Biomedical and Pharmaceutical Application of Fish Collagen and Gelatin: A Review

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Abstract: In last decade, more research has been conducted in order to find the better way for utilizing the wastes product generated from food processing industries. The increasing demand of industrial by-products is one of the main reasons for the conversion of these wastes into valuable products. Among the different valuable products from the waste, the extraction of collagen and gelatin could be a better way of utilizing the wastes, due to their effective applications in biomedical and pharmaceutical industries. The most abundant source of collagen and gelatin are land-based animals, such as cow and pig. However, the extraction of collagen and gelatin from non-mammalian sources such as fish has been high influences in current society due to some religious and disease transmission issues. Many studies have dealt with the extraction and functional properties of collagen and gelatin from fish wastes. The present work is a compilation of information on biomedical and pharmaceutical application of collagen and gelatin from fish processing wastes.

Keywords: Collagen, gelatin, tissue engineering, wound healing, coatings.

INTRODUCTION

Collagen, a well-known protein, constitutes 25% of total proteins in vertebrates. The word "collagen" is derived from the Greek words 'kolla' and 'genos' meaning glue formation. There are more than 25 types of collagen that occur naturally in the body. Hydroxyproline plays an important role in stabilising the collagen triple helix, protecting against proteolytic digestion. Collagen plays an important role in the formation of tissues and organs, and is involved in various functional expressions of cells. Collagen is a good surface-active agent and demonstrates its ability to penetrate a lipid-free interface [1]. Collagen exhibits biodegradability, weak antigenicity [2] and superior biocompatibility compared with other natural polymers, such as albumin wheat gluten, corn zein, soy protein, and peanut protein. The main reason for the usefulness of collagen in biomedical application is it can form fibers with extra strength and stability through its self-aggregation and cross-linking. It has been used in biomedical industries and pharmaceutical applications. Their applications include treatment of pain associated with osteoarthritis, hypertension, use in tissue engineering, implants in human, inhibition of angiogenic diseases [3]. It is also used as dermal filler, as hemostat, for drug delivery, skin substitutes, expandable intra-arterial stents and cell attachment substrate [4].

Gelatin is a partially hydrolyzed form of collagen and mostly, it has been used as a potential agent in food and packaging industry [4]. Film-forming applications of gelatin, which is derived by partial hydrolysis of collagen, in the pharmaceutical and food industries include microencapsulation of ingredients and manufacture of tablet and capsule coatings [5]. The gel-forming property is one of the important properties of gelatin in classical food, photographic, cosmetic and pharmaceutical application. Recently in the food industry, an increasing number of new applications have been found for gelatin in products such as emulsifiers, foaming agents, colloid stabilizers, biodegradable film forming materials and micro-encapsulating agents, in line with the growing trend to replace synthetic agents with more natural ones.

Most of the collagen and gelatin are derived from cow and pig skins. The outbreaks of certain animal diseases such as bovine spongiform encephalopathy (BSE); and foot and mouth diseases (FMD) have caused restrictions on the use of animal collagen as there is a possibility of these diseases getting transmitted to human beings [6,7]. In such circumstances, fish collagen and gelatin are considered as the best alternative sources because of its high availability, no risk of disease transmission and no religious restrictions. Moreover, Fish processing discards, by-catch of unutilized as well as underutilized fish species, are the promising sources for the extraction of fish collagen and gelatin. Fish processing discards include skin, bones, scales and fins. They are generally dumped in-land or hauled into the ocean. Disposal of these wastes also caused environmental problems for seafood processors.

Collagen and gelatin has been recently used in the preparation of biodegradable biomedical products such

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as tablet, shield, films, sheets, microspheres, scaffolds and so on. The main objective of this review was to discuss the biomedical and pharmaceutical application of collagen and gelatin from fish processing wastes.

COSMETICS

Water soluble fish collagen is currently on the market as useful skin care product having a moisture retaining functions. The uses of collagen in cosmetic were well studied [8, 9]. A preclinical study investigated the effects of oral ingestion of hydrolyzed fish collagen, along with vitamin C and glucosamine; and suggested that the moisture content and smoothness of skin had improved [9]. Sun damage (extrinsic aging) and aging (intrinsic aging) causes collagen in the skin to deteriorate. Other than fish source, collagen is derived from animal sources and plant derivatives that act like collagen (pseudo-collagen) used as a cosmetic ingredient. Collagen in cosmetics, regardless of the source, has never been shown to have a direct effect on producing or building collagen in skin.

