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APPLICATION OF ACELLULAR GRAFTS FOR EXTENSIVE SKIN DEFECTS – A REVIEW

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ABSTRACT

Skin healing in extensive skin defects includes stages of granulation and epithelialization, which are the main processes in wound healing in a secondary way. Epithelialization is possible only when the wound cavity is filled with granulation tissue to the level of the skin edges. The source of epithelial cells is the preserved epithelial structures from the skin edges themselves and the hair follicles.

In wounds with a large surface due to a severe inflammatory reaction, burns or in wounds with tissue avulsion, epithelialization processes are complicated due to damage to the structures that are a source of epithelial elements. Wound healing is difficult and takes a long period of time, which causes secondary complications.

The acceleration of this process can be directed by using an auto- allo and xenograft to shorten the healing period. Xenografts must meet several conditions such as biocompatibility, efficiency, accessibility, ease of application and low cost. In recent years, acellular grafts from the skin of certain fish species – *tilapia* and *salmon* – have been increasingly used.

In conclusion, acellular fish skin grafts are making promising advances, offering a natural, biocompatible and cost-effective solution to promote wound healing and tissue repair.

Key words: wounds, acellular fish skin grafts, fish, epithelialization.

Introduction

In veterinary practice, wounds of various nature, arising on the base of physical, chemical and traumatic agents, are common. To achieve a favorable outcome, timely and appropriate treatment of these wounds is important. The standard for their treatment includes surgical treatment, drug antiseptics, and infection prevention by performing biological antiseptics (Zubin *et al.*, 2015).

Based on the size, location and severity of the wounds, healing them in a primary way can be difficult or impossible. Healing of wounds in a secondary way takes a longer period of time, carries a risk of developing infections, as well as a complication of the formation of extensive cicatrixes (Campbell *et al.*, 2015).

Cicatrixes and keloid formation are complications of extensive wounds in the facial area, in the area of the limbs and especially on the plantar and palmar surfaces. Excessive tension of the wound when closing, suboptimal approach of the wound edge can cause poor cosmetic results. Cicatrization of tissues often leads to contractures and limitation of motor capacity in the joints (Prpich *et al.*, 2012).

In order to shorten the healing period in extensive skin defects with tissue avulsion and to avoid possible complications, auto- allo- and xenografts are used (Dreifke *et al.*, 2015).

A transplantation is a surgical operation in which tissues, parts of an organ or an entire organ are taken from one body (donor) to be transplanted for medicinal purposes to another body (recipient) or to another site from the same body (Chapman *et al.*, 1997).

Skin graft transplantation is a method of restoring extensive skin defects in animals resulting from the lack of healthy granulation tissue and epithelialization disorders due to chronic inflammation in bite wounds and burns. The extracellular matrix of the grafts affects the function and success of the graft. Components- *collagen, elastin, GAG (glycosaminoglycans)* and other bioactive molecules, provide structural support, mechanical strength and biochemical signals necessary to maintain cell attachment, angiogenesis and remodeling processes (Schallberger *et al.*, 2008; Shores *et al.*, 2015; Luo *et al.*, 2019).

In autotransplantation, the donor skin is taken from a different part of the body of the same individual. The advantages of this type of transplant are related to biocompatibility– due to the fact that they originate from the same individual, the likelihood of rejection of the transplant is minimal. Autologous skin transplantation has a good restorative effect, but carries the risk of infection, caused secondary injury and pain at the donor site, and in some cases are impossible to obtain (Jeremias *et al.*, 2002; Thornton *et al.*, 2004; Khavari *et al.*, 2014).

In allotransplantation, the donor and recipient are of the same species. Belonging to different individuals triggers an immune response in the recipient and may lead to graft rejection due to biological incompatibility (Centanni *et al.*, 2011).

In xenotransplantation, the donor and recipient are of different types. There is a possibility of an immune rejection response and the risk of transmission of viral diseases to recipient animals. Clinically, only tissue xenografts have been used in world practice so far, due to the inability to achieve tolerance of the immune system during organ transplantation (Chapman *et al.*, 1997; Hermans *et al.*, 2014; Nyame *et al.*, 2014).

Acellular Dermal Matrices (ADM)

In recent years, the use of xenografts as a suitable method of tissue repair, especially for extensive skin defects, has become more and more popular. The main types of xenografts that are used in medical practice are intestinal submucosal or amniotic tissue from various types of donors (pigs, horses or cattle) or acellular graft from fish skin (Boháč *et al.*, 2018).

Successful administration of xenografts requires that they meet several conditions: biocompatibility, lack of antigenicity, efficiency, accessibility, ease of administration, low cost (Shores *et al.*, 2007).

Acellular dermal matrices *(ADM)* are biological materials obtained through the decellularization of tissues, in which the cells are removed while the extracellular matrix and bioactive molecules that stimulate tissue repair and remodeling are preserved. These matrices are used in veterinary medicine for the treatment of wounds with extensive skin defects, ulcers, and burns, providing structural support, promoting cell migration, reducing the risk of infections, and aiding regeneration processes (Bush *et al.*, 2016).

The main types of *ADM* include matrices made from pig, cattle, and fish skin. They are used depending on the specific needs of the patient, with pig and cattle skin matrices providing stability and good mechanical support, but they may trigger immune reactions since they contain higher levels of proteins that can induce inflammatory responses (Kirsner *et al.*, 2020). On the other hand, fish skin *ADM* have significant advantages, especially in animals with heightened immune responses, as these matrices contain fewer cellular antigens, resulting in a reduced risk of rejection (Balsa *et al.*, 2015).

Acellular fish skin grafts show excellent biocompatibility with host tissues, minimizing the risk of rejection or adverse reactions. The preserved structure of the extracellular matrix serves as a scaffold for migration, proliferation and tissue regeneration of host cells. *Fish skin graft (FSG)* has received approval from *the U.S. Food and Drug Administration (FDA)* for use in humans for

treating various types of wounds, including traumatic wounds, partial and total thickness wounds, decubital wounds, diabetic ulcers, surgical wounds, venous ulcers and chronic vascular ulcers (Baldursson *et al.*, 2015; Kirsner *et al.*, 2020).

Baldursson *et al.*, 2015 conducted a study comparing an acellular fish skin graft with an extracellular matrix derived from the submucosal layer of porcine small intestine in the healing of full-thickness wounds in humans, finding that wounds treated with the acellular fish skin graft healed significantly faster. Fish skin *ADM*, compared to mammalian *ADM*, has no potential risk of an autoimmune reaction.

By reason of to the biological properties of fish skin and the content of its extracellular matrix, rich in *collagen, elastin, glycosaminoglycans* and other bioactive molecules, it supports the wound healing process and tissue regeneration. These components contribute to the ability of the graft to support cell attachment, angiogenesis, and remodeling processes. Following processing, *FSG* retains the structure of dermal tissue and contains *omega-3 polyunsaturated fatty acids*, which possess antibacterial, antiviral and anti-inflammatory properties (Bush *et al.*, 2016).

The naturally occurring 3-D microporous structure of acellular fish skin graft is similar to the extracellular matrix of mammalian skin. This enables autologous cells, such as fibroblasts, to infiltrate, colonize, and proliferate in the area, promoting angiogenesis. Consequently, the graft transforms into functional living tissue as it gradually integrates into the viable wound bed through the accumulation of new granulation tissue (Carlsson *et al.*, 2016).

