UNIVERSITÀ DEGLI STUDI DI PADOVA

SCUOLA DI MEDICINA E CHIRURGIA

CORSO DI LAUREA MAGISTRALE IN MEDICINA E CHIRURGIA

U.O.C. CHIRURGIA PLASTICA

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TESI DI LAUREA

A New Boundary in Wound Healing with a Fish-Skin Derived Acellular Matrix (Clinical and Histological Outcomes): a Case Series

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Anno Accademico 2022-2023

SUMMARY

ABSTRACT

Background. If an injury is left untreated or the treatment is not appropriate or the patient has adverse underlying conditions, the outcome of wound healing can be inappropriate, causing to the patient physical and psychological issues. To prevent an unsatisfactory healing, plastic surgeons can employ many tools; one amongst those are Acellular Dermal Matrixes (ADMs) that, as the name itself suggests, will promote the regeneration of soft tissues temporarily replacing the dermis until autologous components will fill the wound.

Aim. The objective of the study was to analyze a novel fish-skin derived acellular matrix for the treatment of wounds, as a possible alternative to the dermal substitutes which have been used thus far. In a 1-year follow-up, the outcome was studied both clinically and histologically.

Methods. 6 patients met the inclusion criteria and were treated with the fish-skin bioinductor, for a total of 8 applications.

Results. In all the patients, for the first week after surgery the fish-skin derived bioinductor was visible at the base of the wound and after it's reabsorption, the wound was covered with granulation tissue. 4 out the 6 treated patients had a favorable outcome and an appropriate healing process; in contrast, 2 patients after the initial promising uptake, 2 weeks after surgery had no withstanding granulation tissue visible but rather did have necrosis or bone exposure.

Conclusions. Fish-skin bioinductor is a novel acellular dermal matrix that showed promising results in wound treatment, for its versatility and for the results obtained with the new formed skin. Further studies will be necessary to learn how to avoid future failure; for Padova's experience, the application is not indicated for patients with high risk of bone exposure or with poorly vascularized ulcers.

INTRODUCTION

Wound Healing Process

Physiological

A wound is an interruption of the skin, the mucosa and eventually the underlying tissues, that can heal through regeneration – with specific substitution of the tissue - or through repair – with fibrosis and scar formation. The healing process can be schematized in phases *(Figure 1)* (1):

The Vascular Response (Hemostasis and Coagulation): an immediate vasoconstriction of the injured vessels associated with blood clots formation is followed by a vasodilatation that allows the invasion of the thrombocytes into the provisional wound matrix (1).

The Cellular Response (Inflammation): neutrophils are the first cells to be recruited to phagocytize local bacteria, secrete proteases, degrade necrotic tissue and act as chemoattractant; in 3 days macrophages invade the wound to support the process with phagocytosis of pathogens and apoptotic cells and debris, promoting the resolution of the inflammation, which determinates the extent of the resulting scars; they also support the cell proliferation, activating the following phase (1).

Proliferation and Repair: local keratinocytes and epithelial stem cells from sweat glands, pilosebaceous units and hair follicles ensure reepithelization through a process called *shuffling*, migrating from the edges of the wound over a connective matrix. Simultaneously, activated endothelial cells migrate in a process called *sprouting* to form new vessels, which will then differentiate into arteries and venules. At the same time, the provisional wound matrix starts to be replaced by granulation tissue, a highly vascular tissue composed mainly of fibroblasts - that produce collagen and ECM substances -, but also granulocytes, macrophages,

capillaries and collagen bundles. (1,2) The healing is assisted by myofibroblast, which attach to ECM and contract to narrow the wound area (3).

Remodeling: from the 21st day for more than 1 year, the ECM undergoes through changes; the wound healing process is abated when endothelial cells, macrophages and fibroblast abandon the injury site, leaving a scar that will never recover the characteristics of the injured skin (1,4).

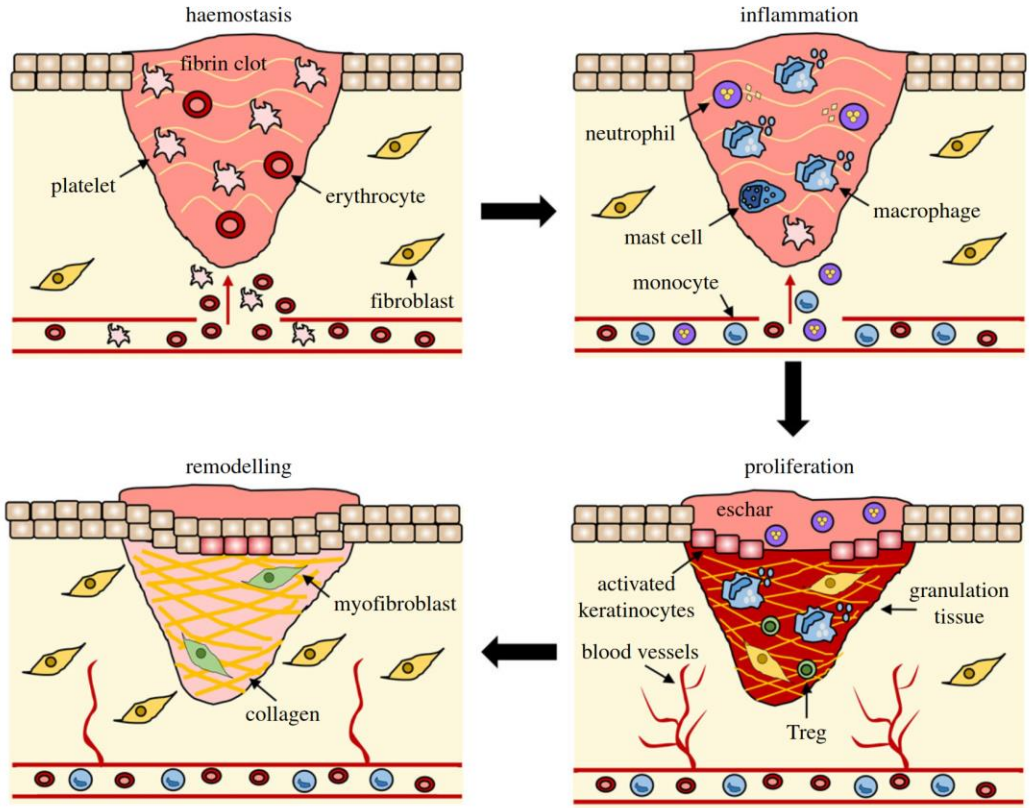

Figure 1 - the phases of the wound healing process (Wilkinson et al., 2020)

Pathological

- **Chronic wounds**

A wound is defined as chronic when it fails to heal within normal time frames or frequently reoccurs; this can be caused by various factors, such as an inadequate vascularization, uncontrolled diabetes, advanced age, infections or ineffective initial

treatment (4,5); the chronic inflammatory state leads to excessive growth of the granulation tissue, which prevents the proper remodeling of ECM and hinders the physiological healing of the wound (1). The burden of chronic wound is humanistic, with a reduced quality of life because of pain and reduced mobility, and economic, because of direct (medical and healthcare) and indirect (productivity losses) costs (6).

- **Hypertrophic scars or keloids**

Abnormal scar overgrowth compromises both functionality and aesthetics. Impairments in re-epithelization and dermal remodeling, coupled with chronic inflammation, result in uncontrolled function of activated connective tissue cells and myofibroblasts, causing an excess of ECM proteins (3,4) that give rise to two different types of fibrotic scars: hypertrophic scars, which are erythematous, pruritic, raised lesions confined within the boundaries of the injury; and keloids, which extend beyond the original wound site and continue to grow resembling benign fibro-proliferative tumor (3).

- **Normal scars on extended or critical areas**

Restoring skin integrity is crucial to protect against dehydration and infections. However, despite regaining the barrier function, the histological composition of a scar differs from that of normal skin: scars are composed of loosely arranged connective tissue that, despite undergoing remodeling, remains weaker and functionally deficient (3). The ability to perform activities of daily living can be compromised because of a movement restriction or severe deformities, associated to pain and itchiness (7). Beyond the functional repercussions, scars also have significant psychological impacts on the affected individual: they can trigger negative emotions related to the trauma, impair self-acceptance and influence behaviors and perceptions (8).

The Role of Plastic Surgery

If an injury is left untreated, or if the treatment is inadequate, or if the patient has underlying adverse conditions, the outcome of wound healing can be unsatisfactory, leading to physical and psychological issues for the patient. To prevent such outcomes, plastic surgeons have various tools at their disposal. One of these tools is Acellular Dermal Matrixes (ADMs). As the name suggests, ADMs promote the regeneration of soft tissues by temporarily replacing the dermis until autologous components can fill the wound.

Acellular Dermal Matrix (ADM)

An acellular dermal matrix is a dermal graft obtained from allogenic (cadaveric skin donor) or xenogeneic (mammalian skin donor) sources, that undergoes decellularization to prevent tissue rejection and graft failure. ADMs provide a collagenous mesh that, when applied to a well-vascularized and infection-free wound, can be repopulated by the patient's cells; the purpose of these products is to reduce the thickness of autologous grafts, thereby minimizing the risks of scarring, seroma formation or wound dehiscence at the donor site. It's important to note that ADMs are not intended to fully substitute the skin as they lack the epidermal layer, but comparing a full-thickness autograft with a thin split-thickness autograft combined with an allograft dermal matrix, studies have shown that the outcomes in wound healing are comparable, with the advantage of reduced morbidities at the donor site with the latter method (9,10).

Based on their impact on the granulation tissue, ADMs are classified into two categories: Permanent Dermal Substitutes and Granulation Tissue Bio-inductors. These categories differ in terms of porosity, surface density, components, and degradation time (11).