EDIBLE COATINGS ON MEATS, POULTRY AND SEAFOODS

Edible coatings from polysaccharides, proteins, and lipids can be extending the shelf-life of foods by improving the properties such as water loss, lipid oxidation and sensory as well as gas and vapor barriers. Although use of edible coatings and films to preserve food quality is not a novel concept, research in this field has intensified recently by many researchers. Factors contributing to renewed interest in development of edible coatings include consumer demand for high quality foods; new storage techniques for food processors; environmental concerns over disposal of nonrenewable food packaging materials; and opportunities for creating new market outlets for film forming ingredients derived from under-utilized agricultural commodities. Almost most of the food industry could utilize appropriately formulated edible coatings to meet challenges associated with marketing safe, nutritious, stable, economical, and high quality foods. Particularly with regard to the meat, poultry, and fisheries industries, the following are potential benefits of using collagen and gelatin edible coatings and films: (i) moisture loss during storage of fresh or frozen meats leads to texture, flavor, and color changes, while also reducing saleable weight. Edible coatings with good moisture barrier properties could help alleviate the problem of moisture loss. (ii) When fresh meat, poultry, or fish cuts are packaged in retail plastic trays, dripping

of product juices occurs making such packages unattractive to consumers. Edible coatings could hold in juices, prevent dripping, enhance product presentation, and eliminate the need for placing absorbent pads at the bottom of trays. (iii) The rate of rancidity-causing lipid oxidation and brown coloration-causing myoglobin oxidation in meats could be reduced by using edible coatings of low oxygen permeability, although not so low as to create anaerobic conditions. (iv) Collagen and gelatin edible coating solutions, which have been heated just prior to application, could reduce the load of spoilage and pathogenic microorganisms and partially inactivate deteriorative proteolytic enzymes at the surface of coated meat, poultry, and fish cuts. (v) Volatile flavor loss from, and foreign odor pick-up by meat, poultry, and seafoods could be restricted with collagen and gelatin edible coatings. (vi) As an application of active packaging, edible coatings carrying antioxidants and/or antimicrobials (e.g. organic acids) can be used for direct treatment of meat surfaces, thereby delaying meat rancidity and discoloration, and reducing microbial loads. (vii) Coatings applied on the surface of fish, poultry, and meat pieces prior to battering, breading, and frying, could improve the products nutritional value by reducing oil uptake during frying. It is evident from the above that edible coatings could substantially improve the quality of meats, poultry, and seafoods.

Jones and Whitmore [10] developed a method, where collagen was mixed with an aqueous mixture of lactic acid and glyceraldehyde; and make a coating for hamburgers capable of withstanding cooking temperatures without melting. Gennadios *et al.* [5] had developed a well-established technology in production of collagen sausage casings from the regenerated orium layer of food-grade beef hides. The application of gelatin in meat coating has been widely studied by several researchers. Edible coatings of gelatin have shown potential as carriers of antioxidants and prevent lipid oxidation of food product during frozen storage at −12 °C for 6 months with better sensory scores. Acidic aqueous solutions of gelatin and metal gelatinates were applied on processed meats such as sausages, Canadian bacon, boned hams. Upon drying, transparent coatings formed, offering protection against mold growth, salt rust, lipid oxidation, grease bleeding, and handling abuse. Moisture loss from frozen or refrigerated meat cuts was reduced by coating with hot melts consisting of gelatin, a polyhydric alcohol. Smoke cured chicken meat treated with an ethylene glycol/gelatin coating had greater moisture content by

15 to 21% than uncoated meat after 7 d at 27 to 31 °C. In addition, gelatin coatings on the surface of battered and breaded fish, poultry, and meat pieces had decreased oil absorption considerably during subsequent frying [11]. Gomez-Estaca *et al.* [12] reported that the edible coating of gelatin-chitosan film containing clove essential oil on Cod (*Gadus morhua*) fillets had inhibited the growth of microorganisms such as *Pseudomonas ssp.*, Lactic acid bacteria, *Enterobacteriaceae* and delay the TVB production up to 11 days.

