

# Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches

Tiago H. Silva,<sup>1,2</sup> Anabela Alves,<sup>1,2</sup> Elena G. Popa,<sup>1,2</sup> Lara L. Reys,<sup>1,2</sup> Manuela E. Gomes,<sup>1,2</sup> Rui A. Sousa,<sup>1,2</sup> Simone S. Silva,<sup>1,2</sup> João F. Mano<sup>1,2</sup> and Rui L. Reis<sup>1,2</sup>

<sup>1</sup>3B's Research Group, Biomaterials, Biodegradables and Biomimetics; University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine; Guimarães, Portugal; <sup>2</sup>ICVS/3B's; PT Government Associate Laboratory; Braga/Guimarães, Portugal

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Biomedical field is constantly requesting for new biomaterials, with innovative properties. Natural polymers appear as materials of election for this goal due to their biocompatibility and biodegradability. In particular, materials found in marine environment are of great interest since the chemical and biological diversity found in this environment is almost uncountable and continuously growing with the research in deeper waters. Moreover, there is also a slower risk of these materials to pose illnesses to humans.

In particular, sulfated polysaccharides can be found in marine environment, in different algae species. These polysaccharides don't have equivalent in the terrestrial plants and resembles the chemical and biological properties of mammalian glycosaminoglycans. In this perspective, are receiving growing interest for application on health-related fields. On this review, we will focus on the biomedical applications of marine algae sulfated polymers, in particular on the development of innovative systems for tissue engineering and drug delivery approaches.

## Introduction

As a response to trauma or tissue disease, the human body tries to remodel the injured tissue, but for many situations, these efforts result in dysfunctionality and then tissue failure.<sup>1</sup>

To address this serious health problem, scientists have dedicated great attention aiming at developing alternative therapeutic solutions, to overcome the drawbacks of the current clinical practices (prosthesis, autografts, allografts, xenografts, with insufficient properties, site morbidity, donor scarcity and risk of immune rejection as main drawbacks). In this regard, regenerative medicine arose as a hot medical topic, with Langer and Vacanti defining tissue engineering, in their seminal paper, as a multidisciplinary approach that uses principles of engineering and life sciences to create a new tissue.<sup>2</sup> Following the general strategy, a porous structure is developed in which cells are seeded/

cultured, under certain conditions, with defined biochemical and mechanical cues, until obtaining a functional tissue substitute to be subsequently implanted in the patient injury site.

Such porous structures—called scaffolds—are requested to exhibit certain properties, such as biocompatibility both in as-implanted form and after degradation, which should occur in controlled manner, present appropriate mechanical properties to meet the needs of the tissue to be regenerated, adequate surface properties to enhance interaction with cells and optimal three-dimensional structure (porosity, pore size and interconnectivity). Thus, different processing technologies have been proposed to prepare such structures,<sup>3,4</sup> for instance foaming,<sup>5,6</sup> freeze-drying,<sup>7-9</sup> fiber extrusion and bonding,<sup>10,11</sup> three-dimensional printing,<sup>12,13</sup> porogen leaching,<sup>14-16</sup> in situ pore forming,<sup>17</sup> particle aggregation,<sup>18-20</sup> electrospinning,<sup>21,22</sup> supercritical fluids technology<sup>23,24</sup> and use of ionic liquids.<sup>25</sup>

Together with several techniques, a wide range of materials have been also proposed, including natural and synthetic polymers or a conjugation of both. More recently, considering their predominant existence in the extracellular matrix, but also because of their low immunogenicity and enhanced interaction with growth factors, attention has been devoted to glycosaminoglycans, namely hyaluronic acid and chondroitin sulfate.<sup>26,27</sup> Despite the incomplete understanding of the interactions between cells and extracellular matrix, namely at molecular level, it is known that glycosaminoglycans modulate the adhesion of progenitor cells and their subsequent differentiation and gene expression. In particular, degree of sulfation, molecular weight and structural composition influence cell behavior, with changes being observed in different physiological but also pathological processes.<sup>27</sup>

In fact, degree of sulfation seems to play such a relevant role that recent efforts have been focusing on the preparation and further use of highly sulfated glycosaminoglycans derivatives, in particular by introducing sulfate features in hyaluronic acid or increasing sulfate degree of chondroitin sulfate. Interesting findings have been observed, as the enhanced binding of growth factors to sulfate hyaluronic acid when comparing with chondroitin sulfate with the same sulfation degree, which has been attributed to differences in the sulfation pattern.<sup>28</sup> This is in agreement with the findings of Gama and coworkers, where the precise position of sulfate groups resulted in specific ligand-

