Mechanical induced wounds in fish – a review on models and healing mechanisms

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Abstract

Skin wounds and injuries are frequently occurring in farmed fish, particularly in more intensive production systems. Any disruptions of the skin, such as mucus removal, scale loss or deeper incisions, are negatively correlated with barrier functions and disease resistance. In this review, the current knowledge on mechanically induced wounds in fish is dedicated to five areas of focus: (i) The anatomy and functions of fish skin, (ii) Techniques to inflict mechanical skin damage in farmed and laboratory fish for experimental purposes, (iii) Healing mechanisms of deep wounds, (iv) *In vitro* models for wound healing studies and (v) Wound care, with focus on factors that may enhance or delay skin regenerative processes. The aim of the review was to presents key points for a better understanding of skin resilience and fish robustness, with focus on Atlantic salmon, *Salmo salar*, in Norwegian coastal production environment.

Key words: Atlantic salmon, barrier function, fish skin, welfare, wound healing.

Introduction

Skin wounds and diseases are emphasized as a primary constraint to the sustainable growth of many farmed aquaculture species (Groff 2001; Roberts 2012; Bruno *et al.* 2013). Disruption of the skin and the associated mucus layer may be caused by mechanical trauma or ulcer-inducing diseases (Groff 2001). A distinction is therefore made between mechanically induced wounds and those caused by pathogens or underlying pathologies triggered by, for example nutrition insufficiency or other dermatological diseases (Roberts 2012; Bruno *et al.* 2013). In this review, a mechanically induced wound is defined as any disruption of the skin integrity including the mucus layer, and we separate between superficial wounds, partial-thickness wounds and deep wounds. Superficial wounds leave the dermis intact, while partial-thickness wounds are extending into the first two layers of skin, the epidermis and dermis. Deep wounds cut through the skin and subcutaneous adipose tissue. Superficial and partial-thickness wounds may heal fast, hours to days, depending on the species and the temperature (Anderson & Roberts 1975; Richardson *et al.* 2016). Healing of deep wounds is more complex, and the process may last weeks to months, depending on wound severity, the fish species and the rearing environment (Roubal & Bullock 1988; Wahli *et al.* 2003; Richardson *et al.* 2013;

Schmidt 2013). However, to which extent the three dimensions of the wound (depth and width) contributes to wound severity, and how environmental parameters and nutritional needs contribute to the healing rate, is still not completely understood.

In the Norwegian Atlantic salmon (*Salmo salar*) production, mechanical trauma is associated with poor management practices, high production intensities, handling operations, aggressive behaviour, predator attacks and acute panic episodes (Tørud & Håstein 2008; Takle *et al.* 2015; Sveen *et al.* 2016; Hjeltnes *et al.* 2018). Skin wounds are often associated with mortality, however rarely reported as the sole cause of losses (Takle *et al.* 2015; Hjeltnes *et al.* 2018). Superficial and deep wounds may develop into bigger lesions, with complex pathology and welfare issues for the fish (Svendsen & Bøgwald 1997; Raj *et al.* 2011). The severity of the wound development is likely related to many factors, such as presence and ability of skin pathogens to cause disease, stress and the general physiological state of the animal (Groff 2001; Takle *et al.* 2015).

This review highlights the fish skin microarchitecture, the regenerative processes and healing cascades of mechanical induced wounds in fish, skin resilience and barrier function. The current knowledge on wound healing mechanisms in teleost fish is summarized in five focus areas (i)

The anatomy and functions of fish skin, (ii) Techniques to inflict mechanical skin damage in farmed and laboratory fish for experimental purposes, (iii) Healing mechanisms of deep wounds, (iv) *in vitro* models for wound healing studies, and (v) Wound care, with focus on factors that may enhance or delay these regenerative processes.

The anatomy and functions of fish skin

In-depth knowledge of fish skin microarchitecture is imperative for the understanding of the repair and regenerative processes involved in wound healing. With more than 34 000 (FishBase 12/2019) known fish species which are adapted to virtually all aquatic habitats, there are of course large interspecies variations in the structure and function of the skin (Fontenot & Neiffer 2004; Elliott 2011). However, some general features are common for most skin types (Figs 1–3): (i) The outer cuticle (mucus layer) built from glycoproteins formed by secretions from cells in the epidermal layer, (ii) An outer epithelial layer (the epidermis) with live epithelial cells and mucous-producing cells, (iii) The intermediate dermal layer that largely contains connective

Figure 1 Atlantic salmon skin. (a) The successive structure of fish skin, with the mucus (mu) layer, epidermis (epi), dermis and hypodermis (hyp). The main structures in the dermis are the dense connective tissue (dct) and loose connective tissue (lct). The scales (sc) are skin appendages anchored in the dermal layer. (b) The skin of Atlantic salmon covers the head (area 1), the main body (area 2) and the fins (area 3). The main body is covered by overlapping scales, and the head and fins are scale less. Photograph: Helge Skodvin, Nofima.

tissue, nerves, blood vessels, pigment cells and adipose tissue, and (iv) The deeper hypodermis which is primarily built from adipocytes but also contributes to skin pigmentation and vascularization.

The cell types in the skin tissues are derived from different germ layers during gastrulation. The epithelial cells are derived from the surface ectoderm, skin pigment cells from the ectoderms neural crest and connective tissues are of mesodermal origin (Schmitz *et al.* 1993; Le Guellec *et al.* 2004; Cooper & Raible 2009; Elliott 2011). After skin damage, each of these cell types have different responses and functions in wound repair and regeneration (Richardson *et al.* 2013; Richardson *et al.* 2016).

The epidermis and the mucus layer

The epidermis and the mucus layer have critical roles during steady conditions and during skin repair (Hawkes 1974; Esteban 2012). The epithelial surface is protected by a mucus gel produced and secreted by epidermal mucous cells (Fig. 2; Wainwright & Lauder 2017). Weak binding of bacteria to the mucus can be beneficial to 'wash off' bacteria and prevent colonization (Easy & Ross 2010; Padra *et al.* 2019). In addition, extracellular proteins, such as enzymes, antimicrobial peptides and immunoglobulins, are active in the mucus gel, protecting the underlying epithelia, reviewed by multiple authors (Esteban 2012; Rakers *et al.* 2013; Dash *et al.* 2018; Reverter *et al.* 2018). The functions and dynamics of the mucus layer are complex with multiple suggested functions related to respiration, reproduction, parental feeding, nest building, as well as innate and adaptive immune functions (Shephard 1994). Wounded fish also exhibit excessive mucus secretion (Fig. 2e,f). In addition to providing physical and antimicrobial protection of the wounded surface, the mucus gel may enhance wound healing through its haemolytic activity and promote vasoconstriction of smooth muscle cells (Thulesius *et al.* 1983; Al-Hassan *et al.* 1985; Al-Hassan *et al.* 1986). Application of fish mucus on mammalian wounds may activate healing (Al-Hassan *et al.* 1983; Al-Hassan 1990; Al-Hassan *et al.* 1991; Akunne *et al.* 2016), and authors suggest that the mucus may reduce inflammation and enhance granulation tissue formation and wound contraction (Al-Hassan *et al.* 1983).

