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Fish Collagen and its Applications in Food and Pharmaceutical Industry: A Review

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Abstract

Marine collagen has gained immense attention in the recent past as an appropriate alternative to mammalian collagen. The marine collagen is extracted from various marine sources and their by-products including processing waste including fish skin, scales, bones, etc. The type and functional properties of these collagen vary with their molecular structure, extraction process, source, and so on. Marine collagen is usually extracted using mild acids such as acetic acid or enzymes, at low temperature, to prevent denaturation and changes in molecular properties. Collagen finds wide application in food, pharmaceutical and biomedical industries.

Keywords: *Marine Collagen; Denaturation; Food; Pharmaceutical; Biomedical*

Introduction

The marine ecosystem is known for their most valuable food resource as shell fish and fin fish. The remarkable evolution of the marine biodiversity has made the marine environment as the world's richest natural resource with wide diversity of specific and potent bioactive compounds. Marine food from very long time has been linked to beneficial health properties and in recent past innumerable research teams were actively engaged in exploring the health benefits of various marine bioactive components. These include polyunsaturated fatty acids (PUFA), collagen, gelatin, marine carbohydrates (polysaccharides/prebiotics), minerals, vitamins, antioxidants, enzymes and bioactive peptides with valuable nutraceutical, pharmaceutical and cosmeceutical potentials. These were reported to display huge nutritional and health benefits. Various marine sources such as fish skin, muscle, bone, intestine, etc. from pre-processing and processing centers are used to isolate many of these bioactive compounds. Fish peptides and algal polysaccharides have also attracted a significant attention due to their anticancer, antidiabetic, anticoagulant, antimicrobial and antihypercholesterolemic activities [1]. Omega 3-fatty acids from fish oils and marine bacteria, polyphenols and pigments from algae, are also currently studied for their potent antioxidant activities [2]. These researches have gained enormous interest in recent past due to the growing need for novel health substances with least side-effects that would not only aid in the prevention and treatment of several communicable and non-communicable diseases but also supplement the diet and are easily obtainable. In this context, marine bioactive compounds are viewed as significant for their noteworthy potentiality as therapeutic and nutraceutical compounds.

Collagen, the major structural connective tissue protein found in skin, tendon, ligaments, bones, is most prevalent in extracellular matrix (ECM). One fourth of the animal total protein is represented by collagen. These are chiefly of bovine and porcine origin (commercial collagens); and associated with these are the risk of transference of zoonotic diseases such as bovine spongiform encephalopathy

(BSE), transmissible spongiform encephalopathy (TSE) and foot and mouth disease (FMD) or the religious constraints. The transmission of avian influenza has also restricted the utilization of collagen of poultry origin. Other best alternative sources are highly debated, and marine collagen has emerged as an appropriate yet relevant and feasible alternative to their mammalian counterparts [3]. Several marine sources such as marine sponges, jellyfish, squid and fish have been recognized for the readily available sources of marine collagen. The under-utilized resources such as, skin, bones, fins, heads and scales constitute about 75% of total fish weight and these marine discards are considered as the sustainable and cost-effective source of collagen [4-7]. Recent biotechnological advancements have discovered the utilization/transformation/incorporation of marine collagen (hydrocolloid) as functional biomaterials or bioactive compounds with pharmaceutical applications [7]. The high potential of collagen and its peptides have taken the medical industry (surgery, orthopedics, ophthalmology and dentistry) by revolution [7]. It has also found applications in cosmeceuticals as moisturizer, natural humectant, anti-aging and anti-wrinkling product [8]. It has also been accredited to have potential application in food industry [9].

The wide range of potentiality of marine collagen in both health and non-health sectors has interested the scientific community on sustainable exploration of marine products and its by-products as a valorization strategy. The increasing demand for health care and nutraceutical supplements is driving the market for marine collagen. The marine collagen market was valued at US $ 581.3 Million in 2017 and is forecasted to reach US $ 897.5 Million by 2023, growing at a CAGR of 7.7% from 2018 [10].

In the present review, the credibility of marine collagen as a potential candidate for medical, pharmaceutical, food, cosmetic and other industrial applications, has been detailed.

Marine collagen

Source, structure and type

The collagen is derived from Greek word “kola” and “gen” which means gum and producing. According to Protein Data Bank (PDB), it is the most abundant fibrillar protein available in the connective tissues including skin, joints, cartilage, teeth (collagen joined to mineral crystals), tendon, bones and others [3]. The mammalian collagen has been widely investigated and accordingly, approximately 25% of the mammal protein are collagen that contributes to stability and structural integrity of tissues and organs. In human, nearly 29 distinct types of collagen, made up of 46 distinct types of polypeptide chains each encoded by separate genes, have been identified. Amongst these, type I to type V are the most common types [3]. The different types of collagen have been grouped into 8 families based on their structure, chain bonding and the distribution in the human body (Table 1). Collagen are primarily fibril-forming, basement membrane, microfibrillar, anchoring fibrils, hexagonal network-forming, fibril-associated collagen with interrupted triple helix (FACIT), transmembrane and multiplexins.

