International Journal of Biological Macromolecules 82 (2016) 315–327

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Marine polysaccharide-based nanomaterials as a novel source of nanobiotechnological applications

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ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 13 October 2015
Accepted 27 October 2015
Available online 30 October 2015

Keywords:
Marine polysaccharides
Nanomaterials
Biomedical applications
Nanoparticles
Drug delivery

ABSTRACT

Research on marine polysaccharide-based nanomaterials is emerging in nanobiotechnological fields such as drug delivery, gene delivery, tissue engineering, cancer therapy, wound dressing, biosensors, and water treatment. Important properties of the marine polysaccharides include biocompatibility, biodegradability, nontoxicity, low cost, and abundance. Most of the marine polysaccharides are derived from natural sources such as fucoidan, alginates, carrageenan, agarose, porphyran, ulvan, mauran, chitin, chitosan, and chitooligosaccharide. Marine polysaccharides are very important biological macromolecules that widely exist in marine organisms. Marine polysaccharides exhibit a vast variety of structures and are still under-exploited and thus should be considered as a novel source of natural products for drug discovery. An enormous variety of polysaccharides can be extracted from marine organisms such as algae, crustaceans, and microorganisms. Marine polysaccharides have been shown to have a variety of biological and biomedical properties. Recently, research and development of marine polysaccharide-based nanomaterials have received considerable attention as one of the major resources for nanotechnological applications. This review highlights the recent research on marine polysaccharide-based nanomaterials for biotechnological and biomedical applications.

1. Introduction

Marine polysaccharides have a great promise for applications in modern medicine and nanobiotechnology and are well known in the biomedical engineering field for several applications, namely gene delivery, drug delivery, tissue engineering, and wound dressing [1]. Marine algae produce a variety of bioactive compounds with various structures and interesting biological activities. Marine algae have been recognized as a rich source of sulfated polysaccharides, including fucoidan, alginate, carrageenan, agarose, porphyran, and ulvan [2]. Chitin, chitosan (CS), and oligosaccharides are derived from the exoskeleton of marine crustaceans. Exopolysaccharides (EPS) produced by marine microorganisms are a complex mixture of biopolymers mainly consisting of polysaccharides as well as nucleic acids, lipids, proteins, and humic substances [3].

Marine polysaccharide-based nanomaterials have attracted attention as one of the most important research in the recent years, particularly, in biomedical and chemical research because of their good biocompatibility, biodegradability, nontoxicity, low cost, and abundance [4]. Nowadays, pharmaceuticals based on marine bionanoparticles of polymers, liposomes, metals or metal oxides, micelles, or dendrimers are actively being considered for combating different diseases, including cancer and bacterial pathogens and for tissue engineering, drug delivery, gene delivery, and wound healing [5]. Marine polysaccharides are easily processed into nanoparticles, nanofibers, microparticles, scaffolds, membranes, gels, beads, and sponge forms, and these forms have been used for various biomedical applications in cancer therapy, drug delivery, tissue engineering, biosensors, wound dressing, and water treatment in the area of nanobiotechnology [6]. This review focuses on marine polysaccharide-based nanomaterials for nanotechnological and biomedical applications.

2. Marine organisms as a source of polysaccharides

Marine polysaccharides are one of the major components of all living organisms. In nature, marine polysaccharides are derived from various resources such as marine algae, crustaceans, and microorganisms [4]. Marine organisms are recognized as the

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http://dx.doi.org/10.1016/j.ijbiomac.2015.10.081
0141-8130/

most abundant source of polysaccharides. For decades, they have been used for various biological and biomedical applications, namely anticoagulant [7], antioxidant [7], anti-inflammatory [8], antiproliferative [7], antiviral [8], antitumor [9], antiparasitic [8], antiangiogenic [10], antimetastatic [11], and immunomodulating [8] activities. The sulfated polysaccharides have been of great interest in recent years because of their potential to produce novel bioactive compounds [12].

2.1. Marine algae

Marine algae are excellent candidates for sulfated polysaccharides and have received a greater interest as natural resources of marine natural products. The sulfated polysaccharides extracted from marine algae have received considerable attention in the nutraceutical, cosmeceutical, and pharmaceutical fields [13]. The marine algae are divided into three major types: green algae (*Chlorophyceae*), red algae (*Rhodophyceae*), and brown algae (*Phaeophyceae*). Laminarin, fucoidan, and alginates are naturally occurring biopolymers extracted from marine brown algae. Carrageenans, agarose, and porphyran are sulfated polysaccharides extracted from red seaweeds [14]. Ulvan is a natural polysaccharide isolated from green algae [15].

2.2. Marine crustaceans

2.2.1. Chitin

Chitin is one of the most abundant natural polymers on earth and is found in the exoskeletons of marine crustaceans and cell walls of marine fungi [5]. The major sources of chitin are shrimp, crab, and lobster shells, which are the most abundantly available waste products of the seafood industry. This biopolymer is easily processed into nanoparticles, nanofibers, microparticles, membranes, scaffolds, gels, beads, and sponge forms in the biomedical field because of their excellent biocompatibility, high biodegradability and low toxicity [16]. These properties, find various biomedical applications such as targeted drug delivery, gene delivery, wound dressing, and tissue engineering in the area of nanotechnology [17].

2.2.2. Chitosan and chitooligosaccharides

CS is a naturally occurring biopolymer isolated by the *N*-deacetylation of chitin. It is the main constituent of the exoskeletons of marine crustaceans, including shrimp and crab. Recently, CS is of a special interest for applications in the chemical, nutraceutical, and pharmaceutical industries [18]. Chitooligosaccharides (COSs) are the depolymerization products of chitin or CS by enzymatic and acidic hydrolysis methods. These methods have attracted special interest because of their ease of control and safety. Several enzymes have been used to prepare COSs. They are highly water soluble and nontoxic have good biocompatibility, excellent biodegradability, and low cost [19]. CS and COSs have a great promise in recent years because of their biomedical applications, namely antimicrobial activity [20], tissue engineering [21], wound healing [22], drug delivery [23], antitumor effects [24], and hypocholesterolemic effects [25].

2.3. Marine microorganisms

Marine microbial EPS are natural biopolymers primarily consisting of polysaccharides produced by bacteria [26], cyanobacteria [27], actinobacteria [28], and fungi [29]. Nowadays, EPS have attracted increasing attention in pharmaceutical, cosmeceutical, and nutraceutical industries as well as in wastewater treatment and detergent applications [30]. Marine microbial polysaccharides have been a growing interest for biological activities such as antiviral, anti-inflammatory, and antitumor activates [31]. Raveendran et al. [1] reported an extremophilic bacterial polysaccharide, mauran (MR), for the first time as a novel biocompatible and stable biomaterial to the world of nanotechnology, pharmaceutics and biomedical technology. Manivasagan et al. [32] studied the production of polysaccharide-based bioflocculant for the green synthesis of silver nanoparticles by *Streptomyces* sp. The biosynthesized silver nanoparticles can be extended as an alternative for the development of novel bactericidal bionanomaterials for wastewater treatment and biotechnological applications.

3. Marine polysaccharide-based nanomaterials

Marine polysaccharide-based nanomaterials have attracted considerable attention of the nanotechnology scientists in recent years because of their good biocompatibility, high biodegradability, low cost, and nontoxic nature, and have received interest as novel carriers for imaging and therapeutic agents due to their unique physicochemical properties. Marine polysaccharides are the most abundant polysaccharide, simple, stable, inexpensive, nontoxic, safe, hydrophilic, biocompatible, and biodegradable [33]. These properties are of special interest in the field of nanotechnology and have a promising future as biomaterials. In recent years, many researchers have investigated on polysaccharides-based nanomaterials for biomedical application such as antimicrobial activity, drug delivery, gene delivery, tissue engineering, cancer therapy, and wound dressing (Fig. 1) (Table 1) [34–36].

