A Comprehensive Review on Ulvan Based Hydrogel and Its Biomedical Applications

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Ulvan is a natural sulfated polysaccharide obtained from marine green algae composed of 3-sulfated rhamnoglucuronan as the main component. It has a unique chemical structure that rich of L-rhamnosa, D-glucuronic acid, and L-iduronic acid. Ulvan has a similar structure to glycosaminoglycans (GAGs) in mammals including chondroitin sulfate, dermatan sulfate, and heparan sulfate that has broad range applications for many years. Here, we provide an overview of ulvan based hydrogels for biomedical applications. Hydrogels are one of ulvan advances in polymer science for application in drug delivery, tissue engineering, and wound healing. This review presented an overview about functional information of ulvan based hydrogels and the promising potential in biomedicals collected from published papers in Scopus, PubMed, and Google Scholar. Other important aspects concerning properties, hydrogel-forming mechanisms, and ulvan based hydrogel developments were reported as well. As conclusion, ulvan showed interesting properties in forming hydrogels and promising advances in biomedical applications.

Key words ulvan; green algae; hydrogel; biomedical application

1. Introduction

In the last few years, the polymeric materials especially polysaccharide from marine algae sources has been impressively used in wide ranging of biomedical applications. In particular, carrageenan from red algae, and alginate from brown algae have been thoroughly extensively studied by medical researchers due to its good an active biomaterial. Typically, alginate is one of the most abundant natural polymers that has been widely used as hydrogels forming polysaccharides than the others. This anionic polysaccharide is composed of two blocks of α-1-guluronic acid (G) and β-1-mannuronic acid (M) residues. The blocks consist of either successive M residues (MMMMMM), G residues (GGGGGG), or alternating G and M residues (GMGMGM). Nonetheless, the gelling capability of alginate varies with the proportion of G and M groups. G monomer helps to form ionic bridges for alginate gelation. Therefore, if alginate rich in G monomer making the gelling strength higher than the alginates rich in M groups. Alginate was successfully fabricated for a wide range of biomedical functions, including wound healing, drug delivery, and tissue engineering. From this point of view, Ulvan, a polysaccharide from green algae (Chlorophyceae) is considered to be an alternative biopolymer, its potential as good as those polysaccharides with different sources to create hydrogels due to the unique structural characteristics that will produce great functional hydrogels. Similar to other polysaccharides from seaweed, abundance in nature as well as having a high functional value in medicine making ulvan becomes more desirable.

Ulvan is a sulfated polysaccharide belong to Ulva genus. Many studies have been reported for its biological activities, including anticoagulant, antioxidant, antihyperlipidemic, antimicrobial, antiviral, and immunomodulatory properties. Same as other sulfated polysaccharides such as carrageenan, alginate, and dermatan sulfate that have several applications in drug delivery, wound healing, and tissue engineering, ulvan has been also proposed as a biomaterial polymer which applied can be used for biomedical application. The presence of distinctive functional groups such as carboxyl, hydroxyl, and sulfate groups on ulvan makes them to be potential and valuable candidates as an active biomaterial. Owing to its chemical structure resemblance with mammalian glycosaminoglycans (GAGs), which is one of the main components of the extracellular matrix (ECM), ulvan has a repetitive disaccharide structure dominated by the bonding of uronic acid with sulfate sugar. Thus, it becomes a favorable candidate for the process and function of modulation.

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The diversity of developing fields of ulvan have come up along with the advancement of this material from nanofibers, membranes, films, matrix tablets, nanoparticles, nanocomposite and hydrogels. Hydrogels, emerging as one of the utilized materials that gained enormous interest by researchers in many fields because of their unique characteristics. It is well known that hydrogels can be produced from natural or synthetic polymer materials. Natural polymers from polysaccharides are accomplished than synthetic polymers because of biocompatibility, biodegradability, relatively low toxicity, and easily recognized by cells.

Furthermore, due to the prominent properties making this polysaccharide extensively potential to be used for many purposes. Consequently, this review highlighted the principal points of ulvan based hydrogels and the potential applications as a biomaterial for biomedical applications.

2. Methodology

This review identified suitable studies from literature databases, such as Scopus, PubMed, and Google Scholar using the keywords “ulvan,” “green seaweed,” “biomedical applications” and “hydrogel.” We excluded opinions and literature in which the application was not appropriate for medical uses such as in food applications. The databases are limited to obtain the specific topic in biomedical applications of ulvan hydrogels. This review summarizes 137 selected articles between 1993–2020. The flowchart of the methodology is shown in Fig. 1.

