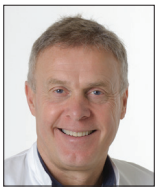
Clinical innovation: fish-derived wound product for cutaneous wounds





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Recent research suggests that wound care products derived from fish skin could improve cutaneous wound healing. A PubMed search and extended literature search identified 392 articles relating to such research. Evidence indicates that fish-derived scaffold materials are biocompatible, biodegradable and do not require frequent dressing change. It also provides properties that could accelerate wound healing, offer antibacterial and antiviral properties, and are not associated with disease transmission or unwanted immunological reactions.

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The prevalence of wounds is increasing annually and is strongly associated with increases in the older population and lifestyle issues including inactivity, poor dietary management and smoking (Margolis et al, 2002; Wild et al, 2004). Wounds impose a huge burden on patients and healthcare systems. Approximately DKK700 million (£83.4 million) is spent every year on the treatment of wounds in the Danish municipal sector and it is estimated this will increase by 31% by 2020 (Hjort and Gottrup, 2010). Wound care accounts for 1.6–1.8% of the total annual hospital budget (Gottrup et al, 2013). As the impact of chronic wounds is increasing over time, there is a need for economic, safe and reliable wound care products.

Fish skin-derived wound care products

Fish skin has been explored to possibly contribute as a new product in the treatment of wounds. By manufacturing scaffold materials form its by-product, the skin, it can provide a cost-effective and ecofriendly treatment option. They are biocompatible, biodegradable and have a high collagen content (Yamada et al, 2014; Tang and Saito, 2015). Fish skin accelerates the wound healing process and could combat local infections (Zhou et al, 2016, Magnússon et al, 2017). So far, no unwanted immunological or allergic reactions have been following its application to wounds. This may be because the main protein responsible for allergic reactions to fish is no longer present after manufacturing and sterilisation (Halim et al, 2010). In addition to this, fish-derived materials do not require frequent dressing changes (DeLuca and Asaad, 2012) and their use does not conflict with religious beliefs (Eriksson et al, 2013).

Scaffolds, or skin substitutes, are classified as cellular or acellular and can be derived artificially or from natural sources, such as bovine and porcine. They can be further modified through a chemical or mechanical tissue engineering process, which modulates it into a finished wound care product (International Consensus, 2011). Cellular scaffolds contain different kinds of cells including those that support reactions and modulators that aid wound healing. Acellular scaffolds are manufactured through a cleansing process, where most cells are removed to minimise the risk of disease transmission and prevent unwanted immunogenic reactions (Rizzi et al, 2010). The different manufacturing methods have benefits and drawbacks; the debate about how to make the ultimate scaffold product is ongoing as the technology continues to develop.

Nevertheless, a scaffold should imitate the basic structure of healthy skin so the host is able to incorporate it into the wound. It should provide structural support, enhance cell adhesion and balance enzymatic reactions in a similar manner to the body's optimal wound healing process. By applying a scaffold into a wound, it becomes an attachment site for metalloproteinases (MMPs), which then breaks down collagen in the scaffold instead of new developing tissue and ECM components in the

Table 1. Average amino acid residues in different types of skin commonly used as scaffold materials (Zhao and Chi, 2009; Shoulders and Raines, 2009; Szpak, 2011; Hu et al, 2017).

	Average residues/1,000		
Amino acid	Bovine	Porcine	Fish skin
Glycine	296–334	330	332–339
Alanine	105–122	110–112	114–132
Proline	105–129	68	108–113
Glutamic acid	70–80	25–72	76–77
Arginine	48–52	48–49	50–52
Hydroxyproline	92	91–97	79–97

wound itself. This leads to a reduction of MMPs affecting the wound and unwanted enzymatic reactions and growth factor levels rebalances in a higher manner leading to increase rate of tissue regeneration. Initially, it also provides a physical barrier to pathogens by covering the wound (Sigurjonsson et al, 2013; Magnússon et al, 2017).

Scaffold materials are typically applied to acute and chronic cutaneous wounds, including pressure ulcers, venous ulcers, necrotic and diabetic wounds (Sigurjonsson et al, 2013). They have also shown promise in the treatment of burns (Magnússon et al, 2017). This article reviews literature relating to the use of fish-derived scaffold material for the treatment of cutaneous wounds.

