Utilisation of fish skin waste as a collagen wound dressing on burn injuries: a mini review

A Afifah \(^1\), O Suparno\(^1\), L Haditjaroko\(^1\), K Tarman\(^2\)

\(^1\)Department of Agroindustrial Technology, Bogor Agricultural University, Bogor, Indonesia, e-mail: amaliaafifah111@gmail.com
\(^2\)Department of Aquatic Products Technology, Bogor Agricultural University, Bogor, Indonesia

Abstract. The fish processing industries produce wastes such as skin, fish scales, stomach contents, and bones. Those organic wastes have not been widely used. The biggest content of fish processing industry is collagen. Fish skin has the highest collagen content (5%-30%). Collagen has been widely used in pharmaceuticals, cosmetics, and food. One of the benefits of collagen is as a wound dressing on burn injuries. Collagen as the most abundant fibrous protein in fish is the main constituent of the extracellular matrix (ECM). ECM is a dynamic substance between cells in a tissue and has the ability to improve the tissue functions. Burn injuries are one of the types of wounds that has a long healing phase, which requires intensive treatment. Severe burn injuries could make people who experience it have traumatic and paralysis that affects the entire organ system. Collagen wound dressing was expected to be able to engineer tissue and accelerate cell regeneration to replace or regenerate damaged tissues. This paper will describe the potency of fish skin waste, problems in healing burn injuries, collagen extraction, and collagen application as wound dressing.

Key Words: burn injuries, collagen, fish skin waste, wound dressing.

1. Introduction
The fish processing industries produce wastes such as skin, fish scales, stomach contents, and bones. Those organic wastes have not been widely used. All of these wastes have high collagen content, and the highest is found in the skins. Collagen has been widely used in pharmaceuticals, cosmetics, and food. Out of all the collagen applications, the highest economic value lies in the field of cosmetics and pharmaceuticals. However, in terms of benefits, collagen in the pharmaceutical field is very useful in its role as healing assistance. In pharmaceutical, collagen acts as a homeostatic agent, regeneration of bone tissue, membrane oxygenator, contraception, implants, and drug delivery systems [1]. In addition, the problem in the pharmaceutical world are about halal. The collagen from fish processing industry is expected to solve this problem due to its halal source.

Collagen is a protein found in vertebrate and invertebrate animals. Collagen is found in the skin, tendons, and bones. The collagen has about 25%-35% of the total protein in the body. In the body, collagen is in the form of fibers and it plays a role in the formation of cell structures in the extracellular matrix to maintain tissue shape. Collagen is also a major component in building bones, teeth, joints, muscles, and skin [2]. Collagen is not toxic and has no side effects, thus it is compatible to be utilized in the pharmaceutical field. According to Kartika [3], the field of regenerative medicine has not focused on biomaterials as a stimulator of cell growth. They are still focusing on substances that trigger tissue...
regeneration. Collagen-based biomaterials can be used as materials to grow cells, combining this function with the biodegradable and biocompatible properties. Burn injury is a wound that has a long healing process, so it requires wound dressing that can help speed up the healing of the wound. With the above-mentioned characteristics, collagen is expected to be applied as a wound dressing to accelerate healing of burns.

Currently, collagen is mostly extracted from the skin of mammals, such as cattle and pigs. Collagen extraction from the skin of these mammals still causes problems related to religious beliefs, such as Islam. Thus, the collagen extraction research from fish has been developed to solve this problem. Kittiphattanabawon et al. [4] conducted collagen extraction on bamboo shark and blacktip shark with acid soluble collagen (ASC) and pepsin soluble collagen (PSC) methods. Singh et al. [5] extracted collagen in the skin of Pangasinosodon hypophthalmus using ASC and PSC methods. Skierka and Sadowska [6] extracted Godus morhua fish skin using ASC and PSC methods. Huang et al. [7] found a new method of extraction using high-temperature water in a short time, on Tilapia fish. The research was conducted to produce type I collagen with good physicochemical properties and high yield [7].

Red snapper, tuna, mackerel, cork, and belida are fish that are widely used as fillets. However, the skin from the waste of these fishes has not been utilized optimally. The collagen extraction process from these fishes needs to be optimized, especially during pretreatment by using NaOH, and during extraction. Pretreatment using NaOH is a process for removing non-collagen proteins contained in the ingredients and is influenced by different types of fish. There are several collagen extraction methods, i.e. acid soluble collagen (ASC), pepsin soluble collagen (PSC), papain soluble collagen (PaSC), and extrusion-hydro-extraction (EHE). However, because collagen utilization as a biomaterial, wound dressing requires high level of collagen purity, the use of enzymes as a method of extracting collagen can produce purer collagen.