The most defining feature of collagen is its structure that gives stability, functional ability, mechanical strength and modulate its physical characteristics. Collagen is constructed from tropocollagen that contains three polypeptide (three α-chain) fibrils with a diameter of 10 to 500 nm, molecular weight ranging between 285 and 300 kD (approximately), and 1400 amino acids with glycine as every third residue, which attributes to the collagen’s characteristic fibrillar helicoidal robust structure [3]. Each collagen monomer is a long cylindrical protein of 2800 A^o length and 14 - 15 A^o diameter. These are generally white, opaque, viscoelastic material with high tensile strength and low extensibility. The isoelectric point is around 5.8 and the shrinkage temperature for mammalian collagen (Ts) has been reported to be between 62 and 65^{o}C, while in fish it ranges between 38 and 54^{o}C. The denaturation temperature (25 - 30^{o}C) of fish collagen is considerably lower than its shrinkage temperature (Ts). The collagen has three parallel polypeptide chains in a left-handed, polyproline II-type (PPII) helical conformation, which coils with each other to form right-handed superhelix to form a rod like molecule. This structural motif is same for all collagen [11]. The repeating units characterized as (Gly-X-Y)n is the vital requirement of the triple helical structure of collagen where ‘X’ is mostly proline and ‘Y’ is hydroxyl-proline/hydroxyl-lysine (post-translational modification), which confers rigidity to the

Family	Type	Distribution	Application
Fibril-forming	I	Skin, bone, tendon (non-cartilage), dermis, cornea, ligament	Membranes for guided tissue regeneration
	II	Cartilage, vitreous humor, nucleus pulposus, lung, cornea, skin, bone	Cartilage repair, arthritis treatment
	III	Extensible connective tissue (skin, lung, vascular system viz. artery), skin, vessel wall, reticular fibers of most tissues (lungs, liver, spleen)	Hemostats and tissue sealants
	V	Co-distributed with Type-I, especially in cornea	Corneal treatment
	XI	Along with type II, vitreous body and cartilage	mAbs development for osteoarthritis
Basement membrane	IV	Basement membrane	Attachment enhancer of cell culture (mouse neuroblastoma) and diabetic nephropathy indicator
Microfibrillar	VI	Muscle, dermis, placenta, lungs, intervertebral disk, cartilage	Hemostat
Anchoring	VII	Dermal epidermal junction, skin, cervix, oral mucous	Treatment of dystrophic epidermolysis bullosa (DEB)
FACIT	IX	Along with type II in cornea, cartilage and vitreous body	
	XII	Tendon, perichondrium and ligaments	Regulator in early stages fibrillogenesis
	XIV	Along with type I in vessel walls, placenta, liver, dermis and lungs	
	XIX	Many tissues, human rhabdomyosarcoma	Antiangiogenic and antitumoral properties
	XX	Corneal epithelium, sternal cartilage, embryonic skin, tendon	
	XXI	Many tissues, blood vessel wall	Contribute to matrix assembly of vascular network during blood vessel formation
Transmembrane	XIII	NM junction, skin, hair follicle, intestine, lungs	Involved in inflammation and vasculogenesis, regulate bone mass
	XVII	Epithelia, skin hemidesmosomes	Teeth formation
Multiplexins	XV	Associated with collagens close to basement membranes, kidney, smooth muscle cells, pancreas	
	XVI	Many tissues including keratinocytes and fibroblasts	Drug target and biomarker
	XVIII	Close structural homologue of XV, liver, lungs	Retinal structure, closure of neural tube
Miscellaneous	VIII	Endothelium	
	X	Hypertrophic cartilage	
	XXII	Tissue junctions	
	XXIII	Limited in tissues, mainly transmembrane and shed forms	
	XXIV	Developing cornea, bone	
	XXV	Brain	
	XXVI	Testis, Ovary	
	XXVII	Embryonic cartilage	
	XXVIII	BM around Schwann cells	

Table 1: *Different types of collagen and their distribution in human body [3].*

molecule. The hydroxyl-proline/hydroxyl-lysine are further modified by glycosylation (addition of galactose and/or galactosyl-glucose). The strong hydrogen bonding between the -NH of glycine and -CO of neighboring chain proline also contributes to the stability of the structure (Figure 1). Collagen strands display a "Quarter Staggered" alignment indicating a longitudinal displacement and slight reduction (< $1/4^{th}$) of chain length with respect to neighboring chain. Covalent cross-links (histidino-dehydro-hydroxyl-mero-desmosine) provide extra stabilization to these fibers. The water molecules present in the structure maintains the conformation of the native collagen. The individual water bridges form and break within few picoseconds and prevent unwinding of imino-region. The water molecules that form single hydrogen bond (backbone), destabilizes the helical structure; however, the hydration shell that covers the entire helix serves as an extensive cylinder of hydration surrounding triple helix and hydroxyproline residues; thus, supporting this connection. This hydration shell, a biological lubricant, determines the inter-helix distance [11] (Figure 1).

