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Marine collagen xenografts accelerate tissue repair in open canine skin wounds: clinical and histopathological evidence

Xenoinjertos de colágeno marino aceleran la reparación tisular en heridas cutáneas abiertas de caninos: evidencia clínica e histopatológica

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ABSTRACT

The use of marine collagen xenografts represents an innovative therapeutic alternative in regenerative veterinary medicine due to their ability to accelerate tissue repair and improve the structural quality of cutaneous wound healing. This biomaterial of ichthyologic origin exhibits high biological compatibility, low immunogenic risk, and strong similarity to mammalian dermal collagen. The present study evaluated its efficacy in the regeneration of open skin wounds in dogs through clinical, photographic, and histopathological analyses, in comparison with a conventional treatment using a healing cream. Five mixed–breed dogs with lesions ranging from 2.4 to 11.07 cm² were used. Three dogs were treated with dehydrated and sterilized marine collagen xenografts applied using the MAHVET technique, and two received daily antiseptic cleaning and healing cream. Clinical follow–up and morphometric measurements, obtained with a high–precision vernier caliper, were performed weekly for six weeks. Dogs treated with xenografts exhibited wound area reductions greater than 98% from the first weeks, achieving near–complete closure (99.27–99.82%) within three to four weeks, while those under conventional treatment reached reductions of 97.05–98.81% by the sixth week. Histopathological analysis revealed an intact epidermis, minimal inflammation, mature fibroblasts, and compact collagen fibers arranged in parallel in the xenograft group, in contrast to persistent inflammation and immature collagen in the control group. The results demonstrate that marine collagen xenografts promote wound closure, optimize tissue organization and maturation, and represent a biocompatible and effective therapeutic alternative for the healing of open cutaneous wounds.

Key words: Xenograft; marine collagen; wound healing; histopathology; regenerative Veterinary Medicine

RESUMEN

El uso de xenoinjertos de colágeno marino constituye una alternativa terapéutica innovadora en la medicina veterinaria regenerativa por su capacidad para acelerar la reparación tisular y mejorar la calidad estructural de la cicatrización cutánea. Este biomaterial de origen ictiológico presenta alta compatibilidad biológica, bajo riesgo inmunogénico y similitud con el colágeno dérmico de los mamíferos. La presente investigación evaluó su eficacia en la regeneración de heridas cutáneas abiertas en perros, mediante análisis clínico, fotográfico e histopatológico, en comparación con tratamiento convencional con crema cicatrizante. Se emplearon cinco perros mestizos con lesiones de entre 2,4 y 11,07 cm². Tres pacientes fueron tratados con xenoinjertos de colágeno marino deshidratado y esterilizado aplicados mediante la técnica MAHVET, y dos con limpiezas antisépticas diarias y crema cicatrizante. El seguimiento clínico y las mediciones morfométricas empleando un calibrador vernier de alta precisión, se realizaron semanalmente durante seis semanas. Los pacientes tratados con xenoinjertos mostraron reducciones del área lesionada superiores al 98 % desde las primeras semanas, alcanzando cierres casi completos (99,27–99,82 %) en tres a cuatro semanas, mientras que los tratados convencionalmente alcanzaron reducciones del 97,05–98,81 % al finalizar la sexta semana. El análisis histopatológico evidenció en el grupo con xenoinjertos una epidermis íntegra, mínima inflamación, fibroblastos maduros y fibras colágenas compactas dispuestas paralelamente, en contraste con el grupo control, donde persistió inflamación y colágeno inmaduro. Los resultados demuestran que los xenoinjertos de colágeno marino favorecen el cierre de la herida, optimizan la organización y maduración tisular, representando una alternativa biocompatible y terapéutica eficaz en la cicatrización de heridas cutáneas abiertas.

Palabras clave: Xenoinjerto; colágeno marino; cicatrización; histopatología; Medicina Veterinaria regenerativa

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INTRODUCTION

The tissue repair of open cutaneous wounds represents a significant clinical challenge in veterinary medicine. Traditional approaches, such as continuous disinfection and the use of conventional dressings, although useful, may contribute to antimicrobial resistance, cause discomfort to the dog, and require frequent handling that delays the healing process [1].

Xenografts derived from marine collagen an abundant and sustainable source, have emerged as an innovative and promising alternative. This biomaterial exhibits antioxidant, antimicrobial, and wound–healing properties, acting on key processes of tissue regeneration such as re–epithelialization, collagen deposition, angiogenesis, and granulation tissue formation [2].

Several studies have explored the potential of this biomaterial in cutaneous repair, consistently demonstrating its modulatory effect on the wound–healing response. In an experimental rat model, topical application of collagen derived from tilapia (*Oreochromis niloticus*) accelerated recovery through the stimulation of fibroblasts and myofibroblasts, as well as by promoting a more organized synthesis of the extracellular matrix [3].

Additionally, a recent meta–analysis reported that marine–derived materials enhance critical stages of regeneration, including re–epithelialization, angiogenesis, and tissue maturation across diverse animal models [2].

