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REVIEW

Marine Collagen: An Emerging Player in Biomedical applications

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Abstract Mammalian collagen is a multifactorial biomaterial that is widely used for beneficial purposes in the advanced biomedical technologies. Generally, biomedical applicable collagen is extracted from the mammalian body, but it can also be derived from marine species. Recently, mammalian tissues collagen proteins are considered a great pathological risk for transmitted diseases, because purification of such protein is very challenging and needs efficient tool to avoid structure alteration. Thus, difficult extraction process and high cost decreased mammalian collagen demands for beneficial effects compared to marine collagen. In contrast, marine collagen is safe and easy to extract, however this potential source of collagen is hindered by low denaturing temperature, which is considered a main hurdle in the beneficial effects of marine collagen. Characterization and biomedical applications of marine collagen are in transition state and yet to be discovered. Therefore, an attempt was made to summarize the recent knowledge regarding different aspects of marine collagen applications in the biomedical engineering field.

Keywords Collagen · Extraction · Biomaterial · Marine

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Introduction

Cells, nutrients and bioactive scaffolds are the key components of tissue engineering for developing natural substitutes to reinstate, reestablish or regenerate malfunctioning tissues. The mammalian collagen was extensively used as a scaffolding material in the regenerative medicine (Ramshaw et al., 2009). Recently, the pathological risk of the mammalian collagen is pinpointed in term of transmitted diseases. It has been shown that bioactive natural organic materials originated from mammalian products such as collagen cannot be used for manufacturing of scaffold, because of severe inflectional problems including bovine spongiform encephalopathy, avian and swine influenzas, and tooth-and-mouth disease in bovine, pig, and buffalo occur all over the world (Song et al., 2006; Addad et al., 2011).

In the recent decade, green revolution of biotechnology not only explored the marine in term of pollution, energy, food but also investigate their biomedical applications. A variety of marine were identified as a safe source of bioactive material such as collagen. Furthermore, marine collagen from fish scales, skin, and bone has been widely used as a scaffold and a carrier due to excellent bioactive properties such as biocompatibility, low antigenicity, high biodegradability, and cell growth potential (Cho et al., 2014, Addad et al., 2011; Phanat et al., 2010). Marine collagen is now replacing mammalian collagen for different biomedical engineering purposes (Table 1). However, marine collagen-based systems have few disadvantages in terms of source dependent composition variation as well as low melting temperature (Yunoki et al., 2004). Therefore, improvement of physicochemical and biological properties of marine collagen, employing various biophysical tools will make marine collagen as effective scaffolds for biomedical applications and can be served as substitute for mammalian collagen in food, cosmetics, and biomedical materials.

Table 1 Comparative studies between mammalian and marine collagen

No	Mammalian	Marine
1	Expensive	Cheap
2	High melting point	Low melting point
3	High viscosity solution	Low viscosity solution
4	Difficult extraction (Low availability)	Easily available (Large amount)
5	Soluble in organic solvent	Soluble in water
6	Risk of transmitted diseases	No risk of transmitted diseases
7	Low contents of GLX and ALA with high PRO	High contents of GLX and ALA with low PRO

In this review paper, we mainly focus on the safety and structural modification of marine collagen and what possible methods can be practiced to improve the denaturing temperature of marine collagen.

Distribution, biosynthesis and molecular structure of collagen

Collagen is the essential structural protein that forms elastic molecular networks, which in turn strengthen the tendons and elastic sheets support the skin as well as internal organs of various species. Like mammalian skin, fish skin also contains a high molecular weight elastic protein, followed by higher concentration of connective tissue protein, known as collagen (Jayathilakan et al., 2012). It has been reported that jellyfish collagen contain relatively high contents of glutamine or glutamic acid (GLX) and alanine (ALA), whereas lower proline (PRO) content (78–83 residues/1000) than calf-skin collagen (122 residues/1000). Importantly, jellyfish collagen has additional cysteine and cystine (CYA) content of 10–13 residues/1000, which is normally not found in calf skin collagen (Addad et al., 2011; Song et al., 2006). The structural analysis showed that fish has a similar type I collagen as mammal and avian type I collagen, which contains three polypeptide chains, each consisting of about 1,000 amino acid residues with 100 kDa, approximately (Braco and Haard, 1995; Ho et al., 1997; Sivakumar et al. 2000; Saito et al., 2001, Addad et al., 2011).

Normally, collagen was extracted with Acid-solubilized collagen (ASC) and pepsin-solubilized collagen (PSC) from *Nile tilapia* skin with comparable high denaturing temperature. In both methods extracted collagen were similar in structural composition of amino acid such as absence of disulfide bond, and composed of α-1 (2 chains) α-2 (1 chain), β, and γ subunits (Potaros et al., 2009).

