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Conceptual Paper

Role of Collagen in Wound Healing of Fishes

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The term 'wound' refers to the break in the continuity of tissue, it can be a varying degree of loss of substance caused by either physical, chemical, microbial, or immunological damage to tissue [8]. In aquaculture, fish are subject to injury from a variety of sources, like viral and bacterial pathogens causing internal hemorrhages, parasites, and predators that disrupt the mucosal surfaces by conspecific biting, abrasion against raceways, or contusions, from sorting procedures [13]. These wounds lead to poor welfare on the part of the fish and lead to downgrading fillet quality mostly due to poor visual appeal to the consumer. Wound healing is a physiological process of repairing proceeding injury to the skin and other soft tissues in humans and other animals. It is a complex cellular event which includes resurfacing, reconstruction and restoration of the tensile strength of the injured tissue. For fishes the epidermis protects from osmotic shock and entry of pathogens from the aquatic environment, any injury to it should be corrected rapidly to prevent further damage thus, re-epithelialization is fast in these water inhabitants.

Fish skin and wound healing

Skin is an important first-line defence system against pathogens, as fish are continuously exposed to multiple microbial challenges in their aquatic habitat [10]. For these reasons, it is important for fish to quickly reconstruct this barrier following an injury. Thus, the process of re-epithelialization is fast in teleosts in comparison with other animals. The mechanism of wound healing differs between wound types; Deep wounds in fish take longer to heal than superficial or partial wounds and the recovery process is similar to mammals.

After skin injury, scaled teleost species typically slough off wound scales due to disruption of the epithelium and the dermis lacks a vascular supply. Therefore, bleeding cannot be observed unless the dermis is destroyed. A blood clot does not have to form if hemorrhage occurs. When present, thrombi do not appear to play an important role in wound healing. The initiation of the inflammatory response is characterized by the infiltration of polymorphonuclear cells into the wound. Phagocytic cells, mostly mononuclear leukocytes, cluster around degenerating tissue. Epithelial cells begin to migrate along the wound edge, and rapid re-epithelialization of large surface areas requires the recruitment and migration of undifferentiated intermediate epidermal cells (IECs) at the wound edge. Despite relatively rapid re-epithelialization, skin tissue is still subject to osmotic shock and potential pathogens. Neutrophils and macrophages are recruited to the site of injury to induce inflammation and activate growth factor signaling that promotes cell proliferation and granulation tissue formation. Proliferation begins with an undifferentiated middle layer of epithelial cells formed during wound re-epithelialization. This one cell-thick layer proliferates to recreate three epidermal layers. Two to three days after injury, the epithelial layer of the wound area is infiltrated with fibroblasts and capillaries, causing avascularization at this location. The wound site resembles immature scar tissue. Collagen deposition occurs later, leaving scar-free tissue.

Phases of wound healing involve different cellular and bio-physiological events consisting of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling [5].

In mammalian wound healing- inflammation, re-epithelialization, granulation tissue formation and neovascularization coincide to a large extent however, in fish, re-epithelialization precedes granulation tissue formation which leads to a rapid closing of the

Phases of wound healing	Cellular and Bio-physiologic Events
Hemostasis	Vascular constriction Platelet aggregation, degranulation and fibril formation
Inflammation	Neutrophil infiltration Monocyte infiltration and differentiation to macrophage Lymphocyte infiltration
Proliferation	Re-epithelialization (birds and mammals)
	Granulation tissue formation Neovascularization Contraction Extracellular matrix formation
Remodelling	Collagen remodelling

Table 1

wound [11]. Collagen is known to play a vital role in the natural wound healing process, from the induction of clotting to the formation and appearance of the final scar.

Collagen- a miracle protein

Collagen is the most abundant protein of animal origin comprising 30% of total animal protein (1). Being a major constituent of the connective tissues collagen plays an important part in increasing the mechanical strength, integrity and rheological properties of the muscles and fillets. Collagen is a key component of the extracellular matrix (ECM) which plays a critical role in all phases of wound healing. In nature, there are at least 27 different types of collagen, named type I – XXVII variants which differ in their macromolecular structure [1]. Collagen is comprised of the primary amino acid sequence, Gly-X-Y. Mostly X and Y positions are filled by proline and 4-hydroxyproline. Collagen molecules are comprised of three chains 2 α and 1 β chain. Every sequence of α chain is composed of more than a thousand amino acids based on the sequence -Gly-X-Y. These three chains assemble together due to their molecular structure to form a triple helix [15].