Permanent Dermal Substitutes

Dermal substitutes are comprised of collagen and glycosaminoglycans, biodegradable materials that, after promoting neovascularization and the formation of a neo-dermis, are then completely reabsorbed in four histological stages:

- Imbibition (minutes), initial adhesion of the dermal substitute to the wound bed;
- Fibroblast migration (day 7), collagen secretion begins as fibroblasts migrate to the wound area;
- Neovascularization (day 14), new blood vessels start to form;
- Remodeling and maturation (day 28), the dermal collagen matrix is gradually replaced by the host's collagen. (12,13)

Dermal Substitutes Most Used in Europe

Integra®

Integra® is a bilayer matrix where the lower layer is composed of crosslinked fibers of bovine Achilles tendon collagen type 1, along with glycosaminoglycan (GAG and shark chondroitine-6-sulfate) and the upper layer consists of a silicone pseudoepidermis. The bovine collagen and GAG in the matrix promote the infiltration of fibroblasts, macrophages and lymphocytes as well as the formation of new blood vessels. Over time, these components are gradually replaced by collagen and elastic fibers produced by fibroblasts. The silicone layer acts as a semi-permeable membrane, preventing moisture loss and reducing bacterial invasion; it can be removed after 2 to 3 weeks – once the wound is revascularized – and replaced with a split thickness skin graft. Due to its similarity to natural skin, Integra® helps achieve desirable cosmetic outcomes (5,14,15).

Integra® is also available as a single layer, without the silicone layer. In this case, it can be covered with a thin autologous graft (0.2-0.3 mm) in a single-step procedure (12).

Integra® has a moist, gelatinous, and diaphanous texture (16).

It has shown great effectiveness in the treatment of conditions such as giant nevi, extensive traumas, full-thickness burns, and the release of burn contractions; it may be indicated for deep wounds of any size and in any part of the body (17).

Histology

Stereological analysis has revealed that the density of granulation tissue and blood vessels remains consistent; among the inflammatory cells, neutrophils were predominant (7).

Biopsies have shown the presence of a well recognizable three-dimensional structure – the neodermis –, invaded by fibroblasts, lymphocytes and macrophages and vascularized; the structure differs from normal skin. In the case of the double-layer substitute, the structure remains identifiable even after 12 months, 24 months and up to 5 years after surgery; for the single-layer substitute, the structure is recognizable after 6 months and continues to be present after 12 months, although it may appear less organized. However, further follow-up controls are needed (12).

Nevelia®

Nevelia® is a bilayer porous resorbable matrix of stabilized bovine collagen type 1, covered with a silicon semi-permeable membrane (13,14) reinforced with a polyester fabric (18); it's porous and mechanically rigid (16).

Nevelia® is used for the treatment of skin loss, particularly in burns ad chronic wounds; traumatology; skin tumor surgery; reconstructive plastic surgery (18).

Histology

As for Integra, incisional punch biopsies at the baseline revealed no evidence of elastic fibers or collagen deposition, instead cellular debris and dermal inflammatory infiltrate were observed; after 2 weeks was noted the presence of granulation tissue (inflammatory infiltrate, collagen deposition and neoangiogenesis), along with

regenerated skin with reactive epidermal hyperplasia; after 3 weeks the results differed from Integra®, as Nevelia® exhibited earlier regenerative properties, coherently associated to an earlier color switch of the collagen layer, transitioning from red to yellow/vanilla, which reflects the process of recellularization and creation of new blood vessels (18).

Upon reabsorption of the dermal substitute, the resulting tissue closely resembles normal dermis (18).

MatriDerm®

MatriDerm® is a highly porous collagen-elastin-template, consisting in type 1 collagen fiber coated with 3% a-elastin hydrolysate derived from cattle's legamentum nuchae; it is available as a 1 mm sheet for single-use procedures or as a 2 mm sheet for multi-step procedures (13).

The use of the 2 mm sheet is based on the aim of maintaining the physiological thickness off the dermis; while a single-step procedure may be convenient, long-term comparisons regarding cosmetic outcomes are still debated (19).

Histology

Myofibroblasts can cause excessive scarring and contractures; while rigid environments promote the differentiation of fibroblast into myofibroblasts, the presence of elastin can reduce it. MatriDerm® is the only dermal substitute that contains an elastin element, which potentially leads to a more pliable scar. Additionally, it promotes the production of native elastin and angiogenesis (20).

Cross-linking MatriDerm® has been shown to accelerate angiogenesis and increase the strength, stability, and durability of the scaffold. However, no significant differences in wound contraction were observed compared to non-cross-linked MatriDerm® (20).

Pelnac®

Pelnac® is a bilaminar membrane: a porcine collagen sponge layer derived from pig tendon is covered with a superficial layer is in silicone (7); it is dehydrated and absorbent (16).

In clinical comparisons with Integra®, it has been observed that the wound treated with Pelnac® shows significant contraction up to 4 weeks postoperatively. However, there were no noticeable differences between Pelnac® and Integra® one year after the surgery (21).

Pelnac® is indicated for superficial wounds of any size in various parts of the body, particularly those prone to shrinkage, such as dermal malignant tumors or fingerprint defects. However, it is not recommended for use in burn injuries (17).

Histology

Stereological studies have shown that the granulation tissue significantly reduces at 3rd and 6th day after surgery; among inflammatory cells, neutrophils are predominant. The number of blood vessels decreases at 3rd day after surgery and increases at the 6th day (7).

Despite Pelnac® demonstrating quicker spontaneous re-epithelization compared to Integra® and Nevelia®, there were no appreciable differences observed 30 days after the removal of the external silicone layer (16).

Granulation Tissue Bioinductors

These scaffolds are composed of hyaluronic acid, collagen and sometimes have a silicone surfacing layer. Unlike permanent dermal substitutes, these scaffolds lack macromolecules and degrade at a faster rate. Due to their inactive nature, they

stimulate the formation of granulation tissue, making them suitable for covering the wound bed before a skin graft or flap is applied (11).

The Choice Amongst ADMs

Regardless of the tissue engineering product used, it has been demonstrated that the healing differences are mainly correlated with wound bed preparation, bacterial burden, edema control, adequate vascular status, and reduced mechanical stress (2); the histological differences among the ADMs were not statistically significant and the production of scaffolding materials exhibited similar characteristics in bovine and porcine collagens (7,19).

Indication and Contraindications

The Food and Drug Administration (FDA) recommends the use of ADMs for acute and chronic reconstructive burn surgery, as well as non-burn-related traumatic and chronic extremity wounds; the decision to apply a dermal substitute should be evaluated on a case-by-case basis, considering factors such as the characteristics of the wound, the patient's co-morbidities and preferences, and the surgeon's skills. (13)

- Burn Injuries; when evaluating a burn, it is important to consider that even a small lesion in certain areas can cause severe functional disability. Therefore, the treatment in areas such as hands, wrists, elbows, or axilla should aim to restore the best possible elasticity, pliability, and mobility. Comparative studies between a treatment with dermal substitutes and skin graft compared to a traditional method, showed similar results, but the use of the dermal substitute improves skin elasticity and allows for a superior active range of motion. Satisfactory outcomes have also been achieved when incorporating ADMs for contractures release and scar resurfacing. (12,13)

- Traumatic Injuries; after degloving or skin avulsion injuries, early restoration of skin continuity is essential to prevent infections and enable early mobilization and rehabilitation, which helps prevent stiffness and disuse atrophy. The use of dermal substitutes directly for traumatic injuries is indicated for patients with comorbidities, multiple injuries, or exposed tendons or bones that are not suitable for skin graft coverage, which would otherwise be the preferred choice; alternatively, ADMs can be used to cover the donor site of a skin graft (12,13).

- Soft-tissue reconstruction after tumor resection; the current standard treatment involves skin grafts, which are prone to contraction, fragile and do not provide the best aesthetical outcome or locoregional flaps, that limit the ability to monitor an eventual recurrence. Dermal substitutes have been identified as a viable alternative with optimal results for reconstructing soft tissue after the resection of suspected malignant tumors such as malignant melanoma, basal cell carcinoma, or squamous cell carcinoma. Five years after reconstruction, the neodermis is filled with collagen and elastin, the skin has well-developed papillae, and it appears more similar to normal skin compared to a split-thickness graft, which can lead to hypertrophic skin (12,13).

- Soft-tissue reconstruction after radial forearm flap harvest; the biggest issue of this flap is the repair of the donor site, since split- or full thickness skin graft often result in aesthetic and functional mobility, while the results with the application of dermal substitutes were encouraging; another alternative is to harvest a suprafascial radial forearm flap, but not all surgeons can provide this option (12,13).

- Dupuytren contracture; after the dermofasciectomy, the resultant defect is usually covered with a full-thickness skin graft but there is a case report where a dermal substitute was used instead, resulting in healing through epithelization without the need for graft harvesting, showing promising results (13).

- Autoimmune lesions; the use of dermal substitutes is desirable in order to avoid additional trauma associated with graft harvesting (12).

A comparison of costs for treating small-sized burns with dermal substitutes versus skin grafts revealed that although the expense for the former was higher, it was not significantly higher due to the significant impact of indirect health-related overheads (13); other comparisons have shown economic benefits of using dermal substitutes instead of well-established but long-lasting and complex treatments (22); however, the high cost is justified when the desired outocome is a three-dimensional reconstruction, while it's not cost-effective when used to stimulate the granulation in the absence of graft take, as can occur in chronic lesions, which tend to be poorly vascularized and are often infected (12). Despite this, dermal substitutes have demonstrated efficacy in the treatment of chronic wounds, as long as the wound bed is properly prepared through debridement, infection and inflammation control and moisture balance (5).

Since ADMs require an adequate vascularization and a wound bed free of necrosis or infection, they are not widely used for the treatment of large areas (10).

Whenever the application of an ADM is considered, it is important to note that the resulting tissue will differ from normal skin due to the absence of sweat glands, hair follicles and for the suboptimal restoration of sensibility, that is particularly important in the palm of the hand or in the volar face of the digits. Additionally, the duration or the required immobilization is considerably increased, with the consequent risk of stiffness (13).

AIM OF THE STUDY

The objective of the study was to analyze a novel fish-skin derived acellular matrix for the wound treatment as a potential alternative to conventional dermal substitutes.

A 1-year follow-up was conducted to evaluate the outcomes both clinically and histologically.

The clinical evaluation assessed the healing time and skin quality, while the histological analysis focused on the characteristics of the granulation tissue and the newly formed skin.

