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Use of micro-grafts in a chronic infected open wound after limb amputation in a cat

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Abstract: In human medicine, skin grafting is an innovative surgical technique widely used in reconstructive surgery to repair skin loss. This case evaluated the effectiveness of a treatment with dermal micro-grafting obtained through the Rigenera® technology in a chronic open wound resulting from a suture dehiscence of a limb amputation in a cat. Significant differences were observed between the aesthetic aspects of the injury using traditional treatments (cleaning and curettage) and the regenerative technology. The results showed that the healing periods were significantly reduced after the Rigenera® treatment and that, moreover, a perfect skin status and a complete reduction in the wound area (100%) were achieved in one month. Given these results, Rigenera® has proven to be a simple yet highly effective method in the treatment of inactivated chronic wounds.

Keywords: chronic wound; feline traumatology; regenerative medicine; Rigenera® system; skin injury

Wound healing is a physiological process that allows the restoration of the tissue after injury. It is defined as the body's attempt to recondition the structural integrity and subsequent normal function of the tissue (Leong et al. 2017). Although all wounds undergo similar repair processes, there are some differences in tissue types and species as well as the time it takes for a wound to completely heal (Velnar et al. 2009). A limb amputation is a surgical procedure that, in some cases, can lead to stump infections and/or wound dehiscence, described in both human (Dunkel et al. 2012) and veterinary medicine (Kaufman and Mann 2013). This procedure is commonly performed in small animals, and it is indicated for unresectable neoplasms, severe fractures, ischemic necrosis, soft tissue infections, paralysis, or congenital deformities (Fitzpatrick et al. 2011; Raske et al. 2015). The main cases described are bone infections (56%), abscesses or haematomas requiring a flap elevation (41%), and a partial (19%) or complete flap loss (15%) (Harris et al. 2009). Specifically, wound dehiscence is often associated with some degree of necrosis of the skin border, suggesting that excessive tension can compromise the vascular supply, therefore increasing the risk of the procedure failing (Cantatore et al. 2014). In veterinary medicine, understanding of subcellular events, including assistance from various signalling mediators, is still incomplete, and many mechanisms have yet to be clarified. However, in the last few years, major progress in wound management has been made, with the support of new regenerative medicine technologies (Vitali et al. 2017; Salvaggio et al. 2020). The standard of care for open wound management is currently to promote a moist wound environment to induce debridement as well as granulation tissue development and epithelialisation, reducing any microbial contamination (Hartoch et al. 2007). For the management of chronic open wounds and ulcers, different treatment strategies have been performed on small animals, such as the use of chitosan (Azuma et al.

https://doi.org/10.17221/197/2020-VETMED

2015), bio-scaffolds (Schallberger et al. 2008), platelet-rich plasma (PRP) (Tambella et al. 2014), and growth factor agents (Karayannopoulou et al. 2015; Marchegiani et al. 2020). Recently, a new treatment based on the application of autologous micro-grafting, obtained through the Rigenera® technology, has been described. This innovative treatment strategy helps to achieve the healing of open wounds through tissue regeneration and to avoid the development of non-aesthetic scars (Trovato et al. 2015). This technology has also been able to stimulate tissue healing with highly viable micro skin grafts of 80 microns obtained via mechanical fragmentation. The micro-grafts are enriched with mesenchymal progenitor cells, extracellular matrix (ECM) components, and growth factors obtained from the patients themselves, initiating biological regeneration processes that enhance the wound healing process (Riccio et al. 2019). The use of micro-graft technologies through Rigenera® is strongly supported by the literature in human medicine with a series of clinical cases and various studies (Giaccone et al. 2014; Svolacchia et al. 2016; De Francesco et al. 2017). This clinical case evaluates the effectiveness of dermal micro-grafting, obtained through Rigenera®, in the treatment of a chronic surgical dehiscence wound in a cat, following a standard conservative treatment.

Case presentation

An adult stray female cat weighing 2.1 kg was sent to the emergency service at the veterinary teaching hospital of the University of Camerino for an unknown trauma.

At the clinical presentation, the cat showed clinical evidence of septic shock secondary to an infection caused by an open fracture grade 2 of the right femur (Figure 1).

After resuscitation therapy and stabilisation, the cat underwent amputation of the injured hind limb (Figures 2 and 3).

