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Marine-origin Polysaccharides for Tissue Engineering and Regenerative Medicine

Chitosan and Fucoidan as Illustrative Examples

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118.1 Introduction

Medical staff, pharmaceutical companies, and other scientists and engineers working in human health-related fields are constantly fostering new approaches to establish or improve therapeutic solutions or design prevention strategies. For instance, in several pathologies, the human body faces the loss of tissues caused by trauma or disease, being unable to heal itself. From a medicine based in substitution, science is evolving to a regenerative paradigm, inspired by nature and the striking examples of a newt's regenerating limbs and a starfish's regenerating arms, to name a few. This regenerative medicine uses **tissue engineering (TE)**, proposed about 25 year ago by Langer and Vacanti (Langer and Vacanti 1993), aiming to overcome the drawbacks of current prosthetic devices and grafts from donors of a different nature, such as limited function, scarcity, or non-biocompatibility. TE is commonly based on the development of biodegradable matrices (mimicking the extracellular matrix) – scaffolds – where cells will be seeded and cultured in the presence of appropriate cocktails of bioactive compounds to induce cell fate, pointing to the production of living constructs to be implanted in the patient body. These constructs will, hopefully, experience an adequate integration in the surrounding tissues and subsequently trigger tissue regeneration by recruiting cells that together with the implanted ones will proliferate (and differentiate) and produce a new extracellular matrix, while the implanted scaffold materials are degrading and being secreted from the body. While several derivative approaches can be designed, including acellular ones (Burdick et al. 2013) or scaffold-free approaches (DuRaine et al. 2015; Syed-Picard et al. 2015), it cannot be denied that materials play a pivotal role in TE. In this perspective, several polymers have been used for the development of scaffolds, be they of natural origin or synthesized, with the combination of proteins and polysaccharides promising to resemble the biochemistry of the native extracellular matrix. One can thus see collagens and silk fibroin being combined with alginate, chitosan, starch, or chondroitin sulfate, which will not only act as structural materials with support function, but will also exert several biological activities that have been reported (Malafaya et al. 2007).

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While proteins have a well-defined primary structure coded on genetic information, polysaccharides can be quite variable and heterogeneous, depending on physiological and environmental conditions, to which add a significant degree of variability caused by the methodologies used for their extraction from natural sources. Thus, although several biological activities have been attributed to polysaccharides grouped under the same name, those activities are not ubiquitous, depending on chemical features, since several of those molecules can be quite different and still be given the same *family* name, from which a validation of the chemical and biological properties is always needed when dealing with a new polysaccharide extract. Indeed, the **structure-activity relationship (SAR)** is one of the active areas of polysaccharide research, with intriguing results being reported in the last years (Jin et al. 2016; Oliveira et al. 2017). This variability can be regarded as a source of flexibility, which can be enriched by the many modifications offered by chemistry, being the variation in functional groups, molecular weight, or grafting of bioactive molecules, such as peptides, resulting in a very versatile class of biopolymers (Alves and Mano 2008). Nevertheless, this versatility is not being fully exploited, namely regarding the biomedical potential of polysaccharides, mainly because their functions in the human body are not yet completely understood. Besides the support and storage roles, played respectively by cellulose and starch in plants, for instance, there are a series of other biological roles assumed by polysaccharides, with cells being literally covered by sugars – the common name given to this class of biopolymers – that are believed to interfere on the growth, function, and survival of our organisms, namely by acting on recognition of toxins, viruses, antibodies, bacteria, and other cells (Pashkuleva and Reis 2010).

The marine environment is quite well represented in the polysaccharide world, being the origin of some of the most explored examples, such as chitosan, agar, and alginate. Besides, sulfated polysaccharides from macroalgae have been receiving increasing attention from the scientific community associated to the innumerable biological activities being identified (Silva et al. 2012), as well as exopolysaccharides from microalgae, which can be isolated as by-products from cultivation for capture of CO_2 (Posada et al. 2016).

The present chapter focuses on two polysaccharides – chitosan and fucoidan – that have been studied as models of glycosaminoglycans, in the first case with similarities to hyaluronic acid given the presence of acetylglycosamine (but no uronic acids) and in the second case given the presence of sulfation and uronic acids. These are illustrative examples of the processability, properties, and applications of marine polysaccharides (Figure 118.1), with chitosan deriving from chitin, the second most abundant polysaccharide in the world, and fucoidan representing the highly biologically active sulfated polysaccharides. Moreover, the former is a polycation due to the presence of free amines protonated in acid medium and the latter is a polyanion due to the presence of ester sulfates. In the following sections we discuss the methodologies used for their extraction from the marine origin biomass and their chemical and biological properties, with emphasis on the ones more relevant in biomedical context.

118.2 Chitin/Chitosan

The exploration of polysaccharides like chitin and chitosan from the marine biomass has attracted considerable interest as an ecofriendly and sustainable strategy (He et al. 2014; Huang et al. 2015). Chitin is the second most abundant natural polymer after cellulose. Structurally, chitin is composed of N-acetyl-D-glucosamine and D-glucosamine monomers, bonded by β-D-(1→4) linkages (Bueter et al. 2013; Cheng et al. 2014). Depending on the source, chitin can be characterized by α and β crystallographic

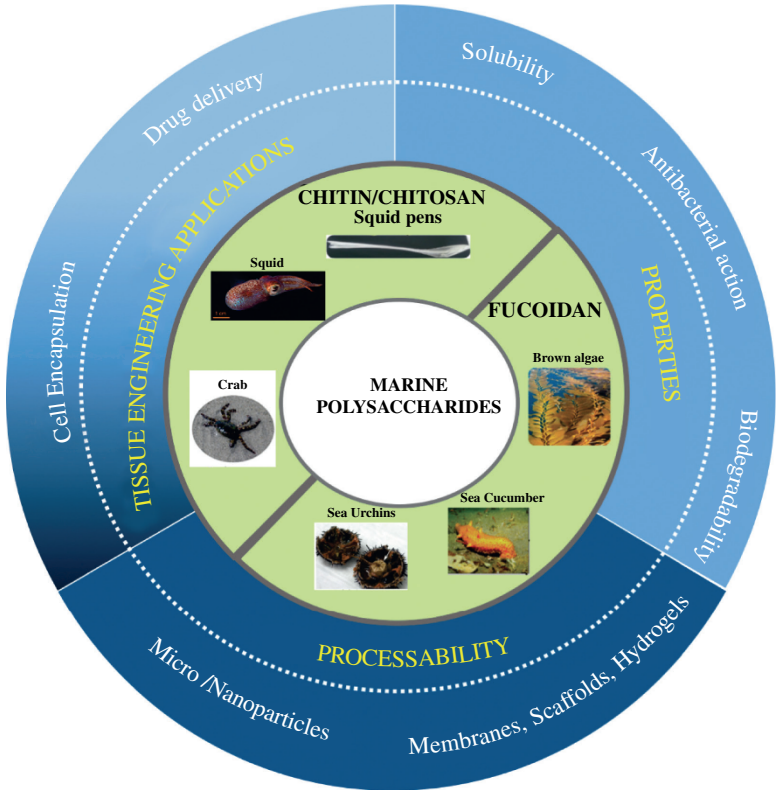

Figure 118.1 Overview of the properties, processability, and tissue engineering applications of marine polymers for biomedical applications (Ribeiro et al. 2011; Bueter et al. 2013; McFall-Ngai 2014; Janakiram et al. 2015).

forms: α-chitin is the most abundant type of chitin in nature, found in the exoskeleton of many crustaceans such as crabs and shrimps, while the β-chitin constitutes the endoskeletons of various mollusks (Langer and Vacanti 1993; Kim and Mooney, Rinaudo 2006; Cheng et al. 2014). In both types of chitins, the chains are organized in sheets and held together by intra-sheet hydrogen bonds (see Figure 118.2). Nevertheless, differing from α-chitin, polymeric chains organized in a parallel way are present in the β-chitin structure, leading to weaker intermolecular bonds, which may explain its higher affinity for solvents and its higher reactivity (understood as susceptibility for chemical modification).

