Porphyridium cruentum: a factory for the production of a new polysaccharide-based biomaterial for tissue regeneration

Marta M. Duarte[a], Artem Suprinovych[a], Inês V. Silva[a,b], Oscar L. Ramos[a], Joana R. Costa[a], Luis Rojo[c,d], Ana L. Oliveira[a,*]

[a]CBQF – Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, 4200-375 Porto, Portugal
[b]Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue Regeneration Department, 6229 ER, Maastricht, the Netherland
[c]Instituto de Ciencia y Tecnología de Polímeros (ICTP), CSIC, c/Juan de la Cierva, 3, 28006 Madrid, Spain
[d]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), 28029, Madrid, Spain

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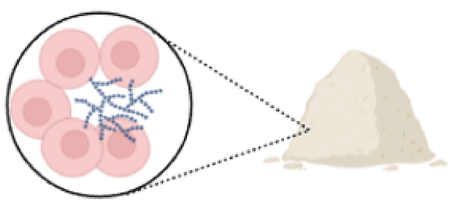
Introduction/Resume

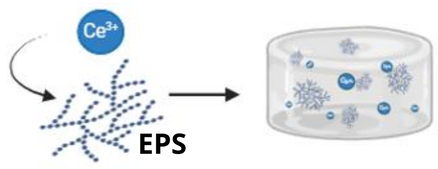
Marine algae and their metabolites have been widely recognized for their bioactive properties with applications in various industries, such as pharmaceutical, cosmetical, and nutraceutical (1). The red unicellular microalgae *Porphyridium cruentum* is a natural source for a variety of bioactive compounds (2), such as exopolysaccharides (**EPS**) and is already cultivated in large scale by several biotechnological companies, mostly for cosmetic applications.

These EPS have been shown to possess biological properties of high interest for tissue regeneration. Such as anti-bacterial, and immunomodulatory properties (3). Currently, there are challenges when it comes to their extraction and purification, which can influence their physicochemical properties. In this study, the best strategy for the processing of EPS from *P. cruentum* was discussed, and the collected, purified EPS fraction was characterized with a focus in maximizing its potential to be used as a new platform for healing and regeneration of chronic wounds.

Objective



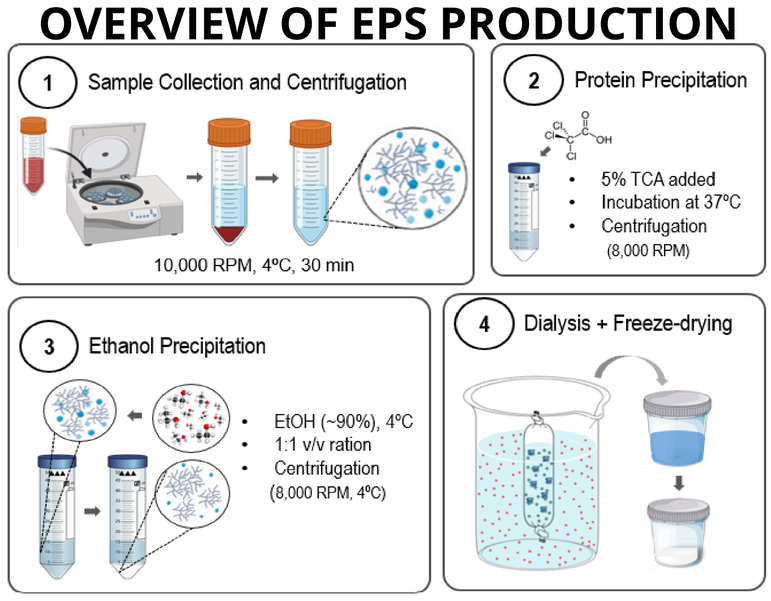
EPS COLLECTION + PURIFICATION



BIOMATERIAL FOR NEW BIOMEDICAL PLATFORM FOR TISSUE REGENERATION

Methods

OVERVIEW OF EPS PRODUCTION



EPS CHARACTERIZATION

Biochemical Composition
- Carbohydrate, protein, lipid, sulphate %

Sugar Composition
- Fourier-Transform Infrared Spectroscopy (FTIR)
- Nuclear Magnetic Resonance (NMR)
- **Structural and Surface Analysis**
- Gel Permeation Chromatography (GPC)
- Scanning Electron Microscopy (SEM)
- Energy Dispersive X-ray Spectroscopy (EDS)
- Differential Scanning Calorimetry (DSC)
- X-ray Diffraction (XRD)

Functional Properties
- Solubility/Water Holding Capacity
- Cytocompatibility

Results

DOES TCA TREATMENT PURIFY EPS SAMPLE?

Table 1. Carbohydrates, proteins, lipids, sulphate, and inorganic content in EPS as percentage of dry weight.