The epithelial compartment of the skin, namely the epidermis, predominantly consists of keratocyte cells (also known as filament containing cells or Malpighian cells) and mucous-producing cells (Fig. 2a; Elliott 2011). Other cell types such as serous cells, club cells, sacciform cells and Merkel cells, venom glands, sensory cells and luminescent organs have been reported for the epidermal layer in different fish species; however, these cells are not present in the skin of Atlantic salmon (Whitear 1970). More detailed
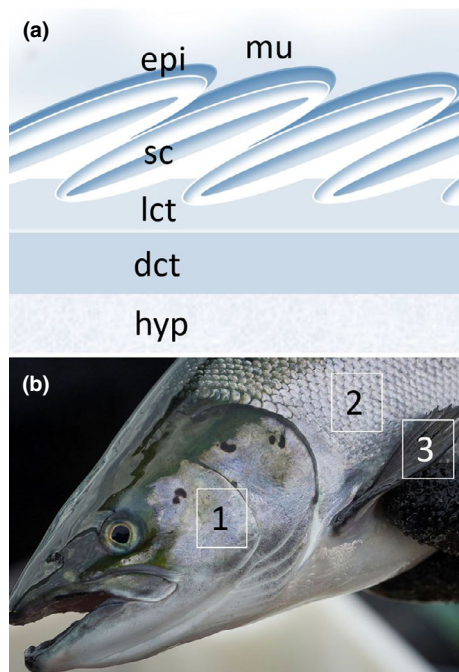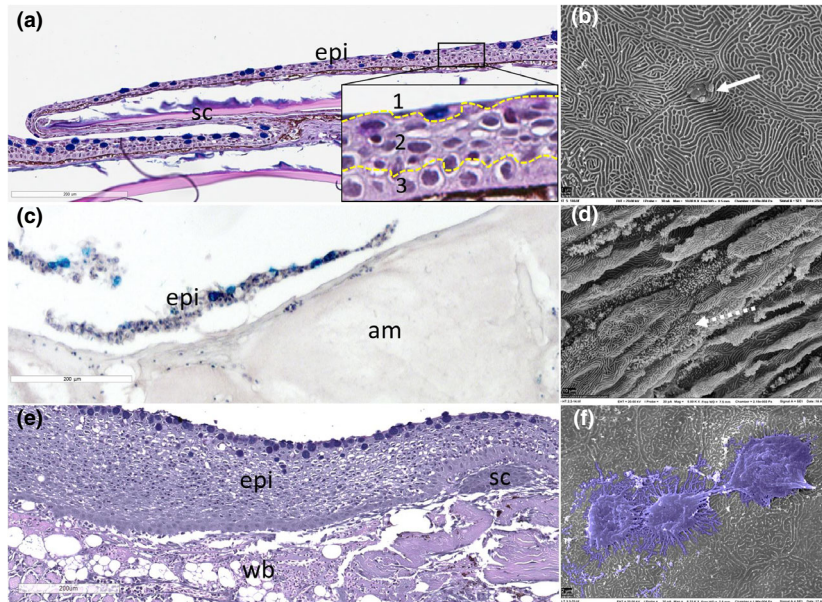
Figure 2 The epidermal layer of Atlantic salmon skin. (a) The main cell type in the epidermis (epi) is the keratocyte cells. The mucous-producing cells are interspersed between the keratocytes, stained blue by AB/PAS staining. Insert picture: the three compartments in the epidermis are marked by dotted lines. In the surface compartment, the keratocytes are flat with elongated nuclei, in the intermediate layer the keratocytes are round, while they are cubical shaped in the basal layer. (b) Atlantic salmon skin surface by scanning electron microscopy. The keratocytes are pentagonal shaped with microridges. One mucous cell is about to empty its content on to the surface (white arrow). (c) Skin surface at one day after punch biopsy wounding, migrating keratocytes and mucous cells on an amorphous (am) substance covering. (d) Skin surface at three days after-punch biopsy wounding. The keratocytes are being remodelled, with uneven shapes (dotted arrow). (e) Mucous cells are displayed apically in the epidermis 14 days after punch biopsy wounding. Regenerating scale (sc) under the epidermal surface. (f) Heavy mucus secretion at the wound surface seven days after wounding. Blue digital colour is added to highlight the mucus
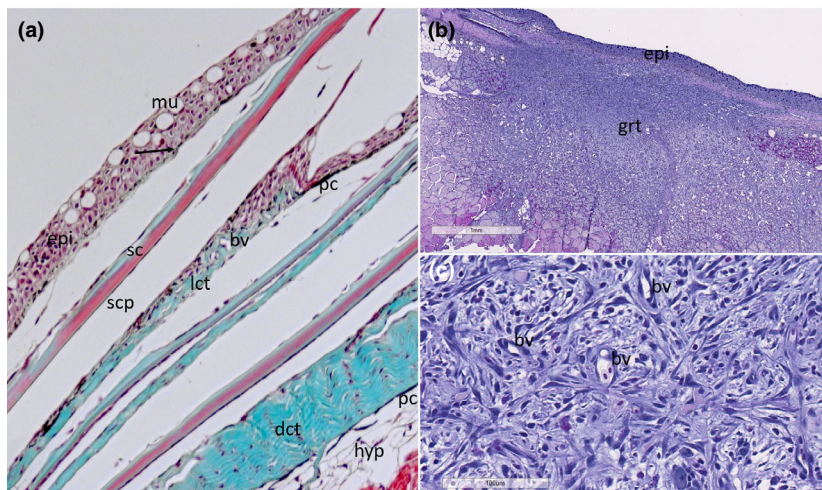

Figure 3 Details of Atlantic salmon dermis. (a) The dermis starts where the epidermis (epi) ends as indicated (arrow). The main structures in the dermis are the dense connective tissue (dct) and the loose connective tissue (lct). In the lct, a variety of tissues and cell types can be found, such as blood vessels (bv) and pigment cells (pc). The scales (sc) are skin appendages anchored in the dermis. Each scale is in its separate scale pocket (scp). Massons trichrome staining, collagens stain blue. (b) Skin wound five weeks after punch biopsy wounding granulation tissue (grt) fills the wound bed. (c) Details of the granulation tissue with multiple blood vessels (bv).

information on these cell types has been provided and reviewed by (Whitear 1986b; Zaccone *et al.* 2001; Groff 2001).

The integrity and continuity of the keratocyte cells are central for the barrier functions of the skin (Chang & Hwang 2011). The presence of intermediate cytoplasmic

filaments comprises an important component of the cytoskeleton of individual keratocyte cells (Keren *et al.* 2008; Elliott 2011). Additionally, the attachment of cytoplasmic proteins to the desmosomal plaques adhere adjacent epithelial cells, which enables the epidermis to respond to mechanical stress (Whitear 1986b; Elliott 2011). The structure and function of the keratocyte cells depend on their localization in the epidermal layer. The superficial keratocytes are flat and display elevated structures called microridges (Fig. 2b; Brown & Wellings 1970). The microridges provide a larger epidermal surface, which is important for mucus retention and ionic and gaseous exchange (Quilhac & Sire 1999). The latter is important during larval development or in fish inhabiting naturally low oxygen level environments (Kirsch & Nonnotte 1977; Fontenot & Neiffer 2004; Esaki *et al.* 2009; Guh *et al.* 2015). The number of cells in the intermediate epidermal compartment varies with age, species and other factors such as temperature (Elliott 2011; Karlsen *et al.* 2018). These cells supply and replace dead cells of the superficial layer. The basal layer consists of a single layer of columnar cells attached to the basement membrane through hemidesmosomes (Whitear 1986a). The basement membrane is counted as a dermal element, although epidermal participation is necessary for its formation (Whitear 1986a).

Other innate immune functions, in addition to the physical and chemical barrier, are assigned to the epidermis. In wounded mirror carp (*Cyprinus carpio carpio*), the keratocytes displayed phagocytic activity, with strong peroxidase content clearing cellular debris during migration (Iger & Abraham 1990). Similarly, in cell cultures, migrating keratocytes may internalize foreign particles such as latex beads (Åsbakk & Dalmo 1998) and bacteria (Lindell *et al.* 2012). This feature, as illustrated in Video S1, may aid in clearing wounds from particulate matter such as opportunistic and pathogenic microorganisms (Iger & Abraham 1990; Åsbakk 2001; Karlsen *et al.* 2012). The phagocytotic process is suggested to involve mannose receptor-mediated uptake of bacteria. As a defence mechanism, the bacteria may utilize O-antigen polysaccharides to avoid phagocytosis (Lindell *et al.* 2012).

The keratocytes also have a role in the first line of defence against parasitic infections. Evidence suggests compartmentalization of immune cells in the epidermal layer (Braden *et al.* 2015; Holm *et al.* 2017). MHC class II molecules are located in the epidermal cell populations in normal Atlantic salmon skin (Braden *et al.* 2015; Holm *et al.* 2017). Also, the interferon (IFN) inducible anti-viral effector protein Mx and CD8α+ cells are identified in the epidermal layer of Atlantic salmon skin (Holm *et al.* 2017). An increase in the lysosomal enzyme alkaline phosphatase content is observed after mechanical skin injury in mirror carp

(Iger & Abraham 1990), and under parasitic infection in Atlantic salmon (Ross *et al.* 2000; Fast *et al.* 2002). The enzyme is produced by epidermal cells, and later appears inside the mucous cells (Iger & Abraham 1990) and is suggested to have a possible protective role in fish during the first stages of wound healing (Iger & Abraham 1990). Further, an epidermal response with hyperplasia and inflammation may play a role in the rejection of salmon lice (*Lepeophtheirus salmonis*) in both coho salmon (*Onchorynchus kisutch*) and chinook salmon (*Onchorhynchus tshawytscha*; Johnson & Albrightz 1992). This process is absent in *L. salmonis* infected Atlantic salmon, illustrating different properties of fish keratocyte cells between species (Johnson & Albrightz 1992).

Dermis and hypodermis

The dermal layer starts where the epidermal layer ends (Figs 1,3). The dermis is further subdivided into two parts, the outer layer (*stratum laxum*) and the deeper layer (*stratum compactum*). The *stratum laxum* contains diverse cell types and tissues (Fig. 3a). These include loose connective tissue, blood vessels, nerve cells, chromatophores, iridophores and peripheral nerve cells (Whitear 1986b; Elliott 2011; Rasmussen *et al.* 2018). The blood vessels located in the dermal layer are part of a secondary vascular system (Burne 1929; Skov & Bennett 2004; Rummer *et al.* 2014), suggested to be involved in nutrient supply, gas transfer and acid-base regulation (Steffensen & Lomholt 1992; Ishimatsu *et al.* 1992; Glover *et al.* 2013). The secondary vascular system arises directly from the primary vasculature (Olson 1996). Under steady conditions, blood flow in the secondary system is low; however, hypoxia or exercise may increase the blood flow to the secondary system, as shown for the glass catfish (*Kryptopterus bicirrhis*; Rummer *et al.* 2014). Partial-thickness wounds such as scale loss will not bleed, but deep wounds that cut through the vascularized hypodermis and/or into the muscle tissue will bleed. Excessive bleeding has been described in experimentally wounded carp, while rainbow trout (*Oncorhynchus mykiss*) responded with limited bleeding to the same treatment (Schmidt 2013), the reason for this interspecies variation is not documented.

