Adhesive proteins from *Bathymodiolus azoricus*: functional and structural characterization

F. Carneiro[1,2], R. Bettencourt[1,2] , R.L. Reis[1,2,3], T.H.Silva[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal;

[2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal;

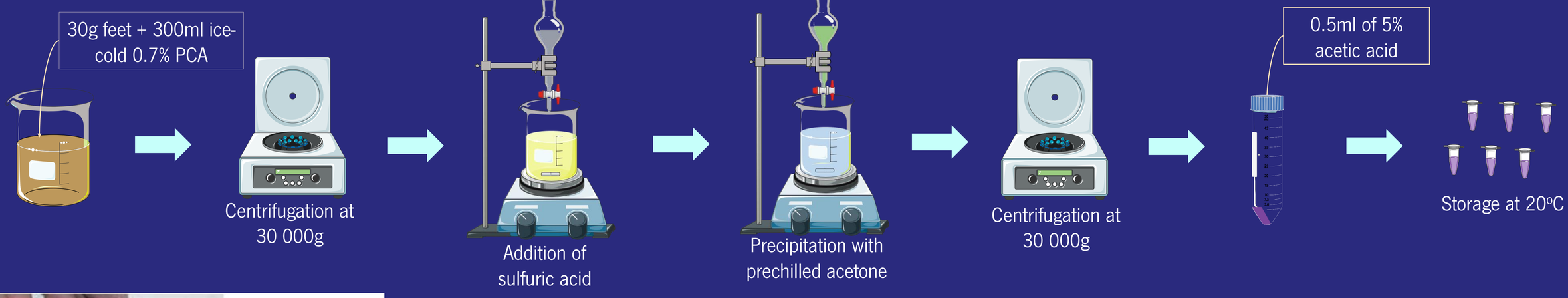
[3] The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal;

INTRODUCTION

The anchoring of mussels to foreign surfaces in seawater is due to role of adhesive proteins known as mussel foot proteins (Mfps). The post-translationally modified tyrosine, 3,4-Dihydroxyphenylalanine (DOPA) [1], present in Mfps, is involved in several types of chemical interactions and catechol crosslinking, which results in the ability of Mfps to solidify and bind tightly to various types of surface substrates [2], originating adhesive plaques with high interfacial binding strength, durability and toughness. Inspired by these remarkable wet adhesive properties, several natural Mfps have been extracted and analyzed from different species of mussels [3] aiming at the creation of biomedical adhesives and drug carriers for therapeutic uses. *Bathymodiolus azoricus* mussel subsists at vent sites in an extreme environment such as unusual levels of heavy metals, pH, temperature, CO_2, methane and sulfide[4]. These conditions require unique anatomical and physiological adaptations. Thus, one may hypothesize that adhesive proteins of *Bathymodiolus azoricus* might show different properties in comparison with homologous proteins from other shallow water mussels. Here, we present some results of the extraction method used to obtain the adhesive proteins from *Mytilus galloprovincialis* in order to optimize the extraction method and to compare with adhesive proteins from *Bathymodiolus aozoricus*. The produced knowledge will be relevant for the design of innovative bioadhesives for biomedical application.

MATERIALS AND METHODS

ADHESIVE PROTEINS EXTRACTION ACCORDINGLY WITH WAITE ET AL. (1995)[5]



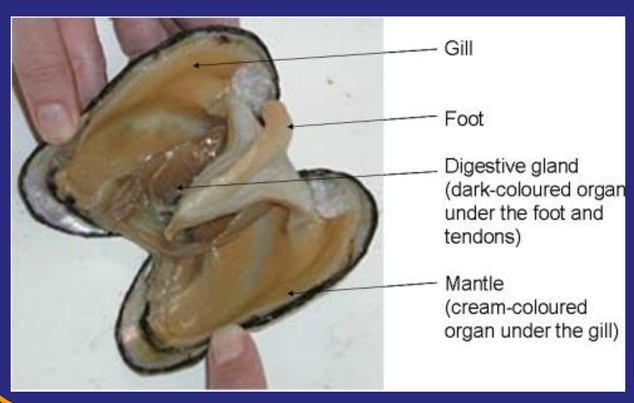


Fig.1- Anatomy of the mussel[6].

The extraction method was applied to two types of tissues from the *Mytilus galloprovincialis* mussel namely, foot and mussel content (gill, digestive gland and mantle – **Fig 1**).

The evaluation of purity, as well as quantification and preliminary characterization of extracted adhesive proteins, were performed by:

- Protein quantification - Pierce™ BCA Protein Assay Kit
- Assessment of possible contaminants, namely, collagen – Soluble Collagen Assay Sircol™
- SDS-PAGE
- FTIR

RESULTS AND DISCUSSION

SDS-PAGE

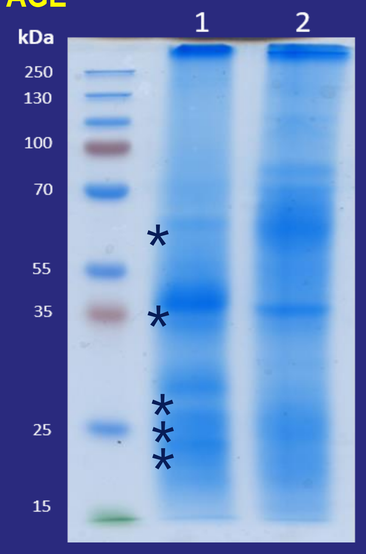


Fig.2- Electrophoresis analysis of adhesive proteins of *Mytilus galloprovincialis.* Lane 1: adhesive proteins extracted from mussel foot. Lane 2: adhesive proteins extracted from a pool of tissues (gill, mantle and digestive gland).