MATERIALS AND METHODS

Materials – Fish-Skin Derived Acellular Matrix

The bioinductor under analysis is derived from wild Atlantic Cod, sourced from a sustainable fish stock in pristine Icelandic waters and processed using renewable energy; it is noteworthy rich in omega3 poly-unsaturated fatty acids – known for their anti-inflammatory properties – and exhibits high similarities with human skin: this similarity is attributed to the absence of viral transfer risk between cold-water fish and humans and consequently, the patented fish skin undergoes gentle processing, ensuring its resemblance to human skin *(Figure 2)*.

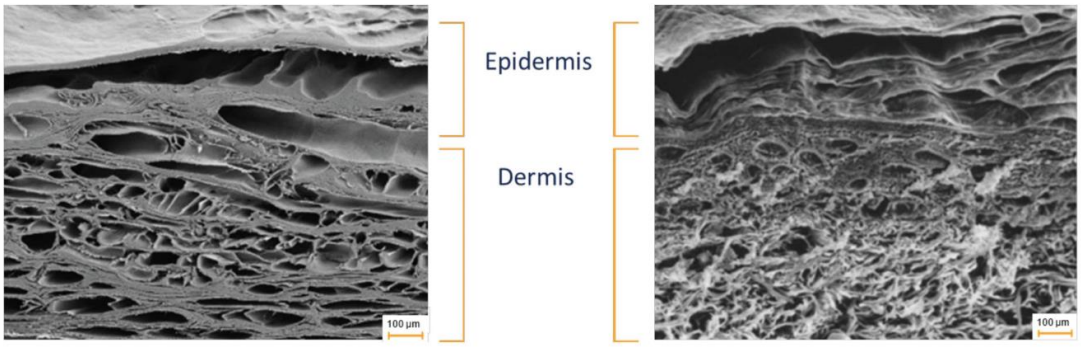

Figure 2 - Scanning electron microscope images of patented fish-skin graft (left) and human skin (right), showing similarities in their 3D structures

The products derived from the bioinductor are available in various formats, including sheets and different dimensions. These formats can be solid, fenestrated (having small openings), or meshed (with a network-like structure). Additionally, there is a micro variant available, which consists of intact fish skin fragmented into tiny particles, which can be rehydrated and directly inserted or poured into complex or irregular wounds.

Population

A total of six patients were enlisted in February 2022 in the Plastic Surgery Ward of Azienda Ospedale-Università of Padova and voluntarily signed an informed consent form prior to participating in the study.

Methods

In February 2022, the six enrolled patients underwent treatment with the fish-skin bioinductor, resulting in a total of 8 applications. During the surgical procedure, the bioinductor was rehydrated in a saline solution. Simultaneously, the wound bed was prepared, and subsequently, the hydrated acellular dermal matrix (ADM) was cut to match the shape of the wound and then applied. Metal staples were used to secure the bioinductor in place. For the secondary dressing, negative pressure wound therapy (NPWT) or polyurethane foam was utilized, and this dressing was maintained throughout the follow-up period.

CASE SERIES

Clinical Case #1

A 26-year-old male patient was transferred from the Orthopedic department of Rovigo to the Plastic Surgery department of Padova two days after sustaining a crush injury to the left foot. The patient presented with a degloving injury to the back-left hindmidfoot, accompanied by tibio-astragalar instability, a fracture of the fibular shaft, Lisfranc injury, and fractures of the base and tuberosity of the second and third metatarsals.

On the same day of admission, the patient underwent debridement surgery, and negative pressure wound therapy (NPWT) was applied to the wound. After 10 days, the patient underwent another debridement procedure, and NPWT was continued *(Figure 3)*.

The following day, the patient underwent further debridement, and a meshed fish-skin bioinductor was applied to the wound, followed by the placement of NPWT *(Figure 4)*.

After 5 days, the patient's condition stabilized, and the wound showed signs of cleanliness. Subsequently, the patient was discharged and closely followed up for dressing changes. Six weeks after the application of the bioinductor, a skin graft was performed *(Figures 5, 6)*.

One year later, an incisional biopsy was conducted to analyze the tissue (*Figure 7)*.

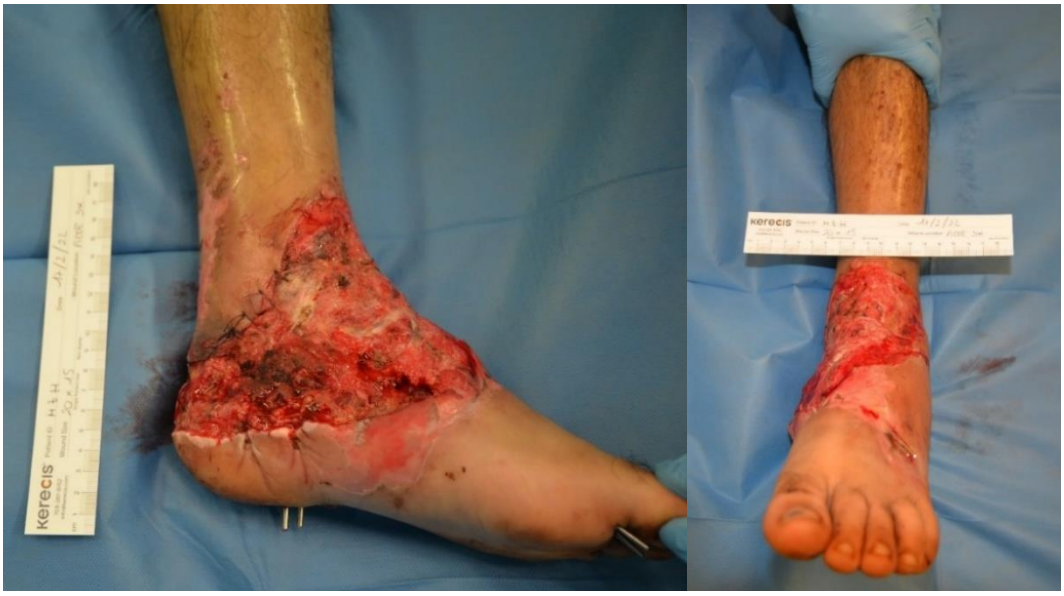

Figure 3 - wound's aspect after NPW, before the application of he meshed fish-skin bioinductor

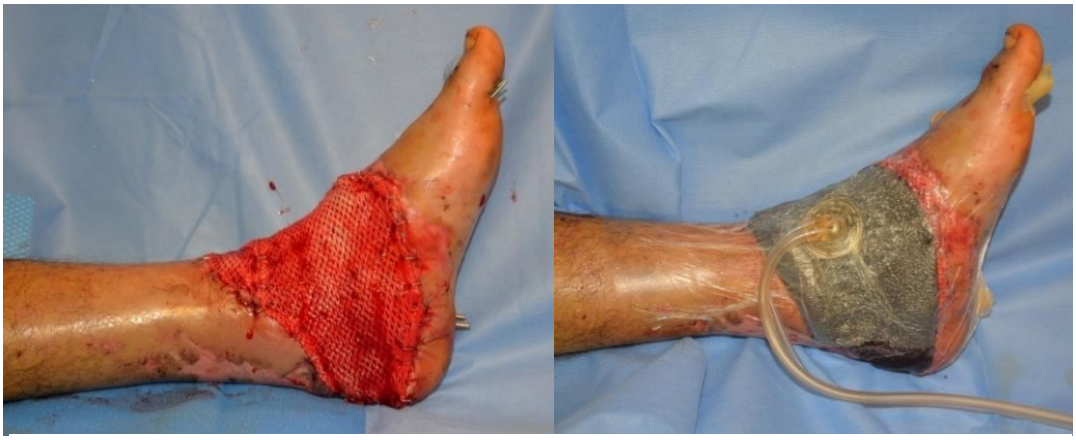

Figure 4 – application of meshed 1:1.5 fish-skin bioinductor (left) and placement of NPWT (right)

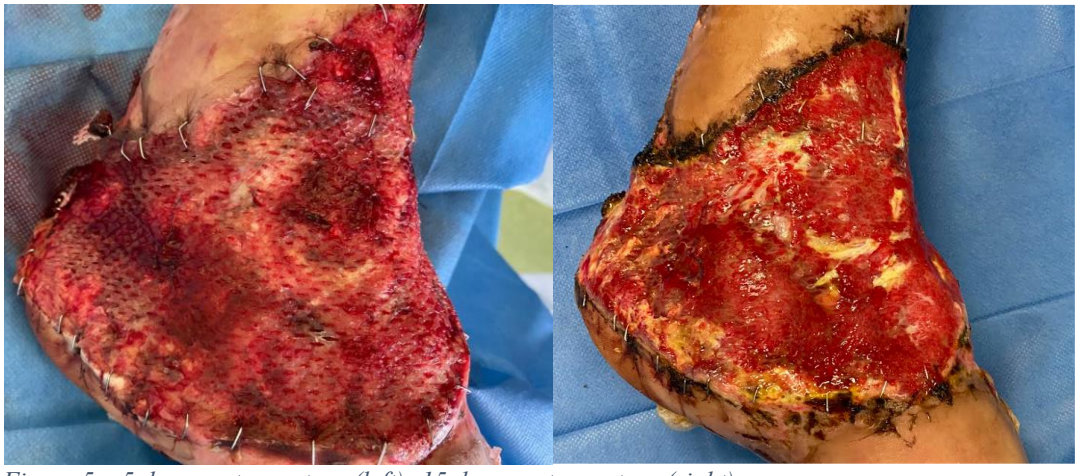

Figure 5 – 5 days postoperatory (left); 15 days postoperatory (right)

