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The Use of Acellular Fish Skin Grafts in Diabetic Foot Ulcers Management – a Systematic Review

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ABSTRACT

Background: Diabetic foot ulcer (DFU) healing and management continue to be a major challenge for patients and healthcare providers, resulting in a considerable socio-economic burden. Lower-limb amputation is a severe clinical condition of DFU due to the presence of a chronic unresponsive diabetic foot ulcer with a high risk of infection, which raises morbidity and mortality rates. Rapid wound healing is necessary to prevent amputation. A recent advance in the development of applicable xenografts was acellular fish skin (AFS) grafts harvested from the North Atlantic cod (Gadus morhua) act as skin substitute, have a substantial lipid profile, primarily composed of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have been shown to result in faster wound epithelialization, provide barrier protection against bacteria and alter the inflammatory profile of wounds. Due to these beneficial wound-healing properties, acellular fish skin might represent an effective treatment approach in chronic diabetic foot ulcer management.

Methods: A systematic review of the literature up to July 2023 was conducted using the electronic databases PubMed, Google Scholar, and ScienceDirect. Titles and abstracts were screened for the following key terms (variably combined): "fish skin", "fish skin grafts", "acellular fish skin grafts", "Omega3 Wound matrix", "Diabetic foot ulcer", "Chronic ulcer", "wound healing".

Results: The present study includes 12 trials that examined the effects of acellular fish skin grafts in diabetic foot ulcers. Compared to the standard of care, the use of acellular fish skin has been shown to accelerate wound healing resulting in reduced risk of amputation, reduced pain, reduced treatment-related costs, fewer dressing changes and improved quality of life.

Conclusions: Acellular fish skin xenografts may represent an effective, low-cost treatment of chronic diabetic foot ulcers.

KEYWORDS: Acellular fish skin; Fish skin grafts; Diabetic foot ulcer

ARTICLE DETAILS

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Highlight

- Lower-limb amputation is a severe clinical condition due to a chronic unresponsive DFU with a high risk of infection.
- Some therapeutic strategies are being employed for wound healing, but there are still various limitations and disadvantages.
- AFS converts the wound from an inflammatory to a healing stage, potentially promoting a normal DFU healing process.
- Using a systematic review, 12 studies reported AFS faster DFU healing, reduced pain and decreased dressing changes.

AFS xenografts may represent an effective, low-cost alternative for treating Diabetic Foot Ulcers.**1.**

INTRODUCTION

Diabetes mellitus (DM) continues to be a global healthcare primary concern due to its substantial socio-economic burden. [1] In 2021, the global prevalence of diabetes was estimated to be 10.5% (536.6 million people), and it is projected to reach 12.2% (783,2 million) by 2045. [2] It has been estimated that between 19 and 34% of diabetic patients will develop foot ulcers (DFU) in their lifetime.[3] The mortality rates associated with the onset of a DFU are estimated to be 5% in the first year and 42% in the next five years. [4]

Diabetic Foot Ulcer (DFU) is among the most severe complications of diabetes mellitus.[5] It is associated with significant morbidity and mortality, and if not recognized and treated promptly, it can result in hospitalization and amputation of the lower extremities.[6] Chronic, nonresponsive DFUs are notoriously difficult to treat, often requiring a strict and costly multitherapy approach.[7] Approximately 20% of people who develop DFU will require lower-extremity amputation. [8] 70% of individuals with a DFU-related amputation will die within five years [9] The total cost of DFU treatment in the United States is estimated to be at least $38 billion. [9,10] The average cost per patient per year was $3368 for ulcer-only treatment, $10468 for minor amputation, and $30131 for major amputation. [11]

Some therapeutic strategies being employed for wound healing based on wound types and intrinsic regenerative capacity include modern wound dressings (hydrogels dressings)[12], topical drug and growth factor delivery [13], hyperbaric oxygen therapy [14], auto/allograft and xenograft [15], cell-based therapy and engineered skin graft [16,17], vacuum-assisted closure[18], electrotherapy[19], negative-pressure therapy [20], ultrasound [21], and exosome-based approach [22] Despite the fact that this treatment modality is well studied and contributes to wound healing, there are still various limitations and disadvantages, such as low efficacy, the need for frequent dress changes, expensive costs, limited availability in some areas or it is an invasive therapy. Therefore, the availability of advanced therapies is crucial to improve healing rates, reduce the risk of amputation, improve patient outcomes, decrease treatment costs and also non-invasive. [10,23]

