


# Marine polysaccharides: therapeutic efficacy and biomedical applications

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**Abstract** The ocean contains numerous marine organisms, including algae, animals, and plants, from which diverse marine polysaccharides with useful physicochemical and biological properties can be extracted. In particular, fucoidan, carrageenan, alginate, and chitosan have been extensively investigated in pharmaceutical and biomedical fields owing to their desirable characteristics, such as biocompatibility, biodegradability, and bioactivity. Various therapeutic efficacies of marine polysaccharides have been elucidated, including the inhibition of cancer, inflammation, and viral infection. The therapeutic activities of these polysaccharides have been demonstrated in various settings, from *in vitro* laboratory-scale experiments to clinical trials. In addition, marine polysaccharides have been exploited for tissue engineering, the immobilization of biomolecules, and stent coating. Their ability to detect and respond to external stimuli, such as pH, temperature, and electric fields, has enabled their use in the design of novel drug delivery systems. Thus, along with the promising characteristics of marine polysaccharides, this review will comprehensively detail their various therapeutic, biomedical, and miscellaneous applications.

**Keywords** Marine polysaccharide · Anti-cancer · Anti-inflammatory · Anti-viral · Biosensor · Tissue regeneration

## Introduction

Polysaccharides are predominantly obtained from various parts of plants, algae, and even animals such as crab and prawn. They have experienced increasing use in the pharmaceutical and cosmetic industries for their clear advantages over synthetic polymers, which include economic benefits, safety, and ease of chemical modification. Polysaccharides can be chemically modified to improve the physicochemical and mechanical properties (Li et al. 2016).

In previous decades, marine-derived polysaccharides have received attention as a potential new class of biomaterials. The polysaccharides can be extracted from marine organisms at a minimal cost in comparison with those from plants owing to the abundance of marine organisms in the ocean (Cardoso et al. 2016; Manivasagan and Oh 2016; Ruocco et al. 2016). Advancements in biotechnology have also led to the *in vitro* production of various marine polysaccharides and production yields have been greatly improved through the optimization of growth conditions (Laurienzo 2010). In addition, marine polysaccharides are generally considered biocompatible and have little or no toxicity. In particular, fucoidan, carrageenan, alginate, and chitosan have attracted enormous attention from the pharmaceutical and biomedical industries owing to their physicochemical and biological properties (Venkatesan et al. 2015).

Marine polysaccharides have been extensively studied as therapeutic agents for the treatment of various diseases. For example, fucoidan extracted from several species of brown algae has shown promising therapeutic efficacies, including anti-cancer and anti-inflammatory activities, as presented in Table 1 (Fitton et al. 2015). Carrageenan obtained from red algae showed anti-viral activities against

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**Table 1** Summary of therapeutic efficacies and their functional mechanisms of fucoidan

Therapeutic efficacy	Functional mechanisms	References
Anti-cancer	Inhibition of angiogenesis	Teng et al. (2015), Huang et al. (2015) and Koyanagi et al. (2003)
	Induction of apoptosis	Xue et al. (2012)
	Downregulation of TGFR	Hsu et al. (2014)
	Upregulation of immune response	Jin et al. (2014)
	Endurance of prolonged chemotherapy	Ikeguchi et al. (2011)
Anti-inflammatory	Inhibition of enzymes related to inflammation	Tsubura et al. (2012, 2015)
	Downregulation of mRNA expression inflammatory chemokines	Yang (2012)
	Blockage of selectin	Myers et al. (2010)
	Induction of immune response and downregulation of inflammatory cytokines	O'Connor et al. (2011)

*TGFR* transforming growth factor  $\beta$  receptor

various types of viruses (Wang et al. 2012). In addition, alginate extracted from brown algae and chitosan obtained from marine crustaceans have been explored for the treatment of hypertension and fungal diseases.

Marine polysaccharides can also be widely applied in the field of biomedical engineering owing to their tissue regenerative properties observed during the process of wound healing (d'Ayala et al. 2008). These materials can be used to improve the stability and sensitivity of biosensors by using tightly affixed biomolecules, such as enzymes and antibodies. For these reasons, various biomedical systems that utilize marine polysaccharides are currently under study for use in tissue engineering applications. Marine polysaccharides are also known to respond to external stimuli, including pH, temperature, and the presence of an electric field (Table 2).

In order to widen the applicability of marine-derived polysaccharides, an understanding of the functions of marine-derived polysaccharides in the pharmaceutical and biomedical industries is crucial. Thus, this review comprehensively discusses the therapeutic activities and biomedical applications of marine polysaccharides, with a particular focus on fucoidan, carrageenan, alginate, and chitosan.

## Therapeutic efficacies of marine polysaccharides

### Fucoidan

#### *Anti-cancer activity*

Cancer is a group of diseases caused by out-of-control cell growth. Although the exact causes of cancer remain unknown, one of the main mechanisms may be the

occurrence of mutations in normal genes or the tumor suppressor gene p53 (Ozaki and Nakagawara 2011). Common treatments for cancer include surgery, chemotherapy, and radiation therapy. However, the main limitation of these therapies is the severe damage caused to normal cells with a high proliferative index, such as those in the gastrointestinal tract and bone marrow (Edris 2007). Thus, marine polysaccharides with various anti-cancer activities have attracted enormous attention as potential alternative treatments (Xue et al. 2012). In particular, fucoidan, a marine polysaccharide derived from brown seaweed, has demonstrated promising anti-cancer activities with various therapeutic mechanisms (Patel 2012).

One of the major anti-cancer mechanisms exhibited by fucoidan is the inhibition of the angiogenesis of tumor tissues through the downregulation of vascular endothelial growth factor (VEGF) (Teng et al. 2015). VEGF activates both vasculogenesis and angiogenesis in response to hypoxia, a unique feature of locally advanced solid tumors (Lin et al. 2004; Vaupel and Mayer 2007). The VEGF gene is one of over 40 genes activated by hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ), a transcription factor controlled by the ERK and PI3K/Akt/mTOR pathways, which are critical pathways in the growth and proliferation of tumor cells. The researchers showed that fucoidan significantly inhibited the phosphorylation of the PI3K/Akt/mTOR cascade in hypoxia-induced mouse hepatocarcinoma Hca-F cells, thereby successfully downregulating VEGF expression and angiogenesis of tumor tissues. Huang et al. (2015) also demonstrated that the anti-VEGF effect of fucoidan could also inhibit lung carcinoma metastases. VEGF is able to regulate vessel permeability, thus promoting tumor metastasis (Shinkaruk et al. 2003). As matrix metalloproteinases (MMPs) are involved in the integrity of the membrane, the disruption of MMPs can lead to tumor