Collagen and its types in tissues

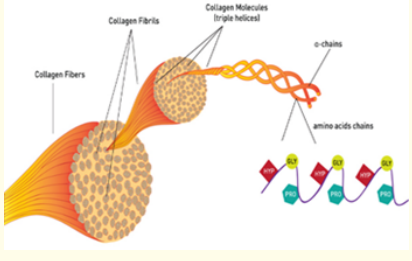

Figure 1

Collagen synthesis in fishes

The process of collagen synthesis occurs both intracellularly and extracellularly in fibroblast cells. During the time of wounding oxygen is scarce due to disruption of the vascular system as there is a high oxygen demand and consumption by cells. Low oxygen levels near the wound stimulate the proliferation of cells via growth factors. As a result, the lactate levels increase due to cell activity, it stimulates the production of enzymes involved in collagen synthesis [4]. Fibroblasts and myofibroblasts present in the dermal cells synthesize most of the ECM molecules (collagens, FN-Fibronectin and TNC-Tenascin C) and proteases (MMP-matrix metalloproteases like MMP9 and MMP13) required for new matrix production and remodelling. The fibroblast and myofibroblast cells produce procollagen through the process of transcription and translation. The procollagen thus formed undergoes modification in the Rough endoplasmic reticulum (RER) to form modified procollagen. Modification of procollagen is done by prolyl 4-hydroxylase (P4H) enzyme which helps in the hydroxylation of certain proline residues of collagen pro-α-chains which is essential for this triple helix formation. The procollagen is further modified in the golgi apparatus and secreted out of the cell by the process of exocytosis [6]. Secretion of collagen from fibroblasts into the ECM only occurs in the triple-helical form.

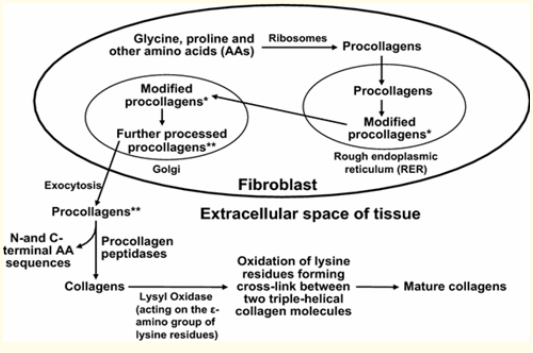

Figure 2

LOX (Lysyl oxidase) is the major collagen cross-linking enzyme which helps in the cross-linking of collagen fibers to increase wound tensile strength [3]. The main function of LOX is the oxidation of lysine residues in collagens and elastin to form spontaneously reacting peptidyl lysines. This facilitates cross-linking of matrix molecules, which provides rigidity and tensile strength to the matrix. Since the mechanical properties of the matrix influence the cells, LOX activity is an important regulator of wound-healing processes [2]. LOX is upregulated during tissue repair and fibrosis and it is induced by TGF-β (Transforming growth factor-β). Cellular recognition of collagens is mediated by a variety of sur-

face receptors, such as integrins, discoidin domain receptors, and immune-related receptors. Upon collagen binding, these receptors may activate other molecules related to remodelling, inflammation and wound-healing processes, such as matrix metalloproteases (MMPs), cytokines and growth factors. Once the provisional matrix has been replaced by a collagen-rich matrix collagen-production returns to normal to form a healed tissue.

During wound healing, the granulation tissue must become properly vascularized as myoblasts are unable to proliferate or differentiate more than 150μm away from a blood vessel. Vascularization of wound tissue may occur by sprouting from existing blood vessels (angiogenesis) or the formation and fusion of new vessels (neovascularization) [14]. FGF-2 (Fibroblast growth factor) and VEGF (Vascular endothelial growth factor) are important growth factors promoting angiogenesis [2]. These are expressed by macrophages and damaged epithelium, and FGF-2 by endothelial cells and nerves, FN and NO (Nitric oxide) also stimulate blood vessel formation. TGF-β on the other hand promotes endothelial quiescence and thus counteracts the effects of FGF-2, VEGF, FN and NO. Once the wound has contracted and sufficient amounts of new ECM have been produced the granulation tissue transits into more mature tissue by apoptosis of endothelial cells, and fibroblasts and by remodelling of the ECM [12]. Remodelling of muscle ECM molecules that form the provisional matrix (such as collagen type III and FN) are gradually replaced by collagen type I. The fibers are aligned along the lines of tension instead of the haphazard arrangement during the earlier stages of wound healing. Wound strength increases rapidly during the first weeks of remodelling, but only slowly afterwards, and it never reaches more than around 80% of the original strength in mammals.

The function of collagen in fishes

Unique characteristics of fish collagen are due to different structures and contents of amino acids. Fibrillar collagen types I and II are the most abundant proteins of ECM, they can form continuous triple-helical domains. These collagens can form stable large fibrils and complex fibrous superstructures that are responsible for the tensile strength of the tissues. Fish collagen has very specific amino acid compositions with a high concentration of glycine, hydroxyproline and proline [7]. When fish collagen is ingested, hydroxyproline peptides are not completely digested to free amino acids and can be detected in the blood. These hydroxyproline peptides stimulate cells in the skin, joints and bones, and lead to collagen synthesis through cell activation and growth. Collagen plays an important role in each phase of wound healing due to its chemotactic role. It attracts cells such as fibroblasts and keratinocytes to the wound, this encourages debridement, angiogenesis and re-epithelialisation. In aquatic organisms the wound closes by a combination of re-epithelialization and contraction thus the epidermis rapidly seals the affected area to regain osmotic homeostasis. The production of new extracellular matrix molecules is essential for the wound healing process, fibroblast and especially myofibroblasts are the main cell types responsible for the production of ECM molecules, including collagen.