After the surgery (T0), the gradual loosening of the suture in the caudal aspect of the wound was reported. After five days (T5), partial dehiscence of the wound was detected; therefore, cleaning and applying ointment based on chloramphenicol and collagenase (Iruxol 1%; Smith & Nephew, Hull, UK) were performed on the open wound, following standard of care techniques. After 10 days (T10) of wound management (cleaning and Iruxol), no improvement was observed, so the surgeon opted to perform a surgical debridement and apply a suture to bring the edges of the wound closer. At the end of the debridement procedure, the area of the wound was calculated (4 327.637 mm^2). After 15 days (T15), the authors verified a new

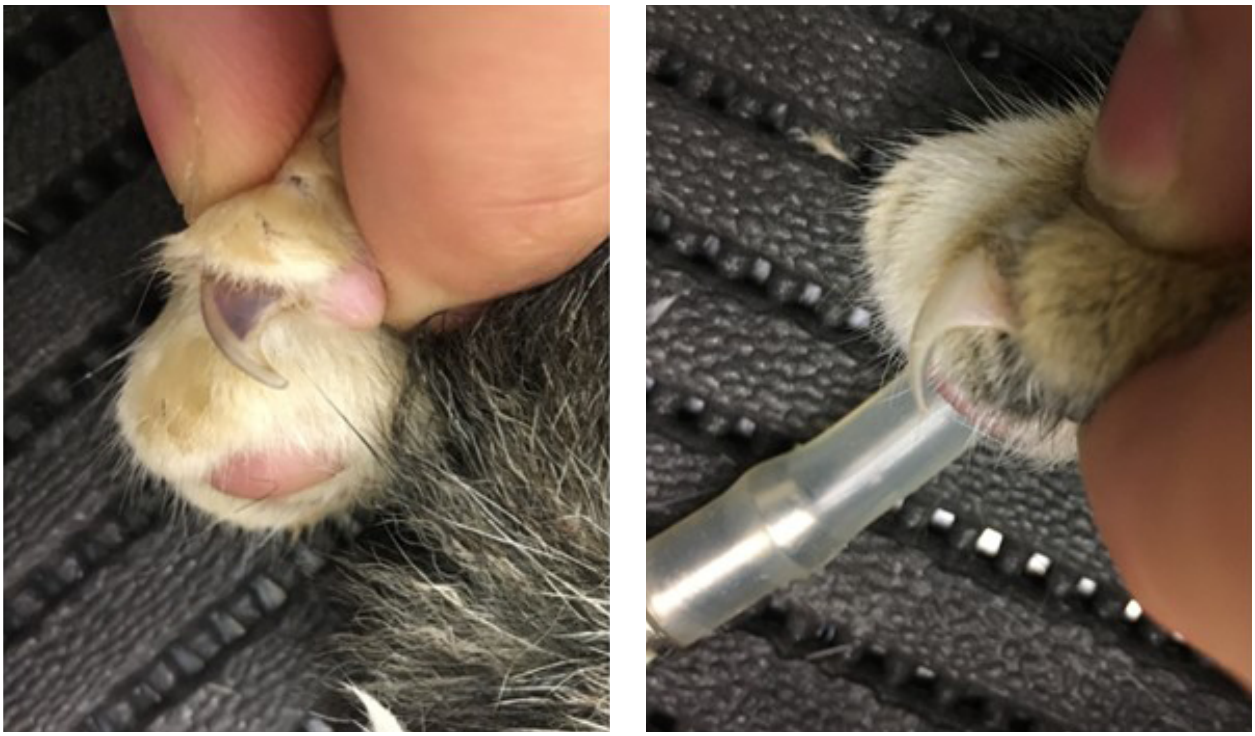

Figure 1. Nail bed vascular outflow arrest evidenced on the right hindlimb at the first clinical presentation

