, physical, and weekly microscopic observations were made 7, 14, and 21 days after surgeries to evaluate the progress of the skin wound healing.

Physical ultrasound evaluation

The linear transducer of 4-13 MHz of Doppler ultrasound was used to detect the presence of cutaneous blood perfusion associated with using both xenografts for the dressing of full-thickness started to be taken since day 12 post-surgery to avoid the separation of the dressing from the underline bed and continued until the end of the experiment on day 21 post dressing (31).

Microscopical evaluation

The progress of cutaneous wound healing following the use of acellular ovine skin and acellular bovine pericardium was further monitored using microscopic criteria, including histopathological and immunohistochemistry.

Histopathological assessment

The histopathological features associated with cutaneous skin wound healing were evaluated on 7, 14- and 21 days following dressing using skin samples collected from the wounds' areas and 1–2 mm of the neighboring normal skin. The transplanted tissues were preserved in neutral-buffered formalin 10% for 24 hours for fixation; then, these specimens were fixed in paraffin, sectioned, stained with hematoxylin-eosin, and examined using light microscopy. Each histological section was assessed microscopically to evaluate the healing features, as shown in (Table 1) (32,33).

Immunohistochemistry of VEGF

Assessment of VEGF visualization and localization was achieved through processing the slides using the Dako-EnVision + System-HRP (DAB) immunoperoxidase staining protocol to investigate tissue viability based on subjective modified scoring criteria (34) (Table 2).

Table 1: Histopathological parameters for semi-qualitative assessment for dressing performance

Criteria	1	2	3	4
Granulation tissue	Not seen	Discrete	Moderate	Strong
Re-epithelization	Not seen	Discrete	Moderate	Strong
Intensity of inflammation	Sever	Moderate	Little	Not seen
Angiogenesis	Not seen	Discrete	Moderate	Strong

Table 2: Immunohistochemical scoring of VEGF

Criteria	0	1	2	3	4
VEGF	Negative -absent 0 cells/field	Very Weak ± 1 to 3 cells/field	Low Positive + 4 to 10 cells/field	Moderate Positive ++ 11 to 25 cells/field	Positive +++ More than 25 cells/field

(0) (-) absent; (1) (±) very weak immunoreactivity; (2) (+) low positive immunoreactivity; (3) (++) moderate positive immunoreactivity; (4) (+++) high immunoreactivity.

Results

All cats survived the surgery and well recovered uneventfully. In addition, the animals were eaten and drank water normally. In both groups, there was some degree of exudate and edema, especially in the 2nd pericardium group, comparable to the 1st ovine skin group. However, the progress of wound healing was better in AOS than in the ABP group. Besides, there were remarkable differences in wound area and morphology between both groups, as shown in (Figures 4 and 5).

Figure 4: Photographic image of acellular ovine skin after 21 days following dressing onto full-thickness skin wounds in cats.

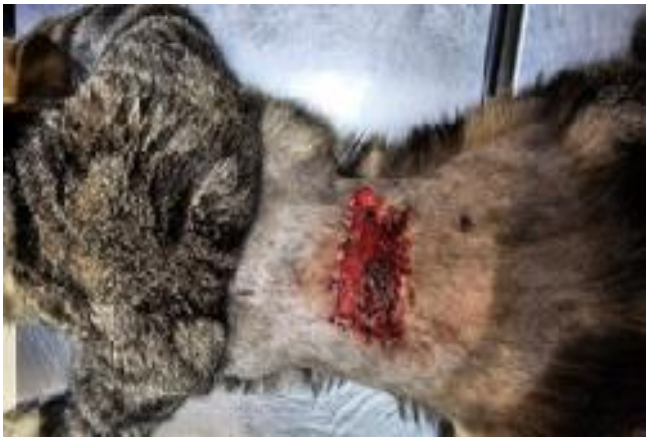

Figure 5: Photographic image for acellular bovine pericardium after 21 days following dressing onto full-thickness skin wounds in cat

Physical parameters doppler ultrasound results

The Doppler ultrasound evaluation to detect cutaneous blood perfusion associated with using both acellular xenografts for the dressing of full-thickness started to be taken on day 12 post-surgery to avoid the separation of the dressing from the underline bed. The Doppler ultrasound examination exhibited the presence of good cutaneous blood perfusion at 12 in acellular ovine skin, comparable to day 14 in the acellular bovine pericardium (Figures 6 and 7), then started to become more evident with the progress of the healing process (Figures 8 and 9).