\*Correspondence to: Tiago H. Silva; Email: tiago.silva@dep.uminho.pt  
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receptor interactions, according to a kind of sulfation code.<sup>29</sup> This specificity results in different cellular behavior: sulfate hyaluronic acid was observed to promote proliferation of dermal fibroblasts,<sup>26</sup> but on the case of rat calvarial osteoblasts such proliferation was hindered.<sup>30</sup> It is noteworthy that the mentioned improved proliferation of fibroblasts was followed by a reduced extracellular matrix expression, which might be quite interesting for skin regeneration, namely by promoting wound closure while hindering scar formation.<sup>26</sup> Besides effects on cell adhesion and proliferation, sulfate groups were shown to also influence cell differentiation: matrices of collagen II and sulfate hyaluronic acid stimulate osteogenic differentiation of human mesenchymal stem cells.<sup>31</sup> In order to shed more light on the mechanism of influence of sulfate groups on cell behavior, the specific effect of sulfate groups, disconnected from the glycosaminoglycan environment, was also assessed. Sulfate-rich surfaces were prepared with self-assembled monolayers of  $\omega$ -sulfatealkanethiols, which were shown to influence cell morphology and mobility with enhanced formation of filopodia in both bone marrow derived mesenchymal stem cells and adipose derived stem cells.<sup>32</sup>

Having in mind this huge potential of sulfate groups, research communities have also been looking at other ways to achieve glycosaminoglycans and sulfated polysaccharides besides chemical modification. In fact, such polysaccharides can be found in marine organisms, in particular macroalgae.<sup>33</sup> Besides being an under-exploited resource, marine environments also possess an enormous chemical and biological variety, thus being an extraordinary source of biomaterials.<sup>34</sup> On the particular case of sulfated polysaccharides bearing glycosaminoglycan-like biological properties, different structures can be found in marine macroalgae, and its marine origin promises potentially safer polymers when compared with mammalian alternatives,<sup>33</sup> since their associated risk of posing diseases to humans is not an issue. In

the present review, focus will be made on the most representative sulfate polysaccharides that can be obtained from each of the three main classes of macroalgae, mainly carrageenans, ulvan and fucoidan from red, green and brown algae, respectively, and the attempts to use them further, particularly in biomedical applications, including tissue engineering approaches.

## Sulfated Polysaccharides from Red Algae

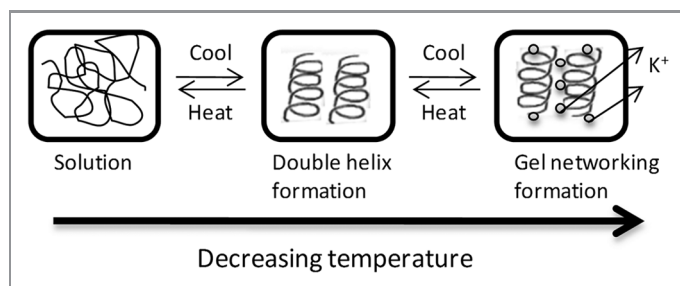
Carrageenans are sulfated polysaccharides that occur as matrix material in several species of red seaweeds (Rhodophyceae), with main sources being *Chondrus crispus*, *Gigartina*, *Euचेuma cottonii* and *spinosum*, and that can be extracted with water or aqueous alkali methods.<sup>35-37</sup> Chemically, these hydrophilic colloids are highly sulfated galactans, with sulfate content varying between 15% and 40%,<sup>38</sup> with a primary structure based on an alternating sequence of  $\beta$  (1-4) and  $\alpha$  (1-3) linked D-galactose residues,<sup>39</sup> resulting in polymers with molecular weight ranging from  $10^5$  to  $10^6$  Da.<sup>40,41</sup> The number and position of sulfate groups in the repeating galactose units allows the classification of carrageenans in three main commercially relevant families: kappa ( $\kappa$ ), iota ( $\iota$ ) and lambda ( $\lambda$ ). Table 1 summarizes the main characteristic features of each of these carrageenan families, as well as a representative scheme of the repeating unit structure.

It should be stressed that carrageenans can be quite heterogeneous, either due to differing molecular structures within the chains, to differing chains within the seaweed (hybrids) or to algae species, ecophysiology and seasonality or even extraction conditions.<sup>42,43</sup> Thus a wide variety of materials can be obtained from them, including hydrogels with a vast range of properties.<sup>44</sup>

In fact, the gel formation is one of the most relevant properties in carrageenans, even though not possible with  $\lambda$ -carrageenan (being although used as thickening agent to control viscosity).