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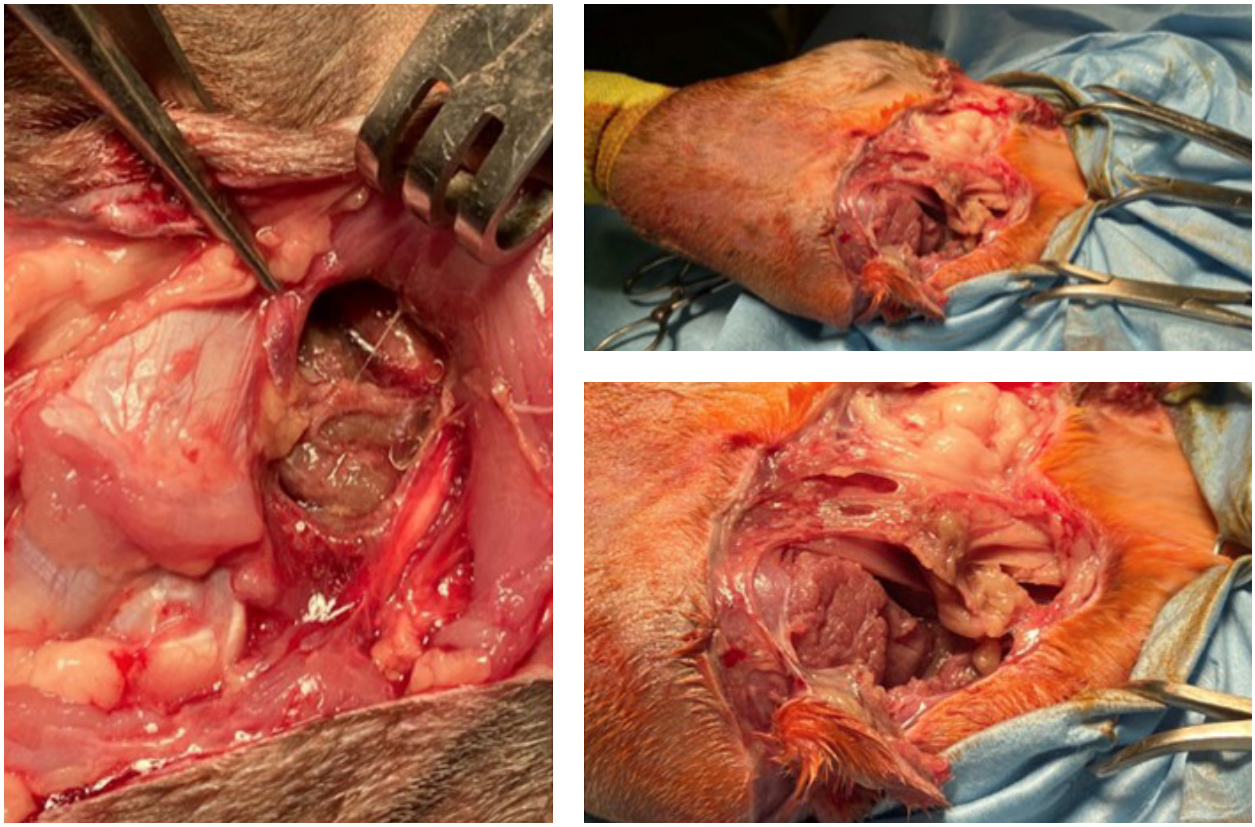

Figure 2. Severe gangrenous necrosis and infection of the hamstring muscle. The black arrow shows the ischiatic nerve of the right limb

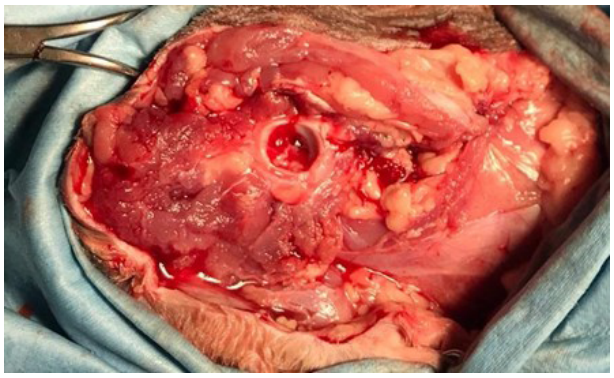

Figure 3. Spread muscle excision after amputation secondary to the surgical debridement of the necrotic tissues

dehiscence with a 31% increase in the injury area (6 272.349 mm^2) (Figure 4).

After 30 days (T30) of medical treatment, the wound showed no improvement, and the area did not change significantly. The wound area (4 025.734 mm^2) showed the presence of an exudate and the absence of granulation tissue.

