Semana de Engenharia 2010
Guimarães, 11 a 15 de Outubro

A BOTTOM UP APPROACH TO ESTABLISH ULVAN AS A BIOMATERIAL

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KEYWORDS
Ulvan, biomaterial, tissue engineering

INTRODUCTION

The successful development of a new nature-inspired biomaterial is largely dependent on the characteristics of the starting material. Looking for better performing materials, ulvan, an algae polysaccharide, is herein proposed as a potential biomaterial. Due to its properties, ulvan can be considered, pure or modified, as a versatile biodegradable polymer for biomedical applications. (Alves et al. 2010; Alves et al. 2010)

METHODS

Ulvan was obtained by extraction from green algae, namely *Ulva lactuca*.

Physical-chemical cararacterization

Elemental analysis (% C, H, N and S content) was performed by combustion to evaluate the content of the major components present in the extracted analyte. To assess the chemical structure of ulvan, powder was analyzed by infrared spectroscopy (IR). Thermal degradation and thermal transitions of the extracted compound were studied by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). X-ray diffraction (XRD) patterns were measured using a powder diffractometer.

***In vitro* cytoxicity**

For all cytoxicity tests performed, latex rubber and standard culture medium were used respectively as positive and negative controls. Hyaluronic acid was used as a biodegradable control material. Mouse C3H/An connective tissue fibroblast-like cells (L929) were used to perform the biological performance studies. Colorimetric MTS assay and dsDNA Quantification were performed to evaluate cell metabolic viability and proliferation.

Processability

Ulvan sponges were produced by freeze drying after crosslinking of ulvan polysaccharide with a FDA approved crosslinker. Inner structure and porosity were evaluated by micro-computerized tomography using a micro CT scanner. Compressive mechanical characterisation of the scaffolds was also performed.

RESULTS AND DISCUSSION

Chemical characterization by chemical analysis (Table 1) and infrared spectroscopy (Fig. 1) indicate that the structure of the obtained polysaccharide is mainly composed of rhamnose and uronic acids, with sulphate groups. (Lahaye et al. 1997)

Table 1: Elemental analysis of ulvan extracted from green algae collected at the portuguese coast.

	% N	% C	% H	% S
Ulvan	1.50±0.01	20.55±0.09	3.23±0.01	9.07±0.58

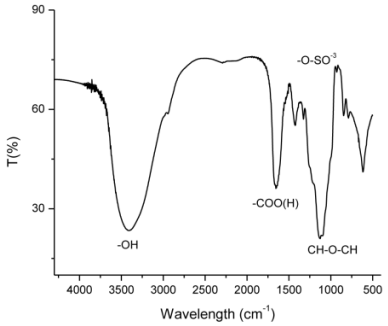

Figure 1: Infrared spectrum of ulvan between 400 and 4000 cm^{-1}.

Thermogravimetric analysis is a simple and accurate method for studying the decomposition pattern and the thermal stability of polymers. According to the thermogravimetric present results (Fig. 2), ulvan undergoes thermal degradation in a very typical manner. On the other hand, ulvan is thermally stable until c.a. 200°C.

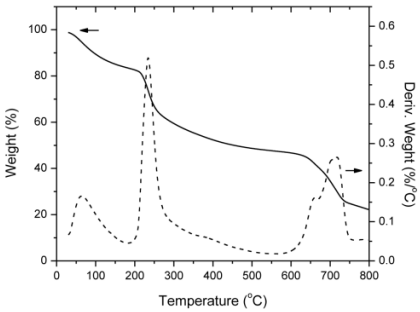

Figure 2: Thermogravimetric curve of ulvan, recording mass loss versus temperature, with its first derivative.

To further understand the thermal behaviour of the polysaccharide ulvan, DSC was performed. Ulvan thermogram in Fig. 3 exhibits a major transition at about 120ºC. This transition could be associated with loss of water, being consistent with the hydrophilic nature of ulvan functional groups. Our DSC experiments did not

allow the identification of a clear occurrence of a glass transition in ulvan in the temperature interval analysed.