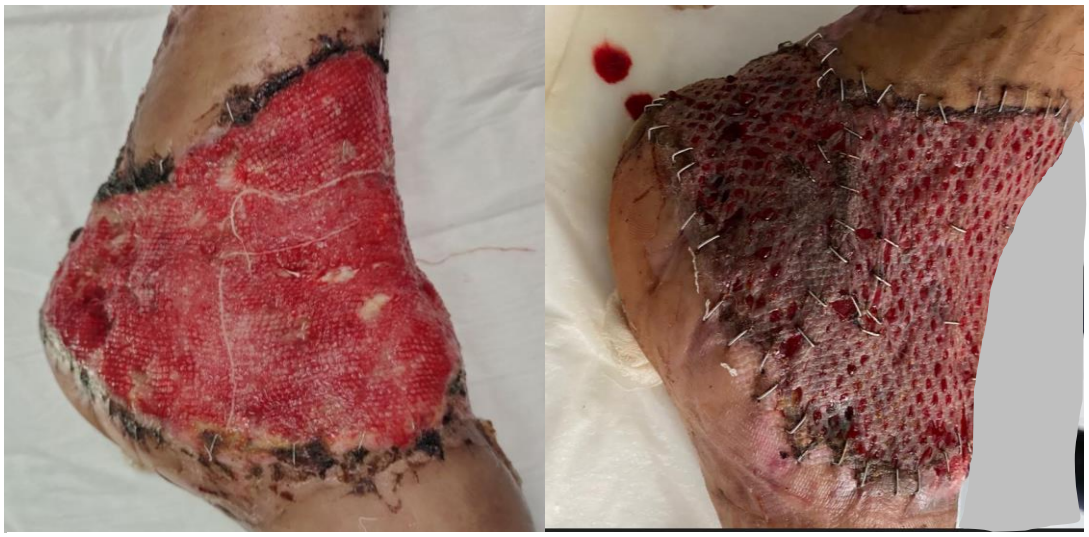

Figure 6 – 3 weeks postoperatory (left); 6 weeks postoperatory application of the skin graft (right)

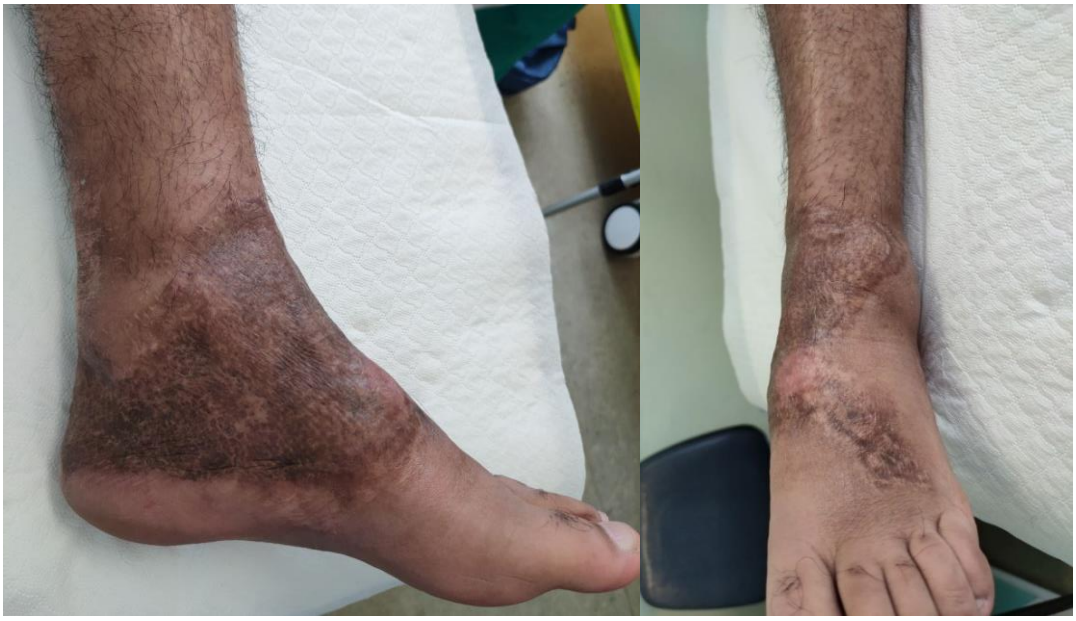

Figure 7 – 1 year postoperatory

Clinical Case #2

A 51-year-old female patient was admitted following a road traffic accident (hit by a truck) with a severe lower limb avulsion (*Figure 8*), subtrochanteric fracture of the left femur, and fragmented fracture of the head and shaft of the left fibula.

She underwent an urgent combined plastic and orthopedic surgical procedure for the approximation of skin flaps *(Figure 9)*.

Two days later, a surgical procedure for reduction and synthesis of the left subtrochanteric fracture was performed using a periarticular plate and screws.

One week after the initial surgery *(Figure 10),* she underwent a debridement procedure and application of negative pressure therapy with instillation.

After another week *(Figure 11),* she underwent debridement again, followed by the application of the bioinductor *(Figure 12)* and negative pressure therapy.

One month after the accident, the patient underwent a surgical procedure for coverage of the skin loss using partial-thickness skin grafts harvested from both thighs using a dermatome and 10 days after, during the check-up, the skin grafts on the lower limb were observed to have taken well, with multiple oozing areas, especially around the knee and the medial region of the calf. There were no signs of infection or subcutaneous collections, and the wound was healing with staples in place. The donor sites appeared reepithelialized with minimal oozing areas.

One year later, an incisional biopsy was conducted to analyze the tissue (*Figure 13)*.

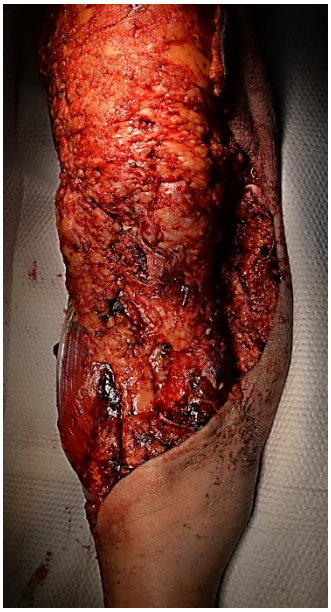

Figure 8 – preoperatory

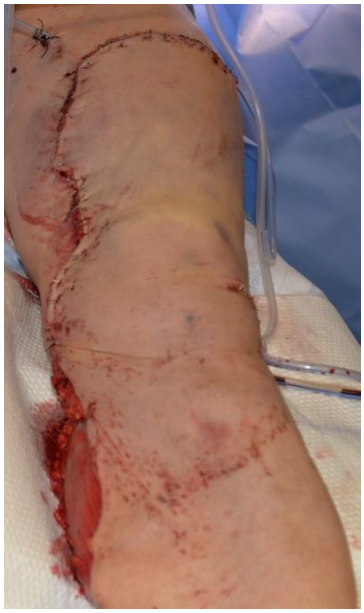

Figure 9 – approximation of skin flaps in emergency

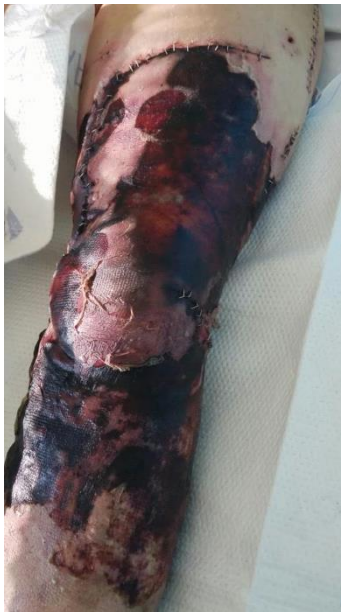

Figure 10 – 1 week after surgery, skin necrosis

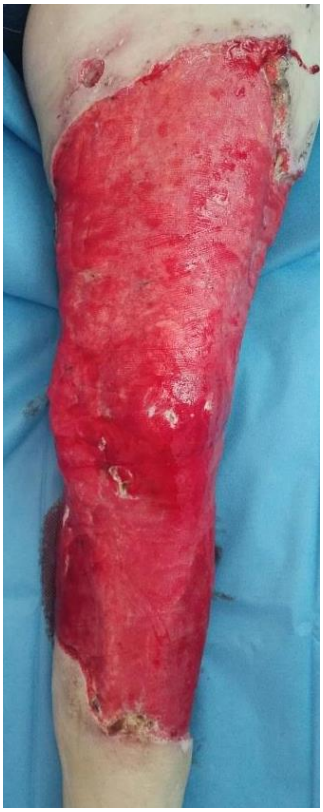

Figure 11 – 10 days after NPWT

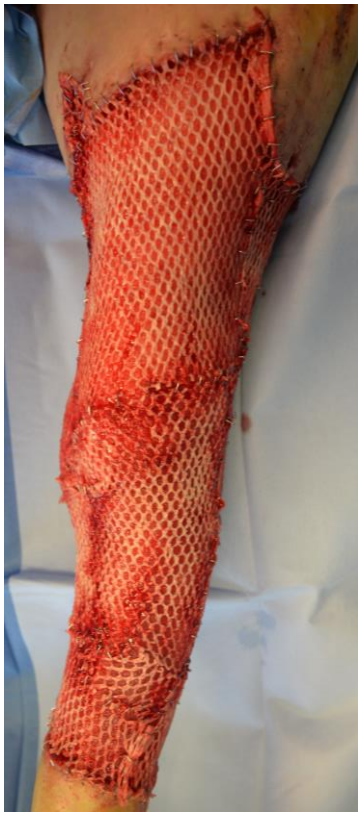

Figure 12 – application of the bioinductor

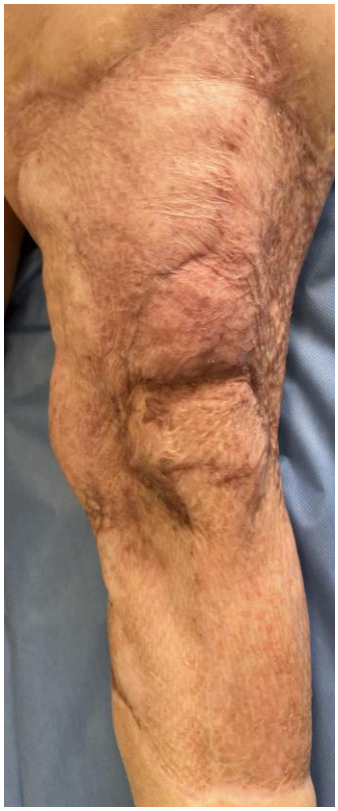

Figure 13 – 1 year postoperatory

Clinical Case #3

A 63-year-old male patient underwent surgery for the removal of a growth in the right nasal ala *(Figure 14)*. The defect resulting from the excision was repaired using the fish-skin bioinductor. Due to the patient's stable condition and favorable local conditions, he was discharged on the same day and received close follow-up care (*Figures 15, 16, 17)*.

One year later *(Figure 18)*, an incisional biopsy was performed to analyze the tissue and assess the outcome of the repair.

