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Facile production of seaweed-based biomaterials with antioxidant and anti-inflammatory activities

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ABSTRACT

New seaweed-based biomaterials have been prepared using a simple method based on the selective dissolution in trifluoroacetic acid (TFA) of specific polymers and bioactive substances from red, green, and brown seaweeds. Depending on the seaweed's origin, the properties were found to be different, especially the mechanical ones. Furthermore, the samples were fully biodegradable in seawater in one month. Moreover, the antioxidant capacity of the biomaterials was highly increased respect to the pristine materials, demonstrating a selective extraction during the process of solubilization. Finally, biocompatibility and anti-inflammatory experiments demonstrated the non-toxicity of the biomaterials prepared from brown seaweed and a similar anti-inflammatory effect to commercial available drugs, confirming the potential application of the prepared biomaterials for the fabrication of biomedical devices.

1. Introduction

Seaweed is the common name for countless species of marine plants and algae that grow in the oceans, rivers, lakes, and other water bodies [1]. Seaweeds can be classified into three broad groups based on their pigmentation: brown, red and green [2]. The chemical composition of seaweeds is very versatile, varying with the species and the harvesting season [3]. In general, they consist of an organic fraction (carbohydrates, proteins, lipids, fibers and ashes) and inorganic substances such as sodium, potassium, calcium, and magnesium salts [4–7]. Similarly to terrestrial plants, some seaweeds contain cellulose, but in a minor proportion, being the anionic polysaccharides (alginates and several sulfated polysaccharides) the main components of their cell wall [7]. Alginates are a family of linear *co*-polymers of 1,4-linked α-L-guluronic and β-D-mannuronic acids with some variations in their relative occurrence and sequential distribution [7,8]. On the other hand, sulfated polysaccharides, characterized by the presence of $-SO_3^-$ functional groups, are named as fucoidans in brown seaweeds, carrageenans in red seaweeds and ulvans in green seaweeds [9–16]. Alginates, carrageenans and agars are commonly known as phycocolloids. Alginates are extracted from brown seaweeds, while agars and carrageenans are extracted from red seaweeds. They represent a high value market with a wholesale of 130 million € only in Europe [17,18]. Alginates are used in the pharmaceutical field, cosmetics, paper industry and processed food [19,20]. Carragenans are largely used in the food industry because of their gelling activity. For instance, carrageenans are present in the ice creams and canned foods. Red seaweeds are also a source of agars [19,20]. Similarly to carrageenans, agars are used in the food industry, but also in the medical field due to its ability to make capsules for different medicines [19,20].

Seaweed biomass washed onto shores by natural processes represents a tremendous resource as renewable feedstock for many applications [21,22]. In fact, seaweeds are rich in sulfated polysaccharides that have been studied in detail due to their biological activities, such as antioxidant capacity [11,23–25], anti-inflammatory activity [16,26], anticoagulant activity [11,27,28], antiviral activity [11,29–31], anticancer activity [11,32] and others [11,33–35]. Nowadays there is an increasing tendency to use natural polymers for the fabrication of medical devices [36,37]. Natural polymers, such as collagen, silk or chitosan have been largely used as biomaterials, but also polysaccharides, mainly alginates because of their high biocompatibility and low toxicity [37–40], are exploited with the same purpose [41].

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Cellulose has been also used for the preparation of scaffolds in the biomedical field [42–44]. However, to the best of our knowledge, there is no information about the full exploitation of all natural substances present in seaweeds for the fabrication of potential biomedical products, most likely as a consequence of the lack of a common solvent for all of them. Recently, trifluoroacetic acid (TFA) has been used as a single solvent for the direct preparation of bioplastics from several agro-wastes [45]. This acid, when is not solved in water, can condense with free hydroxyl groups producing trifluoroacetylated derivatives. In the case of cellulose and other polysaccharides, such derivatives are soluble in TFA [46]. TFA can be easily removed after drop-casting due to the low pressure of the acid and the lability of trifluoroacetylated positions with moisture [45].

In this work, we report the use of seaweeds as raw materials for the preparation of composite biomaterials with tunable properties for biomedical use. In particular, three commercial seaweeds species (*Porphyra yezoensis*, *Ulva lactuca* and *Saccharina latissima*), each one representing one of the three broad seaweed groups, were used to evaluate their potential as biomaterials. The *Porphyra yezoensis* is a red seaweed typically found in cold waters of temperate oceans, the *Ulva lactuca* is a green seaweed widely distributed along the coasts of the world's oceans and the *Saccharina latissima* is a brown seaweed typical from the Atlantic Ocean. The developed biomaterials were easily produced by solution and blending with cellulose in trifluoroacetic acid and subsequent casting and solvent evaporation. The effects of the cellulose content and the nature of the raw material on the thermal, mechanical and structural properties were studied. These new seaweed-based biomaterials were fully biodegradable and presented a high antioxidant capacity, both important characteristics for the preparation of biomaterials. In fact, sustainability of biomaterials is catching the attention of researchers to avoid environmental issues related to traditional petroleum-based materials used so far [47]. On the other hand, the release of antioxidant substances improves the efficiency of the biomaterials since the apparition of reactive oxygen species aggravate inflammatory processes [48]. Moreover, *in vitro* studies performed on human foreskin fibroblasts revealed that the composite biomaterials prepared from brown seaweed have a high anti-inflammatory activity, making them suitable for biomedical devices.