**Table 1.** Structural formula and typical properties of  $\kappa$ -,  $\iota$ -, and  $\lambda$ - carrageenans<sup>196,197</sup>

Idealized chemical structure	Kappa carrageenan	Iota carrageenan	Lambda carrageenan
	one sulfate group	two sulfate groups	three sulfate groups
<b>CHARACTERISTIC FEATURES</b>			
Ionic gel formation	Gels with potassium salts	Gels with calcium salts	No gel formation
Gel texture	Brittle with some syneresis	Elastic with no syneresis	No syneresis
Freeze/thaw stability	No	Freeze-thaw stable	Freeze-thaw stable
Viscosity	Low thixotropic	High, Medium thixotropic	High, Medium thixotropic; Forms highly viscous solutions
Solubility in water	Completely soluble in hot water, partially in cold water	Completely soluble in hot water	Completely soluble in hot water, partially in cold water
Synergism with other gums	Synergistic with locust bean gum	No	No
Acid stability	> pH 3.8, neutral and alkaline pH	> pH 3.8, neutral and alkaline pH	-



**Figure 1.** Gelation model of  $\kappa$ -carrageenan (adapted from refs<sup>194,195</sup>): by decreasing temperature of carrageenan solution, a coil-to-helix conformational transition is enhanced; with further decrease in temperature, in the presence of cations such as potassium, an organization and aggregation of helices is promoted, forming a gel network.

The gelling mechanism is not known in detail, but its high hydration capacity, the structural type, temperature, polymer concentration and the presence of cations are key factors.<sup>45-47</sup>  $\kappa$ -Carrageenan hydrogels are thermoreversible<sup>48</sup> and can be formed by ionic gelation through interaction with cations, particularly with potassium ( $K^+$ ), which inhibits the electrostatic repulsion between the neighboring negatively charged helices, allowing their aggregation (Fig. 1).<sup>49,50</sup> By their turn,  $\iota$ -carrageenan hydrogels exhibit the interesting feature of spontaneously reforming once the mechanical disruption action has stopped,<sup>51</sup> called thixotropy, very useful in certain applications, such as cosmetic emulsions.

The versatility of carrageenans is also patent on its several processing and formulation routes. Besides hydrogels, carrageenans can also be processed into fibers by wet spinning, into membranes by casting and further crosslinking or into porous structures by freeze drying.<sup>52</sup> In addition, the combination of two different families of carrageenans has also been tested, for instance on the production of microscale fibers.<sup>53</sup> Hydrogel systems based on carrageenan and other materials from natural algae origin, namely alginate, have been developed into different formats (beads/fibers);<sup>52,54,55</sup> fibers resulting from wet-spinning carrageenan into chitosan or vice versa are also possible, including together with carbon nanotubes to improve significantly their mechanical properties, in which active ingredients can be trapped;<sup>56</sup> chitosan/carrageenan/tripolyphosphate nanoparticles exhibiting small size and high positive charge have been produced by polyelectrolyte complexation/ionic gelation;<sup>57</sup>  $Fe_3O_4$  nanoparticles with  $\kappa$ -carrageenan<sup>58,59</sup> and spheres of carrageenans crosslinked by paramagnetic ions ( $Ho^{3+}$ )<sup>60</sup> have also been developed.

The chemical reactivity of carrageenans, mainly due to the sulfate groups, as well as the diversity of carrageenan structures just described, can justify the application of carrageenans in numerous applications.<sup>61,62</sup> For example, their reactivity with proteins, due to interactions between the sulfate groups of the carrageenan and the charged groups of the protein, is present in the interaction with casein in milk, extremely important in the dairy industry;<sup>63,64</sup> their ability to bind heavy metals through covalent, electrostatic or redox reaction is useful for removing them from contaminated waters, with clear environmental impact;<sup>65,66</sup> their unique properties of interaction with polyols

can be useful to control the texture of any formulation of polyols.<sup>67</sup> Nevertheless, their major industrial applications is as thickening, emulsifier, gelling and stabilizing agents, such as for example, in salad dressings, processed meat,<sup>68,69</sup> abovementioned dairy industry,<sup>70-73</sup> and personal care products,<sup>74,75</sup> but also in pharmaceuticals formulations,<sup>76,77</sup> being considered a good substitute for gelatin.