Skin grafts offer a promising and effective solution by acting as a temporary barrier as well as providing anti-microbial properties. Skin grafts show a capacity to promote healing effectively compared to traditional wound dressings [24] Many non-allogeneic – non-cellular tissue-based therapies exist, demonstrating key characteristics promoting wound healing. However, none have achieved all characteristics of an ideal skin replacement material. [24] Autografts require the construction of a new wound on a different area of the body, which increases the risk of surgical complications or morbidity. [24] A retrospective study showed diabetic patients who underwent autograft experienced a significantly higher risk of delayed healing time and postoperative complication/ infection and, hence, are more likely to require revisional surgery.[25] Allografts using full-thickness cellular or tissue-based products (CTPs) carry a risk for graft rejection due to the unfortunate potential for the host's immune system to identify the donor skin as foreign and proceed to attack it; although most modern-day human allograft material is made from an acellular dermal matrix that is designed not be recognized by the host's immune system.[24] Xenografts derived from mammalian sources carry the risk of autoimmune response, the risk of transmitting viral diseases requiring these grafts to undergo expensive processing with powerful detergents to decrease that risk and potential cultural or religious issues that may prohibit the use of porcine or bovine products in many countries.[24] [26]

Cost-effectiveness, availability, long-term functionality, conformance to irregular wounds, deep wound applications, low immunogenic reaction, and high bioactivity are highly valued qualities of skin replacement materials. Few materials meet all of these characteristics, indicating that new procedures and materials must be developed for skin grafting technology. Acellular fish skin grafts are a possible candidate for filling this demand gap.[24]

A recent advance in the development of applicable xenografts was acellular fish skin (AFS) grafts harvested from the North Atlantic cod (*Gadus morhua*) have a substantial lipid profile, primarily composed of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have been shown to result in faster wound epithelialization, provide barrier protection against bacteria and alter the inflammatory profile of wounds. [24]

Due to these beneficial wound healing properties, Omega-3-rich AFS may be used in a broad spectrum of applications: Recent studies report good clinical outcomes after application in, e.g., burn wound management [27], calciphylaxis wounds[28], iatrogenic calcinosis cutis [29] or even for neo vaginoplasty in patients with Mayer-Rokitansky-Küster-Hauser syndrome[30]. AFS may also present an effective treatment option in diabetic foot ulcers since studies indicate accelerated wound healing, pain reduction, and decrease in necessary dressing changes as well as treatment-related costs [7]

2. METHODS

This systematic review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting the events evaluated by interventions and healthcare behaviours. Population, intervention, control, and outcome (PICO) questions used in this systematic review were: P (population): Diabetic foot ulcer patient, I (intervention): the use of Acellular fish skin grafts, C (comparison/ control): without or with other treatment and O (outcome): Wound healing / Wound Area Reduction.

2.1. Aim construct

The review aims to summarise the evidence published in peer-reviewed journals on the use of AFS in managing diabetic foot ulcers.

2.2 Eligibility criteria

We included case reports, retrospective studies, prospective studies, cohort studies, randomized controlled trials (RCTs) using only acellular fish skin grafts, and comparisons of acellular fish skin grafts with other treatments of diabetic foot ulcer. Subjects included all patients with Diabetic Foot Ulcer (DFU). Moreover, we only included studies written in English. The exclusion criteria were repeated literature, the subject did not have a diabetic ulcer, the other treatment for diabetes ulcer, fish omega 3 oral supplementations, inability to extract outcome data, animal or cell experimental, and the article that was not written in the English language.

2.3. Search strategy

We reviewed the medical literature to identify all studies investigating the use of Acellular fish skin grafts in diabetic foot ulcers. A systematic search of several international databases, including PubMed, Google Scholar, and ScienceDirect, published until July 2023 was conducted. The keywords used in the search strategy are shown in Table 1

.**Table 1. Search Strategy**

DATABASE	SEARCH STRATEGY	HITS
PubMed	(Acellular Fish skin Grafts OR Fish skin graft OR omega-3 wound matrix) AND (Diabetic foot ulcer OR Chronic ulcer)	15
ScienceDirect	("Acellular Fish skin Grafts" OR "Fish skin graft" OR "omega-3 wound matrix") AND (Diabetic ulcer) AND (wound healing)	7
Google Scholar	("Acellular Fish skin Grafts" OR "Fish skin grafts" OR "omega-3 wound matrix")AND(Diabetic foot ulcer)AND(wound healing)	113

2.4. Screening and study selection

Findings from the search string were transferred to Covidence (www.covidence.org (accessed July 10, 2023)), where duplicates were removed. In Covidence, two authors independently screened and identified the studies (JN, MM). Any disagreement concerning the studies' eligibility was resolved through discussion until a consensus was reached. Studies that met the inclusion criteria were retrieved for a full-text screening by two authors (JN, MM), and the final inclusion was discussed with the third author (AL).