**Table 2** The studied marine polysaccharides and their biomedical applications

Marine polysaccharide	Biomedical applications	References
Alginate	Fundamental material of scaffolds for tissue engineering and improving cardiac function	Alsberg et al. (2001), Landa et al. (2008), Moshaverinia et al. (2012) and Lee et al. (2015)
	Enhancement of sensitivity of biosensor	Abu-Rabeah and Marks (2009) and Han et al. (2014)
	Inhibition of infection on implant sites	Lan et al. (2013)
	Physical barrier against peritoneal adhesion	Cho et al. (2010)
	Stimuli-responsive drug release	Iliescu et al. (2014), Shi et al. (2014) and Wang et al. (2014)
Carrageenan	Scaffolds for cartilage and bone tissue engineering	Santo et al. (2009) and Rocha et al. (2011)
	Temperature-responsive drug release	Li et al. (2014a, b)
Fucoidan	Vascular and bone regeneration	Chabut et al. (2004), Lake et al. (2006), Changotade et al. (2008) and Han et al. (2015)
	Coating material of stent surface to prevent restenosis	Religa et al. (2000) and Kim et al. (2015)
Chitosan	Enhancement of stability, selectivity and sensitivity of biosensor	Khan et al. (2008), Wang et al. (2009), Wu et al. (2009), Safavi and Farjami (2011), Zeng et al. (2011), Kavitha et al. (2013), Su et al. (2013), Şenel (2015), Zhao et al. (2015) and Warner and Andreescu (2016)
	Inhibition of infection on implant sites	Bumgardner et al. (2003), Alex et al. (2008), Swanson et al. (2011), Wiarachai et al. (2012), Honglue et al. (2013) and Pishbin et al. (2014)
	Fabrication of stent and its surface coating	Lauto et al. (2001) and Zhao et al. (2016)
	Physical barrier against peritoneal adhesion	Li et al. (2014b) and Zhu and Zhang (2016)
	Stimuli-responsive drug release	Li et al. (2013), Chandran and Sandhyarani (2014), Naik and Nair (2014) and Saboktakin et al. (2015)

invasiveness. Their findings indicated that fucoidan downregulated VEGF and MMP expression in Lewis lung carcinoma (LLC)-inoculated model mice, which resulted in the inhibition of cancer metastasis. Koyanagi et al. (2003) also reported the anti-angiogenic activities of fucoidan on LLC and B16 melanoma in mice. Therefore, it was demonstrated that fucoidan might prevent the binding of VEGF to vascular endothelial growth factor receptor in cells and downregulate signal transduction.

An alternative anti-cancer mechanism of fucoidan was found to be the induction of apoptosis. Anti-apoptotic proteins such as Bcl-2 and survivin are known to play pivotal roles in the regulation of apoptosis. According to Xue et al. (2012), fucoidan induced a pro-apoptotic effect in mouse breast cancer 4T1 cells through the decreased expression of Bcl-2. Fucoidan also decreased the expression of survivin, the smallest member of the inhibitor of apoptosis protein family. In addition, fucoidan was capable of inducing the release cytochrome C from the mitochondria to the cytosol, which subsequently activates the caspase cascade and, ultimately, leads to tumor cell apoptosis (Fig. 1).

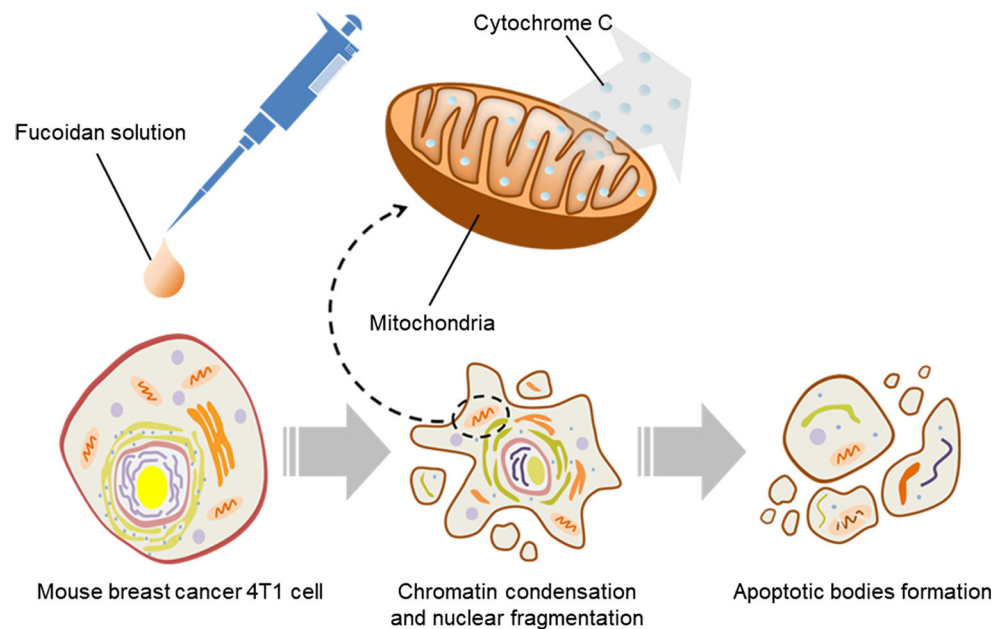
In a study conducted by Hsu et al. (2014), fucoidan exerted anti-cancer activity through the downregulation of transforming growth factor  $\beta$  receptor I and II (TGFRI and TGFRII), which have been reported to induce tumor

growth when over-expressed. The treatment with fucoidan enhanced smurf2-mediated ubiquitination, which resulted in the degradation of the TGFRI. The in vivo experimental results supported the fucoidan-induced marked reduction of tumor volume in male C57BL/6 mice xenografted with LLC1 cells. Fucoidan also reduced the viability of various lung cancer cells, such as human non-small cell lung cancer cells and mouse lung cancer cells.

Other studies have shown that fucoidan exerts a variety of indirect anti-cancer activities as a potential adjuvant, such as the upregulation of immune responses and the enhancement of endurance in patients receiving prolonged chemotherapy. Jin et al. (2014) reported that fucoidan can be used as an adjuvant through the promotion of helper T cell and cytotoxic T cell ( $T_c$  cell) immune responses against the ovalbumin (OVA) antigen. The intraperitoneal injection of OVA with fucoidan showed enhanced production of OVA-specific antibodies and T cell responses in C57BL/6 mice. Moreover, the expression levels of major histocompatibility complex class I and II proteins were upregulated by the injection of fucoidan. It is therefore clear that fucoidan can function as a potential adjuvant. Furthermore, this notable function may contribute to the development of a tumor vaccine.