When a fish is being skinned, the collagenous fibres of the *stratum compactum* are obvious (Fig. 4a). The dense connective tissue makes the bulk of the *stratum compactum*, where collagen-rich fibres are the main element, and crowded between the collagen fibres are rows of fibroblasts that generate the fibres (Fig. 3a). The compacted collagen fibres are arranged in opposing geodesic spirals around the body (Whitear 1986a; Szewciw & Barthelat 2017) and connected to the muscle and skeletal system through the myocommata. (Willemse 1972). It was earlier suggested
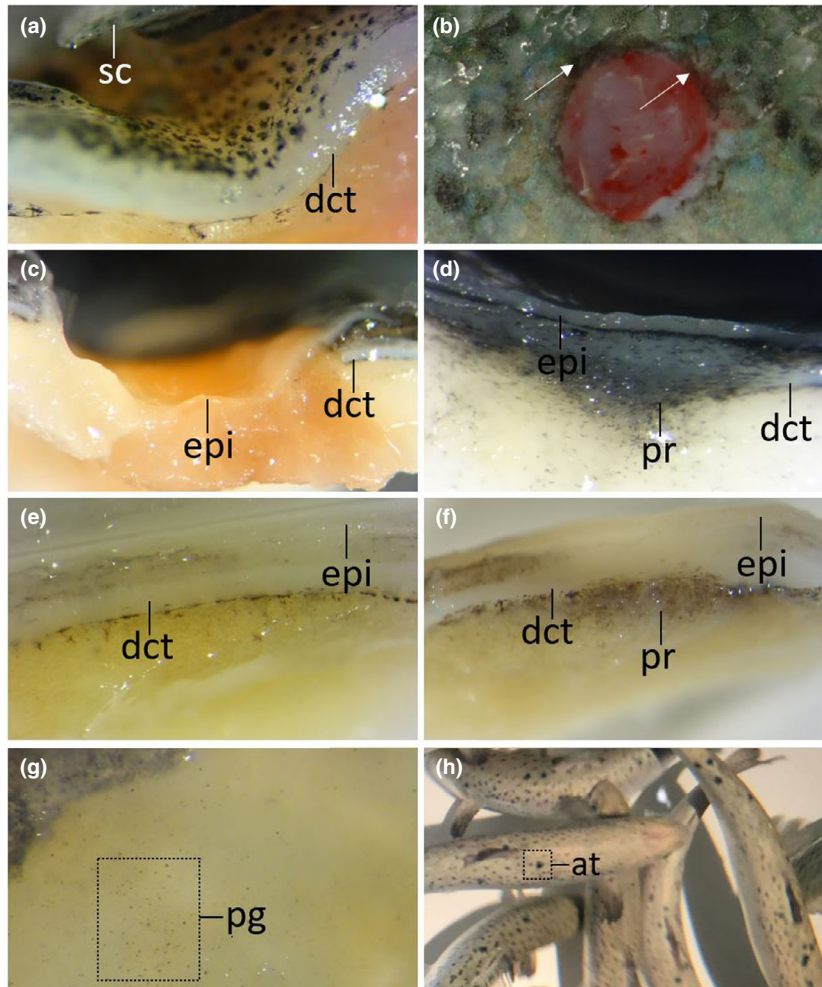
Figure 4 Skin pigmentation in Atlantic salmon. (a) Intact fish skin with scale (sc) and dense connective tissue (dct) and black pigment cells (melanocytes). (b) Punch biopsy wound 1 one day after wounding (dpw) with pigment granules (arrows) at the wound margins. (c) Punch biopsy wound 14 dpw, epidermis (epi) is covering the wound bed. (d) Punch biopsy wound 57 dpw, a strong pigment response (pr) accompanies the formation of connective tissue. (e) Partial-thickness wound 21 dpw where the epidermis and scales were removed by brushing without damaging the dense connective tissue. (f) Similar to (e), however, the dense connective tissue was damaged in the brushing process, resulting in a strong pigment response as the wound heal. (g) The surface of a punch biopsy wound (14 dpw). (h) Skin darkening at the site of *Lepeophtheirus salmonis* attachment. Pigment granules (pg) may be found on the wound surface. (a–h) Photographs of formalin fixated tissue samples. Photograph (h), Steffen Blindheim, ILAB. Photograph (e and f), with contribution from Renate Kvingedal, Cargill.

that skin may act as an external tendon, working in unison with the mechanical movement of the muscle tissue (Wainwright *et al.* 1978; Hebrank 1980; Summers & Long Jr 2005), where the structural arrangement of the collagen fibres promote muscular contraction and produce tendon-like responses in the skin (Szewciw & Barthelat 2017). The puncture resistance of the skin is also shown to be enhanced by the dense connective tissue in the *stratum compactum* (Motta 1977; Szewciw & Barthelat 2017).

The hypodermis is located below the dermal layer and above the musculature (Figs 1,3). This tissue has been described for multiple fish species and is dominated by adipocytes, but also contains chromatophores and leucophores, vascular and neural tissue (Elliott 2011).

Skin pigmentation

The pigmentation and colour pattern in teleost fish have the largest complexity and diversification of all vertebrates (Braasch *et al.* 2007; Braasch *et al.* 2008). The dermis and the hypodermis are the two layers most involved in fish coloration (Fig. 4). Colour is formed by the reflection and absorption of light by chromatophores, iridophores and leucophores. The chromatophores are named after the

colour of their pigment (xanthophores, erythrophores, melanophores and cyanophores). The chromatophore cells have a varying degree of dendritic branching. This is in contrast to the leucophores, which are flattened and elongated cells (Cloney & Brocco 2015). Many fish species have a dark skin colour in the dorsal area, while the ventral surface is paler, this is called countershading and is a type of camouflage. This is also the case in Atlantic salmon, where the dark chromatophores (melanocytes) are found in the *stratum laxum* and hypodermis in the dorsal area (Figs 3,4). In the ventral region, there are few chromatophores, instead iridophores and leucophores give the skin a pale colour with reflective and iridescent effects. In fish, skin coloration also plays a role in mate selection and thermoregulation (Stuart-Fox & Moussalli 2009). Colour changes in fish are mostly autonomous and are effected by the environment, nerve impulses and hormonal releases, UV radiation and temperature (Sugimoto 2002; Kobayashi *et al.* 2012; Cal *et al.* 2017). The skin pigmentation may also change as a result to trauma, parasite attachment and skin infections of fungal, viral and bacterial origin (Fig. 4g; Wildgoose 1998; Roberts 2012).

Scales

The scales are overlapping bony plates located in scale pockets in the *stratum laxum* (Fig. 5 and Video S2). Fish scales may enhance locomotion (Oeffner & Lauder 2012; Wainwright & Lauder 2017) and provide a physical protection to the underlying, softer tissues (Szewciw & Barthelat 2017). Individual scales have a high resistance to sharp penetration, and overlapping scales act collectively to distribute the puncture over large areas (Vernerey & Barthelat 2010; Vernerey *et al.* 2014). The scales consist of two layers: An upper mineralized (ossified) layer (16–59 vol.%) and a non-mineralized matrix and collagen fibres (Fig. 5c; Meunier 1984; Torres *et al.* 2008; Sire *et al.* 2009; Marino Cugno Garrano *et al.* 2012). Bone growth is the result of a coordinated activity between the osteoblasts and osteoclasts, where the bone matrix is secreted and mineralized by the osteoblasts, while the matrix is dissolved by the osteoclasts (de Vrieze *et al.* 2011). These cells are organized in ring structures (Fig. 5b), and the scale grows one ring at a time (Sire *et al.* 1997; Sire *et al.* 2009). Through dissolution by the osteoclasts, the scales may act as a reservoir for calcium and phosphorus (Yasuo 1980; Persson *et al.* 1995; Rotllant *et al.* 2005). The mineral hydroxyapatite ($Ca_5(PO_4)3(OH)$) is important for the strength of the mineralized structures. Availability of calcium and phosphorus is therefore of importance for scale strength (Flik *et al.* 1986; Lall & Lewis-McCrea 2007; Witten *et al.* 2016). Chronic stress is one factor that can delay scale mineralization during wound healing (Sveen *et al.* 2018).