Afterwards, plastic surgery using the dermal micro-grafting technique (Rigenera®) was proposed and accepted by the municipal kennel.

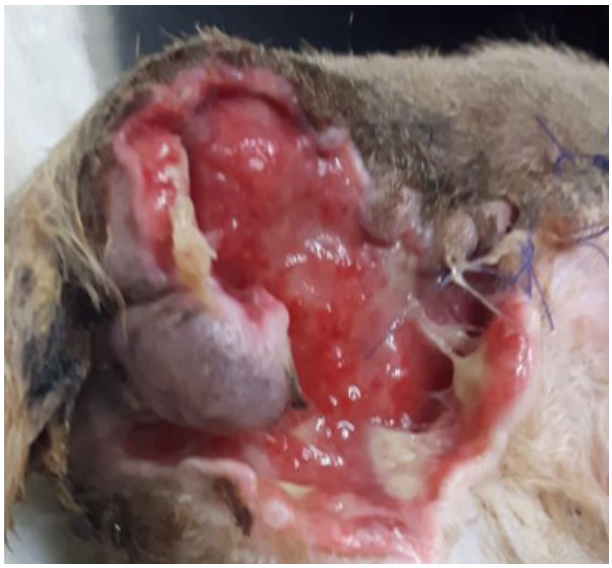

Figure 4. Result of the surgical dehiscence after the surgical curettage (T15)

Treatment

The Rigenera® mechanical disintegration system (CE Class I certificate; Human Brain Wave, Turin, Italy) is a medical device that helps obtain,

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in a standardised way, with high reproducibility and minimal manipulation, micro-grafts from different tissues readily available for clinical practice (Figure 4). The obtained micro-grafts are characterised by a size of about 50–70 µm, which grants them high cell viability and cell nutrition by simple interstitial diffusion. The Rigenera® system consists of a motorised apparatus that allows a sterile and disposable capsule (Rigeneracons®) to mechanically disintegrate the tissue placed inside it (Figure 5). Each Rigeneracons® capsule is internally made up of a helical blade managed by an electric motor that rotates at 80 rpm, thus allowing a precise, uniform, and constant cut. In addition, at the end of each propeller, there is a metal filter containing 100 holes, approximately 50 µm, each of which has 6 micro-scalpels. The disintegrated and filtered tissue is collected at the bottom of the capsule, and thanks to a syringe fitting, the preparation can be aspirated and used. The tissue inserted into the capsule must be previously manually sectioned by the surgeon to obtain a size of about 2 mm, or it must be aspirated, as is the case for adipose tissue, through liposuction with a cannula and subsequently treated with the Rigenera® system. After 31 days (R1), a suture to bring the edges of the wound closer was applied, and a Rigenera® biocomplex protocol for chronic open wounds was performed using three dermal tissue samples obtained from the caudal aspect of the shoulder and processed with a sterile saline solution through the Rigeneracons® (Figure 6). After 60 s, a dermal disaggregate micro-graft was aspirated, and it was ready to use (Figure 6F). The chronic open wound was widely washed (Figure 7A), and sterile swabbing was applied for the microbiological analysis. The syringe sample with the micro-graft solution was subjected to circumferential wound edge infiltration (Figure 7B) and injection on a collagen sponge (Cutanplast®) to create a Rigenera® biocomplex. This biocomplex was placed over the injury, ensuring that the sewn area of the sponge was in contact with the wound bed and fixed on the wound. After the suture and the procedure with the Rigenera® biocomplex, the wound area was 422.924 mm^2 (Figure 7C).

A non-adherent tie-over bandage with a sterile cotton gauze (Pic Solution; Artasana Group, Grandate, Italy) was applied and renewed every 72 h (Figure 7C).

After each new bandage, the wound surface was measured and assessed for contraction. The wound contraction was measured using specific software (Image-J software, ImageJ 1.45 s freeware; National Institutes of Health, Rockville, MD; http://imagej.net/ImageJ; Aragon-Sanchez et al. 2017), expressing the variation of the wound area as a percentage compared to the original area.

