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INVESTIGATING THE HEALING POTENTIAL OF FRESH AMNIOTIC MEMBRANES IN FULL-THICKNESS CANINE SKIN WOUNDS

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ABSTRACT

Wound healing is a multifaceted process that can be interrupted, resulting in chronic wounds and infections. It is essential to develop treatments that are not only effective and maintain cellular bioactivity but are also cost-effective and easy to store. The amniotic membrane (AM) stands out for its high biocompatibility and abundance of bioactive factors, which makes it a promising option in regenerative medicine. This study investigates the efficacy of fresh bovine AM in promoting the healing of full-thickness skin wounds in dogs. Bovine AM was collected, washed, and sterilized. In vivo, two full-thickness skin wounds were created on the backs of 8 dogs and treated with saline (control) or fresh AM. Wound healing was monitored over 3 and 5 weeks, and tissue samples were collected for histological examination. Grossly, wounds treated with fresh AM healed faster than those in control wounds. Histologically, the fresh AM group exhibited accelerated healing compared to the control, with signs of angiogenesis, collagen fiber remodeling, resolution of inflammation, and re-epithelialization. Additionally, hair follicles and their associated glands were observed only in the fresh AM-treated wounds. In conclusion, the application of fresh bovine amniotic membrane greatly promotes wound healing, making it a practical and beneficial choice for managing full-thickness skin wounds.

Keywords: Skin Wound, Amniotic Membrane, Regeneration, Healing.

INTRODUCTION

The treatment of skin wounds is a critical aspect of both human and veterinary medicine, as wound healing can be a lengthy and complex process. In many cases, complications such as infections, delayed healing, and chronic wounds arise, necessitating advanced therapeutic interventions (Guo & Dipietro, 2010). One of the most promising biomaterials in wound management is the amniotic membrane (AM), the innermost layer of the fetal membranes, which has been recognized for its potent regenerative properties (Toda, Okabe, Yoshida, & Nikaido, 2007). The use of fresh amniotic membrane in wound care offers a natural and biocompatible solution that can accelerate healing and reduce complications (Davis, 1909).

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In humans, fresh amniotic membrane has been successfully applied in various clinical contexts, including the treatment of chronic wounds, burns, and surgical wounds (Hao, Ma, Hwang, Kim, & Zhang, 2000; Pfister *et al.,* 2023). Its anti-inflammatory, anti-scarring, and antimicrobial properties make it an attractive option for enhancing the natural healing process (Niknejad *et al.,* 2008). Fresh AM is rich in growth factors, cytokines, and extracellular matrix components, which contribute to tissue regeneration, reepithelialization, and wound closure (Wilshaw, Kearney, Fisher, & Ingham, 2006). Additionally, the membrane serves as a physical barrier that protects the wound from external contamination, further promoting healing (Mermet *et al.,* 2007). Similarly, in veterinary medicine, the application of fresh AM has gained traction for treating wounds in animals (Kim, Choi, Jeong, & Williams, 2009). Common causes of skin injuries in animals, such as traumatic wounds, burns, and surgical incisions, often require treatments that not only accelerate healing but also prevent infection and minimize scarring (Pavletic, 2018). The use of fresh AM in animals has shown promising results, facilitating faster wound closure and improving overall recovery (Fitriani, Wilar, Narsa, Mohammed, & Wathoni, 2023; Murphy *et al.,* 2020). Its ease of use and natural bioactivity make it an ideal option for managing wounds in various animal species (Fairbairn, Randolph, & Redmond, 2014).

However, the availability of human amniotic membrane remains limited, especially in veterinary settings where the need for accessible and cost-effective treatments is paramount. This scarcity has prompted the exploration of xenogeneic alternatives to bridge this gap (Fetterolf & Snyder, 2012). Bovine AM offers similar regenerative properties and can be sourced more easily, making it a viable option for wound treatment in animals (Dadkhah Tehrani, Firouzeh, Shabani, & Shabani, 2020; Octarina, Munadziroh, Razak, & Surboyo, 2022; Yurtal *et al.,* 2023). In this study, we aimed to evaluate the effectiveness of bovine AM on wound healing in canine models to assess its potential as a readily available therapeutic option.