118.2.1 Extraction of Chitin and its Conversion to Chitosan

Chitin present in the exoskeleton of arthropods is associated with minerals, proteins, lipids, and pigments. Therefore, the isolation of α-chitin consists in washing, demineralization, deproteinization, and depigmentation to remove impurities and those other major components: minerals (calcium carbonate), proteins, and pigments, respectively (Reys et al. 2013). In those processes, demineralization is made using strong acids, while deproteinization uses strong bases, e.g. sodium hydroxide (NaOH) solution. A possible alternative to the previous methodologies is the employment of microorganisms and proteolytic enzymes

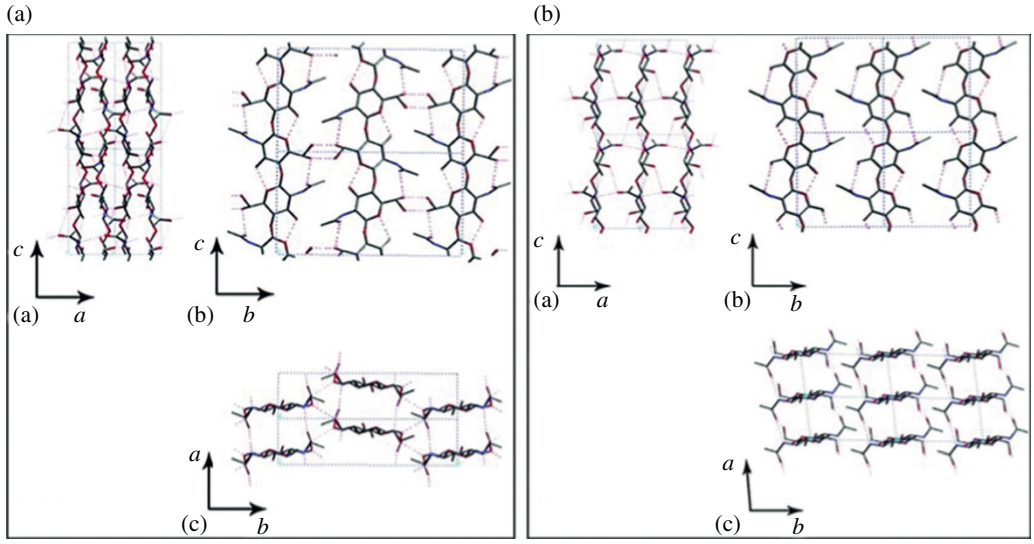

Figure 118.2 Structures of α-chitin (a) and β-chitin (b). Reprinted with permission from Zeng et al. (2012).

(Gasperini et al. 2014; Younes et al. 2014). Later on, the depigmentation process is performed, relying on the use of solvents (acetone or ethanol) for the extraction of red pigments, carotenoids. These solvents will also eliminate the lipids present in the raw material (Hsieh et al. 2003; Maoka 2011). By its turn, β-chitin is isolated from the endoskeletons of squids and cuttlefish (Silva et al. 2008; Jayakumar et al. 2010; Reys et al. 2013). Compared to α-chitin, β-chitin contains a smaller amount of minerals and lipids, while pigments are absent. Thus, chitin is retrieved mainly following a deproteinization procedure.

Chitin has been converted into chitosan by partial deacetylation (Rinaudo 2006; Silva et al. 2010). This process consists of the removal of the acetyl groups using a high temperature (90°–100°C) and strong alkali solutions (e.g. 50% NaOH) during several hours (Evans et al. 2002; Hsieh et al. 2003; Reys et al. 2013). Being harsh conditions, polymer degradation may also be promoted and thus, depending on the reaction conditions used, chitosan with variations in both **molecular weight (MW)** and **degree of deacetylation (DD)** can be obtained. The DD is characterized by the molar fraction of non-acetylated units within the chitosan chain. Both the DD and MW are relevant parameters of chitosan that could influence its performance in pharmaceutical and biomedical applications (Rinaudo 2006). It has been described that chitosan can be only considered when a maximum of 40% N-acetyl-D-glucosamine units remain as part of the polymer structure (Dimzon et al. 2013), although a more empirical approach is commonly used: if soluble in diluted acetic acid, it is chitosan; if not, it is still chitin. A purification process can be made to obtain chitosan with a higher degree of purity, by dissolving chitosan in a diluted solution of acetic acid (1% w/v), followed by filtration to remove insoluble impurities and further chitosan precipitation by the addition of NaOH 3M until pH 8. The pH should be controlled to avoid high alkaline conditions, which could promote the additional deacetylation of the material (Reys et al. 2013). The precipitated chitosan is washed with distilled water and ethanol solutions. Then, the final material is frozen at –80°C overnight and freeze-dried for at least 4 days (Signini and Campana Filho 1998; Reys et al. 2013).

118.2.2 Properties and Processing of Chitin and Chitosan

118.2.2.1 Chitin

Chitin has strong intra- and interchain hydrogen bonding, contributing to the formation of a microfibrillar structure that presents crystalline and amorphous zones (Villares et al. 2014; Deringer et al. 2016). In native chitin, these fibrils with diameters from 2.5 to 25 nm are usually embedded in a protein matrix (Zeng et al. 2012). Chitin microfibers can be hydrolyzed and separated into individual nanofibers, commonly called **nanowhiskers (CHWs)** (Araki and Kurihara 2015). These CHWs have interesting features such as remarkable mechanical properties, positive surface charge, large surface area, relatively low density, high heat resistance, and biodegradability (Araki and Kurihara 2015; Huang et al. 2015; Aklog et al. 2016). The CHWs are used mainly as reinforcing polymer nanocomposites, with the formation of $CaCO_3$/chitin-whisker hybrids, as well as on structuring oil, fueling, and also to produce hydrogels and scaffolds, among other applications (Kadokawa 2013; Pereira et al. 2014; Cui et al. 2016; Valverde Serrano et al. 2016; Zhang et al. 2016; Silva et al. 2017).

Chitin has poor solubility in water and most organic solvents due to its strong intermolecular hydrogen bonding. In fact, only a limited number of solvents, namely N,N-dimethylacetamide/lithium chloride (DMA-LiCl), NaOH/urea, and hexafluoroacetone are able to solubilize chitin (Ravi Kumar 2000; Rinaudo 2006; Silva et al. 2010), representing a drawback for industrial purposes (Deringer et al. 2016; Skołucka-Szary et al. 2016). However, chitin itself has appealing properties for biomedical applications such as biocompatibility, tumor cell growth suppression, acceleration of wound healing, and antimicrobial activity (Ravi Kumar 2000; Silva et al. 2010; Cheung et al. 2015). The mentioned key properties of chitin have stimulated the development of many chitin-based products such as dressing for burns, vascular implants, artificial blood vessels, and tumor inhibitors (Ravi Kumar 2000; Khor and Lim 2003; Muzzarelli 2009).

Recent advances in the processing of chitin into useful materials have been achieved using **ionic liquids (ILs)**, defined as salts that melt below 100°C (Anthony, Brennecke et al. 2002). ILs are composed of different cations and anions and can be tailored to meet the demands of each application, possessing the ability to disrupt strong H-bond interactions. Therefore, the dissolution of chitin in ILs, such as 1-butylimidazolium acetate (BMIMAc) and 1-ethyl-3-methylimidazolium chloride (BMIMCl), has been demonstrated in the production of 2D (films) and 3D-based matrices (sponges, micro/nanoparticles, nanofibers) (Silva et al. 2011; Silva et al. 2017). The produced materials have been designed to act as wound dressing, hemostatic or antibacterial agents, and drug-delivery systems (Silva et al. 2011; Silva et al. 2017). During chemical reactions such as hydrolysis, acetylation, graft copolymerization, and **atom transfer radical polymerization (ATR)** on chitin, imidazolium-based ILs not only reduce dissolution times but are also an excellent catalytic media due to their inherent task-specific properties (Silva et al. 2017). Despite the successful findings, studies involving *in vivo* biocompatibility on chitin-based matrices processed in ILs are scarce. Although more research is needed, the available results suggest that the chitin–IL platform can be an attractive new tool in the processing of chitin-based biomedical devices.

118.2.2.2 Chitosan

Chitosan has fascinating characteristics as a biomedical polymeric material, such as biodegradability, biocompatibility, mucoadhesivity, antibacterial and antioxidant activity, lack of toxicity, hemostatic action, and cationic nature (Rinaudo 2006; Silva et al. 2010). Due to its stable crystalline structure, chitosan is usually insoluble in water, but soluble in diluted acid solutions that promote the protonation of the free amine groups ($-NH_2$) present in the deacetylated units (pKa ≈ 6.3) (Rinaudo et al.