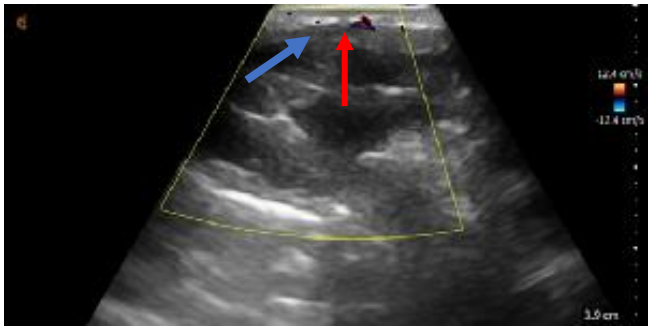

Figure 6: Ultrasonographic image for 1st group (AOS) after 12 days of dressing. Shows the presence of angiogenesis. Red indicates blood flowing towards the beam, while blue indicates blood flowing away from the beam.

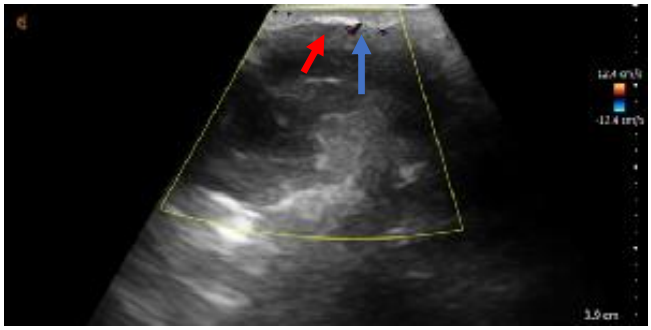

Figure 7: Ultrasonographic image for 2nd group (ABP) after 14 days of dressing. Shows the presence of angiogenesis. Red indicates blood flowing towards the beam, while blue indicates blood flowing away from the beam.

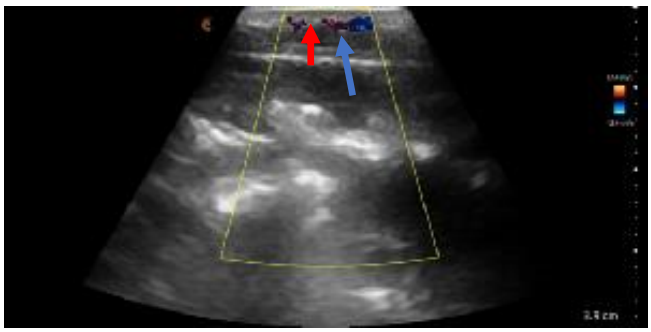

Figure 8: Ultrasonographic image for 1st group (AOS) after 18 days of dressing. Shows the presence of angiogenesis. Red indicates blood flowing towards the beam, while blue indicates blood flowing away from the beam.

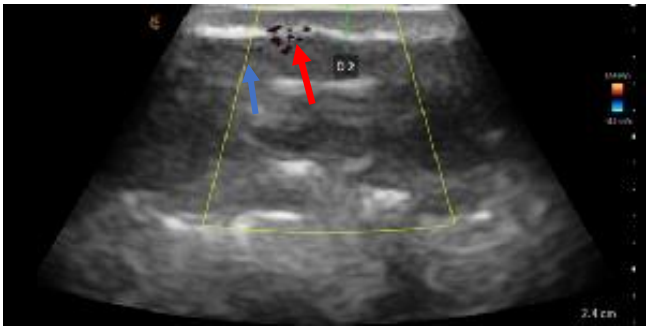

Figure 9: Ultrasonographic image for 2nd group (ABP) after 18 days of dressing. Shows the presence of angiogenesis. Red indicates blood flowing towards the beam, while blue indicates blood flowing away from the beam.

Histopathology at day 7

The histopathological examination for the 1st acellular ovine skin exhibited the presence of close contact between the dressing and wound bed skin margins with severe diffuse infiltration of polymorph nuclear inflammatory cells and mononuclear inflammatory cells (Figure 10). The histological section of the acellular bovine pericardium exhibited the presence of extensive infiltration of inflammatory cells between skin and patches associated with edema and hemorrhage (Figure 11).