Ikeguchi et al. (2011) observed that fucoidan exerted promising protective effects against the toxicity of

**Fig. 1** Schematic diagram illustrating the mechanism of anticancer efficacy of fucoidan against mouse breast cancer 4T1 cells. Cells were treated with different concentrations of fucoidan for 48 h. Apoptotic features such as chromatin condensation and DNA fragmentation were induced in fucoidan-treated 4T1 cells



chemotherapeutic drugs for patients with unresectable advanced or recurrent colorectal cancer. In a trial of 20 patients with unresectable advanced or recurrent colorectal cancer, all patients had been previously administered with anti-cancer drugs such as oxaliplatin plus 5-fluorouracil/leucovorin or irinotecan plus 5-fluorouracil/leucovorin. The participants were randomized into two different groups: a fucoidan-treated group ( $n = 10$ ) and an untreated control group ( $n = 10$ ). It was observed that the fucoidan-treated group endured prolonged chemotherapy without general fatigue and other adverse effects. The results demonstrated that fucoidan might itself exert anti-cancer effects, but the precise mechanism of these effects remains unverified.

#### *Anti-inflammatory efficacy*

The association of inflammation with several chronic diseases is indubitable. For this reason, the regulation of inflammation is highly important. Generally, inflammation can be treated by various medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids.

Topical steroids are the oldest and most frequently used treatments for local inflammation; however, the prolonged use of steroids may cause serious side effects, including epidermal thinning, easy bruising, perioral dermatitis, and skin atrophy (Coondoo et al. 2014). Thus, numerous researchers have sought natural products that exhibit anti-inflammatory response without severe side effects. Among the natural materials investigated, the anti-inflammatory activity of fucoidan has resulted in its widespread use for the treatment of inflammation-associated diseases.

Tsubura et al. (2012) modulated local inflammation by the topical treatment of fucoidan. In this experiment, two Japanese women with persistent and painful recurrent aphthous stomatitis (RAS) applied a fucoidan-containing cream on their lesions twice per day. After 1 week, no side effects were seen in the patients and their symptoms were significantly improved at follow-up after 3 months. Subsequently, the same researchers conducted a similar experiment with a chewable tablet that contained 32% fucose to treat an adult patient with painful symptomatic inflammatory tongue (SIT). This tablet was prescribed because the patient was unresponsive to topical corticosteroid ointment and NSAIDs. After chewing the tablet, the patient did not experience any stinging symptoms and a remarkable increase in healing rate was observed. This result may be attributable to the remodeling and repairing processes exerted by the anti-inflammatory effects of fucoidan (Tsubura et al. 2015). The researchers suggested that fucoidan exerted anti-inflammatory activity through the inhibition of various enzymes such as MMPs, hyaluronidases, and elastases.

Fucoidan was also used for the treatment of atopic dermatitis (AD) owing to its ability to downregulate the expression of AD-associated cytokines and chemokines. Yang (2012) observed that fucoidan relieved AD-like symptoms in 1-chloro 2,4-dinitrobenzene (DNCB) treated Nc/Nga mice. Fucoidan and dexamethasone exhibited similar activity, which was a strong anti-inflammatory effect in comparison with the control groups. In the fucoidan-treated group, the serum levels of inflammatory factors, such as interleukins (ILs) and histamine, were considerably reduced, thereby preventing mast cell

infiltration and immunoglobulin E secretion at the lesion site. Therefore, fucoidan could be used in potential therapeutics for the treatment of AD symptoms in preference to the existing corticosteroid drugs.

Other studies have shown that fucoidan was able to prevent inflammatory damage after ischemic events through its action as a blocker of selectin, a protein that recruits leukocytes to the inflammatory lesions (Koch et al. 1993; Ritter et al. 1998; Cumashi et al. 2007). Myers et al. (2010) performed an open label study on 12 participants with osteoarthritis of the knee. The participants were randomly assigned to groups treated with different doses of fucoidan. Approximately 12 weeks after consumption of the formulation, fucoidan resulted in a dose-dependent decrease in comprehensive arthritis test subscales of pain, stiffness, physical difficulty, and overall symptom severity. The authors suggested that because of its selectin-blocking activity, fucoidan may have reduced leukocyte accumulation at the arthritic region and therefore markedly improved the inflammatory symptoms.

Another study investigated the immunomodulatory effect of fucoidan. O'Connor et al. (2011) conducted an open-label study on the use fucoidan as an immune modulator. Healthy participants ( $n = 12$ ) were randomly assigned to receive different doses of fucoidan. After a 4-week period, the oral administration of fucoidan increased the number of  $T_c$  cells and the phagocytic capacity of monocytes. Fucoidan also decreased IL-6, one of the major inflammatory cytokines. Thus, these results also supported the use of fucoidan as a potential anti-inflammatory drug.

## Carrageenan

### *Anti-viral properties*

Viruses are submicroscopic infective agents that consist of a nucleic acid core and protein coat, and require live host cells to replicate (Orlova 2009). If pathogenic viruses infect human cells, they may cause a variety of symptoms, from mild diseases such as common cold or flu to chronic diseases as infection of human papillomavirus (HPV). Therefore, numerous types of anti-viral medicines have been developed to treat such infections caused by viruses. Recently, marine polysaccharides have received attention because of their anti-viral effects (Ghosh et al. 2008). Specifically, the anti-viral properties of carrageenan have shown promising inhibitory effects on many types of viruses.

Leibbrandt et al. (2010) exploited the anti-viral activities of carrageenan against influenza A virus. The carrageenan treatment resulted in an increased survival rate of influenza virus-infected cells in vitro. The authors discussed that

such a result was obtained because carrageenan effectively blocked the internalization of the virus through the disruption of the interaction between its host cell receptor, through direct binding with the virus particles. In addition, the inhibition of neuraminidase, an essential glycoprotein for the release of the virus from the host cell, also supported this result (Buck et al. 2006; Talarico and Damonte 2007). In consensus with the in vitro results, the in vivo test also indicated that the intranasal application of carrageenan markedly increased the survival rate of mice infected with the influenza virus. This result was similar to that obtained for oseltamivir (brand name Tamiflu), a commercialized neuraminidase inhibitor. Therefore, the authors suggested that carrageenan could be used as a potent anti-viral agent and may replace the marketed drug.

To further elucidate the effectiveness of carrageenan nasal spray, Koenighofer et al. (2014) conducted two double-blinded randomized controlled trials in patients with the common cold infected by human rhinovirus, human coronavirus, and influenza A virus. Patients who received carrageenan nasal spray experienced fewer relapses of symptoms and increased viral clearance during the trial compared with the placebo group. These findings therefore demonstrated the clinical effectiveness of carrageenan as an anti-viral agent against the three virus subgroups.