MATERIALS AND METHODS

Animals

This study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, in accordance with Egyptian laws and OIE animal welfare standards (Approval Number 06/2023/0109).

Amniotic Membrane Harvest

Bovine amniotic membranes were obtained from placentae of five cows following cesarean section at the Obstetrics and Gynecology Department of Assiut University Veterinary Hospital. Placentae were handled aseptically, and the amniotic membranes were isolated and washed 3 times with normal saline containing antibiotics (Vetrocin; El-Nasr Pharmaceutical Chemicals Company, Egypt) for 15 minutes. The membranes were cut into 5 cm × 5 cm pieces, stored in sterile saline with antibiotics, and kept at 4°C until use. All membranes were used within 48 hours and sterilized by UV exposure for 30 minutes before application.

Creation of Full-Thickness Skin Wounds in Dogs

The study involved 8 clinically healthy mongrel dogs (n = 8) of both sexes, weighing between 17–30 kg. Dogs were housed individually with free access to food and water and fasted 12 hours before surgery. Anesthesia was induced using 1 mg/kg xylazine HCl (Xyla-Ject, ADWIA Co., Egypt) and 10 mg/kg ketamine HCl (Sigma-tec Pharmaceutical Industries, Egypt) intramuscularly. Two full-thickness skin wounds (3 × 3 cm) were created on the right side of each dog's back. The wounds were made along the thoracic and lumbar

regions, with at least 4 cm between them. The cranial wound was served as the control, treated with saline and the caudal wound was covered with fresh AM. Fresh AM was secured with four or five interrupted stitches using 3/0 Polyglactin 910 (Vicryl, ETHICON, USA). All wounds were dressed with sterile cotton pads, gauze, and elastic bandages. Dressings were changed weekly. Cefotaxime (Rametax 1 g, Ramida Pharmaceutical, Egypt) was administered intramuscularly in a dose of 20 mg/kg twice daily for 5 days post-surgery. Wounds were examined grossly for signs of infection. Wound closure was calculated using ImageJ software based on percentage reduction from the initial wound size.

Histological Examination

Skin samples were taken at 3- and 5-weeks post-wound induction, fixed, dehydrated, and embedded in paraffin. Sections were prepared and stained with hematoxylin and eosin (H&E) to examine the wound and surrounding area. Samples were taken including the wound and surrounding uncut normal skin under general anesthesia using 1 mg/kg xylazine HCL 2% and 10 mg/kg ketamine HCl 5%.

RESULTS

Gross Appearance

Complete wound closure was achieved over a 5-week period, with wounds photographed at 3- and 5-weeks post-wound induction. The results showed that fresh amniotic membrane-treated wounds healed faster than the control (saline-treated) group as shown in Fig 1. At week 3, the fresh AM group had a higher closure rate (72.1 ± 6.72%) compared to the control group (55.7 ± 4.9%). By week 5, the fresh AM group demonstrated 92.7 ± 3.7% closure rate, while the control group showed 84 ± 2.29% closure rate as shown in Fig 2.

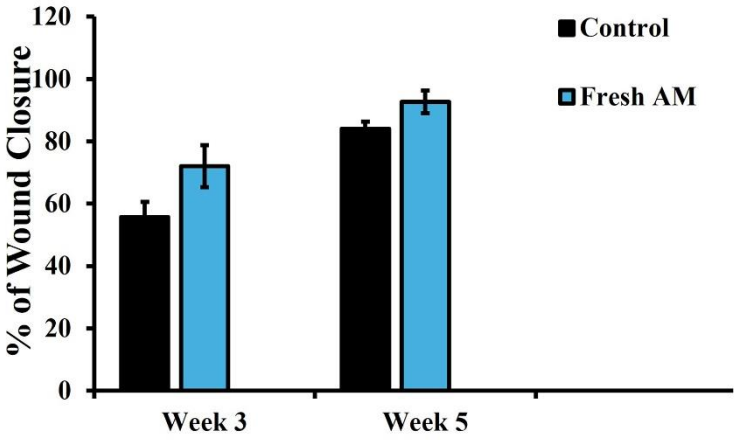

Figure 1: Gross appearance of the skin wound in canine model. Illustrative images of the skin wound areas from the control and fresh AM groups were taken at 3- and 5-weeks post-wound induction.

