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UTILIZATION OF FISH COLLAGEN IN PHARMACEUTICAL AND BIOMEDICAL INDUSTRIES: WASTE TO WEALTH CREATION

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ABSTRACT: Collagen is having enormous applications in biomedical industries while it's use is predominantly limited because of its high cost. With ever-increasing requirement in pharmaceutical industries, exploration of different sources and optimization of the extraction conditions of collagen has attracted the attention of researchers in the last decade. The most abundant sources of collagen are mammalian sources which are having major drawbacks. So, the industrial use of collagen obtained from non-mammalian sources is growing in importance. However, compared to mammalian sources, fish waste can be utilized as cost-effective alternative to produce collagen. Around 50-80% part of fish is discarded as a waste which contains high concentration of collagen. Fish collagen has multiple advantages over mammalian collagen and hence can be a promising alternative for it. This review summarizes the information of collagen, various sources and biomedical applications of collagen.

Keywords: Collagen; Biomedical; Pharmaceutical; Fish waste.

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1. INTRODUCTION

With enormous growth in biomedical sector in past decades, a novel field like research and development of biomaterials is getting very good attention nowadays. With massive growth in the development of science especially in medical fields and with increasing demands due to injuries and diseases; need of newer biomaterials is rising. In current scenario, a sharp incline is observed for the use of biodegradable materials in biomedical industries. Collagen has been potentially dominating in the fields of biodegradable materials. Collagen and its structurally similar form gelatin have been exhaustively exploited in biomedical, cosmetic as well as food industries. It has been predominantly utilized in tissue engineering scaffolds for the synthesis of artificial skin, artificial bone, artificial tendon and artificial cartilage [1]. Collagen is the most abundant protein in mammals representing nearly 30% of total proteins in the animal body. Collagen is the constitutive protein, having a crucial role in the formation of the building block of almost all tissues, organs and hence the complete body. Collagen has been considered as an excellent biomaterial for the development of tissue engineering constructs and wound dressing systems due to its high direct cell adhesion ability, low antigenicity, and exceptional biocompatibility. Collagens are reported to be processed into various forms such as films, injectable solutions, composites, fleeces, sheets, scaffolds, tubes, sponges, membranes, and dispersions [2]. Most imperative applications of collagen and gelatin lies in pharmaceutical industries while collagen/gelatin mediated controlled release drug delivery system is getting attention nowadays. In this review we discuss the structure of collagen, its sources and various biomedical applications.

Collagen and its Structure

Collagen is the complex constituent of extracellular matrix (ECM) and most abundant fibrous structural protein in all higher animals [3, 4]. It is mostly found in fibrous tissues such as tendon, ligament and skin in the form of elongated fibrils and is also abundant in cartilage, intervertebral disc, blood vessels, bone, and cornea [2]. A three-dimensional structure of collagen was proposed by Ramchandran as Madras model using fibre diffraction pattern of kangaroo tail tendon [5]. This model states that, collagen is a coiled coil conformation formed by the three polypeptide chains (Figure 1). This coiling results in a unique tertiary structure which is the most prominent feature of the collagen molecule called “triple helix”. Due to this structural complexity, collagen is very rigid in nature [6].

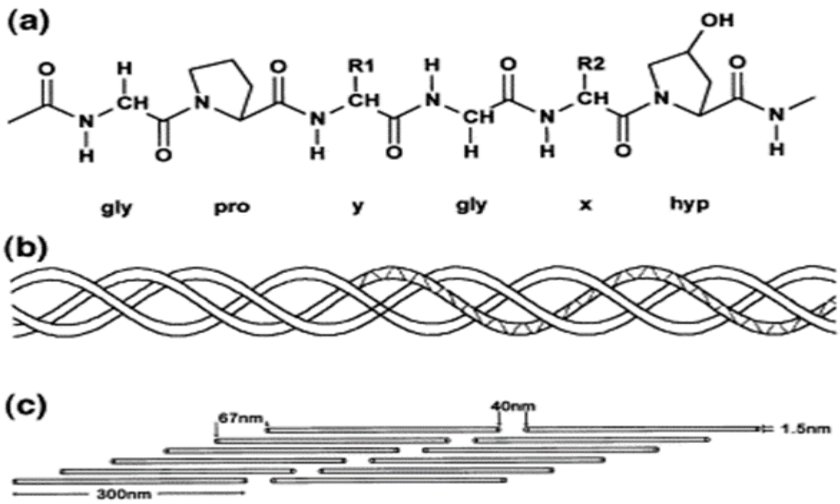

Figure 1: Collagen type I chemical structure. (a) Sequence of amino acids - primary structure, (b) left-handed helix - secondary structure; right-handed triple-helix - tertiary structure and (c) staggered - quaternary structure [7].