1999). Several matrices can be obtained using chitosan. For instance, diluted chitosan acid solutions are the basis for membrane production, while gels can be formed with concentrated polymeric solutions. Particles and fibers can be produced using dripping or direct contact of chitosan solution with a coagulation alkaline bath (Silva et al. 2010). Alternatively, porous structures and tubes can be obtained using freeze-drying. Also, the cationic nature of the chitosan permits the formation of ionic complexes with a variety of anionic polysaccharides, such as alginate or gelatin (Mano et al. 2007; Silva et al. 2010), as well as with **glycosaminoglycans (GAGs)** or proteoglycans (Reys et al. 2013).

The availability of amine and hydroxyl groups present in the chitosan structure offers the opportunity for the introduction of novel moieties and properties (Rinaudo 2006; Alves and Mano 2008). Indeed, this possibility has been widely explored through the synthesis of several chitosan derivatives such as quaternized, N-alkyl, hydroxyalkyl, carboxyalkyl, N-Acyl, O-Acyl, thiolated, sulfated, azidated, or phosphorylated chitosans (Alves and Mano 2008). Among these chitosan derivatives, **carboxymethyl chitosan (CMCS)** has been the most explored for biomedical applications, mainly due to its water-solubility (Upadhyaya et al. 2014). Table 118.1 displays a summary of different chitin and chitosan matrices and their potential biomedical applications.

The combination of chitosan with other polymers, natural or synthetic, is an approach frequently used to create blends and composites with improved mechanical properties or biocompatibility. Although it is much easier to use synthetic polymers since they have reproducible processing, suitable mechanical properties, and thermal stability, natural polymers are also an excellent choice due to their abundance, biocompatibility, and biodegradability. Most of the studies reported in the literature described blended systems composed by chitosan with alginate (Venkatesan et al. 2014: Silva et al. 2015: Conzatti et al. 2017), cellulose (Park et al. 2011; Fu et al. 2017), fucoidan (Murakami et al. 2010; Pinheiro et al. 2015; Huang et al. 2016), chondroitin sulfate (Fan et al. 2017; Nunes et al. 2017), hyaluronic acid (Park et al. 2013; Miranda et al. 2016), gelatin (Dhandayuthapani et al. 2010; Tseng et al. 2013; Nieto-Suarez et al. 2016; Wang et al. 2016), collagen (Sarkar et al. 2013; Wang et al. 2013; Xin et al. 2015; Mahmoud and Salama 2016), and silk fibroin (Silva et al. 2008; Silva et al. 2012), as illustrated by the examples depicted in Table 118.2. These blends can be processed into different forms: membranes can be obtained using solvent casting method (Silva et al. 2008; Silva et al. 2013), porous structures can be processed by freeze-drying technique (Xin et al. 2015; Mahmoud and Salama 2016), while hydrogels can be produced through ion gelation, chemical crosslinking, or copolymerization reactions (Silva et al. 2012), among others. These polymeric matrices have suitable properties and functionalities for biomedical purposes, namely as dermal substitutes, wound dressings, drug delivery, and 3D porous structures for cartilage, bone, and skin regeneration.

Recent research described the positive interaction between chitosan with active phytochemicals found in **aloe vera (AV)**, a medicinal plant, as a strategy to create active wound dressing materials useful for skin repair (Silva et al. 2013; Silva et al. 2013). The chitosan/AV based membranes displayed increased roughness and wettability, while the crosslinking with genipin, a natural crosslinker, promoted the formation of stiffer membranes in comparison to non-modified ones. Moreover, in vitro assays demonstrated that the adhesion and proliferation of human fibroblasts on chitosan/AV membranes were better than chitosan membrane.

Recent studies described the chemical modification of chitosan backbone to convert it into water-soluble CMCS, which has been blended with soy protein (Teng et al. 2013), collagen (Lin et al. 2017), and gelatin (Kanth et al. 2017) to prepare nanoparticles, microspheres, and hydrogel microspheres, respectively. These matrices can be potential candidates for developing controlled release devices for nutraceuticals and drugs.

Table 118.1 Chitin and chitosan matrices for biomedical purposes.

Composition	Matrix type	Relevant properties	Proposed biomedical application	References
Chitin	Nanofibers	Self-assembly	TE	(Rolandi and Rolandi 2014)
	Nanoparticles	Interaction with anticancer drugs	Anticancer therapy (drug delivery)	(Geetha et al. 2016)
	Nanocrystalline films	Protein adsorption	Biological sensors and catalysis	(Wang and Esker 2014)
	Microspheres	Positive charge	Controlled drug release	(Shang et al. 2014)
Carboxymethyl chitin	Injectable hydrogels	Thermosensitivity	Soft tissue regenerative medicine	(Liu et al. 2016)
	Carboxymethyl chitin /PAMAM dendrimer NPs	Grafting potential	Regenerative medicine	(Salgado et al. 2010)
	Microspheres	Emulsification potential	Anticancer therapy (drug delivery)	(Li et al. 2011)
	Porous membranes	Water solubility	TE	(Zhao et al. 2015)
Chitosan	Microparticles	Functionalization potential	Tissue regeneration	(Custódio et al. 2015)
	NPs	Positive charge	Anticancer therapy (drug delivery)	(Van Woensel et al. 2016)
	Coatings	Cell interaction	TE	(Chou et al. 2016)
	Hydrogels	Gelling ability	Bacteria detection	(Sadat Ebrahimi and Schönherr 2014)
	Films	Adhesiveness	Tissue repair	(Barton et al. 2014)
Chitosan-sulfonamide derivatives	Membranes	Antimicrobial activity	Wound dressing	(Dragostin et al. 2016)
Chitin and chitosan	Chitosan-sheath and chitin-core nanowhiskers	Crystallinity and deacetylation potential	Improving the properties of other systems	(Pereira et al. 2014)
N-acetyl-cysteine chitosan	Reinforced mats	Antimicrobicity, hydrating features, crystallinity, and electrospun ability	Wound dressing	(Naseri et al. 2014)

Abbreviations: TE – Tissue engineering; NPs – nanoparticles; PAMAM – Polyamidoamine.

The interactions between chitosan and alginate (Silva et al. 2015; Conzatti et al. 2017) or gelatin (Dhandayuthapani, Krishnan et al. 2010, Tseng, Tsou et al. 2013; Nieto-Suarez et al. 2016; Wang et al. 2016) have been also employed in the preparation of **polyelectrolyte complexes (PECs)**. For instance, the interactions between amine groups of chitosan and carboxylate groups present in gelatin resulted in the formation of hydrogen bonding

Table 118.2 Chitosan blends for biomedical applications.

Composition	Processing methodology	Matrix type	Potential biomedical application	Reference
Chitin/ALG	Dry-jet wet spinning	Fibers	Wound care dressings	(Shamshina et al. 2014)
Chitin/CEL	Gelation in ILs (AMIMBr, AMIMCl)	Composite gel and films	*n.d.*	(Takegawa et al. 2010)
	Wet-spinning process	Fibers	*n.d.*	(Mundsinger et al. 2015)
Chitosan/ALG	PEC formation Drying process (Hot air, freeze-drying, SCC)	Xerogel, alcogel	Gastrointestinal wound dressings	(Conzatti et al. 2017)
	Layer-by-layer	Freestanding membrane	*n.d.*	(Silva et al. 2016)
Chitosan/AG	Freeze-drying	Scaffold	Cartilage TE	(Merlin Rajesh Lal et al. 2017)
	Sol-gel transition	Nanocomposite ionogels	Biotechnology and biomedical applications	(Trivedi et al. 2014)
Chitosan/AV	Solvent casting	Membranes	Wound dressing	(Silva et al. 2013)
	Solvent casting Genipin crosslinking	Crosslinked membranes		(Silva et al. 2013)
Chitosan/CAR	Polyelectrolyte complexation/ ionic gelation	Nanoparticles	Mucosal delivery of macromolecules	(Rodrigues et al. 2012)
	PEC formation	Scaffolds	TE	(Araujo et al. 2014)
Chitosan/CEL	Electrospinning	Nanofibers	Wound repair	(Park et al. 2011)
	Solvent casting	Films	Antibacterial materials	(Fu et al. 2017)
Chitosan/CMC	Spray-drying process	Microparticles	Drug delivery system	(Cerchiara et al. 2016)
Chitosan/COL	Freeze-drying	Scaffolds	Skin regeneration application	(Mahmoud and Salama 2016)
			Therapeutic strategy for ischemic stroke	(Xin et al. 2015)
Chitosan/COL/ ALG	Spray-spinning	Fibrous scaffold	Anticancer drug screening	(Wang et al. 2016)