The potential of replacing plastic meat wrappings with collagen-based edible films had been investigated by the researchers at the U.S. Army Natick Research, Development & Engineering Center (Natick, MA). In continuation, wrapping of collagen films in beef cubes, stored at −18 °C for 20 weeks were not significantly different than plastic films in terms of oxidation, color, microbial growth, and sensory attributes [13]. In addition, coating of edible collagen film on netted roasts, boneless hams, fish fillets, roast beef, and meat pastes, was commercialized in the U.S. Farouk *et al.* [14] reported that both refrigerated and frozen/thawed round beef steaks wrapped in collagen film prior to standard retail packaging (permeable film overwrap) or vacuum packaging exhibited significantly less fluid exudate than unwrapped controls. Moreover, the results of this study was clearly showed no significant effect on meat oxidation, color and sensory properties by the wrapping of collagen film on steaks. From the above report, it has been clearly explained the collagen films as edible coatings were maintained the meat quality for long time without any alter in its natural properties.

Overall, protein films exhibit relatively high water vapor permeability values, i.e. approximately two to four orders of magnitude greater than those of conventional polymeric packaging materials such as polyethylene, polypropylene, polyester, and polyvinylidene chloride [15,5]. The limited resistance of protein films to water vapor transmission is attributed to the substantial inherent hydrophilicity of proteins and to the significant amounts of hydrophilic plasticizers, such as glycerin and sorbitol, incorporated into films to impart adequate flexibility. On the other hand, the good oxygen barrier with better mechanical properties of collagen and gelatin films could be prepared with addition of other biomolecules such as chitosan and organo essential oils. Few studies have been initiated to develop biodegradable edible films using gelatin by incorporation of nanoclay particles [16] and other natural biopolymers like chitosan, starch and cellulose, in order to improve the mechanical and physical properties of the films [17,18].

IN TISSUE ENGINEERING

Different biomedical products of collagen and gelatin such as gel, scaffolds, microspheres and films have demonstrated its usefulness in tissue engineering applications and led to the development of bioengineered tissues, such as blood vessels, heart valves and ligaments [19-21]. Collagen shows hemostatic properties that promote blood coagulation and play an important role in tissue repair process. Collagen sponge or gel initiates adhesion and aggregation of platelets that lead to a thrombus formation [22]. Monomeric collagen does not activate platelet aggregation, while polymeric collagen having a regular arrangement of the molecules does activate it. Arginine side chains of collagen seemed to be responsible for its interaction with platelets [23]. A provisional extracellular support was developed using type I collagen lattice to organize the cells into a three-dimensional structure *in vitro* [24]. A small diameter (4 mm) graft constructed from type I bovine collagen was used earlier to integrate into the host tissue and provide a scaffold for remodeling into a functional blood vessel [25].

A. Collagen and Gelatin as Implants

Collagen based implants have been widely used as vehicles for transportation of cultured skin cells or drug carriers for skin replacement [26, 27]. Since sponge implant was originally developed for recovery of skin and was very efficient in that purpose, various types of artificial skin were developed as a form of sponge. Cultured skin substitutes developed on collagen lattice were also used for skin replacement. Reconstituted type I collagen is suitable for skin replacement due to their mechanical strength and biocompatibility [28]. Chronic wounds resulting from diabetes have been successfully cured with allogenic cultured skin substitutes prepared from cryopreserved skin cells [27]. In the cultured skin substitutes, the contracted collagen lattice was used as a support for epithelial growth and differentiation to replace pathological skin. Allogenic cultured dermal substitute prepared by plating fibroblasts on to a collagen sponge matrix and subsequently freeze dried from a 1% aqueous solution of atelocollagen provided a good environment for epithelialization [29]. The effectiveness of collagen sponges as a substrate for human corneal cells was