3.1. Fucoidan nanoparticles

Fucoidan **(1)** (Fig. 2) is a naturally occurring sulfated polysaccharide extracted from marine brown seaweeds [37,38]. Similar fucan sulfates were obtained from marine invertebrates [39]. Fucoidan is an excellent drug candidate for pharmaceutical applications. Recently, fucoidan has been investigated because of its various biological properties such as anticoagulant [40], antiviral [41], antiangiogenic, antitumor, anti-inflammatory, [42], antioxidant [43], and antiproliferative and immunomodulating properties [41]. In the past few years, a large number of researchers of novel bioactive compounds have taken special interest in fucoidans [44]. Fucoidans have been investigated in the biosynthesis of metal nanoparticles and has been used for cancer treatment and drug delivery.

Lia et al. [45] reported the synthesis and characterization of fucoidan-coated poly (isobutylcyanoacrylate) nanoparticles. The nanoparticles were prepared by anionic emulsion polymerization and by redox radical emulsion polymerization of isobutylcyanoacrylate using fucoidan as a novel coating biomaterial. They exhibited *in vitro* potent cytotoxic activity against J774 macrophage and NIH-3T3 fibroblast cell lines. The IC_{50} (2 μg/mL) of anionic emulsion polymerization nanoparticles on the J774 macrophages was low. Fucoidans are extracted from two different types of marine algae *Cladosiphon okamuranus* and *Kjellamaniella crassifolia.* Fucoidans were used for the green synthesis of gold nanoparticles [46].

Leung et al. [47] reported the biosynthesis of silver nanoparticles using carboxymethylated curdlan or fucoidan as reducing and stabilizing agents. Fucoidan was isolated from marine brown seaweed *C. okamuranus* and encapsulated in nanoparticles using liposomes as nanocarriers. These nanoparticles showed potent *in vitro* anticancer activity against osteosarcoma [48]. To prepare the chemotherapeutic agent-loaded nanoparticles, hydrophobically modified fucoidan was synthesized by the acetylation of

Fig. 1. Various types of sulfated polysaccharides and their applications in nanobiotechnology.

Table 1
Biomedical application of marine polysaccharides-based nanomaterials.

Polysaccharides	Source	Biomedical applications	Reference
Fucoidan	Brown algae	Cytotoxic activity Anticancer	Kimura et al. [48] Lee et al. [49]
Alginate	Brown algae	Drug delivery Anti-tubercular and antifungal Antitumor	Guo et al. [56] Ahmad et al. [58] Zhang et al. [55]
Carrageenan	Red algae	Antioxidant activity Drug delivery	De Souza et al. [62] Grenha et al. [64]
Agarose Porphyran	Red algae Red algae	Antibacterial activity Cytotoxic activity Drug delivery	Kattumuri et al. [69] Venkatpurwar et al. [73] Venkatpurwar et al. [72]
Ulvan Chitin	Green algae Shrimp and crab	Tissue engineering Wound dressing Antibacterial activity Drug delivery	Toskas et al. [74] Song et al. [80] Dev et al. [81] Smitha et al. [82]
Chitosan	Shrimp and crab	Gene delivery Antimicrobial activity Tissue engineering Anticancer activity Wound dressing	Katas and Alpar et al. [96] Ali et al. [98] Peter et al. [122] De Campos [92] Madhumathi et al. [22]
Chitooligosaccharide Mauran	Shrimp and crab Bacteria	Anticancer Drug delivery	Bae et al. [103] Raveendran et al. [1]

fucoidan. Doxorubicin (DOX was used as a model chemotherapeutic agent, and the drug was loaded in the acetylated fucoidan nanoparticles **(11)** (Fig. 3). The nanoparticles were characterized by their morphology and drug release properties [49].

3.2. Alginate nanoparticles

Alginate **(2)** (Fig. 2) is a natural polysaccharide extracted from brown seaweeds and has been widely investigated and used for

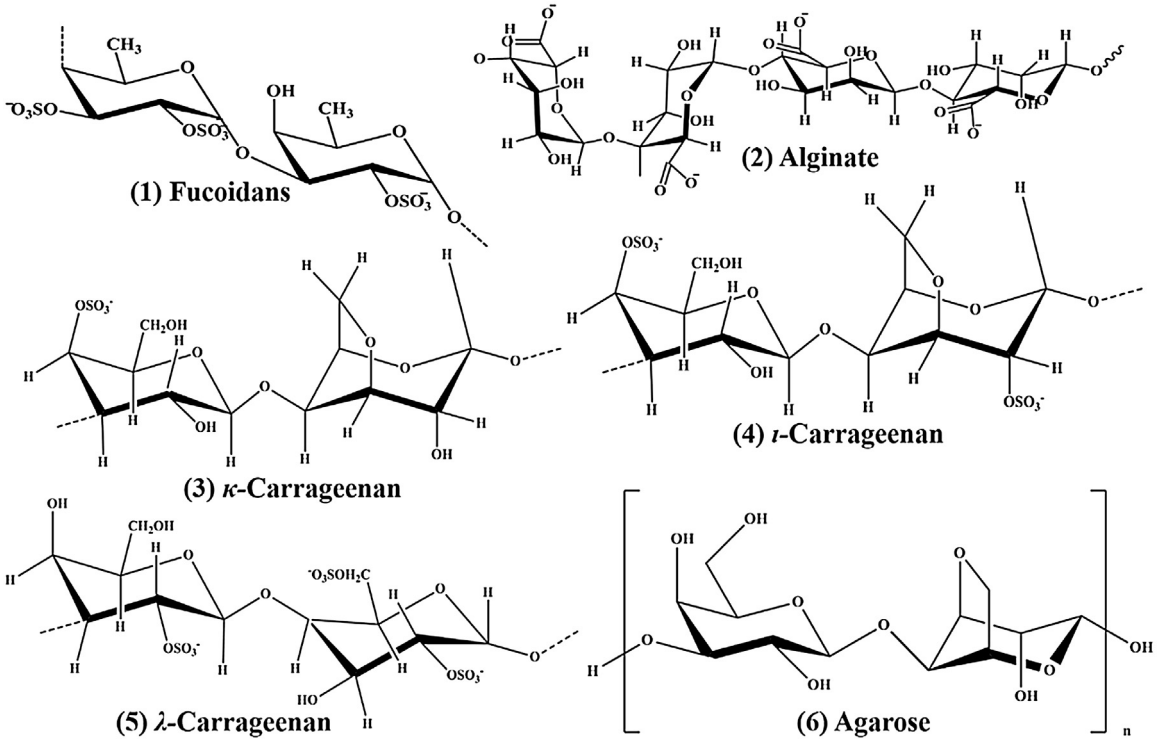

Fig. 2. Chemical structure of fucoidans, alginate, kappa (κ) carrageenan, iota (ι) carrageenan, lambda (λ) carrageenan and agarose.