Collagen is formed of three identical or non-identical polypeptide chains; each of which composed of nearly 1000 amino acids. The unique arrangement of amino acids helps to stabilize the structure of collagen. Gly-X-Y is the most repeating unit in the collagen sequence. Glycine being the smallest amino acid is repeated at every third location in the order, while 35% of the non-glycine positions are occupied by proline at X position and 10% by the hydroxyproline at Y position [8]. Table 1 describes the abundance of amino acid in collagen from different sources such as rat, bovine and codfish [9].

Table 1: Amino acid composition of collagen obtained from the skin of codfish, rat and bovine commercial collagen (per 1000 residues) [9]

Amino Acid	Rat	Bovine	Codfish
Alanine	111.16	102.04	91.48
Arginine	42.24	32.86	30.45
Aspartic acid	45.32	36.65	38.82
Cystein	0.99	1.24	1.28
Glutamic acid	73.33	59.43	56.08
Glycine	333.18	296.44	266.12
Histidine	3.61	3.11	5.01
Hydroxylysine	9.33	8.86	6.65
Hydroxyproline	96.06	78.35	39.6
Isoleucine	7.48	6.74	5.61
Leucine	23.29	17.5	16.51
Lysine	27.07	22.2	19.62
Methionine	8.03	7.81	15.04
N-isobutylglycine	12.7	14.33	13.75
Phenylalanine	14.62	11.58	12.7
Proline	109.21	89.89	62.69
Serine	42.74	32.03	53.87
Threonine	18.79	13.2	16.89
Tyrosine	3.76	1.48	2.25
Valine	17.08	12.86	12.02

Sources of Collagen

In vertebrates, around twenty-eight diverse types of collagen have been identified composed of forty-six distinct polypeptide chains. Most abundant collagens are of type I, II, and III which provides the scaffolding and guide cells to migrate, proliferate and differentiate [2]. Gelatin shares the same physicochemical characteristic as that of collagen, being different form of the same macromolecule obtained by partial hydrolysis of collagen. Most of the times the collagen and gelatin used in the industrial products are obtained from mammalian sources (bovine and porcine) whereas; production of collagen and gelatin from the fish waste has received considerable attention in recent years [10]. Collagen can be obtained from animals and the most common sources are bovine, porcine, fish and human collagen. Other terrestrial sources comprise from rat tail tendons, chicken, frog skin, equine tendon, bird's feet, sheep skin, duck feet, alligator skin and kangaroo tail. [11]. Collagen from mammalian sources (bovine and porcine) are majorly utilized most of the times. The above-mentioned sources are cost-effective and easily available, but after prolonged use and concrete characterization, collagen from these sources tended to be allergenic and responsible for the risk of transmissible diseases like foot/mouth disease, bovine spongiform encephalopathy (mad cow disease), ovine and caprine scrapie and other zoonoses. Another reason for limiting the use of these sources due to certain religious practices which forbid the use of bovine and porcine products. [10]. On the other hand, marine collagen is a reasonable source which is considered as GRAS (Generally recognized as safe) by the FDA [12]. Fish is one of the most-traded food commodities in global market. Marked growth in fisheries and aquaculture sector was observed which was around 154 million tonnes in 2007 and increased up to 171 million tonnes in 2014. India's worldwide share in fishery industry is increasing day by day. India stands at 6th position worldwide in case of fish captures from marine sources and it is at 2nd position in fish captures from inland sources as per the report of Food and Agriculture Organization [13]. Huge amount of fish production and its consumption results in the generation of waste in equal quantities as that of final product. Various products are developed from fish waste such as enzymes, collagen peptides and fertilizers [14]. On the other hand, collagen is the most important product which can be extracted from fish waste [10, 15, 16].

Collagen Extraction

Extraction of collagen from fish waste consist of numerous steps. Fish waste is subjected to various pre-treatments followed by extraction method. Before pre-treatments fish waste is segregated such as bone, scale, skin and swim-bladder. The pre-treatment is given to the segregated fish waste to remove the impurities as well as to increase the quality of extracted collagen. Fish waste contains numerous contaminants such as lipids, non-collagenous proteins, pigments, calcium and other inorganic materials which can be removed in the pretreatment methods. There are number of methods reported for the extraction of collagen which are classified depending on the different methods used such as salt-soluble collagen (SSC), acid soluble collagen (ASC), and enzyme soluble collagen (ESC). The properties of collagen and its recovery are directly affected by the collagen extraction method. Therefore, all procedures must be performed at low temperature (~4 °C) to avoid the degradation of collagen [17]. Methods of collagen extraction from fish waste are summarized in figure 2.

