Biomodulation of Marine Biopolymers for the Preparation of Nutraceuticals and Biomaterials of Healthcare Importance

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Natural bioactive substances are gaining increasing research attention because of their prospective health benefits. Dietary supplements and fortified foods containing biologically active molecules play a major therapeutic role in alleviating several lifestyle diseases. In comparison to the terrestrial counterparts, the marine ecosystem consists of huge diversity of flora and fauna which offer countless resources for human nutrition and health. Owing to the chemical diversity and complexity, marine derived compounds have enormous potential to deliver valuable functional food ingredients and nutraceuticals. These bioactive molecules include polysaccharides, proteins, peptides, phytochemicals, vitamins, fatty acids and antioxidants which can be utilized to produce functional food products and can be formulated into nutraceuticals. Bio-modulation, the reactive or associative alteration, of these biomolecules can lead to the development of novel dietary supplements which will provide health benefits beyond basic nutrition. Apart from providing nutritional benefits, several marine-derived biopolymers are abundantly used in the development of biomedical products. In this chapter, an attempt is made to appraise the bio-modulation strategies of various marine biopolymers and their suitability in nutritional and biomedical applications.

1. Chitin, chitosan and its derivatives

Chitin is found in the exoskeleton of crustaceans, insects, as well as mollusks and fungi and is the second most abundant polymer in nature next to cellulose. Chitosan is a natural biopolymer derived from chitin. Chitosan which is a linear, semi-crystalline polysaccharide is formed by the partial de-acetylation of chitin. The structure of chitin and chitosan is depicted in Figure. 1. The presence of hydroxyl and amino groups on its

backbones makes chitosan an amenable molecule which can be easily altered by various methods for specific applications. Several bio-modulation strategies for chitosan are well documented in literature including chemical, physical, and enzymatic approaches. For example, due to the high molecular weight and high viscosity, the usefulness of chitosan is limited in certain biological applications; hence de-polymerization strategies can be adopted to obtain oligosaccharides and monomers. The de-polymerization can be achieved by enzymatic hydrolysis using chitinase enzyme. N-alkylation, hydroxyl alkylation, carboxy alkylation, thiolation, glycation, etc. are chemical means to tailor chitosan for particular purposes (Chatterjee *et. al.*, 2015; Werle and Bernkop-Schnurch, 2008; Ravi Kumar, 2000). These methods can modify chitosan with enhanced properties such as excellent solubility at different pHs, modulated surface charges, improved absorption properties and new crosslinking sites. Several nano-technological approaches are also recently developed which allow chitosan to be used for the development of novel functional healthcare products in nutrition and in bio-medicine (Mourya and Inamdar, 2008; Gan and Wang, 2007; Malhotra *et. al.*, 2001). Recently, modified forms of chitosan have received increasing attention and their applications have been extensively explored in all aspects of science especially in food science, tissue engineering and drug delivery (Shu and Zhu, 2000; Upadhyaya *et. al.*, 2014; Jeong *et. al.*, 2010; Anitha *et. al.*, 2011). Chitosan is considered as GRAS (Generally Recognizedas Safe) by Food and Drug Administration (FDA). Many advanced applications of chitosan and its derivatives have been developed in food science, including novel chitosan derivatives with enhanced antioxidant and antimicrobial activities, chitosan-based active films for food packaging to extend shelf life, as well as chitosan-based encapsulation and delivery systems for micronutrients.

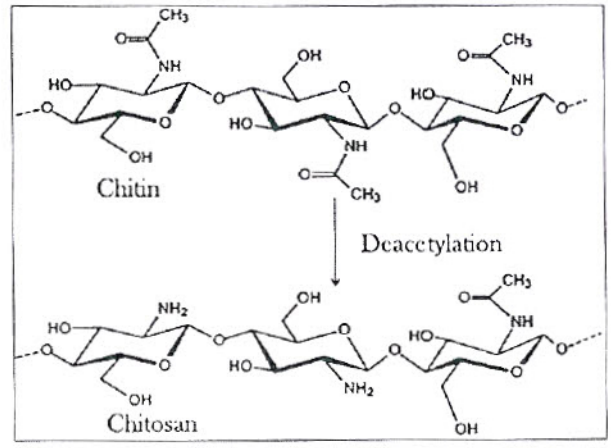

Fig. 1. Chemical structure of chitin and chitosan

2. Collagen and collagen peptides

Collagen is the most abundant structural protein present in the extracellular matrix and connective tissue of both vertebrates and invertebrates. Bioactive collagens

