Carbohydrate Polymers 218 (2019) 355–370

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review

Leto-Aikaterini Tziveleka, Efstathia Ioannou, Vassilios Roussis*

Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece

ARTICLE INFO

Keywords:
Ulvan
Sulphated polysaccharide
Hybrid materials
Biomedical applications

ABSTRACT

Ulvan, a sulphated polysaccharide located in the cell walls of green algae that possesses unique structural properties albeit its repeating unit shares chemical affinity with glycosoaminoglycans, such as hyaluronan and chondroitin sulphate, has been increasingly studied over the years for applications in the pharmaceutical field. The increasing knowledge on ulvan's chemical properties and biological activities has triggered its utilization in hybrid materials, given its potential efficacy in biomedical applications. In the present review, the use of ulvan in the design of different biomaterials, including membranes, particles, hydrogels, 3D porous structures and nanofibers, is presented. The applications of these structures may vary from drug delivery to wound dressing or bone tissue engineering. In this context, general information regarding the structure and chemical variability, extraction processes, physicochemical properties, and biological activities of ulvan is reported.

1. Introduction

Natural polymers, due to their inherent unique properties of biocompatibility and biodegradability, are highly appreciated as valuable ingredients of biomaterials and therefore are widely exploited in the biomedical field (Germershaus, Lühmann, Rybak, Ritzer, & Meinel, 2015; Liu et al., 2018; Ngwuluka, Ochekpe, & Aruoma, 2014; Song et al., 2018). Polysaccharides, incorporating various functionalities in their structures and exhibiting interesting physicochemical properties and significant biological activities, are considered attractive materials for the development of novel systems for bioapplications, such as drug delivery and tissue engineering (Patel, 2012; Shelke, James, Laurencin, & Kumbar, 2014). In this context, natural carbohydrates are increasingly utilized either in their native form or after chemical modification, so as to deliver tailor-made materials for specific applications (Cardoso, Costa, & Mano, 2016; D'Ayala, Malinconico, & Laurienzo, 2008).

Marine algae produce polysaccharides that are regarded as safer and non-immunogenic, in contrast to those of animal origin (Stevens, 2008; Venkatesan, Anil, & Kim, 2017; Venkatesan, Anil, Kim, & Shim, 2016). Many species of green algae are cosmopolitan and are considered as important constituents of the human diet (marine vegetables) in many parts of the world. The algal biomass, commonly harvested from marine eutrophicated coastal areas, is a sustainable and renewable waste material that can gain an added value when used as a source of fine chemicals and biopolymers. At the same time, the fact that green algae can be cultivated in land-based tanks or in open cages at coastal areas, acting as a biofilter that improves the quality of the seawater, can ensure the provision of sufficient quantities of their polysaccharides for industrial utilization.

In particular, the genus *Ulva* (sometimes erroneously referred to as *Enteromorpha*), being consumed as food (marine lettuce), is rich in carbohydrates, vitamins, essential amino acids, minerals and dietary fibers (Fleurence, 1991). Ulvan, a water soluble sulphated polysaccharide, is isolated from the common seaweeds of the order Ulvales (Chlorophyta) (Kidgell, Magnusson, de Nys, & Glasson, 2019; Lahaye & Robic, 2007). Its carbohydrate composition is complex and variable, with rhamnose, glucuronic and iduronic acids, as well as xylose being the main constituents.

The incorporation of rhamnose, being a rare sugar usually found in bacteria and plants, and iduronic acid, which has not been identified in other polysaccharides of algal origin (Alves, Sousa, & Reis, 2013a), along with its content in uronic acids and sulphate, partially reminiscent of glycosaminoglycans, such as hyaluronan and chondroitin sulphate, single out ulvan among other algal sulphated polysaccharides, such as fucoidans and carrageenans.

These unique structural characteristics, in conjunction with the broad spectrum of biological activities exhibited and the tunable physicochemical properties, have recently attracted significant attention to

* Corresponding author.
E-mail addresses: ltziveleka@pharm.uoa.gr (L.-A. Tziveleka), eioannou@pharm.uoa.gr (E. Ioannou), roussis@pharm.uoa.gr (V. Roussis).

https://doi.org/10.1016/j.carbpol.2019.04.074
Received 2 March 2019; Received in revised form 23 April 2019; Accepted 23 April 2019
Available online 29 April 2019
0144-8617/

ulvan as a promising material for applications in the pharmaceutical (Chiellini & Morelli, 2011; Cunha & Grenha, 2016; Ngo & Kim, 2013; Popa, Reis, & Gomes, 2015; Raveendran, Yoshida, Maekawa, & Kumar, 2013; Silva et al., 2012; Venkatesan et al., 2015; Wijesekara, Pangestuti, & Kim, 2011) and agricultural sectors (Stadnik & De Freitas, 2014). These properties of ulvan are to a large degree affected by its chemical composition, charge density and molecular size. The yield and specific composition of polysaccharides derived from green algae depend on the particular species (Lahaye & Robic, 2007), the environmental factors, the collection season (Medcalf, Lionel, Brannon, & Scott, 1975; Rico and Fernandez, 2009) and the employed extraction method (Abdel-Fattah & Edrees, 1972; Robic, Sassi, & Lahaye, 2008).

The utilization of ulvan as a biopolymer has not yet been comprehensively investigated. Hitherto, a number of ulvan-based hybrid scaffolds of various structures, such as membranes, particles, hydrogels, 3D porous structures or nanofibers, have been successfully prepared and described for drug delivery or tissue engineering applications.

In the present review, the structure, physicochemical features and biological properties of ulvan are presented, along with the applications of ulvan in the preparation of diverse biomaterials. The reported examples are not restricted to ulvan isolated from *Ulva* species, but include also ulvan-type polysaccharides isolated from other members of the order Ulvales and Ulotrichales, such as species of the genera *Cacosiphon*, *Monostroma* and *Ulothrix*.

2. Extraction and processing

The name ulvan was first introduced by Lahaye and Axelos (1993) when describing the sulphated rhamnoglycuronans isolated from *Ulva* spp. (Lahaye & Axelos, 1993). It originated from the terms ulvin and ulvacin introduced by Kylin (Kylin, 1946) in reference to different fractions of *Ulva lactuca* water-soluble sulphated polysaccharides (Lahaye & Robic, 2007). Nowadays, it is used to describe the class of sulphated polysaccharides mainly composed of uronic acids and sulphated rhamnose (Ray & Lahaye, 1995a) from members of the order Ulvales. After first report of ulvan (Brading, Georg-Plant, & Hardy, 1954; McKinnel & Percival, 1962a), many attempts have been undertaken targeting its isolation and characterization.

The general procedure to obtain ulvan from green algae starts with selection, collection and identification of the raw material, followed by algae stabilization and grinding. The stabilization process has a considerable impact on the final yield of extraction and can be performed by several alternative procedures, including freezing, freeze-drying, hot-air drying, brining and dry salting (Robic, et al., 2008).

Ulvan extraction is mostly performed with hot water at around 80–90 °C (Percival & Wold, 1963; Yamamoto, 1980) or even up to 120 °C (Peasura, Laohakunjit, Kerdchoechuen, & Wanlapa, 2015; Toskas et al., 2011) and can be further improved by the presence of divalent cation chelating agents, such as ammonium oxalate (Brading et al., 1954; Hernández-Garibay, Zertuche-González, & Pacheco-Ruíz, 2011; Robic et al., 2008). The yield ranges from 8% to 29% of the algal dry weight, affected by the extraction and purification protocols (Abdel-Fattah et al., 1972; Costa et al., 2012; Lahaye & Jegou, 1993; Lahaye & Robic, 2007; McKinnel & Percival, 1962a; Percival & Wold, 1963). It has been shown that the extraction temperature, the pH and the duration of extraction affect both the yield and purity. Indeed, it has been reported that low pH improves the selectivity for ulvan, whereas higher extraction temperatures and increased extraction times can enhance the yield (Kidgell et al., 2019). In order to remove pigments, lipids, amino acids and peptides from the polymer, various procedures have been employed (Alves et al., 2013a; Alves, Caridade, Mano, Sousa, & Reis, 2010; Brading et al., 1954; Chattopadhyay et al., 2007; Gosselin, Holt, & Lowe, 1964; Paradossi, Cavalieri, Pizzoferrato, & Liquori, 1999; Percival & Wold, 1963; Robic, Sassi, Dion, Lerat, & Lahaye, 2009; Sarker, Latif, & Gray, 2005). Recovery of ulvan is generally done by precipitation *via* alcohol or quaternary ammonium salt addition (Alves et al., 2010; Lahaye & Robic, 2007). As a final step, ulvan aqueous extract can be concentrated in a rotary evaporator or dried either by freeze- or hot air-drying.

3. Chemical structure

The first established main constituents of ulvan were rhamnose, xylose, glucuronic acid and sulphate (Brading et al., 1954; McKinnel & Percival, 1962a), with the sulphate group being linked to rhamnose (McKinnel & Percival, 1962a). Iduronic acid was later acknowledged as a constituent of the polysaccharide by Lahaye and coworkers (Quemener, Lahaye, & Bobin-Dubigeon, 1997). Ulvan isolated from numerous Ulvales species (Abdel-Fattah & Edrees, 1972; Bryhni, 1978; De Reviers & Leproux, 1993; Gosselin et al., 1964; Lahaye & Axelos, 1993; Lahaye & Jegou, 1993; Lahaye & Robic, 2007; Lahaye et al., 1999; McKinnel & Percival, 1962a; Quach et al., 2015; Quemener et al., 1997; Ray & Lahaye, 1995a; Tabarsa, Han, Kim, & You, 2012; Tabarsa, You, Hashem Dabaghian, & Surayot, 2018; Thanh et al., 2016; Toskas et al., 2011; Yamamoto, 1980) has been reported to consist primarily of rhamnose (16.8–45.0%), sulphate (14.3–23.2%), glucuronic acid (6.5–19.0%), xylose (2.1–12.0%), iduronic acid (0.7–9.1%) and glucose (0.5–6.8%).

In some *Ulva* extracts, xylose or sulphated xylose residues may occur instead of uronic acids (Barros et al., 2013). Additionally, mannose, galactose and arabinose have been reported in variable amounts according to the investigated species (Abdel-Fattah & Edrees, 1972; 1973; Bryhni, 1978; Gosselin et al., 1964; Lahaye & Axelos, 1993; McKinnel & Percival, 1962a; Tabarsa et al., 2018; Thu et al., 2015; Toskas et al., 2011; Yamamoto, Tadokoro, Imai, & Mita, 1980), but their participation in ulvan structure has not been unequivocally proved (Bryhni, 1978; Lahaye & Robic, 2007). A greater amount of glucose than that reported in the literature (11.9% and 40.9%), which also equaled the rhamnose content, has been measured in the polysaccharide of *Ulva intestinalis* extracted at 80 and 65 °C, respectively (Peasura et al., 2015; Tabarsa et al., 2018). Moreover, in some *Ulva* extracts, notably enhanced amounts of rhamnose have been reported (51.2–60.8%) (Tian, Yin, Zeng, Zhu, & Chen, 2015).

These biopolymers are of branched complex structure, with no defined backbone or simple repeating unit. Actually, the major repeating disaccharide in ulvan was found to comprise two different types of aldobiouronic acid. Moreover, they do not appear to have long chains of the same sugar (Alves et al., 2013a; Lahaye & Robic, 2007; Percival, 1979). The chemical heterogeneity of ulvan results to an essentially disordered conformation of the biopolymer.

These wide variations in the chemical composition of ulvan may be primarily ascribed to differences in species, harvesting region and growth conditions of the analyzed seaweeds. It is well-established that growth conditions affect ulvan biosynthesis and, thus, its chemical structure (Lahaye, Gomez-Pinchetti, Del-Rio, & Garcia-Reina, 1995). Still, to date, specific trends between growth conditions and the chemical composition of these sulphated polysaccharides have not yet been established (Percival & Wold, 1963). Additionally, species identification based on morphological characteristics is not precise and can thus explain the observed compositional variability (Blomster, Maggs, & Stanhope, 1998; Coat et al., 1998).

Seasonal variations in seaweed chemical composition are associated with the exact life stage. Higher carbohydrate content and reduced protein content result from photosynthetic activity, which increases in periods of maximum growth (Rico & Fernandez, 1996). Moreover, seasonal variations in carbohydrate contents (Abdel-Fattah & Edrees, 1973; Lahaye et al., 1999; Lai, Li, & Li, 1994; Medcalf et al., 1975) may actually reflect differences in the content of starch or other cell wall polysaccharides, such as glucuronan, xyloglucan, or some other glycoproteins co-extracted with ulvan (Abdel-Fattah & Sary, 1987; Lahaye,

Ray, Baumberger, Quemener, & Axelos, 1996; Ray & Lahaye, 1995a).

Furthermore, the isolation protocols used, as well as the analytical methods employed can affect the structures proposed. The most efficient procedure employed in the determination of ulvan composition has been proven to be a combination of chemical and enzymatic degradation (Quemener et al., 1997). The accurate determination of the sugar composition of ulvan is difficult, since the aldobiouronic linkage is resistant to acid hydrolysis (Bemiller, 1967; McKinnel & Percival, 1962a) and iduronic acid is partially destroyed during acid hydrolysis (Conrad, 1980; Fransson, Roden, & Spach, 1968).

The sugar sequence determination in ulvan represents a major challenge. Oligosaccharides and oxidation products released after mild acid hydrolysis of native and chemically modified ulvan suggest the presence of rhamnose, xylose, glucuronic acid or glucose, all present in the same chain. Moreover, it has been also indicated that glucuronic acid can occur as branches on C-2 of rhamnose (Lahaye & Ray, 1996; Lahaye & Robic, 2007).

Chemical studies on ulvan isolated from different species indicated that its backbone is mostly composed of α- and β- (1→4)-linked sugar residues, namely of α-1,4- and α-1,2,4- linked L-rhamnose, β-1,4- and terminally linked D-glucuronic acid and β-1,4-linked D-xylose (Gosselin et al., 1964; Haq & Percival, 1966; Lahaye, 1998; Lahaye & Ray, 1996; Lahaye & Robic, 2007; Lahaye et al., 1999). The sulphation sites are localized mainly on C-3 or at both C-2 and C-3 of rhamnose (McKinnel & Percival, 1962b). Indications that sulphate groups are also attached at C-4 exist (Thu et al., 2015), while additional data have also confirmed the presence of xylose-2-sulphate (Lahaye, Inizan, & Vigouroux, 1998; Lahaye et al., 1999; Ray & Lahaye, 1995b). Branching points have been determined on *O*-2 of rhamnose, as well as on *O*-2 of uronic acids (Thu et al., 2015).

More specifically, glucuronic or iduronic acid and rhamnose were proven to occur mainly in the form of aldobiouronic acids, comprising this way the major repeating disaccharides in ulvan (Table 1). The two different types of aldobiouronic acid were named ulvanobiuronic acid 3-sulphate type A (A_{3s}) and type B (B_{3s}). The disaccharide A_{3s} is composed of glucuronic acid and sulphated rhamnose, while type B_{3s} consists of iduronic acid and sulphated rhamnose, mainly associated *via* (1→4) glycosidic linkages. Nevertheless, in some cases, uronic acids are replaced by xylose or sulphated xylose residues. In this case, the disaccharides are named ulvanobioses and symbolized as U_{3s} (ulvanobiose 3-sulphate) and $U_{2'S,3S}$ (ulvanobiose 2',3-disulphate) (Lahaye, 1998).

