A novel polysaccharide for biomedical applications: a bottom up approach to establish ulvan as a biomaterial

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Statement of Purpose: Nowadays, green algae as a source of biomolecules is still rather unexploited. Novel marine molecules can find applicability in different areas, such as foods, cosmetics, dietary supplements, bioactive packaging, industrial products and biomedical devices. In this context, considerable attention has been given to green algae and its polysaccharides, namely ulvan, a polysaccharide present in the cell wall of this algae. Ulvan is mostly composed of rhamnose, glucuronic acid, iduronic acid, xylose, and sulphate. Despite the wealth of potential new uses for ulvan, lack of adequate data on its safety based in standardized toxicologic studies, hamper its acceptability as a consumer oriented product.

Therefore, the aim of the present work is two-fold. First, two different batches of ulvan extract were compared with the purpose of validating the extraction procedure applied for the extraction of ulvan from green algae. Second, the biological performance of ulvan was compared with a well established polysaccharide, hyaluronic acid, which is currently used in many different applications, ranging from cosmetic to the biomedical area.

Methods: Ulvan was obtained by extraction from green algae. 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. To assess the short-term cytotoxicity of the extracted polysaccharide, ISO/EN 10993 part 5 guidelines were followed, adopting a cell exposition period of 24 h.

Cell Line: Mouse C3H/An connective tissue fibroblast-like cells (L929) were obtained from the European Collection of Cell Cultures (ECACC, UK). L929 cells between passages 8 and 10 were used to perform the biological performance studies.

MTS Assay: Colorimetric MTS assay, using CellTiter 96® AQ_{ueous} One Solution Reagent (Promega).

dsDNA Assay: dsDNA Quantification Kit (PicoGreen, Molecular Probes).

Total Protein Assay: Colorimetric protein assay, using Micro BCA™ Protein Assay Kit (Pierce).

Results: In this research work, a direct comparison between two different extracts of ulvan was made in order to assess the influence of batches' variability. Our results suggest that the two batches are similar and possess analogous biological performance. For this reason, we further proceeded with studies on ulvan cytotoxicity, through the evaluation of cell viability and proliferation.

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 many consumer oriented applications. In this study, 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. On the other hand, this marine origin polysaccharide shows a good biological performance when compared to hyaluronic acid.

	Concentration (mg/ml)	0	2,5	5	7,5	10	12,5	15
Cell Viability (%)	Ulvan	100.00	151.07	141.11	111.16	102.93	109.47	100.30
	Hyaluronic Acid	100.00	143.35	132.72	134.89	133.24	135.16	150.05

Figure 1: Effect of different concentrations of ulvan and hyaluronic acid on mouse C3H/An connective tissue fibroblast-like cells (L929) viability, evaluated by MTS assay.

Conclusions: Through the evaluation of two different cellular parameters, we can conclude that ulvan has no detrimental effect on cellular normal functions, within the range of concentrations evaluated, being considered as non-cytotoxic. The results here reported suggest that ulvan extracted from green algae may exhibit large application potential in consumer oriented applications, including biomedical related ones.

References: Lahaye, M., Robic, A. Biomacromolecules 2007, 8, 1765-1774.

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