3. Ulvan

3.1. Source

Ulvan production from Ulva lactuca was firstly discovered from the Sussex coast. Genus of Ulva spp. and Enteromorpha are known as source of ulvan. Green algae from Ulva spp. have many varieties i.e., Ulva rigida, Ulva lactuca, Ulva intestinalis, Ulva armoricana, Ulva fasciata, Ulva ohnoi, Ulva pertusa, Ulva clathrate, Ulva flexuosa, Ulva rotundata, Ulva liase, etc. Those species have high growth rates across diverse geo-climatic conditions.

3.2. Chemical Structure and Composition

In particular, green seaweed contains protein (11%), carbohydrate (36%), ash (53%), and minerals such as calcium, iron, phosphate, and chloride. Synthesis of polymers from Ulva spp. represents around 38–54% of the dry weight and the yield of ulvan content ranges from 8 to 29%. The synthesized ulvan mainly from numerous Ulvales species reported to contain rhamnose (1–60.8%), xylose (0–35.4%), glucose (0.01–87.2%), galactose (0–9.0 mol%), mannose (0–1.05%), arabinose (0–0.93%), uronic acid (9.97–47.1%), glucuronic acid (2.6–52%), iduronic acid (3.8–9.0%), and sulfate (5.8–32.2%). The main polymers of Ulva spp. are ulvan, cellulose and two minor polymers referred to as glucuronan and xyloglucan. Ulvan is spread in the intercellular space and fibral walls of two thick cell layers of the Ulva tallus and having amount up to 45% of the dry weight biomass. Generally, ulvan contains rhamnose and glucuronic acid with different level of iduronic acid, xylose, galactose, and glucose.

The main constituent of the ulvan chemical structure is sulfated rhamnose which is linked to uronic acid (glucuronic acid and/or iduronic acid) by 1,4-glycosidic bonds. It is well known as sulfate type A and type B ulvanobiouronic acid 3-sulfate that refers to β-D-glucuronic acid-(1,4)-α-L-rhamnose-3-sulfate (A3s) and α-L-iduronic-(1,4)-α-L-rhamnose-3-sulfate (B3s), respectively (Fig. 2). However, in some cases, uronic acid is replaced by xylose or sulfated xylose residues. In this context, the disaccharide is called ulvanobiose (ulvanobiose 3-sulfate and ulvanobiose 2/uni2-disulfate). The main constituent of the ulvan chemical structure is sulfated rhamnose which is linked to uronic acid (glucuronic acid and/or iduronic acid) by 1,4-glycosidic bonds. It is well known as sulfate type A and type B ulvanobiouronic acid 3-sulfate that refers to β-D-glucuronic acid-(1,4)-α-L-rhamnose-3-sulfate (A3s) and α-L-iduronic-(1,4)-α-L-rhamnose-3-sulfate (B3s), respectively (Fig. 2). However, in some cases, uronic acid is replaced by xylose or sulfated xylose residues. In this context, the disaccharide is called ulvanobiose (ulvanobiose 3-sulfate and ulvanobiose 2/uni2-disulfate).
Lahaye et al. founded type A and B ulvanobioiuronic acid 3-sulfate among several Ulva species by NMR and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) analysis.25

As previously mentioned, these sugar compositions can vary in ulvan structure and establish different indigenous physicochemical properties. For example, if the content of ulvan is rich with iduronic acid, it will indicate the high proportion of type B ulvanobioiuronic acid.71 The presence of different constituent in ulvan structures, obtain peculiar character to this polysaccharide. Moreover, it has been reported that ulvan contains other monosaccharides such as glucose, galactose, mannose, and arabinose with an estimated amount from the diverse study as mentioned by above.71,84 The composition of ulvan content may vary depending on several aspects such as seasons, species of green algae, extraction method, type of extracting solvent, temperature, extraction time, and purification method.71,72,85