demonstrated and corneal cells exhibited normal cell phenotype when cultured individually on an engineered collagen sponge matrix [30]. Addition of selected antimicrobial drugs like amikacin to the bovine skin implantable collagen managed to control microbial contamination and increased healing of skin wounds [27]. The modified sponge for artificial skin was developed by combining fibrillar collagen with gelatin [30]. Dehydrothermal crosslinks were used to stabilize collagen-based sponge physically and metabolically. Some limitations inherent to cultured skin substitutes, such as deficient barrier function *in vitro* and delayed keratinization after grafting in comparison to native skin autografts, were reported [31]. Supp *et al.* [32] had developed a cultured skin substitutes from collagen with functional epidermis restoration *in vitro*. In the case of collagen and fibrin combination, cultured cells were best grafted directly onto the wound bed or in combination with either a thin layer of collagen or fibrin but not both. To address those limitations, modifications of collagen and gelatin based systems by the combination with other proteins, such as glycosaminoglycan, fibrin, and biotin, were proposed. The role of glycosaminoglycan and difference in its concentrations between pathological and normal tissues were reported earlier [33]. Dermal skin substitutes (membranes) made of collagen and glycosaminoglycan were found to be suitable substrates for the culture of human epidermal keratinocytes [27]. Cultured skin substitutes consisted of human keratocytes and fibroblasts attached to collagen-glycosaminoglycan substrates, which were subsequently crosslinked, decreased the rate of biodegradation and further reduced the engraftment of skin substitutes

B. Bone Substitutes

Among the many tissues in the human body, bone has been considered as a powerful marker for regeneration and its formation serves as a prototype model for tissue engineering based on morphogenesis. Collagen has been widely used as implantable carriers for bone inducing proteins, such as bone morphogenetic protein 2 (rhBMP-2) [34]. Recently, collagen itself was used as bone substitutes due to its osteoinductive activity [35]. Type I collagen crosslinked *N*-telopeptide was used as a marker of bone resorption and clinically used as a marker of bone metastasis of prostate cancer and breast cancer [36,37]. The polymorphisms of collagen type I alpha1 and vitamin D receptor as genetic markers for osteoporotic fracture in women was also reported [38]. This result added to

evidence that interlocus interaction is an important component of osteoporotic fracture risk. The mechanism of direct bone formation by BMPs–collagen complex was ultra-structurally investigated by Nakagawa and Tagawa [39]. The study also proved that direct bone formation is ectopically induced by bone morphogenetic proteins (BMPs) without cartilage formation when atelocollagen type I collagen pellet is used as a carrier. Collagen in combination with other polymers or chemicals was also used for orthopaedic defects. Demineralized bone collagen was used as a bone graft material for the treatment of acquired and congenital orthopaedic defects either by itself or in combination with hydroxyapatite [40]. The result of this study showed that grafted demineralized bone collagen in combination with hydroxyapatite was an excellent osteoinductive material and could be used as a bone substitute. More over, a study showed that addition of 500 IU of retinoic acid to collagen at a site of a bone defect enhanced regeneration of new bone, achieving union across the defect and leading to its complete repair [41]. The effect of type II collagen scaffolds and gel in biosynthesis of articular cartilage were studied by several researchers [42,43]. In addition, sponges made of gelatin by itself in resorbable gel foam were also used as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy [44]. When this gelatin sponge was implanted in an osteochondral defect in the rabbit femoral condyle, gel foam cylinders were observed to be very biocompatible, with no evidence of immune response or lymphatic infiltration at the site.

C. Drug Delivery

Advances in genetic engineering have resulted in a large number of biopharmaceuticals which must be administered by subcutaneous injection or intravenous infusion. Due to the susceptibility of many of these biopharmaceuticals to proteolysis and degradation, as well as due to their large sizes, parenteral administration is the most practical near term route for controlled delivery. While implanted systems (like pumps) provide precise dosages, injectable systems are more desirable because they eliminate surgical implantation. Some limitations of synthetic polymer matrices in parenteral delivery systems have been reported. The majority of investigations of natural polymers as matrices in drug delivery systems have centered on proteins and polysaccharides.