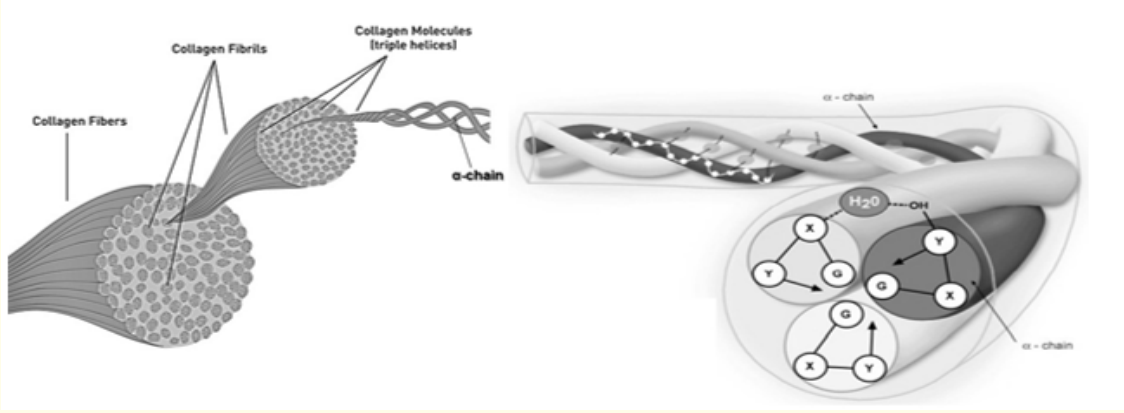

Figure 1: *Structure of collagen fibers and the organization of its polypeptide chains.*

Collagen sources include bovine, porcine, human and marine sources such as fish scale and fish skin [8]. The bovine and porcine collagen are largely used as they are available in plenty and has shown enormous possibility as biomaterial. For instance, the bovine collagen is commonly used as temporary cover for extra-oral wounds and burns on the body; and the porcine collagen matrices are used for grafting soft tissues. Animal terrestrial sources comprising of chicken neck (type I, II, III and V), embryo sternal cartilage (type IX), skin (type I and II) and muscular tissue (type IV), kangaroo tail, rat tail tendon, duck feet, equine skin, cartilage and flexor (type I and type II), alligator bones and skins, bird's feet, sheepskin and frog skin, are the currently used sources of collagen [3]. These sources are cheap and easy to acquire; however, prolonged usage of these have tended to be allergenic and provoke diseases such as osteogenesis imperfecta [3].

As a solution, marine collagen has been recognized as a feasible natural source, which has been reported GRAS (Generally recognized as safe) by FDA. These are economical and have no risk of transmitting diseases. Marine sources include fish (skin, bones, fins, scales), marine sponge and jelly fish. Baltic cod skin, seared fish, black drum and sheepshead seabream collagen are mainly type I, marine sponges such as *Geodia cydonium, Speriditae* and *Icinia fusa* are rich source of type I and IV and 60% jelly fish collagen such as from *Rhopilema asamushi, Stomolophus meleagris, Catostylus tagi* and *Rhizostoma pulmo*. The fish collagen is also called 'Piscean collagen' [6].

Isolation of fish collagen

Collagen, the dominant connective tissue protein, exercises multiple functions; and are extracted from various terrestrial/marine sources. The distribution of molar mass, structure, composition, functional and physical properties of collagen depends highly on the extraction conditions. The main source of collagen extraction are by-products from slaughter house that include achilles tendon, pericardium, bovine inner layer of skin, bovine bones, porcine skin and porcine lung, and poultry slaughter waste such as emu skin, chicken feet, sternal cartilage, skin and tarsus [12]. Fish by-products such as, Japanese sea bass skin (*Lateolabrax japonicus*), clown featherback skin (*Chitala ornata*), yellow find tuna bladder (*Thunnus albacares*), Japanese seer fish skin and bone (*Scomberomorus niphonius*), Japanese sturgeon cartilage (*Acipenser schrenckii*) and fins, scales, skin, bones and swim bladder from bighead carp (*Hypophthalmichthys nobilis*) are considered as finest alternatives for collagen extraction [12]. In recent years, various extraction and characterization techniques for fish collagen from various sources such as pacific cod *(Gadus macrocephalus)*, baltic cod *(Gadus morhua)*, barramundi *(Lates calcarifer)* and red tilapia *(Oreochromis niloticus)*, hake *(Merluccius hubbsi)*, ocellate puffer fish *(Takifugu rubripes)* surf smelt *(Hypomesus pretiosus japonicus Brevoort)*, bighead carp *(Hypophthalmichthys nobilis)*, rainbow trout *(Oncorhynchus mykiss)*, albacore tuna *(Thunnus alalunga)*, dog shark *(Scoliodon sorrakowah)* and rohu *(Labeo rohita)*, black drum and sheephead seabream, golden goatfish *(Parupeneus heptacanthus)*, log barbel catfish *(Mystus macropterus)* and squid (*Loligo duvaucelli*) mantle, tentacle and skin, jumbo squid *(Dosidicus gigas)* [13-15] were described. The acid extraction and enzyme extraction techniques, still remain the prime.