3.3. Physicochemical Characteristics Ulvan constitutes a complex anionic sulfated polysaccharide that dissolves in water. The solubility increases with the increasing temperature (Table 1). At low temperatures, Lahaye and Axelas; Robic et al. examined the formation of gels with low viscosity in solution.34,86 Ulvan solution tends to form a bead-like structure that is partly connected to the filament. This character is associated with the hydrophobic nature that arises among charged polysaccharides, which is mostly related to the presence of methyl groups in rhamnose. At alkaline conditions, the ionic interactions between the carbonyl and sulfate groups resulting in aggregation of the ulvan. Generally, gel formation in ulvan occurs in the presence of boric acid and divalent cations at pH 7.5 and 8.34,37) Besides, Lahaye and Axelos40 reported that gelation kinetics of ulvan in the presence of boron and calcium ions occurred at a certain pH and time. Increasing the concentration of calcium chloride (CaCl₂), significantly increased the viscosity of the sulfated polysaccharide solutions through the interaction between calcium and the other ions in the solutions.96

One of the most substantial characteristics of ulvan forming gels is its favorable viscosity. This property is affected by molecular weight, branching, and sulfate content. Glasson et al.88 reported, that depolymerization and high ash content influence the viscosity of ulvan at low pH. In addition, the rheological properties and various biological activities of these polysaccharides depend on rhamnose, glucuronic, iduronic, sulfate compositions, and high molecular weight of ulvan.71,81,82 These physicochemical characteristics depiction of ulvan is summarized in Table 1.

4. Ulvan Hydrogels

The advantage of using polymers for multipurpose applications arises from their effectiveness of forming a wide variety of three-dimensional (3D) structures called hydrogels. Based on the physicochemical properties, hydrogels have a similar structure to ECM tissue. For polysaccharides polymers, the existence of hydrophilic groups such as sulfate and carboxylate gives the biomaterials an advantage to absorb water or biological fluids and retain a considerable amount of water into the structure and stimulate a 3D network. Therefore, hydrogels can be used to treat skin wounds. Hydrogels are composed of a hydrophilic polymer that can be formed by either physically or chemically crosslinked networks. Meanwhile, hydrogels are designed to provide large water content for the tissue environment and present autolytic debridement which guiding our body to use its enzymes for infected tissue.91–93

4.1. Characterization of Ulvan Based Hydrogels Ulvan based hydrogels are biocompatible, biodegradable, hydrophilicity, easily modified, porous structure, and has high water absorbance. Contrary, the limitation of ulvan hydrogels is poor property of mechanical strength that is also known as the lack of natural polymers.7–9,94–97) To defeat those phenomena, modification of ulvan with chemical reagents or other polymers has risen the desired functionality of ulvan hydrogels, as reported by a group of researchers in Table 2 summarized a different gelation mechanism of hydrogels forming. Based on these approaches, every technique of ulvan hydrogels preparation resulting in specific properties by characterization methods e.g. Fourier-transform IR spectroscopy
(FT-IR), Thermogravimetric Analysis (TGA), X-Ray Diffraction (XRD), rheometer, Scanning electron microscopy (SEM), NMR, and cell culture.

Swelling Degrees
Ulvan hydrogels were fabricated by chemical crosslinking using two methacrylate groups: methacrylate anhydride (MA) and glycidyl methacrylate (GMA). Ulvan-MA derived hydrogels reached 90% of its maximum size within 5 h and continues for 3 d, and then stable for 7 d. In contrast for Ulvan-GMA that reached 90% of maximum size in 4 h but after 2 d become immersed because of poor mechanical strength. Meanwhile, hydrogels produced using polyelectrolyte complex (PEC) (ulvan and chitosan) results in hydrogels swelling to 400% in a medium. This value obtained was lower than UV cross-linked due to neutralization of negative charge and therefore decreases water uptake.9

Water Uptake Capacity
The xylorhamno-uronic acid (XRU) hydrogels demonstrated high water uptake capacity with approx. 30–80-fold increased in weight within 24 h of hydration at room temperature. These samples reached their respective equilibrium water contents (>96%) in less than 3 h. The equilibrium of water content decreases (98.8 to 96.8%) when the XRU concentration increases on the contrary.