Chitosan/CS	Schiff base reaction	Injectable hydrogel	Injectable drug and cell delivery system in cartilage tissue engineering	(Fan et al. 2017)
	Gelation in ILs ([Hmim][HSO_4])	Hydrogel	Many technological purposes	(Nunes et al. 2017)
	PEC formation	Nanoparticles	Controlled release of growth factors and proteins	(Santo et al. 2012)
Chitosan/soy protein/TEOS	Sol-gel process Solvent casting	Membranes	Wound dressing	(Silva et al. 2013)
Chitosan/ Fucoidan	Ionotropic crosslinking	Nanoparticles	Potential carriers in pulmonary delivery	(Pinheiro et al. 2015; Huang et al. 2016)
	Layer-by-layer assembly	Nanocapsules	Delivery system for water soluble bioactive compounds	
Chitosan/GEL	Crosslinking (succinimide-end polyethylene glycol)	Microgels	Delivery vehicle of hydrophobic bioactive molecules	(Wang et al. 2016)
	Crosslinked with glutaraldehyde ice segregation induced self-assembly	Scaffolds	*n.d.*	(Nieto-Suarez et al. 2016)
Chitosan/GG	Crosslinking (PEG)	Hydrogel	Diabetic wound healing	(Shukla et al. 2016)
Chitosan/HA	Photocrosslinking	Injectable hydrogels	Cartilage TE	(Park et al. 2013)
	Gelation/ freeze-drying	Hydrogel scaffold	Periodontal TE	(Miranda et al. 2016)
Chitosan/SF	Gelation	Hydrogels	Skin regeneration	(Silva et al. 2012)

Abbreviations: AG – agarose; ALG – alginate; AV – aloe vera; AMIMBr – 1-allyl-3-methylimidazolium bromide; AMIMCl – 1-allyl-3-methylimidazolium chloride; CMC – carboxymethylcellulose; CAR – carrageenan; CEL – cellulose; CS – chondroitin sulfate; COL – collagen; GEL – gelatin; GG – gellan gum; HA – hyaluronic acid; *n.d.* – not defined; PEG – polyethylene glycol; PLLA – poly(lactic acid); PBS – polybutyrene succinate; PEC – polyelectrolyte complex; SF – silk fibroin; SCC – super critical CO_2 drying; TEOS – tetraethylorthosilicate; TE – tissue engineering.

and electrostatic attraction, enabling the production of blended films (Hamman 2010). In other studies, nanoparticles were produced based on the electrostatic interaction between chitosan and chondroitin sulfate. This system is proposed for the controlled release of protein and **growth factors (GFs)**, specifically **platelet lysates (PLs)** (Santo et al. 2012).

Blending chitosan with synthetic polymers, namely **polyvinyl pyrrolidone (PVP)**, **polyvinyl acetate (PVA)**, **polyethylene oxide (PEO)**, and **poly(butylene terephthalate adipate) (CPBTA)** (Alves da Silva et al. 2011; Sionkowska 2011), can be performed to enhance hydrophilicity, mechanical properties, blood compatibility, or antibacterial properties. The chitosan/synthetic blends can be done in solution or by melting, with Sionkowska describing chitosan-PVP hydrogels produced through crosslinking (Sionkowska 2011), suggested for protein absorption and immobilization.

118.2.3 Applications

The intrinsic properties of chitosan serve as the basis for the utilization of this biomaterial in diverse areas, such as agriculture, environmental protection, food industry, biotechnology, materials science, or pharmaceutical and biomedical industry (Aranaz 2009; Dutta et al.; Silva et al. 2010). Particularly, in **tissue engineering and regenerative medicine (TERM)**, several chitosan-based systems have been used in the development of 3D porous structures to induce and support the regeneration of different tissues, namely bone, cartilage, and skin (Oliveira et al. 2006; Silva et al. 2008; Yilgor et al. 2009; Neves et al. 2011; Jin et al. 2013; Tseng et al. 2013; Francis et al. 2014; Martins et al. 2014).

118.2.3.1 Cartilage Regeneration

Chitosan-based structures have been suggested for cartilage regeneration, since chitosan chemical structure and characteristics have similarities to GAGs. GAGs are known constituents of the cartilage **extracellular matrix (ECM)**, and they have a fundamental role *in vitro* and *in vivo* chondrogenesis (Suh and Matthew 2000). Silva et al. developed porous structures combining chitosan and silk fibroin in which ATDC5 chondrocyte like-cells were able to adhere and proliferate, while producing ECM, during up to 28 days of culture. The biological findings associated with the properties of the developed structures suggest that these systems can be suitable candidates for their use for cartilage regeneration (Silva et al. 2008; Silva et al. 2013).

118.2.3.2 Bone Regeneration

Different authors outlined the use of chitosan-based structures for the treatment of bone and osteochondral defects (Mano and Reis 2007; Muzzarelli 2009; Yilgor et al. 2009; Duarte et al. 2010). This can be achieved by freeze-drying of chitosan–collagen blends (Raftery et al. 2016), processing with supercritical fluids (Duarte et al. 2010), or incorporation of bone-specific growth factors (Yilgor et al. 2009), but the development of biphasic structures are offered as more appropriate to tackle the challenging osteochondral pathologies, since those layered scaffolds supply differentiated regions to promote both bone and cartilage regeneration. Oliveira et al. obtained polymeric biphasic structures combining chitosan with hydroxyapatite, throughout a synthesis and freeze-drying process (Oliveira et al. 2006), with biological results proving that both layers are adequate 3D supports for the adhesion and proliferation of bone marrow stem cells and further enabling their differentiation in osteogenic and chondrogenic lineages.

118.2.3.3 Skin Regeneration

Chitosan has interesting features for skin regeneration, namely wound protection, healing acceleration, and antibacterial action. Therefore, chitosan membranes or blended ones, as well as hydrogels (Santos et al. 2013; Silva et al. 2013), have been proposed as wound dressings or as artificial skin. Some authors

have reached promising results, as for instance Kong et al. with alternately deposited alginate and chitosan on a substrate of solidified gelatin, to form an ultrathin nanomembrane (Kong et al. 2016). This system accelerated wound contraction and epidermalization. By their turn, Lu et al. used a chitosan/ascorbic acid solution blend containing gelatin, followed by crosslinking with tannin acid and freeze-drying, to obtain a chitosan–gelatin sponge (Lu et al. 2016), observing the rapid healing of a treated wound. This wound heal was further promoted with the loading of platelet-rich plasma. Anjum et al. coated a cotton fabric with a blend of chitosan, **polyethylene glycol (PEG)** and PVP (Anjum et al. 2016). By incorporating the drug tetracycline hydrochloride (antibiotic) within the developed system, the drug-loaded dressing demonstrated good antimicrobial nature against both Gram-positive and Gram-negative bacteria. Moreover, *in vivo* studies carried out on full-thickness skin wounds suggested that drug-loaded dressings could provide scar preventive wound healing.

118.2.3.4 Cancer Treatment

Chitosan has multifaceted applications in cancer therapy, namely as assisting in gene delivery and chemotherapeutic delivery, as well as being immunoadjuvant for vaccines (Babu and Ramesh 2017). Chitosan matrices have also been utilized for the development of anticancer therapies. Chitosan-based structures have been designed to mimic the cancerous biologic environment, to both facilitate the study of cancer cell behaviors and to enable the evaluation of the performance of novel therapeutic molecules (Florczyk et al. 2013; Huang and Li 2014; Kievit et al. 2014). For instance, **chitosan nanoparticles (CSNPs)** have drawn considerable attention as anticancer drug delivery carriers due to their easy accessibility, excellent stability, low toxicity, and easy modification (Fu et al. 2016). Jin et al. demonstrated that a nanoparticle-based drug carrier composed of chitosan, ursolic acid (UA), and folate (FA-CS-UA-NPs) could effectively diminish off-target effect and increase local drug concentrations of ursolic acid (Ji et al. 2016). *In vivo* experiments showed that FA-CS-UA-NPs could significantly reduce breast cancer burden in MCF-7 xenograft mouse model, overall suggesting that these NPs might provide a platform to develop an anticancer drug delivery system.