Similarly, oral administration of peptides obtained from chum salmon (*Oncorhynchus keta*) exerted a positive systemic effect, reflected in faster wound closure, improved connective tissue organization, and enhanced neovascularization highlighting the versatility of this resource for both topical and systemic applications [4].

Clinical use of acellular fish–skin grafts has yielded successful outcomes in dogs and cats with open cutaneous wounds. In a series of 17 cases, these grafts effectively promoted wound healing, improved dog comfort, reduced wound manipulation, and demonstrated suitable biological safety [5, 6].

Likewise, hydrogels and composite structures formulated from marine collagen have shown the ability to promote cell proliferation, integrate into the wound bed, stimulate vascularization, and accelerate re–epithelialization in animal models [7].

Moreover, marine collagen has been employed in tissue engineering as a base material for the fabrication of in vitro scaffolds designed for human skin regeneration. Sponges prepared from fish scales supported fibroblast and keratinocyte adhesion and proliferation, demonstrating their biocompatibility and potential for clinical application [8].

The present study aims to evaluate the therapeutic efficacy of marine collagen xenografts specifically derived from fish skin in the repair of open cutaneous wounds in canines, through a comparative clinical, histopathological, and regenerative analysis against conventional treatments. This approach seeks to provide robust scientific evidence regarding the potential of marine–derived xenografts as an innovative, biocompatible, and effective strategy for wound healing, thereby contributing to the development of advanced therapeutic alternatives in regenerative veterinary medicine.

MATERIALS AND METHODS

Animals

Five mixed–breed dogs (*Canis lupus familiaris*) of both sexes, aged 1 to 5 years and weighing 10 to 25 kg, were enrolled in the study. Body weight was determined using a digital scale (OHAUS Scout Pro SP202, OHAUS Corporation, USA). All individuals presented superficial cutaneous wounds with a maximum surface area of 11.3 cm² (length × width), as reported in TABLE I and illustrated in FIG. 1.

Lesion depth ranged from 0.5 to 5 mm, aligning with the anatomical criteria used in reconstructive surgery to classify superficial cutaneous defects. In this framework, depths near 0.5 mm correspond to damage

TABLA I
Macroscopic measurements (length × width) and surface area of cutaneous lesions in the evaluated dogs

Dogs	Measurements L × W (cm)*		Surface area (cm²)
1	1.2	2	2.4
2	3.75	1.35	5.625
3	2.9	1.9	5,51
4	3.2	2.2	7.04
5	4.1	2.7	11.07

*Measurements were performed using a high–precision Vernier caliper (Truper®, model CAL-6P, Mexico). L × W: Length × Width

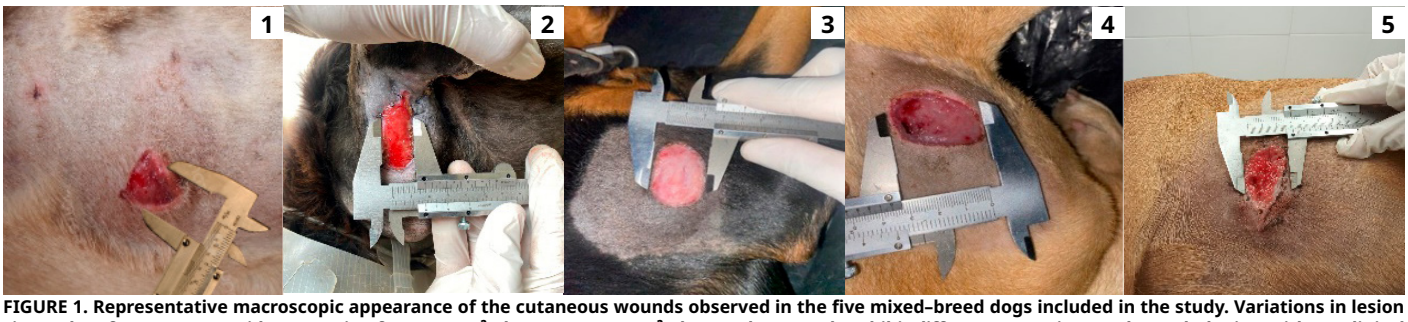

FIGURE 1. Representative macroscopic appearance of the cutaneous wounds observed in the five mixed–breed dogs included in the study. Variations in lesion size and surface area are evident, ranging from 2.4 cm² (dog 1) to 11.07 cm² (dog 5). The wounds exhibit different extensions and morphologies, without clinical signs of systemic infection. Each photograph corresponds to an individual dog, arranged from left to right according to the assigned numbering

confined to the epidermis and superficial dermis, whereas depths approaching 5 mm indicate extension into the deep dermis and superficial subcutaneous tissue without breaching fascia, muscle, or other deeper anatomical layers. According to the classification proposed by Huppes *et al.* [9]. Such defects are compatible with primary closure, local flap techniques, or grafting procedures typically employed in small animal reconstructive surgery. Based on these anatomical considerations, the wounds included in this study met the criteria necessary for standardized evaluation of healing dynamics.