The high effectiveness of acellular fish skin grafts is also due to their antimicrobial properties, due to the presence of natural antimicrobial peptides and other bioactive compounds. This property helps create a protective barrier against infection and can contribute to improved wound healing results. *FSG* integrates into the human wound bed within a time frame of 7 to 10 days and exhibits antimicrobial properties within 1 to 3 days (Magnusson *et al.*, 2017).

Stone *et al.*, 2021 demonstrated that acellular fish skin graft promotes faster epithelialization and vascularization, as well as a more favorable histological response, compared to the use of fetal bovine dermis, in the treatment of second degree burn wounds in pigs (partial-thickness burn wounds).

Acellular fish skin grafts are available in the form of sheets, nets and powdered substances, allowing for flexibility in application. They can be easily applied to wounds of various sizes, shapes and depths, facilitating the grafting process for clinicians. The fish skin possesses distinctive properties that support the inflammatory, homeostasis, proliferative and remodeling stages of wound healing. Its unique three-dimensional structure and natural porosity provide an optimal environment for the proliferation of new cells during the wound healing process (Stone *et al.*, 2021).

Acellular fish skin grafts have been shown to be effective in treating acute and chronic wounds, burns, surgical incisions and skin defects. Clinical studies have reported favorable results, including accelerated wound closure, reduced pain, inflammation and scarring, and improved cosmetic outcomes. Fish skin has been found to have a higher collagen content compared to mammalian skins (Tang *et al.*, 2015; Alves *et al.*, 2018; Meq *et al.*, 2021), resulting in an accelerated wound healing process (Rangaraj *et al.*, 2011).

Chen *et al.*, 2021 used an acellular fish skin graft on rats and proved that wounds treated with acellular fish skin were reepithelialized on day fourteen with minimal cicatrixes, compared to those used by Shores *et al.* al., 2007 modifications of pig skin, including aldehyde cross-linking and impregnation with silver ions to increase antimicrobial properties.

Compared to traditional wound care methods and other biological skin substitutes, acellular fish skin grafts offer a cost-effective alternative with comparable or superior clinical outcomes. Their ease

of use and potential to reduce healing time can lead to overall cost savings for healthcare providers. The manufacturing process is cost-effective and environmentally friendly (Sitje *et al.*, 2018).

FSG has an excellent shelf life, retaining its effectiveness for 3 years after production. The minimally destructive and efficient manufacturing process, low disease transmission capability, and long shelf life of the acellular fish skin graft are excellent features for a biomaterial demonstrating highly marketable characteristics that are effective in clinical settings (Alam *et al.*, 2019).

Acellular fish skin grafts are generally well tolerated and have a favorable safety profile. Side effects such as allergic reactions or disease transmission are rare due to strict processing methods and lack of cellular material. Compared to mammalian acellular grafts (such as those derived from pigs and cattle), acellular fish skin grafts do not pose the risk of transmitting diseases like *bovine spongiform encephalopathy* and a variant of *Creutzfeldt-Jakob disease (CJD)* (Brown, 2001). Additionally, they can undergo a simpler sterilization process while still maintaining their natural content of *omega-3 fatty acids* (Kjartansson *et al.*, 2015; Dorweiler *et al.*, 2017).

The grafts undergo a comprehensive decellularization process to remove cellular components while preserving the structure of the extracellular matrix *(ECM)* and bioactive molecules (Luze *et al.*, 2022).

Acellular grafts derived from fish skin represent an innovative and promising approach to wound healing and tissue repair for the following reasons: *omega-3 fatty acid* content, preservation ability, ideal cell structure, low risk of disease transmission compared to allografts and other xenografts, and a relatively inexpensive resource (Hu *et al.*, 2017; Luze et., 2022*). Omega-3 fatty acids* have anti-inflammatory properties (Lands *et al.*, 2005) and help in the transition of the wound from the inflammatory stage to the proliferation stage (Serhan *et al.*, 2014).

Fish skin is a multifunctional tissue with appropriate physical and mechanical properties and possesses excellent antimicrobial properties against pathogens and can be an ideal alternative for recipient skin regeneration. Compared to hemostatic collagen fungi or hydrogels, *FSG* retains its collagen and natural structure (Rakers *et al.*, 2010).

Obtaining acellular grafts from fish skin

In order to use fish skin in tissue engineering, it is necessary to perform a decellularization process that involves removing cellular components while preserving the structure of the extracellular matrix and bioactive molecules. This process aims to create an acellular scaffold that supports tissue repair and regeneration without triggering an immune response or adverse reactions in the host. Initially, the preparation of the primary material is carried out – fresh fish skin is obtained from selected species, such as *Nile tilapia, North Atlantic cod, salmon, grass carp*, usually originating from aquaculture or intended for the food industry. The skin is gently cleaned to remove contaminants, blood, or tissues (Dorweiler *et al.*, 2018; Kamalvand *et al.*, 2021; Li *et al.*, 2021; Luze *et al.*, 2022).

Decellularization methods are classified into three groups- Chemical, physical and enzymatic (Dussoyer *et al.*, 2020). In the chemical method of decellularization, non-ionic *(Triton X-100)* and ionic *(sodium dodecyl sulfate [SDS])* detergents, as well as acids *(peracetic acid)* and bases *(ammonium hydroxide),* are used to break cell membranes and dissolve cell contents (Miranda *et al.*, 2021).

In the chemical method of decellularization, by using surfactants, cells are lysed by rearranging phospholipids in the cell membrane. *Ionic SDS* is one of the most widely used surfactants due to its effectiveness in removing cells and 90% of *DNA.*

Khajavi *et al.*, 2021 investigated the effectiveness of *SDS* on the decellularization of sturgeon cartilage and proved the preservation of collagen fibers by establishing a high content of hydroxyproline. With this method of decellularization, the disadvantage is that the amount of glycosaminoglycans in the cell matrix decreases.

After the decellularization process, in order to avoid the cytotoxicity of surfactants, the tissue must undergo a washing process using *Phosphate Buffered Saline (PBS).* To aid in the washing process, non-ionic surfactants such as *Triton X-100* are often mixed with *SDS* (García *et al.*, 2020). *Triton X-100* is used to remove remaining fat by breaking the bonds between lipids and proteins (Mohiuddin *et al.*, 2020).

The effectiveness of the decellularization method based on the *Triton X-100* has been studied on the air bladder of *Silver carp* and the skin of grass carp. It has been proven that the created scaffold has a porous structure and stimulates cell proliferation and differentiation. This method of decellularization shows high efficiency, which is based on the significant reduction of cells and fat and the preservation of collagen fibers as the most important component of *ECM* (Wang *et al.*, 2020; Biziar *et al.*, 2022).