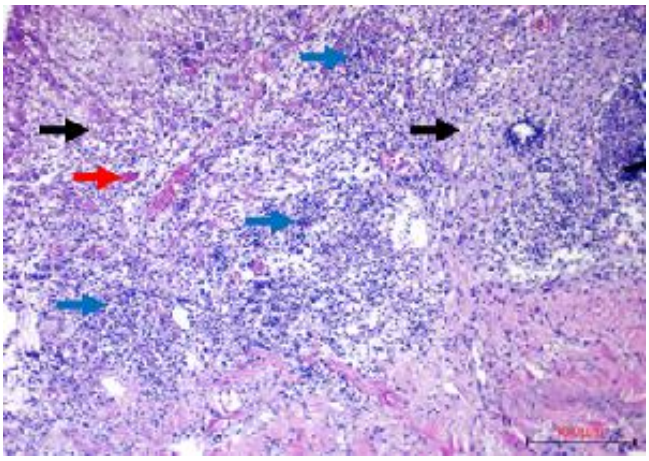

Figure 10: Histopathological section of ovine skin patch graft at day 7 after dressing in a cat skin wound. Shows granulation tissue formation (black arrows) and severe infiltration of polymorphonuclear inflammatory cells between the dressing and skin (blue arrows) associated with congesting of blood vessels (red arrows). (100µm H&E).

Histopathology at day 14

Furthermore, the histological section of the acellular ovine skin patch on day 14 after dressing exhibited that the ovine dressing is closely interwoven with the skin wound bed and attached very well to the skin tissue with infiltration of fibroblast between them, with the presence of granulation tissue. Besides, well collagen fibers are distributed between the patch and skin, forming blood capillaries (angiogenesis). Well, re-epithelialization with few inflammatory cells (Figure 12). Whereas for the acellular bovine pericardium, there was a good close interwoven between skin and pericardium with focal infiltration of mononuclear inflammation, formation of blood capillaries (angiogenesis), re-epithelialization (Figure13).

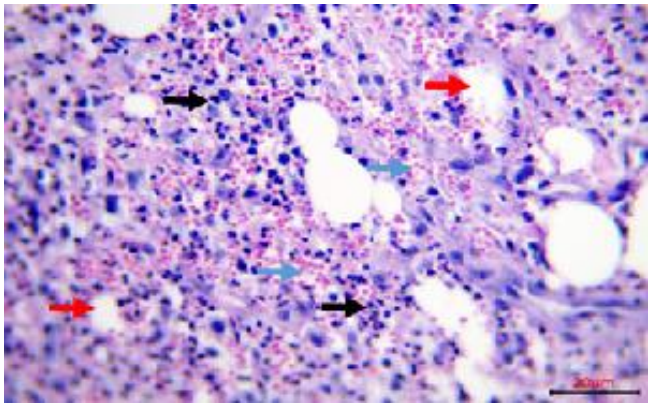

Figure 11: Histopathological section of bovine pericardium patch graft at day 7 after dressing in a cat skin wound. It shows the infiltration of mononuclear inflammatory cells (black arrows), which are associated with edema (red arrow) and hemorrhage (blue arrow) in the bovine pericardium patch. (20 µm H&E).

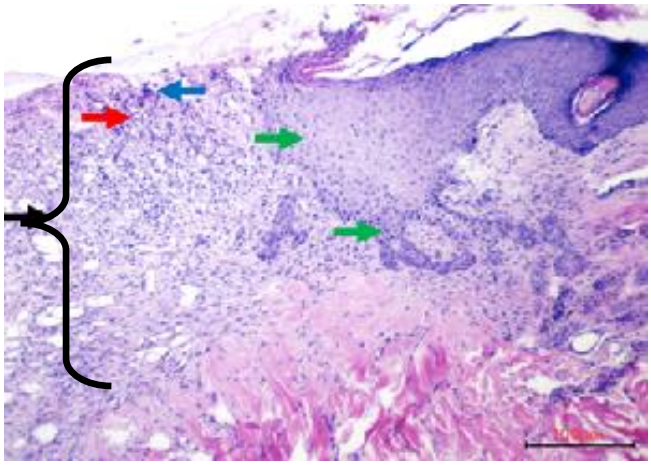

Figure 12: Histopathological section of ovine skin patch at day 14 after grafting in a cat skin wound shows the ovine graft with well-developed granulation tissue (black arrows), with well vascularized (angiogenesis) (red arrow), few infiltrations of inflammatory cells (blue arrows), and well re-epithelialization (green arrow). (100µm H&E).