118.2.3.5 Drug Delivery Systems

Chitosan can be used as an attractive component of different drug delivery systems (DDSs), such as membranes, nanoparticles, tablets, or hydrogels (Hamman 2010, Silva, Alves et al. 2012, Cardoso, Costa et al. 2016). The drug incorporation within these systems can be performed by addition into the solution or posterior entrapment by complexation or adsorption. Bigucci et al. studied complexes formed by chitosan and carboxymethylcellulose (CMC) for the vaginal administration of chlorhexidine, a broad-spectrum antiseptic (Bigucci et al. 2015). Tang et al. developed chitosan films for the administration of ibuprofen in the oral mucosa, with drug incorporation by supercritical impregnation (Tang et al. 2014). Moreover, Calinescu et al. elaborated gastro-resistant tablets destined for the controlled release of therapeutic proteins in the intestine (Calinescu et al. 2012). In other studies, chitosan hydrogels were employed in the sustained release of latanoprost (ocular anti-hypertensive), destined to be used in glaucoma therapy (Cheng et al. 2014). Hermans et al. prepared chitosan films that were used to prolong the release of ciclosporin (immunosuppressive drug) after its ocular administration, as part of the therapeutic treatment of the dry eye (Hermans et al. 2014), while Bhalerao et al. developed chitosan and heparin films for the controlled release of drugs destined for the treatment of the malaria disease (Bhalerao et al. 2015).

118.3 Fucoidan

Fucoidan is an anionic sulfated polysaccharide present in the cell wall of brown algae, although it can represent all polysaccharides

having a polymer backbone composed by sulfated fucose units, which has been found also in sea cucumbers. Fucoidan has been isolated from different species of brown algae, namely *Fucus vesiculosus*, *Ecklonia cava* e *Undaria pinnatifida* (Sezer and Cevher 2011; Wa and Yj 2012). The chemical and structural composition of fucoidan has been much studied over the past few years but it is not yet definitely established (Mak 2012). The main components of fucoidan are L-fucose with ester sulfate groups, with other monosaccharides also being present, like mannose (Duarte 2001), galactose (Rocha et al. 2005), glucose (Nagaoka et al. 1999), xylose (Rocha et al. 2005), and uronic acids (Nagaoka et al. 1999; Ponce et al. 2003). The chemical structure of fucoidan is usually composed of residues of α-L-fucopiranose interconnected by links (1 → 3) or alternating (1 → 3) and (1 → 4), but polymer branching is also possible, including with other sugars, related to the species of brown seaweed (Chizhov et al. 1999), but also to other factors, such as harvest location and season and seaweed maturity (A.Sarah, et al. 2001; Mak 2012), besides experimental conditions used during extraction. In Figure 118.3 we can observe two fucoidan types with varying degrees of sulfation. The interest in this sulfated polysaccharide comes from its intrinsic properties, including antitumor (Li Bo 2008; Shilpi 2011; Oliveira et al. 2017), anticoagulant (Mourão 2004; Li Bo 2008; Shilpi 2011), antiviral (Mourão 2004; Mandal et al. 2007; Li Bo 2008; Shilpi 2011) and anti-inflammatory (Maruyamaa et al. 2005; Li Bo 2008) activities, as well as the capacity to reduce the level of glucose in blood (Li Bo 2008). Based on these features, fucoidan has been used with success in different areas, such as food, cosmetics, pharmaceutical, and medicine (Kitamura et al. 1991) (see examples in Table 118.3). The exploration of fucoidan as a marine origin material has economic and environmental interest, representing a recovery strategy for producing marine-based biomaterials (Ponce et al. 2003). In this perspective, an increasing number of fucoidan-based profitable products have been developed for biomedical application, including controlled drug delivery devices and TE scaffolding (Silva et al. 2012).

118.3.1 Extraction of Fucoidan

Many methods have been investigated to produce high-quality fucoidan, such as using ethanol/water and acidic solutions

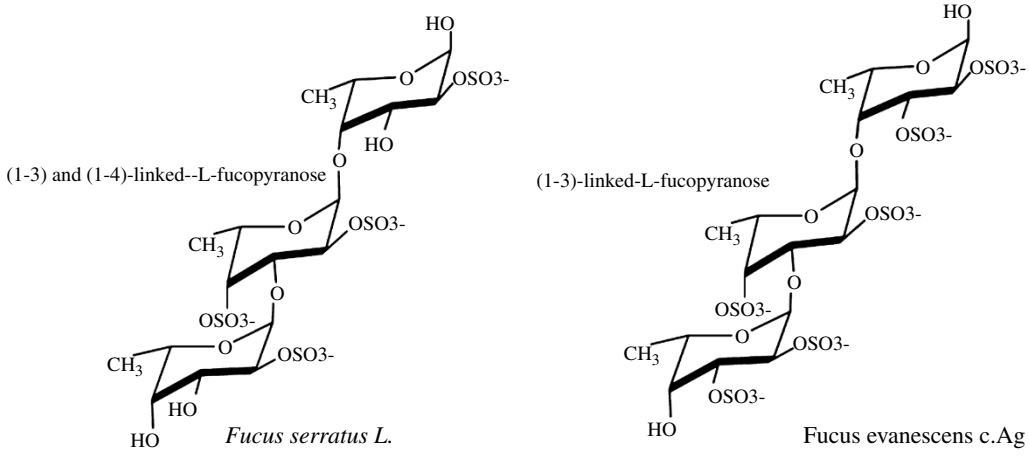

Figure 118.3 Scheme models of fucoidan chemical structure, with different sulfation degrees (higher in right structure, with two sulfate esters in each monomer). Adapted from Hahn et al. (2012).

Table 118.3 Fucoidan bioactive properties and possible biomedical applications.

Biological activity	Source	Applications	References
Antioxidant	*Laminaria japonica* *Sargassum tenerrinum* *Sargassum glaucescens*	Antioxidant activity prevention; helps in diseases that require free radicals	(Wang et al. 2010; Marudhupandi et al. 2014; Huang et al. 2016)
Antiviral	*Cystoseira indica* *Undaria pinnafida* *Sargassum mcclurei* *Sargassum polycystum* *Turbinara ornata*	Antiviral activity against different kinds of viruses (HIV, HSV, and human cytomegalovirus)	(Lee et al. 2004; Mandal et al. 2007; Thuy et al. 2015; Wozniak et al. 2015)
Anti-inflammatory	*Laminaria japonica* *Fucus vesiculosus*	Leukocytes inhibition	(Park et al. 2011; Kyung et al. 2012)
Anticoagulant	*Laminaria japonica* *Fucus vesiculosus*	Anticoagulant activity mediated by antithrombin and heparin	(Irhimeh et al. 2009; Wang et al. 2010; Zhang et al. 2014)
Antitumor	*Undaria pinnatifida* *Fucus vesiculosus* *Cladosiphon navae-caledoniae*	Inhibits cancer cells proliferation; promotes cancer cells apoptosis; enhances activity of chemotherapeutic agents	(Yang et al. 2013; Zhang et al. 2013; Chen et al. 2014; Hsu et al. 2014)
Antihyperglycemia	*Sargassum thumbergii* *Sargassum wighti* *Fucus vesiculosus*	Helps in the treatment of type II diabetes	(Kim et al. 2014; Jiang et al. 2015; Vinoth Kumar et al. 2015; Shan et al. 2016)

(D'Ayala et al. 2008; Mak 2012), assisted with ultrasound or microwave radiation or based in enzymatic processing (Pomin et al. 2005; Rodriguez-Jasso et al. 2011; Hahn et al. 2012; Ale and Meyer 2013; Balboa et al. 2013). In these methods, a set of parameters such as temperature, time of extraction, acid concentration, and pH must be controlled, so as to avoid degradation of the fucose chain but to successfully remove some insoluble components. Indeed, the experimental conditions used during extraction can influence the chemical composition, number of sulfates, chemical structure, and MW of the retrieved biopolymer. The extraction of fucoidan with acids, such as HCl, has a higher yield, but alginates are also extracted and chain degradation may occur (Ale et al. 2011). Thus, calcium solutions are used subsequently to promote the precipitation of alginates (Kitamura et al. 1991; Hahn; et al. 2012), to be separated by centrifugation (Foley et al. 2001). Fucoidan purification can be made using different methods, such as treatment with alcohol/formaldehyde and cetilpiridine chloride, followed by dialysis (Kitamura et a. 1991). Fucoidan extracts usually have a colour varying between yellow and brown (Sezer and Cevher 2011; Mak 2012). Figure 118.4 depicts a scheme with the process commonly used in production plants.