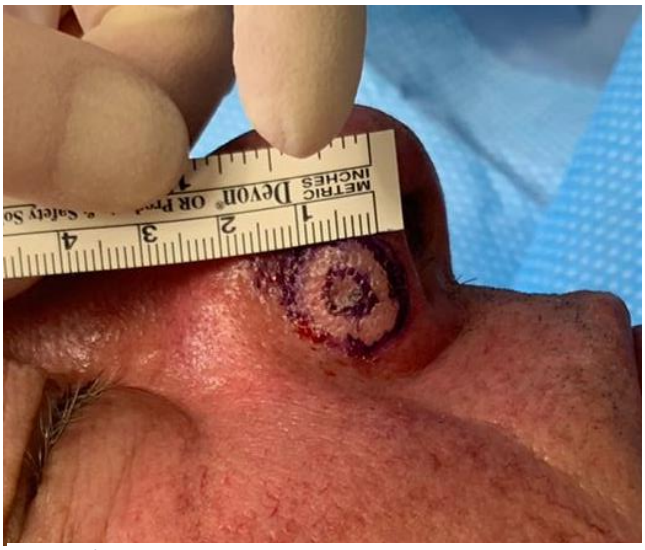

Figure 14 – preoperatory

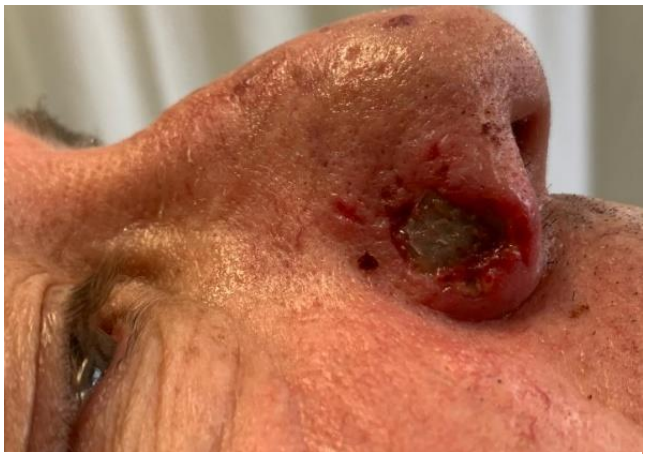

Figure 15 – 2 days postoperatory the fish-skin bionductor is still visible on the base of the wound

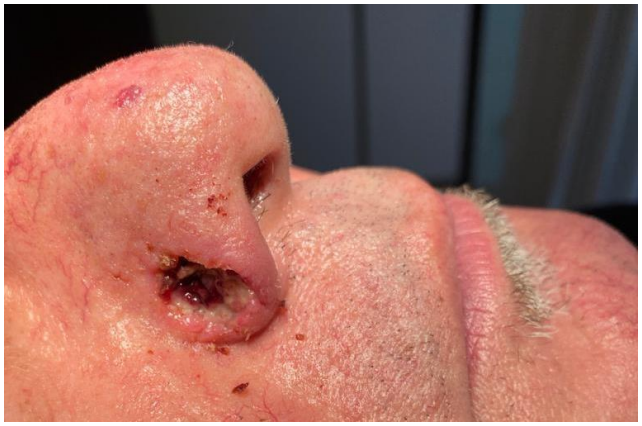

Figure 16 – 1 week postoperatory the fish-skin bionductor was fully reabsorbed; the granulation tissue is visible and there is no cartilagineous exposure)

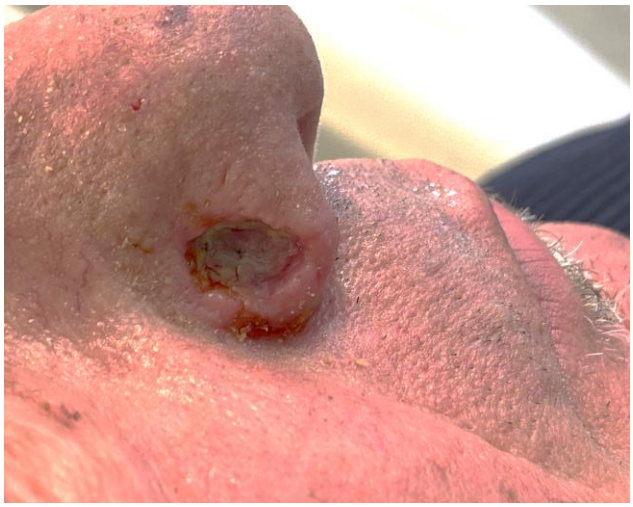

Figure 17 – 3 months postoperatory

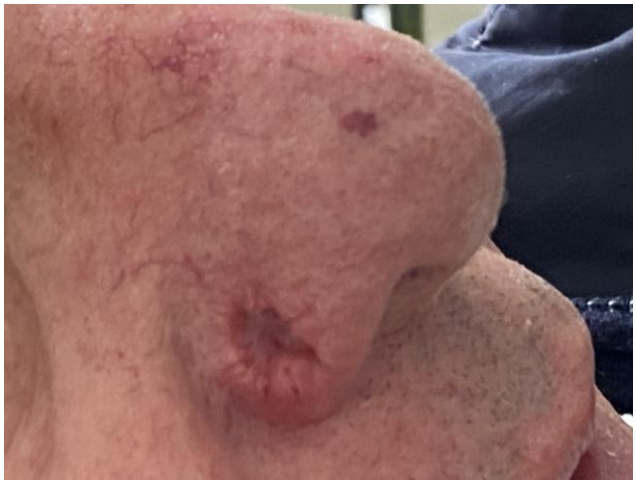

Figure 18 – 1 year postoperatory

Clinical Case #4

A 63-year-old male patient was admitted to the hospital due to an ulcer that exposed the Achilles tendon and a malleolar ulcer *(Figure 19)*. Both ulcers were treated using negative pressure wound therapy (NPWT).

The patient underwent surgical treatment, which involved debridement of the ulcers followed by repair using the fish-skin bioinductor *(Figure 20)*. The bioinductor was medicated with polyurethane foam.

As the patient's condition was stable, and there were no current bleeding issues, he was discharged from the hospital and closely monitored for dressing changes *(Figures 21, 22, 23)*.

One year later *(Figure 24),* an incisional biopsy was performed to analyze the tissue and evaluate the outcome of the repair.

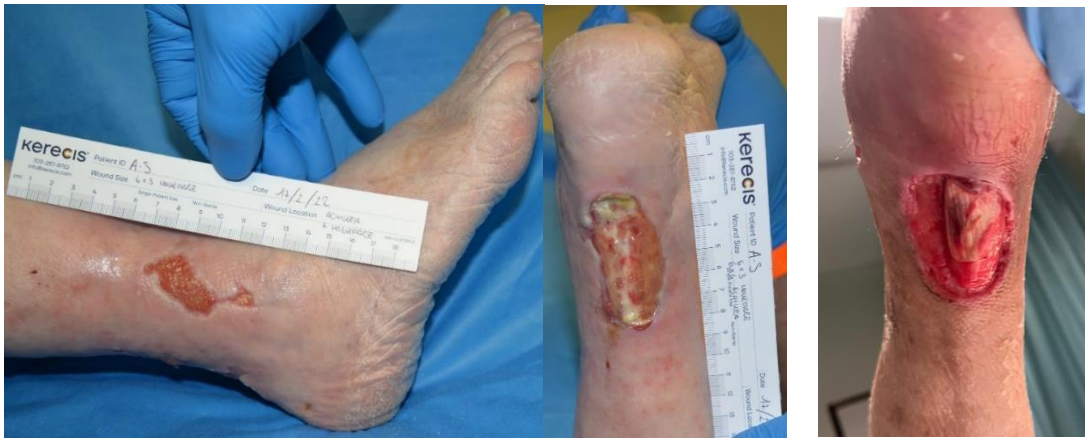

Figure 19 - external malleolar ulcer (left); ulcer with Achilles tendon exposure (middle); ulcer with Achilles tendon exposure after the debridement (right)

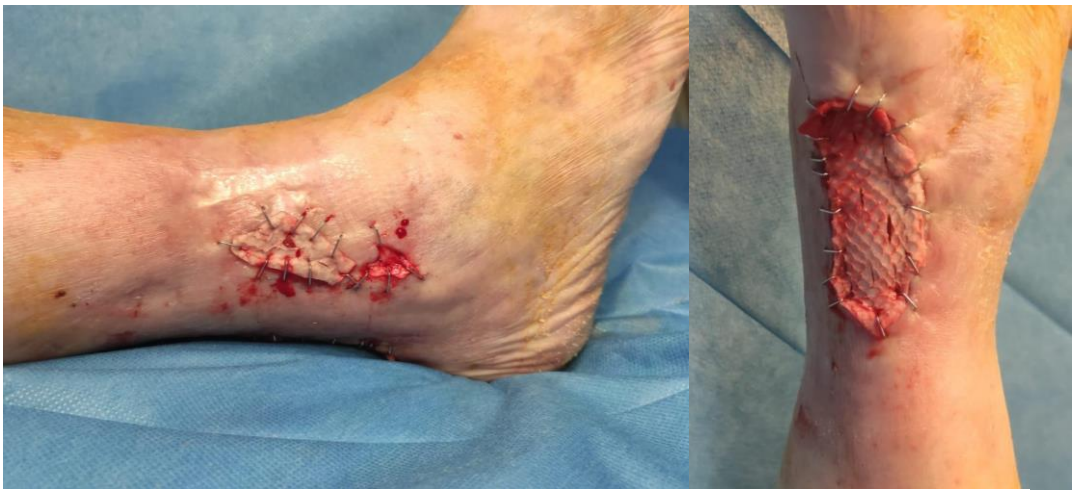

Figure 20 - after the debridement the fish-skin bioinductor is applicated

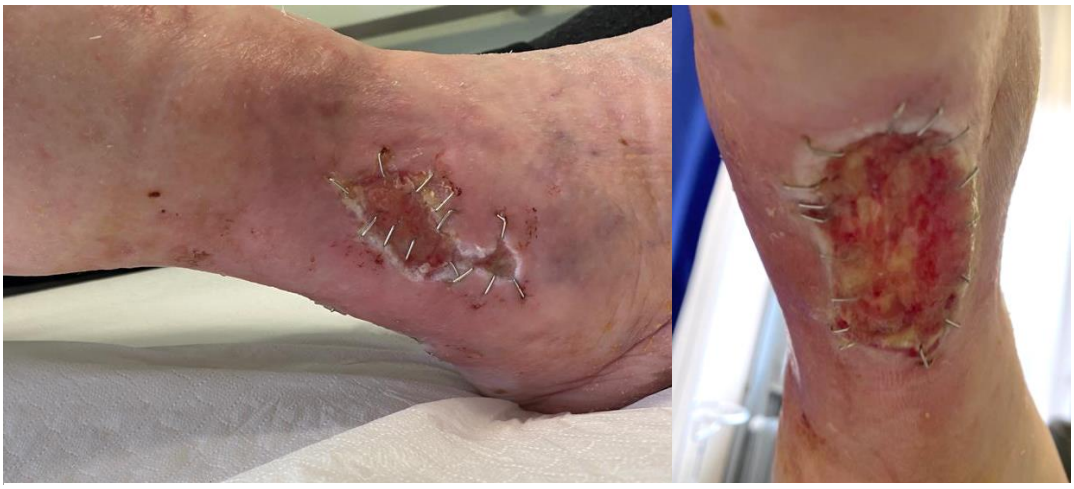

Figure 21 – 4 days after surgery, the wounds produce exudate and the fish-skin bioinductor is still visible