Bases such as sodium hydroxide and acids such as peracetic acid destroy the nuclear material and cell membrane. Proteins are denatured by acids and bases and cells are destroyed as a result of the dissolution of cellular components. The combined use of acids and bases together with other decellularizing agents has better results. When decellularizing tilapia fish, a combination of sodium base with *Triton X-100* and a physical method involving 3 cycles of freezing and thawing were used. It has been proven that the majority of cells and their nuclei are removed in the resulting matrix (Ma *et al.*, 2020). The effectiveness of this method of decellularization of tilapia skin is comparable to the method used by Lau *et al.*, 2019, who administered *SDS* in combination with nuclease. Both methods have proven that the combined use of decellularizing detergents has better results.

Enzymatic methods use proteolytic enzymes such as trypsin, papain, and nucleases to break down cellular proteins and facilitate cell removal (Boccafoschi *et al.*, 2017).

Chelating agents- *ethylenediaminetetraacetic acid (EDTA)* or *ethylenediamine-N, N-disuccinic acid (EDDS)*- can be used to chelate divalent cations and disrupt cell-cell and cell-matrix interactions. This is followed by the machining process. Light shaking, stirring, or centrifugation can be used to facilitate the penetration of decellularizing agents into the tissue and to improve cell removal. Enzymatic methods are difficult to replicate. They significantly increase the possibility of structural alteration of the *ECM* (Miranda *et al.*, 2021).

Nucleases are commonly used in the steps following chemical and physical methods of decellularization. These enzymes break down the nuclear material to effectively remove biological components from the matrix. Nucleases have been used in conjunction with chemical methods of decellularization in grass carp scales. With this protocol, type I collagen and hydroxyapatite remain in the scaffolding of decellularized fish scales (Wu *et al.*, 2021).

Physical methods include freezing-thawing, which achieves a reduction in cellular content (Burk *et al.*, 2014). Freeze-thaw involves intermittent freezing temperatures (~-80°C) with alternating temperatures (~37°C) for an optimized number of cycles. A single freeze-thaw cycle reduces adverse immunological reactions such as leukocyte infiltration into the avascular extracellular matrix structure (Isidan *et al.*, 2019).

This method cannot completely remove cells, but it preserves the mechanical strength and structure of the mold. Freezing-thawing is commonly used in conjunction with other techniques and chemical reagents (García *et al.*, 2020).

After applying some of the above methods (chemical, enzymatic, physical), the decellularized tissue is thoroughly rinsed and washed with buffer solutions to remove residual chemicals and cell residues. It is usually perform multiple washing steps to ensure the full removal of decellularizing agents. Decellularized fish skin is sterilized using methods such as gamma irradiation, ethylene oxide gas, or sterile filtration to eliminate all other microorganisms and ensure the safety of the product for clinical use (Chiu *et al.*, 2005; Alves *et al.*, 2018; Ibrahim *et al.*, 2020).

Decellularized fish skin undergoes rigorous quality control tests to assess its structural integrity, extracellular matrix composition, lack of residual cellular material, sterility and biocompatibility. This may include histological analysis, *DNA* quantification, biomechanical testing, and in vitro or in vivo biocompatibility assessments. Once validated, the decellularized fish skin is packaged in sterile containers and stored under appropriate conditions to preserve its structural and biological properties until clinical use (Dussoyer *et al.*, 2020).

By carefully controlling the decellularization process, it is possible to produce acellular fish skin grafts with preserved extracellular matrix structure and bioactive molecules that can support tissue repair and regeneration in a variety of clinical applications. Optimization of decellularization protocols and quality assurance measures are essential to ensure the safety, efficacy, and reproducibility of decellularized fish skin products for therapeutic use. It is important to note that acellular fish skin grafts are produced industrially and are used as ready-made products in clinical practice, rather than being individually prepared in medical settings (Fig. 1). This ensures the standardization and reliability of the products when applied (Esmaeili *et al.*, 2023).

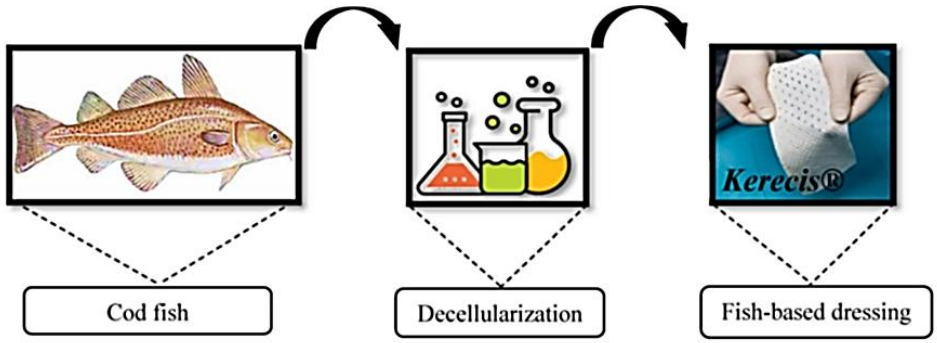


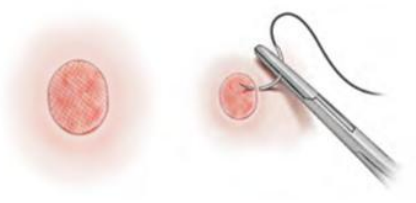
Figure 1: *FSG* by Kerecis ®, made from acellular Atlantic cod skin after the decellularization process (according to Esmaeili *et al.*, 2023).

Method of application of FSG

Acellular fish skin grafts are used to treat complicated or chronic wounds in animals (Dorweiler *et al.*, 2018).

The fish skin undergoes technology, which preserves essential components such as *Omega-3 fatty acids, laminin, collagen, elastin, lipids, fibrin, proteoglycans* and *glycosaminoglycans*. During the healing process of a wound treated with acellular fish skin graft, these components become integrated into the wound bed within 7 to 10 days, promoting effective recovery and regeneration, and the wound defect is filled with granulation tissue to the level of the skin edges, after which an accelerated epithelialization process begins. *FSG* shares similarities with mammalian skin, consisting of both epidermal and dermal components. It accelerates wound healing, functions as an effective antimicrobial barrier, and exhibits anti-inflammatory properties, making it an efficient option for promoting rapid and healthy tissue regeneration (Baldursson *et al.*, 2015; Magnusson *et al.*, 2018).

The method of application of acellular fish skin grafts involves several stages. Initially, surgical treatment of the wound is performed. It is necessary to remove all non-viable and necrotic tissues. Healthy tissue boundaries are established, preferring a slightly bleeding wound before application of the product (Fig. 2a). Next is the underfeeding of the graft. An aseptic technique is used. The process involves cutting the graft according to the wound size and shape with minimal overlap. The graft is hydrated in sterile saline at room temperature for about 30 seconds, then placed in the wound bed so as to ensure a tightly fit (Fig. 2b). The acellular graft can be perforated or fenestrated, making it easier for extravasates to drain. After the graft is placed in the wound bed, fixation is carried out using some of the following methods- sutures, staples or *Steri-Strip* (patches for seamless closure of wounds), followed by a silicone-coated polyurethane dressing (Fig. 2c). Creating a moist environment for healing is essential – the most preferred method is the application of a silicone wound dressing coated with polyurethane. It is possible to provide reinforcement of the bandage in one of the following ways – negative pressure carried out by means of a vacuum pump; dressings – calcium-alginate, hydrofiber, polyurethane foam or wet gauze (Fig. 2d). It usually takes 5–7 days for the acellular fish skin graft to be incorporated into the patient's tissue. If necessary, a new graft is placed after this period (Magnusson *et al.*, 2018; Fertram *et al.*, 2021).