Rheology
Developed ulvan hydrogels in the thermo-gelling system by blending ulvan-acrylate (reacted ulvan with acryloyl chloride as chain initiators) with poly(N-isopropyl acrylamide) (pNIPAAm) through polymerization induced by UV irradiation. The thermo-sensitive injectable hydrogels designated shear viscosity and modulus increase dramatically at 30–37°C, proving hydrogels have elastic behavior. Hereafter, the authors suggest those in situ gelling system materials because it is suitable in biomedical field applications based on rheology and thermal properties.9

Mechanical Properties
Young’s modulus is enhanced by increasing XRU-MA concentration and photo-crosslinking energy. Dash et al. revealed that complex modulus increased in ulvan-chitosan mineralized with alkaline phosphatase (ALP) rather than ALP-free. Authors stated that treating ALP minerals to polymeric system ulvan-chitosan gives an ideal system to form ulvan matrix compare to UV crosslinked.

4.2. Hydrogel Forming Mechanism of Ulvan
Generally, the hydrogel-forming mechanism is divided into two methods; chemical and physical methods. Particularly, ulvan forming hydrogels have been produced by the physical method through ionic crosslinking and phase transitions. Meanwhile, the chemical method is done by enzymatic crosslinking and free radical polymerization crosslinking. As a natural polymer from the polysaccharides class, ulvan becomes the potential to form hydrogels either in physical or chemical methods. Most of the polysaccharides can form hydrogels by physical methods. In contrast, some researchers stated that polysaccharides group are one of the most useful material in chemical method to create hydrogels due to easy to be modified by their ionic groups such as sulfate, phosphate, amide, and carboxylate. The mechanism of ulvan hydrogels formation is presented in Table 2.

4.2.1. Physical Methods

**Ionic Crosslinking**

Ionic crosslinking is a non-covalent interaction between multivalent counterions to form biomaterial hydrogels. Ionic crosslinking of Ulvan hydrogels is a physical interaction forming 3D hydrogel by ionic gelation through crosslinking with boron and calcium ions (Fig. 3a). The gelation mechanism associates the free form of cis-hydroxyl from Ulvan and borate, which is involved rhamnose and uronic acid. It was supported by the chelation of calcium with hydroxyls group of borate. Also, there are ionic interactions between either the carboxylic group of uronic acid and/or sulfate with borate.
through divalent cation due to acidic moieties of Ulvan.\textsuperscript{34} The illustration of the hydrogels forming by ionic crosslinking can be seen in Fig. 3b.

Meanwhile, Siddhanta \textit{et al.}\textsuperscript{101} stated that high levels of uronic acid (27–35\%) influence the viscosity of the solution and the capability of ulvan to form hydrogels. Notably, gel formation depends on intramolecular and intermolecular crosslinking forces that can be inhibited by high negative charges such as carboxylic acids (such as uronic acid), sulfuric groups, and methyl groups (on rhamnose).\textsuperscript{72}

Dash \textit{et al.}\textsuperscript{9} reported that anionic polymers could crosslink with polycations by PEC of ulvan and chitosan, which functionalized using ALP enzyme as mineralization inducer. Chitosan is a cationic polysaccharide with a negative charge that inducing a stronger layer and resulting stabilization of hydrogels-scaffold. Ulvan ionically crosslinked with chitosan comprises the side chain reaction on their macromolecular backbone, particularly between ammonium groups of chitosan and the carboxylate groups of ulvan to form hydrogel. In this study, the hydrogel was prepared by mixing the ulvan solution (using deionized water) to chitosan with a different ratio in an acid condition. As the purpose of this study to produce hydrogels that biofunctionalized by mineralization. Therefore, the PEC hydrogels were treated with varying concentrations of ALP and calcium phosphate. The result showed that the most stable formulation was the ulvan with chitosan complex in ratio of 6:4.\textsuperscript{9}

Combination ulvan with synthetic materials was done by Alves \textit{et al.}\textsuperscript{95} for applications in bone tissue engineering to release dexamethasone from the hydrogel matrix. Poly-\textit{d}-\textit{l}-lactic acid (PDLLA) is a synthetic polymer from the polyester class that is regularly used due to compatibility and desired mechanical properties. Hereafter, before combines with PDLLA, ulvan was complexed with cationic chitosan to stabilize the scaffold form. As a result, dexamethasone released from polymer combined matrix was sustained release delivery.