Method

Articles were initially identified by a broad literature search on PubMed using the terms: (fish or fish skin or piscine) AND (wound product or wound dressing or scaffold or collagen scaffold or collagen) AND (cutaneous wounds or skin wound or burn wounds or chronic wounds or acute wounds). No filters were used. The search identified 100 articles. Further searches were made on PubMed with using the search terms: (antimicrobial peptides) AND (fish skin), (immunological reactions OR safety) AND (fish skin), (wound healing) AND (fish skin), (fish scaffold) AND (immunity OR immunology OR immune responses), (fish scaffold) AND (antimicrobial), (fish scaffold) AND (healing OR healing properties OR regenerative), (fish skin) AND (histology). The search was then extended to *Wounds International*, *Wounds UK*, *Today's Wound Clinic*, *The Brazilian Journal of Burns* (*Revista Brasileira de Queimaduras*), Researchgate, Elsevier and Wilkies online library, using similar search terms. In total, 392 articles were identified.

The authors individually sorted through all of the articles to identify those with relevance to the chosen topic. We then added the results from World Intellectual Property Organization and Patentscope.int patent searches.

Fish skin as a scaffold material

Architecture

Fish skin consists of an epidermis, dermis and hypodermis that share many of the same characteristics as human skin. The differences are mostly due to adaptation to the aqueous environment: scales instead of hair, the secretion of mucus from cells in the epithelium, the lack of a superficial keratinised layer, and two basement membranes (Le Guellec et al, 2004). These adaptations ensure the integrity of the skin and help defend against aquatic pathogenic organisms (Whitear et al, 1980; Magnadottir, 2010). In addition to these differences, fish skin recovers from wounds faster than human skin and does not result in scarring (Richardson et al, 2013).

Composition

Amino acids and collagen are important components of wound healing. They are providers of many necessary supplements and modulates the framework and enzymatic reactions favourable for the healing process (Tracy et al, 2016).

The quantity and composition of amino acids and collagen differ in fish skin compared to other mammalians *[Table 1]*. The high type 1 collagen (COL-1) and amino acid content enhance vital reactions and responses. For instance, proline enhances protein synthesis in areas characterised by a high synthesis rate, such as the intestine and skin, and contributes to wound healing by supporting collagen synthesis (Duval et al, 1991; Phang et al, 2008; Wu et al, 2011). Proline is the derivative of hydroxyproline and affects the physical and thermal stabilisation of collagen (Kotch et al, 2008). Alanine is found in connective tissue and co-induces the production of antibodies. It also buffers pH changes during metabolic processes and enhances the action of carnosine, an amino acid that increases granulation (Nagai et al, 1986).

The architecture of fish skin is largely dependent on the ECM. This is a collection of molecules and fibres responsible for biochemical and structural support. Amino acids are needed to create collagen fibres during the remodelling phase of wound healing. The skin is dependent on a functional ECM in order to receive support from fibroblasts, which in close relation to COL-1 fibres modify and maturates the matrix. Therefore, ECM has a multifactorial