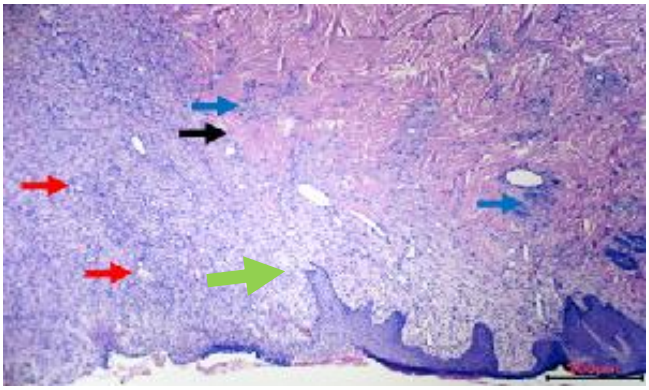

Figure 13: Histopathological section of bovine pericardium patch graft at day 14 after grafting in a cat skin wound shows the pericardium graft is closely interwoven with the skin (black arrow), focal infiltration of inflammatory cells between the graft and skin (blue arrows), formation of blood capillaries (angiogenesis) (red arrows) and re-epithelialization (green arrow) (200µm H&E).

Histopathology at day 21

The histological section of the acellular ovine skin patch on day 21 after dressing exhibited that the acellular ovine skin patch is strictly interwoven with the skin with re-epithelialization and regeneration of wound edges. The repaired epithelium displayed good polarity of normal basal cells, and the differentiation of squamous cells, epidermal outlines, and dermo-epidermal junction started to regain their architecture structure. Besides, the tissue construction, particularly the epidermal borders and dermo-epidermal connection, displayed an excellent histological appearance with good tissue building and organizational integrity. The epidermal integrity and dermal and epidermal junction started to regain their architecture with more deposition of collagen fibers. Few inflammatory cells were found with the distribution of fibroblast cells (Figure 14). On the other hand, the histopathological examination of the acellular bovine pericardium after 21 days showed interwoven between skin and pericardium with re-epithelialization as the rebuilding of epidermis over the pericardium graft, formation of fibrous tissue and infiltration of a cluster of mononuclear inflammatory cells between the dressing and skin. Focal and less edema and increased formation of blood capillaries (angiogenesis), besides epidermal integrity and dermal and epidermal junction, started to regain their architecture with more deposition of collagen fibers (Figure 15).

Immunohistochemistry

The results of vascular endothelium growth factor expression showed its presence within the angioblast cells. The overall trend detected that both exogenous patch dressings showed variable intensity expression and visualization of VEFG; however, decellularized ovine skin showed more staining intensity than decellularized bovine pericardium, which showed mild staining intensity. Furthermore, the intensity of staining and expression gradually increased with time; it was mild on day 7 post-dressing (Figures 16 and 17), then became moderate on day 14 and continued progressing and became stronger on day 21 in both groups (Figures 18 and 19) however that expression was more obvious with ovine skin than bovine pericardium. Furthermore, the statistical analysis showed a significant difference in VEGF localization between decellularized ovine skin and bovine pericardium at different times within each experimental group (Table 3).

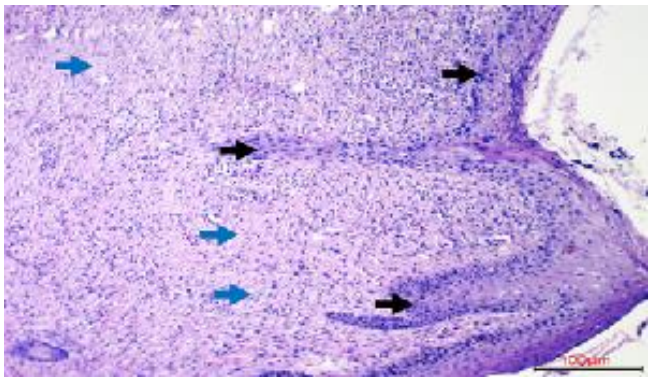

Figure 14: Histopathological section of ovine skin patch at day 21 after dressing in a cat skin wound. The regenerating epithelium showed the polarity of normal basal cells and the differentiation of squamous cells (black arrows), good dermal-epidermal integrity, and a few inflammatory cells were found with the distribution of fibrosis and fibroblast cells (blue arrows). (100µm H&E).

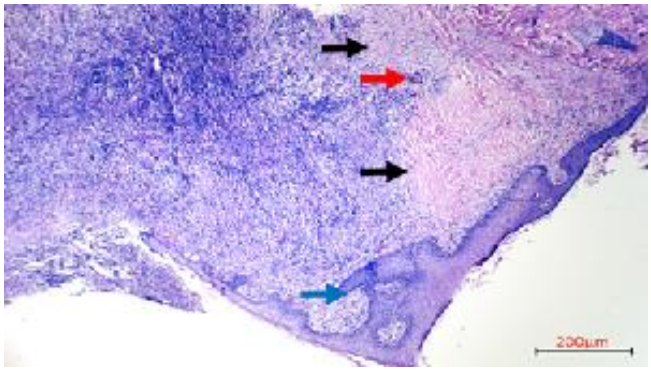

Figure 15: Histopathological section of bovine pericardium patch graft at day 21 after dressing in a cat skin wound. The pericardium patch is strictly interwoven with the skin (black arrow), rebuilding of the epidermis (blue arrow), well-developed blood vessels (red arrow), presence of inflammatory cells (red arrow), infiltration of inflammatory cells (green arrow) (200µm H&E).