The animals were clinically healthy at the time of inclusion; no systemic disease was detected, and none had a history of treatment with immunosuppressive agents. Clinical monitoring and wound progression were documented on standardized technical forms specifically designed for this investigation.

Wound management

Acepromazine maleate (0.05 mg·kg^{-1}, intramuscular route) was administered to induce mild sedation, following the recommendations described by Murrell. These guidelines emphasize the use of low doses of phenothiazine tranquilizers to reduce anxiety, facilitate safe clinical handling, and avoid excessive cardiovascular depression during minor procedures. They also support the intramuscular route due to its predictable onset of action and its ability to provide an adequate level of sedation without compromising respiratory function or hemodynamic stability [10].

The perilesional area (2–3 cm) was shaved with a Wahl® Bravura electric clipper (USA), followed by cleansing with potable water and surgical soap. Subsequently, 2% chlorhexidine was applied to the surrounding tissue, and enzymatic debridement was performed using Protosan® Solution (Lohmann & Rauscher, Rengsdorf, Germany), allowing the product to act for 15 minutes (min) to remove necrotic or damaged tissue. Wound dimensions were measured using a Vernier caliper (Truper®, model CAL-6P, Mexico), ensuring precise documentation of their progression. Finally, the lesions were covered with a sterile bandage, and definitive treatment was initiated after 24 hours. All clinical and procedural activities were carried out at the UDIV–Veterinary Clinic, Veterinary Medicine Degree, Escuela Superior Politécnica Agropecuaria de Manabí “Manuel Félix López”.

Application of xenografts

The xenografts used in this study, donated by Genezing Seven (United Arab Emirates), were composed of dehydrated and sterilized marine collagen. Dogs 3, 4, and 5 were selected, as their wounds presented the largest surface areas (TABLE I). The Advanced Wound Management Technique in Veterinary Medicine (MAHVET) was applied, which consists of placing a collagen xenograft over the wound, covered with a layer of gauze fixed by anchoring sutures and a multilayer bandage [11] (FIG. 2). This technique facilitates biomaterial integration, minimizes wound manipulation, and accelerates recovery, offering an effective and accessible option for the management of complex wounds.

The protocol included replacement of the xenografts every seven days (d) to assess macroscopic progression and record any adverse reactions. At each replacement, wound dimensions were re–measured using a high–precision Vernier caliper (Truper®, model CAL-6P, Mexico).

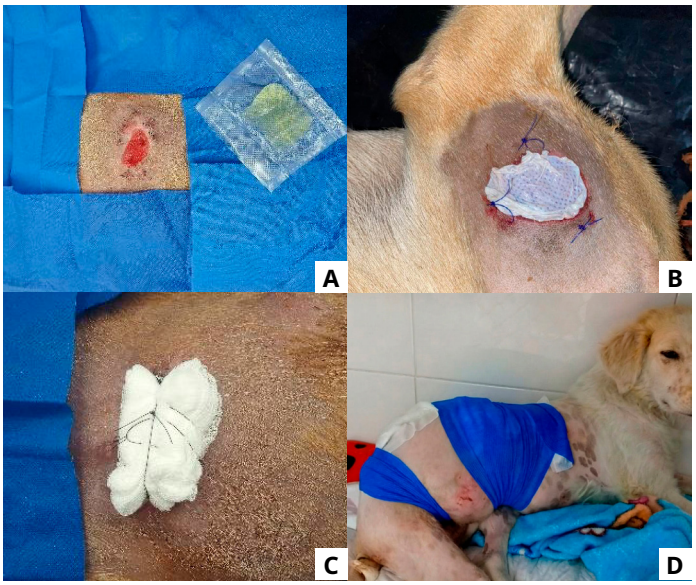

FIGURE 2. Application of a marine collagen xenograft using the MAHVET technique in a cutaneous wound of a mixed–breed dog. A: Placement of the marine collagen xenograft over the wound, B: gauze layer fixed with anchoring sutures according to the MAHVET technique, C: application of a multilayer bandage in which the first layer consisted of elastic or cotton padding, and D: final placement of a self–adhesive blue cohesive layer securing the edges of the initial layer, thereby promoting xenograft integration and wound healing

Conventional treatment (control group)

Dogs 1 and 2 were assigned to the control group. Their wounds received conventional topical management, consisting of daily cleansing with an antiseptic solution (Sablon Germidal) followed by the application of a topical formulation containing phenolic acid, zinc oxide, and pine oil (Crema Alfa®) (FIG. 3). Phenolic acid provides broad–spectrum antiseptic activity, zinc oxide supports epithelialization and tissue repair while offering a protective barrier, and pine oil contributes additional antiseptic and antioxidant effects that help maintain wound hygiene and reduce oxidative stress. These measures were applied according to standard protocols for traditional wound care.