In addition to the mentioned applications, the use of carrageenans in the biomedical field is also being explored, mainly taking advantage of their biological activities mostly related to the sulfate content.<sup>78,79</sup> In this sense, the antioxidant activity of  $\kappa$ -carrageenan has been investigated,<sup>80</sup> as well as the protective activity against viral, fungal and bacterial infections.<sup>81</sup> Based on these known biological activities, carrageenans have been tested as treatments for respiratory weakness, from the common cold until influenza viruses, including the pandemic H1N1 influenza strain,<sup>82</sup> but also against other viruses, such as dengue virus, hepatitis A virus and African swine fever virus,<sup>83,84</sup> or even as topical microbicide targeting HIV and herpes viruses.<sup>85,86</sup> In other works, the in vivo antitumor and immunomodulation activities of carrageenan has been studied, with low molecular weight molecules showing the highest activities.<sup>87</sup> Furthermore, carrageenan has recently been used in a clinical trial to significantly reduce serum cholesterol and triglyceride levels,<sup>88</sup> presenting anticoagulant properties,<sup>89,90</sup> and also a regulatory role, namely on growth factors.<sup>91,92</sup> Nevertheless, carrageenan biocompatibility has been questioned in the literature, with examples of inflammatory response being presented.<sup>93,94</sup> However, it should be noted that food-grade carrageenan and degraded carrageenan (low molecular weight) have completely different toxicological properties,<sup>95,96</sup> and not all the studies of acute toxicity have sufficient details on dose, type of carrageenan, seaweed source or extraction procedures used.<sup>97</sup> Thus, carrageenan biocompatibility is an open debate.

From all these facts, the use of carrageenans in diverse medical applications has been considered, and the tissue engineering field is no exception, with a few works reporting its use in the recent years and more are expected to come.<sup>98</sup> In this perspective, carrageenan has been considered for growth factor/drug delivery systems,<sup>99,100</sup> and for immobilization of enzymes,<sup>101</sup> but also in encapsulation of several cell types for their in vivo delivery,<sup>102,103</sup> envisioning cartilage regeneration. In fact, the structural resemblance of these naturally occurring sulfated polymers to the cartilage extracellular matrix components, glycosaminoglycans (GAGs), may confer biochemical reactivity that urge to determine. Addressing this, hydrogel based on  $\kappa$ -carrageenan was studied for the encapsulation of human-adipose-derived stem cells, human nasal chondrocytes, or a chondrocytic cell line, proving to be a good support for cell culture, viability, cartilage matrix extracellular formation and chondrogenic differentiation.<sup>100,103</sup>

### Sulfated Polysaccharides from Green Algae

As in other macroalgae classes, sulfated polysaccharides can be also found in green algae, but with more complex and diverse

chemistries.<sup>104,105</sup> In fact, different genus or species may synthesize distinct sulfated polysaccharides with distinct sugar composition.<sup>104,106</sup> In this regard, Percival propose a division of these green algae in diverse groups according to the synthesized sulfated polysaccharide, namely (sulfated) glucuronoxylorhamnans, glucuronoxylorhamnolactans and xyloarabinogalactans.<sup>104</sup> However, an accurate division is much more complex: literature is rich in research works reporting sulfated polysaccharides extracted from different green algae (as exemplified in Table 2). For instance, *Codium fragile* is known to possess a sulfated heteropolysaccharide mainly composed of arabinose and galactan moieties.<sup>104,107,108</sup> On the other hand, a pyruvylated galactan sulfate was obtained from *Codium jezoense*<sup>109</sup> and *Codium fragile*,<sup>110,111</sup> whereas a sulfated mannan was extracted from *Codium vermilara*<sup>112</sup> but sulfated arabinan and sulfated arabinogalactan were the ones identified in *Codium dwarkense* Boergs extracts.<sup>113</sup> Within other genus, such variability can also be found.<sup>110,114-117</sup> A genus of green algae receiving particular attention for the extraction of sulfated polysaccharide is *Ulva*, from which ulvan can be obtained, a polysaccharide mostly composed of rhamnose, uronic acid and xylose.<sup>118-125</sup>

This variety of chemistries that can be found in sulfated polysaccharides from green algae is substantiated by the numerous oligosaccharide moieties identified as constituting the basic structural units of these complex polysaccharides. In an interesting review paper,<sup>106</sup> Stengel and coworkers highlight this variability and its relevance and impact in prospect applicative science based on algal origin molecules.<sup>106</sup> In fact, such variability, attributed to taxonomic, ecological or environmental issues,<sup>106,126-128</sup> should always be regarded. Despite this chemical variability, certain biological effects are common. In fact, sulfated polysaccharides are commonly investigated for their biological properties, and the

ones obtained from green algae are no exception. A summary of reported activities demonstrated in these polysaccharides is presented in Table 3.