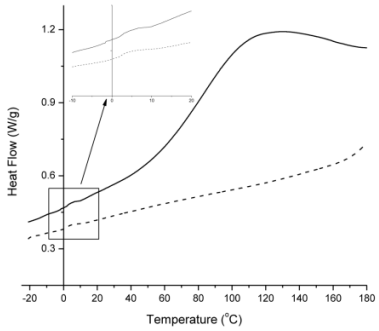

Figure 3: Thermogram of ulvan, on heating: 1st heating (straight line) and 2nd heating (dash line) (Endo up).

The x-ray diffractogram of ulvan (Fig. 4) revealed the semi-crystalline nature of this polysaccharide.

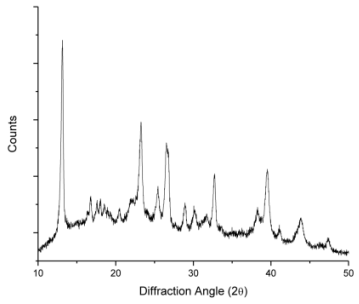

Figure 4: X-ray diffractogram of ulvan powder.

I*n vitro* cytotoxicity assays were carried out to evaluate the effect of different concentrations of ulvan on cellular biochemical functions. In order to benchmark the biological performance of ulvan, its behaviour was directly compared with that of hyaluronic acid, which is already regarded as a gold standard biopolymer in medical applications. In this study (Fig. 5), ulvan exhibited no detrimental effect on cell proliferation, within the range of concentrations evaluated. Our results suggest that ulvan is not harmful to cells and therefore, can be considered as non-toxic. This marine origin polysaccharide shows a good biological performance when compared to hyaluronic acid.

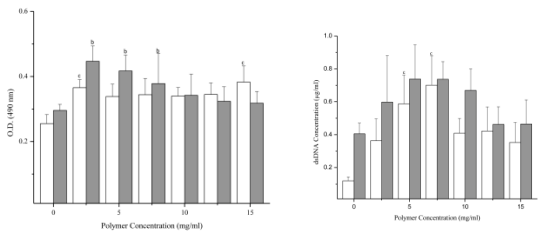

Figure 5: Effect of different concentrations of ulvan and hyaluronic acid on L929 viability (MTS) and proliferation (dsDNA).

In order to fully understand ulvan's potential as a tissue engineering biomaterial, this polysaccharide was used as the basis for the development of cross-linked 3D sponges. Preliminary characterization of ulvan sponges revealed a porosity of approximately 50%, and the majority of pores are of 60 to 80 µm in size (data not shown). Furthermore, they present a compressive modulus of 330 kPa. The results here reported suggest that ulvan extracted from green algae may exhibit large application potential in many consumer oriented applications, including biomedical.

CONCLUSIONS

Ulvan was successfully extracted from green algae and revealed to be a non-meltable semi-crystalline polysaccharide, which is thermally stable before degradation at 220 ºC. Furthermore, this polysaccharide is cytocompatible (under the studied conditions) and processable into scaffolds for different tissue engineering applications. The extraction of ulvan from green algae allowed the valorization of this marine resource, confirming the large application potential of marine derived polymers.

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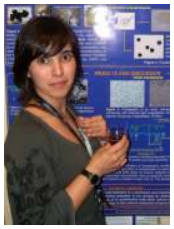
ACKNOWLEDGMENTS

Anabela Alves is grateful for financial support from *Fundação para a Ciência e Tecnologia* through the SFRH/BD/39359/2007 grant.

BIOGRAPHY



ANABELA ALVES was born in Vila Nova de Gaia, in 1981. She moved to Aveiro to get a degree in Biology, at the University of Aveiro. In 2007, she was awarded with a doctoral grant from *Fundação para a Ciência e Tecnologia* to develop her research work in 3 B's Research Group, University of Minho, on 'Development of innovative tissue engineering scaffolds from algae polysaccharides'.