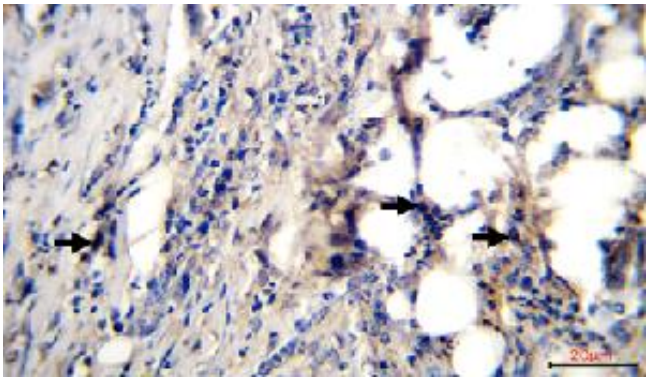

Figure 16: Immunohistochemical staining for VGEF of acellular ovine skin patch graft at day 7 after dressing in a cat skin wound. The weak positive (++) VEGF expression appeared as a nuclear brown stain in the Angioblast cells within the tissue (arrows). IHC. 20µm.

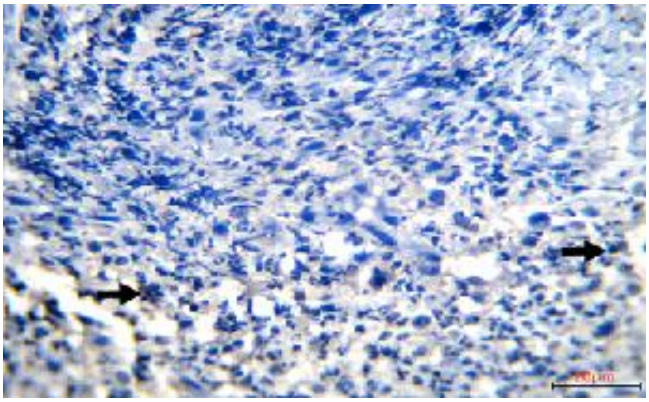

Figure 17: Immunohistochemical staining for VGEF of bovine pericardium patch graft at day 7 after dressing in a cat skin wound. It showed weak VEGF expression, which appears as a nuclear brown stain in the angioblast cells within the tissue (arrows). IHC. 20µm.

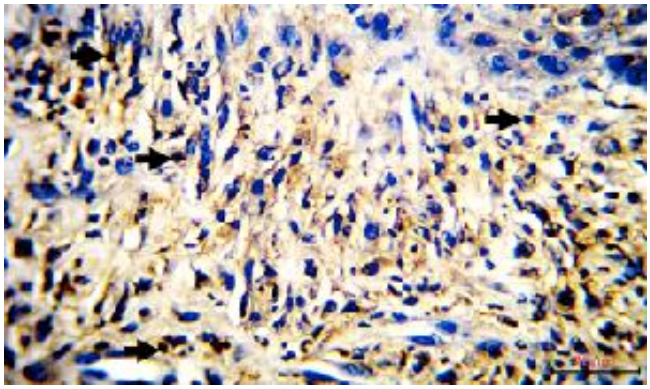

Figure 18: Immunohistochemical staining for VGEF of acellular ovine skin patch at day 21 after dressing in a cat skin wound. Sever (++++) VEGF expression appears as a nuclear brown stain in the angioblast cells within the tissue (arrows). IHC. 20µm.

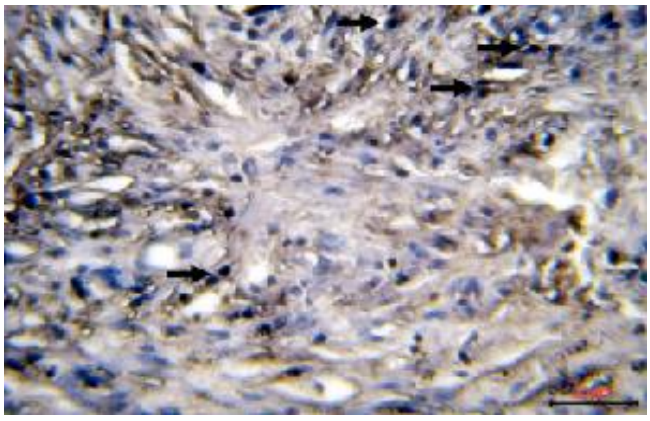

Figure 19: Immunohistochemical staining for VGEF of acellular bovine pericardium patch graft at day 21 after dressing in a cat skin wound. The positive (+++) VEGF expression appeared as a nuclear brown stain in the Angioblast cells within the tissue (arrows). IHC. 20µm.