Phase Transition

Thermally-induced gelation systems (TGS) are uniquely composed of an interpenetrating network of polymeric chains that can undergo phase transitions to the gel-solution state, due to temperature manipulation.\textsuperscript{102}

Morelli \textit{et al.} developed phase transition hydrogel-forming from ulvan–Acrylate conjugate (UA) through photopolymerization UV light-induced with pNIPAAm which playing as chain initiators. The critical gelation concentration (CGC) of UA-NIPAAm was 4–5 wt\% which to obtain the rheological characteristics and thermal behavior that experiencing \textit{in situ} gelling systems, the lower critical solution temperature (LCST) value should be 30–31°C. NIPAAm is a well-known temperature-sensitive polymer having phase transitions at LCST of about 32°C.\textsuperscript{8} Before producing thermosensitive hydrogels, ulvan was grafted with acryloyl chloride. Then, NaOH was added for neutralizing an acid that was produced during the esterification of ulvan and acryloyl chloride, whereas 2-butane was added to increase the compatibility. In this study, the 4°C reduced the hydrolysis process and prevented acryloyl group polymerization. Thereafter, NIPAAm was used to reduce the covalent bond of UA throughout the polymerization.\textsuperscript{48} Preparation and the illustration of these ulvan forming hydrogels were presented in Figs. 4a and 4b, respectively.

Morelli \textit{et al.} investigated the thermo-responsive hybrid nanogels using the ulvan. The hydrogels synthesized begin through UV-initiated radical copolymerization. In this study authors used poly(N-vinyl caprolactam) (PNVCL) as thermo-responsive polymers.\textsuperscript{103} Even though, the method of forming hydrogel in those two studies by Morelli \textit{et al.} was based on physical methods but actually, it was combined with chemical methods by modifying ulvan with acrylate.

4.2.2. Chemical Methods

Preparation of ulvan hydrogel by chemical crosslinked with divinyl sulfone (DVS) was done by Yoshimura \textit{et al.}\textsuperscript{47} They produced biodegradable hydrogels by varying the concentrations of feedstock DVS (5–20 wt\%) to determine the optimal

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Fig. 3. a) Representation Scheme of Preparation Ulvan Hydrogel by Ionic Crosslinking; b) Illustration of Ulvan Hydrogel by Crosslinked Ulvan with Calcium

Fig. 4. a) Representation Scheme of Preparation Ulvan Hydrogel by Phase Transition (Combination with Chemical Crosslinking); b) Illustration of Ulvan Hydrogel by Crosslinked Ulvan with PNIPAAm
condition of hydrogels preparation. The reaction was carried out by mixing the DVS to Ulvan mixture under alkaline conditions at room temperature. Then, after stored for 24h, the reaction terminated by adding HCl. The superabsorbent characteristics and good biodegradability were obtained from DVS 20 wt%. Another chemical method to produce hydrogels includes enzymatic crosslinking and free radical polymerization crosslinking which will be explained further below.

**Enzymatic Crosslinking**

Preparation of enzymatic crosslinked ulvan-based hydrogels was by blending ulvan with tyramine then catalyzing by horse-radish peroxidase (HRP) enzyme and using hydrogen peroxide (H₂O₂) as a reagent. Utilization of enzymes represents a strategy to obtain covalent crosslinking that was used in the development of injectable hydrogels. Varied concentrations of HRP (0.6–5 U/mL) and optimized concentration of H₂O₂ were used in this study to determine the optimum gelation time of in situ hydrogels by the titling method. Ulvan was conjugated with tyramine as a hydroxyphenyl precursor through an amidation reaction while N-(3-dimethylaminopropyl)-N′-ethyl carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) were used as an activator. To form the covalent bonds, the carbon atoms in the ortho position are bonded with either hydroxyl group or oxygen atom of the phenol group. However, it is an effective process of hydrogel formation because it does not require augmenting of chemical agent that can alter the activity of bioactive molecules. In addition, the blended Ulvan/tyramine hydrogels appropriate for in situ injectable hydrogels with the gelation time was 90s at a concentration of HRP and H₂O₂ of 2.5 U/mL and 9.8 × 10⁻³ M, respectively. Preparation of these enzymatic crosslinked hydrogels and the illustration of hydrogels forming are presented in Figs. 5a and 5b, respectively.