2.5. Data extraction and data items

Prior to data extraction, a customized table was developed. One author (JN) extracted the data, including bibliographic information, study goals, study design and trial phase, inclusion and exclusion criteria. The data were then discussed with and validated by a second author (MM) and a third author (AL).

2.6. Quality assessment

To ensure comparability, The Joanna Briggs Institute (JBI) critical appraisal checklist was applied to determine the quality of the studies retrieved and ensure the inclusion of comparable, valid and relevant evidence.

2.7. Outcome data

Studies were eligible for inclusion if they reported any outcome measures. Primary Outcome :

- Wound Area Reduction Time.
- Pain reduction.
- The number of dressing changes.
- Risk of amputations.
- Adverse event
- Disease transmissions
- Autoimmune reactions
- Treatment-related cost.

3. RESULT

3.1 Study selection

A total of 135 studies were initially identified. After removing duplicates, 120 studies remained, of which 90 were excluded based on title or abstract. The remaining 30 studies were retrieved for full-text reading, of which 12 studies met the eligibility criteria as seen in Figure 1.

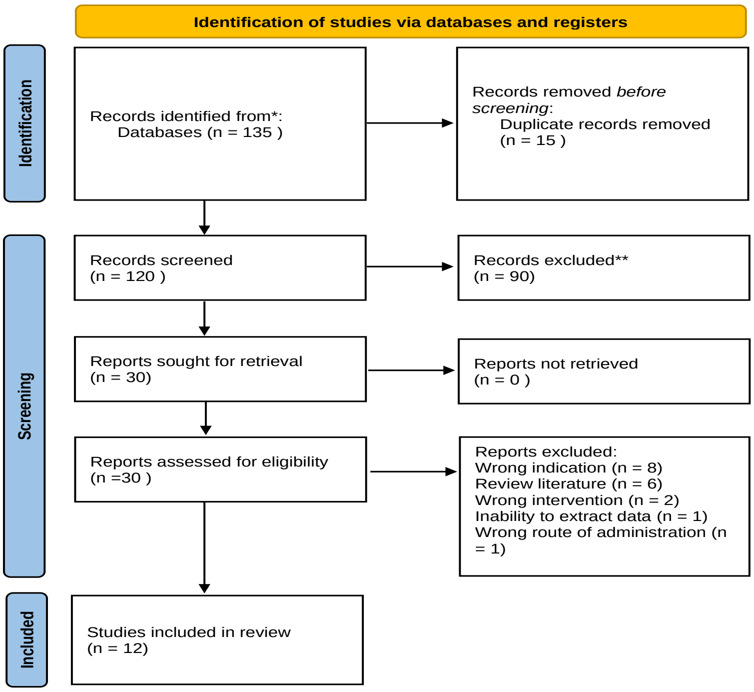

Figure 1. PRISMA 2020 Flow Diagram showing the different phases in searching for relevant publications to Assist the use of fish skin grafts on Diabetic foot ulcers.

3.2. Study characteristics

The included studies were published between 2016 and 2023 and originated from US, UK, Germany, Swiss and France. Study details are depicted in Table 2.

NoP: Number of participants; NR: Not Reported; F: female; M: Male; AFS: Acellular Fish Skin Grafts. DFU: Diabetic foot ulcer.
Table 2. Participants Characteristics.

3.3. Literature review

This systematic review included 12 publications to evaluate the use of acellular fish skin in Diabetic Foot Ulcers (DFU). All trials included were published in English. Study types were defined as follows: a case report (*n* =2), clinical cohort study (n=5), Retrospective cohort study (n=2), and Randomized controlled study (n=3). Study details are depicted in Table 3 and Figure 2.