For instance, these polysaccharides exhibit antioxidant effects, as was recently reported in several research works, describing sulfated polysaccharides with superoxide and hydroxyl radicals scavenging activity, reducing power and able to chelate metals.<sup>129-135</sup> Antitumoral activity and antiproliferative effects have also been described and associated with these polysaccharides.<sup>129,131,136</sup> Another important features of these polysaccharides are their immunostimulating ability, similar to other algal polysaccharides,<sup>137-141</sup> as well as their heparin-like character.<sup>105</sup> Besides, these polysaccharides are largely studied for their antihyperlipidemic activities,<sup>130,142-145</sup> or antiviral effects.<sup>111,131,146-148</sup>

Although common to the several sulfated polysaccharides extracted from green algae, the expression of those biological activities is dependent on different sugar composition, molecular weight and sulfate content,<sup>149</sup> and thus, as abovementioned, on genus, species and ecological and environmental factors. Several studies stress this variability regarding heparin-like behavior according to the genus and species of the studied algae,<sup>115-117,129,131,150-152</sup> but similar variability can be found on anticoagulant<sup>150-152</sup> and antioxidant activities,<sup>133-135</sup> as well as on antiproliferative effect, which was shown to be strongly related with the polysaccharide sulfate content.<sup>129</sup>

Within this scenario, an attractive use and exploitation of green algae would take advantage of these biological properties and translate them into applications with pharmacological and medical relevance. However, among the three main divisions of macroalgae, green algae remain a rather underexploited biomass, particularly in areas where other algal origin polysaccharides have already proven their value. A striking example of commercial success is carrageenan (as discussed in the previous section).

Alongside its biological activity and potential pharmaceutical use, green algae sulfated polysaccharides may also be used for biomedical applications, in areas as demanding as regenerative medicine. In this particular arena, both their biological activities and their resemblance with glycosaminoglycans might position these polysaccharides in an advantageous point. In this regard, some important research work has already been performed related with polysaccharide modification, processing and biomaterial development, particularly using ulvan as a starting material. Described ulvan structures include nanofibers,<sup>153</sup> membranes,<sup>154</sup> particles,<sup>155</sup> hydrogels<sup>156</sup> and 3D porous structures.<sup>157</sup> The applicability of these structures may range from drug delivery to wound dressing or bone tissue engineering.<sup>153-157</sup>

### Sulfated Polysaccharides from Brown Algae

Brown macroalgae are rich in polysaccharides such as alginic acids (alginate) or laminarins (laminarans), but also sulfated fucans, namely fucoidan, which are potential therapeutic agents.<sup>158</sup> Fucoidan is found in the cells walls of these algae, representing 5% to 20% of the algae dry weight.<sup>159</sup> Its history starts in 1913, when it was named fucoidin by Kylin.<sup>160</sup> Later on, McNeely

**Table 2.** Examples of sulfated polysaccharides extracted and identified in green algae

Alga	Polysaccharide(s)	References
<i>Bryopsis plumosa</i>	Rhamnan sulfate	198
<i>Chaetomorpha aerea</i>	Sulfated galactan	147
<i>Codium dwarkense</i>	Sulfated arabinan Sulfated arabinogalactan	113
<i>Codium fragile</i>	Sulfated arabinogalactans Pyruvylated galactan sulfate	104,107,108
<i>Codium vermilara</i>	Sulfated mannan	112
<i>Codium jezoense</i>	Pyruvylated galactan sulfate	109,111,140
<i>Monostroma latissimum</i>	Rhamnan sulfate	115-117
<i>Monostroma nitidum</i>	Rhamnan sulfate	110,114
<i>Ulva lactuca</i>	Sulfated rhaman	118,119
<i>Ulva rigida</i>	Sulfated rhaman	123,124
<i>Ulva rotundata</i>	Sulfated rhaman	120,121
<i>Enteromorpha compressa</i>	Sulfated rhaman	122,125