Outcome and follow-up

After 3 days (R3), the area was 294.450 mm^2, and the wound showed an enhancement in the granulation tissue and a reduction in the exudation, with a contraction of 30.37% of the wound. After

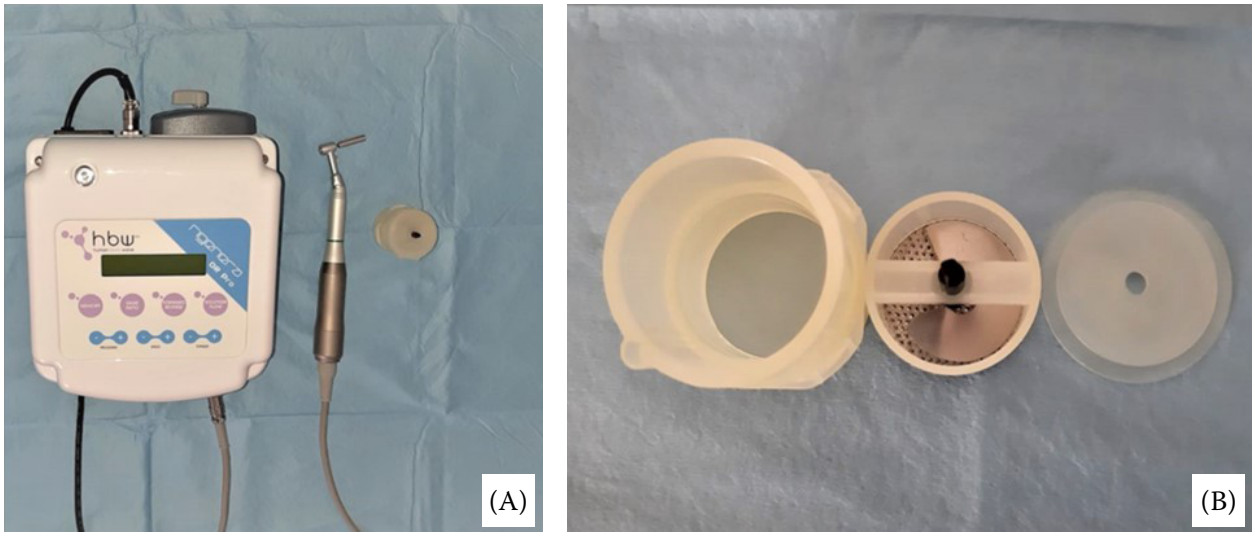

Figure 5. (A) Rigenera® device. The image (B) shows the disposable device (Rigeneracons® or capsule) constituted inside by a helical blade and a metal filter containing 100 holes, each of which has 6 micro blades