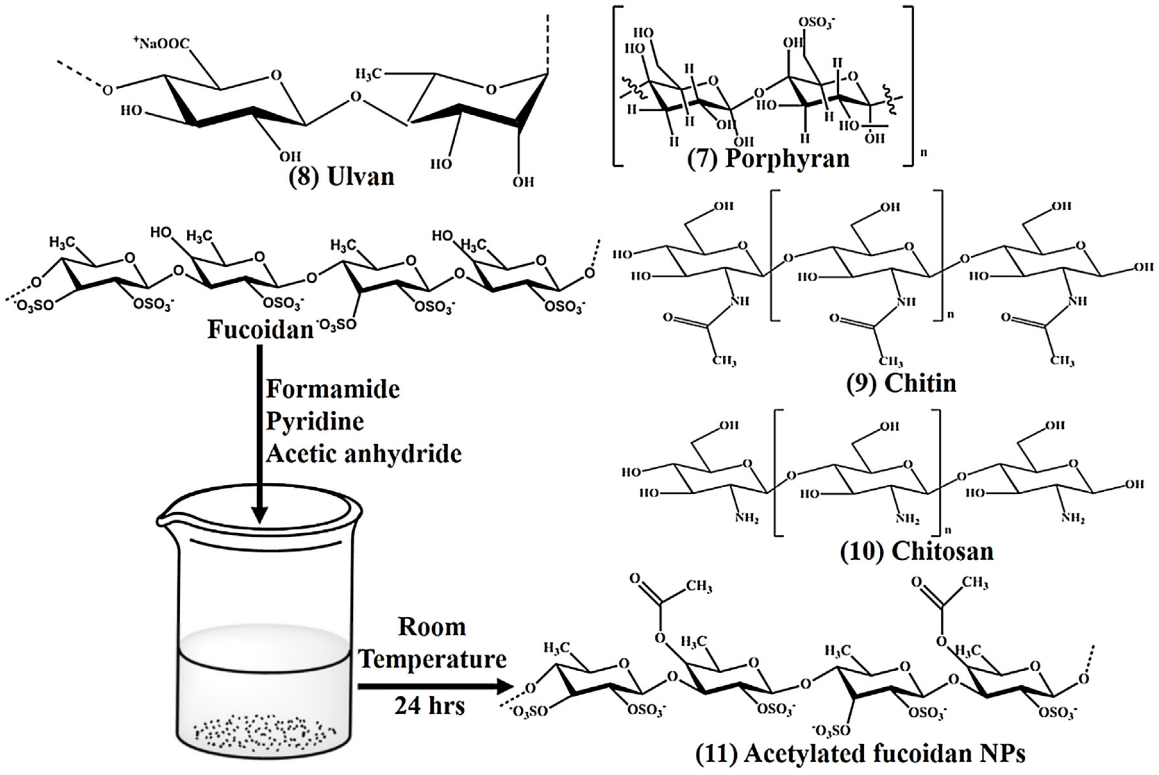

Fig. 3. Chemical structure of porphyran, ulvan, chitin, chitosan and acetylated fucoidan nanoparticles.

biomedical applications because of their excellent biocompatibility, low cost, low toxicity, and mild gelation [50]. Nowadays, a large number of studies for alginate-based nanoparticles have raised greater interest in the medical field because of their potential applications such as in insulin delivery [51] and antifungal and antitubercular drugs [52].

Sarmento et al. [51] prepared insulin-loaded nanoparticles using alginate ionotropic pre-gelation followed by CS polyelectrolyte

complexation. In their study, particles in nanometer size range were obtained under optimized conditions with a loading capacity of 14.3%. In another study using dextran polysaccharide as the complexing agent, again, insulin was loaded in alginate–dextran nanospheres *via* nanoemulsion dispersion followed by triggered *in situ* gelation. The resulting particles ranged in size from 267 nm to 2.76 μm. Particles were prepared with a unimodal size distribution, and insulin encapsulation efficiency of 82.5% was reached. Anh et al. [53] reported an effective γ-irradiation method for the preparation of gold nanoparticles using alginate, a natural polysaccharide, as a stabilizer. The obtained alginate-stabilized gold nanoparticles were characterized using UV–vis spectroscopy and transmission electron microscopy. The results showed that γ-irradiation technique is suitable for the production of alginate-stabilized gold nanoparticles with controllable size and high purity. The alginate-stabilized gold nanoparticles were spherical in nature, with particle size ranging from 5 to 40 nm.

Yang and Pan [54] studied the hydrothermal synthesis of silver nanoparticles using sodium alginate as a reducing and stabilizing agent. It showed that the temperature and incubation time of the reaction played major roles under suitable concentrations of sodium alginate and precursor Ag^+ in the formation of silver nanoparticles with specific shapes. In general, a low temperature and short incubation time of reaction were shown to result in the formation of nanospheres, and increasing the temperature and incubation time of reaction were favorable for the formation of nanoplates.

Zhang et al. [55] studied DOX-loaded glycyrrhetinic acid-modified alginate nanoparticles (ALG NPs) for liver tumor chemotherapy. The nanoparticles showed strong liver-targeting efficiency owing to passive targeting *via* the enhanced permeability and retention effects and the active targeting efficiency of glycyrrhetinic acid (GA). Cardiac toxicity was reduced after the administration of DOX/GA-ALG NPs. In particular, DOX/GA-ALG NPs improved the antitumor activity of DOX against liver tumors *in situ*. GA-ALG/DOX-ALG NPs were planned as a drug delivery system with pH-sensitive and liver-targeted properties. These results showed that GA-ALG/DOX-ALG NPs may act as a therapeutic agents in liver cancer treatment [56].

The therapeutic failure of tuberculosis is mostly due to patient non-compliance, which is recognized to require multidrug administration daily or several times a week for at least 6 months. Nanoparticle-based drug delivery systems are appropriate for mycobacterial infections such as tuberculosis. Interestingly, the biomedical application of nanoparticles-based drug delivery systems offers a new perspective for the treatment of tuberculosis [57]. Ahmad et al. [58] studied the pharmacokinetics and tissue distribution of free and alginate-encapsulated antitubercular drugs in mice at various doses. ALG NP-encapsulating isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) were orally administered to mice. The average size of particles was found to be 235.5 ± 0.0 nm, with drug encapsulation abilities of 70–90%, 80–90%, and 88–95% for INH, RIF, and EMB, respectively.

3.3. Carrageenan nanoparticles

Carrageenan is a naturally occurring biopolymer obtained from marine red algae, *Kappaphycus* sp. and *Eucheuma* sp. The chemical structure of carrageenan is made of D-galactose and anhydrogalactose units combined by glycosidic linkages and containing ester sulfate groups. Depending upon the extraction procedures and resources, carrageenan they are divided into three types, kappa (κ) **(3)**, iota (ι) **(4)**, and lambda (λ) **(5)** (Fig. 2), mostly differing in the substitution degree of the sulfate group. Among the three types of carrageenan, κ- and ι-carrageenan have a high gelling efficiency, with the former being rigid and firm and the latter being elastic and soft in nature [59]. λ-Carrageenan is a non-gelling polysaccharide [60]. Daniel-da-Silva et al. [61] investigated the biosynthesis of magnetite nanoparticles using carrageenan. κ, ι, and λ carrageenans have been investigated that the particle size morphology and chemical stability of nanocomposite. De Souza et al. [62] reported the antioxidant activities of six types of sulfated polysaccharides extracted from marine brown and red seaweeds; λ carrageenan and fucoidan exhibited strong antioxidant activity. Stiles et al. [63] reported the inhibitory effect of λ-carrageenan and a mixture of sulfated polysaccharides isolated from red seaweeds against feline herpesvirus (FHV)-1 in an *in vitro*. The IC_{50} of λ-carrageenan against FHV-1 was found to be 5 μg/mL [63].

Grenha et al. [64] developed a novel formation of carrageenan and CS nanoparticles as a suitable drug delivery system. These nanoparticles are produced in hydrophilic conditions using very mild procedure and avoiding the use of organic solvents and other aggressive conditions. These nanoparticles were demonstrated as appropriate vehicles that can offer sustained and controlled for the drug delivery system. The nanoparticles showed low toxicity against fibroblast cell lines as well as good biocompatibility and high safety. These nanoparticles were indicated to be good candidates for biomedical applications such as drug delivery and tissue engineering.