Table 3: Show the expression of VEGF within both transplanted acellular ovine skin and acellular bovine pericardium during the study period

Protein	7 days	14 days	21 days
VEGF expression ovine skin	2.1±0.14 Ac	3.26±0.22 Ab	4.19±0.16 Aa
VEGF expression bovine pericardium	0.8±0.04 Bc	2.5±0.61 Bb	3.5±0.55 Ba

Discussion

Wound healing is a vital process for repairing skin integrity that inspired many to study it to understand its dynamic events (35-37). It is an energetic physiological phenomenon that happens in the same principle in all animals, except cats, with clinically essential differences from other species (38). The successful wound-healing process requires collaborating and coordinating three basic elements involving the extracellular matrix (ECM), cells, and growth factors (27,39). This experimental study was conducted on 18 cats because (indolent pocket) wounds and pseudo-healing that led to complete wound dehiscence are much more predicted in cats than in other species, especially dogs (40,41).

This study used acellular xenografts derived from ovine skin and bovine pericardium as a compatible biological dressing due to their wide bioavailability, positive applicable history, and relatively less complicated preparation process (27,39). The using of such biological implants has become one of the most acceptable trends for regenerative biomedical applications as a result of their extracellular matrix (ECM) properties that possess the necessary dimensional architecture components to mimic the organizational and physiological properties of the native ECM besides acting as a suitable environment for the host cells to invade and grow through it. These xenograft acellular implants, when placed in the wound bed, their dimensional architecture will act as a temporary suitable scaffold that enables the host cells to occupy, migrate, and proliferate as well as to reinforce mechanical and functional performance that, in turn, enhance the progress of the. Furthermore, these viable scaffolds have several dynamic proteins that protect the healthiness of tissue differentiation and sustain the host response to tissue injury, which becomes a supreme choice for replacing the missing or damaged tissues (42).

This work uses bovine pericardium because of its inherent strength and biocompatibility (23,24). Biomaterials originated from sheep and goats and are broadly accepted by all cultures and religious societies (43). Besides, they have a lower risk of disease transmission (44). To eliminate the immunogenicity usually associated with xenograft implants and thus prevent dressing rejection, we mechanically and chemically processed pericardium and skin through a series of continuous procedures using many chemical agents and protocols, as mentioned previously. Decellularization reduces inflammatory and immunogenic responses associated with original tissues when implanted into recipient tissues (45,46). In this study, the dressing bandage was used over the dressing patches to immobilize them in situ, restrict shear forces, and prevent bacterial contamination. This enhanced the chance of successful dressing, thus yielding better results (47).

The Doppler ultrasound evaluation to detect cutaneous blood perfusion associated with using both xenografts for the dressing of full-thickness started to be taken on day 12 post-surgery to avoid the separation of the dressing from the underline bed. The Doppler ultrasound examination exhibited the presence of good cutaneous blood perfusion since day ten, which became more detectable on day 12 in the first group and day 14 in the second experimental group. The number then continued to increase and reached its peak on day 21. The cutaneous blood perfusion associated with wound healing in cats started within the first week, elevated until day 14, and then declined by day 21 (13,48). Furthermore, the cutaneous blood perfusion was evident since day seven and gradually increased until day 14 (2).

The histopathological manifestations associated with using both acellular ovine skin and bovine pericardium for dressing full-thickness skin wounds of cats revealed significant differences in most of the studied histopathological parameters between them during the whole study period. The acellular ovine skin on day 7 following dressing was much better and exhibited the presence of close contact and interwoven with the wound beds skin with less inflammatory response and infiltration of polymorphonuclear and mononuclear inflammatory cells as compared with acellular bovine pericardium. This could be because the dressing of the 1st group was derived from the skin that resembles the cutaneous wound bed in its histology, anatomy, and even physiology, enabling it to match the full-thickness wound and adhere well to it perfectly and to initiate less inflammatory reaction due to the presence of same tissue elements especially growth factors and cytokines which can only exhibit their action on a particular cell surface receptors that later convey their growth signals as a result of this high attraction to specific surface receptors (49,50). All these features permitted the acellular ovine skin to induce less inflammatory reaction than the acellular bovine pericardium.