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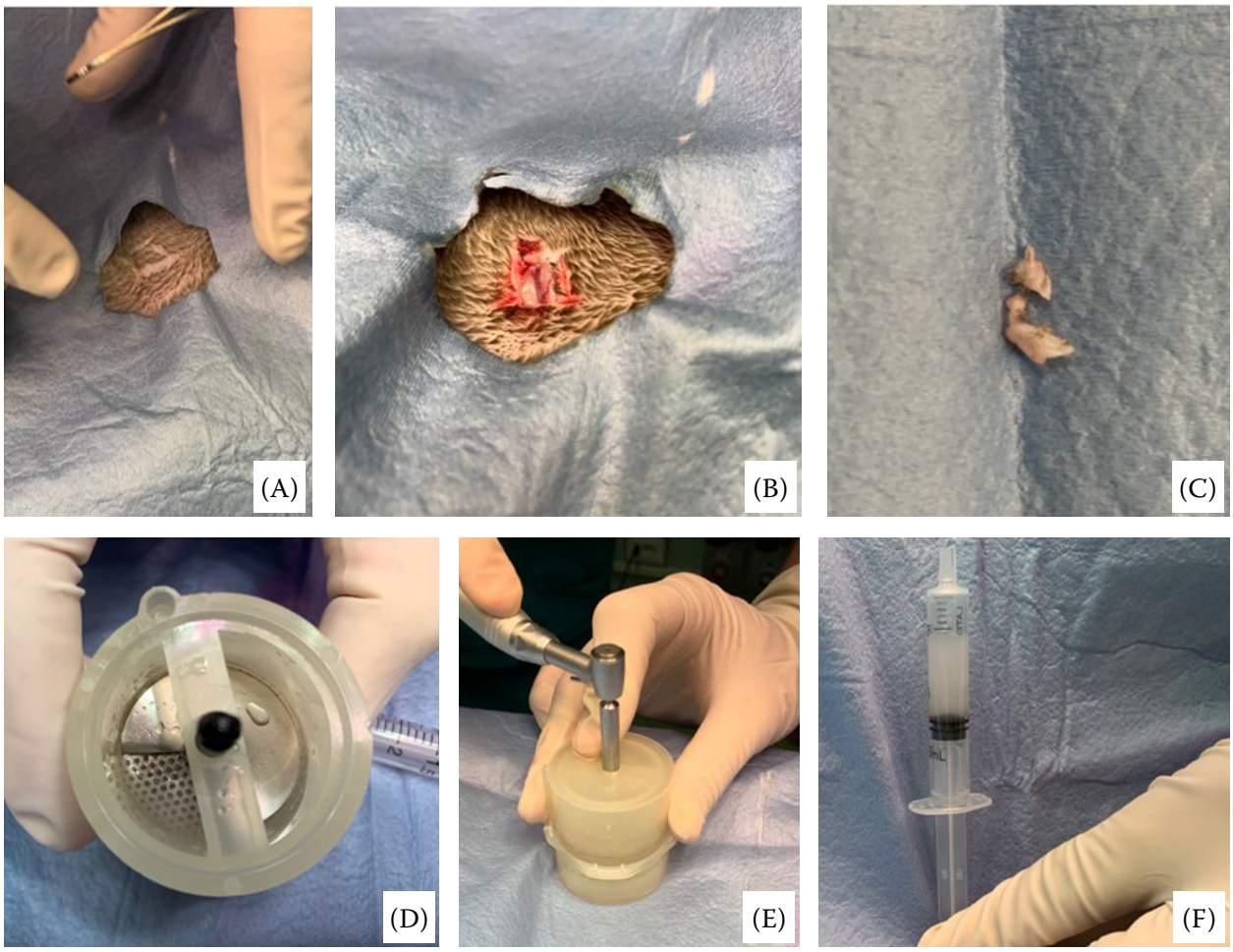

Figure 6. Donor site preparation (A), surgical evidence of the dermal tissue (B) and the samples (C). (D–E) The process of the dermal desegregation using the Rigeneracons®, and the syringe with micro-graft solution ready to use (F)

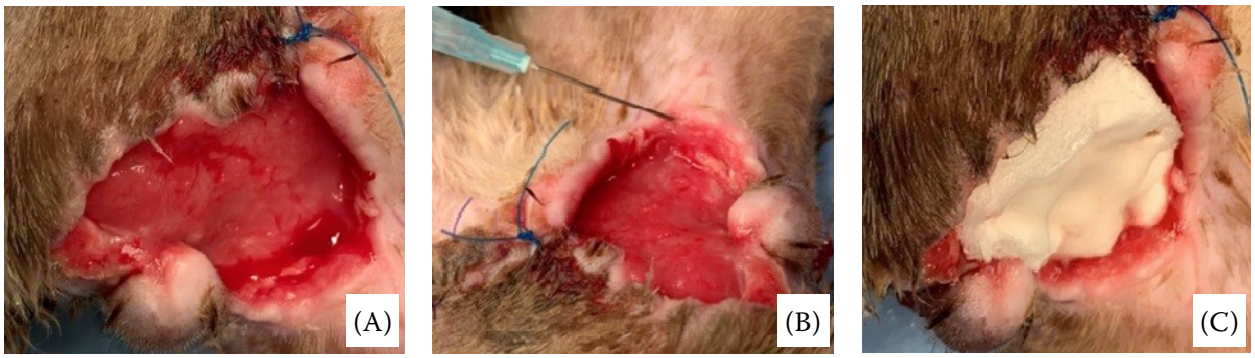

Figure 7. Rigenera® biocomplex procedure: Preparation of the receiving wound and washing (A), skin micro-grafts circumferential infiltration (B), and subsequent suture of the collagen sponge, previously soaked with the micro-graft solution (C)