AUTHORS	STUDY TYPE	STUDY COHORT	RESULTS	CONCLUSIONS
Trinh et al. (2016)	Prospective Evaluation.	5 patients with diabetes mellitus and complicated wounds in the lower limb with exposed bony segments were treated with AFS.	The thigh wound took 26 weeks to achieve wound closure, forefoot wound healed from 13 to 41 weeks. The wound area decreased by 50% within the first third of treatment. Pain reduced within the first 2 weeks.	Fish Skin Grafts represent a viable treatment option in complicated wounds in the lower limb of diabetic patients to circumvent an otherwise necessary proximalization of amputation level.
Yang et al. (2016)	Prospective Evaluation.	18 patients with at least 1 "hard-to-heal" criteria underwent application of the AFS for 5 sequential weeks.	A 40% decrease in wound surface area and a 48% decrease in wound depth were seen with 5 weekly applications of the fish skin graft. (P<0,05) Complete closure was seen in 3 of 18 patients.	The fish skin product appears to provide promise as an effective wound-closing adjunctive ECM.
Dorweiler et al (2018)	Multicenter Experience Report.	23 patients with 25 vascular and/or diabetes mellitus-complicated wounds	The time to heal varied between 9 and 41 weeks. The wound area decreased by 50% within the first fifth of	Fish Skin Grafts represented an effective treatment option in complicated wounds.

AUTHORS	YEAR	COUNTRY	PARTICIPANTS			WOUND CHARACTERISTICS	WOUND SIZE
			NOP	GENDER	AGE (YEARS)		
Trinh et al.	2016	Germany	5	Female:1 Male: 4	67 – 80 (mean age 75 ± 5)	Complicated wounds in the lower limb with exposed bony segments following amputation and impending risk for proximalization of amputation level.	Mean size 15 ± 10 cm2 (Range 6-29 cm2)
Yang et al.	2016	US	18	Female: 4 Male: 14	31 - 84 (mean age 55)	“Hard-to-heal” wound criteria: Full-thickness wound >20cm2 and/or had been present >52 weeks. Mean ulcer age 35 months.	Mean size 8,2 cm2 (Range 1,5 - 25,5 cm$^{2)}$
Dorweiler et al.	2018	Germany	23	Female: 7 Male: 16	50- 101 (mean age 71 ± 8)	The complicated wound on the lower limb following the previous amputation with exposed bone.	3 - 63 cm2
Winters et al.	2018	US	1	Male	56	2 chronic infected diabetic ulcerations of the left foot with exposed bone, cellulitis, osteomyelitis with active bleeding.	I = 4 x 3,5 x 0,3 cm II= 0,3 x 0,2 x 0,5 cm. Post amputation size 5 x3 x1 cm.
Woodrow et al.	2019	UK	8	Female: 3 Male: 5	55 – 88 (mean age 76,5)	< 3 months = 6 > 3 months = 2	Mean size 10,3 cm2 (Range 0.94 - 29,55 cm2)
Michael S et al.	2019	US	51	Female:13 Male: 38	45 - 88 (mean age 66)	Grade 1 = 43 Grade 2 = 11 Grade 3 = 4. Mean ulcer age 18 weeks (1-156 weeks)	Mean size 3.02 cm2 (Range 0,03 - 15 cm2)
Winters et al.	2020	US	55	M/L ratio 1,8	45-88	Grade 1 = 53 % Grade 2 = 37 % Grade 3 = 10 %	Mean size 3.7 cm2 (Range 0.05 – 26.4 cm2)
Lullove et al.	2021	US	49	NR	48,5 - 68	Nonresponsive DFU ulcer	NR
Lullove et al.	2022	US	94	NR	NR	Nonresponsive DFU ulcer	NR
Zehnder et al	2022	Swiss	51	NR	NR	Nonresponsive DFU ulcer	Mean size 3,51 cm2 (Range 0.12 -36.2 cm2).
Dardari et al.	2022	France	1	Male	78	DFU ulcer	NR
Lantis et al.	2023	US	102	Female: 32 Male: 70	55 to 65	Nonresponsive DFU ulcer	Mean size 2,4 cm2 (AFS), 3 cm2 (Control)

		and partially exposed bony segments were treated with the AFS	treatment. A reduction of analgesic intake was noted.	
Winters et al. (2018)	Case Report.	A 56-year-old man with haemophilia, diabetes, and hepatitis C had an infected forefoot ulcer with cellulitis and	At 14 weeks, the wound was healed until it fully healed at 1 year, 10 months. No bleeding episodes. No clinical infection.	Fish Skin Grafts promote quick hemostasis and healing response.