Techniques to inflict skin damage in fish for experimental purposes

Experimental wound healing models have proven powerful in studies investigating feed effects, physiology, rearing conditions and infection routes (Raj *et al.* 2011; Jensen *et al.* 2015; Sveen *et al.* 2018). Models range from non-invasive procedures, such as tissue paper for the removal of the mucus layer to more invasive methods, such as dermal laser and punch biopsy needles (Fig. 6). Depending on which of the skin tissues that are damaged, different healing responses will be triggered. Thus, methodological considerations are required to design and conduct a study capable of detecting appropriate data to support the hypothesis being investigated. Thus, refinement of the hypothesis and methodological approach is required prior to implementation.

Superficial wounds, partial-thickness wounds and deep wounds

There are many ways to induce superficial and partial-thickness wounds in fish (Fig. 6a–d). Raj and colleagues compared tissue paper, tissue swab, sandpaper and brushing (Raj *et al.* 2011). Tissue paper and swabbing resulted in superficial skin damage, with loss of the mucus layer and minor damage to the keratocytes. Sandpaper and brushing resulted in partly or fully loss of the epidermal layer. A different approach was used by Cordero and colleagues (Cordero *et al.* 2017a), where an electric toothbrush was used to remove the epidermal surface. Scale loss is another example of a partial-thickness wound. One or more scales are typically removed from the scale pocket, which results in a breach in the epidermal surface (Video S2). One of the challenges for most wound healing procedures is to fully control the depth of the wound. Dermal healing is different compared with that of epidermal and scale regenerative responses (Richardson *et al.* 2016; Sveen *et al.* 2019). If the deeper dermal tissue is damaged, a strong pigmentation response can be seen in the healing wound, together with the repair mechanisms of fibrous tissue (Fig. 4). Thus, it is of uttermost importance to control the wound depth of partial-thickness wounds to trigger similar healing responses.

Deep wound healing models are used in order to study regeneration and repair of all the layers of the skin, including fish scales, epidermal and dermal structures. Incisional wounds where the skin is cut with a scalpel or a razorblade, typically leaves a narrow cut through the skin (Anderson & Roberts 1975; Roubal & Bullock 1988). A bigger biopsy may be excised with a scalpel blade using a standard template (e.g. 10 × 10 mm; Bello & Emikpe 2013). When using a punch biopsy tool, the resulting wound will be similar in size to the biopsy needle head (Fig. 4b). Depending
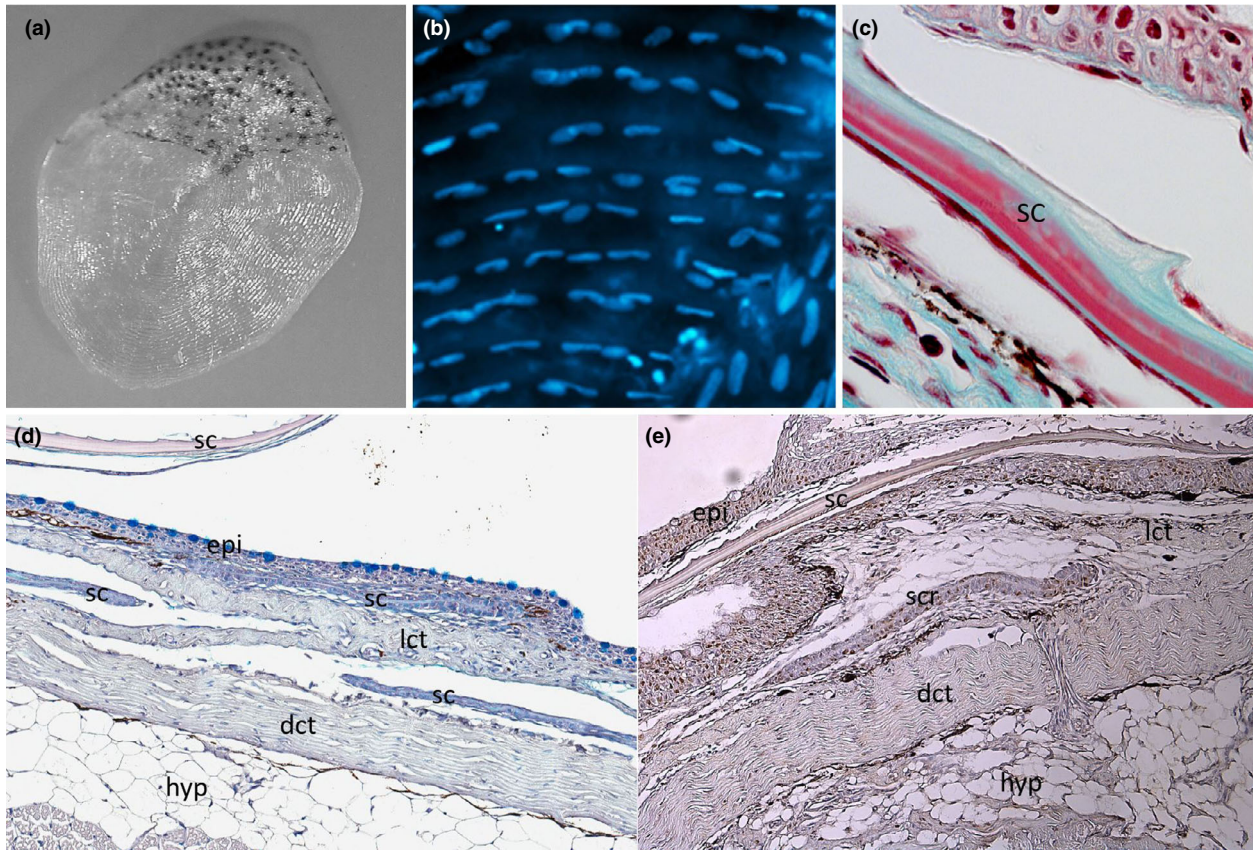
Figure 5 Details of Atlantic salmon fish scales. (a) Fish scale with epidermis and ring structures. (b) Fluorescent nuclei stained (DAPI) of osteoblasts in ring structures on a scale. (c) Fish scale (sc) with an upper mineralized collagen plate (blue) and a unmineralized plate (red). (d) Scale regeneration (scr) in post-smolts (AB/PAS stain), with intact epidermis (epi), loose connective tissue (lct), dense connective tissue (dct) and hypodermis (hyp). (e) Scale (sc) regeneration in post-smolts (stained with PCNA, nuclei of proliferating cells stain brown).

on the species being investigated, the size of the biopsy tools matter. In scaled fish, consider to remove the scales before the skin is punctured (Schmidt 2013; Jensen *et al.* 2015). A third option is the use of a dermal lasers to penetrate the skin (Richardson *et al.* 2013; Richardson *et al.* 2016). By using the same duration and intensity on the laser, a deep wound may be quickly and reproducibly introduced to the fish (Richardson *et al.* 2013; Seo *et al.* 2017). However, the cost and availability of the equipment may limit the implementation of this method.

Combination of techniques

A combination of different wound healing models can be useful under some experimental situations. Comparison of skin swabs to that of abrasion and deep wounds in Indian major carp (*Labeo rohita*) infected with *Cyprinid herpesvirus 3* revealed that the viral infection was dependent on an intact mucus layer (Raj *et al.* 2011). A similar study in Atlantic salmon combined mucus removal and deep wounds with infection with the bacterial species *Vibrio anguillarum* and *Aeromonas salmonicida*. Removing the mucus layer by swabbing resulted in elevated mortalities with *V. anguillarum*, while elevated mortalities were only observed in combination with deep wounds after *A. salmonicida* infections (Svendsen & Bøgwald 1997). In a separate trial, skin subjected to either partial or deep scarification also increased infection and ulcer development of the Atlantic salmon skin pathogens *Moritella viscosa* and *Tenacibaculum* spp. (Olsen *et al.* 2011). Mechanical injuries of the skin can also be used for comparative purposes, such as to separate wound healing from mechanical injuries and parasitic damage. Braden and colleagues (Braden *et al.* 2012) compared abrasion-associated injury to louse-associated injury in the skin of Atlantic salmon and chum salmon (*Oncorhynchus keta*). The infection responses were consistently higher than those caused by abrasion. These studies demonstrate the utility of multiple methods and approaches to reveal the complexity of wound healing mechanisms in fish.
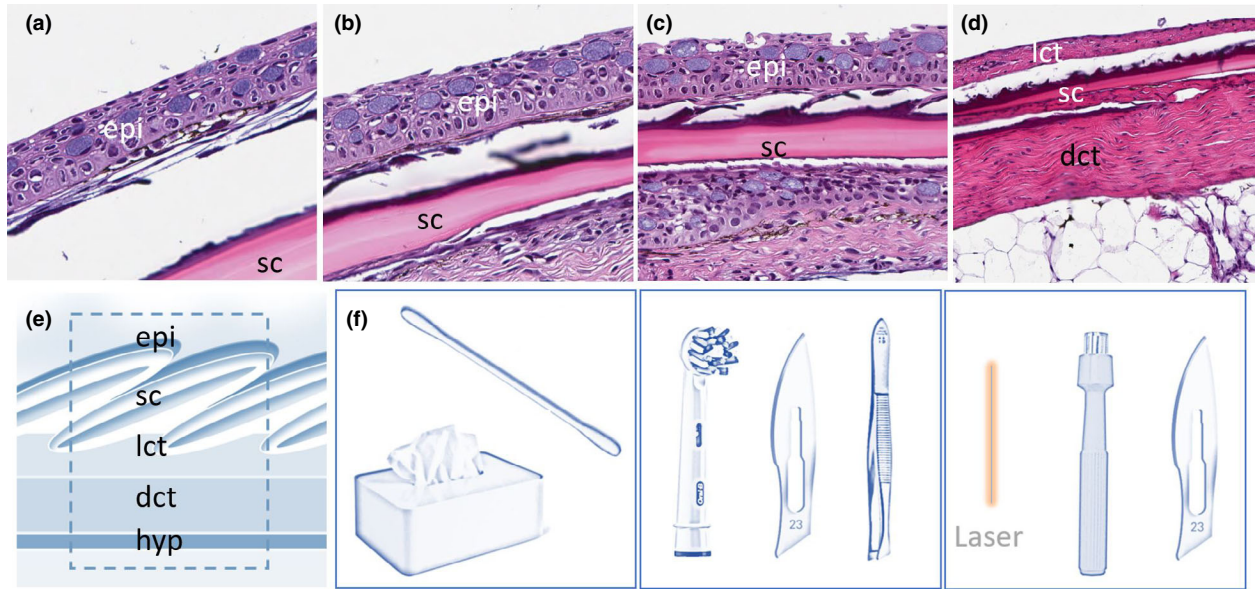
Figure 6 Mechanical induced wounds in fish for experimental purposes. (a) Intact epidermis. (b) The skin surface was wiped with tissue paper damaging the keratocytes at the apical surface. (c) Tissue swab damages the keratocytes at the apical surface and cause mucous cells to empty their content. (d) Scraping with a scalpel results in loss of the epidermis and scales. (e) Dotted lines illustrate skin damage inflicted in deep wounds. (f) Tools used to inflict skin damage for experimental purposes. Tissue paper and swabs remove the mucus layer. Toothbrush, tweezers, and scalpels can be used to induce partial damage. Scalpel blade, laser and punch biopsy tools can be used to inflict deep wounds. (a–d) Histological staining with HE.