6 days (R6), the wound showed a slight widening of the area, reaching 300.258 mm^2, with a loss of almost 1% of the contraction, better quality of the granulation tissue, and a reduction in depth. After 9 days (R9), the slight epithelialisation of the wound edges and excellent granular tissue of the bed, with a slight contraction of the wound, were reported, achieving an area of 284.583 mm^2 and a contraction of 32.71%. After 12 days (R12), the clinical evidence reported a significant reduction in the area (212.314 mm^2), with an improvement of 17% from R9 (49.79% wound contraction). After 15 days

https://doi.org/10.17221/197/2020-VETMED

Figure 8. Percentage of the wound contraction during the different follow-ups until the complete healing. The wound area was detected with ImageJ open-source software

(R15), the granulation tissue filled the wound depth, marking the best result in the wound contraction, reported with an improvement of 21.77% (71.57% of the wound healing, an area of 120.216 mm^2). After 18 days (R18), 81% of the skin tissue was healed, and after 21 days (R21), the patient achieved the complete epithelisation of the wound (Figure 8). One month from the surgery (R30), the cat showed a safe aesthetic wound site with good skin elasticity (Figure 9).

DISCUSSION

The results of this case report showed that using the Rigenera® technology combined with a collagen sponge (Rigenera® biocomplex), a chronic wound can be completely healed in 21 days, with satisfactory aesthetics and a good elastic aspect of the skin after one month. In accordance with Riccio et al.