and their peptides can be extracted from various marine resources such as fishes, sponges, jelly fish, etc. In fish, collagen is extracted mainly from the scales, skin, fins and air bladder. Recent research including preliminary animal and clinical studies has demonstrated that the marine collagen possesses remarkable therapeutic properties that can help promote human health and well-being. Due to excellent antioxidant properties, collagen and its peptides are enormously used in anti-aging and cosmetic products. Although numerous benefits are associated with collagen from animal sources, fish collagen and peptides are known to have enhanced bio-availability owing to the smaller particle size compared to animal collagens. Fish collagens have better nutritional significance as the efficacy of the nutrient ingested is highly related to its bio-availability. Since it is absorbed more efficiently and enters the blood stream more quickly than other animal collagen sources, it is considered as the best collagen source for medicinal purposes. Owing to excellent bio-compatibility, bio-degradability and weak antigenicity, collagen has been widely utilized in nutraceutical, pharmaceutical, cosmeceutical, biomedical, tissue engineering and biopolymer industries. However, compared to the mammalian sources, marine collagen is under-utilized especially in the field of tissue engineering and drug delivery industries.

Type I collagen is the most predominant type of collagen obtained from skin, tendon, bone and muscle (epimysium) of fishes. However, if the source is cartilage Type II collagen can also be obtained. The general methodology of collagen extraction involves removal of non-collagenous proteins using alkaline solution (commonly sodium hydroxide solution) under controlled conditions of time, temperature and concentration of the alkali. Fats and pigments can be removed by using alcohol. If the source of collagen is bone, cartilage or scale, EDTA or dilute hydrochloric acid is used for demineralization. Acetic acid or lactic acid render higher collagen yield during the extraction of acid soluble collagen. Another method of collagen extraction is by enzymatic treatment by pepsin. Pepsin cleaves the peptides specifically in the telopeptide region of collagen and thereby hydrolyze some non-collagenous proteins and thus increases the purity of collagen. This also helps in reduction of its antigenicity.

Unlike other animal sources of collagen, marine collagen is less associated with cultural and religious concerns regarding human use and possesses lower risk of infection causing agents to humans (Goyal *et. al.*, 2013; Brown *et. al.*, 2001). These properties make marine collagen a true alternative source of collagen for a myriad of biomedical and healthcare applications. Since collagen possesses cell recognition sites, it is one of the most preferable biopolymer candidates for tissue engineering and

regenerative medicine applications. Recently the use of marine collagen for bone tissue engineering applications is increasingly reported because along with hydroxyapatite it offers a biomimetic mineralization principle (Hoyer *et. al.*, 2012; Pallela *et. al.*, 2012). In addition, collagen promotes osteogenic differentiation of mesenchymal stem cells. Although marine collagen is considered to possess low antigenicity when considering its use in tissue engineering and regenerative medicine applications, several modifications and combinations of collagen with other biomaterials should be investigated and *in vivo* studies should be performed to evaluate the viability of using the chosen collagen for tissue specific applications.

Marine collagens also have good potential to be used in cosmetics, because it provides elasticity to skin and thus having anti-aging properties. In addition, collagen has been found to have applications in wound healing as it prevents moisture and heat loss from wound tissue and act as a microbial infiltration barrier. Also, marine collagen is gaining increased application in drug delivery systems for delivery of proteins, medicines and genes (Xhauflaire-Uhoda *et. al.*, 2008; Calejo *et. al.*, 2012).

3. Gelatin

Gelatin is the denatured form of collagen. Gelatin produced from fish processing by-products is a potential alternative to mammalian gelatin. Owing to the unique properties of gelatin, it is increasingly used in various applications such as in the food, pharmaceutical, and photographic industries (Ka-Yeon Lee *et. al.*, 2016; Muralidharan Nagarajan *et. al.*, 2015). Since synthetic plastic packaging materials are responsible for serious environmental problems growing interest is generated in biodegradable films which can also act as carriers of antioxidants and antimicrobial agents. These active ingredients not only provide active components but also reduce microbial growth and thereby extend the shelf life of food products. Gelatin, obtained from the hydrolysis of collagen, can be used to prepare biodegradable films. Ka-Yeon Lee *et. al.* (2016) developed a pufferfish skin gelatin film with antimicrobial and antioxidant activities by adding *Moringa oleifera* L. leaf extract and applying the film to the packaging of Gouda cheese. Muralidharan Nagarajan *et. al.* (2015) demonstrated that the incorporation of ethanolic extract from coconut husk enhanced the properties of both gelatin film and nanocomposite film in which better water vapor barrier property could be attained.