Despite its safety and ease in extraction, there are limitations for the application of fish collagen due to its low denaturation temperature. Collagen are basically extracted by chemical and enzymatic hydrolysis. Chemical extraction process is more commonly used; however, for biological applications, enzymatic hydrolysis is more promising as these have high nutritional value and improved functionality [12]. Even though later is expensive, it produces less water and reduce processing time. Extraction of collagen starts with the removal of numerous covalent intra- and intermolecular cross-links, which primarily involves residues of lysine and hydroxy-lysine, ester bonds and other bonds with saccharides that contributes to its complex nature [12]. The steps in collagen extraction include pre-treatment to remove non-collagenous substances, and extraction methods are based on the solubility of collagen in neutral saline and acidic solutions (with/without enzymes) (Table 2).

Fish type I collagen is extremely soluble in dilute acid than avian and mammalian collagen. Furthermore, type I collagen of bony fish and lamprey displayed high degree of structural similarity between species with respect to $\alpha 1$ and $\alpha 2$ chains [16].

Raw material	Pretreatment	Extraction process
Animal source		
Bovine achilles tendon	Pretreatment with 0.15 M NaCl and acetone	Extraction with pepsin in 0.5 M acetic acid (2 d, 20°C) with/without ultrasound (40 kHz, 120 W, pulsed 30/30 min)
Bovine pericardium	Pretreatment with NaOH (0.1 M, 48 h, 4°C)	Extraction with pepsin (1:20 w/v) in HCl (10 mM, 12 h, 4°C)
Avian source		
Emu skin	Homogenization with ethanol (10 %, 4 days), extraction with NaOH (0.1 M, 2 days), washing thoroughly with distilled water (2 d).	Extraction for 4 days with acetic acid (0.5 M, 48 h), NaCl in 0.5 M acetic acid (0.9 M), pepsin in 0.5 M acetic acid (10 %).
Marine source		
Japanese sturgeon skin	Homogenization with NaCl (20 %, 4°C)	Extraction with NaCl (0.45 M, 1: 100, 24 h, 4°C), followed by acetic acid (0.5 M, 24 h, 4°C), followed by pepsin in 0.01 M HCl (48 h, 4°C).
Brownbended bamboo shark and blacktip shark cartilage	Fat removal using 0.1 NaOH (1:10, 6 h, 4°C), decalcification using EDTA (0.6 M, 1: 10 w/v, 40 h, 4°C)	Extraction with acetic acid (0.5 M, 1: 15 w/v, 48 h, 4°C), followed by pepsin (40 units/g of residue) in 0.5 M acetic acid (1:15 w/v, 48 h, 4°C)
Yellowfin tuna swim bladder	Pretreatment with NaOH (0.15 M, 1: 10 w/v, 2h, 4°C) and butyl alcohol (10 %, 1: 10 w/v, 12 h)	Extraction with acetic acid (0.5 M, 1: 10 w/v, 48 h, 4°C)
	Pretreatment with cold distilled water three times	Extraction with enzyme (20 units/g residue) in acetic acid (0.5 M, 1: 10 w/v, 48 h, 4°C)
Japanese seer fish skin	Fat removal with NaOH (0.1 M, 1:10 w/v, 2 d, 4°C), followed by washing with cold water (until neutral pH), followed by butyl alcohol (10 %, 2 d)	Extraction with acetic acid (1:15 w/v, 24 h), followed by extraction with porcine pepsin (750 U/mg dry wt.) in acetic acid (0.5 M, 1:15 w/v, 4°C, 2 d)
Japanese seer fish bones	Pretreatment with NaOH (0.1 M, 1: 20 w/v, 2 d, 4°C), followed by washing with cold water (until neutral pH), followed by descaling with EDTA-2Na (0.5 M, 5d), followed by butyl alcohol (10 %, 2 d)	Extraction with acetic acid (0.5 M, 1: 15 w/v, 3 d), followed by porcine pepsin (20 U/g residue) in acetic acid (0.5 M, 2 d, 4°C)

Table 2: *Extraction of collagen from various sources.*

Characterization of fish collagen

The physico-chemical properties, biocompatibility and biodegradation of fish collagen are the major tools used in the characterization of fish collagen.

The biochemical composition of fish collagen is different from mammalian collagen. Previous studies have demonstrated that the amino acid composition of fish collagen is similar to mammalian collagen with glycine being the most abundant amino acid, accounting for more than 30% of all amino acids. Furthermore, the degree of hydroxylation of proline was estimated to be 35 - 48%, which is comparable with mammalian collagen (approximately 45%). Additionally, a linear relationship between the stability of collagen and the hydroxyproline content was reported. Fish collagen is highly unstable, fail to become stiff with chemical reaction and exhibit biodegradation rapidly [17].

The RGD motif is a representative amino acid sequence with cell adhesion properties that is comprised of arginine (Arg)-glycine (Gly)-aspartic acid (Asp) [16]. Arg-Gly-Asp-serine (Ser) sequences were also identified to contribute in cell adhesion molecule. Collagen contains specific cell adhesion domains. After the integrin receptor on the cell surface binds to the RGD motif on the collagen molecule, cell adhesion is actively induced, which contributes to the promotion of cell growth and differentiation and the regulation of various cell functions. Pure fish collagen has low mechanical stability, which is improved by cross-linking. The utilization of fish collagen in regenerative medicine and tissue engineering is recent and evolving currently [16].