2. Potency of fish skin waste
Fish waste has the potency to be utilized as a product of high economic value considering the national fisheries production in Indonesia in 2016 that reached 23.52 million tons [8]. The level of fish consumption (kg/cap/year) is always increasing every year. The Central Bureau of Statistics [8] predicted the level of fisheries production to reach 33.53 tons, and the level of fish consumption to reach 50.65 kg/cap/year. Of all fish waste, skin waste contains collagen with a yield value of 11%-63%, which is highly dependent on the type of fish, extracting material, and collagen extraction techniques [9]. Collagen can be used as a wound dressing with high economic value. Fish skin consists of two tissues, i.e. epidermal tissue and dermis tissue. Dermis is a layer of skin, which consists mostly of collagen fiber tissue that is constructed by binding fabric [10]. The fish skin composition is presented in table 1 [11].

| Table 1. Chemical composition of fish skin [11]. |
|---|---|
| Composition | Total (%) |
| Water | 69.6 |
| Protein | 26.9 |
| Fat | 0.7 |
| Ash | 2.5 |

3. Collagen
Collagen is a protein that makes up 30% of all protein in the body and is found in vertebrate and invertebrate animals [12]. To date, there are 28 types of collagen identified, i.e. type I to XXVIII and more than 90% as collagen I. Collagen is a fibrin protein (a protein in the form of fibres) that plays a role in the formation of the largest cell structure in the extracellular matrix that maintains tissue shape. Collagen is found in fibrous tissues such as tendons, ligaments, skin, cornea, cartilage, bones, blood vessels, and intestines. Collagen is also a major component in building bones, teeth, joints, muscles, and skin. Collagen can expand due to its weakening binding capacity of the molecular structure when treated at pH below 4 or pH above 10 [2].
Collagen can be utilized in various fields, such as leather manufacturing [13,14,15,16], gelatine, adhesive, food, pharmaceutical and cosmetic industries. The use of collagen in the pharmaceutical and cosmetic fields have high economic values. However, for the field of cosmetics, collagen utilization is still limited to a few groups. In the pharmaceutical field, collagen utilization is needed for people who experience various kinds of large open wounds.

4. Collagen extraction

Commercial collagen is obtained from cow hide, pork skin, or chicken skin, but its use still causes problems considering religious considerations and biological contamination such as MCD (Mad Cow Disease), TSE (Transmissible Spongiform Encephalopathy), FMD (Foot and Mouth Disease), etc. [17]. Therefore, the research is focusing on extracting collagen from fish skin. Collagen can be obtained by chemical hydrolysis or enzymatic hydrolysis [18]. Collagen can be extracted by using three methods, i.e. Acid Soluble Collagen (ASC), using enzyme or Pepsin Soluble Collagen (PSC), and hydro-extraction [7]. Extraction by using enzymes can be conducted not only by using pepsin enzyme but also by enzyme papain. The most used is the chemical hydrolysis method, but enzymatic hydrolysis produces better collagen characteristics with high nutritional value for ingredients that want to maintain the functionality [19]. The enzymatic process also produces less waste and can reduce processing time. However, enzymes are still relatively expensive. Before collagen is extracted, pretreatment must be conducted. The material commonly used for pretreatment is NaOH. Pretreatment is done to eliminate non-collagen substances in order to obtain high collagen yield.

The pretreatment process creates crosslinking bond between collagen and other open substances, thus separating the collagen. In the acid extraction process, there are several types of acids to be utilized, such as acetic acid, citric acid, lactic acid, and inorganic acids. However, organic acids are better in dissolving collagen [6]. The acid that is often used for collagen extraction is acetic acid [20]. ASC extraction is more efficient but some enzymatic extractions can control the hydrolysis of enzymes that results in collagen with a higher level of purity. The extraction method must consider the final characteristics of the collagen produced, such as thermal stability, molar mass, and water holding capacity [21]. The hydro-extraction process begins with pretreatment using a base. Afterward, the process continued with treatment using acid, after which the material containing collagen is extracted by using water at high temperatures and regulated pressure. The results from this extraction show a higher yield compared to acid and enzyme extraction [7]. The current development of collagen extraction from fish skin is not only with one method but is combined between these methods. Kittiphattanabawon et al. [4] extracted collagen in bamboo shark and blacktip shark using acid soluble collagen (ASC) and pepsin soluble collagen (PSC) methods. Singh et al. [5] extracted collagen in the skin of Pangasinodon hypophthalmus using ASC and PSC methods. Skierka and Sadowska [6] extracted Godus morhua fish skin using ASC and PSC methods.