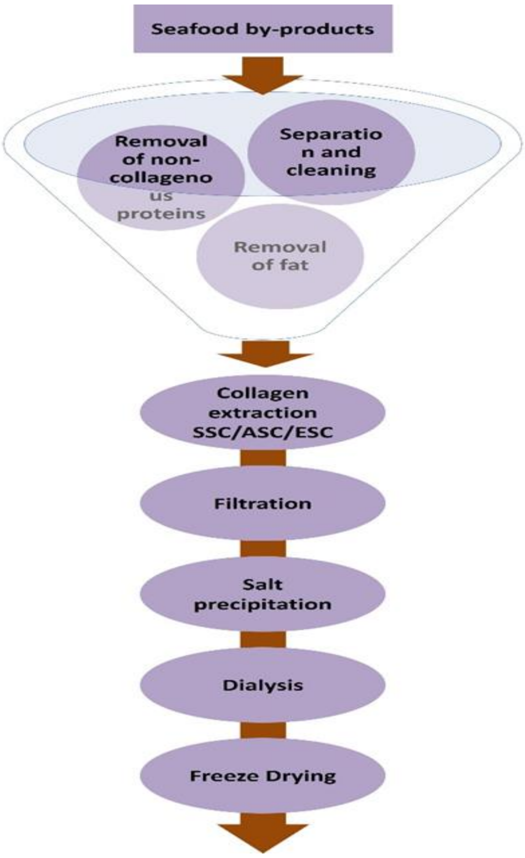

Figure 2. Methods for extraction of fish waste collagen

Collagen Biomaterials

Collagen has been considered as an excellent biomaterial due to its low antigenicity, high direct cell adhesion ability and exceptional biocompatibility. Collagen-based biomaterials can be utilized in two forms based on the degree of their purification: decellularized collagen matrices that maintain the original ECM structure and tissue properties; and more refined scaffolds prepared via extraction, purification, and polymerization of collagen with other biomaterials [18]. Extraction and purification of collagen from natural tissues is carried out in various ways. As solubility of collagen is very low due to its covalent cross-links mediated inert nature; it is insoluble in organic solvents but can dissolve in aqueous solutions, depending on the extent of cross-linking [19]. Collagen based biomaterials are particularly beneficial in wound healing since their wet strength permits their suturing to soft tissue and delivers a template for new tissue growth. Depending on the degree of cross-linking, the collagen biomaterials are degraded by collagenases into peptide and amino acids in 3–6 weeks, and the implant is then replaced by native type I collagen produced by fibroblasts. For medical applications, collagens are reported to be administered in numerous forms (Figure 3). scaffolds [20], fleeces [21], composites [22], dispersions [23], sponges [24], sheets [25], tubes [26], membranes [27], injectable solutions [28] and films [16].

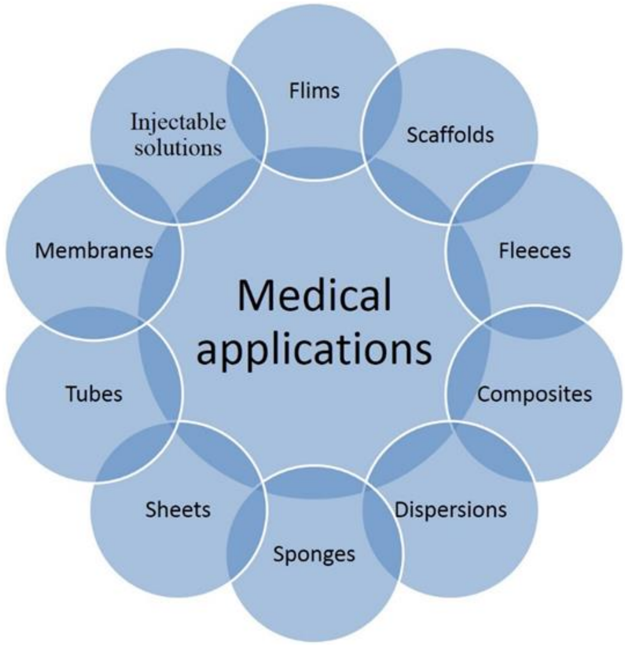

Figure 3. Medical applications of collagen

Scaffolds: Campbell et al. [20] developed collagen scaffolds that have been used to develop analogues of complex tissues in vitro. Anisotropic scaffolds supported the enhanced migration of an invasive breast cancer cell line MDA-MB-231 with an altered spatial distribution of proliferative cells in contrast to invasive MDA-MB-468 and non-invasive MCF-7 cells lines. The provision of controlled architecture in this system may act both to increase assay robustness and as a tuneable parameter to capture detection of a migrated population within a set time, with consequences for primary tumor migration analysis.