The structure of ulvan was further characterized by employing ulvanolytic enzymes, such as an extracellular ulvan-lyase isolated from marine bacteria (Lahaye, Brunel, & Bonnin, 1997; Martin, Portetelle, Michel, & Vandenbol, 2014; Nyvall Collén et al., 2011), an endolytic enzyme that cleaves the (1→4) linkage between sulphated rhamnose and glucuronic or iduronic acid. The released degradation products were identified as oligosaccharides with repeating sequences of -A_{3s}-A_{3s}-, -A_{3s}-B_{3s}-, -A_{3s}-U_{3s}- and-A_{3s}-GlcA-A_{3s}. Isolated oligosaccharide structures of glucuronic acid flanked by A_{3s} proved that an extra 4-linked β-D-glucuronic acid residue disrupts the regularity of the disaccharide pattern, providing that way a cleavage site of ulvan by glucuronan lyase (Delattre et al., 2006).

Evidently, the observed variation in the enzymatic degradation susceptibility of different *Ulva* sp. by the ulvan-lyase demonstrates that other linkages, sugar distributions, branching, and/or sulphation patterns exist. Interestingly, ulvan shares numerous compositional and structural similarities with water-soluble cell wall polysaccharides of members of the Ulotrichales, also belonging to the class of Ulvophyceae, same as Ulvales (Lewis & McCourt, 2004). These are mainly consisted of rhamnose, uronic acid and xylose and characterized as type A ulvanobiouronic acid (Bourne, Megarry, & Percival, 1974; Carlberg & Percival, 1977; Maeda, Uehara, Harada, Sekiguchi, & Hiraoka, 1991; Maeshige, 1962; O'Donnel & Percival, 1959; Synytsya et al., 2015). However, not every polysaccharide isolated from algae belonging to this order resembles ulvan (Rao, Rao, & Ramana, 1991).

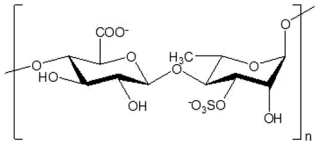
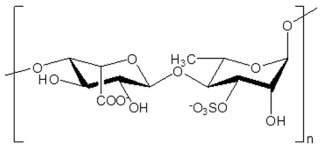
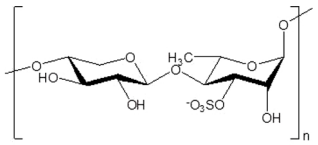
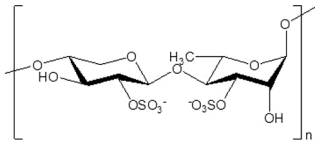
4. Physicochemical properties

Ulvan is considered as a family of chemically related branched molecules of broad distribution of charge density and molecular weight (Percival & McDowell, 1967). Various types of ulvan have been isolated differing in their molecular weights and molecular weight distributions, the determination of which was performed with a number of methods. Molecular weights ranging from 5.3×10^4 to 3.2×10^5 g/mol were determined with sedimentation measurements conducted in ulvan-type polysaccharides isolated from different species (*Ulva pertusa*, *Ulva conglobata*, and *Enteromorpha prolifera*) (Yamamoto, 1980). In the case of ulvan isolated from *U. conglobata*, large variations on the molecular weight values were observed, which were correlated with the temperature at which the polysaccharides were extracted. Specifically, ulvan with molecular weights of 5.3×10^4 and 3.6×10^5 g/mol were obtained after extraction at 30–40 °C and 80–90 °C, respectively, indicative of the high temperature required for the extraction of high molecular weight ulvan. Molecular weights of ulvan isolated from *U. pertusa* and *U. conglobata* were calculated at 9.1×10^4 and 8.2×10^5 g/mol, respectively, when obtained by sedimentation, and 11×10^4 and 7.7×10^5 g/mol by gel permeation chromatography (Yamamoto et al., 1980). Nevertheless, molecular weights as high as 1.6×10^6 and 1.8×10^6 g/mol have been calculated for ulvan isolated from *U. pertusa* and *Ulva rigida*, respectively (Kikionis, Ioannou, Toskas, & Roussis, 2015; Tabarsa et al., 2012). Polydispersity indices equal to 1.5 and 1.3 were calculated for these polysaccharides, indicative of narrow molecular weight distributions. Osmometry measurements of ulvan isolated from *U. rigida* suggested the molecular weight of 1.7×10^5 g/mol (Paradossi et al., 1999), while that of an ulvan-type polysaccharide obtained from *Enteromorpha intestinalis* analyzed by light scattering ranged between 1.9×10^5 to 5.0×10^5 g/mol, depending on the temperature and the nature of the solvent used during the extraction procedure (De Reviers & Leproux, 1993). In this case, the polysaccharide fraction extracted at low temperature had the highest molecular weight, most possibly due to disruption of ionic interactions and interchain bonding at elevated temperatures and acidic pH. On the other hand, ulvan-type polysaccharides isolated from green algae belonging to the order of Ulotrichales exhibited low molecular weights of approximately 5.0×10^3 g/mol (Li, Wen, Sun, Zhao, & Chen, 2018; Synytsya et al., 2015). However, not every polysaccharide isolated from algae belonging to this order is of low molecular weight (Zhang et al., 2008).

It is evident that molecular weight variability of ulvan primarily stems from its origin and mode of extraction. However, variations in molecular weights may also reflect different methods and conditions used for their determination. Moreover, the tendency of ulvan to form microaggregates may largely contribute to the wide disparity in the reported values (Robic, Gaillard, Sassi, Lerat, & Lahaye, 2009).

In general, it has been reported that ulvan solutions exhibit low viscosities and that ulvan demonstrates a viscosity decrease under shear rate increase (pseudoplastic behavior). Specifically, the intrinsic viscosity of ulvan obtained from various species of *Ulva* in saline solutions is in the range of 95–285 mL/g (Lahaye & Jegou, 1993; Percival & Wold, 1963; Yamamoto, 1980; Yamamoto et al., 1980) and is even lower (24–61 mL/g) for ulvan isolated from *Enteromorpha* species (De Reviers & Leproux, 1993; Lahaye & Jegou, 1993; Yamamoto, 1980). Ulvan extracted from *U. pertusa* at 120 °C exhibited a lower viscosity compared to that recorded when extracted at 20–90 °C (Yamamoto, 1980), most likely due to degradability of the polysaccharide at high temperatures. Low viscosities may also reflect the molecular weight variability of ulvan with high proportions of short-chain polysaccharides and/or highly branched structures, as earlier proposed (Percival & McDowell, 1967). However, the structural characteristics and the branching pattern of fragments obtained after lyase treatment support that ulvan is a slightly branched polysaccharide (Lahaye & Robic, 2007; Lahaye et al., 1997). Additionally, the impact of extraction

Table 1
Structures of the main repeating disaccharides constituting ulvan assessed for its biological activity.

Structure and Name	Origin	Reference
 Ulvanobiuronic acid 3-sulphate type A (A_{3s})	*Ulva* sp. (France) *U. armoricana* (France) *U. clathrata* (Mexico) *U. fasciata* (Southeast Atlantic) *U. lactuca* (Vietnam) *U. olivascens* (France) *U. reticulata* (Vietnam) *U. rigida* (Canary islands) *U. rigida* (France) *U. rotundata* (France) *U. scadinavica* (France) *Ulothrix flacca* (China)	Lahaye et al. (1998) Lahaye et al. (1999) Aguilar-Briseno et al. (2015) Paulert et al. (2007) Thanh et al. (2016) Lahaye et al. (1999) Tran et al., 2018 Lahaye (1998) Lahaye (1998) Lahaye et al. (1999) Lahaye et al. (1999) Li, Wen et al. (2018)
 Ulvanobiuronic acid 3-sulphate type B (B_{3s})	*Ulva* spp. (various) *U. armoricana* (France) *U. lactuca* (France) *U. lactuca* (Vietnam) *U. olivascens* (France) *U. reticulata* (Vietnam) *U. rigida* (France) *U. rotundata* (France) *U. scadinavica* (France)	Quemener et al. (1997) Lahaye et al. (1999) Quemener et al. (1997) Tran et al. (2018) Lahaye et al. (1999) Thu et al. (2015) Lahaye et al. (1999) Lahaye et al. (1999) Lahaye et al. (1999)
 Ulvanobiose 3-sulphate (U_{3s})	*U. rigida* (Canary islands) *U. rigida* (France)	Lahaye (1998)
 Ulvanobiose 2′,3-disulphate ($U_{2's,3s}$)	*U. rigida* (Canary islands) *U. rigida* (France)	Lahaye (1998)

procedures on the rheological properties of ulvan extracted from *Ulva lactuca* was recently assessed and established to be of great importance (Yaich et al., 2014).

The repeating aldobiouronic units in ulvan provide local regularity that is believed to be necessary for the formation of transient "junction zones", in turn responsible for the formation of the weak gel (Paradossi et al., 1999). Furthermore, ulvan has been shown to form gels in the presence of Ca^{2+} and B^{+} ions at basic pH, with the gelling ability being enhanced by the presence of divalent cations (Haug, 1976; Lahaye et al., 1996). The influence of pH, nature of buffer and amount of added ions (Ca^{2+} and B^{+}) on the gelling characteristics of ulvan extracted with boiling water after enzymatic treatment of algae, as well as the thermo-reversibility of gel formation during heating and cooling has been systematically investigated (Lahaye & Axelos, 1993). It was shown that ulvan yields viscous aqueous solutions that can form thermo-reversible gels in the presence of Ca^{2+} and B^{+} ions at basic pH. Additionally, gel formation proved to be a time-dependent process, while the viscoelastic behavior of the ulvan solution in the presence of ions at pH 7.5 indicated that the studied extracts led to systems with properties resembling that of solid rather than that of liquid materials. Stabilization treatments, such as freezing, freeze-drying, hot-air drying, brining and dry salting, of *U. rotundata* had great impact on the physicochemical and rheological properties of ulvan that was extracted using sodium oxalate and subsequently water (Robic et al., 2008). Overall, these studies revealed that ulvan gel formation is a thermo-reversible procedure, manipulated by the ion concentration and pH variation, which greatly influence ulvan conformation, and consequently, gel formation. Moreover, being a polyelectrolyte, ulvan's solubility and morphology are greatly defined by both the ionic strength and the pH of the used solvent, since the type and amount of counterions in solution could contribute to the condensation of the polymer (Robic, Sassi et al., 2009).

Ulvan is considered a densely charged polysaccharide, a fact that also determines its water solubility. However, it also possesses a hydrophobic character, originating from the presence of a high number of methyl groups in the repeating unit of rhamnose, resulting to an overall amphiphilic character. Ultrastructural analysis of ulvan in aqueous solutions revealed the presence of spherical-shaped aggregates (Robic, Gaillard et al., 2009). It is well known that surface activity is affected by the chemical structure of biomolecules, especially the balance of hydrophilic and hydrophobic groups. Indeed, good surface activity of two polysaccharides isolated from *U. lactuca* exhibiting high contents of rhamnose (51.2% and 60.8%) was demonstrated (Tian et al., 2015). Although both polysaccharides exhibited critical micelle concentration (CMC) at approximately 1 mg/mL, the polysaccharide with the lower rhamnose and sulphate content (51.2% Rha, 12.0% sulphate) showed better surface activity than that of the one with the higher content (60.8% Rha, 26.8% sulphate).

5. Biological activities

Sulphated polysaccharides exhibit a wide range of biological

Table 2
Biological activities of ulvan or ulvan-type sulphated polysaccharides.

Activity	Species	Reference
Antibacterial	*U. armoricana*	Berri et al. (2016)
Antibacterial	*U. reticulata*	Tran et al. (2018)
Immunostimulating	*U. lactuca*	Lee, Hyun et al. (2004)
Immunostimulating	*U. pertusa*	Tabarsa et al. (2012)
Immunostimulating	*Capsosiphon fulvescens*	Na et al. (2010)
Immunostimulating	*U. armoricana*	Berri et al. (2016); Berri et al. (2017); Bussy et al. (2019)
Immunostimulating	*U. intestinalis*	Tabarsa et al. (2018)
Immunomodulating	*Ulothrix flacca*	Li, Wen et al. (2018)
Anti-inflammatory	*U. lactuca*	de Araújo et al. (2016)
Antitumor	*U. lactuca*	Lee, Hyun et al. (2004)
Cytotoxic	*U. lactuca*	Thanh et al. (2016)
Cytotoxic	*U. lactuca*	Abd-Ellatef et al. (2017)
Antioxidant	*U. pertusa*	Qi, Zhao et al. (2005)
Antioxidant	*U. pertusa*	Qi, Zhang et al. (2005)
Antioxidant	*U. pertusa*	Li, Jiang et al. (2018); Li, Wen et al. (2018)
Antioxidant	*U. intestinalis*	Peasura et al. (2015)
Antihyperlipidemic	*U. pertusa*	Pengzhan et al. (2003)
Antiviral	*U. lactuca*	Ivanova et al. (1994)
Antiviral	*U. clathrata*	Aguilar-Briseno et al. (2015)
Antiherpetic	*Monostroma nitidum*	Lee, Hayashi et al. (2004)
Anticoagulant	*Monostroma latissimum*	Zhang et al. (2008)
Anticoagulant	*Capsosiphon fulvescens*	Synytsya et al. (2015)
Anticoagulant	*Ulothrix flacca*	Li, Wen et al. (2018)
Anticoagulant	*U. fasciata*	De Carvalho et al. (2018)

activities, which are related to their structural characteristics, including chemical composition, amount and position of sulphate esters, uronic acids content and molecular weight. As such, ulvan has been thoroughly investigated for its biological and pharmacological potency (Chiellini & Morelli, 2011; Cunha & Grenha, 2016; Ngo & Kim, 2013; Silva et al., 2012; Wijesekara et al., 2011). Ulvan polysaccharides have been reported to exert a variety of therapeutic activities, including antibacterial, immunostimulating, antitumor, antioxidant, antihyperlipidemic, antiviral, and anticoagulant (Table 2) (Abd El-Baky, El-Baz, & El-Baroty, 2009; Shi et al., 2017; Wijesekara et al., 2011).

Specifically, an aqueous sulphated polysaccharide extract obtained from *Ulva armoricana* collected on the beach at Plestin les Grèves in Bretagne was evaluated for antibacterial activity against 42 Gram-positive and Gram-negative bacterial strains (Berri et al., 2016). The most sensitive pathogens were found to be *Pasteurella multocida*, *Mannheimia haemolytica*, *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus*, and *Streptococcus suis*, with a minimum inhibitory concentration (MIC) within the range of 0.16–6.25 mg/mL. Additionally, an *in vitro* system of differentiated porcine intestinal epithelial cells was used for evaluating the stimulation of the immune response mediators of the host's gut with the extract. The results suggested that the extract activates intestinal epithelial cells to produce cell-mediated immune response cytokines (Berri et al., 2017) that initiate and amplify protective immune responses of the host, and regulate mucosal immunity against intestinal pathogens. Therefore, the use of this extract in animal diets was proposed, for inhibiting the pathogens growth and stimulating the immune response, thus for reducing the occurrence of infections in animal herds and the consequent use of antibiotics (Bussy et al., 2019).