5. Problems in healing burn injuries

The general burn injuries problems are morbidity and long-term disability. Burn wound healing involves several phases, i.e. coagulation, inflammation, granulation, proliferation, matrix synthesis and deposition, fibrogenesis, angiogenesis, wound contractions, and re-epithelization, which are the problems of healing those wound. The use of synthetic drugs in some burn cases causes reactions such as allergies and drug resistance. Thus, the drugs need to be derived from natural ingredients [22]. Healing burns is quite slow and sometimes causes infections, pain, and continuous hypertrophic scarring [23]. Burns are divided into three levels based on the depth of injury, i.e. first-degree, second-degree, and third-degree burns. First-degree burn affects the outer epidermis. Second-degree burn injures deeper layers of the epidermis and part of the dermis, accompanied by blisters. Third-degree burn affects all layers of the epidermis and dermis, and looks like dry wounds [24].
6. Wound healing phase

Wound healing is divided into three phases, i.e. inflammation, proliferation, and maturation. The inflammatory phase occurs from day 0 to day 5. In this phase, there is a direct response in the form of blood clots to prevent blood loss. Hemostasis occurs in this phase and will be prolonged if an infection occurs. This phase is characterized by tumors, rubor, dolor, and calor. The proliferation phase starts from day 3 to day 14. The proliferation or epithelialization phase is also called the granulation phase because of the formation of granulation tissue. This phase is marked by red and shiny wound. Granulation tissue is a combination of fibroblasts, inflammatory cells, new blood vessels, fibronectin, and hyaluronic acid. Epithelialization occurs during the first 24 hours marked by thickening of the epidermal layer at the edges of the wound. The maturation phase (remodeling) lasts from several weeks to two years. In this phase, new collagen formation changes the shape of the wound and increases tissue strength (tensile strength). Scar tissue is formed around 50%-80%, as strong as the previous tissue [3].

According to Nystrom [3], the wound healing process consists of 4 phases unlike Kartika [11]. Before the inflammatory phase, the wound healing phase is preceded by the hemostatic phase. This phase occurs at the beginning of injury. In this phase, the platelets cells play a role, where clots occur to prevent the bleeding from continuing. Cytokines are released by thrombocytes as growth factors that collect cells to help the next healing phase. After this phase is complete, the next phases are inflammation, proliferation, and maturation [3, 11].

7. Wound dressing

Wound dressings have long been used to bandage temporary wounds and burns. Wound dressings are used to replicate skin function to the maximum extent possible during healing. There are various types of wound dressings based on their functions, i.e. as antibacterial, water vapour transmission, and initial healing of wounds. The wound dressing has many advantages, such as its easiness, inexpensive use, plenty of availability, and easy technology. However, wound dressings are inadequate in mimicking skin function, making it difficult to solve the problem of morbidity and mortality that occurs in serious injury to the skin [25]. Biosynthetic dressings are being developed to mimic skin function and replace the epidermis and dermis. Burns are easily infected. Therefore, antimicrobial dressings are widely used to prevent infection and minimise bacteria. Antimicrobial dressing products contain silver, iodine, kadoxomer, and honey, in the treatment of burns [23].

Injuries can occur due to damage to normal tissue structures, both inside and outside the body. The wound is divided into closed wounds and open wounds. Closed wound is a wound that does not damage the surface tissue, such as sprains and broken bones. Open wounds usually occur accidentally due to accidents which are often called traumatic injuries, e.g. penetrating wounds, stab wounds, torn wounds, cut wounds, and burns. Burns have similar conditions as amputation wounds, i.e. its slow healing property [26]. The wound dressing is used to protect the wound from infection and trauma. Wound healing is 50% faster when the wound is moist instead of dry. Humid atmosphere is an optimal condition for accelerating wound healing and it can trigger new tissue growth [27].

8. Collagen application as wound dressing

Shettigar et al. [28] investigated collagen sanitary napkins by extracting collagen from sheep intestines. Collagen is made with a cross-linked technique using ultraviolet light and tested in rats with second-degree and third-degree burns. The collagen applied to the wound makes the epithelial rate faster. There is also a slight inflammatory change in fibre growth in the subdermal layer. However, this study only aimed to test the effectiveness of collagen in healing wounds. Therefore, it is unknown whether the use of collagen can cover the wound faster because the examination of the wound was not conducted every day [28]. Histopathology of wound dressing with collagen after 10 days of dressing is presented in figure 1.
Yang et al. [29] developed the research on collagen dressings. Collagen is extracted from pigskin and made into collagen sheets, which are then applied to burns. The design of dressing in this study is called young collagenous wettable membrane (YCMW). YCMW is in the form of sheet and contains type I and type II collagen, wet but will dry when applied to wounds. The wound dressing does not form antigenicity, more sterile, can help in healing wounds, and cheap. The YCMW’s weakness is that there are maceration and delays in separating eschar, so YCMW is only suitable for skin with shallow scarring and uninfected. YCMW is susceptible to infection, thereby needs additional material to overcome this deficiency [29]. YCMW is only suitable for dry and clean wounds. YCMW illustration can be seen in figure 2.

