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Chapter- 7

Marine Collagen: A Viable and Eco-Friendly Alternative in Pharmaceuticals

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Abstract:
Marine collagen, obtained from the by-products of aquatic invertebrates plays a significant role in several biomedical fields. It is essential for creating biomaterials used in tissue scaffolds, absorbable sutures and wound treatment matrices and for its applications in cosmetics and drug delivery systems. Marine species provide an ideal collagen source due to their lack of religious limitations and the absence of reported transmissible diseases. Studies indicate that collagen derived from fish possesses bioactive properties including regenerative, antioxidant, antibacterial, anti-inflammatory, and immunomodulatory effects along with the ability to inhibit angiotensin-converting enzyme activity. This review explores the scientific advancements surrounding collagen derived from marine organisms and fish by-products.

Introduction:

Collagen is a complex fibrous protein and the primary structural component of the extracellular matrix, present in various connective tissues like skin, bones, ligaments, tendons, cartilage, and interstitial tissues in parenchymal organs. It consists of amino acids like glycine, proline, and hydroxyproline, which comprise 33% and 15-30% of its composition, respectively and prevent the formation of alpha-helix or beta-sheet structures. The basic building block of collagen, tropocollagen, comprises right-handed triple helices made from alpha chains, largely following a repeating Gly-X-Y amino acid sequence. X typically represents proline (Pro), while Y is often hydroxyproline (Hyp), though other amino acids, such as alanine, serine, threonine, lysine, and others, can also be found.

Currently, 29 distinct collagen types have been classified and organized into families such as fibrillar collagens, fibril-associated collagens with interrupted triple helices (FACITs), membrane-associated collagens (MACITs), and others with multiple triple-helix domains (MULTIPLEXINs). Microfibrils are composed of aligned tropocollagen molecules measuring around 300 nm in length and 1.5 nm in diameter, with a molecular weight of 285 kD. These molecules aggregate to form microfibrils, varying between 10 and 125 in number, arranged into fibrils ranging from 10 to 200 nm in diameter. The fibrillar types, such as I, II and III, comprise the majority of vertebrate collagen, contributing to tissue's structural integrity and tensile strength, and are responsible for the skin's elasticity (Lim et al., 2019). The fibrillar

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arrangement of collagens results in a distinctive cross-banded pattern in electron microscopy due to the staggered structure of the helices (Bella and Hulmes, 2017).

FACIT collagens do not independently form fibrils but associate with existing collagen fibrils. Their interrupted helices contain non-collagenous regions that enable joint formation and proteolytic cleavage, which helps overcome the protease resistance of the native helices. MULTIPLEXINs, such as types XV and XVIII, feature hybrid proteoglycan-collagen structures. For example, type XV contains chondroitin sulphate chains, while type XVIII is a heparan sulphate proteoglycan (HSPG), contributing significantly to its molecular weight (Halfter et al., 1998). MACITs, with interrupted helices, are involved in cell adhesion and signalling. Several other less abundant collagen types perform specialized roles in specific organs.

Collagen's versatility extends to its applications in biomedical devices, dermal grafts, implants, food products, beverages, and cosmetics, thanks to its ability to absorb water, flexibility, and potential to form 3D structures. Additionally, its surface properties—such as stability, cohesion, and film formation—make collagen ideal for tissue engineering, drug delivery and regenerative medicine (Lim et al., 2019).

Historically, collagen used for tissue engineering had been sourced from terrestrial animals, mainly pigs and cows. However, due to concerns about diseases like bovine spongiform encephalopathy (BSE) and religious restrictions in certain cultures, marine sources of collagen have become an increasingly desirable and cost-effective alternative (Easterbrook et al., 2008).

In recent years, the extraction and utilization of collagen from marine sources have aligned with the principles of the circular economy (Saha, 2023). Industries can reduce waste by repurposing fishery by-products such as skin, scales, and bones while generating valuable materials for biomedical and cosmetic applications (Mukherjee et al., 2022; Sanyal et al., 2023). This approach promotes resource efficiency and sustainability, contributing to a closed-loop system where waste is minimized and resources are continually reused.

Characteristics of Marine Collagen

Marine environments, covering over 70% of the Earth's surface, contain a vast range of species, representing around half of global biodiversity. This diversity provides a valuable source of collagen and other natural substances for industries such as cosmetics, nutraceuticals, medical devices, and biocompatible materials. Marine collagen can be obtained from various organisms, including sponges, jellyfish, octopuses, sea urchins, squid, starfish and algae. Marine collagen holds several advantages over mammalian collagen, such as enhanced solubility in water, biocompatibility, biodegradability, lower immunogenicity, easier extraction methods, and lower production costs (Lim et al., 2019; Ali, 2024).