Shark collagen gels like other fish collagen gels are difficult to handle due to their low denaturation temperature (Td- approximately 30°C) [18]. Low denaturation temperature of these unable its practice at the actual physical temperature required for human medical application. The Td of chum salmon was reported to be much lower (approximately 19°C), which suggests its instability and unpredictability at physical temperature of human body [19]. The low denaturation temperature of fish collagen causes its melting when in contact with the human body for clinical applications [20]. The collagen derived from ray skin and the scales of tilapia were reported to have a Td of 33 - 34°C and 35°C, respectively. Additionally, chemical cross-linking *in vitro* was proposed to increase the stability of the collagen. The chemical cross-linking of salmon collagen modified its Td (55°C). The degree of hydroxylation of proline of cold sea fish such as chum salmon is lower (35 - 37%) than warm sea fish such as tilapia (43%), which in turn contributes to the Td of the fish collagen [20].

Applications of fish collagen

Fish collagen have numerous applications such as, pharmaceutical/biomedical applications (as anchor in glass, beads for cell culture, biomaterial for vascular prosthesis, microparticles for subcutaneous injection, scaffold in tissue regeneration, as feed/food (gelatin, glue), cosmetics, and to produce collagen hydrolysates (used in oral administration) [3,21]. This review will concentrate on the various pharmaceutical and food applications of fish collagen (Figure 2).

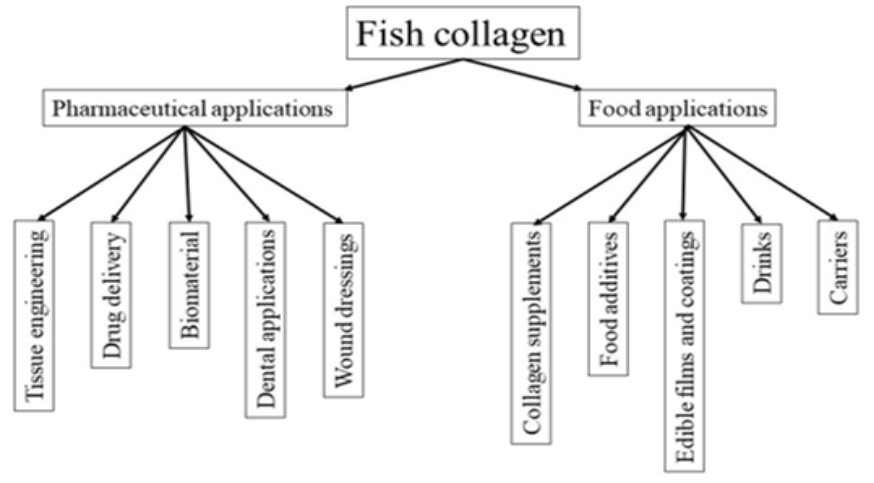

Figure 2: *Various pharmaceutical and food applications of fish collagen.*

The collagen exhibits excellent biocompatibility, weak antigenicity, high level of direct cell adhesion, and high degree of biodegradability compared to other natural polymers such as chitin/chitosan, gelatin, albumin and synthetic polymers. The biocompatibility of collagen aids its usage in tissue engineering. The application of fish collagen as a scaffold for tissue engineering was investigated [22]. Atelocollagen, processed natural biomaterial from bovine type I collagen, was found to be highly biocompatible with low rate of inflammatory responses and high degree of biodegradability; hence, inherits biomaterial characteristics, unlike fish collagen, which is unstable [23]. Collagen scaffolds of jellyfish displayed high porosity with interconnected pore structure, which is convenient for high density cell seeding and provides an efficient nutrient and oxygen supply to the cells cultured in 3D-matrices. Further, jellyfish collagen (as sponge) was found to induce an immune response comparable to that stimulated by bovine collagen and/or gelatin [24]. The chemically cross-linked elastic salmon collagen (SC) vascular grafts induced little/no inflammatory reactions [25]. The physically cross-linked collagen sponges/nanofibers from tilapia demonstrated that it induced no inflammatory responses *in vivo* and was statistically similar to porcine collagen and high-density polyethylene [26]. Tilapia collagen showed degradation after 4 d; however, *in vitro* degradation studies (using collagenase solution) demonstrated a high level of stability among cross-linked scaffolds derived from tropical fresh water fish scale collagen (~50% reduction in mass after 30d). Furthermore, minimal immunological reactions were observed for collagen solutions *in-vivo*. These recommend that fish collagen (skin, scale, muscle and other under-utilized resources) is biocompatible and exhibit bioresorption; and, hence, may have great potentiality in tissue engineering and biomedical applications.