		osteomyelitis treated with AFS.		
Woodrow et al (2019)	Prospective Evaluation.	8 patients with DFU following forefoot surgery. AFS was applied weekly for six weeks.	At 6 weeks, the mean PAR was over 84.9% in six wounds of less than 3 months duration. And <42% in two wounds over 3 months duration. No infection, skin reactions, odor, discharge or itching.	Fish Skin Grafts show promise as an agent to accelerate wound healing.
Michael et al (2019)	Retrospective Study.	51 patients with a total of 58 DFUs were treated with AFS.	At 16 weeks, the mean PAR was 87.57%, and 35 wounds (60.34%) were fully healed. The mean time to achieve wound healing in the 35 fully healed wounds was 10 weeks.	Fish Skin Grafts promote wound healing in DFUs. A rapid increase in wound healing was observed during the initial 4 weeks.
Winters et al. (2020)	Retrospective Comparative Cohort Study.	59 DFUs treated with AFS compared to SOC.	Lower costs ($11 210 vs $15 075 per wound), more wound healing (83.2% vs 63.4%), fewer amputations (4.6% vs 6.9%), and a higher quality of life (0.676 vs 0.605) than the SOC.	Including Fish skin grafts in the SOC for DFU treatment has the potential to reduce cost while improving patient outcomes.
Lullove et al. (2021)	Multicenter, Blinded, Randomized Controlled Clinical Trial.	49 patients with Nonresponsive DFU were randomized to either receiving SOC alone or SOC plus AFS applied weekly for up to 12 weeks.	At 12 weeks, 67% of patients in the AFS healed compared with 32% in the SOC (P value =0 .0152). The mean PAR was 97,3% in the AFS and 76,8% in SOC	The application of AFS to previously nonresponsive DFUs resulted in significantly more fully healed wounds at 12 weeks than SOC alone.
Lullove et al. (2022)	Multicenter, Prospective, Randomized Controlled Trial.	94 patients with Nonresponsive DFU were randomized to either receiving SOC alone or SOC plus AFS applied weekly for up to 12 weeks.	At 12 weeks, 63% of patients in AFS healed compared with 31,3% in SOC. The mean PAR was 87.1% in the AFS and 54.0% in SOC (P =0.0039).	A clinically and statistically significant difference in healing was observed between patients treated with AFS and those treated with SOC.
Zehnder et al. (2022)	Outcome-Based Model.	51 patients were recruited (26 VLUs and 25 DFUs), and 42 wounds were randomized to either receiving SOC or AFS	The majority healed >50% sooner and as early as <10% of the time than was predicted. (> 25% improvement in wound area vs SOC).	Management with AFS resulted in faster healing wounds than SOC predicted, while SOC-treated wounds mostly followed model prediction.
Dardari et al. (2022)	Case Report.	76-year-old male patient with necrotic angiodermatitis in complicated diabetes patient.	Complete epithelization of the lesion after 10 weeks of treatment after the application of the 10th Fish Skin Grafts, reduces pain.	Fish skin grafts present a new therapeutic approach to necrotic angiodermatitis.
Lantis et al. (2023)	Prospective, Multicenter, Randomized Controlled Clinical Trial.	102 patients with a DFU were randomized to either receiving SOC alone or SOC plus AFS applied weekly for up to 12 weeks.	At 12 weeks, The mean PAR was 86.3% for AFS and 64.0% for SOC (P =0.0282). An annualized cost savings of $2818 compared with SOC.	Treatment of DFUs with AFS resulted in significantly more wounds healed and annualized cost saving of $2818 compared with SOC.

DFU: Diabetic foot ulcer; SOC: Standart Of Care; AFS: Acellular Fish Skin Grafts; PAR: Percent Area Reduction

Table 3. Literature review evaluating the use of Acellular fish skin in DFU

Figures 1a (above left) and Figure 1b (above second left). Initial visit – debridement and first application of Kerecis Omega3 Wound.

Figure 2. Wound at week 3.

Figure 3. Wound at week 5.

Figure 4. Wound at week 7.

Figure 5. Wound at week 18.

Figure 6. Wound at week 20.

Figure 7. Wound at week 32.

Figure 2. Fish skin grafts faster wound healing in the infected forefoot ulcer complicated by cellulitis and osteomyelitis with comorbid moderate factor VIII deficiency haemophilia, diabetes, and hepatitis C patient, as seen in a case report by Winters, 2018. [31] On week 0, the ulcer measured 5x3x1 cm and probed close to the distal cut ends of the metatarsal bones. On week 14, the wound healed until it fully healed at 1 year, 10 months.

This study focused primarily on the reduction of total wound area in DFU patients treated with AFS and the potential reduction of pain, dressing changes, and treatment costs. Since not all studies included control or comparison products, it is impossible to make general statements regarding the level of statistical significance. A two-tailed p-value of 0.05 was considered statistically significant when the control or other dressing groups were included in the analysis. Existing clinical trials support the use of AFS in treating DFU, as will be discussed in detail in the following chapters.