2a. Initial surgical wound treatment

2b. Preparation of the graft according to the manufacturer's instructions

2c. Placement and fixation using sutures

2d. External dressings, with the FSG placed directly on the wound defect under the contact layer of the dressing

Figures 2a, 2b, 2c, 2d: Application method of *FSG* by Kerecis ®, made from acellular Atlantic cod skin after the decellularization process (Fertram *et al.*, 2021).

Mechanism of action of acellular grafts from fish skin

Acellular fish skin grafts represent an innovative approach in wound therapy due to their biological and structural characteristics, which create a favorable environment for tissue healing.

These grafts contain basic components of the extracellular matrix, such as *collagen*, *elastin*, and *glycosaminoglycans*, which provide structural support and promote cell migration and proliferation, essential for the processes of tissue regeneration and remodeling (Sandness *et al.*, 2019).

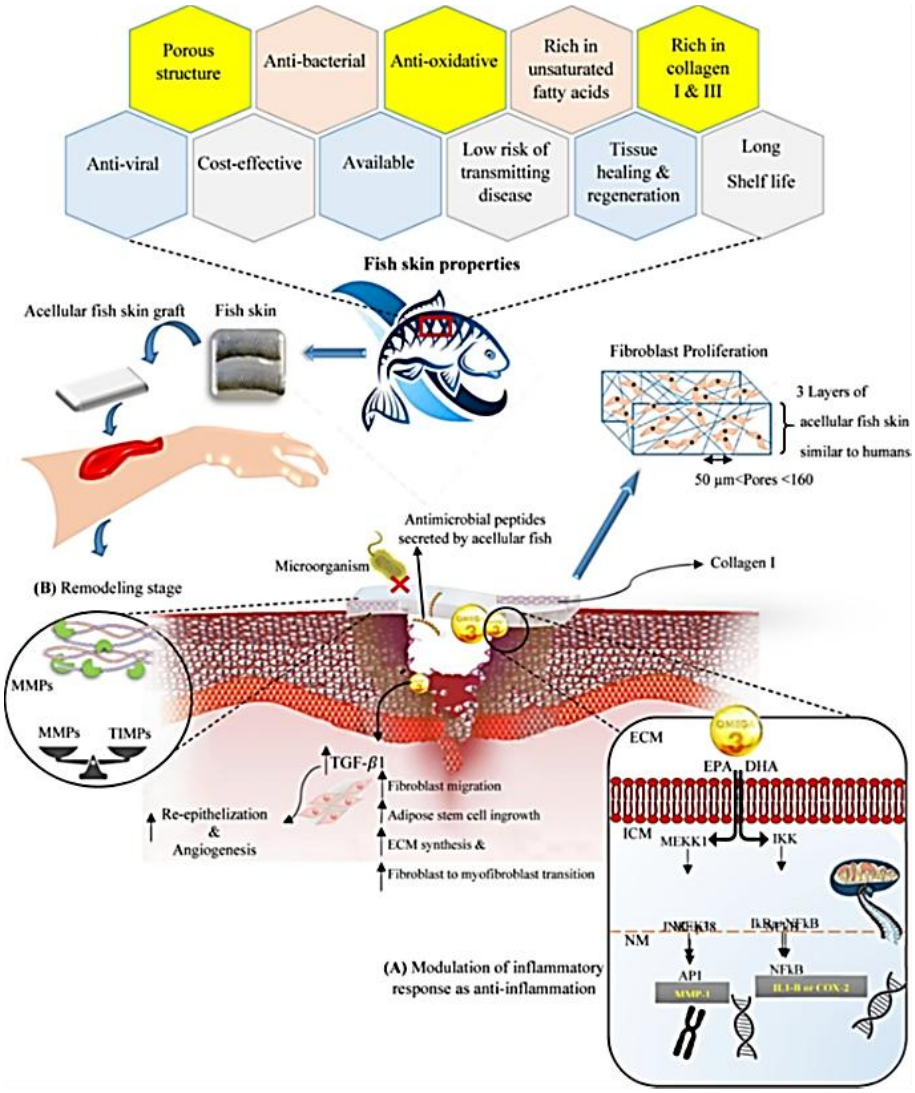


Figure 3: Properties of acellular fish skin grafts and the impact of *omega-3 polyunsaturated fatty acids* on inflammation processes and wound healing stages, as well as their role in tissue remodeling. Mechanism of action of omega-3 fatty acids. (B) Matrix metalloproteinases (MMPs) (Esmaeili et al., 2023).

According to Esmaeili *et al.*, 2023, acellular fish skin grafts provide a structural foundation for the repair of damaged tissues and modulate molecular pathways that accelerate tissue remodeling processes and reduce inflammation. The presence of *omega-3 fatty acids*, such as *eicosapentaenoic acid (EPA)* and *docosahexaenoic acid (DHA)*, enhances the therapeutic effect of acellular fish skin grafts through their impact on inflammatory and regenerative processes. *Omega-3 fatty acids* modulate various molecular pathways by activating proteins and enzymes, including *AP1*

(activator protein 1), COX-2 (cyclooxygenase-2), and *IL-1B (interleukin 1-B),* which regulate inflammation and the synthesis of *ECM* components (Fig. 3A). Through these mechanisms, *omega-3 fatty acids* not only reduce inflammatory activity but also stimulate the activity of *matrix metalloproteinases (MMPs),* thereby accelerating tissue remodeling and wound healing processes (Aristotelis *et al.*, 2021; Esmaeili *et al.*, 2023).

The main mechanism of action of acellular fish skin grafts is related to their ability to regulate the activity of matrix metalloproteinases, which are enzymes responsible for the degradation of *ECM* components (Fig. 3B). *MMPs* take part in a central role in the dynamics of inflammation and tissue remodeling by controlling the breakdown of the old extracellular matrix and the synthesis of new components necessary for the repair of damaged tissue. The balance between the activity of *MMPs* and their inhibitors, known as *tissue inhibitors of metalloproteinases (TIMPs),* is crucial for the proper formation of a new extracellular matrix and for the effectiveness of the healing processes. Acellular fish skin grafts help maintain this balance, promoting optimal *ECM* remodeling and reducing the inflammatory phase of healing (Dussoyer *et al.*, 2020).

Biazar *et al.*, 2022 compared the healing effect of self-administration of acellular fish skin *(AFS);* the combination of *AFS* with fibrin gel containing growth factors *(GF gel)* and self-administration of *GF gel* in skin wounds in a rat model. They prove that 100% is the closure of wounds in the combination of *AFS/GF*. Recovery in wounds treated with *GF gel* is 82%; with an acellular fish skin graft is 85%. The study demonstrated that the combined use of *AFS* and *GF gel* was more effective than their use alone in promoting wound healing in the rat model. The accelerated recovery in the combined group is due to the synergistic action of collagen and *Omega-3 fatty acids* present in *AFS*, along with the growth factors contained in the *GF gel.*

Acellular fish skin graft acts as a natural biological dressing, providing structural support and stimulating cell migration, making it highly effective for the treatment of skin wounds.