Pharmaceutical applications

Biomedical application

Studies have recommended the usage of type I collagen as a biomaterial after chemical cross-linking, as it confer remarkable strength and stability to the matrix; however, they also result in potential cytotoxicity or poor biocompatibility. The physical cross-linking techniques provide adequate stability and are biocompatible. It assists in wound healing, development of cells, angiogenesis, platelet activation, etc. As drug delivery system, it shields ophthalmology, acts as sponges for burns/wounds, mini pellet and tablets for protein delivery, as gel in sustained drug delivery in combination with liposomes, controlling material for transdermal delivery, nanoparticle for gene delivery and basic matrix for cell culture system. It is also used in tissue engineering, skin replacement, bone substitute, and artificial blood vessels and valves [3]. In hydrolyzed form, it helps in weight management. Further, in cosmetic surgery, it is used as dermal fillers for treatment of wrinkles and aging skin [3]. Improving the fish collagen characteristics would make it a suitable biomaterial for biomedical applications.

Tissue engineering requires porous, biodegradable scaffold that resemble the ECM. It organizes the cells spatially, provide environmental signals and direct site-specific cellular regulation. Recently, scaffolds consisting of natural polymers (collagen, gelatin), biodegradable synthetic polymers (polylactic acid, polyglycolic acid), inorganic materials (hydroxyapatite) and composite materials were rapidly developed for tissue engineering. The pore size, pore number, surface area and pore wall morphology of scaffolds significantly determine the desirability of these scaffolds in tissue engineering, cell seeding, migration, growth, new tissue formation, etc. The adhesion domains (RGD motif) in collagen contribute to adhesion [16]. Collagen is the most promising natural biomaterial as scaffold or carrier in tissue engineering, as it is abundant, biocompatible, biodegradable, resembles the components present in ECM and supports the connective tissues including skin, tendon, bone, cartilage, blood vessels, and ligaments. The poor mechanical strength of pure fish collagen limits its utility in tissue engineering; hence, modifying the collagen using a cross-linker is indispensable to make up for this disadvantage [27].

The collagen from jellyfish stimulated the transcription activity and translation activity for enhanced immunoglobulin and cytokine production [28]. Scientists demonstrated that jellyfish collagen containing telopeptides that enhance the production of immunoglobulin M (IgM) in the human hybridoma cell line (HB4C5) and the production of immunoglobulins M and G (IgM and IgG) in human peripheral

blood lymphocytes. These antibodies protect against bacterial and viral infections. The *in vivo* responses of jellyfish atelocollagen was highly porous with interconnected pore structure, which enhanced the high-density cell seeding, provided nutrients and oxygen supply to cells efficiently in the three-dimensional matrix. Further, the immunological responses of these were comparable to that stimulated by bovine collagen and/or gelatin *in-vivo* [16,24].

Advantages	Disadvantages
Abundant availability	High cost of pure type I collagen
Non-antigenic	Variability (cross-links, density, fibre size, trace impurities, etc.) in isolated forms
Biodegradable, bioresorbable, non-toxic, biocompatible	Swelling and rapid release due to hydrophilic nature
Synergic with bioactive compound	Variability in the rate of enzymatic degradation as compared with hydrolytic degradation
Act as biological plastic with high tensile strength and minimal expressibility	Complex in handling
Hemostatic	Side-effects (transmissible diseases such as bovine spongiform encephalopathy, etc. and mineralization)
Formulated in different forms, easily modifiable using its functional groups	
Compatibility with synthetic polymers	

Table 3: *Advantages and disadvantages of collagen as biomaterial [16,24].*

Dental Applications

Advancements in medical arena led to the understanding that collagen with its properties such as elasticity and repair, could have wide application in dentistry. Collagen was first used as medical suture more than 5000 years ago in the form of hair [29]. In dentistry, collagen is used as membrane, bone graft material, drug delivery vehicle (local) and a hemostatic agent [29]. Collagen, as plugs are used for controlling bleeding, dressing oral wounds, as graft and extraction site closure, and promote healing; as membranes are used in periodontal and implant therapy to prevent epithelial migration and regeneration and repopulation of cells in the defective area (Gupta., *et al.* 2012). The collagen was used in the treatment of dental caries/pulpitis through dentine/pulp regeneration using pulp stem cells, granulocyte colony stimulating factor (G-CSF) and collagen solution pumped in patients with pulp extirpated cavities [30]. The piscine collagen and its active peptides in dental gel "COLLGEL", reduced the healing time of soft tissue in the mouth. It also created a protective membrane that regenerates and protects the damaged tissue without causing pressure on the soft tissue. It also relieves pain and soothes irritation immediately after application and adheres to mucous membranes. It further removes the scar [31].

Collagen is also used as bioresorbable membrane in guided tissue regeneration (GTR) in periodontal reconstruction [29] as it is chemotactic for periodontal ligament fibroblasts; and hence, preventing second surgery. These prevent the gingival epithelial cells from migrating to the wound. Some commonly available collagen membranes are BioMend® (Zimmer Dental), Bio-Gide® (Geistlich Pharma), OraMem® (Salvin), RCM6 Resorbable Collagen Membrane and conFORM™ (ACE Surgical), and Periogen® (Collagen Corp.). Rat collagens, Avitene™ [32] and dura mater have also shown potentiality in GTR. Fish collagen membrane [33] is also used in the treatment of periodontal intrabony defects. Collagen also find application as hemostatic sponge (CollaPlug®, CollaCote® and CollaTape® (Zimmer Dental) and Helistat® Absorbable Collagen Hemostatic Sponge (Integra Lifesciences Corp.) to prevent bleeding and cause coagulation [29].