4. DISCUSSION

The patient with diabetes is at high risk for amputation due to a delayed wound-healing response.[32] In a healthy patient, wound healing progresses predictably through 4 stages: hemostasis, inflammation, proliferation, and remodelling. Diabetic wounds do not undergo these 3 typical stages and become stuck in the first stage (i.e., inflammatory stage), resulting in chronic inflammation and delayed wound healing.[33] Chronic wounds can develop infections, drug-resistant microbial biofilms, and unresponsive epidermal cells. [24] The fish skin graft appears to convert the wound from an inflammatory to a healing stage, potentially promoting a normal healing process and effectively allowing normal healing processes to occur. The technology provides a natural structure containing proteins and fats (including omega-3), allowing stem cells and cells to migrate in the fish skin graft, creating new dermal tissue to seal the wound.[34]

Acellular fish skin grafts (AFS), known as piscine xenografts, are a source for facilitating wound healing and are shown to possess antiviral and antibacterial properties as well as faster healing times.[24] AFS is a decellularized fish skin harvested from codfish in the North Atlantic (Gadus morhua) that has undergone a proprietary process to gently preserve the fish skin's original form and chemical composition. The resulting AFS closely resembles human skin structurally and chemically, making it a compatible alternative with the capability to encourage cellular proliferation in wound healing without hypersensitive reactions. [7,24]

The fish-skin graft is a skin substitute containing collagen, fibrin, proteoglycans, and glycosaminoglycans, with the potentially added benefits of bioactive lipid mediators. [26] Its lipid mediator is the most unique feature of AFS grafts, contributing to its effectiveness. [24] The lipid mediator is rich in omega-3 polyunsaturated fatty acids (ω-3 PUFA) with a large concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are associated with anti-microbial and anti-inflammatory properties.[24] Notably, ω-3 PUFA has been shown to affect the inflammatory stage of skin healing by altering the synthesis and activity of proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumour necrosis factor-α which play essential roles in signalling transduction throughout the wound healing process. ω-3 PUFA added protection against pathogenic microorganisms and reduced the activity of bacteria. [35] Numerous in-vitro studies have noted antibacterial and anti-biofilm effects of both EPA and

DHA, including activity against Staphylococcus pyogenes, both Methicillin-resistant Staphylococcus aureus (MRSA) and non-resistant S. aureus, Vibrio vulnificus, Candida albicans, and more. [36] Furthermore, topical application of DHA accelerated wound healing associated with reduced expression of IL-1β and increased expression of IL-6 and transforming growth factor β (TGF- β). This allows for a more regulated inflammatory response and possibly faster wound healing. [36]

The three-dimensional structure of the acellular fish skin graft is also believed to play a crucial part in effective wound healing, offering a favourable environment for cellular ingrowth and a barrier to bacterial invasion of Staphylococcus aureus. The structure is highly porous with approximately 16.7 large diameter pores per 100 m2 allowing for good adhesion to human skin and facilitating the passage of human fibroblasts[35] This porosity with natural structure offers a very large surface area that is exposed to the blood cells and platelets in a bleeding wound and potentially facilitating a rapid haemostatic response.[31]

AFS have very little risk of transmitting viral diseases to humans compared to mammalian sourced skin cellular or tissue-based product therapies (CTPs). For this reason, the manufacturing process of fish skin is far less harsh. [24] These gentler processes make it possible to preserve the structural integrity and molecular components of the skin, such as omega-3 unsaturated fatty acids, soluble collagen, elastin, laminin, and glycoproteins. [37] The milder manufacturing method of Atlantic cod skin also provides a more cost-effective and environmentally friendly option when compared to traditional xenografts.[35] The low cost of piscine CTPs is potentially useful in impoverished areas where advanced wound care is inaccessible. Additionally, acellular fish skin has an excellent shelf life, maintaining effectiveness for 3 years after manufacturing.[24] The minimally destructive and efficient manufacturing process, the low possibility for disease transfer and the extensive shelf life of acellular fish skin are excellent traits for a biomaterial, demonstrating highly marketable characteristics that are effective in a clinical setting [24].

The patent of Atlantic cod AFS graft is Kerecis® Omega3 which has been processed and sterilized for medical use, which available worldwide and was initially approved for the treatment of various wounds by the US Food and Drug Administration in 2013. [33] The Kerecis® Omega3 dressings were used in all 12 trials.