The study by Kherkar *et al.*, 2024, also shows that both fish-based biological dressings and *platelet-rich plasma (PRP)* therapy lead to good healing of skin wounds in cats, with both methods showing significant reductions in wound area and improvements in granulation and epithelialization. However, the fish skin graft provides additional advantages, as it not only supplies biological components that stimulate healing but also offers structural support for the tissues, making it exceptionally effective in accelerating tissue regeneration and remodeling processes.

On the other hand, *PRP* therapy requires preparation time, as it involves the process of venous blood collection and plasma processing. The results can vary depending on the individual characteristics of the patient, such as age, health condition, and immune response, which can lead to unpredictability in the treatment's effectiveness in different patients. The limitations of *PRP* application are, on the one hand, related to the higher number of leukocytes in peripheral blood, which makes it impossible to separate platelet mass. On the other hand, in patients with low body weight, obtaining a sufficient amount of blood (about 8 ml minimum) is not always possible over several consecutive days. The lack of a structural scaffold for building and anchoring the newly formed tissue is the third limitation when using *PRP* for the treatment of slow-healing wounds. *Platelet-rich plasma* does not provide the same structural support and biological components, such as *collagen* and *omega-3 fatty acids,* which are essential for accelerating tissue remodeling.

However, the combination of *AFS* and *GF gel* provides significantly improved results in wound healing, with the synergistic effect between these components speeding up the healing processes and optimizing tissue recovery.

Chen *et al.*, 2021 and Wang *et al.*, 2021 conducted two separate studies to evaluate the effectiveness of different types of acellular dermal matrices in wound treatment in a rat model. Both studies compared an acellular matrix of fish skin with other synthetic or semi-synthetic wound dressings.

In the study by Chen *et al.*, 2021, researchers compared *AFS* with a chitosan-based wound dressing called *SURCHI-fiber*. They found that wounds treated with *AFS* were reepithelialized with minimal cicatrixes, while wounds treated with *SURCHI-fiber* showed more pronounced scarring. This suggests that AFS provides a better environment for tissue regeneration and reduces the process of scar formation compared to *SURCHI-fiber*.

In the study by Wang *et al.*, 2021, the authors compared the effect of using a cell-free dermal matrix from tilapia fish *(TADM)* with bovine fetal acellular dermal matrix *(FBADM)* on wounds in rats. They observed that the degree of erythema and edema in the *TADM*-treated group was significantly less compared to the *FBADM*-treated group. *TADM* shows better properties, such as insulation, mechanical strength, biodegradability, and biocompatibility, which are favorable for cell infiltration, adhesion, growth, and angiogenesis. This research suggests that *TADM* is a more suitable material for wound treatment than FBADM due to its benefits.

Comparing the two trials, both studies support the use of *AFS* as an effective wound treatment material in a rat model. However, while Chen *et al.*'s study highlights scar reduction when using *AFS*, Wang *et al.*'s study focuses on the improved properties of *AFS* compared to another type of dermal matrix. Both studies provide evidence to support the potential benefit of using *AFS* in wound treatment. Three other studies have supported the use of an acellular fish skin graft as an effective material for treating burn wounds. The main differences between these studies include: the type of wound model used (rabbit deep burns of partial thickness, rats with burn wounds and pig burn wounds), the type of collagen peptides used (from salmon, tilapia and a combination of salmon and tilapia) and the additional mechanisms of action investigated in each study (modulating the skin microbiome, modulating the expression of immune receptors and forming specialized lipid mediators). Despite these differences, all studies show that an acellular fish skin graft can be a valuable alternative to traditional dressings in wound healing.

Hu *et al.*, 2017, conducted an experiment with rabbits and experimentally created a partial thickness deep burn model (Second burn degree) and compared the effect of applying lyophilized marine collagen peptides *(MCP)* with a moisturizing ointment for burn treatment (Meibao Pharmaceutical Co., Ltd. (China)). They proved that in the group treated with *MCP*, there was almost complete wound coverage by new epidermis, active proliferation of the hair follicle, complete structure of the muscle layer, fibroblasts and new capillaries due to the structurally determining components of the cell-free skin grafts from fish. The main role is played by *collagen types 1* and *3, elastin, laminin, glycoproteins and Omega-3 fatty acids.*

In the second study, Mei *et al.*, 2020 investigated the effects of collagen peptides derived from the skin of *Salmo salar (Salmon)* and *Oreochromis niloticus (Tilapia)* on the wound healing process in rats. They prove that the degree of wound healing in the groups treated with collagen peptides from *Tilapia* and *Salmon* is significantly accelerated. This is associated with reduced levels of *Tumor Necrotic Factor-α (TNF-α), Interleukin-6 (IL-6) and Interleukin-8 (IL-8)* and increased levels of *Beta-defensin 14 (BD14), Nucleotide-Binding Oligomerization Domain containing 2 (NOD2), Interleukin (IL-10), Vascular Endothelial Growth Factor (VEGF) and Major Fibroblast Growth Factor (β FGF).* The accelerated healing in the groups treated with collagen peptides is also due to the modulation of the skin microflora. *Leuconostoc spp., Enterococcus spp. and Bacillus spp*. have a positive effect on wound healing.

Leuconostoc spp. have the ability to produce lactic acid, which leads to the maintenance of an acidic pH on the surface of the wound and thus favors the healing process. These microorganisms produce substances that inhibit the growth of pathogenic bacteria and thus reduce the risk of infection. *Enterococcus spp.*, like *Leuconostoc spp., Enterococcus spp.* are also gram-positive cocci that produce lactic acid and other antimicrobial compounds. They have the ability to stimulate the production of growth factors and cytokines, thereby promoting wound healing. Bacillus spp. are a group of gram-positive rod-shaped bacteria. They are involved in the production of a wide range of antimicrobial peptides, which again supports the wound healing process.

Other types of microorganisms from the microbiome such as *Stenotrophomonas spp., Bradyrhizobium spp., Sphingomonas spp. and Phyllobacterium spp.* have a negative impact on wound healing due to their ability to produce toxins (exotoxins and hemolysin) that damage tissues and erythrocytes.

The mechanism of action of collagen peptides derived from the skin of Salmon and Tilapia involves changing the composition of the skin microbiome and regulating the expression of *immunoreceptors NOD2* and *BD14*, resulting in faster and more effective wound healing.

In the third study, Aristotelis *et al.*, 2021 used an acellular fish skin graft on burn wounds in pigs and proved that the *omega-3 fatty acids* in *FSG* have antibacterial and anti-inflammatory properties. With this experiment, the authors prove that acellular fish skin grafts are rich in *eicosapentaenoic acid* and *docosahexaenoic acid* conjugated with *phosphatidylcholines. EPA* and *DHA* support wound healing through the formation of *specialized lipid mediators (SPMs) - resolvins, protectins* and *maresins*. They control the inflammation process by stopping excessive neutrophil infiltration and stimulating changes in gene expression that supports antimicrobial protection.

SPMs lead to reduced production of pro-inflammatory cytokines and chemokines *(TNF-α; IL-6; and IL-8)* and support wound epithelialization by increasing the number of receptors for the high expression of *transforming growth factor-beta 1 (TGF-β1), alpha-smooth muscle actin (α-SMA),* and *Platelet/endothelial cell adhesion molecule-1 (CD31)* (Mei *et al.*, 2020).