Healing of mechanical induced wounds in fish skin

In recent years, studies on mechanically induced deep wounds healing have gained increased attention. Table 1 lists 27 studies utilizing 13 different fish species including model species such as zebrafish (*Danio rerio*), and fish farmed for commercial value such as salmonids (*Salmonidae*), carp (*Cyprinidae*), breams (*Sparidae*) and catfish (*Siluriformes*). These studies imply that a deep wound activates a wound healing cascade, with re-epithelialization, inflammation, granulation tissue formation and tissue remodelling (Guerra *et al.* 2008; Richardson *et al.* 2013; Schmidt 2013; Sveen *et al.* 2019). This cascade shares many similarities with the classical wound healing cascade in mammals. However, the initial re-epithelialization step observed in fish is replaced by the initial blood clot formation in mammals (Fig. 7). Unlike mammals, fish are poikilothermic, with a fluctuating internal temperature caused by of the variation in the ambient environmental temperature; thus, wounds heal faster in temperate-species than cold-adapted species (Figs 8,9; Schmidt 2013).

Re-epithelialization and epidermal regeneration

As in mammals, the first response to a deep wound is bleeding, there is however no evidence for blood clot formation in fish (Roubal & Bullock 1988; Richardson *et al.* 2013). Despite this, genes involved in haemostasis and blood coagulation are active during the first days after wound infliction (Sveen *et al.* 2019). An amorphous substrate filling the wound has also been identified by several authors (Roubal & Bullock 1988; Sveen *et al.* 2019). This structure may smoothen the initial wound surface aiding in the early migration of the keratocyte cells (Fig. 2b; Sveen *et al.* 2019). The keratocytes cells rapidly move in a collective sheet to the wound site to cover the exposed area (Figs 2a,10; Anderson & Roberts 1975; Quilhac & Sire 1999). During this process, the keratocytes undergo structural changes depending on where they are in the epidermis (Quilhac & Sire 1999; Richardson *et al.* 2016; Caraguel *et al.* 2016). The keratocytes which initiate migration, belongs to the intermediate layers of the epidermis (Quilhac & Sire 1999). In partial-thickness wounds, these cells spread on the surface from the posterior and anterior side of the wound (Quilhac & Sire 1999), and migration stops when the migrating cell fronts meet each other (Quilhac & Sire 1999; Richardson *et al.* 2016). Fast rearrangements of the keratocyte cells during migration are possible through extensive recruitment of cells from the adjacent epidermis (Quilhac & Sire 1999; Richardson *et al.* 2016). The main reservoir of recruited keratocytes is suggested to originate from the inter-scale pockets, indicating the importance of scales and scale pockets in wound healing and re-epithelialization rates (Richardson *et al.* 2016). In the case of deep

Table 1 Existing literature on mechanical wound healing in fish, showing fish species, topic of the study, tool used to inflict the wound, duration of the experiment in days after wounding (dpw) and reference

Family and species	Studied effect	Wound type	Days	Reference
Cyprinidae				
Common carp (*Cyprinus carpio*)	Beta-glucans	6 mm pb	14	Przybylska-Diaz *et al.* (2013)
Indian Major Carp (*Labeo rohita*)	Role of chromophores	Incisional	20	Rai *et al.* (2012)
Mrigal Carp (*Cirrhinus mrigala*)	Asiaticoside	2 mm pb	30	Verma *et al.* (2017)
Rohu (*Labeo rohita*)	Wound surface	Incisional	4	Rai *et al.* (2012)
Rohu (*Labeo rohita*)	Curcumin	4 mm pb	30	Kumari *et al.* (2017)
Zebrafish (*Danio rerio*)	Characterization	Dermal laser	28	Richardson *et al.* (2013)
Zebrafish (*Danio rerio*)	Re-epithelialization	Dermal laser, scale loss	3	Richardson *et al.* (2016)
Zebrafish (*Danio rerio*)	Silver nanoparticles	Dermal laser	20	Seo *et al.* (2017)
Zebrafish (*Danio rerio*)	Collagen mixture	5% acetic acid injection	1<	Xiong *et al.* (2018)
Cichlidae				
Tilapia (*Oreochromis niloticus*)	Vitamin C	10 × 10 mm cut	16	Jauncey *et al.* (1985)
Channidae				
Striped snakehead (*Channa striata*)	Characterization	4 × 6 mm cut	35	Banerjee and Mittal (1999)
Salmonidae				
Atlantic salmon (*Salmo salar*)	Temperature	Incisional	21	Anderson and Roberts (1975)
Atlantic salmon (*Salmo salar*)	Temperature and zinc	5 mm pb	14	Jensen *et al.* (2015)
Atlantic salmon (*Salmo salar*)	Hydrocortisone implants	Incisional	90	Roubal and Bullock (1988)
Atlantic salmon (*Salmo salar*)	High fish density	5 mm pb	57	Sveen *et al.* (2018)
Atlantic salmon (*Salmo salar*)	Characterization	5 mm pb	57	Sveen *et al.* (2019)
Rainbow trout (*Oncorhynchus mykiss*)	Beta-glucans	6 mm pb	100	Schmidt *et al.* (2016)
Rainbow trout (*Oncorhynchus mykiss*)	Vitamin C	Incisional	21	Wahli *et al.* (2003)
Sparidae				
Gilthead seabream (*Sparus aurata*)	Body site	8 mm pb	7	Ceballos-Francisco *et al.* (2017)
Gilthead seabream (*Sparus aurata*)	Body site	4 mm pb	15	Cordero *et al.* (2017b)
Siluriformes				
African catfish (*Clarias gariepinus*)	Plant extracts	10 × 10 mm cut	14	Bello and Emikpe (2013)
African catfish (*Clarias gariepinus*)	Vitamin C	10 × 10 mm cut	14	Erazo-Pagador and Din (2001)
African catfish (*Clarias gariepinus*)	Characterization	10 × 20 mm cut	30	Guerra *et al.* (2008)
African catfish (*Heterobranchus bidorsalis*)	Probiotic	Incisional	14	Akanmu *et al.* (2016)
African catfish (*Heterobranchus bidorsalis*)	Clove basil	lacerated (1 cm)	14	Abdel-Tawwab *et al.* (2019)
Walking catfish (*Clarias batrachus*)	Characterization	5x3 mm cut	35	Dutta and Rai (1994)
Serrasalmidae				
Small-scaled pacu (*Piaractus mesopotamicus*)	Chromium carbochelate	15 × 10 mm cut	35	Bortoluzzi *et al.* (2017)

pb, Punch biopsy wound.

wounds, scales and clefts may hinder or delay keratocyte migration (Richardson *et al.* 2016). Because of this partial-thickness wounds are re-epithelialized at a faster rate compared with deep wounds which has a more uneven surface compared with most partial-thickness wounds (Fig. 10; Richardson *et al.* 2016). The initial re-epithelialization process is also believed to be independent of cell proliferation and inflammation (Roubal & Bullock 1988; Quilhac & Sire 1999; Caraguel *et al.* 2016; Richardson *et al.* 2016). Evidence for this comes from studies in zebrafish and Atlantic salmon where hydrocortisone treatment results in a reduction of the inflammatory response, but with no apparent effect on the re-epithelialization process (Roubal & Bullock 1988; Richardson *et al.* 2013). When the migrating cell fronts meet, the keratocytes proliferate and as a result the neo-epidermis thickens (Banerjee & Mittal 1999; Jensen

et al. 2015). In a partial-thickness wound, skin regeneration mainly involves the re-epithelialization processes, followed by scale regeneration (Figs 5,10). Several authors have focused on the molecular processes involved in scale regeneration and generated several reviews on this topic (Bereiter-Hahn & Zylberberg 1993; Sire *et al.* 1997; Sire & Akimenko 2004).