Rodríguez et al. (2014) showed that carrageenan could also inhibit HPV. The authors investigated the in vitro and in vivo anti-viral activities of carrageenan in HeLa cells and a mouse HPV pseudovirus (PsV) model, respectively. Gel formulations with different amounts of carrageenan (3.06 and 1.04%, w/v) were used for both in vitro and in vivo tests. All formulations showed non-toxic and anti-viral properties against three types of HPV in vitro: HPV 16, 18, and 45. Also, the intravaginal administration of carrageenan-based gels to BALB/c mice resulted in significant protection from HPV-16 PsV infection. The anti-viral activity of carrageenan gel showed dose-dependent activity in vivo. These effects might be related to the properties of carrageenan in blocking the adhesion of the virus to heparan sulfate expressed on the cell surface, which plays a key role in viral entry and infection (Shukla and Spear 2001; Zhu et al. 2011).

## Alginate

### *Anti-cystic fibrosis activity*

Alginate, which disrupts the mucin-mucin interaction or the chelation of calcium ions (Ambort et al. 2012), can be utilized to reduce the accumulation of intestinal mucus in mice with cystic fibrosis (CF). Vitko et al. (2016) evaluated the therapeutic effect of alginate oligosaccharide, OligoG,



on the CF mouse model. CF is caused by a mutation in the gene that encodes the CF transmembrane conductance regulator protein. This leads to intestinal damage through the accumulation of glue-like and thick mucus within ducts and tubes in the body. In the CF mice, OligoG solution enhanced the intestinal transit time by effectively causing the intestinal contents to move into the small intestine and resulted in higher survival rates than regular water. These results demonstrated that the alginate oligomer, OligoG, may play a key role in the decrease of mucus accumulation through the disruption of mucin cross-linking or calcium sequestration (Taylor Nordgård and Draget 2011; Sletmoen et al. 2012; Pritchard et al. 2016).

#### *Anti-hypertensive efficacy*

Alginate also shows a potent preventive effect against hypertension. Hypertension may result from several causes, such as excess sodium intake or hypokalemia (Adrogué and Madias 2007). In the treatment of hypertension, it is important to reduce sodium retention and increase potassium concentration. Chen et al. (2010) investigated the anti-hypertensive effects of low molecular mass potassium alginate (L-PA) and KCl in hypertensive rats induced by deoxycorticosterone acetate (DOCA) salt. After oral administration for 30 days, L-PA significantly prevented the development of hypertension and mortality in a dose-dependent manner. L-PA also inhibited cardiac and renal hypertrophy derived under hypokalemia through the reversal of both hypertension and hypokalemia in DOCA salt-induced hypertensive rats. The anti-hypertensive effects were much greater than KCl. These findings could be explained mechanistically; L-PA might provide a greater cellular exchange of sodium than KCl and directly regulate vasoconstriction and sodium resorption in renal tubules. All these myriad therapeutic effects make alginate an excellent candidate for the development of potentially promising therapies for various diseases.

### **Chitosan**

#### *Antifungal properties*

One study indicated that chitosan showed potent antifungal activity against *Candida* strains that caused superficial mycoses, including vulvovaginal candidiasis. Although the exact antifungal mechanism of chitosan was not clearly identified, it was likely to result from the disruption of membrane stability through the binding of the positively charged chitosan and the negatively charged cell wall of the fungus (Fig. 2). Alburquenque et al. (2010) explored the possible antifungal properties of low molecular weight chitosan (LMWC) in 105 clinical isolates of *Candida*

species. The in vitro treatment of LMWC showed significant inhibition of the strains and improved antifungal activity at pH 4.0, which suggested that chitosan has strong antifungal properties. In addition, some reports have suggested that the toxicity of chitosan might be much less likely to occur in humans (Park et al. 2008; Vinsova and Vavrikova 2008). Finally, medications containing chitosan exerted appreciably low toxicity and a marked antifungal effect that could potentially be exploited for the treatment of candidiasis, which generally emerges in an environment with a pH of 4.0–4.5.

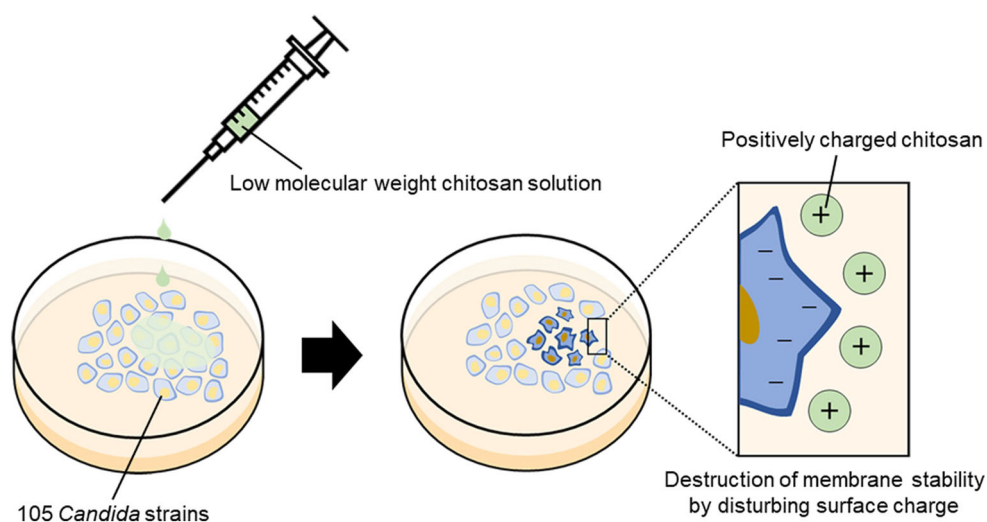
### **Biomedical applications of marine polysaccharides**

#### **Application of marine polysaccharides in tissue engineering**

Damaged tissues can result in severe health problems in humans. For this reason, scientists have continuously attempted to regenerate the damaged tissues by various strategies using platforms containing therapeutic cells, such as stem cells and tissue regenerating agents. In order to confer multifunctionality and biocompatibility to these platforms, the identification of suitable fundamental materials for the fabrication of the platforms is highly crucial. Among the materials investigated for this purpose, marine polysaccharides, such as alginate, fucoidan, and carrageenan, have shown promise owing to their gel formation ability under mild conditions (Gutowska et al. 2001; d'Ayala et al. 2008), biocompatibility (Lee and Mooney 2012), biocompatibility, and non-cytotoxic properties (Martins et al. 2015). These marine polysaccharides have been processed into scaffolds for tissue engineering, cancer therapy (Stephan et al. 2015; Park et al. 2017) and carriers of various tissue-regenerating agents, such as growth factors, DNA, and mRNAs for the production of proteins that contribute to tissue repair.