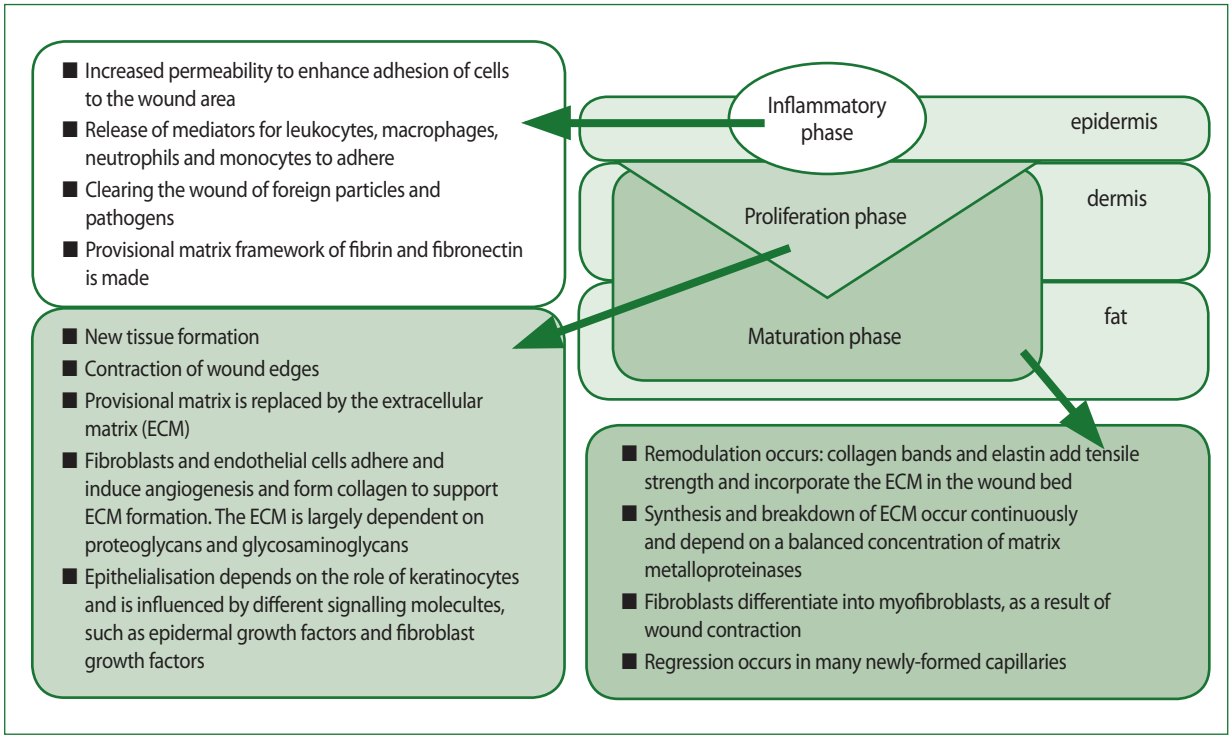

Figure 1. The three stages of normal wound healing in humans (Velnar et al, 2009; Gonzalez et al, 2016).

role for the ingrowth of cells and plays a dominant role in maintaining the biological and structural integrity of the skin (Gould, 2016; Yang et al, 2016).

Influence on wound healing

The wound healing process can be divided into three phases *[Figure 1]*. *In vitro* and *in vivo* studies, as well as microscopic evaluations have been conducted to assess the wound healing properties of fish and scaffolds derived from fish skin. The summary/conclusion from the studies indicated that fish-derived scaffold materials had both an accelerated wound healing time and properties differentiating from research of mammalian-based scaffold materials.

Increased and prolonged MMP concentrations are obstacles to natural wound healing. These proteases occur naturally in wounds and are important in the maturation phase as they enable degeneration of the ECM. In chronic wounds, MMPs are present in higher concentrations than normal and halt wound healing. The use of fish skin in cutaneous wounds reduces and balances MMP levels, allowing wound healing to proceed. The mechanism behind this is unclear, but it has been proposed that the high concentration of collagen and its ability to bind pro-inflammatory cytokines are responsible (Wiegand et al, 2010).

Keratinocytes play an important role in epithelialisation. Keratinocytes from fish skin allow better dispersal and ingrowth of cells into the ECM compared to human amnion/chorion grafting and alginate dressings (Magnússon et al, 2017; Zhou et al, 2017). It is belived this is due to the pore size and three-dimensional structure of fish skin. This structure allows fibroblasts to adhere more rapidly, supports the production of ECM and closes the wound (Magnússon et al, 2017).

Another molecule associated with collagen synthesis and fibroblast proliferation is transforming growth factor beta (TGF-β). Scaffolds derived from fish present modulators responsible for increased TGF- β expression, which may accelerate healing (Zhou et al, 2017). Interestingly, chronic and hard-to-heal wounds often have low concentrations of TGF- β (Kim et al, 2003).

During re-epithelisation, the enzyme transglutaminase-1 links proteins, such as involucrin and filaggrin, to generate the outmost layer of the epidermis. These factors are significantly up-regulated in fish skin, suggesting that fish scaffolds could potentially influence the remodelling phase of wound healing (Zhou et al, 2016).

Antimicrobial properties and pain management

For wound healing to proceed in an uninterrupted manner, foreign particles, non-viable tissue and biofilm should be removed

from the surface of the wound (Wolcott and Fletcher, 2014). Normally, a saline solution is chosen for cleansing, but an antimicrobial solution might be better as studies suggest that non-antiseptic solutions do not remove MMPs and cytokines (Cutting, 2010). Prolonged and high levels of MMPs and proteases are believed to have a significant role in impaired wound healing as they degrade the ECM (Isik et al, 2015). Investigations have shown that fish skin contains antimicrobial peptides (AMPs) — such as hepcidin, defensin-like peptides, specific apolipoproteins and piscidins (Chen et al, 2009; Najafian and Babji, 2012; Peng et al, 2012) — which have broad spectrum antimicrobial action and also modulate innate immunity. AMPs from fish have been investigated for possible clinical application in humans (Najafian and Babji, 2012).