Rodrigues et al. [65] reported the production of CS, carrageenan, and tripolyphosphate nanoparticles with smaller size, strong positive surface, and stability and were used for application in mucosal delivery of macromolecules. Hezaveh and Muhamad [66] reported the effect of metallic nanoparticles on gastrointestinal release from modified κ-carrageenan hydrogels. The effect of genipin cross-linking and metallic nanoparticle loading was also investigated, and the most suitable drug delivery system was successfully introduced. These metallic nanoparticles seem to be a great promising strategy to significantly improve gastrointestinal tract-controlled drug delivery. Salgueiro et al. [67] demonstrated the effect of incorporating spherical and rod-shaped gold nanoparticles in the microstructure and thermomechanical properties of κ-carrageenan hydrogels and in the release kinetics and mechanism of methylene blue from κ-carrageenan nanocomposites. Hydrogel nanocomposites showed enhanced viscoelastic properties as compared to neat κ-carrageenan, when using either gold nanospheres and gold nanorods.

3.4. Agarose nanoparticles

Agarose **(6)** (Fig. 2) is a natural biopolymer extracted from red seaweeds, *Gracilaria* sp. and *Gelidium* sp. Agarose is a linear biopolymer made up of repeating units of agarobiose, which is a disaccharide made up of D-galactose and 3,-6-anhydro-L-galactopyranose. Agarose is commonly used in the biochemistry, molecular biology, and biotechnology fields for the isolation of biomolecules, particularly DNA by electrophoresis. Agarose is generally used for its gel-forming property to make semiconductor and metal nanoparticles. These nanoparticles exhibited strong antibacterial activity against *Escherichia coli.* Interestingly, the agarose composite films can be quickly converted to carbon–metal composites by carbonizing the films in nitrogen atmosphere [68]. Kattumuri et al. [69] reported the use of agarose-stabilized gold nanoparticles for the detection of micromolar concentrations of DNA nucleosides using surface-enhanced Raman spectroscopic detection. These results indicate that agarose-stabilized gold nanoparticles yield higher surface-enhanced Raman spectroscopic detection for DNA nucleosides, which is used for on-chip biosensing applications.

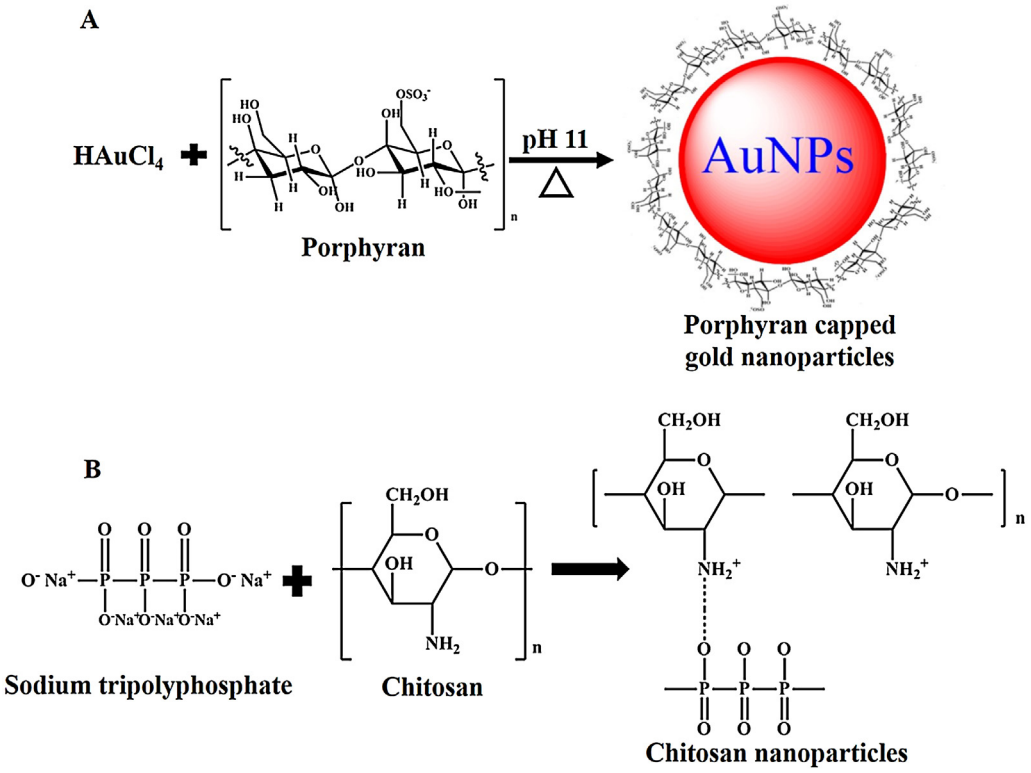

Fig. 4. (A) Schematic representation showing porphyran capped gold nanoparticles. (B) Schematic diagram of the functional structure of chitosan nanoparticles formed during ionic gelation process.

3.5. Porphyran nanoparticles

Porphyran **(7)** (Fig. 3) is a naturally occurring sulfated polysaccharide extracted from marine red seaweed, *Porphyra vietnamensis* [70]. Porphyran comprises the hot-water-soluble portion of the cell wall, is the major constituent (40–50%) of the marine red seaweeds, and has nutritional value. Porphyran is made up of anionic disaccharide units consisting of 3-linked D-galactosyl residues alternating with 4-linked 3,6-anhydro-L-galactose and 6-sulfate residues. The pharmaceutical purposes of porphyran extracted from different porphyra species were reported in various structural and functional studies. It was interesting to note the reports on the anticancer and antioxidant activities of porphyran [71]. Venkatpurwar et al. [72] reported the biosynthesis of gold nanoparticles using a porphyran and subsequent loading of DOX. The porphyran-capped gold nanoparticles (Fig. 4A) and DOX-loaded gold nanoparticles were evaluated using suitable techniques to study morphology, size, surface charge, and drug-loading efficiency. Venkatpurwar et al. [73] demonstrated that the toxicology profile comprises *in vitro* cytotoxic activity and *in vivo* subacute oral toxicity of newly synthesized gold nanoparticles using new sulfated polysaccharide porphyran obtained from marine red seaweed. *In vitro* cytotoxic activity of porphyran-reduced gold nanoparticles (10, 50, or 100 μM) was performed employing normal monkey kidney cell line, which showed a non-toxic nature of nanoparticles.

3.6. Nanofibers of Ulvan

Ulvan **(8)** (Fig. 3) is a complex anionic sulfated polysaccharide isolated from the cell walls of marine green algae (*Ulvales*, *Chlorophyta*). They are still under-exploited, are an abundant resource, and have a low cost of production. The major components of ulvan are sulfated, xylose, rhamnose, glucuronic, and iduronic acids. The potential applications of ulvan have been investigated as an antioxidant, antitumor, anticoagulant, and immune modulator [15]. Presently, ulvan isolated from marine green algae, *Ulva rigida* has been used for the preparation of nanofibers. Nanofibers are of special interest in the biomedical engineering field because of their potential applications in drug delivery, wound dressing, and tissue engineering. Ulvan is a good candidate for nanofiber fabrication and has been successfully introduced into nanobiotechnology.

Toskas et al. [74] reported the spinnability of ulvan-rich, isolated polysaccharides from marine green seaweed *U. rigida* that was used for the fabrication of nanofibers. The nanofiber ability of an ulvan-rich extract in combination with its interesting physicochemical and biological properties can lead to a new biomedical application such as drug delivery, wound dressing and tissue engineering. Kikionis et al. [75] prepared novel fibrous biocomposites of ulvan using the electrospinning technique. These nanofibrous matrices represent novel promising biomaterials in biomedical applications, including tissue engineering, wound dressing, and drug delivery systems.