The variation in the antibacterial activity observed against pathogenic microbes has been attributed to structural differentiation of ulvan obtained from different *Ulva* species (Tran et al., 2018). Earlier, ulvan isolated from *Ulva fasciata* collected in Florianópolis, Santa Catarina, Brazil, with a repeating structure of ulvanobiuronic acid 3-sulphate, was found inactive against several bacteria (Paulert, Smania, Stadnik, & Pizzolatti, 2007). On the other hand, ulvan extracted from *U. reticulata* collected from Nhatrang sea in Vietnam, with ulvanobiuronic acid 3-sulphate branched at *O*-2 of glucuronic acid, was found to be active against *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter cloace* with the highest inhibition activity exhibited primarily against *E. cloace* (Tran et al., 2018).

Additional evidence for ulvan's immunostimulatory effect has been demonstrated in a number of studies. Initially, the water soluble fraction of the methanol extract of *U. lactuca* harvested from the Busan and Anin coastline in Korea was evaluated on macrophages, splenocytes and a human tumor cell line and it was found to stimulate the growth activity of splenocytes, increase the nitric oxide (NO) production of macrophages and exhibit an antitumor effect (Lee, Hyun et al., 2004). In another study, sulphated polysaccharides extracted from *U. pertusa* harvested from the coast of Anin in Korea and fractionated by ion-exchange chromatography, were investigated for their immunostimulatory activities (Tabarsa et al., 2012). The extracted polysaccharides consisted mostly of carbohydrates (61.4%), uronic acid (13.8%), and sulphate (13.9%), with the major monosaccharide being rhamnose (69.8%). Despite their low cytotoxicity against a human gastric carcinoma cell line, these polysaccharides significantly induced NO, as well as pro- and anti-inflammatory cytokine production from macrophage cells, suggesting that they might be potent immunostimulators. Further fractionation yielded three fractions, differing in molecular weight, sulphate and uronic acid content. The fraction with the lowest anti-inflammatory activity was also the one with the lowest amount of sulphate and lower molecular weight. Complementary, in a previous work (Schepetkin et al., 2008), a relationship between the Mw and NO production from macrophages was observed, with the higher molecular weight polysaccharides inducing greater production of NO from RAW264.7 cells. Moreover, water-soluble sulphated polysaccharides (5.84% uronic acid, 30.18% rhamnose, 40.88% glucose and 18.4% sulphate) isolated from *U. intestinalis* collected from the coast Noor in Iran, were fractionated into two distinct polysaccharide groups with different chemical compositions (Tabarsa et al., 2018). In this case, the fraction with the lowest molecular weight and sulphate content could effectively proliferate RAW264.7 macrophage cells and stimulate them to release NO and pro-inflammatory cytokines, its stimulatory activity being positively correlated with its lower molecular weight compared to the crude polysaccharide. A structural characteristic of this polysaccharide was that its backbone consisted of (1 →2)-linked rhamnose and (1 → 2)-linked glucose residues with random branches at C-4. In another study, a water soluble polysaccharide was isolated from the Korean *C. fulvenscens* (Na et al., 2010) and although it was originally characterized as glucuronogalactomannan, subsequently it was proven to be an ulvan-type glucuronorhamnoxylan (Synytsya et al., 2015). It was shown that the sulphated polysaccharide was able to activate RAW264.7 macrophages to produce pro-inflammatory mediators, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), NO and prostaglandin E2 (PGE2), and hence, could be used as immune stimulator. Immunomodulatory properties have also been reported for an ulvan-type sulphated low molecular weight polysaccharide, isolated from *Ulothrix flacca* from the eastern coastal area of Zhoushan in China (Li, Wen, et al., 2018).

Even though all studies on the immunomodulating properties of ulvan demonstrated an obvious relation between the molecular weight of the polysaccharides, their sulphate content and the level of immunostimulating activity, the compositional and structural heterogeneity of the polysaccharides renders the elucidation of a direct correlation of the immunomodulating properties and the structural characteristics of ulvan rather complicated.

Recently, the structural features of polysaccharides from *U. lactuca* obtained from the coast of Brazil and their effects on the classical models of nociception and inflammation have been investigated (de Araújo et al., 2016). A fraction of the crude extract obtained by enzymatic digestion decreased significantly the antinociception, with its analgesic activity most probably occurring through a peripheral mechanism. Additionally, the same fraction of polysaccharides exerted a

vascular anti-inflammatory effect, with bradykinin being the major target, thus suggesting that the antinociceptive and anti-inflammatory responses result from activity on the bradykinin pathway.

Thanh and coworkers showed the significant cytotoxic activity of ulvan from *U. lactuca* collected from Nha Trang sea of Vietnam against hepatocellular carcinoma (IC_{50} 29.67 ± 2.87 μg/mL), human breast cancer (IC_{50} 25.09 ± 1.36 μg/mL), and cervical cancer (IC_{50} 36.33 ± 3.84 μg/mL) in a dose dependent manner (Thanh et al., 2016). The ulvan studied was mainly composed of rhamnose, while its sulphate content (18.9%) and uronic acid content (21.5%) were at the lower and higher limit, respectively, in comparison to sulphated polysaccharides from other sources (Alves et al., 2013a; Alves, Sousa, & Reis, 2013b; Alves, Sousa, & Reis, 2013c; Lahaye & Robic, 2007). *In vitro* bioassays on human breast cancer cell line and an *in vivo* animal model of breast carcinogenesis were employed for assessing the potential chemopreventive properties of polysaccharides isolated from *U. lactuca* collected from the Mediterranean Sea coast in Egypt (Abd-Ellatef, Ahmed, Abdel-Reheim, & Abdel-Hamid, 2017) to reveal that ulvan has potential chemopreventive effects both at the level of initiation and promotion. These preventive effects have been proposed to be mediated through the induction of apoptosis, suppression of oxidative stress and inflammation, as well as the enhancement of antioxidant defense system.

Several studies have reported the antioxidant activity of ulvan extracted from *U. pertusa* collected from the coast of Qingdao in China, which is strongly dependent on its molecular weight and sulphate content (Li, Jiang et al., 2018; Qi, Zhang et al., 2005; Qi, Zhao et al., 2005; Zhang et al., 2010). Different molecular weight ulvans demonstrated significant antioxidant activity, with the lower molecular weight ones exhibiting the highest levels of activity (Qi, Zhao et al., 2005). Furthermore, ulvans with different sulphate content, either due to chemical modification or chromatographic fractionation, were proven to exert variable antioxidant activity, with the strongest activity being observed for ulvan possessing the highest sulphate and uronic acid contents (Qi, Zhang et al., 2005). This antioxidant activity shows the potential of ulvan as a natural antioxidant protecting the liver from oxidative stress induced by cholesterol-rich diet (Li, Jiang et al., 2018). Moreover, the *in vitro* antioxidant activity of natural, acetylated and benzoylated ulvans has been comparatively investigated (Qi et al., 2006). Acetylated ulvan exhibited the strongest scavenging activity against hydroxyl radicals and chelating ability, whereas benzoylated ulvan exhibited the strongest reducing power. Laohakunjit and coworkers investigated the effect of the extraction duration and the nature of the solvent used on the chemical composition and antioxidant characteristics of the sulphated polysaccharides extracted from *U. intestinalis* collected from Pattani Bay in Thailand (Peasura et al., 2015). Different types of solvents (distilled water, acidic and alkaline solutions) and extraction times had a significant influence on the chemical characteristics, molecular weight and antioxidant activity of the obtained polysaccharides. The sugar backbone of the polysaccharides from all extraction processes was composed of rhamnose and glucose, containing sulphate at the C-2 or C-3 of rhamnose, however polysaccharides extracted with acid exhibited higher levels of antioxidant activity than those extracted with distilled water and alkaline solutions. The acid-extracted polysaccharides had lower molecular weight, rhamnose and sulphate content and were the only ones containing arabinose, probably important for the observed scavenging effect (Hernández-Garibay, Zertuche-González, & Pacheco-Ruíz, 2010).

Pengcheng and coworkers isolated ulvan (151.6 kDa) from *U. pertusa* collected from Qingdao coast in China, as well as two low molecular weight fractions (64.5 and 28.2 kDa) resulting from degradation treatment, and incorporated them in the feed of rats on a hypercholesterolemic diet for 21 days to evaluate their antihyperlipidemic action (Pengzhan et al., 2003). The results indicated that ulvans with different molecular weights exhibited distinct effects on lipid metabolism. The high molecular weight ulvan reduced serum total cholesterol and Low Density Lipoprotein (LDL) cholesterol, whereas the low molecular weight fractions were effective in decreasing triglycerides and increasing High Density Lipoprotein (HDL) cholesterol. In comparison to the high molecular weight ulvan, the fractions were found to be more beneficial against hyperlipidemia associated with diabetes.

Ulvans isolated from *U. lactuca* collected from the Bulgarian Black sea coast and from cultivated *Ulva clathrata*, along with a mixture of ulvan with fucoidans isolated from *Cladosiphon okamuranus* have been reported to exhibit *in vitro* antiviral activity against a number of human and avian influenza viruses (Aguilar-Briseno et al., 2015; Ivanova et al., 1994; Shi et al., 2017). The effect was dose-dependent and strain-specific, the latter supporting the selectivity of the antiviral action (Ivanova et al., 1994). In an attempt to elucidate the mechanism of action, ulvans were found to inhibit viral fusion by interacting with the intact F0 protein but not with the mature F protein (Aguilar-Briseno et al., 2015; Shi et al., 2017). Furthermore, ulvans showed better anti-cell-cell fusion effects compared to fucoidans, while combined administration resulted in stronger effects. Likewise, a rhamnan-type polysaccharide isolated from *Monostroma nitidum* collected from Japan showed high and specific activity against herpes simplex virus (HSV-1) (Lee, Hayashi, Maeda, Hayashi et al., 2004), polysaccharide isolated from *Enteromorpha compressa*, also collected from Japan, did not show any anti-HSV-1 activity.

An ulvan-type polysaccharide isolated from the Korean green alga *Capsosiphon fulvescens*, in addition to its reported immunomodulatory properties (Na et al., 2010), exhibited significant anticoagulant activity (Synytsya et al., 2015). The results of the study suggested that the polysaccharide inhibited in a dose-dependent manner intrinsic and common pathways, but not extrinsic pathways, of the blood coagulation cascade and the thrombin activity or conversion of fibrinogen to fibrin. The high degree of *O*-3-sulphation of its glucuronic acid units increased the density of negative charge in this polysaccharide and thus enhanced its anticoagulant activity, almost reaching that of heparin and heparan sulphates. Additionally, a sulphated polysaccharide with high rhamnose content was isolated from the cell walls of *Monostroma latissimum* collected on the coast of Zhejiand province in China (Zhang et al., 2008). The parent polysaccharide and five H_2O_2 degradation products (725.4, 216.4, 123.7, 61.9, 26.0 and 10.6 kDa) were found to inhibit both the intrinsic and/or common pathways of coagulation and thrombin activity or conversion of fibrinogen to fibrin. The molecular weight of the sulphated polysaccharide obtained from *M. latissimum* had an important effect on the anticoagulant activity and a relatively high molecular weight was necessary to achieve thrombin inhibition. In contrast, a low molecular weight (5 kDa) ulvan-type sulphated polysaccharide isolated from *U. flacca* from the eastern coastal area of Zhoushan in China demonstrated significant anticoagulant activity (Li, Wen et al., 2018). It was presumed that it acts on the intrinsic coagulation pathway and that it affects thrombin activity or conversion of fibrinogen to fibrin (Qi et al., 2012). Compared to heparin, this ulvan-type polysaccharide possessed mild anticoagulant activity, similar to those of low molecular weight heparins (LMWHs) (Zhao et al., 2017). LMWHs, deriving from heparin by polydisperse depolymerization and having molecular weights under 6 kDa, despite their lower activity as compared to that of heparin, are widely used as anticoagulants, since they exert lower bleeding risk (Hirsh et al., 2001). Thus, this polysaccharide could be further investigated as an alternative to LMWHs. It is worth mentioning that anticoagulant activity was detected so far in ulvan-type polysaccharides isolated from algae belonging to the order Ulotrichales. Although, in the case of *M. latissimum* the anticoagulant activity positively correlates with the molecular weight increase, the other two active ulvan-type polysaccharides had notably lower molecular weights (5.0 and 5.8 kDa), thus resembling LMWHs. Recently, a series of polycarboxyl ulvans, produced *via* periodate-chlorite oxidation of ulvan, have been reported (de Carvalho et al., 2018), with anticoagulant activity 7.7–17.9 times higher than that of the parent ulvans, initially isolated from *U. fasciata* collected from Santa Catarina State in

Brazil. The increased number of carboxyl groups present in the polycarboxyl ulvans, regardless of their position, was positively correlated with the increased levels of anticoagulant activity.

The results of all studies on the biological activities of ulvan and ulvan-type polysaccharides suggest that they are affected by their sugar composition, molecular weight and sulphate content, characteristics that are defined by genetic, as well as environmental factors. Yet, no clear correlation has been established, since the carried biological studies have not been supported by systematic structure elucidation of the ulvans investigated.

6. Hybrid biomaterials

In the recent years, there is an ever increasing need for novel biomaterials with innovative properties for applications in the biomedical field, including systems for tissue engineering, regenerative medicine and drug delivery. Polysaccharides are considered potential candidates, not only because of their natural origin, inherent biocompatibility and biodegradability, but also due to their high availability at a relatively low cost. The presence of glycosidic bonds that can be easily cleaved by hydrolases, thus contributing to their biodegradation, in conjunction with the presence of negatively charged sulphate and carboxylate groups that potentiate polyelectrolyte behavior and permit functionalization, renders sulphated polysaccharide-based biomaterials valuable in the sector of pharmaceutical biotechnology. Additionally, chemical modification of these polymers is generally easily accomplished due to the abundant hydroxyl groups. Introduction of multiple functionalities into the polysaccharidic backbone facilitates the modification of the biopolymers' properties, enabling precise tailoring towards the envisaged objectives (Chiellini & Morelli, 2011; Cunha & Grenha, 2016).

The sulphated polysaccharide ulvan has been proven cytocompatible at concentrations up to 1.5 mg/mL (Alves et al., 2013b). Comparison to hyaluronic acid, used as a non-cytotoxic control, demonstrated the non-cytotoxic behavior of ulvan, suggesting that ulvan can be considered as non-toxic within the studied concentrations' range. As a consequence of the increasing knowledge on ulvan's chemical properties and biological activities, ulvan has attracted great attention and has been recently proposed as a potential biomaterial. Hitherto, the described ulvan-based structures, presented below in detail, include membranes, particles, hydrogels, 3D porous structures and nanofibers.

6.1. 2D structures: membranes and films

The use of ulvan for the preparation of polymeric membranes was first reported in 2012 by two individual groups (Alves, Pinho, Neves, Sousa, & Reis, 2012; Toskas et al., 2012). Ulvan membranes insoluble in water and stable at physiological conditions were prepared by employing chemical crosslinking, achieved by the epoxide 1,4-butanediol diglycidyl ether (BDDE), *via* the formation of ether bonds on ulvan's hydroxyl groups (Fig. 1A) (Alves, Pinho et al., 2012). The prepared membranes (Fig. 1B) revealed remarkable ability to uptake water (up to ~1800% of its initial dry weight) and increased mechanical performance related to the crosslinking. Moreover, using dexamethasone as a model drug, an initial steady release of the drug (nearly 49% within the first 8 h) was observed, followed by slower and sustained release for up to 14 days. The properties of ulvan membranes supported their potential use as drug delivery systems or as medicated wound dressings.