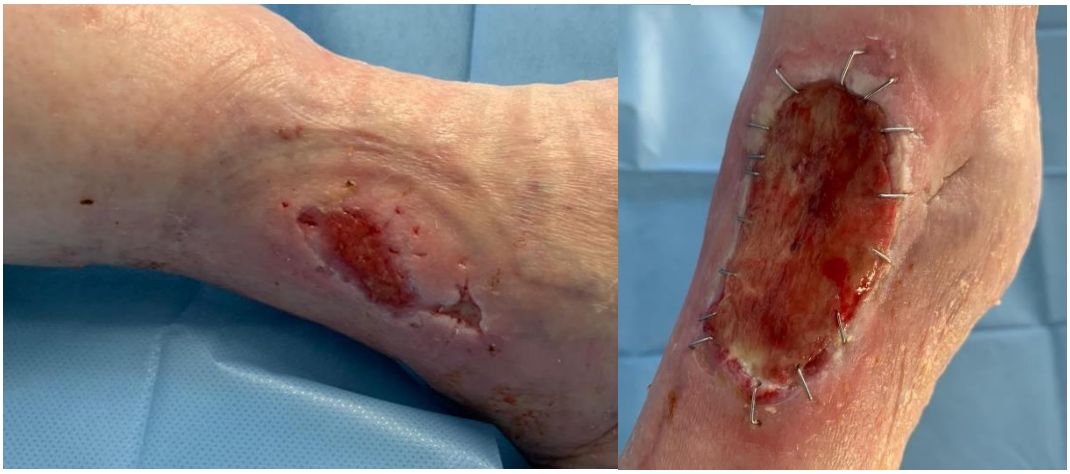

Figure 22 – 1 week after surgery, the wounds produce exudate, the base is covered with granulation tissue and it's ready for the skin graft

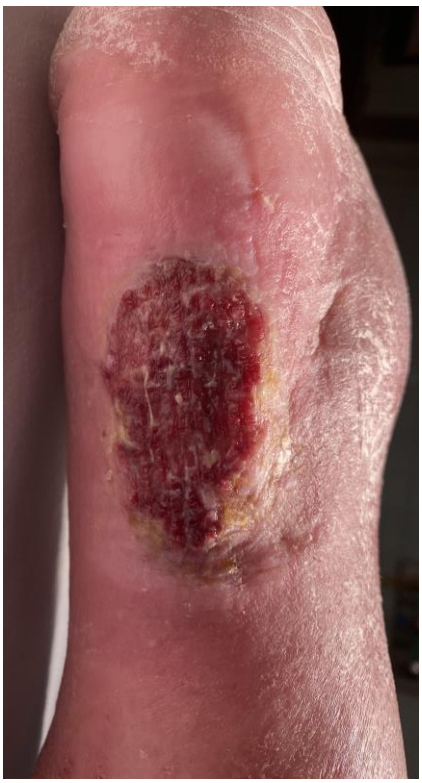

Figure 23 – 6 months after surgery

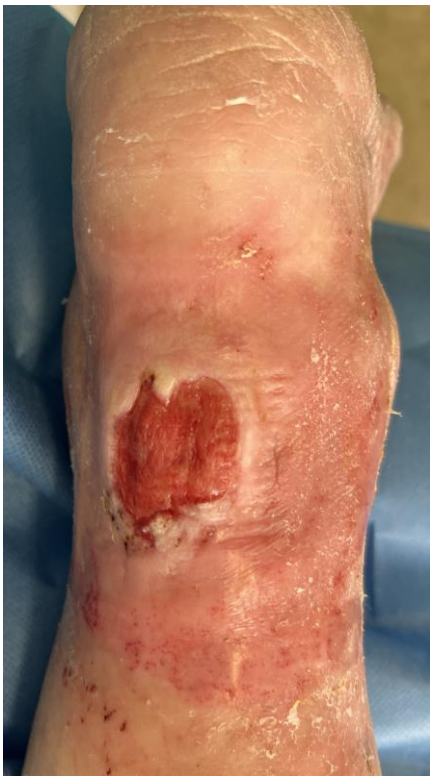

Figure 24 – 1 year after surgery

Clinical Case #5

A 63-year-old male who became paralyzed following a trauma that occurred 4 years ago underwent treatment for a post-traumatic calcaneal ulcer on the lymphedematous left foot.

The treatment involved the application of a double layer of fish-skin bioinductor at the sites where an ALT (Anterolateral Thigh) microsurgical flap was performed *(Figure 25)*.

After the patient was discharged, he was closely followed up for dressing changes *(Figures 26, 27)*.

After 2 weeks, wound was still being cleansed, and the depth of the lesion had reduced. However, after one month, necrosis developed *(Figure 28)*, and it became necessary to perform a flap procedure to close the wound.

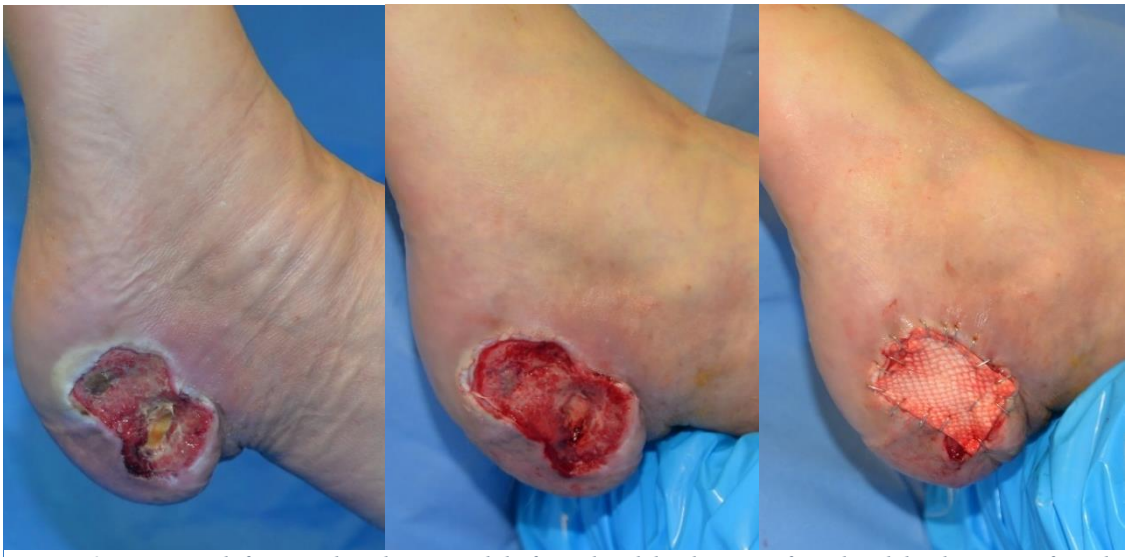

Figure 25 - From left to right: the wound before the debridment; after the debridement; after the application of the fish-skin bioinbuctor in double layer

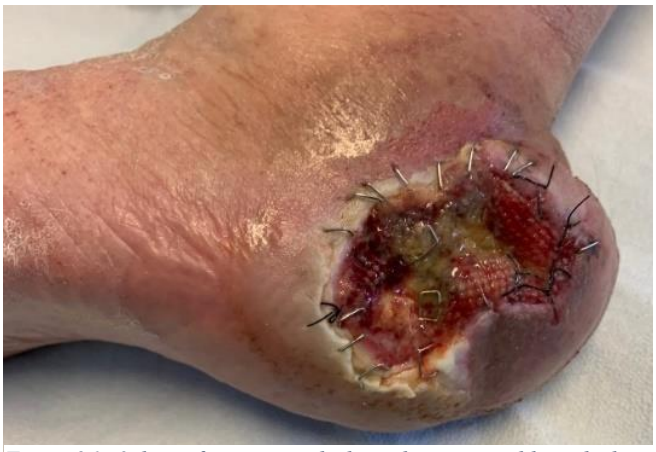

Figure 26 - 3 days after surgery the bioinductor is visible at the base of the wound, that produces a lot of exudate, the edges are erythematous and macerated, with suspect of superinfection

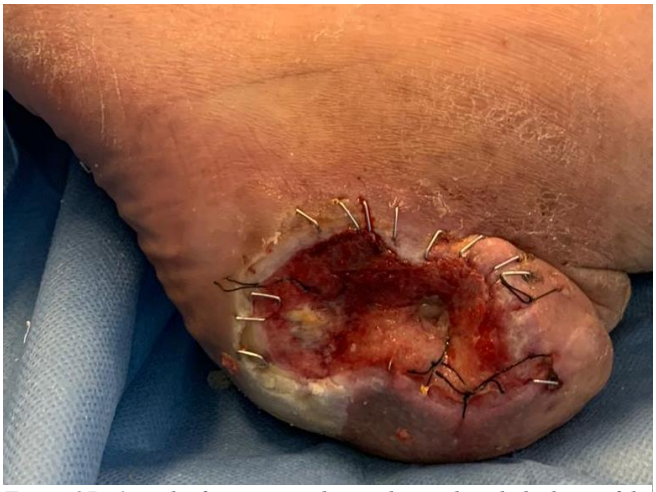

Figure 27 - 1 week after surgery the exudate reduced, the base of the wound is cleansed and granulating