In a deep wound, the neo-epidermis initially contains few mucous cells. However, as the keratocytes proliferate and the epidermal layer thickens, the number of mucous cells increase (Fig. 2e; Guerra *et al.* 2008; Kumari *et al.* 2017; Sveen *et al.* 2019). In the newly formed epidermis of Atlantic salmon, the mucous cells are not randomly dispersed, but displayed apically as beads on a string (Figs 2,10; Jensen *et al.* 2015; Sveen *et al.* 2019). The apical position of these cells is likely an early innate defence system,

Cutaneous wound healing in mammals

Cutaneous wound healing in mammals follows a cascade with hemostasis, inflammation, proliferation, formation of granulation tissue, re-epithelialization, wound contraction and tissue maturation. Hemostasis begin with vasoconstriction and blood coagulation with thrombocytes and platelets in a fibrin network. Fibrin clot formation is absent in fish. Inflammatory cells invade the blood clot triggering a local inflammatory response. Re-epithelialization in adults is driven by an inflammatory response, typically initiated 5 – 10 days post wounding. Embryonic models can re-epithelialize small skin wounds in the absence of inflammatory responses, like that observed in fish. Simultaneously granulation tissue is formed and the wound contracts. The transition from granulation to scar tissue occurs between 10 - 15 days post-injury. In the following months (and years) the scar tissue slowly remodels. Among mammals, there are differences in the regenerative abilities. The African spiny mouse can regenerate full-thickness wounds, while human skin has limited ability to regenerate.

Figure 7 Skin healing in fish and mammals, a comparison. The figure is based on the following literature (Seifert *et al.,* 2012; Gonzalez *et al.,* 2016; Richardson *et al.,* 2016; Sorg *et al.,* 2017).
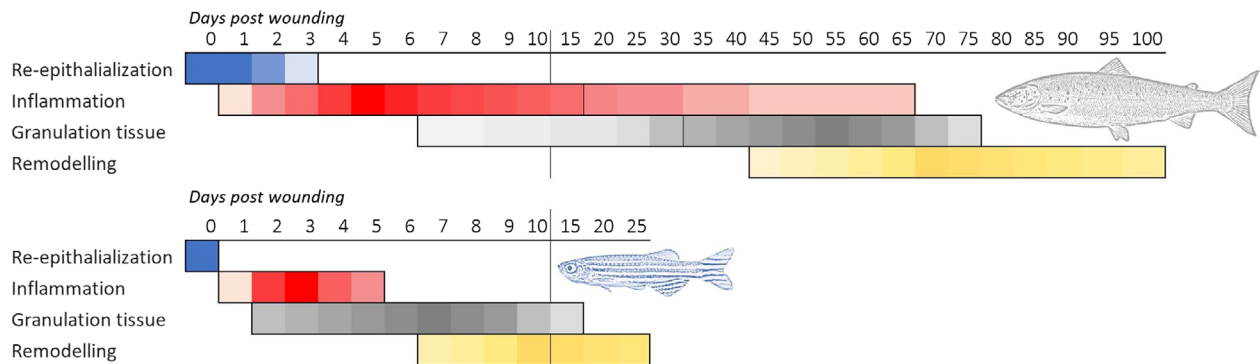

Figure 8 The wound healing cascade. Mechanical induced deep wounds in fish activate a conserved wound healing cascade with four overlapping phases, re-epithelialization, inflammation, granulation tissue formation and tissue remodelling. Wound healing rate in warm water species such as zebrafish is faster compared with cold-water species. Fish is poikilothermic with a fluctuating internal temperature because of the variation in the ambient environmental temperature. As with all chemical processes, the wound healing rate is to a large degree temperature dependent. Life stage, environment and diet may also influence the wound healing rate. The figure is based on publications listed in Table 1.

which acts to protect the neo-epidermis and wound bed. The secreted mucus may also change its viscosity and adhere to the wound surface (Fig. 2f; Sveen *et al.* 2019),

and evidence suggests a different glycosylation pattern with less acidic charges; however, the results require further verification (Sveen 2018). The epidermal layer continuous to
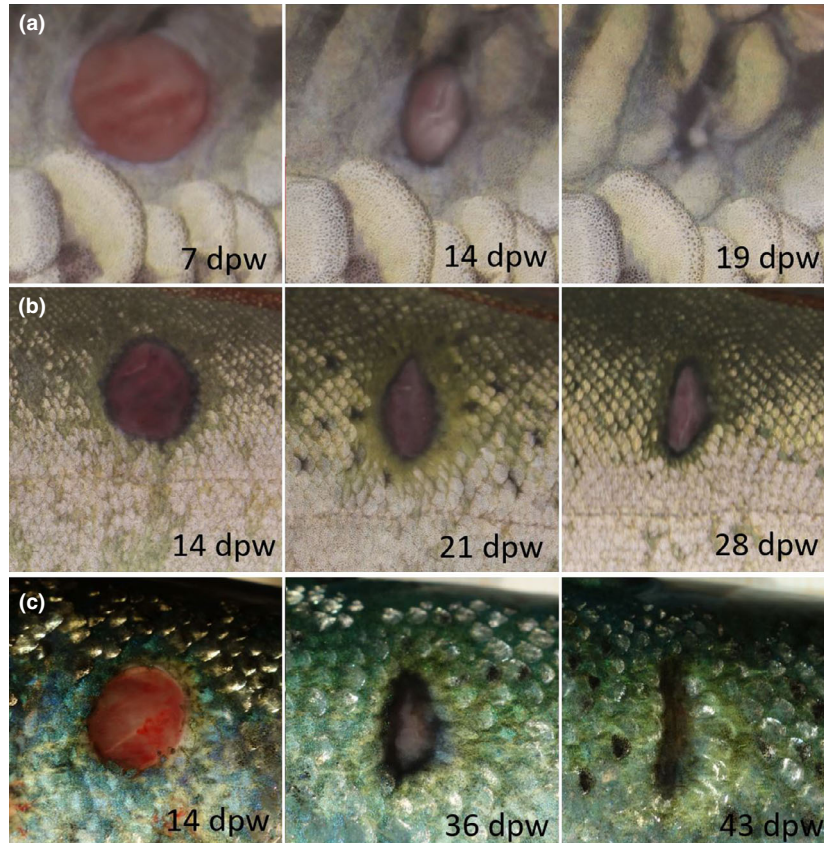
Figure 9 Photographs of healing punch biopsy wounds. (a) Carp (*Cyprinus carpio*), temperature 21°C. (b) Rainbow trout (*Oncorhynchus mykiss*), temperature 15°C. (c) Atlantic salmon (*Salmo salar*), temperature 10°C. The wounds slightly expand before contraction. Panel (a and b), Photograph: Jacob Schmidt (Schmidt, 2013; Sveen *et al.,* 2019).

reconstruct during the entire wound healing process (Rai *et al.* 2012b; Sveen *et al.* 2019), but eventually the epidermis is able to fully regenerate including the initial pigmentation pattern (Richardson *et al.* 2013).