The omega-3 polyunsaturated fatty acids (PUFAs) present in fish skin also have antiviral and antibacterial properties. They have been used to treat multidrug-resistant bacteria in cystic fibrosis patients (Mil-Homens et al, 2012; Desbois and Lawlor, 2013; Imai, 2015). Similar anti-bacterial properties have also been confirmed in *in vitro* studies, were fish skin scaffolds with residuals of Omega-3 PUFAs had the ability to withstand bacterial invasion in a high manner (Magnússon et al, 2017). *In vivo* studies have found oral omega-3 to be anti-nociceptive, preventing tolerance to morphine and reducing the incidence of neuropathic pain (Ko et al, 2010; Escudero et al, 2015). Clinical trials assessing the thermal pain sensation in rats, concluded that Omega-3 supplementation was able to reduce pain response by inhibiting part of the neurosensory pathways through cyclooxygenase and activating microglia (Nobre et al, 2013).

Safety

Scaffold materials derived from fish skin have demonstrated biocompatibility with human tissues and have a bacterial count within acceptable limits (Yamada et al, 2014; Magnússon et al, 2015; Lima-Junior et al, 2016). These results are achieved with less severe processing than mammalian-derived scaffold material. For instance, one method used to create safe porcine-derived scaffold material involves viral inactivation with peracetic acid and aqueous ethanol mixture for periods of up to 2 hours. This is to remove the risk of disease transmission (Hodde and Hiles, 2002; Tang and Saito, 2015). Scaffold material from fish does not carry this risk and, therefore, no viral inactivation is required (Yang et al, 2016). The manufactures of Kerecis™ Omega3® Wound, a fish-based scaffold, propose that this results in more of its wound-healing attributes being retained (Sigurjonsson et al, 2013; Magnússon et al, 2015). In a parallel-group, non-inferiority, double-blinded, randomised controlled trial (RCT)including 162 participants, the scaffold was not rejected by any patients and there were no pathological effects (Baldursson et al, 2015). The risk of an allergic reaction is low as the protein parvalbumin, is responsible for 90% of all allergic reactions to fish, is removed from fish scaffold during manufacturing (Saptarshi et al, 2014)

In vivo trials

Acute wounds

In a clinical setting, scaffold material from fish skin has shown promising results in the treatment of wounds. A RCT (n=162) was conducted to compare scaffold material (Kerecis Omega3® Wound) with the more established porcine scaffold (Oasis® Wound Matrix). Two full-thickness punch wounds were made 2 cm apart on participants' non-dominant arm. Wound healing was evaluated by a dermatologist once a week over a 28-day period. The primary outcome was epithelisation and the secondary outcomes were time to heal, the incidence of wound irritation, infection leading to antibiotic treatment and measurements of auto-antibodies. Fish-derived scaffold material had many similar properties to the porcine-derived type; however, it showed accelerated wound healing in the early stages of the trial. At day 21, 72.5% of wounds in the fish group had healed compared to 56% in the porcine group. Four days later, 77.5 % of wounds were healed in the fish group versus 65% in the porcine group, possibly indicating that scaffolds from fish increase early healing compared to porcine scaffolds (Baldursson et al, 2015).

Similar results were found in RCT trials in animals. In one study, the wound healing properties of tilapia fish skin collagen sponge were compared to Kaltostat® alginate dressing. Initially, fibres from the fish skin were separated and applied to a collagen sponge though an electrospinning method in purpose of working as a ECM in the wound. The first trial included 12 rats with full-thickness skin defects which were covered with either collagen sponge or Kaltostat. One other group was left untreated for control comparison. There was accelerated healing in the collagen group versus to the other groups at 7 and 14 days. Keratinocyte proliferation had increased significantly, with epidermal differentiation and up-regulated

expression of involucrin, filaggrin and type 1 transglutaminase, which stimulate re-epithelisation. In the second trial, eight rats were randomly implanted with collagen sponge containing fish skin fibres or allocated to a control group. After 28 days, blood analyses showed that the fish skin fibres had not affected IgG, IgM, CD4+ or CD8+ levels compared to the control group. These results suggest that the application of fish scaffold material does not induce an immunological response (Zhou et al, 2016).

Chronic wounds

Scaffold material derived from fish has been applied in patients with diabetic wounds. It is thought that impaired healing in people with diabetes is related to the dysregulation of MMPs and reduction in TGF-β. This has in part been confirmed an *in vitro* study, where patients showed accelerated healing when healthy MMP regulation and TGF-β concentrations were achieved (Liu et al, 2009).