Sandness *et al.*, 2019, Arham *et al.*, 2022, Zeller *et al.*, 2024 conducted independent studies, to evaluate the effectiveness of acellular fish skin matrices in the treatment of various types of wounds in animals. All studies have reported positive results, such as improved wound healing, reduced pain and inflammation, and faster recovery.

Arham *et al.*, 2022 use Nile tilapia skin as a means of closing skin wounds in cats. They prove that the sewn biological dressing made of *Nile tilapia* skin favors faster, safer and aseptic healing.

Zeller *et al.*, 2024, used an acellular matrix of fish skin in a horse, with chronic traumatic wounds in the hoof border area and cracks in the heel area. They prove that with the use of an acellular fish skin graft. Pain, inflammation and healing time are significantly reduced. The formation of new tissue is stimulated and overall recovery is improved.

Sandness *et al.*, 2019 used a fish skin acellular matrix (Kerecis™ Omega3 Burn), for the treatment of second-degree burns in a dog. After a week monitor a well-formed healthy granulation tissue in the wound bed. They note that the graft quickly integrates without complications.

All these studies support the use of acellular matrices from fish skin as an effective treatment of various types of wounds in animals. The ability of these matrices to promote wound healing, reduce pain and inflammation, and improve overall recovery makes them promising alternatives to traditional wound dressings. Additional research is necessary to comprehensively elucidate the mechanisms of action of these matrices and establish the most suitable conditions for their application in clinical settings, ensuring their efficacy and safety in promoting wound healing and tissue regeneration.

Conclusion

In recent years, the use of a decellularized matrix of fish skin, created by tissue engineering, has found application in the treatment of extensive skin defects. Extracted from the skin of selected fish species, such as *Nile tilapia (Oreochromis niloticus)* or *North Atlantic cod (Gadus morhua)* (Kerecis ® Omega3, Kerecis, Iceland), these grafts undergo a comprehensive decellularization process to remove cellular components while preserving the structure of the extracellular matrix and bioactive molecules (Crapo *et al.*, 2011; Balestrini *et al.*, 2015; Sakina *et al.*, 2020; Luze *et al.*, 2022).

ECM contains growth factors and collagen fibers necessary for protein deposition in the process of angiogenesis and epithelialization (Schallberger *et al.*, 2008; Balsa *et al.*, 2015). *ECM* confers elasticity and resilience to recipient skin tissue, as it is mainly composed of *collagen* species *(I and III), glycosaminoglycans, proteoglycans, adhesive glycoproteins (fibronectin, laminin, and vitronectin),* and *elastic fibers* (Theocharis *et al.*, 2019; Pfisterer *et al.*, 2021).

The preservation of these components after cell removal is essential to ensure cell proliferation, differentiation, and tissue repair (Oliveira *et al.*, 2010).

The preserved *ECM* in decellularized skeletons has structural properties that can provide an appropriate environment for cell migration, growth, and proliferation in the injured area, improving the healing process, wound closure in extensive skin defects, and reducing cicatrix formation (Brigido *et al.*, 2004; Tabata *et al.*, 2008; Brown *et al.*, 2014).

Compared to acellular fish skin grafts, bovine or pig grafts require a longer preservation process, which often denatures the matrices and removes much of the collagen, which can aid in wound healing (Magnusson *et al.*, 2017; Dorweiler *et al.*, 2018).

Depending on the method of preparation of the acellular fish skin graft, it is possible that it can be stored at room temperature for up to three years (Alam *et al.*, 2019), which makes it more affordable product, especially in resource-poor areas, such as war zones or low- to middle-income countries (Lima *et al.*, 2020).

The mechanism of action of acellular skin grafts from fish includes their ability to create a moist environment, provide structural support, promote cell migration and proliferation, exhibit antibacterial properties, and reduce pain and inflammation. One of the most important requirements and conditions for the replacement effect of the graft is its porosity, which allows fibroblasts, fibrocytes and keratinocytes to pass through these small openings in order to build a new network in the damaged tissue (Dorweiler *et al.*, 2018).

In a study done by Magnusson *et al.*, 2017, it was found that there were 1.7 holes per 100 μm in the *dHACM (dehydrated human amnion/chorionic membrane)* allograft compared to 16.7 holes in acellular grafts from fish skin.

The structure of the acellular skin graft from fish is highly porous with a high degree of hydrophilicity, which contributes to creating a moist environment in the wound and facilitates the passage of nutrients and oxygen through cell membranes (Chen *et al.*, 2021; Biazar *et al.*, 2022; Ali *et al.*, 2023).

Fish skin acellular grafts are a promising new alternative to support the healing process in wounds and skin burn cases (Hu *et al.*, 2017; Luze *et al.*, 2022). Although there is still no robust evaluation supporting the benefits of using the acellular fish skin graft, its positive qualities make it a preferred material in the treatment of wounds with large skin defects (Hu *et al.*, 2017; Luze *et al.*, 2022).

Fish skin has excellent antimicrobial properties against pathogens and can be an ideal alternative for recipient skin regeneration. The benefits of applying this type of grafts are a longer shelf life, relatively easy processing and method of administration, healing and antibacterial properties

(Lands *et al.*, 2005; Magnusson *et al.*, 2017), lower risk of disease transmission and significantly lower cost compared to other xenografts (Kirsner *et al.*, 2020).