The histopathological appearance associated with using both acellular tissues as a dressing xenogenic cover of feline full-thickness cutaneous wounds showed severe infiltration of inflammatory cells, especially mononuclear during the first two weeks, which indicates that both of them provoked more immunogenicity and less acceptance because both of them were derived from xenogenic sources, i.e., ovine and bovine (51,52). This intense spread of this inflammatory cell was also noticed with existence of severe mononuclear cells on day 14 during his work on the transfer of peripheral nerve xenograft (53). Furthermore, other inflammatory cells, like polynuclear inflammatory cells, were also recognized in both cellular tissues, indicating foreign body reactions (54). Macrophages are the main responsible cells for the innate immune system, and thus during xenograft transplantation, they display phagocytic action and modify adaptive immunity through participating in cell recruitment and antigen exhibition (55). Furthermore, inflammatory reaction was less severe with acellular ovine skin comparable to acellular bovine pericardium which in turn helped in the better performance of ovine skin dressing especially its pliability that survived longer than bovine pericardium. The skin dressing survival is significantly associated with the drops in its associated inflammatory infiltration (56). Besides this, the intense inflammatory reaction increased from day 0 to day 14 in both groups, then started to decline until day 21, which was in agreement with the studied cutaneous wound healing in cats (2).

Other histopathological indicators associated with applying both acellular tissues revealed the presence of good angiogenesis and new blood vessels since day 14 following dressing. However, this angiogenesis was detected earlier from day 10-12 using Doppler ultrasonography. Besides, this revascularization became more noticeable as the healing process progressed forward, which is regarded as the foundation for the ideal healing process because

angiogenesis and the formation of new blood capillaries are the basic steps for any normal tissue renewal and regeneration as these recently molded blood vessels would carry the essential nutrient elements with oxygen supply to the dynamically active renewing tissue which is critical for bioactive regeneration of the injured tissue as it needs an adequate huge vascular support as several studies have found that angiogenesis is the key for wound repair that stimulate healing rates. At the same time, its decline can impair healing rates (57,58).

Lastly, well-developed granulation tissue related to the acellular ovine skin group is comparable to the decellularized bovine pericardium, which has been evident since day 14 after dressing. However, its rate and quantity are much lower than that which is usually seen with other species, especially dogs, because wound healing in cats is different from that of other animals, making this cardinal phenomenon (lower granulation rate) as one of the apparent manifestation of wound healing (7,8), which indicating that the ovine skin suited wounds bed tissue much better than acellular bovine pericardium as the presence of granulation tissue with its all histological elements such as inflammatory cell infiltration, the proliferation of fibroblasts, endothelial cells, and new thin-walled blood vessels is considered as the revealing parameters for the good progress of the healing course within the dressing area (59-61).

On day 21 of the experiment, the presence of all the cardinal necessary healing elements of the perfect healing process associated with using acellular ovine skin has led to improved histological appearance, better tissue construction, and organizational integrity, especially epidermal integrity and dermo-epidermal junction. In the current study, the staining expression of VEGF in both experimental groups was inspected to assess the preserved tissue viability, which depends on their capacity to express this active growth factor crucial for the success of any wound healing process. Angiogenesis is one of the major contributing factors in the wound repair process, and it is thought to permit the sending of necessary nutrients and oxygen to the rapidly multiplying reparative cells at the wound site. VEGF is the greatest substantial mediator for wound angiogenesis, and its manufacturing inspires new capillary growth to deliver adequate nutrients, oxygen, and inflammatory cells. Many studies have found angiogenesis to be supportive of wound repair. Angiogenesis motivation can stimulate healing rates, while a decline in angiogenesis can spoil healing rates (57,58). During the wound healing process, new blood capillaries are gradually produced into the wound bed, forming a rich network of new blood vessels up to 10 times thicker than within normal tissue. The beginning of angiogenesis is entirely controlled by several growth factors, especially VEGF, the leading proangiogenic element in healing wounds. VEGF acts as a powerful proangiogenic moderator that can elevate vascular permeability and participate in wound edema (62).