Alginate can be used to prepare scaffolds for bone tissue engineering because of its intrinsic biocompatibility and low toxicity (Alsberg et al. 2001; Lee et al. 2004). The mannuronic acid/guluronic acid ratio of alginate was shown to highly affect the biocompatibility of alginate; Klöck et al. (1997) demonstrated that alginate with 68% mannuronic acid did not elicit any inflammatory responses when it was implanted in rats and Mushollaeni et al. (2014) observed the low toxicity of alginate extracted from various seaweeds, such as *Sargassum* and *Padina*, in mice. These results indicated that many different kinds of alginate did not exhibit significant toxicological effects, although one limitation of alginate is that it cannot be degraded in the human body. However, alginate has been oxidized to confer biodegradability and this strategy has

**Fig. 2** Schematic diagram of the antimicrobial mechanism of low molecular weight chitosan (LMWC) against 105 *Candida* isolates. 105 *Candida* strains were markedly inhibited by LMWC dissolved in acetic acid. Chitosan with positive charges is capable of interacting with anionic charged cell walls of the *Candida* isolates, resulting in destabilization of the walls



been used for some tissue engineering applications (Gao et al. 2009). Moshaverinia et al. (2012) compared the degradation of alginate hydrogels with different oxidation levels in PBS and observed that the oxidation rate of alginate hydrogels was proportional to the degradation rate of the polysaccharide.

Carrageenan has been used to fabricate carriers for the delivery of tissue regenerating agents. The marine polysaccharides have been proven to exhibit good biocompatibility and non-toxicity (Cohen and Ito 2002; Rocha et al. 2011). Eccles et al. (2010) evaluated the efficacy and safety of carrageenan in human volunteers. In the carrageenan-treated group, carrageenan reduced the level of pro-inflammatory mediators, such as fibroblast growth factor-2 (FGF-2), ILs, and interferon- $\alpha$ 2. Similar results were also reported by McKim et al. (2016), who evaluated the cytotoxicity of carrageenan in human intestinal and hepatic cell lines. The results showed that carrageenan did not induce pro-inflammatory factors, such as IL-8, IL-6, and monocyte chemoattractant protein-1, in human intestinal and hepatic cells. These studies demonstrated the biocompatibility, safety, and non-toxicity of carrageenan. Moreover, the gel-forming ability of carrageenan can be exploited to control the release of the cargo. Carrageenan hydrogels containing platelet-derived growth factor (PDGF) with bone tissue regenerating property through the promotion of angiogenesis (Battagay et al. 1994) have been investigated (Santo et al. 2009). The research group evaluated the encapsulation efficacy and release rate of PDGF incorporated into carrageenan hydrogels. They observed that the sustained release of PDGF from the carrageenan hydrogels resulted from the crosslinking of the hydrogels by  $K^+$  ions and the formation of the ionic bonds between the sulfate group of the cations and the carrageenan. This process enhanced the mechanical strength of the hydrogel, which might be a crucial factor for the sustained release of

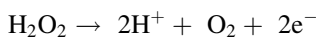
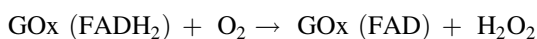
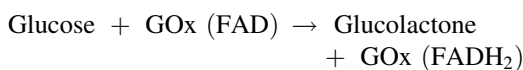
PDGF. Furthermore, the encapsulation efficiency of PDGF in the carrageenan hydrogels was controlled by the cross-linking time of carrageenan by  $K^+$  ions.

In addition, fucoidan assisted the tissue regeneration processes by the modulation of physiological processes. Han et al. (2015) used the natural antioxidant property of fucoidan to protect mesenchymal stem cells (MSCs) from ischemia-induced apoptosis. The group suggested that fucoidan promoted the expression of manganese superoxide dismutase, which enhanced the survival of MSCs through the inhibition of the generation of intracellular reactive oxygen species. Moreover, low-molecular-weight (LMW) fucoidan has been reported to increase the expression of growth factors such as FGFs and vascular endothelial growth factors (Chabut et al. 2004; Lake et al. 2006). In a study, LMW fucoidan promoted the proliferation of human osteoblasts through an increase in the production of the growth factors and the subsequent promotion of the regeneration of bone tissues (Changotade et al. 2008). These applications were possible because fucoidan did not cause any acute or subchronic toxicity. Li et al. (2005) demonstrated that fucoidan showed no signs of toxicity after the oral administration to rats. At a dose of 300 mg/kg body weight per day, fucoidan showed no significant toxicity and no adverse effects. Chung et al. (2010) also reported a low toxicity of fucoidan in various in vitro and in vivo tests. In the Ames test, no mutagenicity was observed in the fucoidan-treated group. In addition, fucoidan did not induce toxicological changes in rats after 28 days of oral administration. These results confirmed that it was possible to use fucoidan for the applications of tissue engineering.

### Immobilization of biomolecules for improvement of biosensor sensitivity

A biosensor is a diagnostic device that detects specific biochemicals generated by the reaction between targeted analytes and the immobilized biomolecules, such as enzymes, antibodies, or receptors on the electrode. Compared with other diagnostic tools, biosensors offer advantages of high specificity, fast response time, user convenience, and portability. For example, patients with diabetes can relieve potential complications, such as heart disease, kidney failure, and blindness, by the personal management of blood glucose concentration using a glucose biosensor (Wang 2008). Biosensors generally consist of biological elements that react with the target analyte and a transducer that transforms the signal that results from the reaction between the biomolecules to measurable or quantifiable values. The immobilization of the sensing element to the electrode is therefore crucial in the design of an efficient biosensor. After the immobilization process, the biological elements are required to maintain their chemical structure to react with the analyte and to remain on the electrode to ensure high stability of the biosensor and reproducible results (Sassolas et al. 2012).

The glucose biosensor typically detects the products from the reaction of immobilized enzymes; in general, the reaction of glucose oxidase (GOx) with glucose. The immobilized GOx oxidizes glucose, producing hydrogen peroxide. Hydrogen peroxide is then oxidized by catalysts, such as palladium and platinum anode, to produce electrons, which are detected to produce results.