3.7. Mauran nanoparticles

Mauran is a naturally occurring sulfated polysaccharide with high sulfate, phosphate, and uronic acid content. It is isolated from a moderately halophilic bacterium, *Halomonas maura*, and has been used for the biosynthesis of metal nanoparticles. It has been reported that they constitute mannose, galactose, glucose, and glucuronic acid. Moreover, they are well known for their viscoelasticity. The pseudoplastic and thixotropic natures of mauran make it an ideal molecule for material science applications [76]. Raveendran et al. [1] reported the synthesis of sulfated polysaccharide-based nanoparticles as a good biocompatible material for anticancer activity, drug delivery and bioimaging.

3.8. Chitin nanoparticles

Chitin **(9)** (Fig. 3) is also a biopolymer that is abundant in nature [77]. It is isolated from crab, shrimp, and lobster shells as a by-product of the seafood industry. Worldwide, several million tons of chitin are generated annually as waste by the seafood industry [78]. Chitin has been successfully prepared by several methods such as enzymatic methods, methods using hydrolytic conditions of boiling HCl and vigorous stirring, and methods using chitin whiskers of slender parallelepiped rods. Chang et al. [79] reported the preparation of presumably functional chitin nanoparticles and their application as reinforcing fillers in polymeric matrices.

Carboxymethyl chitin (CMC) is a nontoxic, water-soluble anionic derivative of chitin containing carboxyl groups. It is used as a constituent of wound-healing dressings, exhibits good biocompatibility and can be synthesized from chitin [80]. Dev et al. [81] prepared CMC nanoparticles using the cross-linking approach with $FeCl_3$ and $CaCl_2$ that was used for controlled drug delivery applications. These nanoparticles exhibited *in vitro* cytotoxic activity against mouse L929 cell lines. The nanoparticles showed strong antibacterial activity against *Staphylococcus*. Smitha et al. [82] demonstrated novel amorphous chitin nanoparticles (AC NPs) that were widely used for colon cancer drug delivery. Paclitaxel (PTX) was used as the main chemotherapeutic agent, and the drug was loaded into AC NPs through ionic cross-linking approach using pentasodium tripolyphosphate. The PTX-loaded AC NPs had an average size of 200 ± 50 nm. These nanoparticles were confirmed to be hemocompatible and *in vitro* drug release exhibited a sustained release of PTX.

Drug delivery into intracellular compartments is a big challenge in the present treatment systems. To deliver drugs into the intracellular compartments, numerous drugs encapsulated in nanoparticles and microparticles were prepared, which showed enhanced efficacy in delivering the drug into the intracellular compartments [83]. Smitha et al. [84], designed rifampicin (RIF)-loaded AC NPs for intracellular delivery of RIF inside polymorphonuclear leukocytes. The RIF molecules entrapped in the amorphous chitin polymer remained in the bioactive form, and the antibacterial activity was retained upon their release. Furthermore, sustained drug delivery could reduce dosing frequency, lower toxicity, enable long-term treatment, and prevent potential side effects related to the free drug. RIF-loaded AC NPs could be used for an effective delivery of RIF into the intracellular compartments of the polymorphonuclear leukocytes to resolve serious bacterial infections faster and more effectively.

3.9. Chitosan nanoparticles

CS **(10)** (Fig. 3) is a naturally occurring biopolymer family of linear polysaccharide that is composed of glucosamine and *N*-acetyl glucosamine units *via* β-(1→4) linkages randomly or block-spread throughout the polymer chain, depending on the extraction procedures to derive CS from chitin. The deacetylation degree is well defined as the molar ratio of glucosamine to *N*-acetyl glucosamine, which is a significant factor determining its physicochemical properties and industrial applications [59]. After the deacetylation process, CS is able to dissolve in an acidic medium and becomes the only sulfated polysaccharide that possesses a high density of positive charges, owing to the protonation of amino groups on its backbone. Besides this unique characteristic, CS has been evidenced to have various other intrinsic properties, namely good biocompatibility and biodegradability and non-toxicity [85]. Nowadays, CS has attracted considerable scientific interest and nanotechnological attention in the biomedical sector for various applications, including tissue engineering [86], drug delivery [87], and nutrition [88].

Rather et al. [89] reported the CS- and CS-gold nanoconjugates of salmon leutinizing hormone releasing hormone (LHRH) of desired size, dispersity, and zeta potential were synthesized and evaluated at half the dose rate against full dose of bare LHRH for their reproductive efficacy in female fish, *Cyprinus carpio*. Because the reaction involves complex formation between oppositely charged species (negatively charged groups of the pentasodium tripolyphosphate and the positively charged amino groups of CS), CS undergoes ionic gelation and precipitates to form spherical particles. The functional structure of CS nanoparticles is depicted in Fig. 4B.

Janes et al. [90] reported the potential of CS nanoparticles as a delivery system for DOX. Evaluation of the cytotoxic activity of DOX-loaded cell lines suggested that addition of dextran sulfate enabled the maintenance of cytotoxic activity, which is relative to free DOX, whereas DOX complexed with CS before nanoparticle formation exhibited slightly decreased activity. Mitra et al. [91] prepared dextran-DOX encapsulated in CS nanoparticles using reverse microemulsion and studied their antitumor activity in murine tumor models. De Campos et al. [92] demonstrated the potential use of CS nanoparticles as a novel carrier for the targeted delivery of drugs to the ocular mucosa, using the immunosuppressive peptide cyclosporin A as a model drug. CS nanoparticles cross-linked with glutaraldehyde have been prepared in AOT/*n* hexane reverse micellar system. The particle size of these CS nanoparticles was mainly influenced by the degree of cross-linking. The particle size at infinite dilution was 30 nm, when 10% of the amine groups in the polymeric chains were cross-linked and it shoots up to 110 nm when all the amine groups were cross-linked [93].

Qi et al. [94] showed the *in vitro* antibacterial activity of CS nanoparticles and copper-loaded nanoparticles against pathogenic microorganisms. These nanoparticles showed antibacterial activity against *E. coli*, *Salmonella choleraesuis*, *Salmonella typhimurium*, and *Staphylococcus aureus*. These results indicated that CS nanoparticles and copper-loaded nanoparticles could inhibit the growth of pathogenic bacteria. Gan et al. [95] studied polyanion-initiated gelation process in fabricating CS–tripolyphosphate nanoparticles in the size range from 100 to 250 nm, intended to be used as carriers for the delivery of gene or protein macromolecules. Katas and Alpar [96] developed CS nanoparticles as a delivery system for double-stranded small interfering RNA (siRNA). *In vitro* studies in two different types of cell lines such as CHO K1 and HEK 293 have exposed that the preparation technique of siRNA association to CS plays a major part on the silencing affect. Chitosan–tripolyphosphate nanoparticles with entrapped siRNA were shown to be good vehicles for siRNA delivery compared with CS–siRNA complexes, possibly because of their excellent binding capacity and high loading efficiency.

Min et al. [97] showed that hydrophobically modified glycol chitosan (HGC) nanoparticles encapsulated camptothecin (CPT) with a high loading efficiency and CPT–HGC nanoparticles demonstrating sustained CPT release maintained the stability of the active lactone form of CPT by protecting the lactone from hydrolysis. Compared with free CPT, CPT–HGC nanoparticles exhibited a longer circulation time in the blood, a better targeting of the drug to tumor tissue and a substantial enhancement of antitumor activity. Ali et al. [98] demonstrated the synthesis and characterization of CS and silver-loaded CS nanoparticles for antimicrobial textile applications. Silver loading on the synthesized CS nanoparticles exhibited synergistic antibacterial activity against *S. aureus.* These results suggested that the preparation of novel CS nanoparticles for antimicrobial textile applications as CS in nano form is highly active due to their high surface area and very low concentration. Lima et al. [99] showed the evaluation of the hemagglutination activity of CS nanoparticles using human erythrocytes. CS nanoparticles presented hemolytic activity ranging from 186.20% to 223.12%,

whereas neutralized solutions ranged from 2.56% to 72.54% compared with distilled water.