Structurally, marine collagen is similar to mammalian collagen with glycine as the predominant amino acid, accounting for over 30% of its composition. Although marine collagen generally has lower concentrations of amino acids like proline and hydroxyproline, cold-water fish species often exhibit higher levels of amino acids such as serine and threonine. The hydroxyproline content influences collagen's rigidity and denaturation temperature, contributing to the overall stability of its triple helix. Freshwater fish such as tilapia, have shown amino acid profiles and thermal stability similar to mammalian collagen. Hydroxyproline and hydroxylysine help maintain collagen's structural integrity and heat resistance, thus influencing its thermal equilibrium (Oslan et al., 2022).

Extraction and Purification of Marine Collagen

Collagen extraction typically employs three key methods **:** neutral salt solubilization, acid solubilization and pepsin solubilization (Li et al., 2020). The neutral salt method involves extracting loosely cross-linked collagen molecules using salt solutions, followed by purification processes such as dialysis, sedimentation, and centrifugation. In contrast, dilute acidic solvents like citrate buffer, acetic acid, or hydrochloric acid (pH 2-3) are generally more effective for collagen extraction. However, collagen from sources like bones, cartilage, or older organisms, which have higher levels of keto-imine cross-links, displays lower solubility in mildly acidic solutions (Blanco et al., 2017).

Hydrolysed collagen exhibits a range of beneficial biological activities, making it valuable for nutritional, medical, and food industries. It has been reported to assist in treating conditions such as brittle bone disease, diabetes, gastric ulcers, hypertension and skin hydration and it functions as a preservative (Barzkar et al., 2019). Given its extensive industrial applications, collagen is increasingly utilized in pharmaceuticals, food products, beverages, cosmetics, tissue engineering and healthcare. Its outstanding properties—haemostatic activity, biodegradability, and low antigenicity—are the main reasons for its use in therapeutic and pharmacological fields (Lim et al., 2019).

Collagen hydrogels form through polymerization of collagen suspensions, typically at room temperature for about 15 minutes, then transition to a non-transparent state. Once incubated at 37°C for 45-60 minutes, a medium is added, and the gels are detached gently, often with a pipette, to ensure they float within the medium (Govindharaj et al., 2019). These hydrogels create intricate 3D networks capable of absorbing large volumes of water, enhancing their biocompatibility, flexibility, and suitability for biotherapeutic applications. Collagen hydrogels are particularly useful in tissue and cell cultures, tissue regeneration, biofabrication, drug delivery and soft gelatine gel development (Wang et al., 2015).

Marine-derived polymers have gained significant attention recently as natural, eco-friendly alternatives for developing biocompatible materials (Barzkar et al., 2019; Sankarapandian et al., 2023). Collagen biopolymers extracted from aquaculture products such as fish skin or jellyfish offer increased value and functionality for biomaterials while aligning with circular economy strategies. Marine collagen also reduces infection risks and enhances immune response (Govindharaj et al., 2019).

Marine Collagen in Bone and Cartilage Regeneration

Alves et al. explored the immune response to collagen and gelatine derived from blue sharks and codfish by characterizing these materials and evaluating endotoxin levels. After exposing bone marrow-derived macrophages to the collagen, they measured gene expression and protein levels of pro-inflammatory and anti-inflammatory cytokines. The results showed that shark collagen produced the lowest immune response as indicated by reduced levels of pro-inflammatory cytokines, inducible nitric oxide synthase (Nos2), and increased Arginase 1 (Arg1). Although shark gelatin induced higher pro-inflammatory responses, it also boosted IL-10 (an anti-inflammatory cytokine) and Arginase, markers of M2-like macrophages.

In mouse models, both materials resulted in temporary neutrophil recruitment, mostly subsiding within 24 hours. When mouse osteoblast cells (MC3T3-E1) were treated with fish collagen peptides (FCP), they showed increased gene expression of collagen-modifying enzymes such as lysyl hydroxylase (LH) 1-3, particularly LH2 and lysyl oxidase-like proteins (LOXL) 2-4. This was accompanied by enhanced collagen deposition and matrix mineralization, as *in vitro* mineralization assays demonstrated. FCP also increased lysine hydroxylation, boosted hydroxylysine-aldehyde cross-link formation, and accelerated cross-link maturation compared to controls, indicating the potential of FCP in bone regeneration (Yamada et al., 2013).