2. Materials and methods

2.1. Materials

Microcrystalline cellulose from cotton linters, anhydrous trifluoroacetic acid (TFA), 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH·), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate were purchased from Sigma Aldrich and used as received. Seaweeds were purchased from Algamar S. A. (Galicia, Spain).

For the biocompatibility and anti-inflammatory tests, lipopolysaccharides (LPS) from *Escherichia coli* (serotype 026:B6) and dexamethasone (DXM) were purchased from Sigma Aldrich. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine and penicillin–streptomycin were from Euroclone (Milan, Italy). CellTiter-Glo reagent was purchased from Promega (Madison, WI, USA). Human foreskin fibroblasts (HFF-1) were from ATCC®. RNA extraction kit (PureLink RNA Mini Kit) and Super-Scripts® VILO™ cDNA Synthesis Kit were purchased from Ambion by Life Technologies (USA). Gene-specific primers using fluorogenic probes (TaqMan) and TaqMan® Universal PCR Master Mix, No AmpErase® UNG were from Applied Biosystems (USA).

2.2. Preparation of biomaterials

Cellulose and seaweeds were dried in an oven at 40 °C overnight in order to remove the adsorbed water even if these products were already dehydrated. Cellulose solutions with a final concentration of 1 wt% were prepared by dissolving microcrystalline cellulose powder in TFA. The complete dissolution took three days under room conditions. Similar solutions were prepared with the three types (red, green, and brown species) of seaweeds in TFA. Depending on the seaweed species, the time for obtaining a clear solution was different, being 7 days in the case of brown seaweed, 4 days for the red seaweed and 5 days for the green seaweed. To standardize the production of biomaterials, all the films were aged 7 days in TFA maintaining a constant temperature of ~30 °C and shaking continuously. Then, solutions were centrifuged. Films were prepared by blending predetermined volumes of cellulose and seaweed solutions in order to obtain a final concentration of 75 wt % of seaweed in the composite biomaterials. This concentration of cellulose was the minimum necessary to handle the films. Control samples of pure cellulose with no seaweed contribution were also prepared. The solutions, after centrifugation and filtering, were cast in plastic Petri dishes and the solvent was allowed to evaporate during 1 day to make free-standing films. All samples were stored at 44% RH for 7 days before analysis to ensure the reproducibility of the measurements. The removal of TFA was assessed by X-ray photoelectron spectroscopy (XPS), as illustrated in Fig. S1. Samples were labelled as CB, CR and CG followed by the number 25 according to the concentration of 25 wt% of cellulose. Abbreviations C, B, R and G make reference to cellulose, brown, red, and green seaweed, respectively.

2.3. Morphological characterization

The morphology of the seaweed-based biomaterials was characterized by scanning electron microscopy (SEM), using a JEOL JSM-6490LA microscope working in high vacuum mode, with an acceleration voltage of 15 kV.

2.4. Chemical and structural characterization

X-ray photoelectron spectroscopy (XPS) was carried out with an Axis Ultra DLD spectrometer under 10^{-9} mbar pressure. Monochromatized Al Kα source with photon energy 1486.6 eV was used with an emission current of 20 mA and an operating voltage of 15 kV. High-resolution spectra were acquired with a step of 0.1 eV and an analyzer pass energy of 10 eV. Surface charging was neutralized with low-energy electrons (4 eV) and energy calibration was performed by setting the C—C/C—H component of the C 1s spectrum to a fixed binding energy value of 284.5 eV. Data analysis was performed with CasaXPS software. The concentration of Na, K, Ca and Mg was determined by elemental analysis using an inductively coupled plasma-atomic emission (ICP-AES) spectrometer (iCAP 6500, Thermo). Selected samples were digested in aqua regia. Specifically, 50 mg of each sample was mixed with 2.5 mL of aqua regia and left overnight to ensure a complete digestion of the material. Then, the solution was further diluted with Milli-Q water up to 25 mL and filtered through 0.45 μm PTFE filters prior to elemental analysis. The crystallinity of the films was analyzed by X-ray diffraction (XRD) using a Rigaku SmartLab X-Ray Diffractometer equipped with a copper rotating anode. The X-ray source was operated at 40 kV and 150 mA. A Gobel mirror was used to obtain a parallel beam and to suppress Cu Kβ radiation (1.392 Å). The measurements were performed using a 2θ scan.

2.5. Mechanical characterization

Mechanical properties of the films were measured by uniaxial tensile tests on a dual column Instron 3365 universal testing machine. Dog-bone shaped samples were stretched at a rate of 5 mm/min. All the stress-strain curves were recorded at 25 °C and 44% RH. Ten measurements were conducted for each sample and the results were averaged to obtain a mean value. The Young's modulus, yield stress and elongation at break values were calculated from the stress-strain curves.