	EPS	EPS w/ TCA
Carbohydrates (neutral)	14.6 ± 0.4%	38.6 ± 3.9%
Proteins	28.9 ± 1.8%	11.3 ± 0.9%
Lipids	10.6 ± 0.68%	4.8 ± 0.24%
Sulphate	18.0 ± 1.5%	6.0 ± 1.2%

Results show that the protein precipitation protocol (**EPS w/ TCA**) was successful in decreasing the protein fraction of EPS and increasing carbohydrate content (**Table 1**).

The addition of this purification step also led to differences in the **functionalization** of the EPS powder, increasing solubility and water holding capacity (**Table 3**).

Table 2 and 3. (Left) Number Average Molecular Weight (Mn), Weight Average Molecular Weight (Mw), Polydispersity index (Mw/Mn), and **(Right)** Moisture, Solubility, and Water Holding Capacity values for EPS and EPS with TCA samples.

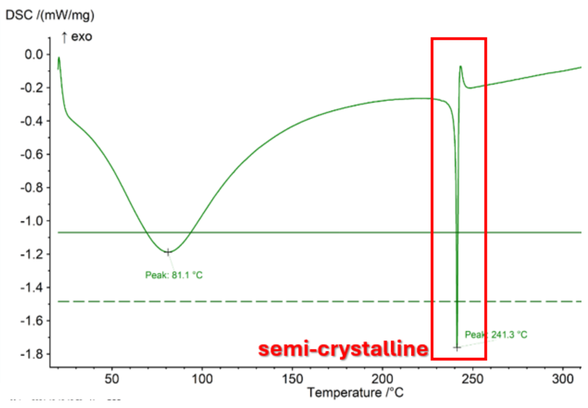
	EPS	EPS w/ TCA
Mn	7.1 x10^5	3.1 x10^6
Mw	2.6 x10^6	3.9 x10^6
Mw/Mn	3.7	1.2

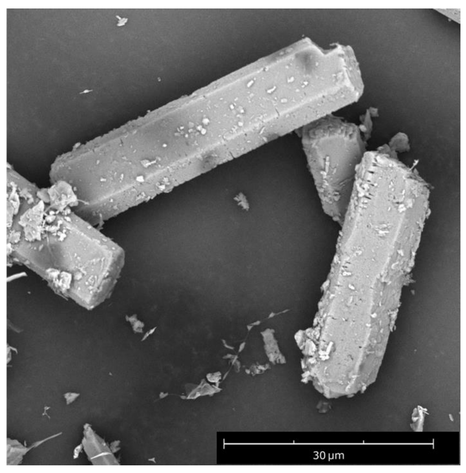
	EPS	EPS w/ TCA
Moisture (%)	7.3 ± 0.2	8.0 ± 0.3
Solubility (%)	16.7 ± 2.7	50.4 ± 1.4
WHC (%)	635.6 ± 1.6	5,632.5 ± 2.4

CHARACTERIZATION OF EPS SAMPLE

Elemental analysis showed that purified EPS composition was of 37% **Carbon**, 49% **Oxygen**, 6% **Sulphate**, and 7% **Calcium** (wt.%).

Their crystallinity was **61%**.





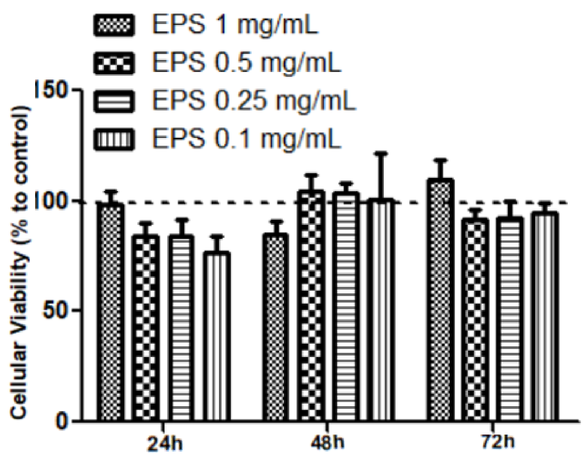


Figure 2 – (a) DSC curve for EPS w/ TCA (b) SEM close-up of EPS particles (4100x), (c) Cytotoxicity assay on hDFs

Conclusions

The results show that impurities can be significantly reduced by the TCA treatment, followed by dialysis, resulting in a more purified EPS fraction with a higher carbohydrate content and solubility. The obtained EPS fraction was of high molecular weight (**Table 2**), presented a high crystallinity index, was thermally stable (**Figure 2, a**) and cytocompatible within the range of tested concentrations (**Figure 2, c**). These results show its potential to be used as a new platform for the healing and regeneration of chronic wounds.

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[3] Casas-Arrojo V, Decara J, de los Ángeles Arrojo-Agudo M, Pérez-Manríquez C, Abdala-Díaz RT. Immunomodulatory, Antioxidant Activity and Cytotoxic Effect of Sulfated Polysaccharides from Porphyridium cruentum. (S.F.Gray) Nägeli. Biomolecules; 11(4):488

Acknowledgements

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