Natural biomaterials such as collagen [45], gelatin [46] have been widely used for drug delivery. The

major advantage of employing these biomaterials for controlled protein delivery is their excellent biocompatibility, compared to that of the synthetic polymers [46]. Type I collagen is a triple helical peptide molecule, which can self-associate into fibers and are biodegradable upon the actions of collagenases [47,48]. The effect of protein films, gels, microspheres and nanosphere have been widely studied in natural polymer drug delivery system

Microspheres

Gelatin microspheres were used as a drug carrier for parenteral delivery of cancer drugs, such as methotrexate [49]. Gelatin microspheres crosslinked with 20% glutaraldehyde had no difference in release rate of apomorphine compared than noncrosslinked microspheres [50]. Highly crosslinked collagen–gelatin microspheres could be useful for sustaining the release of highly hydrophillic drugs [51]. The microspheres showed zero order kinetics in the release profiles of incorporated drugs, due to a small size, a large surface area, high adsorptive capacity, and ability to disperse in water to form a clear colloidal solution.

Collagen based microspheres have been employed to sustain the release of IL-2, crosslinked collagen has been used to retard the release of insulin, and collagen hydrogels have been used to deliver cisplatin for cancer treatment. Collagen-heparin hydrogels have also been used as delivery vehicles in wound healing applications. Collagen gels loaded with liposomes have sustained the release of insulin and growth hormone for over a week. Chemically modified collagen (succinylated collagen) has been investigated for of gentamicin and pilocarpine *in vivo* [52]. Release of the drug into the systemic circulation was slower from liposomes sequestered in collagen gel than from vesicles alone [53]. Collagen molecules decrease liposome permeability by an antioxidant effect and also by a specific interaction with phospholipids [54].

Nevertheless, there is lack microsphere fabrication system available for collagen because of its poor mechanical and shape stability [55]. Apart from the lack of suitable fabrication method, the major challenge to adopt collagen as protein drug delivery system is that the meshwork is too open to retain the encapsulated protein in the matrix, therefore a secondary mechanism of retention must be employed [45]. It is also demonstrated that photochemical crosslinking can be used as a secondary retention mechanism for proteins in a collagen matrix. The above statements were

clearly demonstrated that a novel collagen and gelatin based protein drug delivery system were excellent protein compatibility than other proteins.

Capsules

Different types of collagen and gelatin capsules had been developed to understand the mechanism and better drug delivery by several researchers [56-58]. As a result, It has been concluded that a rod with a diameter and a length of 1 mm and 1 cm, respectively, is a useful shape as a drug delivery device, because this rod (minipellet) is small enough to be injected into the subcutaneous space through a syringe needle and still spacious enough to contain large molecular weight protein drugs, such as interferon [22] and interleukin-2 [57]. A single subcutaneous injection of a mini pellet caused a prolonged retention of interleukin- 2 and decreased its maximal concentration in the serum. This pellet carrier was used for local delivery of minocycline and lysozyme for the treatment of periodontitis symptoms. An attempt to produce a pellet type controlled release delivery vehicle made of purified type I collagen for water soluble osteogenic proteins was described by Lucas *et al.* [59].

On the other hand, Ochiya *et al.* [60] reported that a minipellet with a cylindrical shape (0.6 mm in diameter and 10 mm in length) containing 50 g of plasmid DNA and human HST-1/FGF-4 cDNA was evaluated as a controlled delivery system for plasmid DNA. This gene transfer method allowed a sustained release and expression of plasmid DNA in normal adult animals through the use of atelocollagen, a biocompatible polymer, as a carrier.

Water soluble proteins from a collagen based system induced cartilage and bone growth with a success rate of 76%, demonstrating the feasibility to formulate controlled release delivery systems for soluble bioactive factors, which interact with local responsive cells. Kohmura *et al.* [61] was investigated, the effect of atecollagen based mini pellet on the mRNA expression and functional status of facial nerve in the rat model. The facial nerve transaction and immediate repair was accelerated by this system and the facial nerve regeneration was ultimately achieved. The solid nature of atelocollagen *in vivo* seems to have a great potential for site- or tissue-specific transportation of target genes. The controlled gene transfer using atelocollagen in a form of pellet has allowed a prolonged systemic circulation of target products and has facilitated a long term use of naked

plasmid vectors for somatic gene therapy [60]. Further studies are needed to assess the applicability of atelocollage-based pellet systems for the augmentation of the bioavailability of low molecular weight materials, such as antisense oligonucleotides and biologically active oligopeptides, or virus vectors.