118.3.2 Fucoidan Properties

Fucoidan has interesting properties, namely antioxidant, antivirus, anti-inflammatory, anticoagulant, antitumor and antihyperglycemia (Silva et al. 2012). In Table 118.3, the biological activities, sources, and possible

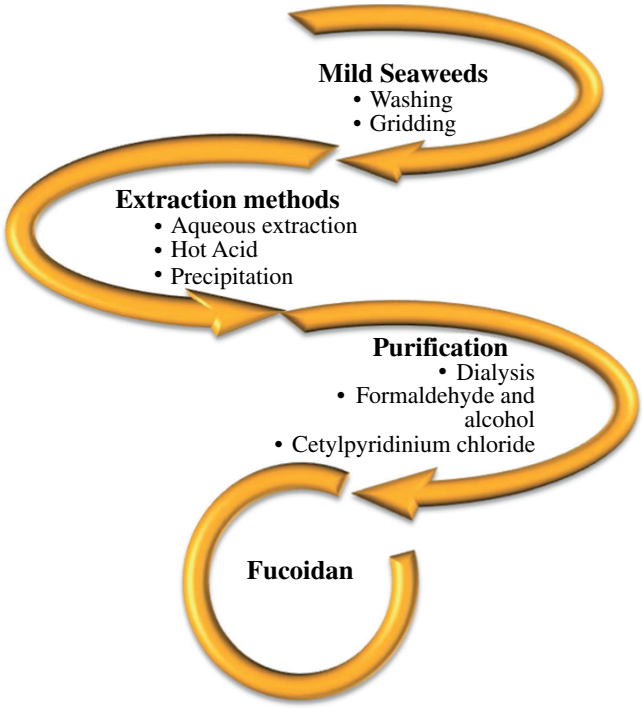

Figure 118.4 Procedure for production of fucoidan from seaweeds.

applications of fucoidan are summarized. Bioactive properties of fucoidan may be influenced by its MW, polymer branching, sugar content, sulfation degree, and sulfates distribution (Ale et al. 2011).

In recent studies, fucoidan has shown significant antioxidant activity in *in vitro* experiments. It has been demonstrated that fucoidan is an excellent natural antioxidant and has great potential for preventing free radical-mediated diseases. This antioxidant activity is often associated with fucoidans' MW and sulfate content (Wang et al. 2008; Marudhupandi et al. 2014). Another interesting feature is the antiviral activity that fucoidan (like other sulfated polysaccharides) exhibits. Its low cytotoxicity, when compared with other antiviral drugs currently used in a clinical setting, is of considerable interest (Li et al. 2008). It has been reported that different fucoidans obtained from different species showed inhibition of leucocyte recruitment in the inflammatory process, with fucose contents and sulfation degree seeming not to affect its efficacy regarding this property (Cumashi et al. 2007; Li et al. 2008). The anticoagulant effect of fucoidan has been also addressed, associated with a higher sulfation degree and sulfates position (Li et al. 2008; Jin et al. 2013). Antitumor and antihyperglycemia properties will be analyzed in more detail in the following sections.

118.3.2.1 Antitumor Properties

In vitro and *in vivo* studies indicate that fucoidan protects the organism against different types of cancer, namely leukemia, colon, breast, and lung (Ale et al. 2011; Boo et al. 2011; Boo et al. 2013; Moussavou et al. 2014). The mechanism of action of fucoidan in cancer therapies is not yet clearly understood. However, some studies reveal that fucoidan inhibits the growth of tumors, inducing cytotoxicity and apoptosis of cancer cells, and interrupts the cell cycle by targeting key apoptotic molecules (Lee et al. 2012; Senthilkumar et al. 2013). It also inhibits metastasis formation and enhances the toxic effects of other chemical therapies and compounds. It can also

affect cancer cells by controlling the angiogenic activity (Kwak 2014; Atashrazm et al. 2015).

Lower MW and higher sulfated fucoidans have been associated with higher toxicities over tumor cells (Koyanagi et al. 2003; Kasai et al. 2015); however, the results are not fully reproducible and different behaviors have been observed, showing that not all fucoidan extracts present this anti-tumor activity (Kwak 2014). The reasons behind this fact are not yet clarified but may be related to different factors such as the MW, the sulfation degree, the sugar compounds, polymer branching, and sulfation pattern (Ale et al. 2011; Oliveira et al. 2017), by their turn dependent on the source and the extraction method.

Keeping in mind all these properties, it is of the utmost importance to characterize the fucoidan extracts to try to reveal the mechanisms of action and understand which factors(s) play a major in role in this antitumor behavior.

When considering a cancer therapy based in fucoidan, there are some concerns about its toxicity to non-cancer cells. Indeed, the fundamental aspect of a cancer therapy is based on eliminating cancer cells without affecting the surrounding healthy tissues. For this reason, it is essential to take into consideration specific and targeting therapies to affect only the tumors, envisioning a more effective treatment.

118.3.2.2 Blood Glucose Reduction

Some *in vitro* and *in vivo* studies propose the application of fucoidan in reducing blood glucose (Kim et al. 2014; Vinoth Kumar et al. 2015). In fact, some studies show the antidiabetic potential of extracts of brown algae (Wang et al. 2014). Kim et al. (2012) have shown that the low MW fucoidan prevents hyperglycemia in diabetic rats, pointing out that the reduction of glucose is dependent on the MW of fucoidan (Kim et al. 2014). Moreover, it has been referred that the lowering of blood glucose is related to inhibition of enzymes α-amylase and α-glycosidase during the digestion of carbohydrates (Ali et al. 2006; Kim et al. 2014). In the process of digestion of a diet rich in carbohydrates, enzymes α-glycosidase and α-amylase play a vital role and the inhibition of these enzymes would reduce the digestion of oligosaccharides and disaccharides, leading to a delay in the production of glucose. The action of fucoidan on the cited enzymes, when fully understood, can play a major role in the treatment of diabetes mellitus type I and II.

118.3.3 Fucoidan-based Biomaterials and Biomedical Applications

The applications of fucoidan have been centered mainly in Japan, Australia, and the United States (Shibata et al. 2000), mostly relying on its biological activities. In this perspective, fucoidan is building a successful story in different industries, such as agriculture (fertilizers for plants), food (dietary fiber, cholesterol reduction, and sports drinks), cosmetics (skin exfoliate, acne treatment, hair moisturizing, and toothpaste) and biomedical (anticoagulant, antiviral, and immune control action) (Courtois 2009; Choi 2010). Additionally, its use with a support role may be also explored, particularly in the development of biomaterials for the biomedical area. However, fucoidan has high solubility in water, which could be a problem for the production of stable structures in aqueous media using fucoidan alone. Some studies have been reported involving fucoidan composites or mixtures with other natural or synthetic macromolecules, such as silk (Cheng 2009), chitosan (Sezer et al. 2007; Murakami et al. 2010), hydroxyapatite (Jeong et al. 2013), gelatin (Ko et al. 2012), poly(ε-caprolactone), PCL, (Gyuhyun Jin 2011; Ji Seok Lee et al. 2012; Venkatesan et al. 2014), polycarbonates and polyethylene terephthalate (PET) (Mandal et al. 2007). These systems could be processed as 2D and 3D structures such as hydrogels (Ali Demir Sezer 2008), microspheres (Sezer 2006), fibers (Ji Seok Lee 2012), polyelectrolytes complexes, and 3D porous structures (Gyuhyun Jin 2011).