In another approach, anionic ulvan and cationic chitosan, polymers with oppositely charged backbones, were combined and novel supramolecular structures of stabilized membranes were formed through electrostatic interactions (Fig. 2A and B) (Toskas et al., 2012). The structure of the membranes was altered according to the weight ratio of the employed polyelectrolyte components. Weight ratio changes of the two polysaccharides resulted also to variations on the porosity. The observed excellent attachment and proliferation of the 7F2 osteoblasts to ulvan and ulvan/chitosan membranes (Fig. 2C–F) was attributed to the micro- / nanofibrous structure of these constructs, mimicking the fibrous part of the extracellular matrix. The combined properties of the ulvan/chitosan membranes were considered a promising basis for the development of scaffolds for cell cultivation.

Recently, ulvan dopant anions were employed for the electrochemical polymerization of poly(3,4-ethylenedioxythiophene) (PEDOT) films (Molino et al., 2018). PEDOT films incorporating ulvan produced composite materials (PEDOT-ULV) of the greatest surface roughness (Fig. 3A and B), and demonstrated significantly lower shear modulus values relative to all other PEDOT films examined. Ulvan was proven a suitable biopolymer for the doping of PEDOT films, showing appropriate biocompatibility and electrochemical properties. Moreover, electrical stimulation of PC-12 cells on PEDOT-ULV has shown that this significantly enhances cell differentiation (Fig. 3C–F), further demonstrating the feasibility of using large naturally occurring polysaccharide molecules as dopants in PEDOT biomaterials for cell and tissue electrical stimulation.

6.2. Nano- and microparticles

The development of particulate carriers has opened new avenues for the design of novel drug delivery systems with improved pharmacokinetic and pharmacodynamic properties. In comparison to conventional dosage forms, particulate delivery systems exhibit many advantages, such as availability for delivery through various routes of administration, tailoring of particle size and surface characteristics and, even the potential for controlled and sustained release of the drug at the sites of interest (Nikam, Mukesh, & Haudhary, 2014).

Sulphated polysaccharides have been used for the production of nanoparticles and microparticles, mainly because of their ionic nature. The interaction of oppositely charged polyelectrolytes results in the formation of complexes that can be utilized in the production of nanosized drug carriers, since polyelectrolyte complexes allow the association of drugs with the polymer matrix at a molecular level (Lankalapalli & Kolapalli, 2009). Drug encapsulation in such structures is accomplished either during precipitation of the complex, or through absorption on the already formed complexes. Alternatively, the drug can be chemically linked to the polymers and thus incorporated in the complex. In such cases, the drug can be released from the complex either by ion exchange mechanisms, charge interactions, or by polymer degradation and dissolution of the complex (Liu, Jiao, Wang, Zhou, & Zhang, 2008; Saravanakumar, Jo, & Park, 2012).

In this perspective, and in order to produce a novel scaffold for bone tissue engineering applications, the combination of ulvan with poly-d,l lactic acid (PDLLA) has been reported (Alves, Duarte, Mano, Sousa, & Reis, 2012). Initially, ulvan-based microparticles (Fig. 4A) were produced employing the extrusion–dripping method, by a drop-wise addition of an aqueous ulvan solution to a chitosan solution, affording after thorough washing and drying the stabilized ulvan-based particles. 3D scaffolds of PDLLA loaded with ulvan-based particles were prepared by subcritical fluid sintering with CO_2. The incorporation of these particles within the PDLLA matrix resulted in a scaffold with desirable morphometric features, suitable mechanical performance and adequate cytocompatibility. Furthermore, entrapment of the ulvan-based particles loaded with dexamethasone as a model drug within the PDLLA matrix was used for proving the applicability of this scaffold as a system capable to sustain the delivery of relevant bioactive agents. The observed sustained release of dexamethasone from ulvan-based particles embedded within the PDLLA matrix (Fig. 4B) revealed the potential use of the designed systems for localized drug delivery.

Taking into consideration ulvan's polyelectrolyte properties, in another study ulvan was allowed to interact with the positively charged lysozyme, a ubiquitous enzyme responsible for bacterial cell wall lysis (Tziveleka et al., 2018). A series of lysozyme/ulvan complexes were prepared under various charge ratios at physiological pH, the size of which was found to depend on the charge ratio employed, with the

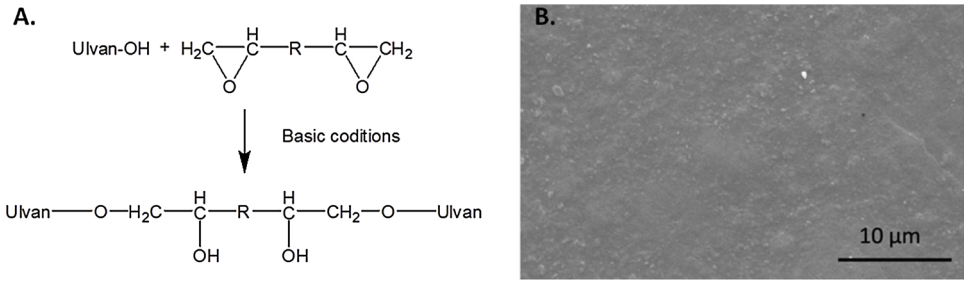


Fig. 1. (A) Structure of the fully BDDE crosslinked ulvan polysaccharide. (B) Surface topography of produced crosslinked ulvan membranes (magnification 3000×). Adapted from Alves, Pinho et al., 2012 with permission from Elsevier.

highest complexation efficiency being observed for charge ratios close to 1 (+/-), while the protein structure of lysozyme after complexation was retained. At the charge ratio of 0.8 full complexation occurred, resulting in a nanoparticulate structure of 186 nm size. Bacterial growth studies showed that lysozyme, once complexed with ulvan, not only maintained its antibacterial activity against the Gram positive strain *Staphylococcus aureus*, but actually exhibited increased levels of activity, thus highlighting the potential of ulvan as a particulate nanocarrier for positively charged bioactive molecules.

6.3. Hydrogels

Hydrogels are crosslinked 3D networks of hydrophilic polymer chains, capable of absorbing large quantities of water and consequently swelling extensively in water media. Since water is the most abundant component of the human body, hydrogels are considered as promising biomaterials for biomedical applications (Chai, Jiao, & Yu, 2017; Venkatesan et al., 2015). Therefore, the applicability of hydrogels has been investigated in the fields of tissue engineering, drug delivery, self-healing materials, biosensors, and hemostasis bandages. Specifically, they have attracted considerable attention as drug delivery systems, mainly because of their highly porous structure that permits drug loading into the gel matrix and subsequent diffusion coefficient-dependent drug release through the gel network. Hydrogels, once in contact with water or other biological fluids, swell without dissolving and can be easily manipulated by controlling the crosslinking extent in the gel matrix (Hoare & Kohane, 2008). Hydrogels are advantageous when compared with other types of biomaterials because of their increased biocompatibility, tunable biodegradability, and porous structure. However, due to the low mechanical strength and fragile nature of the hydrogels, their applicability is limited and novel hydrogels with stronger and more stable properties are still needed.

Regarding ulvan, one exceptionally interesting feature is its ability to form thermoreversible gels in the presence of B^{+} and Ca^{+2} ions at a pH between 7.5 and 8.0. Therefore, in order to exploit its inherent gel forming ability, ulvan derivatives containing functional groups sensitive to UV photopolymerization were successfully prepared using different methacryloyl precursors [methacrylic anhydride (MA) or glycidyl methacrylate (GMA)] (Morelli & Chiellini, 2010). The MA-based ulvan hydrogels were proven to be stable under physiological conditions, while the UV crosslinking of GMA-based ulvan resulted to unstable hydrogels. These materials were considered as appropriate matrices for cell encapsulation due to the radical quenching ability of ulvan (Qi, Zhang et al., 2005) and the protection it can offer against the radicals produced during UV crosslinking. The observed softness of such materials is fundamental for the preparation of cytocompatible scaffolds, since soft matrices are expected to promote cell spreading.

Following another approach to develop thermosensitive hydrogels based on ulvan, poly(*N*-isopropylacrylamide) (pNIPAAm) was grafted onto the polysaccharide backbone (Fig. 5A) (Morelli, Betti, Puppi, & Chiellini, 2016). pNIPAAm is a thermosensitive polymer and its aqueous solutions exhibit phase transitions from solution to gel at a lower critical solution temperature of about 32 °C (close to the human body temperature), attributed to the structural rearrangement of the polymeric chains (Schild, 1992). The difference between the body and room temperature is considered an excellent stimulus commonly used for the engineering of *in situ* gelling systems. In this context, pNIPAAm was grafted onto ulvan by UV irradiation-induced radical polymerization of *N*-isopropylacrylamide onto pendant acryloyl groups conjugated to the polysaccharide to act as chain initiator. The thermal behavior and the rheological properties of the developed materials satisfied the necessary requirements for *in situ* gelling systems for biomedical applications (Fig. 5B).

In the same year Yoshimura and coworkers reported the preparation of biodegradable hydrogels based on the crosslinking of ulvan with divinylsulfone (DVS) under alkaline conditions (Yoshimura, Hirao, & Fujioka, 2016). Gelation was observed consistently when ulvan obtained at 80 °C was crosslinked with DVS, but not always when ulvan obtained at room temperature was used. Both biodegradation rate and water absorbency of hydrogels were found to depend on the feed amount of DVS, with the highest water absorbency being observed for the sample with DVS feed ratio of 20% (w/w). Maximum absorbency in pure water, however, was 80 g/g, which is considerably lower than that of the commercially available crosslinked sodium polyacrylate (300 g/g).

6.4. 3D porous structures

A 3D porous structure with stability in physiological conditions, controlled water-uptake ability and improved mechanical properties was developed by Alves and coworkers, to be applied as a medical device, particularly for tissue engineering applications (Alves et al., 2013c). For the chemical modification, BDDE, an agent widely used to crosslink hyaluronic acid for the production of commercially available stable fillers for dermatological purposes (Baumann, 2009), was employed due to its acceptability and applicability in biomedical, pharmaceutical and cosmetic applications. The produced ulvan matrices (Fig. 6) after lyophilization presented variable mechanical properties, with a remarkable ability to uptake water (up to 2000% of its initial dry weight) and were characterized by a highly porous and interconnected structure (Fig. 6). These ulvan-based substrates underwent *in vitro* nontoxic degradation, as confirmed by the fact that mouse fibroblast cells remained viable in the presence of the matrices during various culture time intervals. According to the authors of this report, these ulvan structures can be used as prospective blocks that could be further chemically modified in order to obtain the desired stability and biological interactivity for tissue engineering scaffolds.

In another study, the role of enzymatic mineralization on polymeric structures bearing anionic groups was investigated (Dash et al., 2014), since anionic proteins are known to play a significant role in mineral phase deposition on bones and affect mineralization in simulated body fluids. In general, mineralization offers advantages, such as enhancement of bioactivity post implantation, osteoblastic differentiation through increased stiffness and enhanced binding of growth factors

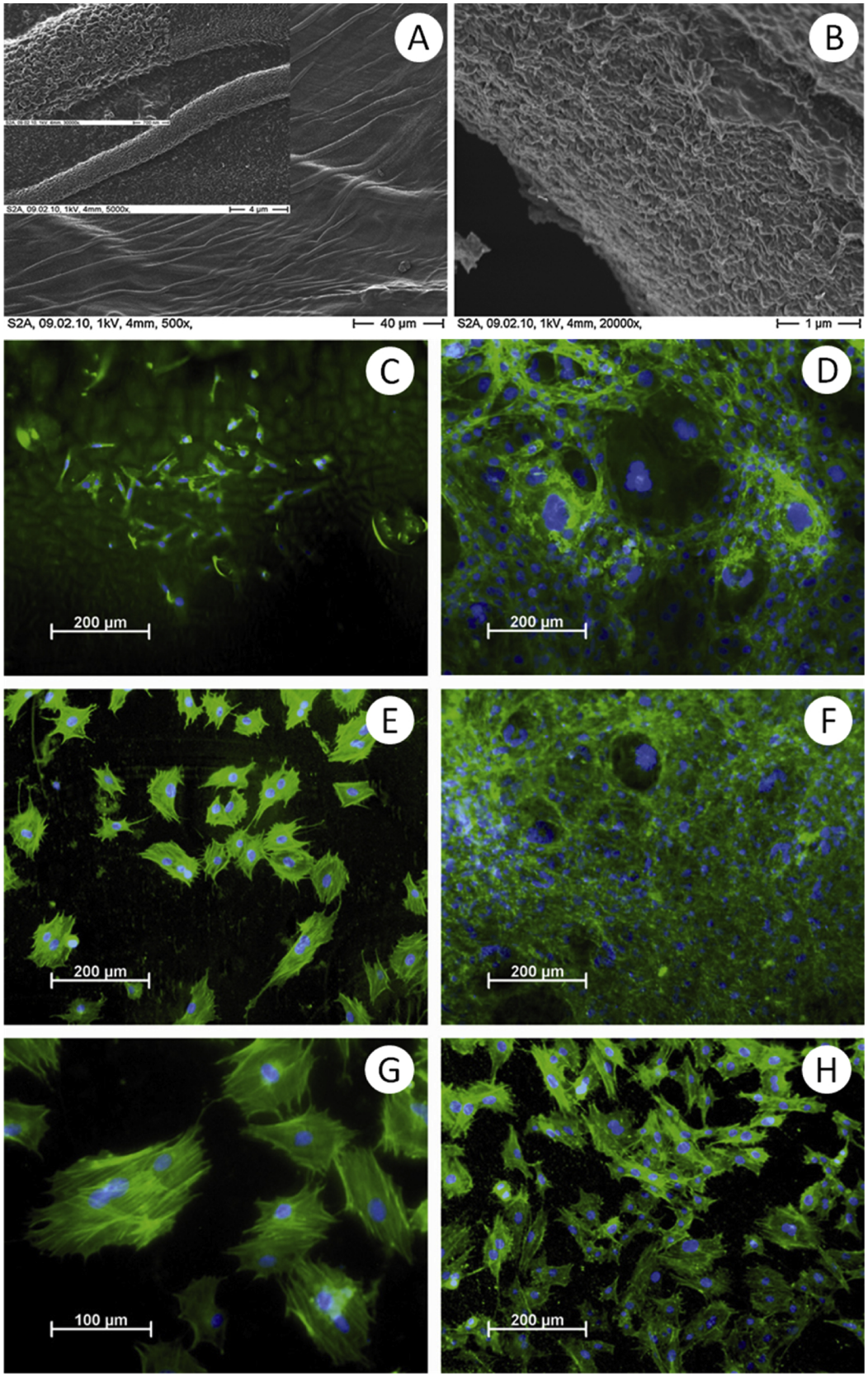


Fig. 2. (A) Rough surface with open pores on fibrous structured membranes from a ratio of ulvan/chitosan 1:2 (magnification 500×; inserts: magnification 5000× and 30,000×). (B) Porous cross-section (magnification 20,000×). (C) Fluorescence microscopic images of 7F2-cells on chitosan-film after 6 days culture time; (D) ulvan-film after 6 days; (E) ulvan/chitosan-film (4:5) after 1 day and (F) 6 days culture time; (G) ulvan/chitosan-film (4:5) after 6 days, more detailed image; (H) pure titanium after 1 day as a reference. Adapted from Toskas et al., 2012 with permission from Elsevier. (The reader is referred to the web version of this article for a coloured version of Fig. 2).