Further research supports marine collagen's positive impact on bone marrow stem cells in rats. Liu et al. reported that a fish collagen concentration of 0.2 mg/mL promoted cell survival and increased the expression of osteogenic markers (RUNX2, ALP, OPN, and OCN) and endothelial markers (CD31, VE-cadherin, and VEGFR2) (Liu and Sun, 2014). Another study revealed that hydrolyzed fish collagen enhanced human periodontal ligament cell viability and osteogenic differentiation, as shown by increased expression of osteogenic markers and proteins like alkaline phosphatase and osteocalcin (Liu and Sun, 2015). Interestingly, hydrolysed fish collagen also stimulated rat bone marrow mesenchymal stromal cells to produce anti-inflammatory markers (IL-6, TGF-β1, and PGE2), demonstrating that the immunomodulatory properties of collagen remain intact even in osteogenically differentiated cells (Liu and Sun, 2019). Additionally, marine collagen-derived bioactive peptides have been shown to enhance calcium and zinc absorption, acting as antiosteoporosis agents. Marine collagen hydrolysates reduce pro-inflammatory markers associated with osteoarthritis while promoting collagen synthesis in articular chondrocytes (Bourdon et al., 2021).

Marine Collagen in 3D Culture

Three-dimensional (3D) cell cultures provide a more realistic environment for cellular growth, allowing cells to interact with the extracellular matrix (ECM) in all directions. This contrasts conventional two-dimensional (2D) cultures, where cells are confined to a flat surface. 3D cultures, which can be created with or without scaffolds, offer a more accurate representation of biological environments, simulating in vivo conditions. Due to its biocompatibility and capacity to support cell adhesion, proliferation, and tissue formation, Marine collagen holds considerable potential for 3D cell culture applications (Urzi et al., 2023). Its similarity to the ECM makes it an ideal candidate for developing physiologically relevant environments for various cellular processes.

Marine collagen has found increasing applications in tissue engineering, regenerative medicine, and pharmaceutical testing. However, challenges such as high production costs and material variability persist, requiring further research. Marine-derived polymers like alginates, carrageenans, fucoidans, and chitosans, sourced from organisms such as algae, crustaceans, and fish, are being incorporated into bio-inks for bioprinting. These marine-derived biomaterials are becoming promising scaffolds for 3D cell and tissue cultures (Zhang et al., 2019). The use of 3D systems to evaluate marine-derived materials is expanding, offering benefits over traditional 2D models.

Marine Collagen for Skin Regeneration and Wound Healing

Marine collagen peptides (MCPs), derived from aquatic invertebrates through biochemical and enzymatic hydrolysis, are characterized by their lower molecular weight, enhancing water solubility and making them easier to absorb in biological systems. Wang et al. showed that MCPs extracted from salmon skin improved dermal wound tensile strength in rats, largely due to an increase in hydroxyproline levels. Additionally, collagen from marine tilapia skin and bovine collagen nanofibers accelerated wound healing in collagen-treated rats compared to controls (Chen et al., 2019).

A randomized triple-blind clinical trial on women aged 45-60 assessed the effects of freshwater fish-derived collagen on skin wrinkles, elasticity, and flexibility. After three months of supplementation, a 35% reduction in wrinkles was observed (Wang et al., 2015). Six weeks post-treatment, participants exhibited significant improvements in skin elasticity, particularly on the cheeks (Evans et al., 2021). Marine collagen, known for its potent free radical scavenging capabilities, neutralizes reactive and unstable free radicals that can damage cellular membranes, degrade dermal macromolecules, and harm DNA, all factors linked to skin aging (Geahchan et al., 2022).

Conclusion

Collagen, a crucial protein that makes up approximately 30% of the body's total protein, is found primarily in skeletal tissues, cartilage, the gastrointestinal tract, teeth, dermis, and adventitia. Due to its favourable properties, such as low immunogenicity, marine collagen has become widely used in biopharmaceuticals and cosmetics. Marine species are rich in biologically active compounds that show promise for applications in medicine, tissue engineering, and cosmetics. Notable sources of marine collagen include fish (dermis, skeleton, scales, and cartilage), molluscs, and marine invertebrates like jellyfish, sea cucumbers, sea urchins, polyps and squid.

Research indicates that marine collagen promotes the migration of keratinocytes and fibromuscular tissue, stimulating angiogenesis within the skin. It has also been effective in preventing and treating age-related metabolic bone diseases such as osteoarthritis and osteoporosis, by increasing bone mass and tensile strength. Due to its dynamic pharmacological applications, marine collagen has emerged as a superior alternative to terrestrial collagen sources. As demand for natural products rises, collagen is now used in emulsifiers, foaming agents, stabilizers, hydrogels, microencapsulation and more. Advances in analytical techniques have allowed for more detailed exploration of marine collagen's properties, making it a promising candidate for various biomedical and industrial uses.

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