The major observed difference between fish and mammal collagens includes compatibility to crosslinking as well as thermostability at high temperatures (Yunoki et al., 2004; Cho et al., 2014). The denaturing temperature of marine collagen mainly depends on the habitat and composition of amino acids (proline and hydroxyproline) of various species. In line with this, shark skin collagen had a higher denaturing temperature than other cold-water fish skin, including jellyfish collagens (29 °C), Atlantic cod (15 °C), deep-sea red fish (*Sebastes mentella*) (16.1 °C), big eye snapper skin (31 °C). Although, aquatic collagen has a lower denaturing temperature than mammalian collagen calf skin collagen (40.8 °C) and porcine skin collagen (37 °C). Previously, it has been reported that low denaturing temperature of marine collagen is due to the lower amino acid contents (Proline and hydroxyproline) than mammalian collagen, which normally contain higher amino acid residues (Addad et al., 2011; Phanat et al., 2010). The amino acid composition of fish collagen is dependent on the type of the species, because difference has been noted in concentrations of glycine, Gly-X-Y amino acid sequence within the olive flounder, black rockfish, sea bass, and red sea bream were 17.5, 17.9, 18.6, and 16.9 g/100 g, respectively (Cho et al., 2014) (Table 1).

Methods for improving cross linking and thermal stability

Recently, various methods have been adopted for improving cross linking and thermal stability of marine collagen. In line with this, denaturing temperature (Td) of *salmon atelocollagen* (SC) can be increased by using a modified method. Normally, the natural Td of the SC solution was formed at 18.6 °C. The neutral buffer such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) was mixed with acidic SC solution at 4 °C during fibril formation of collagen. This method surprisingly increased the Td of SC from 18 to 47 °C. Additionally, the proliferation rate of human periodontal ligament cells was observed higher with this modified collagen as compared to pure fibrilar SC collagen (Yunoki et al., 2004). Also, cross linking of jellyfish extracted collagen with 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) was carried out by using freeze dryer for wide spread applications in tissue engineering. This study revealed that marine or jellyfish collagen EDC/NHS-cross-linked scaffolds exhibited higher cell density and enhanced cell proliferation as compared to

other naturally derived biomaterials such as bovine collagen (Song et al., 2006; Addad et al., 2011). The Td of marine collagen was also increased by employing a modified method for extraction with acid-solubilized collagen (ASC) and pepsin-solubilized collagen (PSC) from skin of *Nile tilapia*. These two different techniques increased the Td of marine collagen, which is 34.29 and 34.43 °C with ASC, 34.32 and 34.61 °C with PSC, respectively (Potaros et al., 2009). In another study, it has been reported that use of ASC and PSC for extraction of soluble collagen from the skin wastes of marine eel fish (*Evenchelys macrura*), increased total yields based on dry weight. It is also showed that ASC and PSC from eel fish skin exhibited higher thermal stability at 39 °C and 35 °C, respectively (Veeruraj et al., 2013). The amino acids analysis revealed that both ASC and PSC contained 190 and 200 amino acid residues per 1000 residues, respectively. Also, the hydrolysis of pepsin has no remarkable effect on the secondary structure of marine collagen as confirmed with Fourier Transform Infrared (FTIR) spectra results (Veeruraj et al., 2013). Recently, genipin has been investigated under high pressure of carbon dioxide to improve marine collagen cross linking and increased Td. In this method, collagen extracted from shark skin was used to prepare pre-scaffolds by freeze-drying, followed by genipin cross linking, under dense CO_2 atm for 16 h. This method produced a highly porous collagen scaffold with greater adherence properties and enhanced proliferation of Chondrocytes (ATDC5) cells (Fernandes-Silva et al. 2013).

The hybrid of marine collagen with chitosan and hydroxyapatite using a freeze-drying and lyophilization method for cross linking were also analyzed for its thermostability. It is revealed that the hybrid of marine collagen with chitosan and hydroxyapatite using a freeze-drying and lyophilization method shows a higher thermal stability of marine collagen hybrid (Pallela et al., 2012). Furthermore, it has been reported that PSC extracted from the body wall of crown-of-thorns starfish (COTS) (*Acanthaster planci*) using pepsin digestion in acetic acid, has shown greater biocompatibility. It is known that pepsin extracted collagen has a modified structure as compared to other marine and mammalian collagen. FTIR analyses have shown that the triple helical structure of PSC was well preserved during extraction of collagen from COTS. Collagens extracted from COTS by this method have slightly higher Td (33.0 °C) as compared to other marine extracted collagen, which was comparable with mammalian collagen (Tan et al., 2013). In another study, porous scaffolds from jellyfish collagen, refibrillized under optimized conditions, were fabricated by using freeze-drying and subsequent chemical cross-linking was carried out. Scaffolds possessed an open porosity of 98.2 % and these scaffolds were stable under cyclic compression and displayed an elastic behavior (Hoyer et al., 2014).