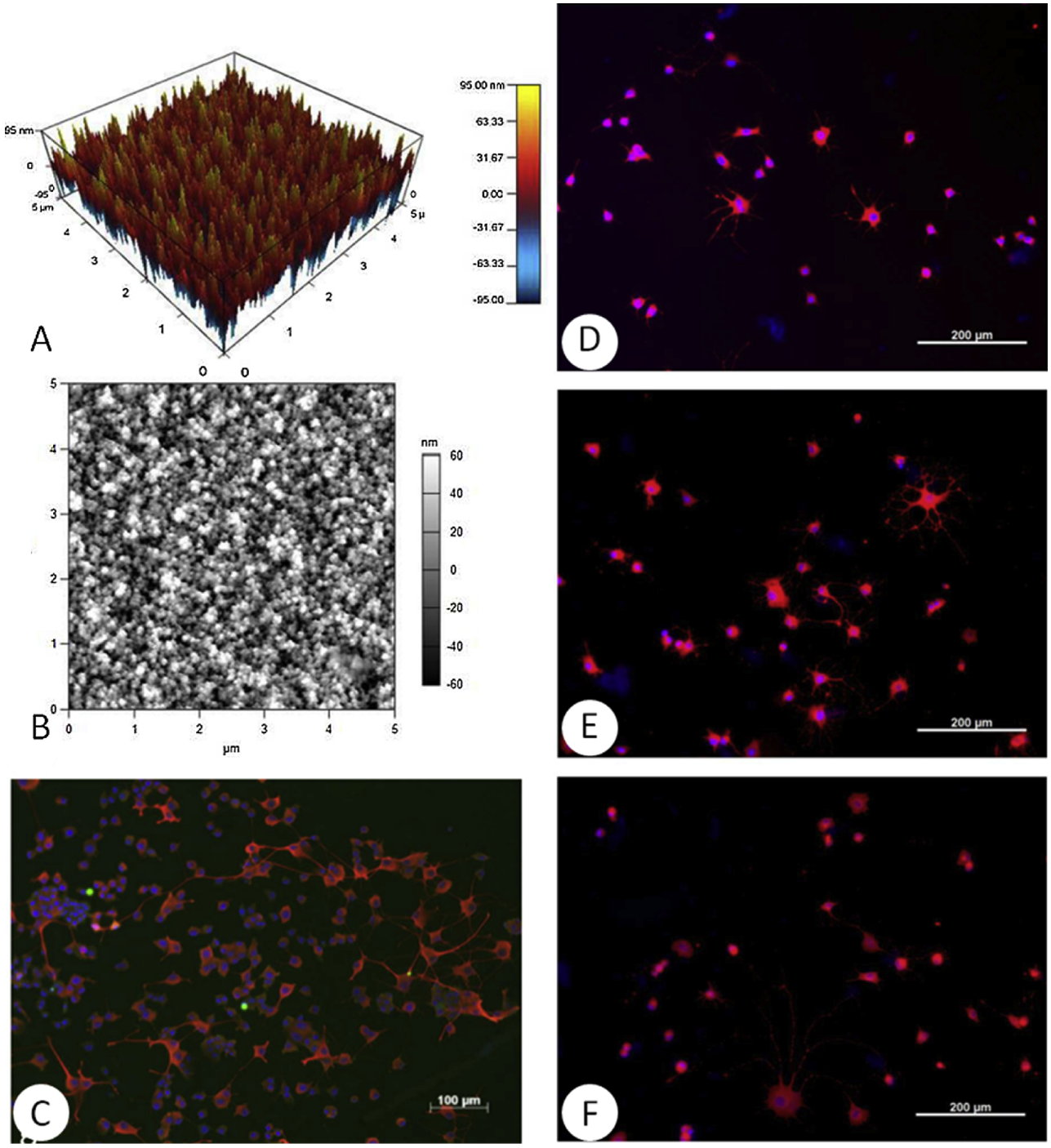


Fig. 3. (A, B) Representative 2D and 3D High Magnification (5 µm × 5 µm) AFM images of poly(3,4-ethylenedioxythiophene)-ulvan composite materials (PEDOT-ULV). Color coded scale bars for both 2D and 3D images are to right of figure. (C) Immunocytochemical characterization of PC-12 cells on PEDOT-ULV substrates following 5 days of cell differentiation (scale bar represents 100 µm). Immunocytochemical characterization of PC-12 cells on stimulated and unstimulated PEDOT-ULV substrates and TCP control (scale bar represents 100 µm). Cells were under stimulated conditions for 8 h per 24 h for 3 days in an incubator at 37 °C with a humidified 5% CO_2 environment. Substrates: (D) TCP control, (E) PEDOT-ULV, (F) Stimulated PEDOT-ULV. Immunostaining markers include; βIII tubulin (red) for neuron-specific cytoplasmic staining, DAPI (blue) for nuclear staining and GAP43 (green) for neural growth cone staining. Adapted from Molino et al., 2018 with permission from The Royal Society of Chemistry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

which stimulate bone healing. In this context, photo-crosslinked polymeric ulvan scaffolds were initially produced and subsequently enzymatically treated for further calcium phosphate deposition. The crosslinked ulvan scaffolds were treated with alkaline phosphatase (ALP) and mineral formation was successfully induced (Fig. 7A–D), leading to homogeneous mineralization at ambient temperature, while the formed minerals were proven to contain apatite. The mineralized scaffolds were non-toxic and the presence of the minerals improved the activity of osteogenic cells (Fig. 7E and F), thus suggesting their potential application as resorbable bone graft substitutes.

The same group recently evaluated the activity of osteogenic cells in scaffolds based on polyelectrolyte complexes of chitosan and ulvan,

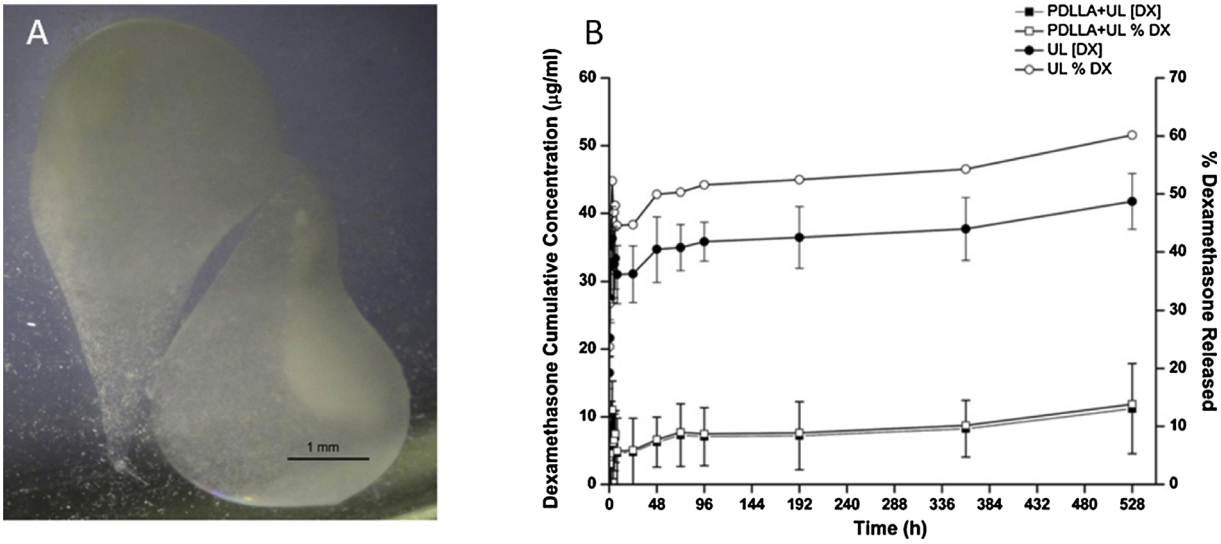


Fig. 4. (A) Magnified ulvan particles loaded with dexamethasone presenting a tear-drop like morphology: Diameter ~3 mm, height ~4.5–5 mm. (B) Dexamethasone concentration and percentage of drug released from the produced scaffolds and ulvan particles. Adapted from Alves, Pinho et al., 2012 with permission from Elsevier.

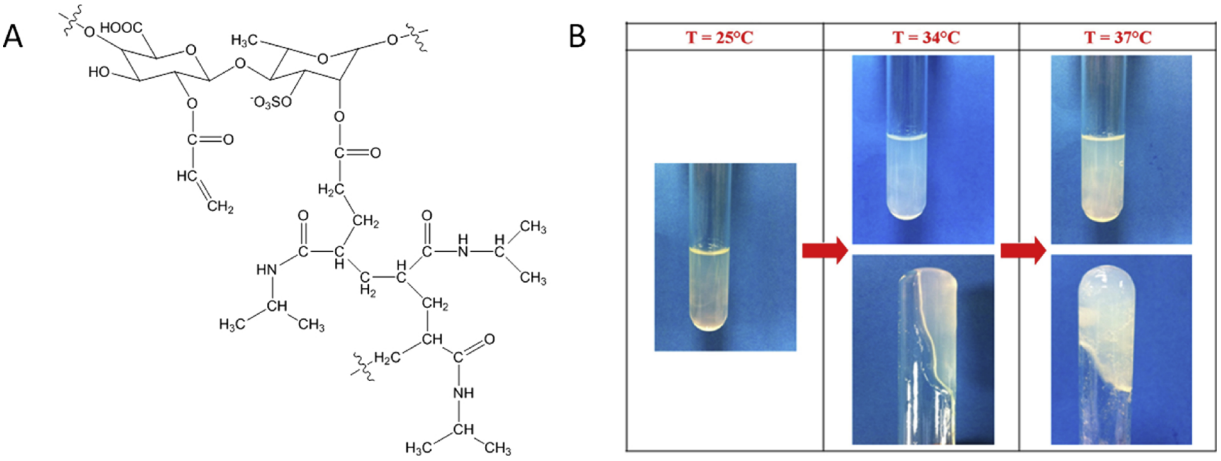


Fig. 5. (A) Structure of the poly (*N*-isopropylacrylamide)-*g*-Ulvan (UA-NIPAAm) copolymer. (B) Thermogelling behavior of UA-NIPAAm solution (4%, in 10 mM PBS, pH 7.4) tested by tilting method at different temperatures. Adapted from Morelli et al., 2016 with permission from Elsevier. (The reader is referred to the web version of this article for a coloured version of Fig. 5).

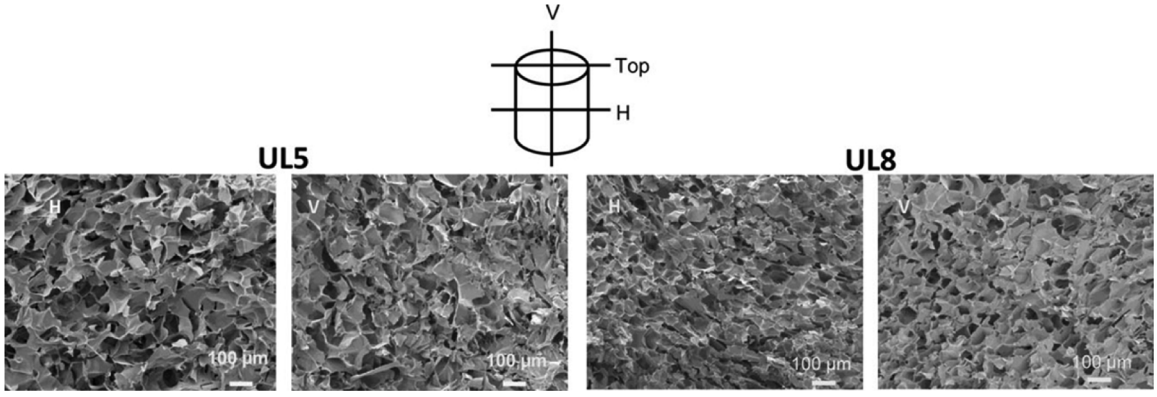


Fig. 6. Scanning electron microscopy micrographs of the two crosslinked ulvan-produced structures (UL5 and UL8) from different perspectives—top: top view; H: horizontal cross-section; V: vertical cross-section. (magnification 250×). Adapted from Alves et al., 2013c with permission from John Wiley and Sons.

further functionalized by employing ALP as mineralization inducer (Dash et al., 2018). Scaffolds of the polyelectrolyte complexes were subjected to ALP and successful apatitic mineral formation was achieved with the deposited minerals observed as globular structures, which promoted cell attachment, proliferation and extracellular matrix formation. These polyelectrolyte complexes and their successful calcium phosphate-based mineralization offer a greener route towards the development of resorbable materials for tissue engineering.

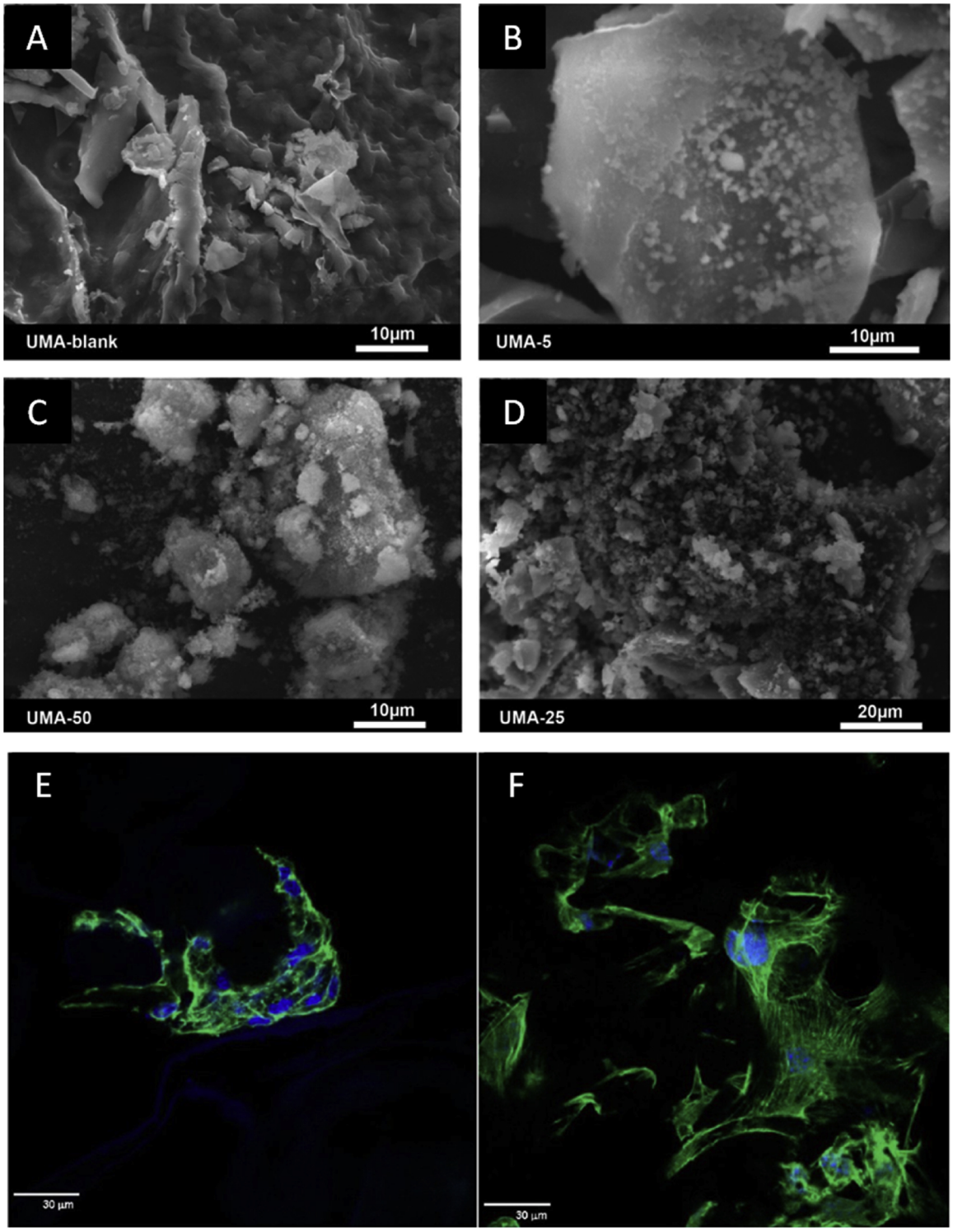


Fig. 7. SEM images of UMA scaffolds with increasing concentration of ALP (A to D). CLSM micrographs of MC3T3 $\times 10^{-1}$ cells cultured on (E) UMA blank scaffolds and on (F) UMA 5 scaffolds at 20× magnification. Adapted with permission from Dash et al. (2014). Biofunctionalization of ulvan scaffolds for bone tissue engineering. *ACS Applied Materials & Interfaces*, *6*, 3211 − 3218. Copyright 2014 American Chemical Society. (The reader is referred to the web version of this article for a coloured version of Fig. 7).