Nanosphere

Nanosphere formation is driven by a combination of electrostatic and electropic forces with sodium sulfate employed as a dissolving reagent to facilitate greater charge– charge interactions between plasmid DNA and collagen [62]. The molecular weight of collagen or gelatin has a decisive influence on the stability of the manufactured collagen/gelatin nanoparticles [63]. The molecular weight profile of the collagen solution was affected by pH and temperature, both of which further influenced the noncovalent interactions responsible for the molecular structure of collagen [64]. The relationship between electropic forces and gene factors was also evaluated. Polyion complexation between basic fibroblast growth factor and gelatin was studied by turbidity change of a mixed solution and isoelectric electrophoresis [65]. It was found that an electrostatic interaction was the main driving force for the complexation between acidic gelatin and basic fibroblast growth factor. The biodegradable collagen based nanoparticles or nanospheres are thermally stable, readily achieving their sterilization [66]. Moreover, nanoparticles can be taken up by the reticuloendothelial system [67], and enable an enhanced uptake of exogenous compounds, such as anti-HIV drugs, into a number of cells, especially macrophages [68], which may be an additional advantage of collagen based nanoparticles as a systemic delivery carrier. Thus, nanoparticles were used as a parenteral carrier for cytoxic agents and other therapeutic compounds, such as campthocin and hydrocortisone [69,70]. Collagen based nanoparticles have demonstrated their potential to be used as a sustained release formulation for anti-microbial agents or steroids [71]. Collagen nanoparticles were used to enhance dermal delivery of retinol [66]. The retinol in the system was very stable and facilitated a faster and higher transportation of the incorporated drug through the skin than the freshly precipitated drug.

Scaffolds

Collagen/gelatin scaffolds, one of the most representative extracellular matrix (ECM) proteins, have been used in different tissue engineering applications. The presence of signalling molecules, such as growth factors (GFs), within ECM-mimicking scaffolds is also critical for tissue repair, guidance and development [72]. Nevertheless, the use of GFs in a tissue engineering approach raises important issues: (i) GFs must target the desired cell population, thus minimizing signal propagation to non-target tissues and cells; (ii) GFs, even if bioactive at very low doses, are rapidly degraded once secreted; (iii) tissues should frequently be exposed to GFs for relatively long timeframes to obtain the desired effect. Therefore, a sustained and spatially controlled delivery of GFs should result in a more effective neo-tissue growth [73]. Recently, Jeevithan *et al*. [74] reported that addition of chitosan and calcium acetate had increased the mechanical properties (TS and YM) and functional properties (swelling ratios and shrinkage factor) as well as reduced biodegradation rate of fish gelatin scaffolds, which make the scaffolds more suitable for biomedical applications.

Films

The unique properties of collagen films make them a viable system for drug delivery. As indicated by the transport behavior of model drugs (retinoic acid, retinol palmitate, ascorbic acid 6-palmitate, and tocopherol acetate), collagen films are a suitable carrier. Crosslinking with appropriate concentration of chemical and biological plasticizers, further modifies the diffusion characteristics of collagen films and can be used to manipulate the desired release rate for model drugs.

D. Wound Healing

Healing is a complex process involving co-ordinated interactions between diverse immunological and biological systems. The various processes of acute wound repair, which are triggered by tissue injury, may be united into a sequence of four time-dependent phases: (i) coagulation and haemostasis, beginning immediately after injury; (ii) inflammation, which begins shortly thereafter; (iii) proliferation, which starts within days of the injury and encompasses the major healing processes; and (iv) wound remodelling, in which scar tissue formation takes place [75].

The process of wound repair involves the timed and balanced activity of inflammatory, vascular, connective tissue and epithelial cells. All of these components need an extracellular matrix to facilitate the healing process. To minimize scar formation and to accelerate healing time, different techniques of skin substitution have been introduced in the last decades [76]. Autologous skin grafting is still a gold standard.

However, in cases in which skin grafts are used, a new wound is created on the donor side. Thus, there is a need to eliminate a "new" wound to close the "old" one, and to close as many tissue defects as possible without the risk of large area infection, necrosis, tissue hypertrophy and contraction, as well as deformation of wound borders.