4.1 Wound Area Reduction

The total wound reduction time of chronic DFU wounds treated with AFS represents the main focus in trials published to date. Fish skin grafts can be a potential option for healing chronic DFU wounds, which have complications even with the bone exposed which don't even heal with other treatments. A case study by Winters et al. reported A 56-year-old male with an infected forefoot ulcer with cellulitis and osteomyelitis with comorbid moderate factor VIII deficiency haemophilia, diabetes, hepatitis C and high risk of further amputation, which did not heal within 2 years, successfully healed at 14 weeks application of 6 patches Acellular fish skin grafts combined with treating the underlying shortage of clotting factors, control infection and blood glucose. [31] Dardari et al. also reported a 76-year-old male patient with an ulcer on the right leg in complicated diabetes associated with arterial hypertension, dyslipidemia, and hyperuricemia. Complete epithelization of the lesion was seen at 10 weeks of treatment after the application of the 10th fish skin graft.[34]

This evidence is supported by a larger trial conducted by Trinh et al., Yang et al. and Dorweiler et al. trials with a total of 48 patients with complicated DFU wounds in the lower extremity with exposed bony segments and “hard-to-heal” wounds criteria treated with fish skin grafts. Trinh et al. and Dorweiller et al found a significant reduction of wound area of 50% within the first-third and first-fifth treatment duration. Similarly to them, Yang et al found 40% and 48% of wound surface area and wound depth had decreased at 5 weeks. Also at 5 weeks, Yang et al noted 3 of 18 wounds (16,67 %) healed. Trinh et al and Dorweiler et al. found the time to complete healing in the complicated DFU with a bony segment exposed between 9 weeks and 41 weeks depending on the severity and extent of the DFU wound.[26,38,39]

Woodrow et al. and Michael et al. focused on following 8 and 58 DFUs in order to assess the time to complete wound closure. Although there was no control to compare the results with, the study focused on the importance of rapid wound healing in the case of DFUs to find a treatment that minimizes the number of amputations seen in patients with treatment-resistant DFUs.[33,40] The results are in line with the three articles in this review that compared AFS grafts with SOC (collagen alginate dressings) in the treatment of DFUs. From 2021 to 2023, Lullove et al. and Lantis et al. performed a Randomized control trial. They found that using AFS grafts to treat DFUs resulted in faster healing and an increased number of wound closures compared to the SOC (Collagen alginate dressings). At 6 weeks, the mean Percent Area Reduction (PAR) was 69,3% to 72,8% on AFS compared to 44,2 % to 51,6% on SOC (Collagen alginate dressing). At 12 weeks, mean PAR was 86,3% to 97,3% on AFS compared to 54% to 76,8% on SOC (Collagen alginate dressing), and 56,9% to 67% of patients completely healed in the AFS arm compared to 31,3% to 32% in the SOC (Collagen alginate dressing). They found that 25.5% (1,8 times) more wounds healed with AFS compared to SOC (P-value = 0,0163). There is a statistically and clinically significant difference in more wounds healed in the use of AFS compared to SOC (Collagen Alginate dressing). [7,10,41] All the trial reports by Lullove et al. and Lantis et al. are similar to Zehnder et al. trial. They agree there is more than 25% improvement in wound areas treated with AFS compared to SOC. [7,10,41,42]

4.2 Pain reduction.

Management of chronic pain needs to address all of the unpleasant physical and psychological conditions that lead to pain.[43] The known effects of EPA and DHA include inflammatory modulation, skin barrier and anti-microbial enhancement, adjustments in perceived pain, and more. [44] Existing evidence to date reports promising effects of AFS in pain reduction. Trinh et al. reported a reduction level of local pain within the first two weeks of treatment and a reduction of analgetics intake was noted when the treatment with the Fish skin grafts was initiated. [38] Yang et al. assessed the visual pain scale of 1-10 and found a nonstatistically significant reduction in reported pain was noted.[26] Dorweiler et al. reported a reduction of analgesics intake was noted when the treatment with the Fish skin grafts was initiated. [39] Woodrow et al. assessed pain intensity using a linear visual analogue scale (VAS), and no patient reported increased pain.[40] Dardari et al. reported significant pain reduction in the patient. [34] The other study did not report any increase in pain. They agree using fish skin grafts reduces overall pain intensity and reduction of analgesic intake.