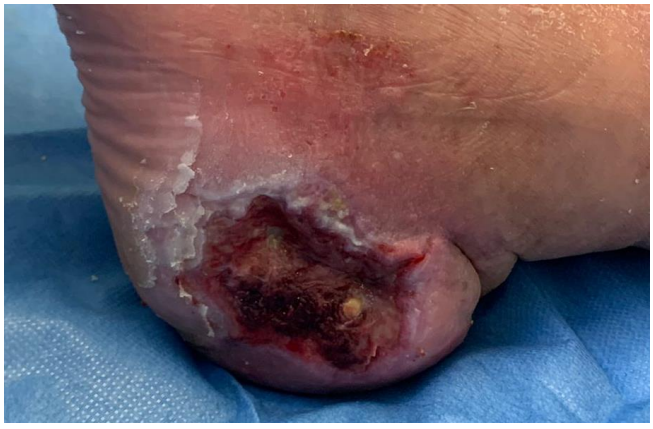

Figure 28 – 1 month after the necrosis developed

Clinical Case #6

An 82-year-old male underwent surgery to remove a squamous cell carcinoma (SCC) on the scalp *(Figures 29, 30)*. The surgical site was then repaired using a fish-skin bioinductor *(Figure 31)*.

Due to the favorable conditions, the patient was discharged and closely followed up for dressing changes *(Figure 32)*.

1 week after the surgery, the wound edges were granulating, but the center of the wound exposed the cranial theca; he fish-skin bioinductor was applied again and dressed with polyurethane foam.

1 month later, the bone was completely exposed without any presence of granulation tissue *(Figure 33)*. At this point, a flap procedure was performed to address the situation.

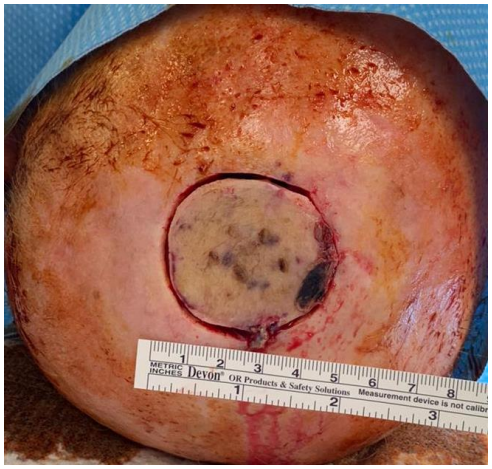

Figure 29 - preoperatory

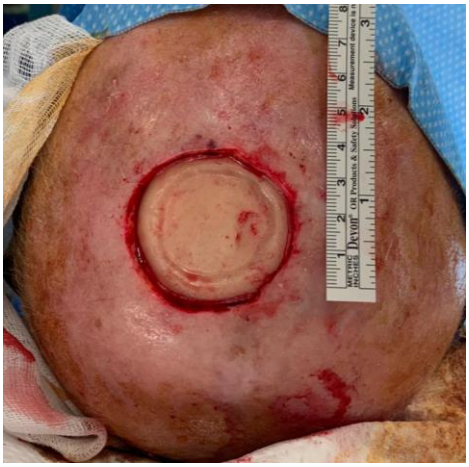

Figure 30 - removal of the previous skin-graft and milling of the cranial theca

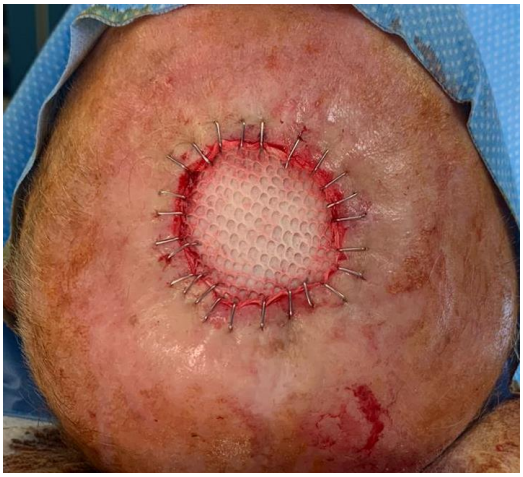

Figure 31 - application of fish-skin bioinductor fixed with ahraphes and secondarily dressed in polyurethan foam

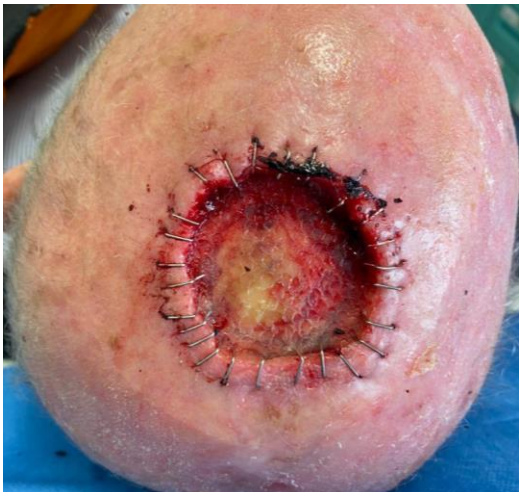

Figure 32 – 3 days postoperatively the wound is poorly exudating, the bioinductor is visible on the base

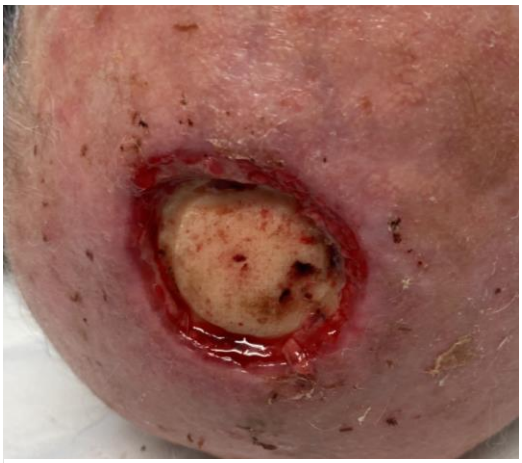

Figure 33 - the bone completely exposed without the presence of granulation tissue

RESULTS

Clinical outcomes

In all the patients, the fish-skin derived bioinductor was initially visible at the base of the wound during the first week after surgery; following its reabsorption, the wound was then covered with granulation tissue. 4 out the 6 treated patients had a favorable outcome and an appropriate healing process; in contrast, two patients after the initial promising uptake, 2 weeks after surgery had no withstanding granulation tissue visible but rather did have necrosis or bone exposure.

Histological outcomes

1 year after the surgery, biopsies were performed to the four patient who had a successful healing with the fish-skin derived bioinductor *(Figures 34-37, Table I)*

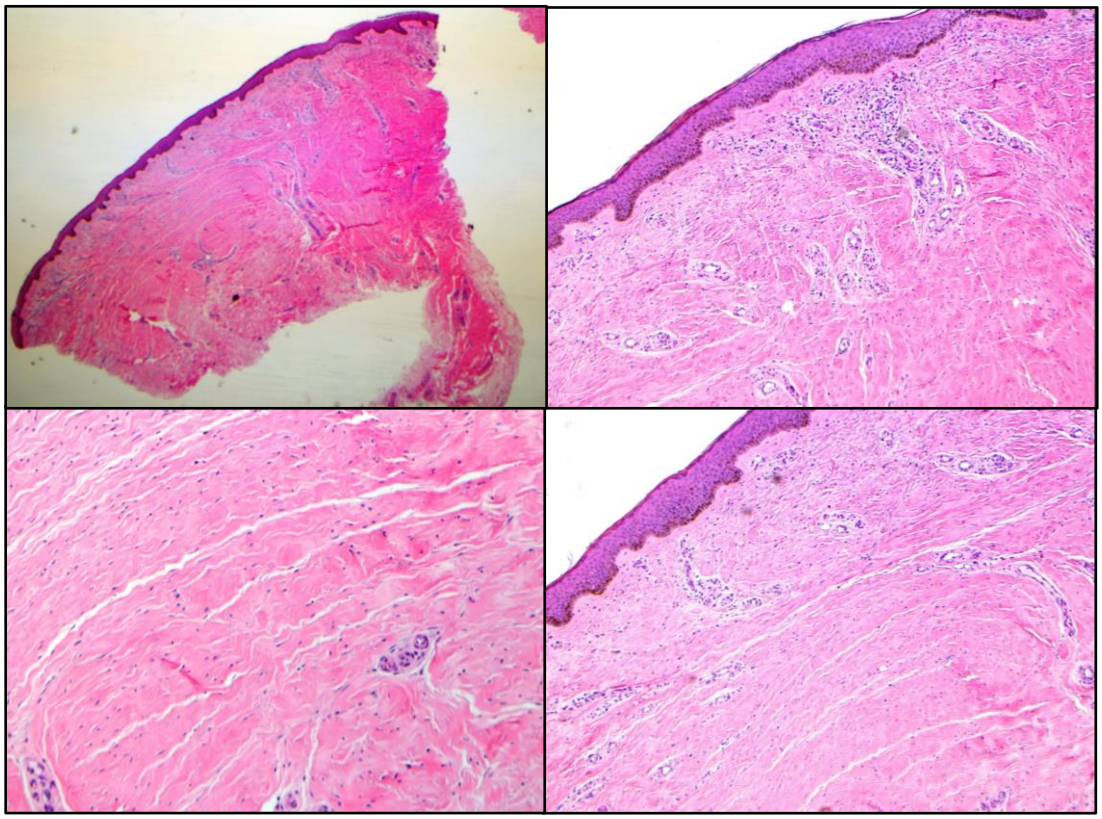

Figure 34 - Post-traumatic foot ulcer. absence of scaffold, neoangiogenesis, horizontal and vertical thick collagen fibers, no pilosebaceous unit