4. Fucoidan

Fucoidans are a class of sulfated fucose-rich polysaccharides found in brown marine algae, echinoderms and some of the sea grasses. These molecules possess an

attractive array of bioactivities and potential applications in biomedical field such as in immune modulation, cancer prevention, pathogen destruction etc. Currently, fucoidans are approved for use in cosmetics, functional foods, dietary supplements and aquaculture feed supplements. However, the recent research demonstrates the promising potential of fucoidan in drug delivery and tissue regeneration applications. The chemical structure of fucoidan is depicted in Figure 2.

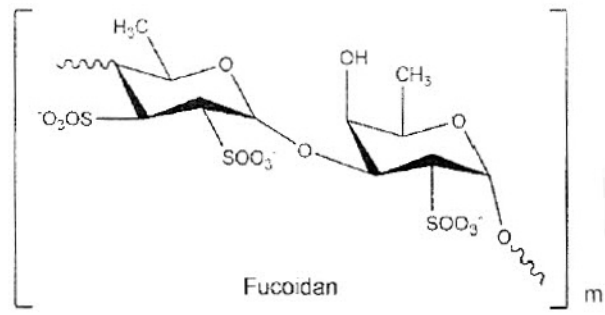

Fig. 2. Chemical structure of fucoidan

Fucoidan contains substantial percentages of L-fucose and sulfate ester groups. Due to their versatile biological properties, fucoidan from various species have been extensively investigated and shown to possess anticoagulant, antithrombotic, antivirus, anticancer, immuno-modulatory, hypolipidiemic, antioxidant and anti-inflammatory properties (Janet Helen Fitton, 2011; Li *et. al.*, 2008). Since the biological activities are directly related to the molecular weight and sulfate content of fucoidan, the biomodulation approaches should be based on these properties.

5. Carrageenan

Carrageenan is a nutritionally important red algal polysaccharide derived from macroalgae in the orders *Gelidiales*, *Gigartinales* and *Gracilariales*. Recent research reports show that carrageenan possesses anti-viral activities against herpes simplex, herpes zoster, dengue-2, vaccinia, rabies, and vesicular stomatitis virus (Baba *et. al.*, 1988). Although carrageenan possesses anti-cancer properties, the potential benefits and negative effects of including carrageenan products in the diet require continued research as the harmful nature of low molecular mass carrageenans.

6. Alginate

Alginate is a polysaccharide frequently obtained from brown seaweeds, including *Laminaria hyperborea*, *L. digitata*, *L. japonica*, *Ascophyllum nodosum* and *Macrocystis pyrifera*. Alginate is a commonly investigated biomaterial that has found numerous applications in biomedical science and tissue engineering because of its favorable properties, such as bio-compatibility and ease of gelation. Since alginate hydrogels have structural similarity to the extracellular matrices of soft tissues, this is an attractive

polymer in wound healing, drug delivery, and tissue engineering applications. There are various approaches to cross-link alginate chains to prepare gels, and these approaches alter the hydrogel properties relevant to specific biomedical applications (Lee and Mooney *et. al.*, 2012). These gelling methods include ionic cross-linking, thermal gelation, covalent cross- linking and cell cross-linking. The conventional role of alginate in pharmaceutics includes serving as thickening, gel forming, and stabilizing agents, as alginate can play a significant role in controlled-release of drug products. Alginate is used in the pharmaceutical industry for the delivery of small drug molecules and proteins.

7. Ulvan

Ulvan is a green seaweed polysaccharide extracted from the members of the Ulvales. Due to the high production of Ulvan, *Ulva* spp. is the best studied of the green seaweed polysaccharides. The biological properties of Ulvan are attributed to the unusual hydrophilic polyanionic features and structural analogies with animal glycosaminoglycan regulators (dermatan sulfate, heparin/heparin sulfates) and L-rhamnose specific lectins in humans. The reported bioactivities of Ulvan extracts *in vitro* include antibacterial, anticoagulant, antioxidant, antiviral, anti-tumor, anti-hyperlipidemic, and immuno-regulatory effects (Mark *et. al.*, 2017). Moreover, it is an important marine drug, prescribed in the Chinese Marine Materia Medica (Guan and Wang, 2009). High sulfate content Ulvan has demonstrated to improve lipid profiles through upregulation of FXR and PPARγ and downregulation of LXR (Huimin Qi and Jiwen Shen, 2015).

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