The hard tissue regenerative medicine in the field of dentistry is another area where the collagen has been found to be of great significance as it treats defects of the alveolar and/or jaw bone instigated from diverse etiologies. Bone graft placement, guided tissue/bone regeneration, use of various growth factors and/or host-modulating agents (emdogain and parathyroid hormone) are some of the treatment techniques currently used [34]. HEALOS® bone graft replacement (DePuy Spina, Inc.), Integra Mozaik® (Integra OrthoBiologics), Infuse® bone graft, MasterGraft® Putty, MasterGraft® Strip, Progenix® Plus, Progenix® Putty (Medtronic Spinal and Biologics), Vitoss® (Orthovita), OP-I® implant and OP-P® Putty (Stryker Biotech), and CopiOs® Bone void filler (Zimmer) are some of the collagen bone substitutes currently in commercial application [21].

It was reported that the degradation/denaturation of salmon atelocollagen by γ-irradiation modulated the mouse osteoblastic cells (MC3T3-E1) proliferation [16]. Human periodontal ligament fibroblasts showed growth and exhibited a highly differentiated activity on salmon collagen gel and porcine collagen [16]. The intricate 3D mesh composed of salmon collagen-coated fibers act as functional scaffold and is an improved graft biomaterial both *in-vitro* and *in-vivo*. Fish collagen peptides promote post-transcriptional modification for collagen maturation and the gene expression for cell differentiation in osteoblastic cells. Hence, fish collagen application *in situ* promotes hard tissue formation as scaffold for seeded cells and as bone inducing nutritional factors.

Drug delivery system

Collagen-based membranes have been successfully tested in local drug delivery. Grover., *et al.* [35] described formaldehyde cross-linked by co-protein matrix conjugated with chlorhexidine. PerioCol® is most commonly used collagen-based drug delivery system for chlorhexidine [33]. The collagen membranes developed using air bladder, skin and scales of fresh water fish (*Labeo rohita*) were predominantly used to develop particles, sponges and sheets that showed potentiality in drug delivery [29]. Another collagen-based drug delivery system used commonly is resorbable Periodontal Plus AB™ (EnColl) comprising of tetracycline fibers impregnated in collagen fibers, exhibiting controlled drug release [36].

Collagen dressings

The hydrophilic nature, molecular structure and functional significance of collagen provide surface geometry for cell adhesion and wound repair (seawater immersion wound healing using shark skin collagen), which has enabled its application as collagen dressings (sponges and films) in dentistry [3,29]. Collagen sponge, a 3D structure to enhance tissue infiltration and cell growth *in-vivo*, is used as temporary and permanent coverings. Helisorb® sponge and GelSpon (Sterile absorbable haemostatic gelatin sponges) [37], OraPLUG® [38], Avitene® Ultrafoam® Collagen sponge® [32] etc. are some of the collagen dressings (developed using bovine collagen) currently used in dentistry and surgery. The fish collagen wound dressings (an algae-based wound dressing), Kaltostat, developed from the discarded tilapia skin had shown accelerated healing. The human trials and commercialization of this product is still in progress. Fish collagen nanofibers, collagen patch derived from fish scale are few other examples of utilization of marine collagen in wound healing [26,39].

Food applications

Alike pharmaceutical/biomedical applications, collagen finds wide application in food industry either as collagen polypeptide/collagen peptide or gelatin (denatured collagen containing low molecular weight peptides and proteins). Collagen is currently in demand as healthy food ingredient and is blended with variety of food and beverage products. Collagen supplementation through diet is considered as a substitute for collagen loss in the body due to increased age and poor diet [9]. Currently, there are many available commercial collagen products from different sources marketed locally (Colageno manufactured by JBS, Brazil and Cosen by Jiangxi Cosen

Biochemical, China. Meanwhile, Ovinex is type I and III food grade ovine collagen manufactured using enzymatic process by Hollista CollTech, Australia, Peptan by Rousselot SAS, France, Ni-Kollagen by Bionic Life Science, Malaysia). The latter two are marine collagen suggested for applications in dietary supplements, functional food, drink and beverage, confectionery and desserts; and others are from bovine sources [9].