References

1. Alam K., Jeffery L., (2019). *Acellular fish skin grafts for management of split thickness donor sites and partial thickness burns: a case series.* Mil. Med., 184 (Supplement 1), p. 16–20, 2019, 184:16–20.
2. Ali E., Biazar E., Ebrahimi M., Keshel S., Kheilnezhad S., Farzaneh L., (2023). *Acellular fish skin for wound healing*. Int. Wound J., 20(7): 2924–2941, doi: 10.1111/iwj.14158, PMCID: PMC10410342, PMID: 36924081.
3. Alves N. et al., (2018). *Study of tensiometric properties, microbiological and collagen content in Nile tilapia skin submitted to different sterilization methods*. Cell Tissue Bank. 19(3), 373–382.
4. Arham M., Uzma D., Asim M., Muhammad I., Khizer K., Amber F., Abdul M., (2022). *Nile Tilapia Skin as Dermal Wound Healing Promoter in Cats.* Department of Clinical Sciences, College of Veterinary and Animal Sciences (UVAS Sub-Campus), Jhang, Pakistan, doi10.18805/IJAR.BF-1602.
5. Aristotelis K., Lomana A., Karvelsson S., Heijink., Stone R., Giera M., Rolfs O., (2021). *Prostaglandins, Leukotrienes and Essential Fatty Acids. Lipid mediator profiles of burn wound healing: Acellular cod fish skin grafts promote the formation of EPA and DHA derived lipid mediators following seven days of treatment*. Volume 175, 102358.
6. Baldursson B., Kjartansson H., Konradsdottir F., Gudnason P., Sigurjonsson G., Lund S., (2015). *Healing rate and autoimmune safety of full-thickness wounds treated with fish skin acellular dermal matrix versus porcine small-intestine submucosa: a noninferiority study*. Int. J. Low Extrem Wounds, 14, 37–43.
7. Balestrini L., Gard L., Liu A., Leiby L., Schwan J., Kunkemoeller B., Calle A., Sivarapatna A., Lin T., Dimitrievska S. et al., (2015). *Production of decellularized porcine lung scaffolds for use in tissue engineering*. Integr. Biol., 7, 1598–1610.
8. Balsa M., Culp N., (2015). *Wound Care*. Vet. Clin. North Am. Small Anim. Pract., 45, 1049–1065.
9. Biazar E., Heidari Keshel, Rezaei Tavirani, Kamalvand M., (2022). *Healing effect of acellular fish skin with plasma rich in growth factor on full-thickness skin defects.* Int Wound J., 19(8): 2154–2162.
10. Boccafoschi F., Botta M., Fusaro L., Copes F., Ramella M., Cannas M., (2017). *Decellularized biological matrices: an interesting approach for cardiovascular tissue repair and regeneration*. J Tissue Eng Regen Med., 11(5): 1648–1657.
11. Boháč M., Danišovič Ľ., Koller J., (2018). *What happens to an acellular dermal matrix after implantation in the human body? A histological and electron microscopic study*. Eur. J. Histochem, 62(1): 2873.
12. Brigido A., Boc F., Lopez C., (2004). *Effective management of major lower extremity wounds using an acellular regenerative tissue matrix: A pilot study*. Orthopedics, 27, S145–S149.
13. Brown P., (2001). *Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease: Background, Evolution, and Current Concerns*. Emerg. Infect. Dis.,7:6–16, doi: 10.3201/eid0701.010102.
14. Brown N., Badylak F., (2014). *Extracellular matrix as an inductive scaffold for functional tissue reconstruction*. Transl. Res., 163, 268–285.
15. Burk J., Erbe I., Berner D., et al., (2014). *Freeze-thaw cycles enhance decellularization of large tendons*. Tissue Eng Part C Methods., 20(4): 276–284.
16. Bush K., Gertzman A., (2016). *Process development and manufacturing of human and animal acellular dermal matrices*. In: Albanna MZ, Holmes JH, eds. Skin Tissue Engineering and Regenerative Medicine. Elsevier, 83–103.

17. Campbell G., (2015). *Moist wound healing in dogs and cats: using MRDs to improve care*. Today 's Vet Pract, https://todaysveterinarypractice.com/moist-wound-healing-the-new-standard-of-care/.

18. Carlsson H., Gronet M., Rose F., et al., (2016). *Clinical applications of acellular dermal matrices in reconstructive surgery*. In: Albanna MZ, Holmes JH, eds. Skin Tissue Engineering and Regenerative Medicine. Elsevier, 109–124.

19. Centanni M., Straseski A., Wicks A., Hank A., Rasmussen A., Lokuta A., Schurr J., Foster N., Faucher D., Caruso M., (2011). *StrataGraft skin substitute is well-tolerated and is not acutely immunogenic in patients with traumatic wounds: Results from a prospective, randomized, controlled dose escalation trial*. Ann. Surg., 253, 683.

20. Chen H., Yin B., Hu B., Zhang B., Liu J., Jing Y., Fan Z., Tian Y., Wei X., Zhang W., (2021). *Acellular fish skin enhances wound healing by promoting angiogenesis and collagen deposition*. Biomedical Materials, 16(4): 045011.

21. Chiu T. & Burd A., (2005). *"Xenograft" dressing in the treatment of burns*. Clin. Dermatol. 23(4), 419–423.

22. Crapo M., Gilbert W., Badylak F., (2011). *An overview of tissue and whole organ decellularization processes*. Biomaterials, 32, 3233–3243.

23. Dorweiler B., Trinh T., Dünschede F., et al., (2018). *The marine Omega3 wound matrix for treatment of complicated wounds*. Gefasschirurgie, 23(2): 46–55.

24. Dreifke B., Jayasuriya A., Jayasuriya C., (2015). *Current wound healing procedures and potential care*. Mater. Sci. Eng. C. Mater. Biol. Appl., 48, 651–662.

25. Dussoyer M., Michopoulou A., Rousselle P., (2020). *Decellularized scaffolds for skin repair and regeneration*. Appl Sci (Basel), 10(10): 3435.

26. Esmaeili E., Biazar E., Ebrahimi M., Heidari K., Bahareh K., Farzaneh S., (2023). *Acellular fish skin for wound healing*. Int Wound J., 20(7): 2924–2941, doi: 10.1111/iwj.14158.

27. Fertram S., Baldursson T., Kjartansson H., Baldvin B., Kirsner R., Flanagan M., Margolis D., (2021). *Kerecis® Omega3 VET TM Skin Structure, Kerecis graft Management Guide VET*. KM-21-0145.1 Copyright © Kerecis 2021.

28. García E., Abduldaiem Y., Sawadkar P., Kyriakidis C., Lali F., Greco V., (2020). *Decellularised scaffolds: just a framework? Current knowledge and future directions*. J Tissue Eng.,11: 1–18.

29. Hermans E. et al., (2014). *Porcine xenografts vs. (cryopreserved) allografts in the management of partial thickness burns: Is there a clinical difference?* Burns, 40, 408–415.

30. Ibrahim A., Hassan D., Kelany N., Kotb S., Soliman M., (2020). *Validation of three different sterilization methods of Tilapia skin dressing: Impact on microbiological enumeration and collagen content*. Front. Vet. Sci. 7, 597751.

31. Isidan A., Liu S., Li P., et al., (2019). *Decellularization methods for developing porcine corneal xenografts and future perspectives*. Xenotransplantation., 26(6): e12564.

32. Jeremias E., Bedaiwy A., Gurunluoglu R., Biscotti V., Siemionow M., Falcone T., (2002). *Heterotopic autotransplantation of the ovary with microvascular anastomosis: A novel surgical technique*. Fertil. Steril., 77, 1278–1282.

33. Kamalvand M., Biazar E., Joupari M., Montazer F., Tavirani M., Keshel S., (2021). *Design of a decellularized fish skin as a biological scaffold for skin tissue regeneration*. Tissue and Cell, Volume 71, 101509, https://doi.org/10.1016/j.tice.2021.101509.

34. Khajavi M., Hajimoradloo A., Zandi M., Pezeshki M., Bonakdar S., Zamani A., (2021). *Fish cartilage: a promising source of biomaterial for biological scaffold fabrication in cartilage tissue engineering*. J Biomed Mater Res A.,109(9): 1737–1750.

35. Kherkar T. et al., (2024). *Comparative study of platelet rich plasma therapy and tilapia fish skin biological bandages for wound healing in cats*. International Journal of Veterinary Sciences and Animal Husbandry, VET 2024; 9(4): 38–43.

36. Kirsner R., Margolis J., Baldursson T. et al., (2020). *Fish skin grafts compared to human amnion/chorion membrane allografts: a double-blind, prospective, randomized clinical trial of acute wound healing*. Wound Repair Regen 28(1): 75–80. https://doi.org/10.1111%2Fwrr.12761.

37. Kjartansson H., Olafsson H., Karason S, et al., (2015). *Use of acellular fish skin for dura repair in an ovine model: a pilot study*. Open J Mod Neurosurg, 5(04):124–136.