**Free Radical Polymerization Crosslinking**

Free radical polymerization also known as photo-crosslinking is one of the chemical crosslinking methods. Polymerization was done by utilizing free radical initiators like methacrylate, benzoyl peroxide, and ammonium peroxodisulphate. To achieve a stable hydrogel, the methacrylate polysaccharides are photo-crosslinked by irradiation with UV rays (245 nm), argon ion laser (514 nm), gamma rays, or electron beam with the presence of a photo-initiating system and produced covalent bond pairs. Morelli and Chiellini reported that ulvan was modified by chemical crosslinking using precursors, methacyryloyl (MA and GMA), in hydrogel preparation by photopolymerization with UV light. Functionalized ulvan with MA produce stable hydrogels, while GMA resulted in unstable hydrogels. MA is less toxic than other reagents and could form hydrogels with ulvan through nucleophilic attack of hydroxyl groups in ulvan to anhydride linkage of MA. Therefore, it could be considered...
as a matrix encapsulation due to the excellent radical capability of Ulvan-methacrylate. Light irradiation is considered as a suitable method in hydrogel formation because it is a simple and quick method.

Moreover, Dash et al.7) showed modifications of ulvan using MA and functionalized by polymerizable UV crosslinking using photo-initiator, IRGACURE 2959 while the ALP enzyme was used as a biomineralization inducer. A preparative process of the photo-crosslinking method to produce hydrogels presented in Fig. 6a and the illustration of hydrogels-forming can be seen in Fig. 6b. The result was implied degradability of hydrogel due to the presence of carboxyl ester groups in the crosslinks. Whereas, ALP supported to relieve phosphates from calcium glycerol phosphate medium for precipitation of the calcium phosphate crystals at the pore of ulvan scaffolds. Variations of ALP concentration 5, 25, and 50 mg/mL used in that study and influence the morphology of ulvan. The scaffolds were non-toxic and improve cellular activity.

Chen et al. reported that modified XRU extract with methacrylic anhydride and UV crosslinked produced XRU hydrogels. The mixture variation of XRU and methacrylic anhydride (MA) (5, 7.5, 10% (v/v)) promote cell proliferation by in vitro analysis. The hydrogels were showed high water uptake capacity and feasible mechanical properties. Moreover, increasing XRU-MA concentration and photo-crosslinking energy will increase the Young modulus. Therefore, the result suggested their application in skin repair or tissue regeneration.96)

5. Biomedical Applications of Ulvan Based Hydrogels

Hydrogels have found numerous usefulness in tissue engineering, drug delivery as well as in wound healing. Design hydrogels from biomaterials widely explored over the years. As such, ulvan, a biomaterial from marine resource also noticed by the researchers because of peculiar configuration structure related to function in biomedical due to pharmacological activities (anti-inflammatory, antioxidant, antibacterial, and antiviral).107) Figure 7 represents the main advantages of ulvan hydrogels in the biomedical fields.

5.1. Tissue Engineering

Tissue-engineering systems are created to mimic the ECM using biocompatible and protective material either with or without therapeutic compounds for tissue formation.2,108,109) Biomaterial like polysaccharides continuously used in many studies as tissue regenerating agents related to biocompatibility nature which let those materials match onto the body without negative response. Marine polysaccharides such as fucoidan, alginate, and carrageenan, have been applied for the preparation of hydrogel scaffolds as a biomaterial of tissue regeneration. These marine materials have good scaffold strength within bio-composite.2)

Alves et al. prepared hydrogels through chemical crosslinking with 1,4-butanediol diglisidil eter (BDDE) under alkaline conditions. The crosslinkers may undergo chemical modification to produce specific hydrogels scaffold due to its capability to react with hydroxyl and carboxyl groups of ulvan backbone. The result showed proliferation and differentiation of cells were not detected due to limited pore interconnection and weak adhesion from cells on the surface of the scaffold host. Water uptake was up to 2000% of its initial dry weight with a porous and interconnected structure. Thus, the authors summarized those results to be potentially applied in tissue regeneration.31)

Dash et al.7,9) fabricated hydrogels scaffold-based mineralization. Mineralization of scaffold has been done by incorporated ALP to polymer UMA scaffold. This study showed the UMA sample incorporated ALP at 14 d of MC3T3-E1 cell culture, was significantly higher on cell proliferation and collagen production than untreated UMA.7) Meanwhile, another work by Dash et al. prepared ALP and calcium phosphate mineralization was subjected to PEC of chitosan and ulvan scaffold. Results showed the hydrogel scaffolds promote cell adhesion and differentiation towards an osteogenic phenotype.9) From those two works done by Dash et al. indicated the mineraliza-
tion scaffold hydrogels could be used in the future in tissue engineering applications.