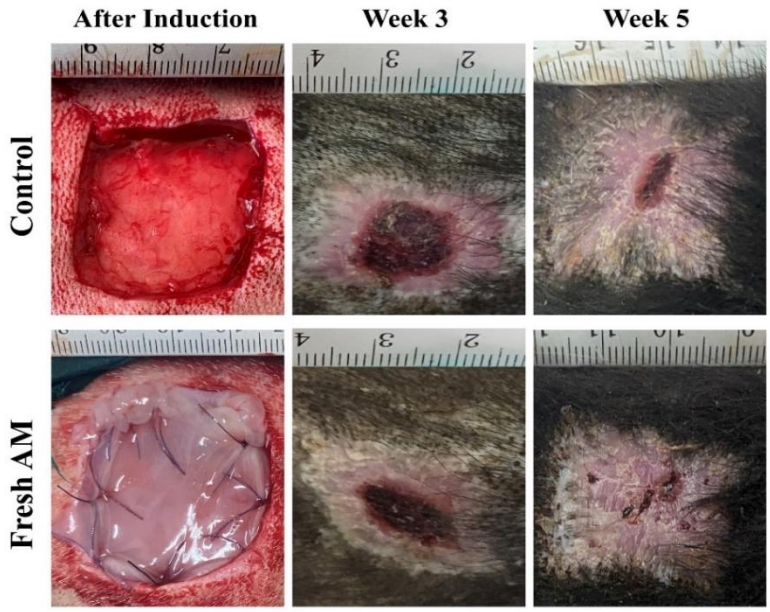

Figure 2: Percentage of wound closure. Wound closure percentage measured using ImageJ, compared to the initial wound area. Data are expressed as mean ± standard deviation.

Histological Findings

The histological paraffin section revealed that the control groups still had necrotic tissue and scabs after three weeks of the wounds, which intermingled with inflammatory cells (Fig. 3 A, B). On the other hand, the wound region exhibited granulation tissue with proliferative epithelium and extensive neovascularization (angiogenesis, or the formation of new blood vessels) in the groups that received fresh amniotic membrane treatment. Furthermore, compared to the control groups, the collagen fibers in the fresh AM's dermis were well-organized (Fig. 3 C, D).