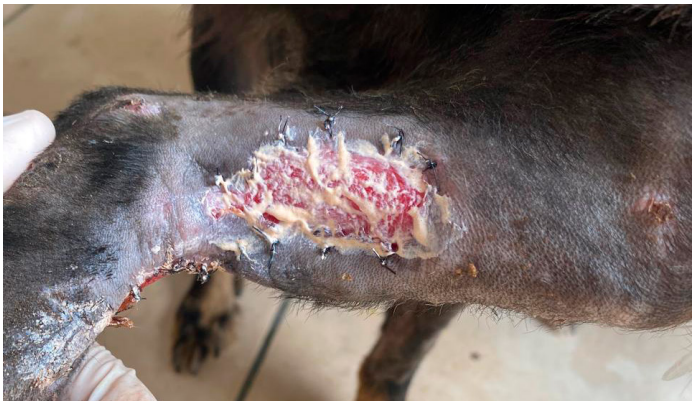

FIGURE 3. Open cutaneous wound treated with conventional therapy using Crema Alfa® (phenolic acid, zinc oxide, and pine oil). The superficial lesion on the canine limb shows exposed granulation tissue, active wound margins, and early re–epithelialization

Histopathological evaluation

Upon completion of the treatment protocols, dog 2 (control group) and the three dogs treated with xenografts were selected for biopsy sampling using the punch biopsy technique described by Huppes *et al*. [9]. Tissue samples were immediately fixed in 10% neutral–buffered formalin (Merck KGaA, Darmstadt, Germany) for 48 hours (h) and submitted to the "Laboratorio Anato–Patológico" H & E Laboratory, a certified center specialized in histological, immunological, and molecular biology techniques, located in the city of Cuenca (Aurelio Aguilar V. 1-85, 010203), Ecuador, where the entire histopathological procedure was carried out.

At the laboratory, samples were dehydrated through a graded series of ethanol (70%, 80%, 95%, and 100%; Sigma–Aldrich, St. Louis, USA) and cleared in Xylene (PanReac AppliChem, Barcelona, Spain). The tissues were then embedded in paraffin wax with a melting point of 56–58°C (Leica Biosystems, Nussloch, Germany) using an automatic tissue processor (Leica TP1020, Leica Biosystems, Germany). Paraffin blocks were sectioned at 4–5 µm thickness with a Leica RM2235 rotary microtome (Leica Biosystems, Germany), and sections were mounted on poly–L–lysine–coated slides (Thermo Fisher Scientific, Waltham, USA).

Sections were stained with Harris hematoxylin (Biopack, Buenos Aires, Argentina) and 1% alcoholic eosin (PanReac AppliChem, Spain) following the standard H&E protocol. Microscopic observations were performed using an Olympus CX43 trinocular optical microscope (Olympus Corporation, Spain) equipped with a digital camera Olympus SC50 and CellSens Standard v3.2 software. Histological images were documented to evaluate inflammatory, proliferative, and remodeling processes, as well as the morphological organization of fibroblasts, angiogenesis, and collagen deposition.

RESULTS AND DISCUSSION

TABLE II presents the dogs treated with marine collagen xenografts, who exhibited a rapid and sustained reduction in wound area. The dog 3 achieved a cumulative reduction of 99.27%, dog 4 of 98.86%, and dog 5 of 99.82%. These findings indicate that the use of marine collagen xenografts promoted accelerated and nearly complete healing from the early weeks of treatment. FIG.4, shows the photographic sequence that objectively documents the progressive reduction of the wounds, visually confirming the clinical efficacy of the xenograft throughout the healing process.

TABLE III shows the dogs treated with Crema Alfa® (phenolic acid, zinc oxide, and pine oil), who exhibited a progressive wound–healing process. Dog 1 achieved a cumulative reduction of 97.05%, whereas dog 2 reached 98.81%. The results demonstrate that the application of Crema Alfa® (phenolic acid, zinc oxide, and pine oil) promoted a significant reduction in wound area, achieving outcomes close to complete closure by the end of the follow–up period. These findings are corroborated by FIG. 5, which presents a photographic sequence documenting the progressive decrease in wound area and complete re–epithelialization at the end of treatment. It is important to note that dog 1 was excluded from the final comparative analysis because the initial lesion size did not represent equivalent severity or extension compared with the other cases, which could have affected the uniformity of the evaluation.

The results revealed differences in the rate of wound healing between treatments. Dogs treated with marine collagen xenografts (TABLE II) achieved reductions greater than 98% within a shorter period, reaching near–complete resolution by the third week (dog 3) and the fourth week (the dog with the largest lesion surface area). In contrast, dogs treated with Crema Alfa® (phenolic acid, zinc oxide, and pine oil) (TABLE III) achieved cumulative reductions of 97.05% and 98.81% by the end of the six–week follow–up period. Taken together, these results demonstrate that although both treatments promoted significant wound healing, the marine collagen xenografts accelerated the tissue repair process, particularly in larger lesions, when compared with conventional therapy using Crema Alfa® (phenolic acid, zinc oxide, and pine oil).