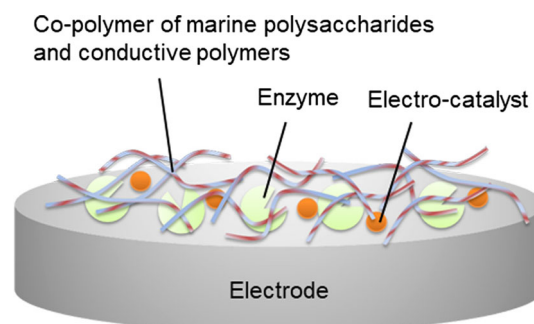
The biocompatibility and non-toxic properties of alginate and chitosan (Kang et al. 2015) provide the biomolecules with a suitable microenvironment for the reactions. Moreover, the hydrophilic properties and gel-formation ability of the polysaccharides can improve the stability of the immobilized biomolecules. When the pH is above its  $\text{pK}_a$ , chitosan becomes deprotonated, soluble, and subsequently forms a viscous hydrogel matrix. Alginate, owing to its anionic properties, can also form a viscous matrix through crosslinking with divalent cations or in an acidic environment. These properties of the marine polysaccharides can be used to fix the biomolecules and maintain their activity. However, as the marine polysaccharides exhibited

low catalytic activity, hydrogels fabricated with the polysaccharides and electrocatalysts have been investigated, as described below. Moreover, co-polymers of the marine polymers and conductive polymers, such as polypyrrole, polyaniline, and polythiophene, can improve the sensitivity of biosensors (Fig. 3).

A chitosan matrix improved the stability and selectivity of the biosensor through the provision of a stable environment to the oxidation catalysts, such as magnetite (Kavitha et al. 2013), platinum (Wu et al. 2009), and palladium (Zeng et al. 2011). Prussian blue (PB), a redox mediator for the selective detection of hydrogen peroxide, forms a hybrid film with chitosan. In neutral pH and a weakly alkaline medium, PB deposited in chitosan films showed improved stability (Wang et al. 2009). An alginate hydrogel prepared with calcium also increased the response efficiency between the biomolecules, which increased the sensitivity of the biosensor (Han et al. 2014).

In acidic conditions, positively charged chitosan can easily adsorb gold nanoparticles with catalytic properties and its amino groups can form amide linkages with conductive polymers, such as polypyrrole (Şenel 2015). The carboxyl residues of alginate can be used to form covalent bonds with conductive materials such as *N*-(3-aminopropyl) pyrrole, which leads to an increase in the conductivity and an improvement of the sensitivity of the biosensor (Abu-Rabeah and Marks 2009). Nanofibers prepared with chitosan and polyvinyl alcohol can also promote the enzyme-analyte response owing to a decrease in the diffusion resistance of the substrates and the high surface area of the matrix (Su et al. 2013).

Chitosan has also been used for other types of biosensors. Similar to a glucose biosensor, a cholesterol biosensor detects the hydrogen peroxide generated by the oxidative reaction of cholesterol oxidase. Chitosan-based films have been applied to the cholesterol biosensor to improve its



**Fig. 3** Improvement of function of biosensor by immobilizing detecting biomolecules on electrode using marine polysaccharides. To increase conductivity of the biosensor, conductive polymers can be compositely used with marine polysaccharides. Incorporation of electro-catalyst in the biosensor can promote bioreactions among the biomolecules



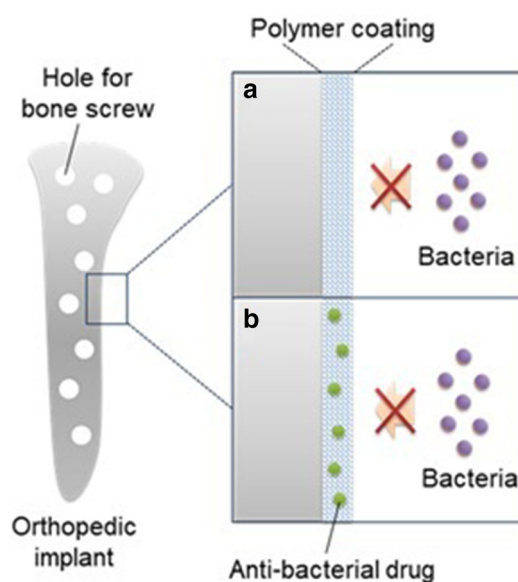
sensitivity (Khan et al. 2008; Safavi and Farjami 2011). In addition, the chitosan films (Warner and Andreescu 2016) and nanocomposites of chitosan with PB (Zhao et al. 2015) were also used to improve pesticide biosensors. Thus, the use of marine polysaccharides as fundamental materials for various biosensors have demonstrated a strong potential for the improvement of biosensor functions.

### Inhibition of infection at orthopedic or dental implant sites

Surgical implants are used for the repair or replacement of damaged organs or tissues. Such implants include orthopedic implants, dental implants, and stent. Despite the benefits of the surgical implants, the frequent occurrence of infections at the site of surgery has limited the usefulness of the implants. To resolve this problem, the implant surface has been coated with various materials for the prevention of the proliferation of bacteria such as *Staphylococcus aureus* (Jiri et al. 2014). To further inhibit the infection of bacteria, drug-loaded polymers have also been used to coat the surface of the implants, as shown in Fig. 4 (Noreen and Irving 2012).

The materials used for the surface coating of surgical implants include hydroxyapatite (HA), polymethylmethacrylate (PMMA), poly(lactic acid) (PLA), and poly(glycolic acid) (PGA). In comparison with the materials mentioned above, marine polysaccharides such as chitosan and alginate exhibited high a bacteriostatic property. In addition, the polysaccharides did not exhibit problems related to the toxicity of acidic degradation products generated by the degradation of PLA and PGA (Alex et al. 2008). The in vitro toxicity tests also demonstrated that the different molecular weights and degrees of deacetylation of chitosan exhibited low cytotoxicity to human lymphoblastic leukemia and human embryonic lung cells (Kean and Thanou 2010). In addition, the results of several tests, such as acute systemic toxicity test and irritation tests on eyes and skin, showed that chitosan did not cause any undesirable toxicity in mice, rabbits, and guinea pigs (Rao and Sharma 1997). The low toxicity, biocompatibility, and ease of film formation of the marine polysaccharides contributed to the attractiveness of the polysaccharides for the surface coating of surgical implants.

The electrophoretic deposition of positively charged chitosan was used to fabricate films that contained gentamicin for the prevention of the infection of the implant surface (Pishbin et al. 2014). The aldehyde groups of titanium formed by silane reactions also formed covalent bonds with the amine groups of chitosan, which could be exploited to coat the titanium surface of the implants with chitosan (Bumgardner et al. 2003) and cause the local drug



**Fig. 4** Coating surface of orthopedic implant with marine polysaccharides for inhibiting proliferation of bacteria on the implant surface. **a** Marine polysaccharides coated on the implant surface can inhibit the proliferation of bacteria by preventing the formation of biofilm by bacteria. **b** Anti-bacterial drugs can be loaded in the matrix of marine polysaccharides to further inhibit the proliferation of bacteria

delivery of antimicrobial drugs such as vancomycin (Swanson et al. 2011). Because chitosan is only positively charged below its  $pK_a$ , the antibacterial activity of the polysaccharide is only applicable in an acidic environment. To overcome this limitation, quaternized chitosan (QCh), which contains positively charged quaternary ammonium groups, has been used to increase the aqueous solubility and the antibacterial activity of chitosan. The antibacterial activity of QCh at a wide range of pH values has been successfully used for orthopedic implants (Wiarachai et al. 2012; Honglue et al. 2013).

