Bioadhesion in the marine crustacean *Pollicipes pollicipes*

Mariana Almeida[1,2], Miguel Rocha[1,2], Tiago H. Silva[1,2] and Rui L. Reis[1,2,3]

[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

BACKGROUND

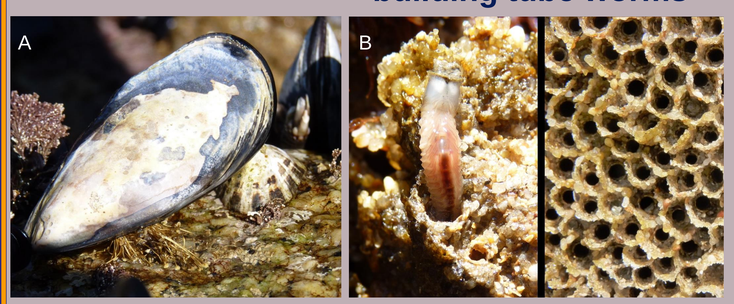
Biomimetic adhesives

- ✓ Natural adhesives produced by marine organisms are of interest to the biomedical field because of their effectiveness in aqueous environments.
- ✓ Generally, marine bioadhesives are functional systems with the purpose of attachment, temporary or permanently, of a organism to a surface. Natural underwater adhesives are able to displace the bound water layer on the surface to which the animal attaches, maintaining a strong and stable bond between the animal and the substrate [1].
- ✓ The development of marine bioinspired adhesives needs the understanding of the diversity of molecular mechanisms of marine bioadhesion [2,3].

Protein based adhesives from marine invertebrates - General characteristics

Mussel byssus

Tubes from the reef building tube worms



- ✓ DOPA
- ✓ Phosphorilated serine (pSer)
- ✓ Involvement of sequence motifs

Fig. 1 A) Mussel *Mytillus* sp. and B) the tubeworm *Sabellaria alveolata*.

Barnacles are sessile crustaceans with mineralized plates found in rocky shore environments. They attach permanently to natural and man made substrates by producing an adhesive called cement.

Fig. 2 Example of a acorn barnacle.

Interest on barnacles cement

- ✓ Strong adhesive capable of resist to wave exposure and flutuations in water temperature and salinity [4].
- ✓ The adhesive, a low viscosity fluid, flows through the ducts of the cement apparatus and hardens underwater in contact with the substrate to form a cement that is insoluble in water.

- ✓ No DOPA detected
- ✓ No phosphorylation
- ✓ No evidence of repeating sequence motifs
- ✓ Apparently no post translational modifications

Different molecular system of adhesion?

AIM

Taking into account that the development of bioadhesives is most effective when using molecular tools to a better understanding of the key features and mechanisms of natural adhesives, we propose to apply protein chemistry tools to characterize the adhesive proteins from the marine sessile crustacean *Pollicipes pollicipes* (Crustacea Scalpelliformes).

APPROACH

Study organism

✓*Pollicipes pollicipes*, a species with high economic value found in the Portuguese coastline, is a stalked barnacle which attaches permanently to rocks in littoral environments by means of strong adhesive substances (Fig. 3).

✓The body of stalked barnacles consists of the capitulum (ca) containing the appendages and most organs typically covered by calcareous plates, and a muscular peduncle (pe) protected by a cuticle containing the ovary and the cement apparatus (Fig. 3), whereas in acorn barnacles (Fig. 2), the most studied barnacle group in the context of bioadhesion, the peduncle is absence and the calcareous plates are directly attached to the substrate [5].

Fig. 3 Stalked barnacle *Pollicipes pollicipes* (Crustacea Scalpelliformes), *Praia da Memória*, NE coast of Portugal.

Ongoing work

- ✓ Adult specimens (2.5 to 3 cm) and adhesive material were collected in *Praia da Memória*, NW coast of Portugal in April 2018.
- ➢ After solubilization of secreted adhesive material and isolation of the proteins, protein characterization will be assessed by mass spectrometry. The amino acid sequences will be compared to other related species to find for protein homologues or putative novel proteins.

EXPECTED OUTCOMES

It is expected that the development of this research may contribute to biotechnological advances in the design of new surgical adhesives or biohybrid and biomimetic materials. Due to the fouling problems that barnacles cause, it may also be applied to the development of toxic-free antifouling compounds and also in the aquaculture of this species, as an alternative to supply the market and for re-stocking programmes.

References:

[1] Power AM, Klepal W, Zheden V, Jonker J, McEvilly P, von Byern J (2010) Mechanisms of adhesion in adult barnacles. In: von Byern J, Grunwald I (eds) Adhesion phenomena in nature. Springer, New York, pp 153–167; [2] Waite JH, Qin X (2001) Biochemistry 40, 2887–2893; [3] Zhao H, Sun C, Stewart RJ, Waite JH (2005) J Biol Chem 280, 42938–42944; [4] Lobo-da-Cunha A, Alves A, Oliveira E, Cunha I (2017) Mar Bio, 164:11; [5] Walker G (1978) Bull Mar Sci 28, 205–209.

Acknowledgments:

This work was partially funded by European Union Transborder Cooperation Programme Interreg España-Portugal 2014-2020 (POCTEP) under projects 0245_IBEROS_1_E and 0302_CVMAR_I_1_P.