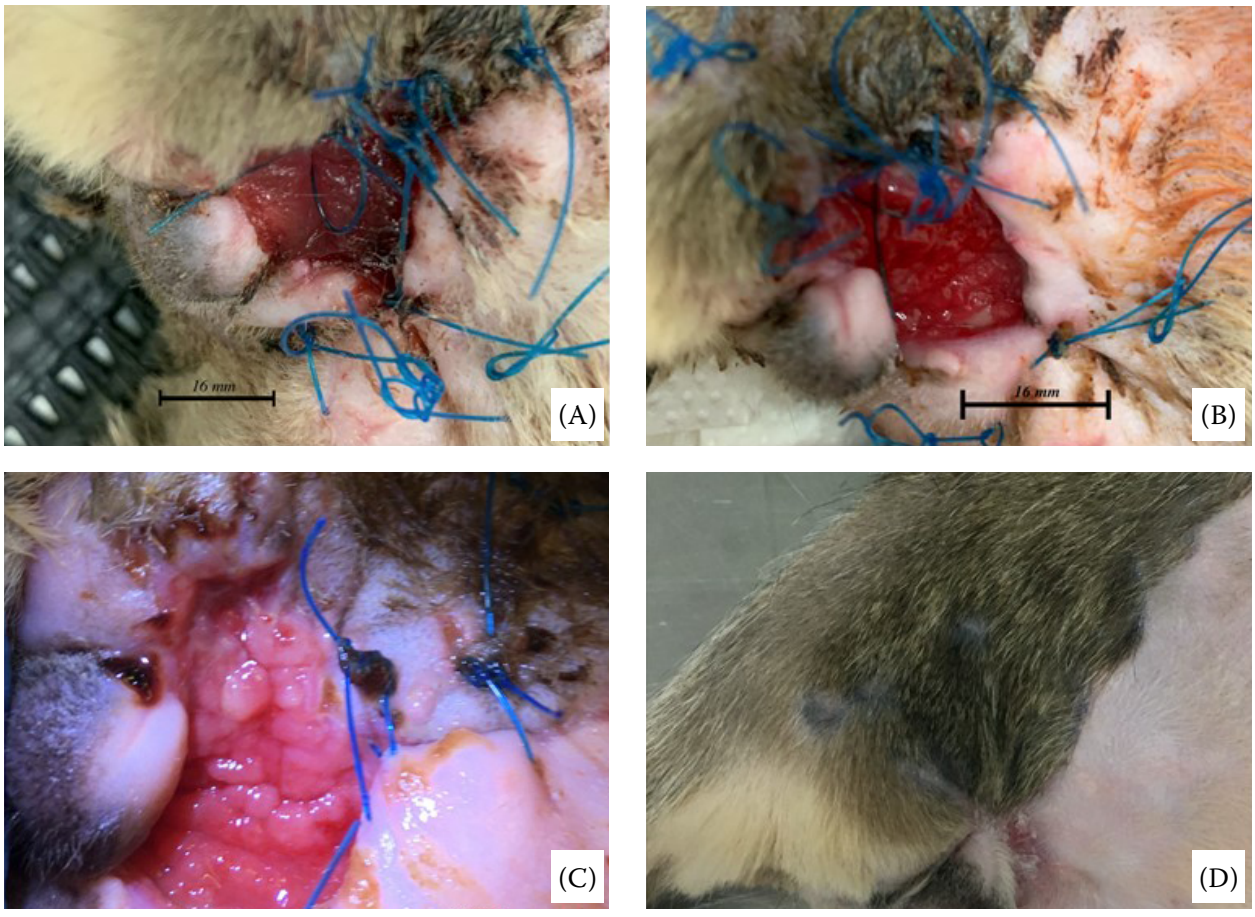

Figure 9. Healing process and clinical outcome versus time – starting at the first follow up (R3) (A) with a good vascular wound bed and a reactivation granulation tissue. (B) (R9) starting the partial epithelisation of the wound edge. (C) Filling the wound depth by granulation tissue after 15 days (R15). (D) Complete epithelisation after 30 days from surgery (R30)

https://doi.org/10.17221/197/2020-VETMED

(2019), the use of the Rigenera® system accelerates the healing time of skin wounds in terms of granulation tissue formation, inflammation reduction, and rapid re-epithelisation compared to traditional techniques. In this case, the authors observed significant differences in the aesthetic aspect of the injury between the traditional treatment (cleaning and curettage) and the regenerative technology. The healing period was significantly reduced in the latter, with the result corresponding to the perfect status of the skin and a complete reduction of the wound area (100%). No complications were detected, and the harvesting and processing procedure was simple and easily repeatable. We detected an immediate effect on the vascular wound bed with an important filling of the wound depth and, probably because of circumferential infiltration, the rapid contraction and epithelialisation of the treated wound. In this case, the Rigenera® biocomplex helped restore the normal healing process in a disrupted chronic wound. Free skin autogenous grafts (Pavletic 2010) normally produce evident scars, and they are incapable of promoting tissue healing. The micro-graft concept is founded on the principle that this can help a wound heal faster than original-size grafts. Therefore, the best way to achieve good graft performance is to increase the surface area, leading to faster cell migration to the wound and reducing the cell death itself. Since then, the micro-graft concept has been applied in many procedures in human medicine (Sardari et al. 2011; Tambella et al. 2014). Over the past two years, several experimental, prospective studies have demonstrated the successful application of Rigenera® in chronic post-traumatic skin defects (Riccio et al. 2019) and burns (Andreone and de Hollander 2020) as well as the repair of cancer wounds (Baglioni et al. 2016) in human medicine, with promising results for future applications, even in veterinary medicine. However, our study contains some limitations: (1) it is a single-case report, which must be spread to a bigger population; (2) it involves only a clinical evaluation, with the necessity to use a histologic evaluation for assessing the quality of the wound repair; and (3) it requires a control group in which a collagen sponge can be used alone because an enhanced wound-healing effect using collagen itself was reported. The rationale of this device is founded on the use of a biological disruptor capable of disaggregating little portions of connective tissues and selecting a specific cell population, including progenitor cells, based on the size of the cells. This cellular population, together with the ECM and growth factors derived from the original tissue, form ready-to-use autologous micro-grafts, applicable to several injured areas alone or in combination with different biological scaffolds, such as collagen. This method may lead to a relatively innovative management process for injury treatment and the improvement of tissue regeneration. The versatility of this technology could provide an opportunity for various applications in plastic surgery in veterinary medical practice, such as preparing better wound beds before standard surgical techniques.

For several years, the veterinary teaching hospital at the University of Camerino has performed research for different strategies to manage and treat chronic wounds as well as apply regeneration technologies. To the authors' knowledge, this is the first micro-grafting technique used on a chronic open wound in a cat as well as the first use of Rigenera® in small animal practice. Despite many limitations bound to a single clinical case, Rigenera® has proven to be a simple and highly effective method in the treatment of inactivated chronic wounds. The results obtained have led the authors to believe that prospective research will be needed as the next step.

Conflict of interest

The authors declare no conflict of interest.

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