Pires and coworkers employed ulvan for the development of a polyacrylic acid (PAA)-free glass-ionomer bone cements formulation (Barros et al., 2013). With this aim, carboxymethylation of both chitosan, extracted from the squid-pen *Loligo forbesis*, and ulvan, extracted from *U. lactuca*, was performed, since acidity enhancement is believed to contribute to faster curing kinetics of glass-ionomer bone cements and improved mechanical performance, in comparison to those of the unmodified polysaccharides. A high degree of carboxymethylation of ulvan and chitosan (98% and 87%, respectively) was achieved and mixtures of these modified polysaccharides were applied in the formulation of PAA-free glass-ionomer bone cements. The inclusion of carboxymethylated ulvan in the cement formulation, as shown by

mechanical and *in vitro* bioactivity tests, enhanced its mechanical performance, generating non-cytotoxic cements and inducing the diffusion of Ca- and/or P-based moieties from the surface to the bulk of the cements.

6.5. Nanofibers

Nanofibers represent another nanosized carrier with potential biomedical applications. Due to their large surface-to-volume ratio, nanofibers are considered appropriate substrates in applications where high porosity is desirable. Unlike conventional rigid porous structures, nanofibrous structures are dynamic systems where the pore size and shape can change and either a flexible or a rigid crosslinked structure can be prepared. The most versatile process for producing nanofibers with relatively high productivity is electrospinning that produces porous, nanofibrous matrices appropriate for numerous applications. Generally, nanofibers find applications in tissue engineering, as well as the encapsulation and controlled release of drugs, growth factors or other bioactive molecules.

The poor rheological properties of ulvan solutions, in combination with its limited solubility in various solvent systems, restrict its successful electrospinning for the formation of fibers, when ulvan is used as the sole polymer. Thus, improvement of the rheological properties and the charge carrying capacity of the ulvan electrospinning solution are crucial for fiber formation.

The first fabrication of ulvan-based nanofibers was reported by Toskas et al. in 2011 who successfully electrospun ulvan blended with poly(vinyl alcohol) (PVA) in the presence of boric acid and Ca^{+2} ions to obtain uniform nanofibers with diameters as small as 84 nm (Fig. 8A and B) (Toskas et al., 2011). The produced nanofibers were described as a nonwoven membrane without interconnections that presented a high degree of orientation, attributed to the presence of ulvan.

Subsequently, the preparation of electrospun ulvan-based nanofibers blended in different ratios with biodegradable polymers, such as polycaprolactone (PCL) and polyethylene oxide (PEO) was reported (Kikionis et al., 2015). Morphological examination of these biocomposite nanofibers showed that the fiber average diameters decreased when the ulvan content increased, while the strong interaction and good compatibility between ulvan and the two copolymers was observed. It is worth-noting that blends of ulvan/PCL spinning solutions yielded highly interconnected spider-web-like structures (Fig. 8C), whereas ulvan/PEO afforded spindle-like nanofibers (Fig. 8D).

6.6. Other drug delivery systems

Matrix tablets, one of the most conventional formulations, are widely used for the modification of sustained release of drugs. In the last decade the inclusion of simple excipients as inert substances in the formulations has changed and the approach of multifunctional compounds has been adopted. To this end, new excipients are expected not only to contribute on the stabilization and controlled release of drugs, but also to provide biocompatibility and targeting ability. Ulvan's presence in such matrices can contribute benefits derived from its bioactivities instead of being a plain inactive matrix material (Gupta, 2001).

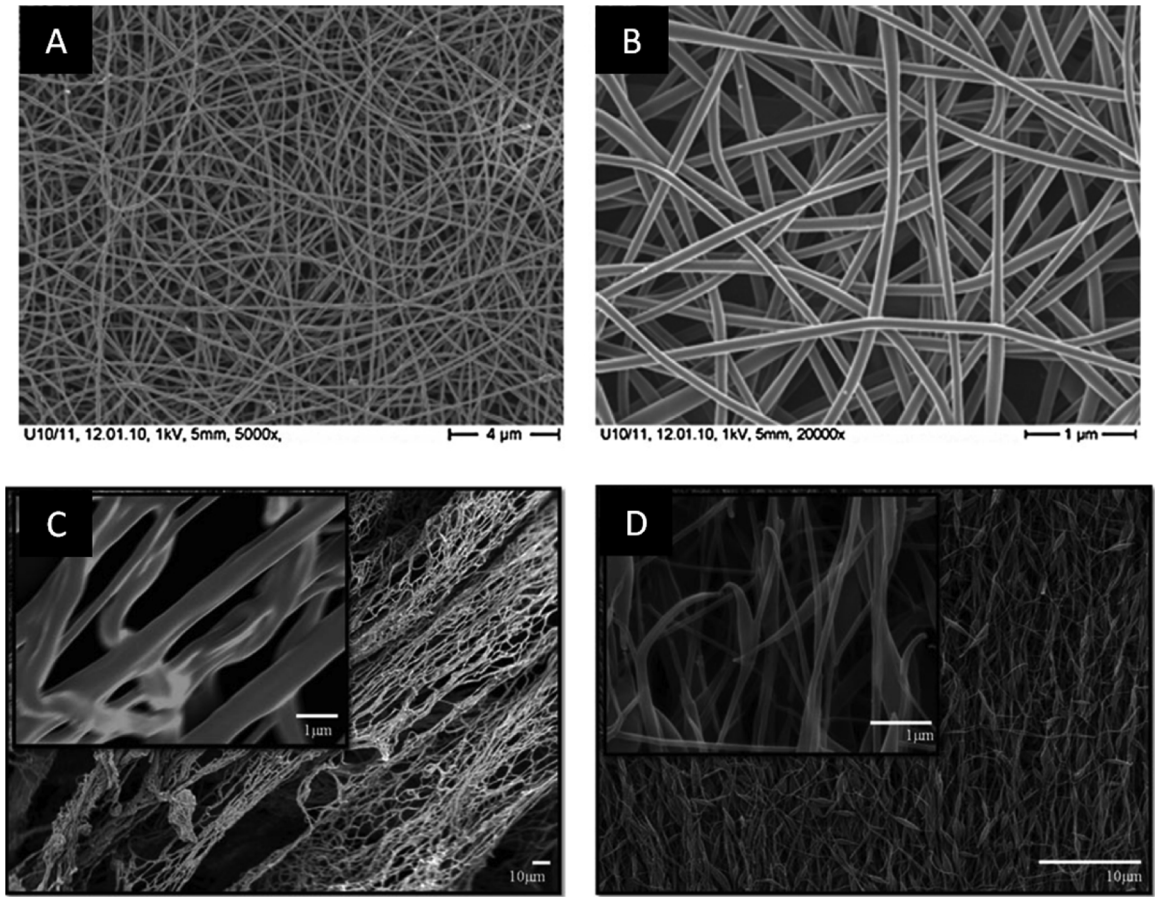


Fig. 8. SEM images of fibers of (A, B) ulvan/PVA (1:1). Adapted from Toskas et al., 2011 with permission from Elsevier, (C) ulvan/PCL (3:8) and (D) ulvan/PEO (1:1). Adapted from Kikionis et al., 2015 with permission from John Wiley and Sons.

In this context, ulvan was used as a formulant in hydrophilic matrix systems, containing the chronobiotic hormone melatonin (MLT) (Vlachou et al., 2018). The MLT's *in vitro* modified release profile in gastrointestinal-like fluids was probed and the release profile of MLT from ulvan-based tablets was compared to that of the market drug Circadin® at pH 1. The observed release of most of the ulvan-based formulations was relatively higher than that of the commercial drug and followed a sigmoidal pattern which denotes that the drug release is controlled by polymer relaxation and/or erosion.

Recently, ulvan extracted from *U. armoricana* was employed as an active ingredient in the preparation and stabilization of silver nanoparticles by means of eco-friendly processes (Massironi et al., 2019). Ulvan provided great stability to the synthesized silver nanoparticles which was significantly higher when compared to traditional stabilizers used in commercially available formulations, such as citric acid, due to the formation of a thick polysaccharide shell around the inorganic nanoparticles-based core. The strong and fast antibacterial action of ulvan-stabilized silver nanoparticles against clinically relevant Gram-positive and Gram-negative bacteria suggested that ulvan can be useful for the preparation of antimicrobial materials for cosmetic and biomedical applications.

The very first study to produce hybrid ulvan-based materials described the combination of montmorillonite with ulvan (Laza et al., 2007) in order to develop new exfoliated and intercalated products with innovative properties. Depending on the drying process, air- or freeze-drying, ulvan was inserted in the interlayer space or adsorbed on both sides of the inorganic layers. The crystallization of water molecules bound to ulvan induced the delamination of the layers during lyophilization. The adsorption of ulvan in Na-montmorillonite and the formation of nanocomposites *via* clays delamination are considered suitable for nanotechnological applications (pharmaceuticals, protective materials, or the cosmetic industry).

7. Conclusions

Ulvan is a polysaccharide of great versatility and potential. The dependence of its physicochemical properties and biological activities on its structural features, such as molecular size and degree of branching, which in turn depend on the algal raw biomass (species and geographic origin) and extraction / isolation conditions, is acknowledged. Nevertheless, the exact correlations remain unclear, due to the lack of systematic studies on the structure-activity/properties relationships of structurally well-characterized ulvan. The significant biological activities of ulvan, in combination with its tunable physicochemical and rheological properties have triggered high interest for its utilization in hybrid biomaterials. Furthermore, taking into account its natural origin, the abundant and renewable sources, as well as the ease of functionalization of its hydroxyl and carboxyl groups, ulvan can be considered as a versatile functional material for tailor-made applications in the field of biomedicine. Towards this end, polysaccharide modification, processing and material design and/or material–cell interactivity is required in order to achieve the successful development of novel biomaterials. So far, ulvan has been used as is or after complexation, crosslinking or chemical modification for the development of hybrid materials of various structures, such as membranes, particles, hydrogels, 3D porous structures or nanofibers, aiming towards drug delivery or tissue engineering applications. Nevertheless, a thorough investigation concerning the biocompatibility and applicability of the developed hybrid biomaterials contextualized with specific targets is required prior to any targeted therapeutic application.

Acknowledgements

The present work was partially co-funded by the European Union and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH-CREATE-INNOVATE" (project code: T1EΔK-00232, BIOALGAFOOD).