Inflammation follows re-epithelialization

In the wound bed, an inflammatory response with recruitment of leucocytes and macrophages is necessary to clear the wound from damaged tissue and drive the repair processes (Richardson *et al.* 2013). Initially, neutrophils remain behind the leading edge of the re-epithelializing epidermis (Richardson *et al.* 2013). Later, more neutrophils and macrophages are recruited to the wound site (Roubal & Bullock 1988; Iger & Abraham 1990; Richardson *et al.* 2013). In zebrafish, the number of neutrophils and macrophages peaked at 8 h after wounding and gradually dropped over a four-day period leaving some macrophages in the wound bed (Richardson *et al.* 2013). Transcriptional analysis in Atlantic salmon and rainbow trout suggest similar mechanisms, with a small delay in early innate immune responses (Schmidt *et al.* 2016; Sveen *et al.* 2018; Sveen *et al.* 2019). Early recruitment and later maturation of B- and T-cells are also suggested; however, these findings need further verification (Sveen *et al.* 2019). Transcription of *matrix metalloproteinases* (9 and 13) and acute phase effector genes showed a short lag phase, with peak transcription levels between 3 and 14 days after wounding (dpw; Schmidt *et al.* 2016; Sveen *et al.* 2018; Sveen *et al.* 2019). Further, the transcription profiles of *matrix metalloproteinase 9* in common carp (*C. carpio*), Atlantic salmon and Japanese flounder (*Paralichthys olivaceus*) suggest a critical role of these enzymes during the inflammatory response (Murakami *et al.* 2006; Skugor *et al.* 2008; Braden *et al.* 2012; Krasnov *et al.* 2012; Schmidt *et al.* 2016). In mammals, the matrix metalloproteinases are secreted by both keratinocytes and macrophages (Schultz *et al.* 2005), and they degrade extracellular matrixes such as fibrillar collagens, and control inflammation by regulating the activity of cytokines and chemokines (Xue & Jackson 2015). In this context, it is relevant to mention that enhanced activity of matrix metalloproteinases is reported as a key factor in
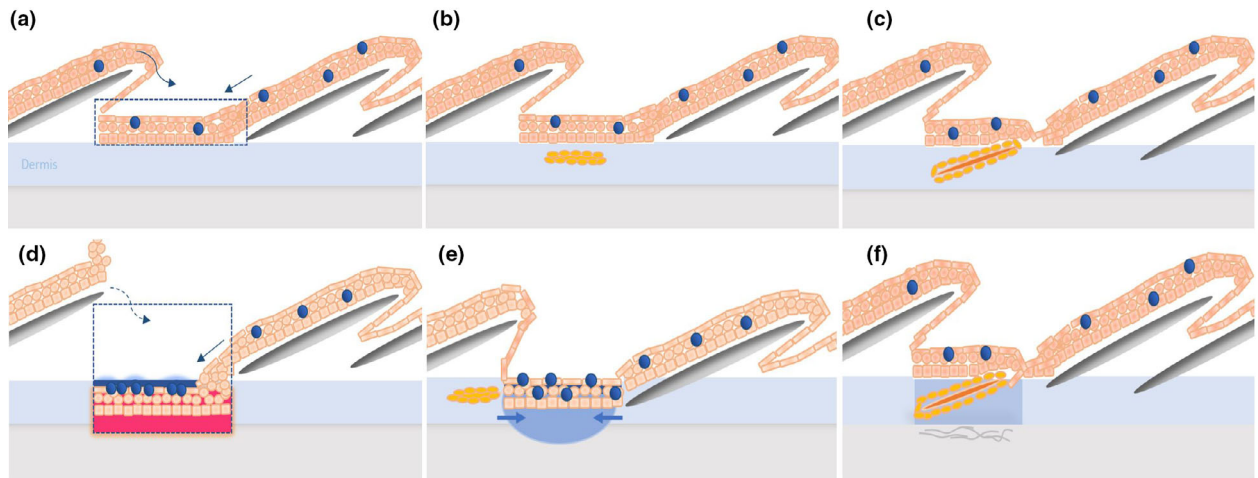
Figure 10 Simplified schematic presentation for the healing of partial-thickness wounds and deep wounds in fish. (a) In the case of partial-thickness wounds, keratocytes migrate to cover the damage from all directions and the incision closes in the middle of the wound. (b) Formation of papilla (developing scale) beneath the epidermal surface. (c) Extension of the developing scale. (d) In the case of deep wounds, keratocyte migration may be hindered (dotted arrow) and as a result re-epithelialization happens from the posterior part of the wound. A strong inflammatory response is triggered in the wound bed. (e) As wound healing progress inflammation subside, and granulation tissue is formed. The wound contracts. The scales develop at the wound edges. (d) The wound matures and the skin regenerates, while the muscle tissue heals with fibrotic repair. The figure is inspired by Richardson *et al.* (2016), Schmidt *et al.* (2016) and Sveen *et al.* (2019). Dotted square marks wound area.

chronic wounds (Reiss *et al.* 2010), where the fine tuning of the inflammatory responses is crucial for successful healing (Landén *et al.* 2016). This is also likely the case in fish, where inflammation and fibroblast growth factor signalling is necessary to drive the proliferation of cells and the subsequent formation of the repair tissue (Roubal & Bullock 1988; Richardson *et al.* 2013). In zebrafish treatment with silver nanoparticles, enhanced wound contraction was detected, which somewhat correlated with the induction of *matrix metalloproteinase 9* and *13* and pro-inflammatory cytokines (Seo *et al.* 2017). However, in Atlantic salmon reared at high fish densities, enhanced transcription of *matrix metalloproteinases* was on the other hand correlated to a delay in the formation of repair tissue (Sveen *et al.* 2018). This suggests that it is possible to skew the inflammatory response in fish, and by doing so, alter the outcome of the healing process.

Granulation tissue formation and wound contraction

The repair tissue, also known as granulation tissue, consists of connective tissue, fibroblasts, myofibroblasts, immune cells and small blood vessels (Roubal & Bullock 1988; Richardson *et al.* 2013; Sveen *et al.* 2019). The tissue typically grows from the wound borders and with time it replaces the damaged tissue (Richardson *et al.* 2013; Sveen *et al.* 2019). Zebrafish exhibit rapid granulation tissue formation, with tissue being present already at 2 dpw, with maximum size at 4 dpw (Richardson *et al.* 2013). In

comparison, granulation tissue and scale development were prominent 14 dpw in Atlantic salmon, with maximum size at 36 dpw (Sveen *et al.* 2018; Sveen *et al.* 2019). Comparable results were reported for rainbow trout and juvenile Atlantic salmon (Roubal & Bullock 1988; Wahli *et al.* 2003). In zebrafish, the granulation tissue is gradually cleared over a six-day period. Within a month from the original injury, the skin is almost fully regenerated with scales, subcutaneous adipocytes and skin pigmentation (Richardson *et al.* 2013). However, in the case of deep wounds, the muscle tissue does not fully regenerate. In this sense, skin regeneration of deep wounds in salmonids are comparable to that in zebrafish (Schmidt *et al.* 2016).

A deep cut through the fish skin is associated with immediate wound expansion (Schmidt 2013; Sveen *et al.* 2019). Later, granulation tissue is what drives the contraction (Sveen *et al.* 2019). The contracted area becomes elongated, in a dorsal-ventral direction, a phenotype observed in Atlantic salmon, rainbow trout, zebrafish, seabream and common carp (Schmidt 2013; Seo *et al.* 2017; Sveen *et al.* 2019; Fig. 9). It is likely that this contraction pattern could be caused by the angles of the cross-ply of the alternating collagen fibres in the *stratum compactum* (Szewciw & Barthelat 2017); however, this needs further verification. Wound position can also influence the contraction rate. In seabream, wounds located in the ventral region contract faster than wounds located in the dorsal region (Ceballos-Francisco *et al.* 2017; Cordero *et al.* 2017b). In African catfish, wounds in the caudal region healed faster compared

with the lateral region (Abdel-Tawwab *et al.* 2019). According to the authors, contrasts in immune responses and tissue texture led to the observed differences. In mammals, it is well documented that tissue tension and mechanical movement influence scar formation and tissue regeneration (Aarabi *et al.* 2007; Seifert *et al.* 2012; Harn *et al.* 2019). Similar responses might influence wound repair in fish.

Alterations in skin pigmentation

In the case of a deep wounds, there is a rapid release of melanin granules which slightly alters the skin pigmentation (Rai *et al.* 2012a; Schmidt 2013; Sveen *et al.* 2019; Fig. 4b). This rapid change in the skin melanocytes to injury suggest that these processes is under neural control (Rai *et al.* 2012a). A similar darkening of the skin is also observed directly after salmon lice (copepodite) attachment on Atlantic salmon skin (Fig. 4g).

As granulation tissue forms, the deep wound will obtain a dark colour which first appear at the wound edges (Fig 9). The hyperpigmentation is caused by melanocytes infiltrating the collagen-rich repair tissue (Fig. 4d,f; Sveen *et al.* 2018; Sveen *et al.* 2019). Wound hyperpigmentation is observed in multiple fish species including, bagrid catfish (*Rita rita*) (Mittal & Munshi 1974), common carp (Iger & Abraham 1990), striped snakehead (*Channa striata*) (Banerjee & Mittal 1999), African catfish (Guerra *et al.* 2008), Indian major Carp (Dutta & Rai 1994), Atlantic salmon (Sveen *et al.* 2019) and rainbow trout (Schmidt 2013). In humans, skin trauma may also result in dark spots, known as post-inflammatory hyperpigmentation (Davis & Callender 2010; Cardinali *et al.* 2012). The pigment itself is a polymer produced and secreted by the melanocytes, and it may act as an electron acceptor being important in protection against UV radiation and oxidizing agents (Różanowska *et al.* 1999; de Cassia & Pombeiro-Sponchiado 2005). During the biosynthesis of melanin, many toxic intermediates are produced, which together with melanin may exhibit antibacterial and antifungal activity (Mackintosh 2001; Burkhart & Burkhart 2005; Correa *et al.* 2017). One study in laboratory rats suggests that topical administration of a 'melanin-gel' with natural antibacterial and antifungal properties improved the initial wound healing in laboratory rats (Tab urets *et al.* 2016). The role of melanin and the melanocyte cells during skin regeneration is relatively unexplored in fish.