The comparison between the photographic sequences shown in FIGS. 4 and 5 reveals notable differences in both the healing time and the quality of tissue repair achieved. In the group treated with marine collagen xenografts (FIG. 4), the wounds exhibited a significant reduction in lesion area and an almost complete re–epithelialization process within three weeks.

This behavior is associated with the ability of collagen to act as a highly functional biological scaffold that provides both structural and biochemical support during tissue repair. Its tridimensional matrix facilitates the migration, adhesion, and proliferation of fibroblasts, keratinocytes, and endothelial cells key components in skin regeneration [12].

Moreover, collagen participates in the modulation of the local inflammatory response by reducing the infiltration of neutrophils and pro–inflammatory macrophages, thereby promoting a

TABLE II
Progression of cutaneous wound surface area and cumulative percentage reduction in three canine dogs treated with marine collagen xenografts

Week	Dog 3 Wound area (cm^2)	Cumulative reduction (%)	Dog 4 Wound area (cm^2)	Cumulative reduction (%)	Dog 5 Wound area (cm^2)	Cumulative reduction (%)
1	5.51	–	7.04	–	11.07	–
2	0.22	96.01	1.04	85.23	3.45	68.83
3	0.04	99.27	0.08	98.86	0.84	92.41
4	–	–	–	–	0.02	99.82

Surface area (cm^2) and cumulative reduction (%) in three dogs treated with marine collagen dressings. The cumulative reduction was calculated relative to the initial wound surface area superficie

TABLE III
Progression of cutaneous wound surface area and cumulative percentage reduction in two canine dogs treated with conventional therapy

Week	Dog 1 Wound area (cm^2)	Cumulative reduction (%)	Dog 2 Wound area (cm^2)	Cumulative reduction (%)
1	2.40	–	5.06	–
2	1.00	58.33	3.91	22.86
3	0.20	91.67	2.39	52.89
4	0.06	97.05	1.08	78.67
5	–	–	0.27	94.67
6	–	–	0.06	98.81

Surface area (cm^2) and cumulative reduction (%) in two dogs treated with conventional therapy consisting of cleansing and Crema Alfa® (phenolic acid, zinc oxide, and pine oil). The cumulative reduction was calculated relative to the initial wound surface area

microenvironment dominated by M2 macrophages. This immunological balance enhances angiogenesis, the orderly deposition of collagen fibers, and the maturation of granulation tissue, accelerating re–epithelialization and improving the histological organization of the newly formed dermis [12,13].

In contrast, conventional treatment based on daily antiseptic cleansing and topical application of healing cream (FIG. 5) exhibited a more prolonged repair process, achieving complete healing only by the sixth week. Although this approach enabled functional tissue regeneration, the process was slower and accompanied by persistent signs of local inflammation, dense granulation tissue, and the formation of a fibrous scar with lower histological organization.

These findings suggest that, unlike treatment with marine collagen xenografts, conventional management limits the modulation of the inflammatory response and fails to efficiently stimulate angiogenesis or orderly re–epithelialization, resulting in a less structured dermal architecture and a longer recovery period. The persistence of inflammation has been described as a critical factor that compromises tissue repair by hindering the orderly transition to the proliferative phase; in experimental models, prolonged inflammatory responses significantly delayed wound closure and reduced granulation tissue formation, thereby affecting angiogenesis and cell migration required for re–epithelialization [14].

Immune dysregulation leads to excessive extracellular matrix deposition and the formation of disorganized scars, whereas M2 macrophage dysfunction delays regeneration and increases susceptibility to secondary infections [15, 16]. Collectively, these mechanisms explain the limited inflammatory resolution and greater fibrosis observed with conventional topical therapies, underscoring the need for biomaterials capable of modulating inflammation and promoting structured skin regeneration.