Alginate hydrogels can also be used for the inhibition of the infection of surgical implants. A research group prepared ring-shaped dental implants with composite hydrogels of alginate and poly- $\epsilon$ -caprolactone (PCL) containing metronidazole. Through a variation of the ratio of alginate and PCL, the mechanical properties of the ring implants could be modified to achieve the sustained release of metronidazole (Lan et al. 2013).

### Alternative materials for stent fabrication and surface coating agents for the inhibition of restenosis

A stent is a tubular, mesh-structured medical device that is commonly used to recover the narrowed coronary artery caused by atherosclerosis. However, problems such as plaque reoccurrence over the stent, known as in-stent

restenosis, and the movement of the stent to other sites, known as migration, limit the usefulness of stents. To resolve the problems, stents have been modified in various ways to create self-expanding, biodegradable, or drug-eluting stents (Mani et al. 2007). The biodegradability, biocompatibility, adhesive properties, and anti-coagulant activity of chitosan can be exploited for this purpose.

Chitosan was also used to fabricate a self-expanding stent (Lauto et al. 2001). When the chitosan stent contacted with the moisture of the target tissue, it self-expanded and performed the stent function. In another study, thiolated chitosan, known to exert mucoadhesive properties through the formation of disulfide bonds with mucin layers, was coated onto the surface of the stents (Zhao et al. 2016). The stents coated with thiolated chitosan showed enhanced mucoadhesion, thereby preventing the unwanted migration of the stent. Fucoidan has also been coated onto the surface of stents to prevent restenosis that occurred through the growth of smooth muscle cells (SMC) over the stent (Kim et al. 2015). Fucoidan, a sulfated polysaccharide, inhibited the proliferation of rat SMC *in vitro*. The mechanism of inhibitory effect of fucoidan on restenosis has not been fully understood, but it may occur through the binding of fucoidan to growth factors, which affects the expression of fibronectin and thrombospondin and thereby inhibits the proliferation of SMC (Religa et al. 2000).

### Physical barrier for prevention of peritoneal adhesion

Peritoneal adhesion is a postoperative disease that is experienced by approximately 93% of patients who have undergone abdominal surgery. Its complications, which include small bowel obstruction, female infertility, and abdominal and pelvic pain, influence the patient's quality of life and burden the patients with huge surgical and hospital expenses (Ergul and Korukluoglu 2008).

Peritoneal adhesion is caused by cytokines, coagulation factors, and proteases. These molecules cause inflammation, angiogenesis, tissue repair, and ultimately lead to peritoneal adhesion (Schnüriger et al. 2011). One of the possible strategies for preventing peritoneal adhesion is the application of a physical barrier to inhibit the peritoneal adhesion at the damaged surface through the separation of the damaged site and other tissues.

The physical barrier is generally composed of natural polymers, such as gelatin, collagen, hyaluronic acid, and chondroitin sulfate, or synthetic polymers, such as silicone, poly(tetrafluoro ethylene), poly(vinyl alcohol), and poly(hydroxyl acid). These polymers have been shown to effectively reduce the peritoneal adhesion. However, rapid clearance of the barriers from the applied site is largely problematic and may require an additional suturing process

(Cho et al. 2010). The mucoadhesive properties of alginate and chitosan can be used to resolve the problem (Ahuja et al. 2016).

The carboxylic groups of alginate can form hydrogen bonds with the hydroxyl groups of adjacent tissue proteins. Therefore, un-crosslinked alginate hydrogels, which have many free carboxyl groups than crosslinked alginate gels, were used to prepare a mucoadhesive anti-adhesion film (Cho et al. 2010). The un-crosslinked hydrogels were also transparent and flexible, which are useful for the application to the injured site. The un-crosslinked alginate hydrogels successfully reduced the peritoneal adhesion.

N,O-carboxymethyl chitosan (NOCC) is a water-soluble, negatively charged polymer. Although the mechanism of anti-peritoneal adhesion of NOCC has not been fully elucidated yet, the inhibitory activity of NOCC on peritoneal adhesion and cardiac surgery adhesion was determined (Zhu and Zhang 2016). An injectable crosslinked NOCC-hyaluronic acid hydrogel was found to considerably reduce the formation of peritoneal adhesion and showed biocompatibility and biodegradability (Li et al. 2014b).

### Scaffolds for the improvement of cardiac function

During the progression of heart failure, the left ventricle (LV) wall thickness of the heart generally decreases and the shape of the LV changes from an ellipse to a sphere. This deformation of the LV cavity leads to increase in the LV contraction volume. The increased LV contraction volume causes increased stress to the LV wall, which is followed by the deterioration of heart failure. For this reason, the increased LV wall thickness and decreased LV cavity can prevent the aggravation of the heart failure. In clinical studies, alginate hydrogels have been found to possess a similar structure to that of the extracellular matrix (ECM) (Lee et al. 2015). Alginate hydrogels injected into the LV wall can increase the thickness of the LV wall. The structural restoration of the LV wall led to decrease in LV cavity and mechanical stress onto LV wall. The decreased mechanical stress onto the LV wall improved cardiac function (Landa et al. 2008; Lee et al. 2015).

### Stimuli-responsive drug delivery systems

The distinct advantages of marine polysaccharides have been exploited for the design of drug delivery systems. In particular, the conformation of the marine polysaccharide chains is considerably changed by the pH, temperature, and ionic strength of the surroundings. Thus, marine polysaccharides have been used to prepare stimuli-responsive drug delivery systems. In stimuli-responsive drug delivery systems, drugs can be released in response to stimuli from the

surrounding environment. These systems can control the release and distribution of drugs at the target site, which thereby reduces the systemic side effects of drugs and enhances the drug delivery efficiency.

#### *pH-responsive drug release*

The pH values of various organs and tissues in the human body differ. In addition, some tissues associated with diseases such as tumors will generate an acidic environment. Thus, pH-responsive drug delivery systems can selectively release drugs to the target tissues and exhibit improved drug delivery efficiency.