In vitro models as systems to investigate epithelial repair processes

Researchers should always strive to reduce, refine and replace (3R's) animals for experimental purposes. *In vitro* models offer the possibility of research wound healing without conducting experiments on live fish. Fish skin keratocytes may be cultured by extracting a scale from the skin on to a cell culture plate with medium (Video S1). Some fish species are however scale less and other methods for cultivating keratocytes need to be established. The keratocytes migrate from the fish scale and onto the surface of the culture plate, resulting in confluent cell sheets. These sheets may further be used in more detailed *in vitro* studies (Keren *et al.* 2008; Rapanan *et al.* 2014; Sveen *et al.* 2018). For some fish species, single-cell systems have also been applied to investigate keratocyte morphology and mechanism of cell motility (Keren *et al.* 2008; Wilson *et al.* 2010). During single-cell migration, the keratocytes have several shapes, all shapes are characterized by the main body and the lamellipodium (Keren *et al.* 2008). Rapid molecular dynamics and turnover of actin subunits in the lamellipodium allow for fast migration (Theriot & Mitchison 1991). In fact, fish keratocytes are among the fastest moving animal cells, with a migratory speed up to 1 micron/sec (Cooper & Schliwa 1986). Like other physiological rate processes, keratocyte motility is dependent on temperature (Ream *et al.* 2003). Keratocytes in general migrate faster at higher temperatures, but cell motility is also linked to the thermal tolerance of the species. The thermal limits of keratocyte motility appear to exceed upper and lower limits of the whole-organism in warm water species, but not for Antarctic species (Ream *et al.* 2003). Slow rates of keratocyte locomotion in cold-water species could influence and delay the healing processes.

The keratocyte ability to respond quickly to epidermal abrasion and their extraordinary rapid rate of migration is likely to constitute an important part of the wound healing progression. Thus, in terms of wound healing or re-epithelializing studies, the use of collective cell-sheet migration is of particular interest (Rapanan *et al.* 2014). In zebrafish, keratocyte explant cultures have been characterized as a wound healing model both by differential gene expression and morphological changes (McDonald *et al.* 2013). The keratocyte system appears to be a good model for studying central physiological processes in epithelial wound healing, and to understand the directional motile responses of the keratocyte including tissue remodelling. Such an *in vitro* model may also be used to supplement data from wound healing trials. As an example, keratocytes in cell cultures at low and high temperatures showed similar phenotypes as keratocytes in the epidermis of Atlantic salmon reared at high fish densities (Sveen *et al.* 2018). Electric fields direct cell migration and manipulate wound healing *in vivo* (Zhao *et al.* 2006), with similar results in *in vitro* studies (Sun *et al.* 2013). *In vitro* models could be used to screen for therapeutics and toxic substances to pre-select candidates prior to a big wound healing trial. Keratocyte cell cultures from Atlantic salmon skin was recently used to investigate

the importance of long-chained omega-3 in keratocyte mobility (master thesis by Martine Trorrisen, Nofima/ Karolinska Institutt), further confirming consistency between *in vitro* and *in vivo* trials for studies with Atlantic salmon skin (Ytteborg *et al.* 2018).

Wound care

For farmed fish, the goal of wound care is rapid wound closure. Wound therapy and preventive action could be addressed by improvements and optimization of abiotic water quality parameters such as pH, dissolved CO_2 and O_2, temperature and total ammonia nitrogen (Fontenot & Neiffer 2004). Optimizing stocking density to reduce social and chronic stressors could also facilitate wound healing (Sveen *et al.* 2016; Mateus *et al.* 2017; Sveen *et al.* 2018).

Antibiotics can be used to treat bacterial skin diseases such as infections with *Aeromonas hydrophila, A. salmonicida, Flavobacterium, Vibrio* and *Pseudomonas species* (Yanong 2003; Grave & Helgesen 2018). Here, it is relevant to mention that the use of antibiotics in the Norwegian fish farming industry is low (Grave & Helgesen 2018). Literature on the effect of antibiotics as a treatment of mechanical induced wounds is scarce, but a recent study investigated the effect of the enrofloxacin on the healing rate of surgical wounds in rainbow trout (Hjelmstedt *et al.* 2020). Contrary to the authors' hypothesis, enrofloxacin did not decrease the prevalence of infection or reduced the post-surgical recovery time. Instead, the treatment induced potentially adverse effects on both the heart rate recovery following the surgery and on transcription of the pro-inflammatory cytokine TNFα. A large review on antibiotic use in mammals also concluded that antibiotics to treat wounds should only be used when there is a risk of infection and not as an elective therapy for wound treatment (Altoé *et al.* 2019).

Functional feeds are supplemented with feed additives which is beyond the basal requirements for normal growth and development. Beta-glucans are naturally occurring indigestible carbohydrates found in the yeast cell wall. Common carp reared in water supplemented with two different types of beta-glucan showed faster wound contraction (Przybylska-Diaz *et al.* 2013). However, a similar treatment had limited effect in rainbow trout (Schmidt 2013). A combination of cochromium carbochelate and the yeast (*Saccharomyces cerevisiae*) had some effect on re-epithelialization and organization of dermal structures in small-scaled pacu (*Piaractus mesopotamicus*; Bortoluzzi *et al.* 2017). These findings may indicate that there may be differences between fish species and the effect of feed additive on wound healing. Another functional ingredient, curcumin an active component of the herb turmeric (*Curcuma longa*), is noted for having anti-inflammatory and antioxidant properties and suggested as a promising candidate in mammalian wound therapy (Mohanty & Sahoo 2017; Emiroglu *et al.* 2017). Indian major carp, fed curcumin in a 30 days wound healing trial showed evidence for early epidermal and dermal repair (Kumari *et al.* 2017). In rat, potential mechanisms of curcumin-induced wound healing may be linked to stimulation of fibroblast proliferation and migration (Dai *et al.* 2017) and modulate immune functions through stimulation of TNF-α and suppression of *matrix metalloproteinase 9* (Yen *et al.* 2018).

In African catfish, other unconventional ingredients such as clove basil extract (Abdel-Tawwab *et al.* 2019), plant extracts (Bello & Emikpe 2013) and probiotics (Akanmu *et al.* 2016) are suggested to enhance wound healing. Verma *et al.* (2017) tested the effect of the therapeutic asiaticoside on wound healing in Mrigal carp (*Cirrhinus mrigala*; Verma *et al.* 2017), while silver nanoparticles (Seo *et al.* 2017) and administration of collagen mixture have been tested in zebrafish (Xiong *et al.* 2018). More trials are however needed to conclude on the effects of these treatments on production fish.

There are surprisingly few studies on the effect of essential micro- and macro nutrients on wound healing. A sufficient level of ascorbic acid is necessary for normal development of granulation tissue, as demonstrated in African catfish, tilapia and rainbow trout (Jauncey *et al.* 1985; Erazo-Pagador & Din 2001; Wahli *et al.* 2003). Another study showed enhanced epithelial repair in Atlantic salmon fed a diet supplemented with zinc (Jensen *et al.* 2015). Transcriptomic results from our wound healing trial in Atlantic salmon also showed up-regulation of zinc transporters and genes related to the metabolism of arginine, glutamate, glutamine and lipid signalling (eicosanoids; Sveen 2018; Sveen *et al.* 2019). In mammals, suboptimal nutrition can alter immune function and collagen synthesis (Quain & Khardori 2015). This is not surprising, as successful wound healing is dependent on an interplay of signal molecules, enzymes and structural proteins which functions are directly dependent on the availability of micro and macro nutrients (Molnar *et al.* 2014). In mammals, administration of isolated nutrients beyond recommended amounts may have a pharmacologic effect to help wounds heal (Molnar *et al.* 2014). Based on the presented results, we suggest that treatment of fish skin wounds through nutritional manipulation of essential nutrients is one area of research that deserves further attention.

Concluding remark

Research on the skin surface of fish is essential to understand its role for fish health and the immediate interaction with the rearing environments during farming. However, the research field is faced with significant challenges. First,

cross-species comparisons may be challenging as environment and habitat conditions may vary greatly between species. Secondly, commercial production of fish compared with experimental fish may perform differently, as fish produced commercially will be exposed to environmental fluctuations and various management practices. Despite the amount of work on the topic, basic knowledge on long-term effect of environmental conditions, dietary treatments, the effect of handling, pharmaceuticals and other operational procedures remains to a large extent as knowledge gaps that need to be addressed.

Acknowledgements

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Video S1. Time-lapse light microscopy images of Atlantic salmon primary keratocyte cell culture exposed to the bacterium *Aliivibrio wodanis*.

Video S2. Scale loss results in a partial-thickness injury in the skin.