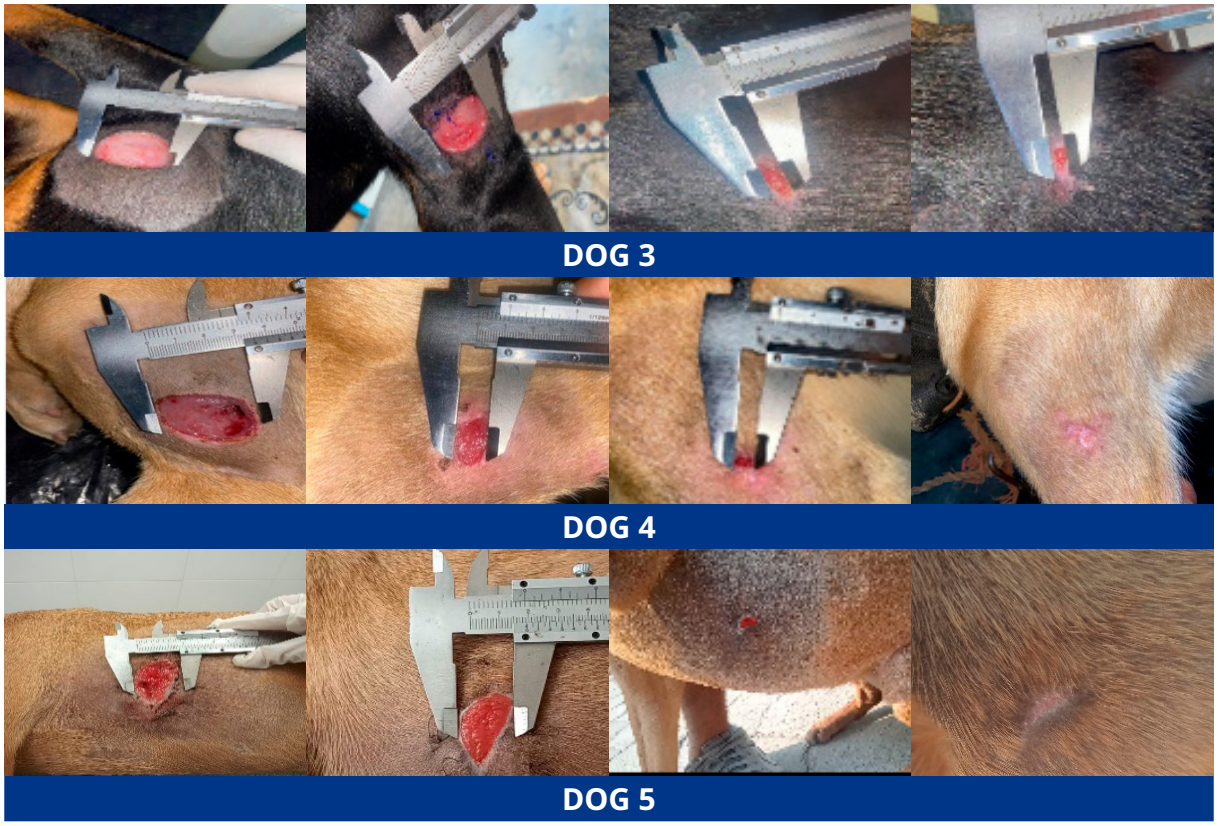

FIGURE 4. Photographic sequence of cutaneous tissue repair in dogs treated with marine collagen xenografts. A progressive reduction in wound area, granulation tissue formation, and re–epithelialization can be observed, leading to complete closure and formation of a functional scar at the end of the follow–up period

FIGURE 5. Photographic sequence (dog 2) showing the evolution of a cutaneous wound in a dog treated with antiseptic cleansing and topical application of Crema Alfa® (phenolic acid, zinc oxide, and pine oil) over a six-week period. A progressive reduction in wound surface area, granulation tissue formation, and complete re-epithelialization can be observed, resulting in scar closure at the end of the follow-up

The application of marine collagen xenografts thus represents a clinically relevant advantage in veterinary medicine, as it significantly reduces wound closure time, improves the histological quality of the healed tissue, and minimizes complications associated with chronic inflammation. These properties position marine collagen xenografts as a promising biotechnological alternative to conventional treatments for cutaneous injuries in dogs.

The results of the present study confirm the efficacy of marine collagen as a therapeutic biomaterial, consistent with previous research that demonstrated its rapid adherence to the wound bed, early granulation tissue formation, reduced infection risk, and orderly tissue regeneration [17, 18]. In particular, the use of tilapia skin as a biological xenograft in dogs with cutaneous discontinuity allowed immediate graft adherence, early granulation tissue formation, and progressive reduction of the wound area, with no evidence of infection or rejection and adequate restoration of epidermal integrity [19].

In another clinical case, the application of tilapia skin to an extensive bite wound in a dog resulted in optimal graft adherence, rapid epithelialization, and complete closure within three weeks, with minimal inflammatory response [20].

Furthermore, composite membranes of tilapia (*Oreochromis niloticus*) collagen and thermoplastic polyurethane (Col–TPU) have been developed, showing high structural compatibility, thermal stability, and improved mechanical strength while maintaining the integrity of the collagen triple–helix structure.

These materials exhibited no cytotoxicity and favored fibroblast adhesion and migration, demonstrating favorable cellular behavior. Such findings confirm the potential of marine collagen as a biocompatible, biofunctional, and structurally stable biomaterial [21], in accordance with the healing efficacy observed in the present study.

Recent studies further support the regenerative potential of fish–skin xenografts in the treatment of chronic and complex wounds. In a clinical investigation, fish–skin grafts were shown to promote rapid granulation tissue formation, stimulate angiogenesis, and induce orderly epithelialization, leading to complete resolution without local complications [22].

Similarly, ichthyologic xenografts have been reported not only to accelerate wound closure but also to preserve the underlying tissue and affected limb, due to their anti–inflammatory effect and capacity to induce structured tissue regeneration [23].

