How do sea cucumber Cuvierian tubules become sticky? A comparative histological approach

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INTRODUCTION: Marine bioadhesive research has been gaining increasing interest because of its high potential for the development of novel adhesives for biomedical applications like in surgery or in dentistry. Both fields indeed require adhesives working in wet environment where a "classical glue" cannot operate. Currently permanent adhesion is the most investigated type of adhesion but in the search for new models, we are studying Cuvierian tubules, a specialized defense system occurring in some species of sea cucumbers. When these animals are stressed, they expel a few tubules, which lengthen considerably and become sticky upon contact with any object, immobilizing potential predators. The material secreted by theses tubules has the particularity to become immediately sticky unlike permanent adhesion material. In this study, we investigated the interface between tubules and the substrate to show how the glue is produced and released.

METHODS: Individuals of *Holothuria forskali* and *Holothuria maculosa* were collected by scuba diving in Roscoff (France) and by snorkeling in Toliara (Madagascar), respectively. Cuvierian tubules were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer at three different stages: (*i*) before expulsion, (*ii*) after expulsion but before contact and (*iii*) after expulsion and contact. They were then postfixed with 1% osmium tetroxide in 0.1M cacodylate buffer, embedded in Spurr resin, sectioned transversally or longitudinally and observed in transmission electron microscopy (TEM). For scanning electron microscopy (SEM), Cuvierian tubules were forced to be expelled on different substrates (glass, Teflon®, mica) and then detached manually leaving Cuvierian tubules glue prints on the surface. These prints were fixed in Bouin's fluid and prepared by critical-point drying and sputter-coating before observation.

RESULTS AND DISCUSSION: Cuvierian tubules were observed at different stages during their elongation and adhesion process. Although the tubules from the two species present differences in their quiescent state, these tend to disappear as soon as tubules elongate. Indeed, in *H. forskali*, the mesothelium, the layer responsible for adhesion, is composed of two cell layers: an inner folded layer of granular cells and an outer layer of peritoneocytes. *H. maculosa*, on the other hand, possesses a mesothelium with well-developed peritoneocytes but reduced granular cells. Moreover, an additional layer of a third voluminous cells type is sandwiched in-between. When Cuvierian tubules elongate without contact, peritoneocytes, and the third cell type in *H. maculosa*, disintegrate and granular cells become the most external. Finally, when they make contact with a surface, granular cells release the content of their granules and the glue is formed. Cuvierian tubule glue prints from the two species were observed in order to understand the structure and formation of the adhesive. In *H. forskali*, prints present a heterogeneous aspect due to variations in the quantity of deposited material within a same patch. This can be explained a contamination of the glue with other tubule components (collagen fibres, granules of granular cells) which may occur when tubules are detached. The glue itself seems to be composed of 60-80 nm globular nanostructure deposited as a thin film. No difference was observed between the substrates regarding glue structure but Cuvierian tubules were less sticky on Teflon than on glass or mica, which is reflected by the lower quantity of adhesive attached on this material. In *H. maculosa*, glue prints present the same structure but a fibrillar network was frequently observed covering the glue and "contaminant" granules from granular cells were not as abundant as in *H. forskali*. Thus, although peritoneocytes possess mucous granules which are labelled by anti-glue antibodies, only the contents of granular cells seem to enter in the composition of the glue in both species. The role of peritoneocytes and of the new cell type is still unknown.

REFERENCES: P. Becker and P. Flammang (2010) Unravelling the sticky threads of sea cucumbers – A comparative study on Cuvierian tubule morphology and histochemistry in *Biological Adhesive System* (eds J. von Byern & I. Grunwald) Spinger, pp 87-98.