5.2. Drug Delivery  It is well known that hydrogels use as the development delivery systems. The incorporation of bioactive agents can be modified to deliver specific purposes to prolong the action. The bioactive molecule must reach the site of action at a certain concentration and the therapeutic dose range should remain constant over time. Therefore, several factors decrease the effective enforcement of pharmacetical ingredients, including degradation of the drug, lack of penetration into tissues, and interaction with cells. Thus, biopolymeric devices as drug delivery carriers using polysaccharides are raising concerns because they could produce preferable pharmacological activities. Their porous structure allows drugs to be loaded and then released. However, there are some controlling factors like swelling force, mechanical strength, diffusion release (matrix or reservoir system), and viscosity that need to be considered to release the drug from the matrix.

Ulvan has been modified with lysozyme that has cationic charged. The complex formation results in stable nanoparticles and increasing antibacterial activity. The study recommended that ulvan was potentially applicable as a nanocarrier for positively charged bioactive molecules. Massironi et al. investigated antimicrobial properties of silver nanoparticles (AgNPs) in thick ulvan shells. As a result, the IC_{50} of AgNPs aligned ulvan shell of 10 \mu g/mL in Balb/3T3 mouse embryo fibroblasts showed good antimicrobial properties toward Gram-positive and negative bacteria. Bang et al. prepared curcumin-loaded acetylated ulvan nanogel to enhance the solubility of hydrophobic curcumin by 20,000 times. Indeed, the system effectively altered curcumin into nano-size with a diameter of 293 nm and prevented from re-aggregating. The study emphasized that modifying-ulvan properties turn into hydrophobic using acetic anhydride which appears as a carrier to deliver water-insoluble bioactive compounds.

A study by Morelli et al. showed that hybrid nanogels were synthesized by UV-initiated radical polymerization of NVCL. This study using BSA as a model for protein encapsulating carrier showed that by increasing the ulvan concentrations, thus loading efficiency in nanogels will increase. Alves et al. have prepared a 3D scaffold porous combination of ulvan-PDLLA complex loaded with dexamethasone. In vitro cytotoxicity of the ulvan-PDLLA scaffold demonstrated good cytocompatibility and cell viability after 72 h of culture. Feasibility of ulvan as a carrier system of dexamethasone through the fast release from scaffolds proved steady release during the first 3 h up to 52% then followed by sustained release for 21 d.

5.3. Wound Healing  Hydrogels have been applied as wound dressing material due to its capability to absorb and retain water in the network structure. The aqueous environment of hydrogels resembles the condition of cells in the body so that it can be utilized especially in repairing tissue. Hydrogels could contain about 90% (w/v) water and 10% (w/v) polymer, causing it to be very suitable for the treatment of necrotic and dry wounds. Also, permeable hydrogel structure does not inhibit the exchange of CO_{2}, O_{2}, and H_{2}O.

The optimum management of wound treatment can be achieved by utilizing materials exhibited pharmacological activity in wound healing, for example, antimicrobial, antioxidant, and anti-inflammatory agents. As mentioned before, ulvan has therapeutic activities for supporting those functions. The presence of sulfated groups is reported to determine the antioxidant activity of ulvan. The antioxidant effect is characterized by radical damping and metal plating. This activity was reportedly responsible for the antiproliferative effects of ulvan. Ulvan also reported have protective activity against microorganisms and its antimicrobial effect is mainly through inhibition of biofilms. In wound conditions, bacteria will attack leukocytes resulted in amplification of cytokines, proteases, and reactive oxygen species (ROS) which will prolong...
the inflammatory process, cause ECM and growth factors degradation, interfere with cell migration, and inhibit closure to the wound.126 In general, the antibacterial mechanism of polysaccharides caused by the activity of glycoprotein-receptors on the surface of polysaccharide cells that bind to components on bacterial cell walls, cytoplasmic membranes, and DNA.125 Investigation of antimicrobial activity of several species from green algae has been carried out by the researcher toward the inhibition of Gram-negative bacteria (Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa) and Gram-positive bacteria (Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, and Bacillus spp.).126–129