The evolution of biologic and synthetic wound dressings began with the recognition that any skin wound requires a barrier protection to prevent infection, desiccation and cell guidance by dermal elements to maximize healing. Any successful artificial skin or skin-like material should replace all of the functions of skin and therefore consist of a dermal portion and an epidermal portion. To achieve this goal, different types of scaffolds have been developed with different physical properties and a specific, unique host response by several researchers [74, 76, 77]. Scaffold materials can be either synthetic or naturally occurring. Synthetic materials such as poly(L)-(lactic acid) and poly(glycolic acid) have received considerable attention for tissue engineering applications and have shown promise in preclinical animal studies and some early human clinical trials. Synthetic materials have predictable and reproducible mechanical and physical properties (e.g., tensile strength and pore size) and can be manufactured with great precision. However, synthetic materials tend to elicit a foreign material type of response in the host, specifically, a fibrous connective tissue deposition leading to formation of dense scars and fibrosis. Therefore, naturally occurring materials such as hyaluronic acid and purified collagen have been investigated as alternatives to synthetic scaffolds. Collagen is a natural substrate for cellular attachment, growth and differentiation in its native state. In addition to its desirable structural properties, collagen has functional properties. Certain sequences of the collagen fibrils are chemotactic and promote cellular proliferation and differentiation. Collagen provides considerable strength in its natural polymeric state.

The source of collagen either purified from animal or fish sources and its treatment prior to use are important variables in the design of tissue-engineered devices. Biomaterials made of non-mammalian sources such as fish collagen/gelatin offers several different advantages: They are biocompatible and nontoxic to tissues (including neural and brain tissue) and have well documented structural, physical, chemical, biological and immunological properties. Different approaches to utilize fish derived collagen for tissue

substitution have been developed in the past 20 years:(a) the collagen gel, made of a mixture of fibroblasts and bovine collagen, (b) the collagen sponge based upon the production of a lyophilized collagen matrix in which fibroblasts are cultured and migrate, (c) the synthetic mesh composed of a nylon ora polyglactic acid mesh on which fibroblasts are cultured, (d) the collagen membrane used alone or with reconstructed epidermal sheet, and (e) the *in vitro* reconstructed skin like products based on collagen matrix [76].

The acellular collagen–chondroitin sulfate material was represented one of the first attempts at engineering dermal component [78,79]. Bell *et al*. [80] proposed a bilayered model of skin using contracted collagen lattices containing living dermal fibroblasts coveredin a second-step procedure with *in vitro* reconstructed epidermal sheets. Native collagen and collagen containing products have been proposed for covering superficial wounds, for tissue augmentation or as hemostatics in visceral surgery. The practical use of soluble collagen for wound healing is limited due to problems with storage stability and the time required to prepare enriched collagen solutions. Traditional collagen pads or vlieses manufactured from solubilized collagen material are not suitable for these purposes because of their high compression after application onto the wound surface and their lack of transparency. Recently, a novel collagen spongy matrix containing oxidized regenerated cellulose (ORC) named PromogranR has been introduced to both US and EU market [77]. PromogranR has been designed to treat exuding wounds including diabetic, venous and pressure ulcers. Since metallo-proteases may be elevated in chronic wounds and contribute to degradation of important extracellular matrix proteins and inactivate growth factors, their binding into the ORC/collagen matrix may have positive effect on physiological wound healing process [81].

CONCLUSION

The extraction of collagen and gelatin from non-mammalian species had grown in importance, as a way to convert by-products from fish processing industries waste. The properties of collagen and gelatin are varied with the origin of fish species and different part of fish as well as living environments of fish such as tropical and sub-tropical warm-water. An increasing demand of collagen and gelatin have been found in many applications in food industries as emulsifiers, foaming agents, colloid stabilizers, hydrogels, fining

agents; in food packaging industries as biodegradable edible film, micro-encapsulating agents; and in pharmaceutical, biomedical industries as capsules, bioactive peptides. Recently, many studies were urged to identify the impact of collagen and gelatin bioactive peptides on anti-cancer, anti-oxidant, anti-diabetic, anti-aging, cholesterol lowering agent, anti-arthrosclerosis activity. Therefore, many modern analytical methods have been developed to characterize the collagen, gelatin and its bioactive peptides properties from all new sources. Due to the excellent bioavailability and ''natural- like'' incorporation into the host tissue, fish collagen and gelatin could be used as a suitable biopolymer in biomedical and pharmaceutical applications.

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