In-vitro and in-vivo safety analysis of fish collagen

Various in-vitro and in-vivo analysis have confirmed the safety of marine collagen. In line with this, SC collagen has been used in order to promote human periodontal ligament cells growth. Because, modified TD of SC collagen exhibited higher proliferation rate of human periodontal ligament cells as compared to pure salmon fibrillar collagen (Yunoki et al., 2004). Previously, in-vitro study has shown the cytotoxicity analysis of jellyfish collagen, and concluded that it can be used as a scaffold material and carrier due to its excellent bioactive properties as well as potential for cell growth. Also, jelly fish collagen is non-toxic and had higher cell viability (fibroblastic, epithelial, osteoblastic and fibrosarcoma), as compared to bovine collagen (Song et al., 2006; Addad et al., 2011). The freeze-dried EDC/NHS cross-linked porous jellyfish collagen scaffolds were characterized for its high porosity and an interconnected pore structure. Cytotoxicity tests were also performed, which includes the measurements of pro-inflammatory cytokine secretion, antibody secretion, and the population change of immune cells after in-vivo implantation. This study demonstrated that jellyfish collagen induced a comparable immune response and it has more potential than bovine collagen (Song et al., 2006; Addad et al., 2011). The tilapia (*Oreochromis aureus*) scales extracted collagen was analyzed in culture medium for facial fibroblast cell proliferation and its production ability of procollagen. The proteases extracted collagen of fish-scale collagen peptides (FSCPs) was shown to induce cell proliferation of facial fibroblast cells and procollagen synthesis in a time- and dose-dependent manner (Chai et al., 2010). The extract of fish collagen gel was also examined for sterility (bacteria and viruses), cell toxicity, sensitization; chromosomal aberrations, intracutaneous reaction, acute system toxicity, pyrogenic reaction and hemolysis. This study concluded that all aforementioned parameters were found negative. The atelocollagen prepared from tilapia is known to be a promising biomaterial to use as a scaffold in regenerative medicine (Yamamoto et al., 2014). Another study has also reported the potential of porous scaffolds from jellyfish collagen for human mesenchymal stem cells (hMSCs) proliferation and expression of Chondrogenic markers. This study determined that it has no cytotoxic effect and promote hMSC proliferation. Chondrogenic-induced hMSCs stimulation revealed viable cells and upregulation of Chondrogenic markers at mRNA level from day 1 to 21 on jellyfish collagen scaffolds (Hoyer et al., 2014). The hybrid of marine collagen with chitosan and hydroxyapatite using a freeze-drying and lyophilization method for cross linking were also analyzed for cell viability and proliferation. Biophysical staining experiments have showed that proliferation of MG-63 cell lines was relatively higher in composite scaffolds than pure chitosan. This study further confirmed the therapeutic role of marine

collagen for bone repair and bone augmentation along with other biomedical applications (Pallela et al., 2012).

The marine collagen peptides from *Chum Salmon* skin was tested for its wound healing effect in rat model. Marin collagen was administered (2 g/kg) in wound-induced rats in comparison with control group. The results showed faster wound healing and improved tissue regeneration at the wound site after administration of marine collagen as well as enhanced angiogenesis and formed thicker and better organized collagen fiber deposition compared to vehicle-treated group (Zhang et al., 2011). In another study, the marine collagen was tested for nasal tissue engineering both in-vitro and in-vivo of rat model explants. Histological and immunological tests confirmed that marine collagen has no cytotoxic reaction in-vitro and in-vivo experiments. The adoptability experiment has shown greater adherence of culture Chondrocytes on marine collagen by expressing cartilaginous matrix proteins, such as collagen type II. Similarly, in-vivo septal cartilage defects repair experiment was performed on marine collagen scaffold, which showed a significant repairing effect as compared to non collagenous scaffold transplant in rat model (Bermueller et al. 2013).

Summary

The aforementioned description clearly narrates that marine collagen has potential uses in tissue engineering and regeneration. However, all fishes collagen have lower denaturation temperatures compared to vertebrate's collagen, indicating that fish collagen is generally less stable than mammalian counterparts. The lower thermal stability of fish collagen might be due to short amino acid (proline and hydroxyproline) residues as compared mammalian collagens. The denaturing temperature of fish collagen could be increased by modified extraction method as well as by making a hybrid composite for biomedical application. The future study needs to investigate the detailed characteristics of marine collagen as well as different methods should be employed for improving thermal stability of marine collagen. The modification of thermal stability of marine collagen will explore a new era of potential application of collagen in tissue engineering and will provide a base for tissue regeneration in near future.

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