These structures can be produced using standard processing technologies namely electrospinning technique (Ji Seok Lee et al. 2012), rapid prototyping (Gyuhyun Jin 2011), freeze-drying (Venkatesan et al. 2014), solvent evaporation (Sezer et al. 2007) and polyelectrolyte complexation (Nakamura, et al. 2008) (see examples in Figure 118.5).

118.3.3.1 Fucoidan Blends for Wound Healing

Fucoidan hydrogels can be made by swelling the materials in acidic solution to form a gel that can be applied to burn injuries healing on rabbits. Sezer et al. described that fucoidan/chitosan hydrogels applied on the burn wound induced significant wound contraction and healing (Sezer et al. 2008). In a similar study, Nakamura et al. produced fucoidan/chitosan hydrogels as a carrier for controlled release of heparin-binding fibroblast growth factor (FGF)-2 (Nakamura et al. 2008). The *in vivo* studies demonstrated that chitosan/fucoidan hydrogel could promote neovascularization by FGF-2 release.

In other studies, membranes and hydrogels based on fucoidan or blended systems have been proposed as a wound dressing or as artificial skin (Bhatnagar and Bhatnagar 2015). Sezer et al. described that coating of chitosan films with fucoidan induces significant wound contractions, and accelerates the migration of fibroblasts, wound closure, and healing process (Sezer et al. 2007). After seven days of treatment, fibroplasia and scars were observed on wounds treated with the fucoidan-chitosan film. The films healed the wound within 14 days, while the control groups took longer healing time (Sezer et al. 2007). Yanagibayashi et al. (Yanagibayashi et al. 2012) developed a hydrocolloid sheet composed of alginate, chitin/chitosan, and fucoidan (ACF-HS) to treat healing impaired wounds, such as diabetic wounds. The results demonstrated that ACF-HS could effectively protect a healing-impaired wound in diabetic db/db mice, providing a good moist healing environment with exudate. Additionally, ACF-HS absorbed serum and FGF-2 were found to be proliferative for fibroblasts and endothelial cells, respectively, and ACF-HS-absorbed serum appeared chemoattractive for fibroblasts. The authors concluded that ACF-HS wound dressing showed properties

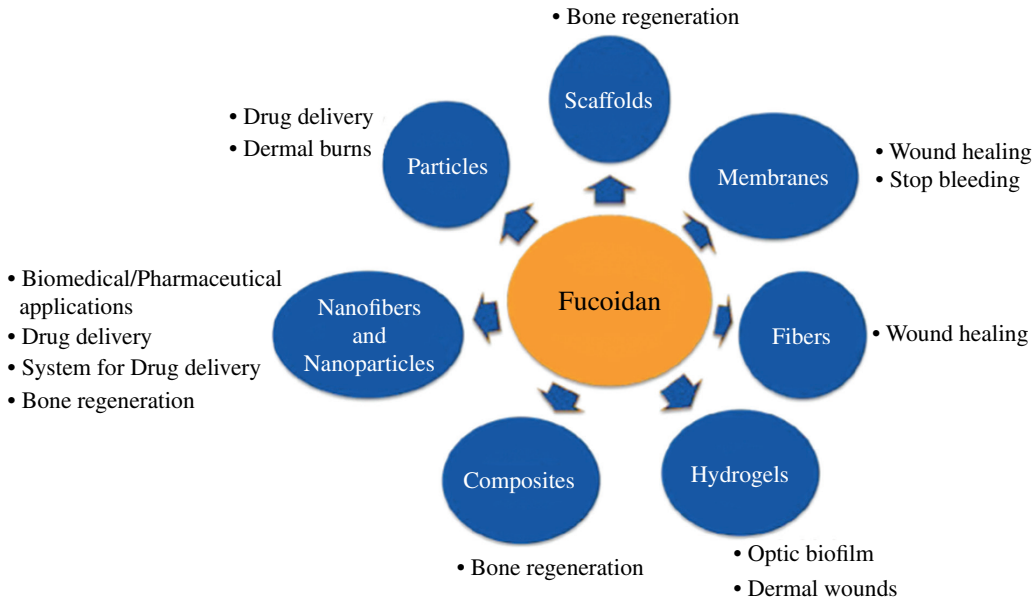

Figure 118.5 Biomedical applications of fucoidan.

such as ease of application, removal, and excellent adherence that could make it a useful application for treatment of diabetes-related wounds (Renn 1984; Yanagibayashi et al. 2012).

118.3.3.2 Fucoidan Composites for Bone Tissue Engineering

Some authors outlined the use of fucoidan-based composites and fibers meshes for bone regeneration (Jeong et al. 2013). This approach consisted of the development of fucoidan structures by different techniques, combining it with other materials, namely PCL and nano-hydroxyapatite, which will promote bone regeneration. Jeong et al. observed two times higher mineralization in fucoidan/nano-hydroxyapatite scaffolds than in nano-hydroxyapatite scaffolds, suggesting that the former matrices could be promising biomaterials for bone tissue engineering (Jeong et al. 2013). Jin et al. developed structures of PCL/fucoidan using rapid prototyping technique (Gyuhyun Jin 2011), with mechanical properties and mineralization promotion superior to the ones composed by pure PCL (Gyuhyun Jin 2011; Venkatesan and Kim 2015). A recent approach to treat bone diseases involves the effect of fucoidan on human mesenchymal stem cells, observing that the fucoidan improved the stem cell behavior regarding osteogenic differentiation, namely by registering an increase in the expression of ALP, osteopontin, type I collagen, RUNX-2, and osteocalcin (Park et al. 2012; Venkatesan and Kim 2015).

118.3.3.3 Nanoparticles for Controlled Delivery of Bioactive Agents

Over the past years, nanoparticles have been widely studied for the delivery of drugs and macromolecules (Liu et al. 2008). Nanoparticles have interesting properties such as the small size that allow them to pass through the capillary vessels. They can also pass cells and tissue gaps to arrive at specific organs, showing controlled-release behavior (Singh et al. 2014). The surface properties of the nanoparticles are also noteworthy, since it is possible to functionalize the nanoparticles to target and be recognized by specific cells (Steichen et al. 2013; Loureiro et al. 2014), envisaging more efficient systems with reduced side effects.

Polymeric materials are often used to prepare nanoparticles, with polysaccharides being among the most promising building blocks with a cost-effective processing (Silva et al. 2012; Cardoso et al. 2016; Manivasagan and Oh 2016). Moreover, the high number of reactive groups found in polysaccharides, namely carboxyl and amino groups, facilitate its modification and functionalization (Liu et al. 2008). Polysaccharide-based nanoparticles are often prepared by polyelectrolyte complexation, self-assembling and ionic or covalent cross-linking (Santo et al. 2012). Polyelectrolyte complexation takes advantage of the electrostatic interactions between two opposite charged polymers (Hamman 2010; Ramasamy et al. 2014), with chitosan being often used as polycation, combined with negatively charged polymers, such as fucoidan, chondroitin sulfate, carrageenan, hyaluronic acid, and alginate, among others. To optimize the production of the nanoparticles, different conditions need to be tested, namely polymer concentrations and ratio, pH, stirring time, and also the solution ionic strength (Jonassen et al. 2012; Jain et al. 2013), which affects particles average size and distribution, as well as surface charge (Couvreur 2013).

Chitosan-fucoidan nanoparticles (NPs) have been reported as potential carriers for different applications. The release of gentamicin from chitosan-fucoidan NPs has been studied for pulmonary delivery in pneumonia treatment (Huang et al. 2016). Another possible application is the successful release of basic FGF for nerve tissue regeneration (Huang and Yang 2013). Chitosan-fucoidan NPs were also reported as effective in delivering antibiotics to the lungs, showing the antioxidant activity of the prepared nanoparticles (Huang and Li 2014). Poly-L-Lysine was also released from chitosan-fucoidan nanocapsules, making this

a promising delivery system for water-soluble bioactive compounds, showing a great potential for application in the pharmaceutical industries (Pinheiro et al. 2015). Moreover, Huang and Li reported that fucoidan-chitosan NPs had a highly antioxidant effect by reducing the concentration of reactive oxygen species (Huang and Li 2014). In another study, pH-sensitive chitosan-fucoidan NPs were produced to protect curcumin, an antitumor drug, from deterioration (Huang and Lam 2011), envisaging their use in oral delivery since they can resist the low pH of the gastric medium in the stomach, which is one of the major barriers to oral delivery approaches (Huang and Lam 2011; Jeong et al. 2013).