3.10. Chitooligosaccharide nanoparticles

COS, a low molecular weight depolymerization product of CS, has attracted increasing attention in pharmaceutical and biomedical applications because it not only is water-soluble, biocompatible, biodegradable, and nontoxic in nature, but also demonstrates unique biological activities such as antimicrobial, immune-enhancing, and antitumor activities. COS is particularly suitable for developing polymer-drug conjugate because of its availability for coupling with the primary amino groups and hydroxyl groups of each polymer subunit and the cationic nature that allows ionic crosslinking [100]. Hydrophobically modified amphiphilic COS derivatives can fabricate self-assembled polymeric nanoparticles and therefore facilitate drug delivery in cancer therapy by improving the solubility of insoluble drugs, drug targeting, and absorption [101]. Lopez-Cruz et al. [102] prepared magnetic nanoparticles consisting of iron oxide cores modified with covalently linked COS that was colloidally stable in water and buffers. The enhanced colloidal stability of these COS-coated nanoparticles, owing to the covalent linking of COS to the nanoparticle, makes them attractive for biomedical applications.

Bae et al. [103] prepared CS oligosaccharide-stabilized ferrimagnetic iron oxide nanocubes (Chito-FIONs) as an effective heat nanomediator for cancer hyperthermia. Chito-FIONs showed superior magnetic heating efficiency with a high specific loss power value (2614 W/g) compared with commercial superparamagnetic Feridex nanoparticles (83 W/g). They exhibited strong antitumor activity on an animal tumor model without any severe toxicity. Liu et al. [104] investigated a novel multidentate dithiolane lipoic acid and phosphorylcholine conjugated CS oligosaccharide derivative that can serve as a ligand to effectively stabilize gold nanoparticles. These nanoparticles could be widely used for biological applications. Lin et al. [105] prepared the COS nanoparticles by the formation of polyelectrolyte complexes. These nanoparticles exhibited strong inhibition of the proliferation of HeLa and B16 melanoma cells. Lu et al. [106] prepared the synthesis of COS-based multidentate ligand for ultrastable, cytotoxicity and biocompatible nanoparticles.

4. Biomedical and biotechnological applications of marine polysaccharide-based nanomaterials

Marine polysaccharide-based nanomaterials are considered as nanomedicine presenting high potential for diagnosis and therapeutical purposes. Recently, the studies on the biotechnological and biomedical applications of marine natural products, particularly marine polysaccharides, are attracting more and more attention all over the world [107]. Marine polysaccharides and their lower molecular weight oligosaccharide derivatives have been shown to possess a variety of biomedical activities. Some of the important nanobiotechnological applications of polysaccharide-based nanomaterials are in the field of antimicrobial activity, drug delivery, gene delivery, tissue engineering, cancer therapy, wound dressing biosensors, and water treatment (Fig. 5).

4.1. Biomedical applications

4.1.1. Antimicrobial activity

The ability of pathogenic microorganisms to resist antimicrobial agents has evolved since the start of antimicrobial therapy and it still constitutes a serious problem in clinical practice, thus limiting the arsenal of drugs available and requiring the development of new substances to combat them [108]. In this context, inorganic agents have been considered excellent candidates for research because of their high antimicrobial potential [109]. Among them, silver compounds and their derivatives are one of the most extensively studied, and a current focus of research involving this metal is the synthesis and evaluation of their antimicrobial activity in the form of nanoparticles [110,111]. Shukla et al. [111] reported the synthesis and characterization of silver nanoparticles and nanocomposite material using gar extracted from the red alga *Gracilaria dura*. The antibacterial effect of silver nanoparticles showed greater bactericidal effect, with 99.9% reduction of bacteria over the control value. The silver nanocomposite film with proven antibacterial activity may find applications in food preservation and wound dressing. Venkatpurwar et al. [112] reported the green synthesis of silver nanoparticles using marine polysaccharide isolated from marine red algae, *P. vietnamensis*. The dose-dependent effect of biosynthesized silver nanoparticles revealed strong antibacterial activity against Gram-negative bacteria compared with Gram-positive bacteria.

4.1.2. Drug delivery

The potential use of nanobiotechnology in medicine and more efficient drug delivery is set to spread rapidly. Recently, many bioactive compounds are under-exploited for targeted drug delivery, although more efficient for cancer treatment. Interestingly, biomedical sciences are using nanomaterials to reduce toxicity, dosing frequency, and avoiding potential side effects and until now do not understand that delivery systems themselves may impose risks to the patient [113]. De Salamanca et al. [114] studied the *in vitro* and *in vivo* effects of CS nanoparticles as a novel drug delivery system for the ocular mucosa. Fuente et al. [115] developed a delivery platform based on CS nanoparticles, which suits the requirements of the topical ocular route. These nanoparticles have been exactly modified for the delivery of hydrophilic and lipophilic drugs as well as polynucleotides onto the eye surface. Venkatpurwar et al. [72] studied the biosynthesis of gold nanoparticles using porphyran and the subsequent loading of DOX. The DOX-loaded gold nanoparticles demonstrated strong cytotoxicity on LN-229 cell line.

Wu et al. [116] reported a nanocarrier based on CS and fucoidan for oral delivery of berberine (Ber). A sulfonated fucoidan, fucoidan–taurine (FD–Tau) conjugate, was synthesized and characterized by Fourier transform infrared spectroscopy. The FD–Tau conjugate was self-assembled with berberine and CS to form Ber-loaded CS/FD–Tau complex nanoparticles with high drug–loading efficiency. A schematic diagram of the preparation of Ber-loaded CS/FD–Tau nanoparticles is shown in Fig. 6A.

4.1.3. Gene delivery

Gene therapy is the use of genes for correcting genetic disorders. In case of gene delivery, the plasmid DNA has to be successfully introduced into the target cells, which should get transcribed and the genetic information should ultimately be translated into the corresponding protein. To achieve this aim, a number of hurdles are to be overcome by the gene delivery device [100]. Increasingly, nucleic acids are being applied for both vaccination and therapeutic gene expression, and CS nanoparticles have been recommended as promising nonviral gene carriers. The core-shell structured CS-ALG NPs (Fig. 6B) were made using a water-in-oil reverse microemulsion template. These nanoparticles were used to encapsulate a plasmid DNA for gene delivery *via* the cell endocytosis pathway [117]. However, as has been stated above, CS–DNA nanoparticles may be readily formed by complex coacervation between the positively charged amine groups on CS and negatively charged