Duraipandy et al. [30] studied the easy contamination in the dressing. In this study, collagen was combined with plumbagin, which is a naphthoquinone compound with pharmacological properties. Plumbagin is cross-linked with nanosilver, which also has antimicrobial properties called PCSN (caging plumbagin on silver nanoparticle). Plumbagin that has been cross-linked with nanosilver, is then cross-linked with collagen. Cross-linking between collagen and PCSN can improve physical, thermal, and mechanical properties between collagen molecules, and improve their antimicrobial properties. SEM test result (figure 4) shows a uniform pore structure. This pore structure determines the ability to transport substances on the scaffold. The result of antibacterial activity testing (figure 3) shows cross-link of PCSN and collagen that were able to inhibit Escherichia coli and Bacillus subtilis bacteria, with a minimum concentration of 1.25 M. PCSN-collagen has more effective wound healing ability than pure collagen [30].
Collagen dressing continues to be developed to improve its properties and improve its capabilities. Xie et al. [31] conducted research by developing composites from chitosan, collagen, and alginate (CCA). CCA creation aims to act as wound healing and to inhibit the sea water. The application of dressing from collagen is developing towards the specific use. Collagen has low antigenicity, low inflammation, good biocompatibility, and good cell growth stimulation and cell proliferation. Chitosan has good properties, such as biocompatible, non-toxic, antibacterial, and hemostatic. Alginate is used in dressings because of its ability to absorb water and trigger wound healing. CCA composites are able to absorb exudate wounds well and create a relatively moist wound environment. CCA composites can also prevent injury from immersion in seawater for at least 4 hours [31]. The wound healing process and the rate of wound healing by CCA in mice are presented in figure 5 and figure 6.
Figure 6. Wound healing rates by CCA, pure chitosan, and gauze [31].

9. Conclusion
Utilization of fish skin solid waste can produce products with high economic values. The wound dressing as an application of collagen has advantages compared to conventional wound dressing, i.e. its practicality. The wound healing process is relatively faster, and it does not cause pain. The wound dressing made from collagen using cross-linking and other ingredients have better mechanical and antibacterial properties. Wound treatment using collagen wound dressing will make the wound heal faster than the use of conventional wound dressing. Burns is one of the wounds that takes a long time to heal. Thus, collagen wound dressing combined with other material may be used to heal burns in a better way.