Additionally, fish skin has been explored as a regenerative biomaterial in the management of equine (*Equus caballus)* wounds, yielding favorable clinical responses. In donkeys (*Equus asinus*), the use of tilapia skin as a biological dressing for full–thickness metacarpal wounds promoted early epithelialization, accelerated wound contraction, and reduced bacterial proliferation, resulting in effective cutaneous regeneration without local complications [24].

Likewise, in humans with partial–thickness burns, tilapia skin xenografts achieved faster healing, significant pain reduction, and superior functional and aesthetic results compared with conventional treatments [25]. These findings confirm the capacity of ichthyologic collagen to stimulate dermal repair mechanisms and consolidate its clinical potential as a cross–species biomaterial for regenerative medicine.

The results of this study, supported by the reviewed scientific evidence, confirm the efficacy of marine collagen xenografts as an innovative and effective therapeutic alternative for managing open skin wounds in veterinary medicine.

Comparative histopathological assessment of wound healing between marine collagen xenografts and conventional therapy

In dog 2, treated with the healing cream (Crema Alfa®), histopathological analysis revealed an ongoing tissue repair process. The epidermis exhibited partial re–epithelialization, and the connective tissue showed features consistent with an intermediate proliferative phase, characterized by the predominance of active fibroblasts arranged in a disorganized pattern, the presence of immature and sparse collagen fibers, and a persistent inflammatory infiltrate (FIG. 6).

The FIG. 7, shows that treatment with a xenograft (dog 5), corresponding to the wound with the largest diameter (11.07 cm^2), exhibited an advanced stage of tissue repair with proper implant integration. This was characterized by the presence of a continuous

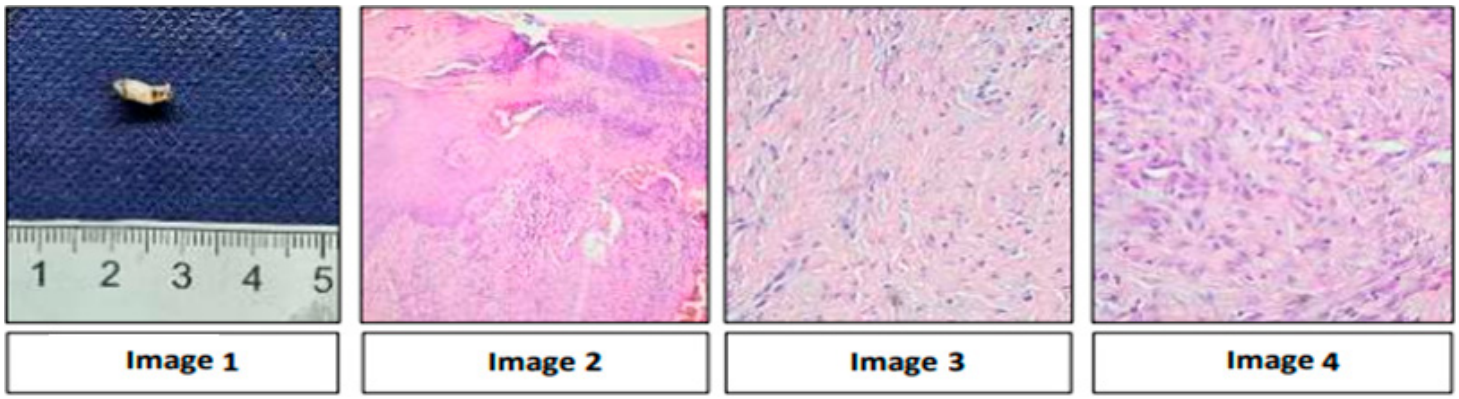

FIGURE 6. Histopathological progression of a superficial wound treated conventionally with a healing cream (Crema Alfa®). Macroscopically (Image 1), the punch biopsy sample was adequate in size and quality. Histology at low magnification (Image 2, 4×, panoramic field) reveals partial re–epithelialization with evident keratinocyte migration from the wound margins, indicating an active epithelial repair phase. At intermediate magnification (Image 3, 10×, dermal field), the connective tissue exhibits numerous active fibroblasts with elongated nuclei, associated with irregular and loosely organized collagen bundles, characteristic of granulation tissue. At high magnification (Image 4, 40×, detailed dermal field), a moderate inflammatory infiltrate composed mainly of lymphocytes, plasma cells, and scattered macrophages persists, suggesting that the remodeling phase is ongoing. The arrangement and density of collagen fibers indicate that the tissue has not yet reached complete structural maturation