References

Abd El-Baky, H. H., El-Baz, F. K., & El-Baroty, G. S. (2009). Potential biological properties of sulphated polysaccharides extracted from the macroalgae *Ulva lactuca* L. *Academic Journal of Cancer Research, 2*, 1–11.
Abdel-Fattah, A. F., & Edrees, M. (1972). A study on the polysaccharide content of *Ulva lactuca* L. *Qualitas Plantarum et Materiae Vegetabiles, 22*, 15–22.
Abdel-Fattah, A. F., & Edrees, M. (1973). Seasonal changes in the constituents of *Ulva lactuca*. *Phytochemistry, 12*, 481–485.
Abdel-Fattah, A. F., & Sary, H. H. (1987). Glycoproteins from *Ulva lactuca*. *Phytochemistry, 26*, 1447–1448.
Abd-Ellatef, G.-E. F., Ahmed, O. M., Abdel-Reheim, E. S., & Abdel-Hamid, A. –H. Z. (2017). *Ulva lactuca* polysaccharides prevent Wistar rat breast carcinogenesis through the augmentation of apoptosis, enhancement of antioxidant defense system, and suppression of inflammation. *Breast Cancer - Targets and Therapy, 9*, 67–83.
Aguilar-Briseno, J. A., Cruz-Suarez, L. E., Sassi, J.-F., Ricque-Marie, D., Zapata-Benavides, P., Mendoza-Gamboa, E., et al. (2015). Sulphated polysaccharides from *Ulva clathrata* and *Cladosiphon okamuranus* seaweeds both inhibit viral attachment/entry and cell-cell fusion, in NDV Infection. *Marine Drugs, 13*, 697–712.
Alves, A., Caridade, S. G., Mano, J. F., Sousa, R. A., & Reis, R. L. (2010). Extraction and physicochemical characterization of a versatile biodegradable polysaccharide obtained from green algae. *Carbohydrate Research, 345*, 2194–2200.
Alves, A., Duarte, A. R. C., Mano, J. F., Sousa, R. A., & Reis, R. L. (2012). PDLLA enriched with ulvan particles as a novel 3D porous scaffold targeted for bone engineering. *The Journal of Supercritical Fluids, 65*, 32–38.
Alves, A., Pinho, E. D., Neves, N. M., Sousa, R. A., & Reis, R. L. (2012). Processing ulvan into 2D structures: Crosslinked ulvan membranes as new biomaterials for drug delivery applications. *International Journal of Pharmaceutics, 426*, 76–81.
Alves, A., Sousa, R. A., & Reis, R. L. (2013a). A practical perspective on ulvan extracted from green algae. *Journal of Applied Phycology, 25*, 407–424.
Alves, A., Sousa, R. A., & Reis, R. L. (2013b). *In vitro* cytotoxicity assessment of ulvan, a polysaccharide extracted from green algae. *Phytotherapy Research, 27*, 1143–1148.
Alves, A., Sousa, R. A., & Reis, R. L. (2013c). Processing of degradable ulvan 3D porous structures for biomedical applications. *Journal of Biomedical Materials Research Part B, Applied Biomaterials, 101A*, 998–1006.
Barros, A. A. A., Alves, A., Nunes, C., Coimbra, M. A., Pires, R. A., & Reis, R. L. (2013). Carboxymethylation of ulvan and chitosan and their use as polymeric components of bone cements. *Acta Biomaterialia, 9*, 9086–9097.
Baumann, L. (2009). *Cosmetic dermatology, principles and practice.* New York: McGraw-Hill.
Bemiller, J. N. (1967). Acid-catalyzed hydrolysis of glycosides. *Advances in Carbohydrate Chemistry, 22*, 25–208.
Berri, M., Olivier, M., Holbert, S., Dupont, J., Demais, H., Le Goff, M., et al. (2017). Ulvan from *Ulva armoricana* (Chlorophyta) activates the PI3K/Akt signaling pathway *via* TLR4 to induce intestinal cytokine production. *Algal Research, 28*, 39–47.
Berri, M., Slugocki, C., Olivier, M., Helloin, E., Jacques, I., Salmon, H., et al. (2016). Marine-sulfated polysaccharides extract of *Ulva armoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. *Journal of Applied Phycology, 28*, 2999–3008.
Blomster, J., Maggs, C. A., & Stanhope, M. J. (1998). Molecular and morphological analysis of *Enteromorpha intestinalis* and *Enteromorpha compressa* (Chlorophyta) in the British Isles. *Journal of Phycology, 34*, 319–340.
Bourne, E. J., Megarry, M. L., & Percival, E. (1974). Carbohydrates metabolised by the green seaweed *Urospora penicilliformis*. *Journal of Carbohydrates Nucleosides and Nucleotides, 1*, 235–264.
Brading, J. W. E., Georg-Plant, M. M. T., & Hardy, D. M. (1954). The polysaccharide from the alga *Ulva lactuca*. Purification, hydrolysis, and methylation of the polysaccharide. *Journal of the Chemical Society*, 319–324.
Bryhni, E. (1978). Quantitative differences between polysaccharide compositions in normal differentiated *Ulva mutabilis* and the undifferentiated mutant lumpy. *Phycologia, 17*, 119–124.
Bussy, F., Le Goff, M., Salmon, H., Delaval, J., Berri, M., & Collén, P. N. (2019). Immunomodulating effect of a seaweed extract from *Ulva armoricana* in pig: Specific IgG and total IgA in colostrum, milk, and blood. *Veterinary and Animal Science, 7*, 100051.
Cardoso, M. J., Costa, R. R., & Mano, J. F. (2016). Marine origin polysaccharides in drug delivery systems. *Marine Drugs, 14*, 34.
Carlberg, G. E., & Percival, E. (1977). The carbohydrates of the green seaweeds *Urospora wormskioldii* and *Codiolum pusillum*. *Carbohydrate Research, 57*, 223–234.
Chai, Q., Jiao, Y., & Yu, X. (2017). Hydrogels for biomedical applications: Their characteristics and the mechanisms behind them. *Gels, 3*, 6–20.
Chattopadhyay, K., Mandal, P., Lerouge, P., Driouich, A., Ghosal, P., & Ray, B. (2007). Sulphated polysaccharides from Indian samples of *Enteromorpha compressa* (Ulvales, Chlorophyta): Isolation and structural features. *Food Chemistry, 104*, 928–935.
Chiellini, F., & Morelli, A. (2011). Ulvan: A versatile platform of biomaterials from renewable resources. In R. Pignatello (Ed.). *Biomaterials - Physics and chemistry* ISBN: 978-953-307-418-4, InTech.
Coat, G., Dion, P., Noailles, M. C., De Reviers, B., Fontaine, J. M., Berger-Perrot, Y., et al. (1998). *Ulva armoricana* (Ulvales, Chlorophyta) from the coasts of Brittany (France). II. Nuclear rDNA ITS sequence analysis. *European Journal of Phycology, 33*, 81–86.
Conrad, H. E. (1980). The acid lability of the glycosidic bonds of L-iduronic acid residues

in glycosaminoglycans. *The Biochemical Journal, 191*, 355–363.
Costa, C., Alves, A., Pinto, P. R., Sousa, R. A., Da Silva, E. A. B., Reis, R. L., et al. (2012). Characterization of ulvan extracts to assess the effect of different steps in the extraction procedure. *Carbohydrate Polymers, 88*, 537–546.
Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs, 14*, 42.
D'Ayala, G. G., Malinconico, M., & Laurienzo, P. (2008). Marine derived polysaccharides for biomedical applications: Chemical modification approaches. *Molecules, 13*, 2069–2106.
Dash, M., Samal, S. K., Bartoli, C., Morelli, A., Smet, P. F., Dubruel, P., et al. (2014). Biofunctionalization of ulvan scaffolds for bone tissue engineering. *ACS Applied Materials & Interfaces, 6*, 3211–3218.
Dash, M., Samala, S. K., Morelli, A., Bartoli, C., Declercq, H. A., Douglas, T. E. L., et al. (2018). Ulvan-chitosan polyelectrolyte complexes as matrices for enzyme induced biomimetic mineralization. *Carbohydrate Polymers, 182*, 254–264.
de Araújo, I. W. F., Rodrigues, J. A. G., Quinderé, A. L. G., Silva, J. D. F. T., Maciel, G. D. F., Ribeiro, N. A., et al. (2016). Analgesic and anti-inflammatory actions on bradykinin route of a polysulfated fraction from alga *Ulva lactuca*. *International Journal of Biological Macromolecules, 92*, 820–830.
de Carvalho, M. M., de Freitas, R. A., Ducatti, D. R. B., Ferreira, L. G., Gonçalves, A. G., Colodi, F. G., et al. (2018). Modification of ulvans *via* periodate-chlorite oxidation: Chemical characterization and anticoagulant activity. *Carbohydrate Polymers, 197*, 631–640.
De Reviers, B., & Leproux, A. (1993). Characterization of polysaccharides from *Enteromorpha intestinalis* (L.) Link, Chlorophyta. *Carbohydrate Polymers, 22*, 253–259.
Delattre, C., Michaud, P., Keller, C., Elboutachfaiti, R., Beven, L., Courtois, B., et al. (2006). Purification and characterization of a novel glucuran lyase from *Trichoderma* sp. GL2. *Applied Microbiology and Biotechnology, 70*, 437–443.
Fleurence, J. (1991). L'habilitation des algues en alimentation humaine: Le point sur la réglementation française. *Industries Alimentaires et Agricoles, 108*, 501–502.
Fransson, L. A., Roden, L., & Spach, M. L. (1968). Automated ion-exchange chromatography of uronic acids and uronic acid containing oligosaccharides. *Analytical Biochemistry, 23*, 317–330.
Germershaus, O., Lühmann, T., Rybak, J.-C., Ritzer, J., & Meinel, L. (2015). Application of natural and semi-synthetic polymers for the delivery of sensitive drugs. *International Materials Reviews, 60*, 101–130.
Gosselin, C. C., Holt, A., & Lowe, P. A. (1964). The polysaccharides of *Enteromorpha* species. *Journal of the Chemical Society,* 5877–5880.
Gupta, V. (2001). Controlled-release tablets from carrageenans: Effect of formulation, storage and dissolution factors. *European Journal of Pharmaceutics and Biopharmaceutics, 51*, 241–248.
Haq, Q. N., & Percival, E. (1966). In H. Barnes (Ed.). *Some contemporary studies in marine science* (pp. 355–368). London: Allen and Unwin Ltd.
Haug, A. (1976). The influence of borate and calcium on the gel formation of a sulfated polysaccharide from *Ulva lactuca*. *Acta Chemica Scandinavica B, 30*, 562–566.
Hernández-Garibay, E., Zertuche-González, J. A., & Pacheco-Ruíz, I. (2011). Isolation and chemical characterization of algal polysaccharides from the green seaweed *Ulva clathrata* (Roth) C. Agardh. *Journal of Applied Phycology, 23*, 537–542.
Hernández-Garibay, E., Zertuche-González, J. A., & Pacheco-Ruíz, I. (2010). Isolation and chemical characterization of algal polysaccharides from the green seaweed *Ulva clathrata* (Roth) C. Agardh. *Journal of Applied Phycology, 23*, 537–542.
Hirsh, J., Warkentin, T. E., Raschke, R., Granger, C., Ohman, E. M., & Dalen, J. E. (2001). Heparin and low-molecular-weight heparin: Mechanisms of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. *Chest, 119*, 64S–94S.
Hoare, T. R., & Kohane, D. S. (2008). Hydrogels in drug delivery: Progress and challenges. *Polymer, 49*, 1993–2007.
Ivanova, V., Rouseva, R., Kolarova, M., Serkedjieva, J., Rachev, R., & Manolova, N. (1994). Isolation of a polysaccharide with antiviral effect from *Ulva lactuca*. *Preparative Biochemistry & Biotechnology, 24*, 83–97.
Kidgell, J. T., Magnusson, M., de Nys, R., & Glasson, C. R. K. (2019). Ulvan: A systematic review of extraction, composition and function. *Algal Research, 39*, 101422.
Kikionis, S., Ioannou, E., Toskas, G., & Roussis, V. (2015). Electrospun biocomposite nanofibers of ulvan/PCL and ulvan/PEO. *Journal of Applied Polymer Science, 132*, 42153.
Kylin, H. (1946). Über den Zuwachs der Keimlinge von *Ulva lactuca* in verschiedenen Nährflüssigheiten. Kungliga Fysiografiska Sällskapets i Lund. *Förhanlingar, 16*(1–5), 102–105.
Lahaye, M. (1998). NMR spectroscopic characterisation of oligosaccharides from two *Ulva rigida* ulvan samples (Ulvales, Chlorophyta) degraded by a lyase. *Carbohydrate Research, 314*, 1–12.
Lahaye, M., & Axelos, M. A. V. (1993). Gelling properties of water-soluble polysaccharides from proliferating marine green seaweeds (*Ulva* spp.). *Carbohydrate Polymers, 22*, 261–265.
Lahaye, M., & Jegou, D. (1993). Chemical and physical-chemical characteristics of dietary fibres from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev. *Journal of Applied Phycology, 5*, 195–200.
Lahaye, M., & Ray, B. (1996). Cell-wall polysaccharides from the marine green alga *Ulva* "*rigida*" (Ulvales, chlorophyta). NMR analysis of ulvan ogigosaccharides. *Carbohydrate Research, 283*, 161–173.
Lahaye, M., & Robic, A. (2007). Structure and function properties of ulvan, a polysaccharide from green seaweeds. *Biomacromolecules, 8*, 1765–1774.
Lahaye, M., Gomez-Pinchetti, J. L., Del-Rio, M. J., & Garcia-Reina, G. (1995). Natural decoloration, composition and increase in dietary fibre content of an edible marine algae, *Ulva rigida* (Chlorophyta), grown under different nitrogen conditions. *Journal of the Science of Food and Agriculture, 68*, 99–104.
Lahaye, M., Ray, B., Baumberger, S., Quemener, B., & Axelos, M. A. V. (1996). Chemical characterisation and gelling properties of cell-wall polysaccharides from species of *Ulva* (Ulvales, Chlorophyta). *Hydrobiologia, 327*, 473–480.
Lahaye, M., Brunel, M., & Bonnin, E. (1997). Fine chemical structure analysis of oligosaccharides produced by an ulvan-lyase degradation of the water-soluble cell-wall polysaccharides from *Ulva* sp. (Ulvales, Chlorophyta). *Carbohydrate Research, 304*, 325–333.
Lahaye, M., Inizan, F., & Vigouroux, J. (1998). NMR analysis of the chemical structure of ulvan and of ulvan-boron complex formation. *Carbohydrate Polymers, 36*, 239–249.
Lahaye, M., Cimadevilla, E. A. C., Kuhlenkamp, R., Quemener, B., Lognone, V., & Dion, P. (1999). Chemical composition and ^{13}C NMR spectroscopic characterisation of ulvans from *Ulva* (Ulvales, Chlorophyta). *Journal of Applied Phycology, 11*, 1–7.
Lai, M. F., Li, C. F., & Li, C. Y. (1994). Characterization and thermal behavior of six sulphated polysaccharides from seaweeds. *Food Hydrocolloids, 8*, 215–232.
Lankalapalli, S., & Kolapalli, V. R. M. (2009). Polyelectrolyte complexes: A review of their applicability in drug delivery technology. *Indian Journal of Pharmaceutical Sciences, 71*, 481–487.
Laza, A. L., Jaber, M., Miehé-Brendlé, J., Demais, H., Le Deit, H., Delmotte, L., et al. (2007). Green nanocomposites: Synthesis and characterization. *Journal of Nanoscience and Nanotechnology, 7*, 3207–3213.
Lee, J.-B., Hayashi, K., Maeda, M., & Hayashi, T. (2004). Antiherpetic activities of sulfated polysaccharides from green algae. *Planta Medica, 70*, 813–817.
Lee, D.-G., Hyun, J.-W., Kang, K.-A., Lee, J.-O., Lee, S.-H., Ha, B.-J., et al. (2004). A potential seaweed for tumor treatment and immune stimulation, *Ulva lactuca*. *Biotechnology and Bioprocess Engineering, 9*, 236–238.
Lewis, L. A., & McCourt, R. M. (2004). Green algae and the origin of land plants. *American Journal of Botany, 91*, 1535–1556.
Li, W., Jiang, N., Li, B., Wan, M., Chang, X., Liu, H., et al. (2018). Antioxidant activity of purified ulvan in hyperlipidemic mice. *International Journal of Biological Macromolecules, 113*, 971–975.
Li, P., Wen, S., Sun, K., Zhao, Y., & Chen, Y. (2018). Structure and bioactivity screening of a low molecular weight ulvan from the green alga *Ulothrix flacca*. *Marine Drugs, 16*, 281.
Liu, F., Chen, Q., Liu, C., Ao, Q., Tian, X., Fan, J., et al. (2018). Natural polymers for organ 3D bioprinting. *Polymers, 10*, 1278.
Liu, Z., Jiao, Y., Wang, Y., Zhou, C., & Zhang, Z. (2008). Polysaccharides-based nanoparticles as drug delivery systems. *Advanced Drug Delivery Reviews, 60*, 1650–1662.
Maeda, M., Uehara, T., Harada, N., Sekiguchi, M., & Hiraoka, A. (1991). Heparinoid-active sulfated polysaccharide from *Monostroma nitidum* and their distribution in the Chlorophyta. *Phytochemistry, 30*, 3611–3614.
Maeshige, S. (1962). Chemical studies on the green alga *Monostroma nitidum* L. Component sugars of the mucilage. *Bulletin of the Japanese Society of Scientific Fisheries, 28*, 326–334.
Martin, M., Portetelle, D., Michel, G., & Vandenbol, M. (2014). Microorganisms living on macroalgae: Diversity, interactions, and biotechnological applications. *Applied Microbiology and Biotechnology, 98*, 2917–2935.
Massironi, A., Morelli, A., Grassi, L., Puppi, D., Braccini, S., Maisetta, G., et al. (2019). Ulvan as novel reducing and stabilizing agent from renewable algal biomass: Application to green synthesis of silver nanoparticles. *Carbohydrate Polymers, 203*, 310–321.
McKinnel, J. P., & Percival, E. (1962a). The acid polysaccharide from green seaweed, *Ulva lactuca*. *Journal of the Chemical Society,* 2082–2083.
McKinnel, J. P., & Percival, E. (1962b). Structural investigations on the water-soluble polysaccharide of the green seaweed *Enteromorpha compressa*. *Journal of the Chemical Society,* 3141–3148.
Medcalf, D. G., Lionel, T., Brannon, J. H., & Scott, J. R. (1975). Seasonal variation in the mucilaginous polysaccharides from *Ulva lactuca*. *Botanica Marina, 18*, 67–70.
Molino, P. J., Garcia, L., Stewart, E. M., Lamaze, M., Zhang, B., Harris, A., et al. (2018). PEDOT doped with algal, mammalian and synthetic dopants: Polymer properties, protein and cell interactions, and influence of electrical stimulation on neuronal cell differentiation. *Biomaterials Science, 6*, 1250–1261.
Morelli, A., & Chiellini, F. (2010). Ulvan as a new type of biomaterial from renewable resources: Functionalization and hydrogel preparation. *Macromolecular Chemistry and Physics, 211*, 821–832.
Morelli, A., Betti, M., Puppi, D., & Chiellini, F. (2016). Design, preparation and characterization of ulvan based thermosensitive hydrogels. *Carbohydrate Polymers, 136*, 1108–1117.
Na, Y. S., Kim, W. J., Kim, S. M., Park, J. K., Lee, S. M., Kim, S. O., et al. (2010). Purification, characterization and immunostimulating activity of water-soluble polysaccharide isolated from *Capsosiphon fulvescens*. *International Immunopharmacology, 10*, 364–370.
Ngo, D.-H., & Kim, S.-K. (2013). Sulfated polysaccharides as bioactive agents from marine algae. *International Journal of Biological Macromolecules, 62*, 70–75.
Ngwuluka, N. C., Ochekpe, N. A., & Aruoma, O. I. (2014). Naturapolyceutics: The science of utilizing natural polymers for drug delivery. *Polymer, 6*, 1312–1332.
Nikam, A. P., Mukesh, P. R., & Haudhary, S. P. (2014). Nanoparticles—An overview. *Journal of Drug Delivery and Therapeutics, 3*, 1121–1127.
Nyvall Collén, P., Sassi, J.–F., Rogniaux, H., Marfaing, H., & Helbert, W. (2011). Ulvan lyases isolated from the *Flavobacteria persicivirga* ulvanivorans are the first members of a new polysaccharide lyase family. *The Journal of Biological Chemistry, 286*, 42063–42071.
O'Donnel, J. J., & Percival, E. (1959). Structural investigations on the water-soluble polysaccharides from the green seaweed *Acrosiphonia centralis* (spongomorpha arcta). *Journal of the Chemical Society,* 2168–2178.
Paradossi, G., Cavalieri, F., Pizzoferrato, L., & Liquori, A. M. (1999). A physico-chemical study on the polysaccharide ulvan from hot water extraction of the macroalga *Ulva*. *International Journal of Biological Macromolecules, 25*, 309–315.