References
[1] Schrieber R dan Gareis H 2007 Gelatin handbook: theory and industrial Germany (DE). Wiley-VHC
[2] Peranginangin R, Murniyanti, Nurhayati W, Rahmad 2014 Processing collagen from tilapia skin. (Jakarta: Penebar Swadaya) (In Indonesian)
[3] Kartika RW 2015 Chronic wound care with modern dressings. CDK. 42(7) 546-550. (In Indonesian)
[4] Kittiphattanabawon P, Benjakul S, Visessanguan W and Shahidi F 2009 Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (Chiloscyllium punctatum) and blacktip shark (Carcharhinus limbatus). LWT-Food Science and Technology 43(5) 792-800
[5] Singh O, Benjakul S, Maqsood S, Kishimura H 2011 Isolation and characterization of collagen extracted from the skin of striped catfish (Pangasianodon hypophthalmus). J. of Food Chemistry 124(1) 97-105
[6] Skierka E, Sadowska M 2007 The influence of different acids and pepsin on the extractability of collagen from the skin of Baltic cod (Gadus morhua). Food Chemistry 105(3) 1302-1306
[7] Huang CY, Kuo JM, Wu SJ, Tsai HT 2016 Isolation and characterization of fish scale collagen from tilapia (Oreochromis sp.) by novel extrusion-hydro-extraction process. 190 997-1006
[8] Central Bureau of Statistics [Indonesia]. 2017. Fisheries production. Jakarta: CBS
[9] Nagai T and Suzuki N 2000 Isolation of collagen from fish waste material-skin, bone, and fins. Food Chemistry (68) 277–281
[10] Padmasari R 2002. Utilization of red tilapia skin waste (Oreochromis sp.) As raw material for making siomay (Bogor, Indonesia: Bogor Agricultural University) (In Indonesian).
[11] Oosten JV 1969 Skin And Scale. (New York: Academic Press Inc.).
[12] Nystrom A 2016 Collagens in wound healing, In: Wound healing biomaterial-volume 2. (Cambridge (USA): Woodhead Publishing Series i Biomaterials).
[13] Covington AD, Evans CS, Lilley TH, Suparno O 2005 Collagen and polyphenols: new relationships and new outcomes. Phenolic reactions for simultaneous tanning and coloring. Journal of the American Leather Chemists Association 100(9) 336-343.
[14] Suparno O, Covington AD, Evans CS 2007 Novel combination tanning using diphenols and oxazolidine for the production of high stability leather. Journal of the Society of Leather Technologists and Chemists 91(5) 188-192.
[15] Suparno O, Kartika IA, Muslich 2009 Chamois Leather Tanning Using Rubber Seed Oil. Journal of the Society of Leather Technologists and Chemists 93(4) 158-161.
[16] Suparno O 2010. Optimization of Chamois Leather Tanning Using Rubber Seed Oil. Journal of the American Leather Chemists Association 105(6) 189-194.
[17] Setyowati H dan Setyani W 2015 The potential of nanocolagen for fish scales waste as cosmeceutical. J. of Science and community pharmaceutical 12(1) 30-40. (In Indonesian).
[18] Zavareze ER, Silva CM, Mellado MS and PrenticeHernández C 2009 Funcionalidade de hidrolisados proteicos de cabrinha (Prionotus punctatus) obtidos a partir de diferentes proteases microbianas. Química. Nova 32(7) 1739-1743.
[19] Martin L, Wilson CG, Koosha F, Tetley L, Gray AI, Senel S 2002 The release of model macromolecules may be controlled by the hydrophobicity of palmitoyl glycol chitosan hydrogels. J Control Release (80) 87-100.
[20] Liu D, Wei G, Li T, Hu J, Lu N, Regenstein M. and Zhou P 2015 Effects of alkaline pretreatments and acid extraction conditions on the acid-soluble collagen from grass carp (Ctenopharyngodon idella) skin. Food Chemistry (172) 836-843.
[21] Schmidt MM, Dornelles RCP, Mello RO, Kubota EH, Mazutti MA, Kempa AP, Demiate IM 2016 Collagen extraction process. Int. J of Food Research 23(3) 913-922.
[22] Zhang H, Chen J, Cen Y 2018 Burn wound healing potential of a polysaccharide from sanguisorba officinalis L. in mice.
[23] Wang Y, Beekman J, Hew J, Jackson S, Fisher ACI, Parungao R, Lajevardi SS, Li Z, Maitz PKM 2017 Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring.
[24] Sabiston 1992 Surgical Textbook (Jakarta: Buku Kedokteran EGC) (In Indonesian).
[25] Garfein ES, Orgill DP, Pribaz JJ 2003 Clinical application of tissue engineered constructs. J. of Clin. Plast Surg 30(4) 485-498.
[26] Stevens PJM, Bordui F, Weyde JAG 1992 Nursing Science (Jakarta: Penerbit EGC) (In Indonesian).
[27] Noer MS, Perdanakusuma DS, Saputro ID, Rizaliyana S, Basoeki AP, Airlangga PS, Semedi BP 2014 Emergency management of major burn (Surabaya: PT Revka Petra Media).
[28] Shettigar UR, Jagannathan R, Natarajan R 1981 Collagen film for burn wound dressings reconstituted from animal intestines. J. of Artificial Organs 6(3) 256-260.
[29] Yang JY 1990 Clinical application of collagen sheet, YCMW, as a burn wound dressing. J. of Burns 16(6) 457-461.
[30] Duraipandy N, Lakra R, Vinjimur SK, Korrapati PS, Ramamoorthy U, Kiran MS 2012 Plumbagin caged silver nanoparticle stabilized collagen scaffold for wound dressing. J. of Materials Chemistry B 00(1-3) 1-11.
[31] Xie H, Chen X, Shen X, He Y, Chen W, Luo Q, Yuan W, Tang X, Hou D, Jiang D, Wang Q, Liu Y, Liu Q, Li K 2017 Preparation of chitosan-collagen-alginate composite dressing and its promoting effects on wound healing. Journal of Biological Macromolecules, p 1-37.
Acknowledgments
The authors highly appreciate The Indonesian Ministry of Research, Technology and Higher Education
(Kementerian Riset, Teknologi dan Pendidikan Tinggi R.I.) for its research funding and support.