Collagen Supplements

The moisture absorption characteristics of collagen and its fractions have recognized it as a valuable nutritive fiber and protein source [40]. The collagen synthesis decreases, and the tissues get thinner, weaker and less supple, as human being age; these supplements maintain the skin, hair, nails and body tissues [41]. The collagen metabolites attracting fibroblasts that generate the synthesis of new collagen; thereby aid in assembling bone, skin and ligaments. The diameter of the dermal collagen fibrils and cohesion are also improved, which in turn enhances the hydration properties of tissue and make it thick, supple and resilient [41]. Collagen supplementation could also be in the form of nutricosmetics that are offered in the form of liquids, pills or functional foods. The Munchy's Wheat Krunch Collagen, a nutraceutical, also promote the collagen supplementation. The wheat-based baked crackers were augmented with marine collagen (1200 mg) [9]. Collagen supplements also find high demand in sports nutrition. It improves the lean muscle gain, decrease recovery time, reconstruct damaged joint structure and improve cardiovascular performances through creatine formation, which is an essential amino acid in new muscle growth following workouts. Arginine from hydrolyzed collagen also encourages muscle mass [41]. Oral collagen supplementation showed potentiality in preventing joint pain osteoarthritis and rheumatoid arthritis [42].

Food additives

Collagen as food additives improves the rheological properties of sausages and frankfurters. It enhanced the technological and rheological properties and reduced the fat consumption. Gómez-Guillén., et al. [43] suggested that the heat-treated collagen fiber could be used as an emulsifier in acidic products. It produced electrostatically stable emulsion at acidic pH, improved the creaming index and emulsion rate [43]. These heat-stabilized collagen fibers could be a natural alternative to synthetic emulsifiers in acidic food and drink formulations [44]. Wenther [45] suggested the use of food grade collagen in bologna formulations to replace lean meat in a model emulsion system and frankfurters. Duck feet collagen improved the physicochemical properties of threadfin bream and sardine surimi. The gel strength, gel hardness, folding test score and the color lightness were also improved considerably. The addition of chicken feet collagen in jelly production also improved the jelly characteristics and had good acceptability [46].

Edible films and coatings

Collagen sausage casings (preformed casings) were developed from the corium layer of food grade beef hides and were co-extruded around sausage meat batter [47]. An edible collagen film (used on netted roasts, boneless hams, fish fillets, roast beef and meat pastes) reduced the cook shrinkage, enhanced the product juiciness and was easy to remove after heat treatment [48]. The collagen coatings also act as protective barrier against poultry fat oxidation that lead to the musty-moldy aroma. The collagen based edible films may replace the plastic coatings as it prevents fat oxidation, discoloration, microbial growth and preserve the sensory attributes [49,50]. The use of marine collagen in developing edible films and coatings will be a novel innovation.

Drinks

The collagen-infused drinks (soy collagen, cocoa collagen, cappuccino collagen, juice with collagen, bird nest drink with collagen) are another trend in global market. These stimulate the collagen building mechanism in the body; thus, promote the body tissues and reduce the skin wrinkles and sagging [51,52].

Malaysia Dairy Industries (MDI) has added collagen peptides in their nutritious probiotic drink [53]. It contains prebiotic fibre and collagen peptides (500 mg) and 30 mg of vitamin C. This "Vitagen Collagen" drink stimulates the growth of beneficial gut bacteria and radiate beauty from beyond skin deep. Similarly, Avon also formulated the Avon Life Marine Fish Peptide Collagen Drink, a revolutionary drink from natural and high-quality fish peptide collagen from salmon fish skin, vitamin C and fructo-oligosaccharides [54]. The Nestle Malaysia has also released Nescafe Body Partner, Kacip Fatimah and Collagen Coffee that contained collagen from fish source [55]. The Nutrova collagen+antioxidants (cranberry flavor) is yet another nutraceutical drink, rich in marine collagen and antioxidants for healthy skin [56].

Collagen could also be used as clarifying agent in the cloudy alcoholic beverages by aggregation of the yeast and other insoluble particles [57]. Similar to bovine collagen solutions, fish collagen/isinglass were also reported to show efficient refining properties in beer and yeast preparations [58]. The distinct caprylic taste of collagen in food and drink can be significantly improved by blending sucralose and stevia extract and acesulfame potassium [9].

Carriers

Collagen as films or coatings could function as carriers in the delivery of active substances such as, antioxidants, antimicrobials, colors and flavors [59]. The collagen mini pellets/tablets/discs/films could be used for protein delivery or other bioactive components [60]. The fish collagen fibers were reported to act as carrier of rosemary extract in processed meat industry. These augment its antioxidant properties in the product [43].

Conclusion

Collagen is the major structural component of connective tissues found in the body. Due to its low antigenicity, biocompatibility and bioresorption, the collagen from bovine, ovine, porcine and marine resources find wide possibility as natural biomaterial in various industries. Due to the threat of transmissible diseases, marine sources are considered appropriate alternative for the terrestrial collagen. Marine collagen is extracted from skin, bones, scales, etc. of sponges, fish and jellyfish. Unlike mammalian collagen, marine collagen is less stable and need to be stabilized/strengthened using chemical or physical cross-linking techniques. The collagen finds vast applications in pharmaceutical and biomedical industry as medical devices, scaffolds for tissue regeneration and in drug delivery system. In food industries, the collagen is used as carriers of food additives, clarifying agents, etc. The wide applications collagen has opened novel venues for the appropriate modifications and utilization of marine collagen. This sustainable utilization of under-utilized marine by-products will also restrict fishery wastage and protect the environment.

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