The analysis of the two extracts shown different protein profile suggesting that foot extracts was richer in adhesive proteins.

QUANTIFICATION OF PROTEINS

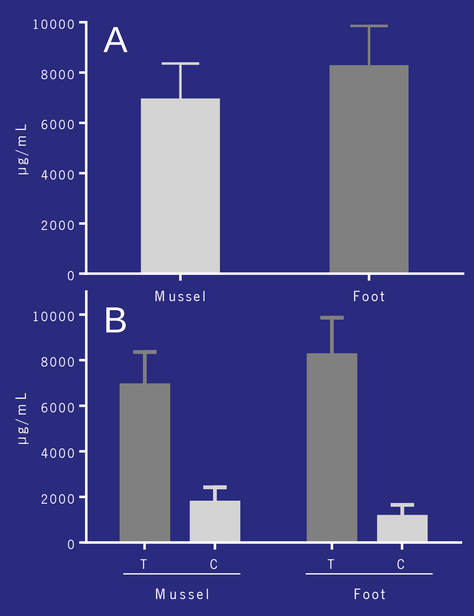


Fig.3- Protein quantification and collagen quantification as a possible impurity.

Protein quantification shown that foot extract have a higher quantity of total protein than the extract obtained from a pool of tissues, being also less contamination with collagen, coherently to the interpretation that it is richer in adhesive proteins.

FTIR SPECTROSCOPY

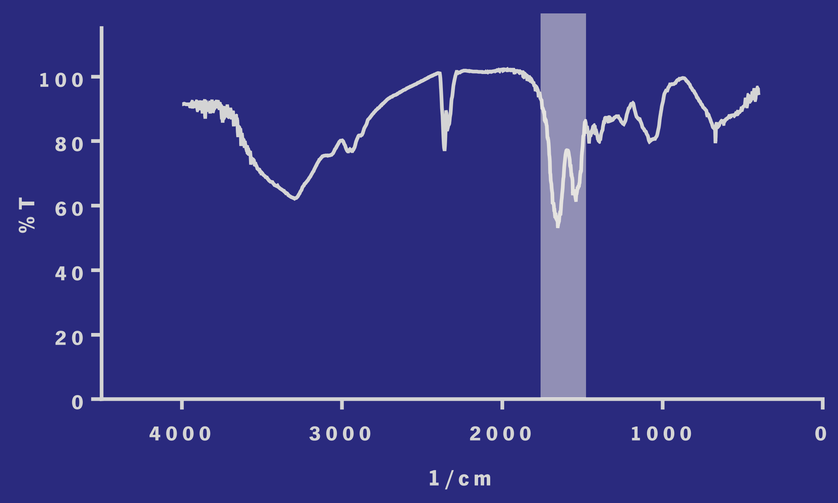


Fig.4- Fourier Transform InfraRed spectroscopy (FTIR) spectra of protein extract from foot mussel.

FTIR spectrum of adhesive proteins from foot extract exhibited a characteristic peak of L-DOPA amino acid as described by Suci and Geesey (2001).

CONCLUSIONS

- Waite (1995) method proved to be the ideal to extract adhesive proteins from the foot of mussel and will be the method used to extract adhesive proteins from *Bathymodiolus azoricus* mussel.
- Adhesive proteins from *Mytilus galloprovincialis* were successfully extracted; however, due to contamination with collagen, further purification is necessary to separate the different mussels foot proteins from other non-adhesive proteins and from each other.
- Further assays will be necessary to fully characterize mussel foot proteins, envisaging the establishment of innovative bioadhesives, as surgical glues, or drug carriers for therapeutic uses.

References:

1. Danner, E. W., Kan, Y., Hammer, M. U., Israelachvili, J. N. & Waite, H. J. Adhesion of Mussel Foot Protein Mefp-5 to Mica: An Underwater Superglue. Biochemistry 51, 6511–6518 (2012).
2. Forooshani, P. & Lee, B. P. Recent approaches in designing bioadhesive materials inspired by mussel adhesive protein. Journal of Polymer Science Part A: Polymer Chemistry 55, 9–33 (2017).
3. Bettencourt, R. et al. High-throughput sequencing and analysis of the gill tissue transcriptome from the deep-sea hydrothermal vent mussel Bathymodiolus azoricus. BMC Genomics 11, 559 (2010).
4. Ninan, L., Monahan, J., Stroshine, R. L., Wilker, J. J. & Shi, R. Adhesive strength of marine mussel extracts on porcine skin. Biomaterials 24, 4091–4099 (2003).
5. Waite, J. Precursors of quinone tanning: dopa-containing proteins. Methods Enzymol 258, 1–20 (1995). 6. Craft, J. A. et al. Pyrosequencing of Mytilus galloprovincialis cDNAs: Tissue-Specific Expression Patterns. Plos One 5, e8875 (2010).
7. Suci, P.A. and G.G. Geesey, "Use of attenuated total internal reflection Fourier Transform Infrared Spectroscopy to investigate interactions between Mytilus edulis foot proteins at a surface," Langmuir, 17(8):2538-2540 (2001).

Acknowledgments:

This work is being partially funded by European Regional Development Fund (ERDF), through European Union Transborder Cooperation Programme Interreg España-Portugal 2014-2020 (POCTEP), under the scope of project 0302_CVMAR_I_1_P, and through NORTE 2020, under the Portugal 2020 Partnership Agreement, under the scope of Structured Project NORTE-01-0145-FEDER-000021.