The immunohistochemistry results of vascular endothelium growth factor expression detected that both exogenous dressings showed variable intensity expression and visualization of VEFG. This output data could be due to the presence of well-preserved ECM within both decellularized ovine skin and bovine pericardium, indicating the efficiency of our decellularization protocol that preserved the intact tissue construction that made both of them still have the talent to express and deliver this vital growth factor. The presence of the well integral ECM within both xenograft dressings made these biomaterials still hold the aptitude to release growth factors and other cytokines to the wound area as this intact matrix, in turn, acts as a vital direct pool for various growth factors and other cytokines that enhance the healing process course (63,64). The active relations between growth factors and ECM deeply influence wound healing. These relations have many levels and forms that could be categorized into direct or indirect levels. The extracellular matrix can directly connect to and secrete certain growth factors that may help sequester and keep growth factors from breakdown and degradation or indirectly improve their activity (65,66). The presence of strong expression of VEGF as a regenerative indicator for the degree of tissue viability that the VEGF is highly expressed during wound healing (67,68).

Conversely, the decellularized ovine skin displayed more staining intensity than the decellularized bovine pericardium. Besides, statistical analysis displayed a significant difference in VEGF expression between them. This could be due to the assumption that ovine-derived tissues are more suitable for cats, or it could also be due to the difference in the type of tissue used as xenografts as biomaterial of the first group, which was taken from the skin. In contrast, the dressing of the second group was prepared from the pericardium. that made ovine skin dressing biomaterial more active in stimulating the release of VEGF because it derived from the same tissue type, i.e., skin that completely resembled the kind of tissue that is injured while dressing biomaterial of the second group was derived from the pericardium, which is completely different from the anatomy, histology and even function from the skin that used to reconstruct it that in turn influence on the proper functions of these tissue elements especially growth factors which can only exhibit their action on particular cell surface receptors that next convey their growth signals as a result of their high attraction to a specific surface receptors (49,50).

Furthermore, the intensity of staining and expression was gradually increased with time as a result of the progress in the healing process, the negative immunohistochemical staining of VEGF at early stages of wound healing (0-3 days) that then elevated in association with progress of wound healing in a time-dependent manner (51-70), that can be even possible to suggested that they would be useful indicators for the determination of wound age. Besides, following the injury, the levels of proangiogenic factors increase gradually

in association with the progress of the healing process, then reach a slight peak and finally diminish (62).

Conclusions

The clinical, physical, and microscopic data of this study revealed that using a bioactive dressing derived from acellular ovine skin and acellular bovine pericardium can efficiently promote the healing process of large cutaneous wounds in cats, with the superiority of acellular ovine skin.

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Conflict of Interests

The researchers of this work announce that there is no conflict of interest associated with the publication of this article.

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مقارنة بين جلد الأغنام اللاخلوي وتامور الأبقار اللاخلوي على التئام فقدان الجلد المستحدث في القطط المحلية

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الخلاصة

كان الهدف من هذه الدراسة هو المقارنة بين جلد الأغنام اللاخلوي وتامور الأبقار اللاخلوي في التئام فقدان الجلد الكبير في القطط. تم استخدام ثمانية عشر قطًا محليًا بالغًا والتي قسمت إلى مجموعتين متساويتين تضم ٩ حيوانات في كل منها. تم إنشاء جروح مربعة كاملة السمك بأبعاد ٤*٤ سم على ظهور جميع القطط. في المجموعة الأولى، تم استخدام جلد الأغنام اللاخلوي برقعة ٣*٣ سم لإغلاق جروح الظهر كاملة السماكة وخياطتها بحواف الجرح ومن ثم ضمد الجرح بينما تم استخدام التامور البقري اللاخلوي بنفس الحجم في المجموعة الثانية. قيمت الجروح مجهرياً وفيزيائياً وسريرياً بشكل يومي في حين اجري الفحص النسيجي المرضي في الأيام ٧ و ١٤ و ٢١ بعد العملية. أظهرت النتائج السريرية وجود فرق معنوي بين المجموعتين في سرعة التئام الجرح. أظهر التقييم الفيزيائي باستخدام الموجات فوق الصوتية الدوبلر وجود تروية دموية جيدة في الجلد منذ اليوم ١٢ في المجموعة الأولى واليوم ١٤ في المجموعة التجريبية الثانية. أظهرت المظاهر النسيجية وجود اختلافات معنوية في معظم المؤشرات النسيجية الملحوظة خاصة التشابك مع طبقات الجرح، تكوين الأوعية الدموية، وجود أنسجة حبيبية متطورة وبنية أنسجة جيدة مرتبطة بمجموعة جلد الغنم اللاخلوية. كشفت نتائج الكيمياء المناعية للتعبير عن عامل نمو بطانة الأوعية الدموية أن المجموعة الأولى أظهرت كثافة وجود عامل نمو البطانة الوعائية أكثر من المجموعة الثانية. باختصار، يعتبر جلد الأغنام اللاخلوي أفضل بكثير من تامور الأبقار اللاخلوي كضمادة لشفاء الجروح الكبيرة.

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