A small *in vivo* trial investigated complicated lower limb wounds with visible bony elements secondary to diabetes and peripheral vascular disease (*n*=7). Following debridement and treatment with Kerecis Omega3® Wound, there was full re-epithelialisation, no sign of maceration or sealing problems, the wounds had halved in size within 11 weeks healed at 33 weeks. There was also a reduced need for analgesics; however, this benefit has not been systematically reviewed and further research is needed (Dorweiler et al, 2018).

Successful outcomes following the application of Kerecis Omega3® Wound to chronic leg wounds have been reported in seven case studies from Danderyds Hospital in Sweden and Stockholm County Innovations. Prior to treatment with fish skin scaffold, these wounds had been conservatively treated with absorbent dressings changed two to three times a week and high-pressure therapy. No debridement was performed beyond normal saline cleansing and no offloading was initiated. One patient's wound, which had persisted for 25 years, healed 2 months after the application of fish skin scaffold (Bentling, 2015). Three-quarters of another patient's chronic leg ulcers healed after 6 months of fish skin treatment, preventing the patient from needing amputation (Bentling, 2017).

Skin burns

Scaffold material from fish has proven effective in the treatment of burns in animals. Being highly susceptible to infection, early coverage of burns is important (Saaiq et al, 2012). In a prospective, qualitative and quantitative study, 40 Wistar rats were given burns before being treated with saline water or an open dressing containing silver sulfadiazine or scaffold material derived from Nile tilapia. On day 21, the rats were euthanised and the wounds histologically analysed. Compared to the rats treated with saline water or silver sulfadiazine dressing, those treated with scaffold material from Nile tilapa had improved wound healing, fibrous connective tissue lacking epithelial lining, and no ongoing inflammatory processes (LIma-Junior, 2017). The reason for this may lie in the architecture of fish skin, which has a highly porous microstructure compared to dehydrated human amnion/chorion membrane (Magnússon et al, 2017).

Discussion

The prevalence of wounds is increasing in parallel with the increasing number of people with conditions such as diabetes and peripheral vascular disease, putting an ever-greater burden on both healthcare providers and patients. Research suggests that the use of scaffold material derived from fish skin is safe and reliable in the treatment of wounds. As this material originates from fish skin waste, it could prove to be an ecofriendly and cost-effective treatment option in future (Yamada et al, 2014; Tang and Saito, 2015; Zhou et al, 2016; Magnússon et al, 2017). Fish-skin scaffold material accelerates the wound healing process, has antimicrobial properties and may also have anti-nociceptive properties.

Due to the gentle manufacturing process, fish-skin-derived scaffold retains AMPs, which contribute to the first line of defence against infection (Najafian and Babji, 2012). We were unable to identify a study looking at AMPs in an isolated clinical setting; however, the use of fish-skin scaffold resulted in reduced inflammation compared to porcine-derived scaffold, which may be the result of the anti-inflammatory properties of omega-3 PUFAs (Baldursson et al, 2015).

The trial conducted by Dorweiler et al (2018) found a reduced need of analgesia by applicating fish skin-derived scaffolds. Other *in vivo* trials found similar results by investigating dietary omega-3 supplementation with results of reduced neuropathic pain. The anti-nociceptive properties in fish skin scaffolds is interesting, but needs more assessments in a clinical *in vitro* setting before a solid conclusion can be made.

The fish-skin scaffold material that is currently available has demonstrated biocompatibility

with human tissue and requires less processing than bovine- and porcine-derived scaffolds (Halim et al, 2010; Yang et al, 2016). It should be borne in mind, however, that some species of fish may pose a risk of zoonosis.

Limitations

This is a fairly new area of study and few *in vivo* trials have been conducted using humans. These trials have had positive results, but have mostly been conducted by researchers affiliated with the companies that produce fish scaffold materials (Baldursson et al, 2015; Yang et al, 2016). The authors also found that the patent for the scaffold material from Kerecis was published in 2013 and the research was conducted after this period (Sigurjonsson et al, 2013), which may lead to bias; however, the study protocol was a double-blinded RCT and follows the recommended guidelines for reducing bias.

Conclusion

Fish-derived scaffold material shows promise in the future treatment of acute and chronic cutaneous wounds. However, human trials are few in number and have included a small number of participants. Further, larger human studies are, therefore, needed to confirm the discoveries made to date and answer questions that remain. WINT

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