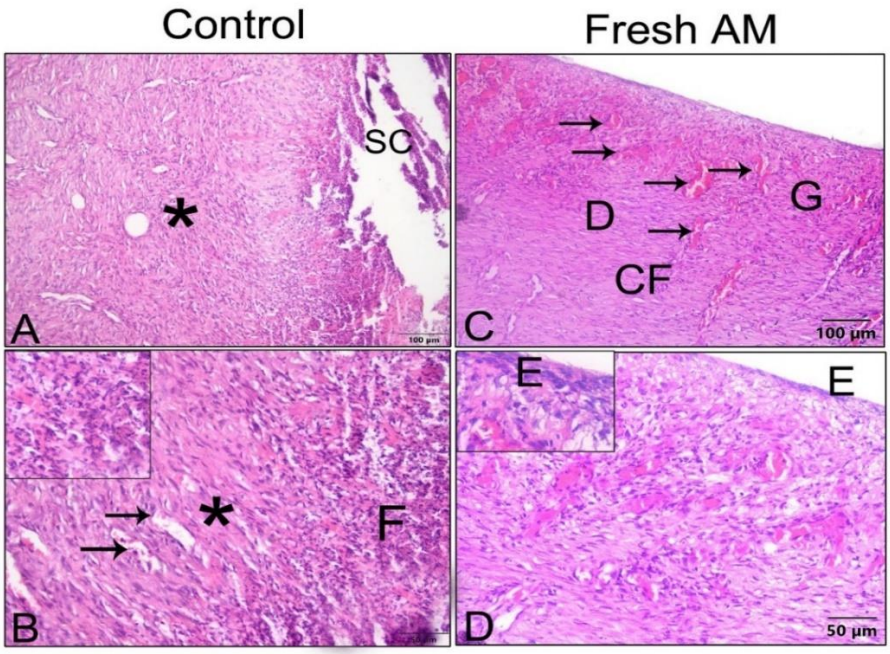

Figure 3: Paraffin section-stained with H and E showing the wound healing after 3 weeks of wound induction. A, B: The control groups showed scabs (SC) intermingled with inflammatory cells (F, inset) and few newly formed blood vessels was noticed (arrows). The collagen fibers were dis-organized (asterisk). C, D: The fresh AM groups showed granulation tissue (G) with excessive neovascularization (arrows). The collagen fibers (CF) were well organized. E: new re-epithelization was seen (E).

Following 5 weeks of wound induction, the control group's skin in the area that was wounded healed normally, but no hair or glands were observed (Fig. 4 A, B). On the other hand, the fresh AM groups displayed almost total healing of the skin wound. The epidermis with its associated structure including the hair follicle, sebaceous and sweat glands were investigated (Fig. 4 C, D).

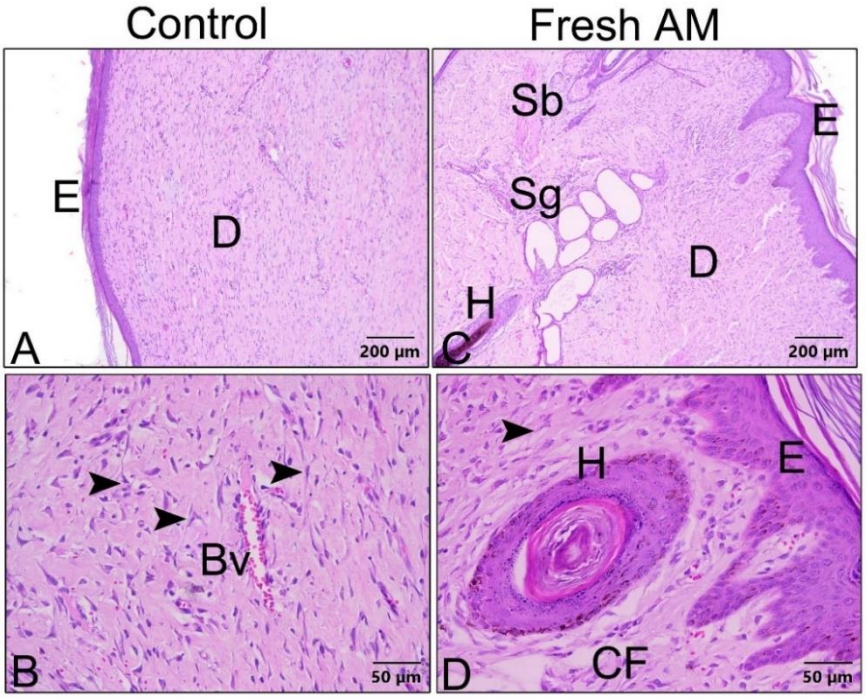

Figure 4: Paraffin section-stained with H and E showing the wound healing after 5 weeks of wound induction. A, B: The control groups showed nearly normal epidermis (E) and dermis (D). the dermis region showed remodeled collagen fiber with proliferation of fibroblast cells (arrowheads). Few blood vessels (Bv) were seen to be congested. C, D: Normal skin with hair in (C) and hair follicles in (D) was displayed by the fresh AM groups. The epidermis (E) with its associated glands such as sweat glands (Sg) and sebaceous glands (Sb) was seen. Arrowhead refers to fibroblast cells. Note the hair (H) and collagen fibers (CF).

DISCUSSION

This study demonstrates the efficacy of fresh amniotic membrane (AM) in treating full-thickness wounds and promoting rapid wound closure, primarily through enhanced epithelialization. Histological analysis supported these findings, showing accelerated healing and the formation of a mature epidermis and dermis similar to healthy skin.