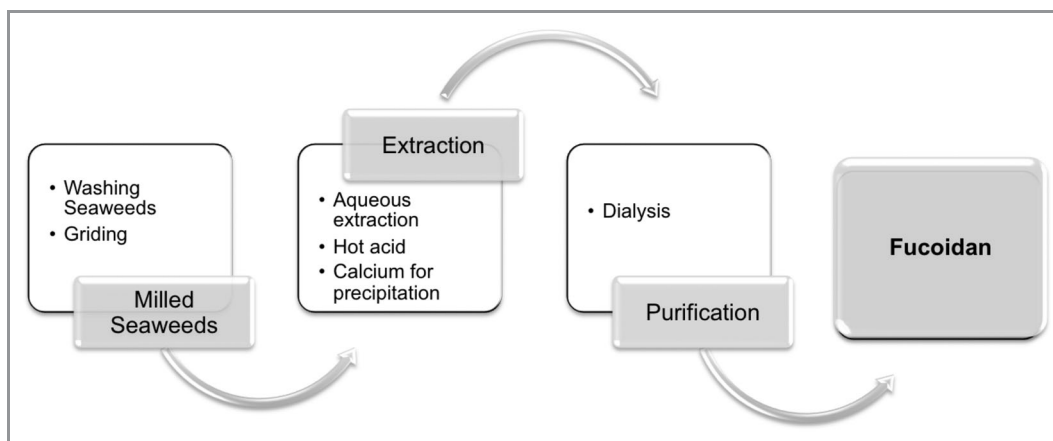
**Table 3.** Biological effects associated with sulfated polysaccharides from green algae

Activity	Alga	References
Antioxidant	<i>Caulerpa cupressoides</i> <i>C. prolifera</i> <i>C. sertularioides</i> <i>Chaetomorpha moniligera</i> <i>Codium fragile</i> <i>C. isthmocladum</i> <i>Enteromorpha intestinalis</i> <i>Ulva pertusa</i> <i>U. lactuca</i>	129-135
Antitumoral and antiproliferative activities	<i>Caulerpa cupressoides</i> <i>C. prolifera</i> <i>C. sertularioides</i> <i>Codium isthmocladum</i> <i>Enteromorpha intestinalis</i> <i>Ulva lactuca</i>	129,131,136
Immunostimulating	<i>Codium tomentosum</i> <i>C. fragile</i> <i>Enteromorpha</i> sp <i>Ulva rigida</i>	137-141
Anticoagulant	<i>Bryopsis plumosa</i> <i>Boodlea composite</i> <i>Caulerpa cupressoides</i> <i>C. prolifera</i> <i>C. sertularioides</i> <i>C. okamurai</i> <i>C. brachypus</i> <i>C. racemosa</i> <i>C. taxifolia</i> <i>C. scalpelliformis</i> <i>C. veravalensis</i> <i>C. peltata</i> <i>Chaetomorpha media</i> <i>C. torta</i> <i>Cladophora fascicularis</i> <i>Codium isthmocladum</i> <i>C. divaricatum</i> <i>C. adhaerence</i> <i>C. latum</i> <i>C. fragile</i> <i>Enteromorpha clathrata</i> <i>E. compressa</i> <i>Monostroma latissimum</i> <i>M. nitidum</i> <i>Ulva lactuca</i> <i>U. fasciata</i> <i>U. reticulata</i> <i>Valoniopsis pachynema</i>	115-117,129,131,150-152
Antihyperlipidemic	<i>Ulva pertusa</i> <i>U. lactuca</i>	130,143-145
Antiviral	<i>Codium fragile</i> <i>Gayralia oxysperma</i> <i>Monostroma nitidum</i> <i>Ulva</i> sp <i>U. lactuca</i>	110,111,131,146-148

named it fucoidan following polysaccharide nomenclature,<sup>161,162</sup> but according to a simple PubMed<sup>TM</sup> search, only around the 1970s this polymer was mentioned for the first time in the medical literature.

The extraction of fucoidan from brown algae, as of other sulfated polysaccharides from the respective macroalgae, is hot-water based, with precipitation with salts or organic solvents.<sup>34</sup>

Nevertheless, the production of valuable polysaccharides follows a more complex procedure, adding additional steps to render more pure materials, dependent on their further use. In the case of fucoidan, its extraction procedure has been methodically studied to develop economically and industrially viable systems (Fig. 2). The process can be divided in three steps: milling seaweeds, extraction/purification (involves multiple, extended aqueous



**Figure 2.** Extraction of fucoidan from brown seaweeds.

extractions and acidic solutions and may include calcium to promote the alginate precipitation and obtain fucoidan) and drying/careful storage. This type of extraction can obtain yields of fucoidan (%) ranging from 0.26% to 20% of algal biomass dry weight.<sup>163</sup> The physico-chemical characteristics of the extracted fucoidan are dependent on the severity of the treatments in the extraction such as temperature, reaction time, concentration of the chemicals, as well as on inherent factors of algae, such as species and size of algae, local climate and environmental factors.<sup>164-166</sup>