Above pH 4, the carboxyl groups of alginate are ionized and the polymer becomes soluble. Therefore, drugs can be selectively released from alginate gels in response to alkaline environments, such as that found in the intestine. The pH-sensitivity of alginate gels can also protect drugs labile to the acidic environment from stomach acids, thereby increasing the bioavailability of such drugs. For example, Iliescu et al. (2014) used an ionotropic gelation process to formulate alginate nanoparticles that contained irinotecan. In colorectal tumor tissues, the nanoparticles adhered to mucosal membranes of the colorectal tumor, swelled, and released the cargo. Another study using a pH-sensitive konjac glucomannan/sodium alginate hydrogel was performed by Wang et al. (2014). 5-Fluorouracil contained in the hydrogel was selectively released in the alkaline pH environment of the colorectal cancer site.

The pH-responsive property of chitosan, conferred by the protonization of the amino groups at acidic pH values, was also exploited for pH-responsive drug delivery systems. For example, Saboktakin et al. (2015) developed a supramolecular chitosan gel for the delivery of insulin. The chitosan gel protected insulin from the acidic environment of the stomach and released the protein in the intestine in a sustained manner.

#### *Temperature-responsive drug release*

Temperature-responsive drug delivery systems have been used to release drugs to target sites in response to body temperature. Chitosan and carrageenan have been used for this purpose, but owing to their lack of temperature-dependent responses, they must be used in combination with other temperature-responsive materials. The most frequently used temperature-sensitive polymer is poly(*N*-isopropylacrylamide) (PNIPAM). PNIPAM exhibits a lower critical solution temperature (LCST). When the ambient temperature becomes lower than the LCST, hydrogen bonds are formed between the polymer chains and water molecules, and the polymers dissolve in water. When the

temperature increases, the hydrogen bonds collapse, and the polymer becomes insoluble in water.

Li et al. (2013) developed thermo-sensitive hollow spheres by using the self-assembly properties of chitosan-graft-PNIPAM (CS-g-PNIPAM). In the study, chitosan was grafted with PNIPAM and crosslinked by sodium tripolyphosphate, an ionic crosslinking agent. PNIPAM formed the core of the hollow spheres and the shell was composed of chitosan. 5-Fluorouracil was loaded in the inner cavity of the CS-g-PNIPAM hollow spheres. At the temperature above LCST, 5-fluorouracil was released from the CS-g-PNIPAM hollow spheres owing to the destruction of interactions between the polymer and drug.

Another research group also developed thermo-sensitive chitosan-based formulations with weakly basic glycero-phosphate (GP). The addition of GP to a chitosan solution could prevent the precipitation of chitosan at high pH values owing to the strong electrostatic attraction between the phosphate groups of GP and the ammonium groups of chitosan. When the temperature increased, the strong interaction decreased and the dehydration of chitosan chains occurred, which transformed the solution to gels. Through this mechanism, the formulations gelled immediately at body temperatures and reverted to sol status during the cooling process. This thermo-sensitive gelling system efficiently delivered doxepin to the brain via the intranasal route (Naik and Nair 2014).

Carrageenan can also be used for thermo-sensitive systems in combination with other thermo-sensitive polymers. For example, Li et al. (2014a) delivered ketorolac tromethamine via an intranasal route by using a thermo-sensitive gel system composed of carrageenan and poloxamer 407. For a poloxamer-based gel, the lack of mucoadhesiveness is a critical obstacle, especially when delivered via the cavity mucous membrane. As carrageenan exhibited significant bioadhesive property owing to its hydrogen bond-forming groups, the incorporation of bioadhesive carrageenan in the poloxamer gels improved the mucosal delivery of drugs.

#### *Electric field-responsive drug release*

The controlled drug release can be achieved by electric field-responsive drug delivery systems, which can release the drugs by the regulation of the strength of current and voltage of electric field. In general, the electric field-sensitive systems contain two redox states, of which one allows electrostatic interaction with ionic drugs. The electric field-responsive release of drugs is commonly accomplished through a series of reduction/oxidation processes by electric field. By the control of the electric field between the electrodes, drug release from the systems can be regulated. Marine polysaccharides, such as chitosan and

alginate, can be used to fabricate electric field-responsive drug delivery systems owing to their biocompatibility and biodegradability, but the electric field responsiveness should be provided by other electrically conductive materials.

Chandran and Sandhyarani (2014) developed an electric field-responsive chitosan-gold nanocomplex for the controlled delivery of 5-fluorouracil to the cervical cancer cells. In their study, gold nanoparticles with a strong electrical conducting ability were integrated into the chitosan matrix. By embedding an electrode into the site of a chitosan-gold nanocomplex beside the tumor cells and the application of electric field, the drug could be released in a controlled manner.

Shi et al. (2014) developed hybrid hydrogels composed of bacterial cellulose nanofiber and sodium alginate containing ibuprofen. As alginate has numerous ionizable carboxyl groups, alginate was selected as the main component of hydrogels. After the application of electrical stimulus, hydrogen ions move from the alginate matrix to the cathode surface. The absence of hydrogen ions resulted in ionized alginate, leading to the swelling of the hydrogels and drug release. Therefore, the drug release could be controlled through the regulation of electric stimulus.

## Conclusion

The versatile properties of marine polysaccharides have been extensively investigated in the pharmaceutical and biomedical fields. In particular, fucoidan, carrageenan, alginate, and chitosan have demonstrated various therapeutic efficacies, such as anti-cancer, anti-inflammatory, and anti-viral activities, and have been exploited as fundamental or supporting materials in biomedical engineering and pharmaceutical formulations owing to their suitable physicochemical and biological properties. The efforts made to elucidate the therapeutic efficacies and identify novel roles of marine polysaccharides have illustrated the possibilities of polysaccharides as substitutes for existing therapeutics and conventional synthetic polymers that have exhibited undesirable side effects or poor biocompatibility.

However, major challenges remain that should be overcome for the maximization of the potential of the polysaccharides. Although the mechanism of the therapeutic efficacies of marine polysaccharides have been gradually elucidated, poor correlations among *in vitro*, *in vivo*, and clinical investigations exhibited by some studies have limited the clinical translation of the polysaccharides. The differences between the studies in different environments may be predominantly attributed to the absence of appropriate delivery systems for efficient

transport of the therapeutic polysaccharides to the desired site. Thus, future studies should focus on the maximization of the therapeutic potentials of marine polysaccharides through the design of novel delivery systems that are highly compatible with the polysaccharides. In addition, further precisely designed investigations on therapeutic mechanisms of the marine polysaccharides should be conducted to elucidate the exact biomolecular features and biochemical pathways of the therapeutic actions of the polysaccharides. Given the continuous efforts to maximize the potential of marine polysaccharides as therapeutics and fundamental materials for application in biomedical engineering and the pharmaceutical industry, the range of applications of the polysaccharides will be broadened, providing novel advantages that have not been achieved with existing polymers.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest.

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