Skin repair involves a complex series of biochemical and cellular events. Traditional therapies and natural products have been used to stimulate regeneration and prevent healing failure, with the amniotic membrane emerging as a notable therapeutic option due to its rich stem cell content and regenerative properties (Vecin & Kirsner, 2023). Compared to other tissues, the placenta has low immunogenicity and is abundant in growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which are crucial for angiogenesis and fibroblast proliferation and other regenerative components (Wassmer & Berishvili, 2020).

The superior healing observed with fresh AM is consistent with its known benefits in tissue repair. Histological analysis revealed

that AM treatment accelerates the formation of a mature epidermis and dermis, comparable to healthy skin. This improvement over control groups, which displayed necrotic tissue and scabs even after three weeks, is supported by similar findings in the literature. For instance, fresh AM has been shown to accelerate wound healing and improve tissue regeneration in various studies. Sledge *et al.* (2020) demonstrated that AM significantly enhanced the healing of diabetic foot ulcers by promoting epithelialization and granulation tissue formation (Sledge *et al.,* 2020). Similarly, in a study by Chandra *et al.* (2005), fresh AM was used effectively to treat corneal epithelial defects, resulting in rapid epithelial regeneration and reduced inflammation (Chandra *et al.,* 2005). Our study's findings align with the results of previous research that fresh AM treatment improved wound healing outcomes by promoting tissue regeneration and reducing scar formation

CONCLUSION

Amniotic membrane is a highly advantageous biomaterial due to its availability, cost-effectiveness, and ease of use. These qualities make it particularly suited for tissue engineering and regenerative medicine, especially in skin and soft tissue repair.

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التحقيق في القدرة العلاجية للأغشية الأمنيوسية الطازجة في جروح جلد الكلاب كامله السماكة

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التئام الجروح هو عملية متصلة متعددة الأوجه، وإذا تعرضت هذه العملية للخلل فقد يؤدى هذا إلى جروح والتهابات مزمنة. لذلك من الضروري تطوير علاجات ليست فقط فعالة وتحافظ على النشاط الحيوي الخلوي فحسب، بل تكون أيضًا غير مكلفه وسهلة التخزين. يتميز الغشاء الأمنيوسي بتوافقه الحيوي العالي ووفرة العوامل النشطة بيولوجيًا، مما يجعله خياراً واعداً في الطب التجديدي.

تتحقق هذه الدراسة من فعالية الغشاء الامنيوسي البقري الطازج في تعزيز شفاء الجروح الجلدية كاملة السماكة في الكلاب. تم جمع الغشاء الامنيوسي من الأبقار وغسلها وتعقيمها. تم إنشاء جروح جلدية كاملة السماكة في الجسم الحي على ظهور ٨ كلاب. تم علاجها بمحلول ملحي (مجموعه ضابطه) او الغشاء الامنيوسي الطازج. تم رصد التئام الجروح على مدى ٣ و ٥ أسابيع. تم جمع عينات الأنسجة للفحص النسيجي، بشكل عام.

الجروح المعالجة بـ الأغشية الأمنيوسية الطازجة تلتئم بشكل أسرع من تلك الموجودة في الجروح الخاضعة للمجموعة الضابطة .من الناحية النسيجية، أظهرت مجموعة الأغشية الأمنيوسية الطازجة شفاءً سريعًا مقارنةً بالمجموعة الضابطة، مع ظهور علامات تكوين الأوعية الدموية، وإعادة تشكيل ألياف الكولاجين، واختفاء الالتهاب، وإعادة التشكل الظهاري، بالإضافة إلى ذلك، لم تتم ملاحظة بصيلات الشعر والغدد المرتبطة بها إلا في الجروح المعالجة بالغشاء الامنيوسي الطازج. في الختام، فإن تطبيق الغشاء الامنيوسي البقري الطازج يعزز بشكل كبير التئام الجروح، مما يجعله خياراً عملياً ومفيداً لعلاج جروح الجلد كاملة السماكة.