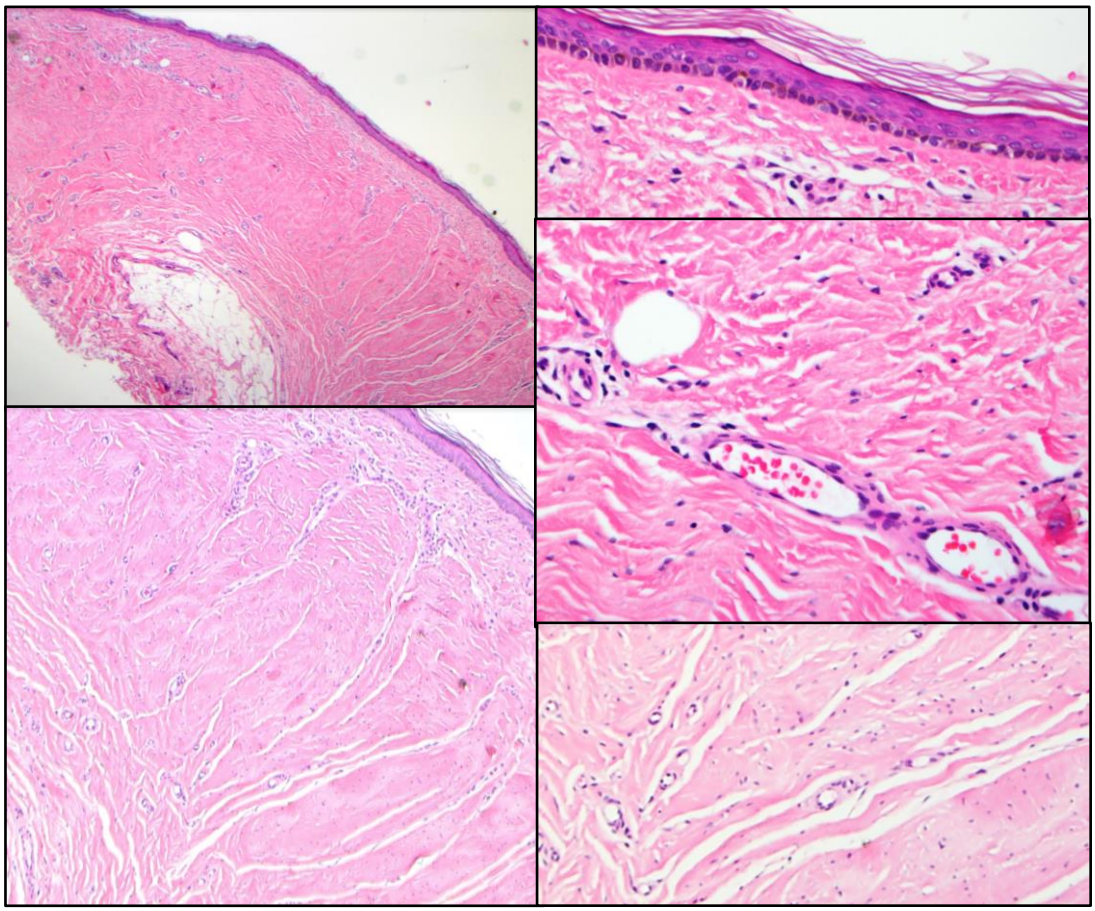

Figure 35 - Degloving Trauma; absence of scaffold, atrophic epidermis, neoangiogenesis, vertical collagen fibers, no pilosebaceous unit

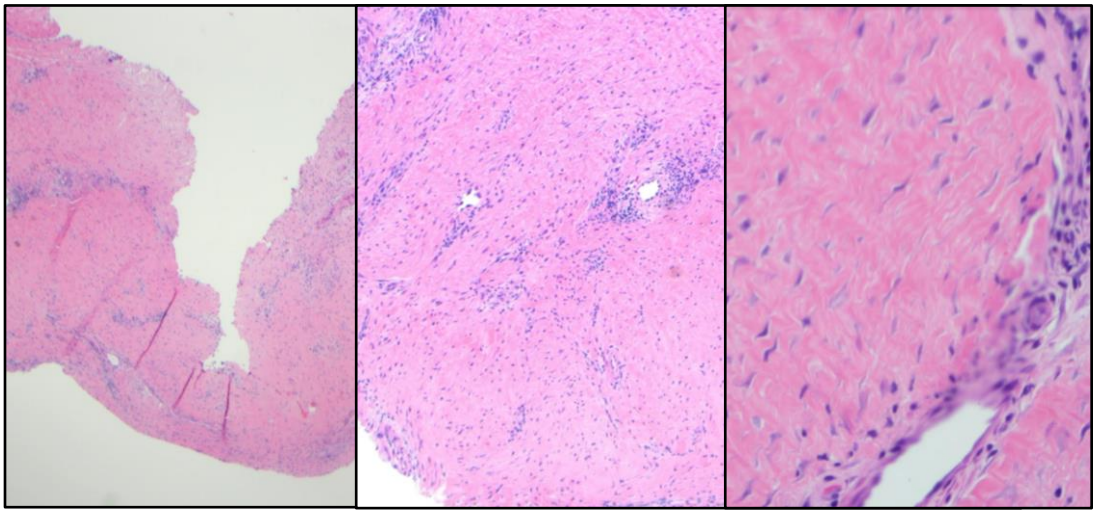

Figure 36 - Foot diabetic ulcer; absence of scaffold, well organized and advanced granulation tissue

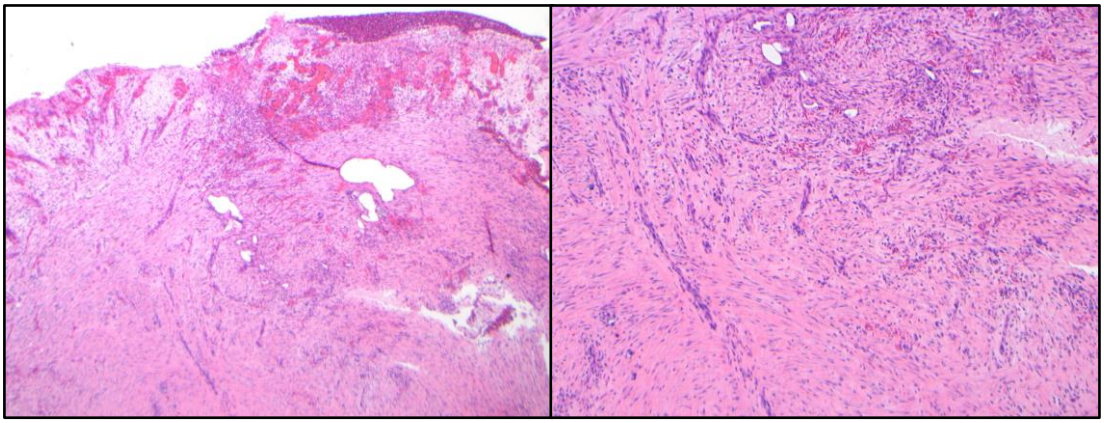

Figure 37 - Basal Cell Carcinoma excision; absence of scaffold, neoangiogenesis

Patient	Wound Localization	Wound Etiology	Histological Outcome
M, 26	Right foot	Post-traumatic foot ulcer	Absence of scaffold, neoangiogenesis, horizontal and vertical thick collagen fibers, no pilosebaceous unit
F, 51	Left leg	Degloving Trauma	Absence of scaffold, atrophic epidermis, neoangiogenesis, vertical collagen fibers, no pilosebaceous unit
M, 63	Right foot	Two diabetic ulcers, one with tendon exposure	Absence of scaffold, well organized and advanced granulation tissue
M, 63	Right nasal alae	Basal Cell Carcinoma excision	Absence of scaffold, neoangiogenesis
M, 69	Right foot	Chronic ulcer in lymphoedema in plegic	Necrosis
M, 82	Scalp	Post oncological excision (radicalization of Squamous Cell Carcinoma)	Bone exposure; absence of granulation tisssue

(Table I)

DISCUSSION

After the application of the acellular fish matrix, each treated patient began to develop granulation tissue at the base of the wound within 1 week. This characteristic indicates that the acellular fish matrix acts as a bioinductor.

Biopsy results revealed that the fish-skin bioinductor can guide spontaneous reepithelization; in fact, the biopsies showed remarkable similarity to naturally healed skin, without the presence of a scaffold. However, the newly formed skin lacked hair follicles and sebaceous glands, likely due to the patients' age or phototype. Additionally, the collagen fibers in the new skin had a tendency to align vertically, possibly due to the load-bearing nature of the areas.

Out of the 6 treated patients, 4 had a favorable outcome: this allowed for successful skin-graft surgeries in 2 of them, resulting in appropriate healing characterized by stability, good skin pliability, absence of scar adhesions, and no limitations in joint movement; the remaining 2 patients experienced good reepithelialization without requiring a skin graft.

However, 2 patients, despite the initial promising progress, exhibited signs of necrosis or bone exposure two weeks after surgery, indicating a lack of sustained granulation tissue formation. This suggests that these particular cases did not respond as positively to the treatment, highlighting the need for further investigation into the factors that may contribute to varying outcomes.

The application of the bioinductor also demonstrated its analgesic effect. Each patient reported a preoperative pain score of 9-10 on the Visual Analogue Scale (VAS), which decreased to 0-2 after the bioinductor application.

The fish-skin bioinductor is highly versatile: it can be used in multiple sessions for the same patient, thanks to its reabsorption time of 7-14 days (compared to 14-21 days required for dermal substitutes); it can be combined with other therapies such

as Negative Pressure Wound Therapy (NPWT); moreover, it can be used alone without the need for a skin graft to close less extensive wounds.

In one case, a patient had exposed tendons, making primary treatment with a skin graft impossible. However, the use of the bioinductor created a suitable wound bed for subsequent closure with a skin graft.

CONCLUSIONS

The fish-skin bioinductor has emerged as a novel and promising acellular dermal matrix for wound treatment due to its versatility and the positive outcomes observed with the newly formed skin.

However, further studies are needed to explore strategies to prevent potential failures. Based on the experience in Padova, it is not recommended to use the bioinductor in patients who have a high risk of bone exposure or poorly vascularized ulcers. This highlights the importance of careful patient selection and individualized treatment approaches in wound management.

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