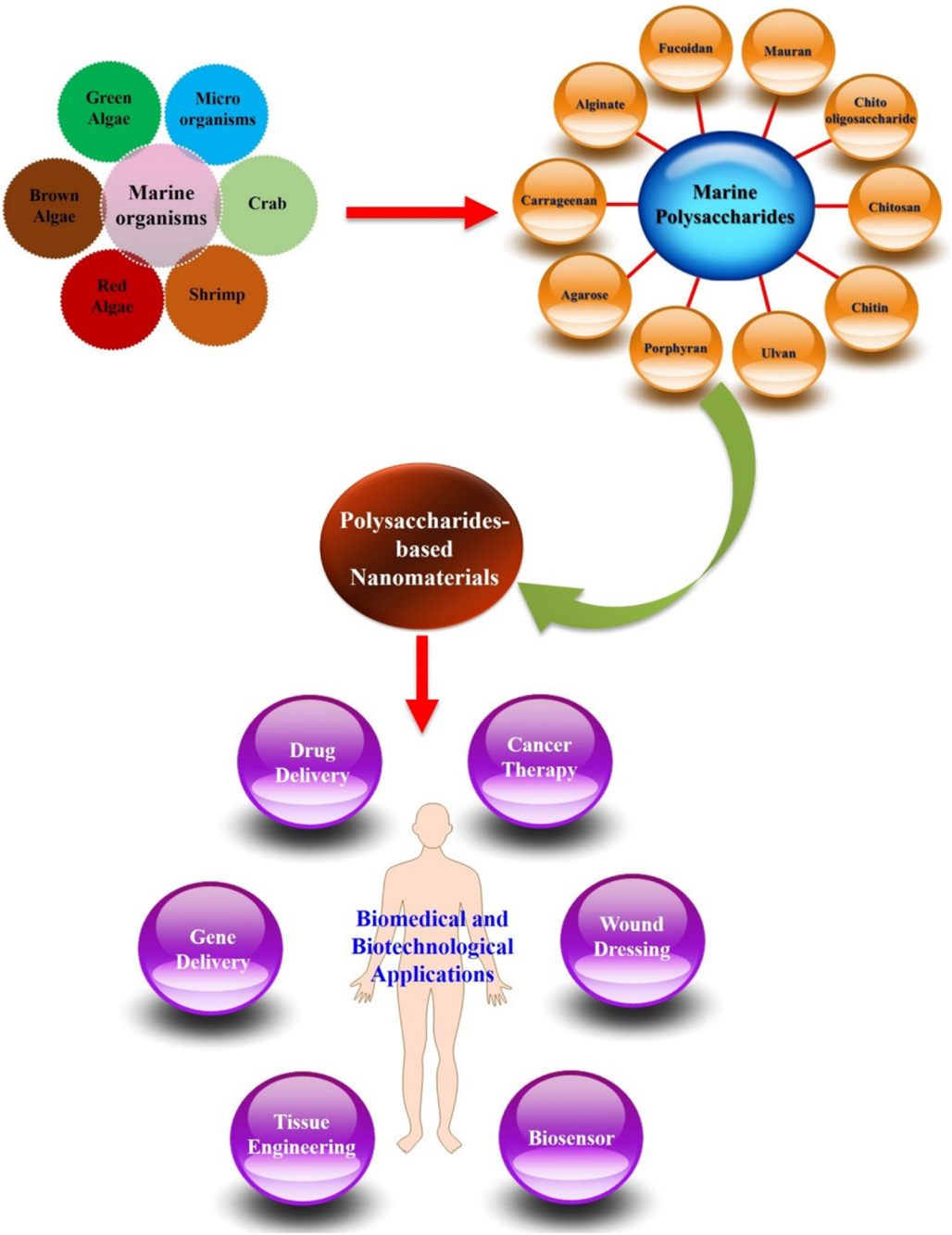

Fig. 5. Procedures for marine polysaccharides-based nanomaterials and their applications.

phosphate groups on DNA [118]. Protection of encapsulated plasmid DNA from nuclease attack offered by CS nanoparticles was confirmed by assessing degradation in the presence of DNase I, and the transformation of the plasmids with incubated nanoparticles were studied by β-galactosidase assay. Model pDNA existed as a mixture of both supercoiled (84.2%) and open circular (15.8%) forms.

The use of siRNA as an important therapeutic agent for the treatment of several diseases is limited owing to its quick degradation and low intracellular association *in vitro* and *in vivo*. Lee et al. [119] investigated that CS–polyguluronate nanoparticles exhibited low cytotoxicity and were useful in delivering siRNA to HEK 293 FT and HeLa cells. These nanoparticles have a great promise for siRNA delivery because of their low cytotoxicity and ability to transport siRNA into cells [119].

4.1.4. Tissue engineering

Tissue engineering is one of the fastest growing multidisciplinary field connecting the development of bioartificial implants and/or foster remodeling of tissues with the purpose of repairing, replacing, maintaining, or enhancing tissue or organ function. Bioartificial constructs mostly consist of living cells and biomaterials; therefore, tissue engineering draws from both living cell and biomaterial science and technology. Noh et al. [120] studied the cytocompatibility of chitin nanofibers. Chitin nanofiber matrix produced by electrospinning was introduced to tissue engineering. The study showed that the chitin nanofibers could be potential candidates for the cell attachment and spreading of normal human keratinocytes and fibroblasts.

Shalumon et al. [121] prepared a novel fibrous membrane of carboxymethyl chitin (CMC)/poly (vinyl alcohol) (PVA) blend using

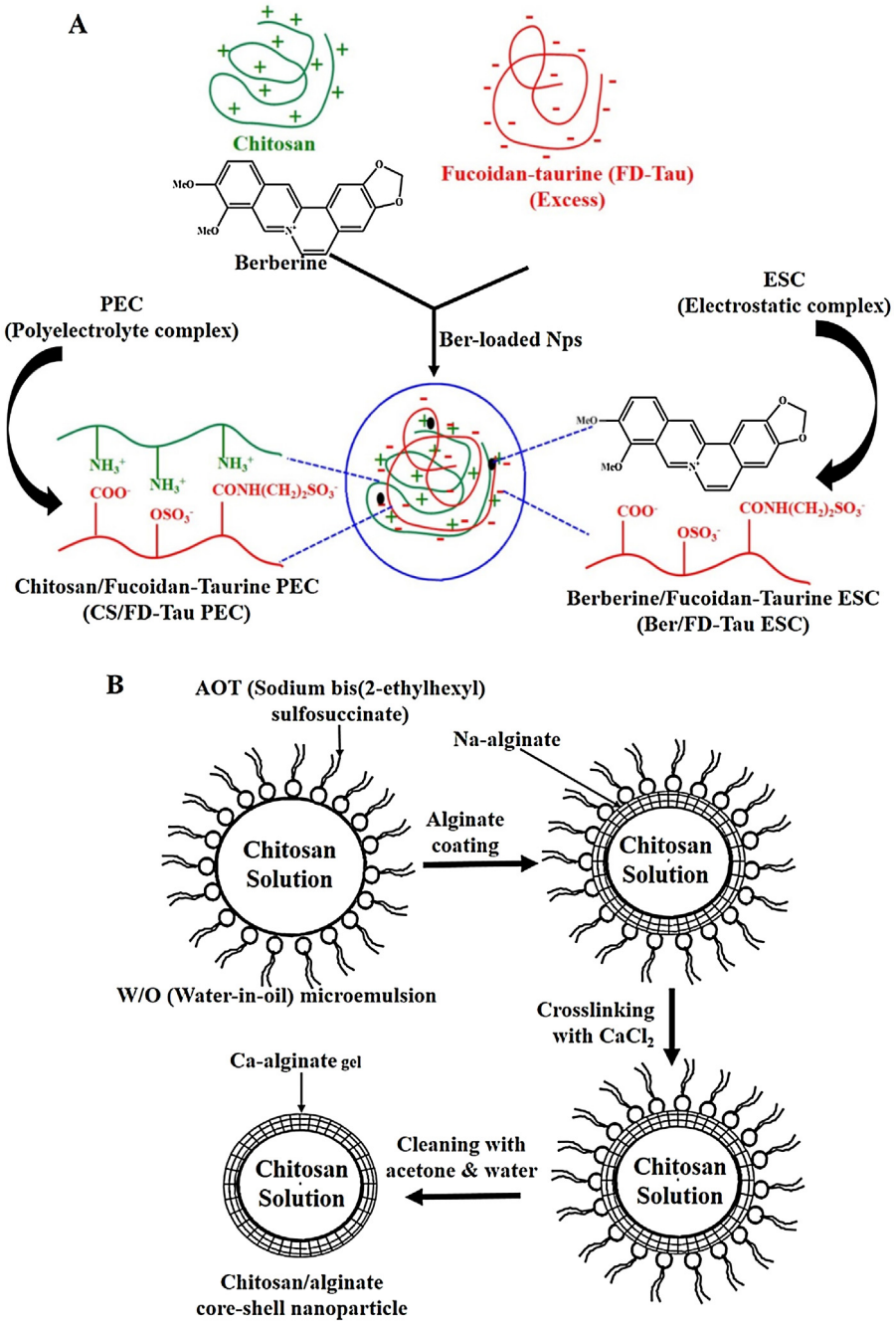

Fig. 6. (A) Schematic diagram of the preparation of berberine (Ber)-loaded chitosan/fucoidan-taurine nanoparticles. (B) Schematic illustration of chitosan-alginate core–shell nanoparticles prepared by reverse microemulsion template.