118.3.3.4 Photocrosslinking for Processing of Hydrogels for Cell Culture

The lack of processability of fucoidan is associated with its water solubility, as already mentioned, from which results an absence of studies using pure fucoidan matrices. A few works reported the modification of polymers by methacrylation to obtain structures further suggested for application in the biomedical area (Baier Leach et al. 2003; Li et al. 2004; Amsden et al. 2007; Mihaila et al. 2013). The methacrylation of natural polymers with anhydride methacrylate (MA) is a strategy to incorporate methacrylic groups that can be further photocrosslinked using photoinitiator solutions, rendering cohesive polymeric matrices. In our group, this strategy was successfully used for processing several natural polymers, namely gellan gum (Silva-Correia et al. 2011) and κ-carrageenan (Mihaila et al. 2013). More recently, methacrylation was also used to functionalize fucoidan, followed by photocrosslinking with visible light to obtain hydrogel-like beads (Reys et al. 2016) (see Figure 118.6 for a simplified production scheme). In particular, methacrylated fucoidan solution was combined with photoinitiators and added dropwise onto superhydrophobic surfaces (Rial-Hermida 2014) that enable the droplets to sustain their quasi-spherical geometry. By irradiation of visible light, crosslinking is promoted, yielding particles or beads with diameters ranging from 0.6 to 1.3 mm. These hydrogel-like particles may find application as drug delivery devices or polymeric matrices for cell culture and/or encapsulation. In this regard, L929 mouse fibroblast-like cells were cultured onto those particles, revealing good adhesion and proliferation. Remarkably, cells

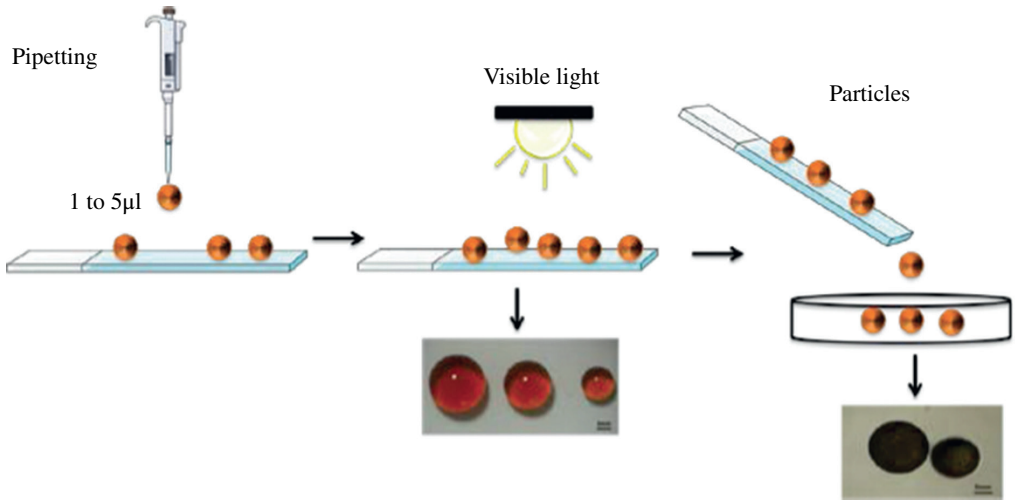

Figure 118.6 Scheme of the methodology to produce fucoidan-based particles by photocrosslinking triggered by using visible light.

were also able to migrate into the particle core (see Figure 118.7), illustrating the capability of these systems to support cell culture regarding further biomedical application.

118.4 Final Remarks

Chitosan and fucoidan have been addressed in particular during the present chapter as illustrative examples of marine polysaccharides mainly with a support role. Both share similarities with GAGs found in the extracellular matrix: while chitosan has N-acetylglycosamine units in common with hyaluronic acid, fucoidan exhibits sulfated sugars and the presence of uronic acids alike chondroitin sulfate and others. Since GAGs are particularly abundant in cartilage extracellular matrix, a biomimetic strategy would propose the use of the referred marine polysaccharides on TE scaffolding.

The chemical composition of chitosan and fucoidan is not only similar to the one observed in specific GAGs, but it is also responsible for interesting properties exhibited by the former. Due to the presence of free amines in the non-acetylated glycosamine units, chitosan is the only natural polycation known and the protonation of amines in acidic pH make it soluble in diluted acid solutions. On the other hand, the presence of sulfated fucose and a highly negative charge density furnish fucoidan with several biological properties of great interest of healthcare.

Both polysaccharides have been explored in the development of particles, membranes, hydrogels, and porous structures as matrices to support cell culture envisaging tissue regeneration, namely on wound healing and bone therapies, among others. Given its abundance, chitosan has been widely used, but fucoidan is receiving increasing attention because it can associate biological activity to the support role, resulting in functional biomaterials equipped with antioxidant, antiviral, anti-inflammatory, and anticoagulant features. Moreover, their potential role in cancer therapies should be also highlighted, being as selective drug (fucoidan) or as component of drug delivery devices.

The share of marine polysaccharides on biomedical science was undoubtedly demonstrated, with several innovative therapeutic solutions being currently proposed and studied. Nevertheless, their ubiquitous use in clinical practice is still farfetched. Firstly, the interaction between scientists, engineers, and clinicians needs to be reinforced and established as common practice toward tissue

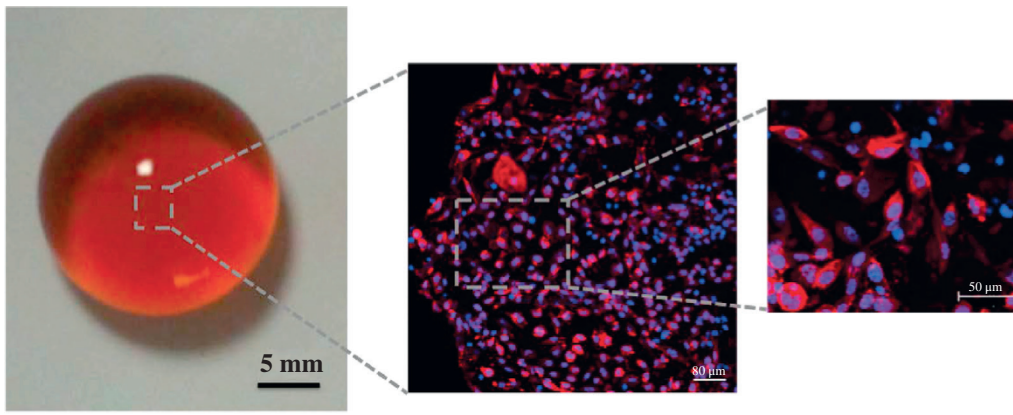

Figure 118.7 Photocrosslinked methacrylated fucoidan beads laden with L929 fibroblast-like cells, observed under fluorescence confocal microscope after 7 days of culture (cross-section is depicted, illustrating the presence of cells in the particles core).

regeneration, in a synergy capable to develop precise and personalized therapies, thus improving patients' quality of life. This will come with the success of clinical trials currently ongoing using different materials, such as hydroxyapatite, collagen, polycaprolactone, among others, which will set the basis for the use of other materials. On the other side, there is still a need to establish production methodologies capable of rendering biopolymers with high purity and reproducible properties in an environmentally, economically, and technically sustainable way. The acceptance ranges for biomedical areas are much narrower than for others and thus current protocols still need to be optimized and validated. Moreover, reproducibility and sustainability need to be also verified in polymer processing rendering biomedical-relevant matrices. Only with this, associated with adequate industrial property rights, will it be possible to raise the investment needed to entrepreneurial initiatives based in marine origin materials be successful. This is a long road but some actors are around, with products in the market in a business-to-business model. The momentum is there and we are contributing to them, believing that industrial efforts to overcome the identified production bottlenecks will allow the achievement of a production scale supporting affordable costs, associated with the scientific efforts to demonstrate the preclinical and clinical efficacy of marine origin polysaccharides based biomaterials, and bring the future closer.

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