38. Lau S., Hassanbhai A., Wen F., et al., (2019). *Evaluation of decellularized tilapia skin as a tissue engineering scaffold*. J Tissue Eng Regen Med., 13(10): 1779-1791.

39. Li D., Sun Q., Wang T., et al., (2021). *Evaluation of a novel tilapia-skin acellular dermis matrix rationally processed for enhanced wound healing*. Mater Sci Eng C Mater Biol Appl., 127: 112202.

40. Luze H., Nischwitz P., Smolle C. et al., (2022). *The use of acellular fish skin grafts in burn wound management- a systematic review*. Medicina (Kaunas) 58(7): 912. https://doi.org/10.3390%2Fmedicina58070912.

41. Ma P., Wang Y., Li B., Hou H., (2020). *Cross-linking effects of carbodiimide, oxidized chitosan oligosaccharide and glutaraldehyde on acellular dermal matrix of basa fish (Pangasius bocourti)*. Int J Biol Macromol., 164: 677-686.

42. Magnusson S., Baldursson B., Kjartansson H. et al., (2017). *Regenerative and antibacterial properties of acellular fish skin grafts and human amnion/chorion membrane: Implications for tissue preservation in combat casualty care*. Mil Med 182(S1):383–8. https://doi.org/10.7205/milmed-d-16-00142.

43. Magnusson S., Kjartansson H., Baldursson B., et al., (2018). *Acellular fish skin grafts and pig urinary bladder matrix assessed in the collagen-induced arthritis mouse model*. Int J Low Extrem Wounds.,17(4):275–281.

44. Mei F., Liu J., Wu J., Duan. Z., Chen. M., Meng. K., Chen. S., Shen. X., Xia. G., Zhao. J., chemistry f., (2020). *Collagen peptides isolated from salmo salar and tilapia nilotica skin accelerate wound healing by altering cutaneous microbiome colonization via upregulated NOD2 and BD14*. 68(6): 1621–1633.

45. Meq V. et al., (2021). *Nile tilapia skin (Oreochromis niloticus) for burn treatment: Ultrastructural analysis and quantitative assessment of collagen*. Acta Histochem. 123(6), 151762–151762.

46. Miranda C., Leonel L., Cañada R., et al., (2021). *Effects of chemical and physical methods on decellularization of murine skeletal muscles*. An Acad. Bras. Cienc., 93(2), e20190942.

47. Mohiuddin A., Campbell B., Poche N., et al., (2020). *Decellularized adipose tissue: biochemical composition, in vivo analysis and potential clinical applications.* Adv. Exp. Med. Biol., 1212: 57–70.

48. Nyame T., Chiang A., Orgill P., (2014). *Clinical Applications of Skin Substitutes*. Surg. Clin., 94, 839–850.

49. Oliveira S., Nascimento M., Crusoé M., Bahia P., Rosa P., (2010). *Avanços e aplicações da bioengenharia tecidual*. Rev. Ciências Médicas e Biológicas, 9, 28.

50. Pfisterer K., Shaw E., Symmank D., Weninger W., (2021). *The Extracellular Matrix in Skin Inflammation and Infection*. Front. Cell Dev. Biol., 9, 1578.

51. Prpich Y., Santamaria C., Simcock O., Wong K., Nimmo S., Kuntz A., (2012). *Second intention healing after wide local excision of soft tissue sarcomas in the distal aspects of the limbs in dogs: 31 cases (2005–2012)*. J Am Vet Med Assoc, 244(2):187–194.

52. Rakers S., Gebert M., Uppalapati S., et al., (2010). *'Fish matters': the relevance of fish skin biology to investigative dermatology*. Exp Dermatol.,19(4): 313-–324.

53. Rangaraj A., Harding K. & Leaper D., (2011). *Role of collagen in wound management*. Wounds uk 7(2), 54–63.

54. Sakina R., Llucià-Valldeperas A., Henriques A., Harichandan A., Gelsomino S., Wieringa P., Mota C., Moroni L., (2020). *Decellularization of porcine heart tissue to obtain extracellular matrix based hydrogels*. Methods Cell Biol., 157, 3–21.

55. Sandness B., (2019). *Use of an acellular Fish skin graft rich in Omega-3(Kerecis™ Omega3 burn) in canine burn wound*. Surgery resident for the MSU Veterinary Medicine Center, https://cvm.msu.edu/vetschool-tails/kerecis-graft-canine-burn-wound.

56. Schallberger P., Stanley J., Hauptman G., Steficek A., (2008). *Effect of porcine small intestinal submucosa on acute full-thickness wounds in dogs*. Vet. Surg., 37, 515–524.

57. Shores T., Brandacher G., Lee A., (2015). *Hand and upper extremity transplantation: An update of outcomes in the worldwide experience*. Plast. Reconstr. Surg., 135, 351e–360e.

58. Shores T., Gabriel A., Gupta S., (2007). *Skin substitutes and alternatives: a review*. Adv Skin Wound Care;20: 493–508.

59. Sitje S., Grøndahl C., Sørensen A., (2018). *Innovation Clinical. fish-derived wound product for cutaneous wounds*. Wounds Int., 9(4): p. 6, 9: 6.

60. Stone R II, Saathoff EC, Larson DA, et al. *Accelerated wound closure of deep partial thickness burns with acellular fish skin graft*. Int J Mol Sci. 2021;22(4):1590.

61. Sun K., Siprashvili Z., Khavari A., (2014). *Advances in skin grafting and treatment of cutaneous wounds*. Science, 346, 941–945.

62. Tang J. & Saito T., (2015). *Biocompatibility of novel type I collagen purified from tilapia fish scale: an in vitro comparative study*. BioMed Res. Int. https://doi.org/10.1155/2015/139476.

63. Tabata Y. et al., (2008). *Current status of regenerative medical therapy based on drug delivery technology*. Reprod. Biomed. Online, 16, 70–80.

64. Theocharis D., Manou D., Karamanos K., (2019). *The extracellular matrix as a multitasking player in disease*. FEBS J., 286, 2830–2869.

65. Thornton F., Gosman A., (2004). *Skin grafts and skin substitutes*. Sel. Read. Plast. Surg., 10, 1–24.

66. Wang J., Chen J., Ran Y., et al., (2020). *Utility of air bladder-derived nanostructured ECM for tissue regeneration*. Front. Bioeng. Biotechnol.,8: 553–529.

67. Wu W., Zhou Z., Sun G., Liu Y., Zhang A., Chen X., (2021*). Construction and characterization of degradable fish scales for enhancing cellular adhesion and potential using as tissue engineering scaffolds*. Mater Sci Eng C Mater Biol Appl., 122:111919.

68. Zeller A., Meier C., (2024). *Wundtherapie mit medizinischer Fischhaut*. Kavallo, Nr. 3/2024, 114. Jahrgang, CHF 10– Das Schweizer Pferdemagazin. 9, 772296–624000.

69. Zubin E., Conti V., Leonardi F., Zanichelli S., Ramoni R., Grolli S., (2015). *Regenerative therapy for the management of a large skin wound in a dog*. Clin. Case Rep., 3, 598.