electrospinning technique. Cytotoxicity of the nanofibrous scaffold was evaluated using human mesenchymal stem cells by the MTT assay. These results indicate that the nanofibrous CMC/PVA scaffold supports cell adhesion/attachment and proliferation, and hence, these scaffolds are useful for tissue engineering applications. Peter et al. [122] prepared CS–gelatin/nanophase hydroxyapatite composite scaffolds by blending CS and gelatin with nanophase hydroxyapatite. The scaffolds exhibited well swelling characteristic, which could be modulated by varying the ratio of chitosan and gelatin. The biomedical response of MG-63 cells on nanocomposite scaffolds was superior in terms of improved cell attachment, higher proliferation, and spreading compared with CS–gelatin scaffold.

4.1.5. Cancer therapy

Cancer is a dreadful human disease. Nanomaterials are frequently considered in the parenteral injection of aqueous insoluble drugs for drug targeting issues because their particle sizes are less than 1000 nm. DOX incorporated nanoparticles prepared using methoxy poly (ethylene glycol)-grafted carboxymethyl chitosan nanoparticles (CMCPED) were developed to study antitumor activity. Because DOX has positive amine groups, it can interact with the carboxymethyl group of CMCPED and can form nanoparticles. The antitumor activity of DOX-incorporated nanoparticles *in vitro* was tested with DOX-resistant C6 glioma. These nanoparticles exhibited higher cytotoxicity to DOX alone [123]. CS nanoparticles were used as vehicles for the mitotic inhibitor paclitaxel. PTX-loaded CS

nanoparticles were prepared by a solvent evaporation and emulsification cross-linking method. Cytotoxic activity exhibited that the paclitaxel-loaded CS nanoparticles had higher cell toxicity than individual paclitaxel, and confocal microscopy analysis confirmed strong cellular uptake efficiency [124]. Lee et al. [49] studied the DOX-loading fucoidan acetate nanoparticles for immunotherapy and chemotherapy in cancer treatment. Acetate nanoparticles have important functions, namely immunomodulation and drug efflux pump inhibition [49].

4.1.6. Wound dressing

Nowadays, the research and development of antibacterial treatment-resistant pathogens is a major problem, and the newly designed wound dressing has provided a main breakthrough for the treatment of infection and wounds. Recently, many researchers have focused on developing novel antibacterial treatments to treat wounds infected with antibacterial treatment-resistant pathogens. Currently, silver nanoparticles have come up as a more efficient bactericidal agent and are finding various biomedical applications ranging from silver-based dressing to silver-coated therapeutic devices [125]. A lot of studies have investigated the use of CS scaffolds and membranes to treat patients with wounds, deep burns, etc. Chen et al. [126] prepared the collagen-CS complex nanofibers by electrospinning. These nanofibers are well known for their positive effect on wound healing. Madhumathi et al. [22] have incorporated silver nanoparticles into chitin scaffolds for wound-healing applications. These α-chitin/nanosilver composite scaffolds were found to possess good antibacterial activity against pathogenic bacteria, combined with excellent blood-clotting ability. These *in vitro* results indicated that α-chitin/nanosilver composite scaffolds could be useful for wound-healing applications.

4.2. Biotechnological applications

4.2.1. Biosensors

Biosensors have been of special interest in recent years because of their advantageous properties as analytical tools such as simple, portable, low cost, well-established, and laboratory-based methods as well as allows miniaturization [127]. The CS nanofibrous membrane was explored as support for enzyme immobilization owing to the characteristics of good biocompatibility, high surface, and large porosity. The CS nanofibrous membrane was up to 63.6 mg/g, and the activity retention of the immobilized lipase was 49.8% less than the optimum condition. This system can be used for biosensors [128]. Wang et al. [129] investigated a new electrochemical tyrosinase biosensor for determining phenolic compounds on the basic of the use of a glassy carbon electrode modified with tyrosinase–Fe_3O_4 magnetic nanoparticles-CS nanobiocomposite film. The tyrosinase biosensor shows good repeatability and stability. Such novel tyrosinase biosensor exhibits great promise for fast, simple, and eco-friendly methods of phenolic contaminants in environmental samples [129]. Chauhan et al. [130] developed an amperometric biosensor for the determination of glutathione (GSH) by covalently immobilizing a glutathione oxidase (GSHOx) onto the surface of gold-coated magnetic nanoparticles (Fe@AuNPs)-modified Pt electrode. CS was used to introduce amino groups onto the surface of Fe@AuNPs. The present study exhibited that GSHOx/CS/Fe@Au-modified Pt electrode was an excellent candidate for the construction of highly sensitive glutathione biosensor.

4.2.2. Wastewater treatment

CS has received considerable attention in water treatment because of the presence of reactive amino groups. Chitin and CS have been widely studied in the removal of heavy metal ions from wastewater [131]. Fierro et al. [132] investigated and found that the CS bead-immobilized algae system with *Scenedesmus* sp. was more effective in removing phosphate and nitrate from water than the conventional free cell system. Manivasagan et al. [32] reported the biosynthesis of silver nanoparticles using polysaccharide-based bioflocculant by *Streptomyces* sp. MBRC-91. The biosynthesized silver nanoparticles exhibited strong antibacterial activity in sewage water, and this result can make a new avenue in the wastewater treatment.

5. Patents on marine polysaccharide-based nanomaterials

More than 50 patents are available on marine polysaccharides-based nanomaterials and their applications. In all these patents, only very few patents specifically claim that nanocomposite materials based on metallic nanoparticles were stabilized with branched polysaccharides, in particular alditolic or aldonic mono-saccharidic and oligosaccharidic derivatives of CS, and their preparation was obtainable with the aqueous solutions of these polysaccharides in the presence or absence of reducing agents. The properties associated with the nanometric dimensions and the presence of biological signals on polymeric chains may be exploited in applications with antimicrobial activities and molecular biosensors [133]. The nanocomposite materials in the form of three-dimensional structure formed by a polymeric matrix consisting of a polysaccharidic composition of neutral or anionic polysaccharides and branched cationic polysaccharides, in which metallic nanoparticles are uniformly dispersed and stabilized, has also been claimed [134].

6. Conclusions

In the present review, marine polysaccharides-based nanomaterials are an excellent source for nanotechnological applications such as drug delivery, gene delivery, tissue engineering, cancer therapy, wound dressing, biosensors, and water treatment. This method is simple, inexpensive, eco-friendly, and highly recommended to be used in the large-scale production of bionanomaterials. Marine polysaccharides are highly stable, safe, biocompatible, biodegradable, nontoxic, low cost, and abundant. However, most of the industrial applications are still at the laboratory level. In addition, *in vivo* studies and clinical applications are needed to develop new commercial nanoproducts. Marine polysaccharide-based nanomaterials have a great promise in biomedicine, fabric, food, and pharmaceutical industries for the future.

Acknowledgements

This research was supported by a grant from Marine Biotechnology Program (20150220) funded by the Ministry of Oceans and Fisheries, Republic of Korea.

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