Different chemical structures have been proposed for this polysaccharide, since its discovery by Kylin. Fucoidan is a heteropolysaccharide and its composition differs depending on the sources and seasonality. The chemical composition of most fucoidan is complex. Fucoidan structure can be divided into two groups depending on their sources: one group includes *Laminaria* species that have their central chains composed by (1→3)-linked  $\alpha$ -L-fucopyranose residues; a second group includes fucoidan

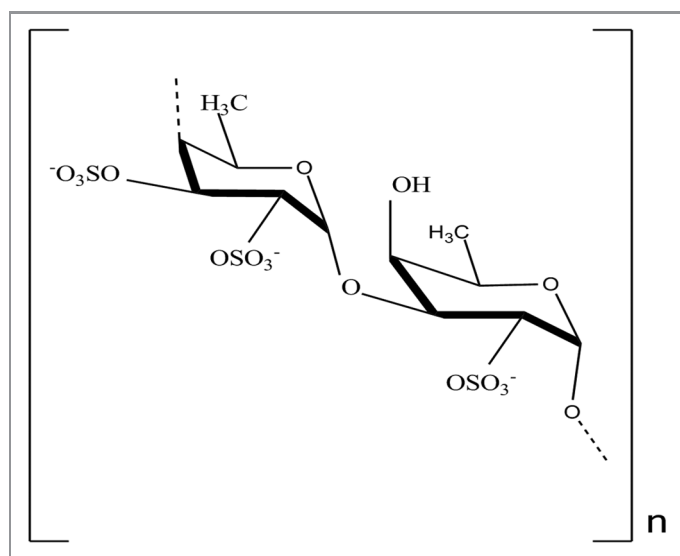
isolated from *Ascophyllum* and *Fucus* species that have their central chains composed of repeating (1→3) and (1→4) linked  $\alpha$ -L-fucopyranose residues.<sup>158,167</sup> Besides fucose and sulfate, the presence of monosaccharide residues such as mannose, galactose, glucose, xylose and uronic acids were also identified.<sup>165</sup> Nevertheless, the chemical structure of fucoidan can be schematically illustrated as showed in **Figure 3**.

The molecular weight of fucoidan varies from 13 kDa to 950 kDa<sup>164</sup> and it depends also on many factors such as source, season and extraction method from which they are obtained. In addition, knowledge on the solubility and rheological properties of fucoidan is important for different applications.<sup>168</sup> In this perspective, recent studies reported that rheological characteristics and viscosity of fucoidan are different according to the algae species, but independent of the molecular weight and proportion of sulfates and uronic acids.<sup>169,170</sup> One of those studies, developed by Rioux and coworkers, report that fucoidan may not be capable to form a gel, but a viscous solution.<sup>170</sup>

As for the previously reviewed sulfated polysaccharides, several different bioactivities have been attributed to fucoidan and its oligosaccharides. These bioactivities include anti-tumoral effects,<sup>171</sup> anti-coagulant,<sup>172-175</sup> anti-viral<sup>176-179</sup> and anti-inflammatory activities.<sup>171</sup> These properties are related to molecular size, type of sugar, sulfation degree and molecular geometry.

Based on the reported activities, fucoidan has been founding application mainly in cosmetic industry (skin exfoliation, acne treatment, hair hydration and tooth paste), food industry (dietetic fibers, cholesterol reducer, functional fibers, sports beverage and processed meat products) and biopharmaceutical industry (immunologic, antiviral and anticoagulant).<sup>180-182</sup> Moreover, fucoidan is presently emerging as a popular potential and natural ingredient to be used in cosmeceuticals industry. Some epidemiological studies suggested that fucoidan has some skin protecting, antioxidant and anti-aging activities.<sup>183</sup>

The interest in fucoidan has also been extended to biomedical-related fields, such as drug delivery, nanomedicine and tissue engineering applications. For instance, Sezer and coworkers reported the development of a new microspheres delivery system based on cross-linking of fucoidan with chitosan (Fucosphere),



**Figure 3.** Chemical structure of fucoidan (Adapted from ref<sup>180</sup>).

with extent of drug release being dependent on the concentrations of the polymers and protein.<sup>184</sup> Alternatively, other authors reported the development of chitosan/fucoidan pH-sensitive nanoparticles for oral administration of drugs.<sup>185</sup> The complexation of fucoidan with chitosan has been explored on the development of nanoparticles with increased potential for the delivery of anticoagulant agents taking advantage of its antithrombotic agents.<sup>185</sup> In addition to delivery of bioactive agents, it was also reported the ability of fucoidan to stimulate the production of hepatocyte growth factor (HGF),<sup>186</sup> thus reinforcing the potential of this algae-derived biomaterial for health-related applications.