4.3 The number of dressing changes.

Fewer dressing changes may have been required due to good adherence of the biomaterial to the wound bed. [9] The fish-skin graft is available in sizes 3 cm x 3.5 cm, 3 cm x 7 cm, and 7 cm x 10 cm, and it is indicated for partial-thickness and full-thickness wounds and skin loss injuries as well as superficial and second-degree burns. The fish-skin graft was moistened with normal saline and applied directly to the wound. It was held in place with surgical adhesive and surgical strips. A secondary dressing that delivers ongoing moisture or is moisture-retentive was necessary. The fish-skin graft can be reapplied weekly and does not require the removal of the previously applied product since it is gradually resorbed and remodelled in the wound.[26] When compared to conventional treatment options, AFS led to fewer dressing changes, with the median number of applications was 6 for the AFS group and 17 for the SOC group. [7] Treatment with fish skin grafts reduces the number of dressing changes by more than 60%. The reduction of necessary dressing changes and, therefore, inpatient treatment days can be associated with a reduction of treatment-related costs and simultaneously increase the patients' comfort.

4.4 Risk of amputations.

Lullove et al. and Lantis et al. found a statistically and clinically significant difference in more wounds healed in the use of AFS compared to SOC (Collagen Alginate dressing).[7,10,41] Lantis et al. reported of the 102 participants with AFS or SOC, only 1 participant in the SOC arm underwent an amputation, none in the AFS arm. [7] Lullove et al. reported of the 94 participants treated with AFS or SOC, there was no amputation. [41] Woodrow et al. reported one amputation from the 8 DFUs' chronic wounds.[40] Winters et al. reported fewer amputations of 4,6% in Fish skin grafts and 6,9% in SOC.[9] All authors agree that Fish Skin Grafts represented an effective treatment, promoting wound healing in DFU and faster healing compared to SOC. It is indicated that AFS reduces the risk of amputation.

4.5 Adverse event

Lantis et al. reported that of the 102 participants, there was one infection in the AFS arm and five infections (1 necessitating amputation) in the control arm. [7] Lullove et al. reported of the 94 participants, there was no infection.[41] Woodrow et al. reported no patient in the trial reported skin irritation, odour and significant new infection[40] All the authors agree there is no significant adverse event in the treatment of AFS. The incidence of infection in the use of AFS is less than SOC.

4.6 Disease transmissions

The fish skin is minimally processed compared to CTPs from mammalian sources since there is no disease transmission risk from Atlantic cod to humans. [31] Similar to the data, no studies reported any disease transmissions.

4.7 Autoimmune reactions

Fish skin is homologous to human skin, sharing structural and functional anatomy of the epidermis, dermis and subcutis, making it a compatible alternative with the capability to encourage cellular proliferation in wound healing without hypersensitive reactions. [24] Dorweiler et al. report there are no autoimmune reactions.[39] There are no studies that report any autoimmune reactions.

4.8 Treatment-related cost.

One study showed costs for patients with DFUs to be more than triple that of patients with DM but without ulcers. In addition to direct costs, a substantial indirect cost is incurred due to loss of productivity, disability, and premature mortality.[9] Winters et al. conducted the study using a Markov model with a 1-year time horizon to compare utility and cost from the payer's perspective of SOC alone versus treatment with fish skin grafts. The main result was that fish skin therapy was more effective and incurred a lower total cost ($11 210 in Fish skin grafts vs $15 075 per wound in SOC).[9] A sensitivity analysis demonstrated that the fish skin therapy was 93.6% likely to be cost-effective and 71.4% likely to be cheaper compared with SOC. [9] Lantis et al. also reported an annualized cost savings of $2818 in AFS compared with SOC. [7]

5. LIMITATION

Due to the small number of study cohorts, the applicability of existing trials and data cannot be combined. In addition, the majority of studies on AFS are conducted by the same organization, and conclusions are limited geographically due to the limited availability of AFS. These findings may be applicable to treatment with Kerecis® Omega3, but additional clinical comparison trials with a significant number of participants are required to determine the full potential of this intriguing strategy. Our review is limited to publications obtained from PubMed, Google Scholar and ScienceDirect, and there is a small chance that we missed further studies.

6. CONCLUSION

The new strategy of AFS xenografts may represent an effective, low-cost alternative for the treatment of DFU, as evidence suggests faster wound healing, decreased pain and the number of dressing changes, reduced risk of amputation, low adverse events, no disease transmission, no immune reaction, and the treatment-related cost is less than the Standart of Care. Future large cohort studies are necessary to determine the full potential of this intriguing method.

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Conflicts of Interest

The authors declare no conflict of interest.

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