Fleeces: Zirk et al. [21] studied the effectiveness of collagen fleeces to prevent the post-operative bleedings. No standard protocol in prevention of bleeding events has met general acceptance among surgeons yet while this study gave very good results suggesting effectiveness of collagen fleeces. Sufficiently performed local hemostyptic measures, like the application of collagen fleeces in combination with atraumatic surgery, bears a great potential for preventing heavy bleeding events in hemostatic compromised patients, regardless of their anticoagulant therapy.

Composites: Fu et al. [22] studied the collagen-chitosan composite which were used as the cell matrix for smooth muscle cells, fibroblasts and BMSCs. The seeded cells retained their biological activity after being cultured in vitro and seeded into the collagen-chitosan composite material. Formed composite can be potentially exploited for biomedical applications as well as in novel field of 3-D bioprinting.

Dispersions: Artery buckling has been proposed as a possible cause for artery tortuosity associated with various vascular diseases. Since microstructure of arterial wall changes with aging and diseases, it is essential to establish the relationship between microscopic wall structure and artery buckling behavior. Mottahedi and Han [23] developed arterial buckling equations to incorporate the two-layered wall structure with dispersed collagen fiber distribution. Parametric studies showed that with increasing fiber dispersion parameter, the predicted critical buckling pressure increases. These results validate the microstructure-based model equations for artery buckling and set a base for further studies to predict the stability of arteries due to microstructural changes associated with vascular diseases and aging.

Sponges: Konstantelias et al. [24] carried out a systematic search for the effectiveness of gentamicin-collagen sponges (GCS) for the prevention of surgical site infections (SSIs). They stated that gentamicin-collagen sponges were associated with a lower risk of SSIs suggesting further high-quality randomized studies which are needed to confirm the benefit of GCS for lowering mortality rates.

Sheets: Peripheral nervous system injuries result in a decreased quality of life, and generally require surgical intervention for repair. Currently, the gold standard of nerve autografting, based on the use of host tissue such as sensory nerves is suboptimal as it results in donor-site loss of function and requires a secondary surgery. Nerve guidance conduits fabricated from natural polymers such as collagen are a common alternative to bridge nerve defects. Alberti et al. [25] stated that collagen sheets support directional nerve growth and may be of use as a substrate for the fabrication of nerve guidance conduits.

Tubes: Fujimaki et al. [26] developed a new scaffold material - oriented collagen tubes (OCT) and evaluated the potential of OCTs combined with basic fibroblast growth factor (bFGF) to repair of a 15 mm sciatic nerve defect in rats. Their findings demonstrated that OCT alone or in combination with bFGF accelerates nerve repair in a large peripheral nerve defect in rats.

Membranes: Nakahara et al. [27] assessed the impact of collagen membrane application and cortical bone perforations in periosteal distraction osteogenesis. Collagen membrane were found to be beneficial where cortical bone perforations have more impact on the osteogenic process.

Injectable solutions: Recently, stimuli-responsive nanocomposite-derived hydrogels have gained prominence in tissue engineering because they can be applied as injectable scaffolds in bone and cartilage repair. Due to the great potential of these systems, Moreira et al. [28] aimed to synthesize and characterize novel thermosensitive chitosan-based composites, chemically modified with collagen and reinforced by bioactive glass nanoparticles (BG) on the development of injectable nanohybrids for regenerative medicine applications. The results demonstrated that the addition of collagen and bioactive glass increases the mechanical properties after the gelation process. The addition of collagen increased the stiffness by 95%. Injectable nanohybrids demonstrated no toxic effect on the human osteosarcoma cell culture (SAOS) and kidney cells line of human embryo (HEK 293T). So, formed nanohybrids have the promising potential to be used as thermo-responsive biomaterials for bone-tissue bio-applications.

Films: Bhuimbar et al. [16] extracted collagen from skin of *Centrolophus niger* and used for the preparation of collagen-chitosan film. Films incorporated with 5% pomegranate peel extract declined solubility in water remarkably and showed antibacterial effect against food borne pathogens. So, this formed film can be potentially exploited for numerous pharmaceutical and food applications.

2. CONCLUSION

Collagen plays a crucial role in many pre-operative and post-operative surgical procedures. Intrinsic biodegradability by endogenous collagenases and higher biocompatibility make externally administered collagen ideal for use in biomedical applications. For several decades, dermatological defects have been treated with subcutaneous injections of collagen solutions. This application is a commercial success, particularly in the area of plastic and reconstructive surgery. Threat of allergic reactions and cost of collagen from mammalian sources are the two main reasons drastically limiting their use in biomedical applications. Collagen from fish sources are better alternative to mammalian sources which can overcome both the limitations of mammalian collagen. Large quantities of fish processing waste which act as a serious environmental pollutant are compelling source of collagen. Reports on the use of fish collagen in the preparation of pharmaceutical products are scanty. So, future research must be directed to utilize collagen from fish waste in various biomedical applications.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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