Patel, S. (2012). Therapeutic importance of sulphated polysaccharides from seaweeds: Updating the recent findings. *3 Biotech, 2*, 171–185.
Paulert, R., Smania, A., Stadnik, M. J., Jr., & Pizzolatti, M. G. (2007). Antimicrobial properties of extracts from the green seaweed *Ulva fasciata* Delile against pathogenic bacteria and fungi. *Algological Studies, 123*, 123–130.
Peasura, N., Laohakunjit, N., Kerdchoechuen, O., & Wanlapa, S. (2015). Characteristics and antioxidant of *Ulva intestinalis* sulphated polysaccharides extracted with different solvents. *International Journal of Biological Macromolecules, 81*, 912–919.
Pengzhan, Y., Ning, L., Xiguang, L., Gefei, Z., Quanbin, Z., & Pengcheng, L. (2003). Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). *Pharmacological Research, 48*, 543–549.
Percival, E. (1979). The polysaccharides of green, red and brown seaweeds: Their basic structure, biosynthesis and function. *British Phycological Journal, 14*, 103–117.
Percival, E., & McDowell, R. H. (1967). *Chemistry and enzymology of marine algal polysaccharides.* London: Academic Press.
Percival, E., & Wold, J. K. (1963). The acid polysaccharide from the green seaweed *Ulva lactuca*. Part II. The site of the ester sulphate. *Journal of the Chemical Society,* 5549–5550.
Popa, E. G., Reis, R. L., & Gomes, M. E. (2015). Seaweed polysaccharide-based hydrogels used for the regeneration of articular cartilage. *Critical Reviews in Biotechnology, 35*, 410–424.
Qi, H., Zhang, Q., Zhao, T., Hu, R., Zhang, K., & Li, Z. (2006). *In vitro* antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). *Bioorganic & Medicinal Chemistry Letters, 16*, 2441–2445.
Qi, X., Mao, W., Chen, Y., Chen, Y., Zhao, C., Li, N., et al. (2012). Chemical characteristics and anticoagulant activities of two sulfated polysaccharides from *Enteromorpha linza* (Chlorophyta). *Journal of Ocean University of China, 12*, 175–182.
Qi, H. M., Zhang, Q. B., Zhao, T. T., Chen, R., Zhang, H., Niu, X. Z., et al. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) *in vitro*. *International Journal of Biological Macromolecules, 37*, 195–199.
Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z., & Xing, R. (2005). Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta). *Journal of Applied Phycology, 17*, 527–534.
Quach, T. M. T., Truong, H. B., Nguyen, T. N., Dang, V. L., Bui, M. L., Tran, T. T. V., et al. (2015). Structural determination of ulvan from green seaweed *Ulva reticulata* collected at central coast of Vietnam. *Chemistry Letters, 44*, 788–790.
Quemener, B., Lahaye, M., & Bobin-Dubigeon, C. (1997). Sugar determination in ulvans by a chemical-enzymatic method coupled to high performance anion exchange chromatography. *Journal of Applied Phycology, 9*, 179–188.
Rao, E. V., Rao, N. V., & Ramana, K. S. (1991). Structural features of the sulphated polysaccharide from a green seaweed, *Spongomorpha indica*. *Phytochemistry, 30*, 1183–1186.
Raveendran, S., Yoshida, Y., Maekawa, T., & Kumar, D. S. (2013). Pharmaceutically versatile sulfated polysaccharide based bionano platforms. *Nanomedicine Nanotechnology Biology and Medicine, 9*, 605–626.
Ray, B., & Lahaye, M. (1995a). Cell-wall polysaccharides from the marine green alga *Ulva"rigida"* (ulvales, chlorophyta). Extraction and chemical composition. *Carbohydrate Research, 274*, 251–261.
Ray, B., & Lahaye, M. (1995b). Cell-wall polysaccharides from the marine green alga *Ulva* "*rigida*" (Ulvales, Chlorophyta). Chemical structure of ulvan. *Carbohydrate Research, 274*, 313–318.
Rico, M. J., & Fernandez, C. (1996). Seasonal nitrogen metabolism in an intertidal population of *Gelidium latifolium* (Gelidiaceae, Rhodophyta). *European Journal of Phycology, 31*, 149–155.
Robic, A., Gaillard, C., Sassi, J. F., Lerat, Y., & Lahaye, M. (2009). Ultrastructure of ulvan: A polysaccharide from green seaweeds *Biopolymers, 91*, 652–664.
Robic, A., Sassi, J. F., Dion, P., Lerat, Y., & Lahaye, M. (2009). Seasonal variability of physicochemical and rheological properties of ulvan in two *Ulva* species (chlorophyta) from the Brittany coast. *Journal of Phycology, 45*, 962–973.
Robic, A., Sassi, J. F., & Lahaye, M. (2008). Impact of stabilization treatments of the green seaweed *Ulva rotundata* (Chlorophyta) on the extraction yield, the physico-chemical and rheological properties of ulvan. *Carbohydrate Polymers, 74*, 344–352.
Saravanakumar, G., Jo, D.-G., & Park, J. H. (2012). Polysaccharide-based nanoparticles: A versatile platform for drug delivery and biomedical imaging. *Current Medicinal Chemistry, 19*, 3212–3229.
Sarker, S. D., Latif, Z., & Gray, A. I. (2005). Natural products isolation. In S. D. Sarker, Z. Latif, & A. I. Gray (Eds.). *Methods in biotechnology* (pp. 1–26). Totowa, NJ, US: Humana Press.
Schepetkin, I. A., Xie, G., Kirpotina, L. N., Klein, R. A., Jutila, M. A., & Quinn, M. T. (2008). Macrophage immunomodulatory activity of polysaccharides isolated from *Opuntia polyacantha*. *International Immunopharmacology, 81*, 455–1466.
Schild, H. G. (1992). Poly(*N*-isopropylacrylamide): Experiment, theory and application. *Progress in Polymer Science, 17*, 163–249.
Shelke, N. B., James, R., Laurencin, C. T., & Kumbar, S. G. (2014). Polysaccharide biomaterials for drug delivery and regenerative engineering. *Polymers for Advanced Technologies, 25*, 448–460.
Shi, Q., Wang, A., Lu, Z., Qin, C., Hu, J., & Yin, J. (2017). Overview on the antiviral activities and mechanisms of marine polysaccharides from seaweeds. *Carbohydrate Research, 453–454*, 1–9.
Silva, T. H., Alves, A., Popa, E. G., Reys, L. L., Gomes, M. E., Sousa, R. A., et al. (2012). Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. *Biomatter, 2*, 278–289.
Song, R., Murphy, M., Li, C., Ting, K., Soo, C., & Zheng, Z. (2018). Current development of biodegradable polymeric materials for biomedical applications. *Drug Design, Development and Therapy, 12*, 3117–3145.
Stadnik, M. J., & De Freitas, M. B. (2014). Algal polysaccharides as source of plant resistance inducers. *Tropical Plant Pathology, 39*, 111–118.
Stevens, M. M. (2008). Biomaterials for bone tissue engineering. *Materials Today, 11*, 18–25.
Synytsya, A., Choi, D. J., Pohl, R., Na, Y. S., Capek, P., Lattová, E., et al. (2015). Structural features and anti-coagulant activity of the sulphated polysaccharide SPS-CF from a green alga *Capsosiphon fulvescens*. *Marine Biotechnology, 17*, 718–735.
Tabarsa, M., Han, J. H., Kim, C. Y., & You, S. G. (2012). Molecular characteristics and immunomodulatory activities of water soluble sulfated polysaccharides from *Ulva pertusa*. *Journal of Medicinal Food, 15*, 135–144.
Tabarsa, M., You, S.-G., Hashem Dabaghian, E., & Surayot, U. (2018). Water-soluble polysaccharides from *Ulva intestinalis*: Molecular properties, structural elucidation and immunomodulatory activities. *Journal of Food and Drug Analysis, 26*, 599–608.
Thanh, T. T. T., Quach, T. M. T., Nguyen, T. N., Luong, D. V., Bui, M. L., & Tran, T. T. V. (2016). Structure and cytotoxic activity of ulvan extracted from green seaweed *Ulva lactuca*. *International Journal of Biological Macromolecules, 93*, 695–702.
Thu, Q. T. M., Bang, T. H., Nu, N. T., Luong, D. V., Ly, B. M., Van, T. T. T., et al. (2015). Structural determination of ulvan from green seaweed *Ulva reticulata* collected at central coast of Vietnam. *Chemistry Letters, 44*, 788–790.
Tian, H., Yin, X., Zeng, Q., Zhu, L., & Chen, J. (2015). Isolation, structure, and surfactant properties of polysaccharides from *Ulva lactuca* L. from South China Sea. *International Journal of Biological Macromolecules, 79*, 577–582.
Toskas, G., Hund, R.-D., Laourine, E., Cherif, C., Smyrniotopoulos, V., & Roussis, V. (2011). Nanofibers based on polysaccharides from the green seaweed *Ulva rigida*. *Carbohydrate Polymers, 84*, 1093–1102.
Toskas, G., Heinemann, S., Heinemann, C., Cherif, C., Hund, R.-D., Roussis, V., et al. (2012). Ulvan and ulvan/chitosan polyelectrolyte nanofibrous membranes as a potential substrate material for the cultivation of osteoblasts. *Carbohydrate Polymers, 89*(997), 1002.
Tran, T. T. V., Truong, H. B., Tran, N. H. V., Quach, T. M. T., Nguyen, T. N., Bui, M. L., et al. (2018). Structure, conformation in aqueous solution and antimicrobial activity of ulvan extracted from green seaweed *Ulva reticulata*. *Natural Product Research, 32*, 2291–2296.
Tziveleka, L.-A., Pippa, N., Georgantea, P., Ioannou, E., Demetzos, C., & Roussis, V. (2018). Marine sulfated polysaccharides as versatile polyelectrolytes for the development of drug delivery nanoplatforms: Complexation of ulvan with lysozyme. *International Journal of Biological Macromolecules, 118*, 69–75.
Venkatesan, J., Lowe, B., Anil, S., Manivasagan, P., Al Kheraif, A. A., Kang, K.–H., et al. (2015). Seaweed polysaccharides and their potential biomedical applications. *Starch/Stärke, 67*, 381–390.
Venkatesan, J., Anil, S., & Kim, S.-K. (2017). *Seaweed polysaccharides. Isolation, biological and biomedical applications.* Netherlands: Elsevier.
Venkatesan, J., Anil, S., Kim, S.-K., & Shim, M. S. (2016). Seaweed polysaccharide-based nanoparticles: Preparation and applications for drug delivery. *Polymer, 8*, 30–54.
Vlachou, M., Tragou, K., Siamidi, A., Kikionis, S., Chatzianagnostou, A. – L., Mitsopoulos, A., et al. (2018). Modified *in vitro* release of the chronobiotic hormone melatonin from matrix tablets based on the marine sulfated polysaccharide ulvan. *Journal of Drug Delivery Science and Technology, 44*, 41–48.
Wijesekara, I., Pangestuti, R., & Kim, S.-K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate Polymers, 84*, 14–21.
Yaich, H., Garna, H., Besbes, S., Barthélemy, J.-P., Paquot, M., Blecker, C., et al. (2014). Impact of extraction procedures on the chemical, rheological and textural properties of ulvan from *Ulva lactuca* of Tunisia coast. *Food Hydrocolloids, 40*, 53–63.
Yamamoto, M. (1980). Physicochemical studies on sulfated polysaccharides extracted from seaweeds at various temperatures. *Agricultural and Biological Chemistry, 44*, 589–593.
Yamamoto, M., Tadokoro, Y., Imai, H., & Mita, K. (1980). Physicochemical characterization of sulfated polysaccharides from green seaweeds: *Ulva pertusa* and *Ulva conglobata*. *Agricultural and Biological Chemistry, 44*, 723–729.
Yoshimura, T., Hirao, N., & Fujioka, R. (2016). Preparation and characterization of biodegradable hydrogels based on ulvan, a polysaccharide from green seaweeds. *Polymers from Renewable Resources, 7*, 33–42.
Zhang, H. J., Mao, W. J., Fang, F., Li, H. Y., Sun, H. H., Ghen, Y., et al. (2008). Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*. *Carbohydrate Polymers, 71*, 428–434.
Zhang, Z., Wang, F., Wang, X., Liu, X., Hou, Y., & Zhang, Q. (2010). Extraction of the polysaccharides from five algae and their potential antioxidant activity *in vitro*. *Carbohydrate Polymers, 82*, 118–121.
Zhao, J., Liu, X. Y., Malhotra, A., Li, Q. H., Zhang, F. M., & Linhardt, R. J. (2017). Novel method for measurement of heparin anticoagulant activity using SPR. *Analytical Biochemistry, 526*, 39–42.