Regarding tissue engineering, in particular considering cell support systems (since drug delivery and growth factor regulatory roles are also relevant), most of the studies investigate fucoidan in combination with different polymers as e.g., chitosan,<sup>187</sup> alginate<sup>188</sup> or polycaprolactone (PCL),<sup>189,190</sup> processed into hydrogels, scaffolds, films and nanofibers. This may relate to the great solubility of fucoidan in water and the difficulty in forming gels. In this regard, the mixture with natural or synthetic polymers, modified structure or chemical cross-linking is critical for its further application. For instance, Sezer and coworkers propose a fucoidan-chitosan hydrogel to be applied as burn injuries healing accelerator on rabbits.<sup>191</sup> The authors report the use of chitosan due to its hydrogel forming properties and advantageous use in applications as wound dressing material, adding to the anti-coagulant activity of fucoidan, among other properties. By their turn, Murakami and coworkers developed a hydrogel sheet by blending alginate, chitosan and fucoidan, aiming to stimulate rapid wound healing in rats.<sup>188</sup> Besides polysaccharides, fucoidan has been also blended with polyesters, namely PCL, following a general melt-plotted process.<sup>189</sup> This rapid-prototyping methodology provided a system with an appropriate pore structure for bone tissue regeneration, where low molecular weight fucoidan was used to induce not only cell proliferation but additionally influence on osteoconductive properties including alkaline phosphatase activity, collagen Type I expression and mineral deposition. Alternatively, micro/nanofibrous scaffolds of PCL and fucoidan have been produced by using an electrospinning process, aimed for application in bone regenerative medicine.<sup>190</sup>

### Final Remarks

Since ancient times, human efforts to treat and recover tissues have been continuously made.<sup>192</sup> Among these, regenerative medicine has brought new hopes for the treatment of numerous diseases and conditions. Although the tissue engineering and stem cell industry is now close to breaking even,<sup>193</sup> technological challenges remain high.

Many tissue-engineered products (TEPs) under development rely on the use of medical devices and/or materials to assure its efficacy. So, the quest for new and improved materials remains a pivotal pillar for the development and marketing authorization of many products. Modulating and controlling cell response through novel biomaterials chemistry and surface is an attractive approach

for development of new technology platforms that can, in principle, sustain intellectual property development strategies. In this regard, biomaterials that can bring exquisite properties and improve biological performance of TEPs will continue to justify research and investment.

Like never before, marine species constitute an inspirational template for development of new biomedical technology. The evolution of animal and vegetal species in marine ecosystems has originated an extensive library of biomolecules with enormous human application potential.

Marine polysaccharides remain an untapped reservoir for development of novel biomaterials.

Sulfate groups can effectively modulate cell behavior in tissue regeneration contexts, which may constitute an opportunity for exploiting the clinical potential of marine origin sulfated polysaccharides. These polymers do not have a true mammalian analog and exhibit a high application potential across many different regenerative medicine applications.

The realization of clinical potential of these polysaccharides will be a long and challenging road, as the regulatory context of medical devices and advanced therapy medicinal products, in particular, are very demanding. The lack of industrial scale extraction and purification of many of these molecules remains an obstacle for their application development. In fact, a fundamental requirement for any clinical application will be related with development and validation of manufacturing methods. In some cases, the extraction route may not be a possible manufacturing strategy, as the scarcity of the raw materials, attainable purity levels or final cost may be incompatible with industrialization. Synthesis of surrogate or close analog molecules may be, in some cases, the only cost effective approach.

In spite of the manufacturing strategy adopted, the natural provenience and novelty of these materials imposes a strict control of their purity, stability and safety, which imply extensive and, above all, expensive studies. More than proving additional and or incremental benefits in discrete application contexts, the challenge ahead for any sulfated polysaccharide will be to gain its status as a new biomaterial. For that, sulfated polysaccharides will have to demonstrate outstanding application performance, scalable manufacturing, remarkable cost-benefit potential, while addressing a tangible market opportunity.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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