The administration of ulvan polysaccharides has been investigated as anti-inflammatory agent for wound healing by some researchers. To describe the mechanism action of ulvan especially in the inflammation phase of wound healing, the RAW264.7 macrophage cells were used. As shown in Fig. 8, administration of ulvan giving a significant release enhancement of pro-inflammatory cytokine secretions namely interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrotizing factor-alpha (TNF-α) which can trigger fibroblasts for collagen production and promote angiogenesis. Also, nitric oxide production is increasing due to the inducible nitric oxide synthase (iNOS) enzyme expression. Meanwhile, prostaglandin-E2 production is decreasing because of the inhibition of cyclooxygenase-2 (COX-2) enzyme.130–132 Kdigell et al. reported that high concentration (100 µg/mL) of ulvan with high molecular weights increased the promotion of IL-1β, IL-6, and IL-12, while decreased prostaglandin E2 (PGE2) at lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells for in vitro assessment.130 Expression of TNF-α and NO is dose-dependently increased in polysaccharides from Enteromorpha intestinalis species.132

In addition, Ulvan has similar structure and function to GAGs such as heparin and chondroitin sulfate. Heparin is frequently used for the healing process by inducing fibroblast proliferation, inhibiting thrombin establishment, and improving fibrinolytic function.135 Meanwhile, chondroitin sulfate has been reported to act in the wound healing process through influencing fibroblast adhesion.135 Besides, polysaccharides rich in rhamnose compounds stimulate cell proliferation and collagen biosynthesis.136 Sacran from Aphanothece sacrum, a macro-molecular polysaccharide that contains sulfate and carboxyl group, also various sugar like rhamnose, xylose, glucose, galactose, mannose, fucose, glucuronic acid and galacturonic acid which is similar to ulvan displayed anti-inflammatory effect by inhibiting pro-inflammatory cytokines (interleukin-5 (IL-5), interferon (IFN)-γ and TNF-α) and some chemokines release that respond to inflammation. Wathoni et al.134 revealed that sacran hydrogels film accelerated wound healing comparing Na-alginated hydrogel film. Specifically, curcumin complexes with 2-hydroxypropyl-γ-cyclodextrin in sacran hydrogel was proved to increase wound healing efficacy at inflammation phases.135 In the presence of γ-cyclodextrin in sacran hydrogels could enhance swelling power, porosity, and moisture content of hydrogels.136 Followed by another study, the incorporation of keratinocyte growth factor in sacran hydrogels film accelerated wound healing on diabetic mice model.137

The study of hydrogels-rich rhamnose and its development in 3D printed XRU was done by Chen et al. resulting in excellent cytocompatibility with human dermal fibroblasts (HDFs). Specifically, the HDF culture was showed high viability and cell proliferation on XRU hydrogels than alginate hydrogels as a comparator in that study. The same result is also seen on 3D printed XRU scaffolds. Hence, the author’s conclusion was that those hydrogels have present promising prospects as a biomaterial for wound healing treatment.96

The potential of ulvan for wound treatment also have been recommended in a study by Alves et al. that reported 2D ulvan for dexamethasone delivery which showed the amount of drug release in the first 1h was 49% and then followed by sustained release, which reached 75% for 14d. Based on the result of this study, researchers recommend ulvan applications as wound treatment material.138

6. Conclusions and Future Perspective
Ulvan based hydrogels is a potential underexploited biomaterial for biomedical applications. Ulvan shows a close similarity with mammalian glycosaminoglycans which also make ulvan as a heparinoid agent that could substitute the GAG substances for wound management.

Ulvan based hydrogels have several promising features in the biomedical field. This is supported by the unique chemical structure of ulvan that is not possessed by other polysaccharides sourced from algae, the distinctive gelling mechanism, and high water uptake. Also, ulvan are compatible and degradable biomaterials making it easy to apply to the body and have extensive usage in biomedical devices such as tissue regeneration, drug delivery, and wound healing. Even though exploration about the biomedical application of ulvan is limited especially in vivo studies but from ulvan activities and its ability to form hydrogels create innovative development for biomedical applications. Nevertheless, ulvan has mechanically inferior related to physical properties. It seems that the future of this biopolymer needs to be developed or modified.

Based on our investigations, there is still no in vivo studies yet related to biomedical applications of ulvan hydrogels. Accordingly, we need many studies on the varied form of hydrogels in the biomedical application for future challenge. Finally, the prospects of developing ulvan hydrogels for biomedical applications are presented with many challenges but also offer new opportunities.

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Conflict of Interest The authors declare no conflict of interest.

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