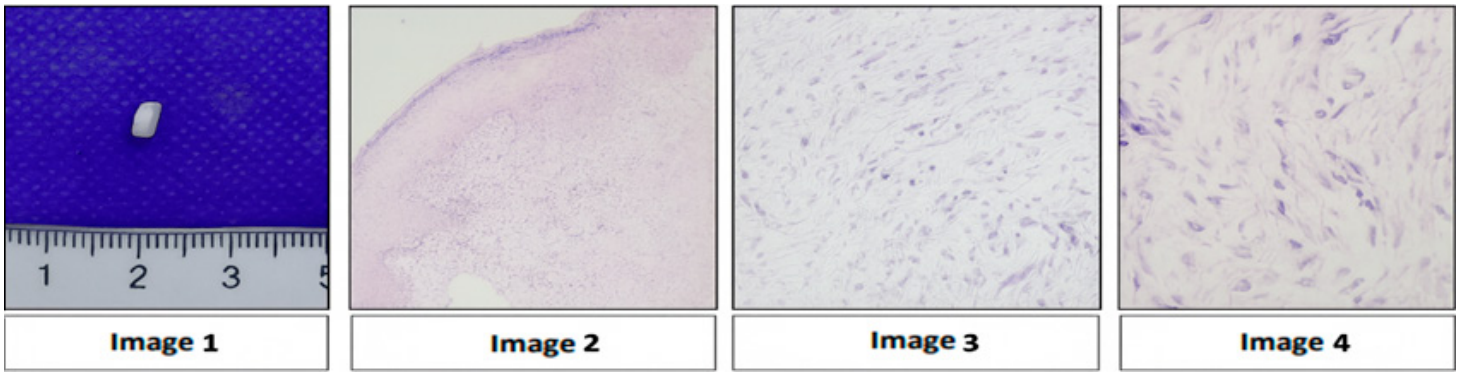

FIGURE 7. Histopathological progression of a superficial wound treated with marine collagen xenografts. Macroscopically, (Image 1) the punch biopsy sample presented similar size characteristics but was clinically obtained from a wound treated with a xenograft. Histology at low magnification (Image 2, 4×, panoramic field) shows a fully re–epithelialized epidermis, continuous and well adhered to the dermal bed, with no evidence of necrosis or ulceration. At intermediate magnification (Image 3, 10×, dermal field), the connective tissue exhibits mature fibroblasts with elongated nuclei and abundant cytoplasm, aligned parallel to the surface, indicative of advanced tissue remodeling. At high magnification (Image 4, 40×, detailed dermal field), compact and well–organized collagen fibers are observed, arranged in parallel bundles, accompanied by minimal inflammatory infiltrate and scarce angiogenic activity, reflecting a late remodeling phase with structural and functional restoration of the dermal matrix

epidermis, minimal inflammatory response, and abundant mature fibroblasts indicative of active tissue remodeling. This dog was selected as the representative case for histopathological evaluation because it presented the most extensive lesion, allowing clearer observation of the complete dermal regeneration process.

The findings were consistent with those observed in dogs 3 and 4, whose histological sections revealed analogous tissue organization, including neovascular formation, alignment of collagen fibers, and uniform re–epithelialization, confirming the reparative efficacy of the marine collagen xenograft.

Histopathological analysis of the punch biopsy samples revealed relevant differences between the conventional treatment with healing cream and the treatment with collagen xenografts. In the conventional treatment group, a partial re–epithelialization process was observed at the epidermal level, with persistence of chronic inflammatory infiltrate and irregularly arranged collagen fibers. Fibroblasts appeared active but displayed a disorganized pattern, corresponding to an intermediate proliferative phase of the healing process.

In contrast, the group treated with the xenograft exhibited an intact epidermis well adhered to the dermal bed, with minimal inflammation and absence of necrosis. The connective tissue showed aligned mature fibroblasts, compact collagen fibers arranged in parallel, and newly formed blood vessels, indicating a more advanced phase of tissue remodeling. These findings suggest that the application of xenografts promotes faster and more organized tissue repair, reduces the inflammatory response, and enhances the overall quality of healing.

These results are consistent with reports in the literature, which have demonstrated that collagen xenografts act as biocompatible matrices capable of stimulating cell migration, promoting angiogenesis, and supporting orderly collagen deposition, thereby accelerating the wound–healing process compared with conventional topical treatments [26, 27, 28, 29, 30].

CONCLUSION

The findings of this study suggest that marine collagen xenografts constitute a biocompatible and promising therapeutic option for enhancing closure and regeneration of open cutaneous wounds in dogs. Their use was associated with appropriate tissue integration and improved structural organization and differentiation of the connective matrix relative to conventional therapy. Nevertheless, given the limited sample size, additional studies with larger cohorts are required to validate these preliminary observations and more robustly determine their clinical effectiveness.

ACKNOWLEDGMENT

The authors express their sincere gratitude to the staff and the company Genezing Seven (United Arab Emirates) for kindly providing the marine collagen xenografts used in this study and for their technical support during the experimental phase.

Ethical approval and animal welfare

This study was conducted in accordance with national and international animal welfare guidelines and was approved by the Bioethics Committee of the Escuela Superior Politécnica Agropecuaria de Manabí "Manuel Félix López" (ESPAM MFL). All procedures were performed with measures to minimize pain and stress, and informed consent was obtained from the owners of the participating dogs.

Conflict of interest

The authors declare that they have no conflict of interest.

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