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Biomimetic Materials as Potential Medical Adhesives – Composition and Adhesive Properties of the Material Coating the Cuvierian Tubules Expelled by *Holothuria dofleinii*

Yong Y. Peng[1], Veronica Glattauer[1], Timothy D. Skewes[2],
Jacinta F. White[1], Kate M. Nairn[1], Andrew N. McDevitt[3],
Christopher M. Elvin[3], Jerome A. Werkmeister[1],
Lloyd D. Graham[4] and John A.M. Ramshaw[1]

1. Introduction

Novel, distinct adhesive systems have been described for a wide range of marine species (Kamino, 2008). These highly effective, natural materials provide a link between biological science and material science, and can serve as models on which new, bioinspired synthetic materials could be based. These various adhesive systems have developed independently, on many occasions, and provide a wide range of opportunities for the development of new, biologically-inspired adhesives. The natural adhesives include, for example, the marine mussel (*Mytilus sp.*) (Lin et al., 2007), barnacle (Nakano et al., 2007) and stickleback (Jones et al., 2001) adhesives, which are protein-based, as well as sponge, certain algal and marine bacterial adhesives (Mancuso-Nichols et al., 2009) that are polysaccharide-based.

In the present paper, we examine the adhesive system found associated with the Cuvierian tubules of a holothurian species (sea cucumber), *Holothuria dofleinii*. This is an example of the particularly rapid marine adhesive that is found on the surface of Cuvierian tubules when they are expelled (DeMoor et al., 2003; Müller et al., 1972; VandenSpiegel & Jangoux, 1987). The unique nature of this natural adhesive system, especially its rapid action under water, has suggested that if the mechanism can be understood, then it may prove to be possible to mimic the adhesive through biotechnology and/or synthetic chemistry. An adhesive that functions readily in an aqueous environment would be particularly valuable, especially in medical applications, as the majority of existing adhesives bind to dry surfaces more strongly than the same surfaces when wet.

Cuvierian tubules provide a host defence mechanism for certain species of holothurians (Lawrence, 2001; VandenSpiegel & Jangoux, 1987). It has long been known that, on expulsion, the Cuvierian tubules fill with liquid and lengthen, become sticky and rapidly

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[1]*CSIRO Materials Science and Engineering, Bayview Avenue, Clayton, VIC 3169, Australia*
[2]*CSIRO Marine and Atmospheric Research, Middle Street, Cleveland, QLD 4163, Australia*
[3]*CSIRO Livestock Industries, Carmody Road, St Lucia, QLD 4067, Australia*
[4]*CSIRO Food and Nutritional Sciences, Julius Ave, North Ryde, NSW 2113, Australia*

immobilise most organisms with which they come into contact (VandenSpiegel & Jangoux, 1987). The tubules, once expelled, are immediately adhesive on contact with a solid surface (VandenSpiegel & Jangoux, 1987), such as the exoskeleton or skin of a predator. Crabs, molluscs and sea stars can stimulate tubule expulsion, and the tubules stick to these species. This adhesion happens entirely under water, and does not need the mixed environment of the intertidal zone where many of the other potential adhesives are sourced.

Sticky tubules are found only within the family Holothuridae within the order Aspidochirotida, and mostly in the genus Bohadschia and the genus Holothuria. Various authors have described the ultrastructure of the tubules, especially for *H. forskåli* (Lawrence, 2001; VandenSpiegel & Jangoux, 1987; VandenSpiegel et al., 2000), as well as their expulsion and release (Flammang et al., 2002) and the timeframe of regeneration (Flammang et al., 2002; VandenSpiegel et al., 2000).

Flammang and Jangoux (2004) suggested, from the differences in the surface (adhesive) protein types and compositions in *H. forskåli* and *H. maculosa*, that the adhesion proteins and mechanism may differ between species. Other studies showed that adhesive strengths varied between species, with the adhesion in *H. leucospilota* being several times greater than for six other species (Flammang et al., 2002). A limited number of studies have probed the mechanism of adhesion, focusing on *H. forskåli* and *H. leucospilota* (De Moor et al., 2003; Müller et al., 1972; Zahn et al., 1973). These studies have shown that best adhesion is found at temperatures, salinity and pH similar to those found in the marine environment in which the organism flourishes, and is most effective with hydrophilic surfaces (Flammang et al., 2002; Müller et al., 1972; Zahn et al., 1973). Increasing concentrations of urea led to a loss of adhesion, suggesting that native protein structure(s) or interctions(s) may be required for effective bonding (Müller et al., 1972). Later biochemical studies have also suggested that the adhesive mechanism involves protein components (DeMoor et al., 2003).

In the present study, we have extended the information on Cuvierian tubule adhesion. In this study we examined the tubules of a different species, *H. dofleinii* Augustin, 1908. We have examined the distribution of the adhesive substance on the surface of expelled tubules, along with the molecular weights and amino acid compositions of its main protein components. We have estimated the strength of adhesion of *H. dofleinii* tubules to different substrata, and examined the effects of salinity, pH, ionic strength and denaturants on the adhesive properties.

2. Materials and methods

2.1 Collection of materials

Individual *H. dofleinii* were obtained from shallow subtidal seagrass banks in Moreton Bay, Queensland, at a depth of about 1-2 metres at low tide, close to the western side of Stradbroke Island (153° 26.4' E 27° 25.13' S to 27° 25.68' S), and were held for up to 5 days prior to use in filtered, recirculating seawater tanks at 21.5 – 22 °C. The identification of the animals was based on morphology, spicule shape and size and 18S-RNA sequencing (Peng & Skewes, unpublished data).

2.2 Sample preparation

To collect expelled Cuvierian tubules, *H. dofleinii* individuals were held and gently stimulated underwater until tubules were expelled. Immediately after expulsion, a tubule was individually collected using polytetrafluoroethylene-tipped forceps, and was allowed

to drain briefly (<20 sec), but not by squeezing as had been proposed by others (Zahn et al., 1973). Intact tubules prior to expulsion were obtained by dissection of animals that had been euthanised by freezing at minus 20 °C.

2.3 Microscopy

To look for the presence of glycoprotein on the surface of expelled tubules, samples were treated with fluorescently-labelled lectins; fluorescein isothiocyanate (FITC)-labelled concanavilin A (ConA), FITC-labelled *Datura stramonium* agglutinin (DSA), and FITC-labelled *Lycopersicon esculentum* agglutinin (LEA) (all from Sigma, St Louis). All FITC-labelled lectins were applied as 20 μg/mL solutions in Tris-buffered saline (TBS) for 60 min, followed by 3 × 5 min washes in TBS. Samples were examined using appropriate narrow pass filters on an Olympus BX61 fluorescence microscope.

To examine the distribution of adhesiveness on tubules, individual freshly expelled tubules after draining (see above) were transferred to a wash solution in a plastic trough which contained a suspension of 0.5% w/v Bio-Gel P2 (45-90 μm particle size) in 3.5% w/v NaCl, 10 mM sodium phosphate, pH 7.6. After 5 sec immersion, the tubules were washed 3 times in 3.5% NaCl, 10 mM sodium phosphate, pH 7.6 and were then drained and placed onto glass slides. After air drying, the tubules were examined by microscopy.

For scanning electron microscopy (SEM) expelled tubules were examined using a Philips XL30 FESEM microscope at an accelerating voltage of 2 kV.

2.4 Gel electrophoresis analysis

Freshly expelled and drained tubules were allowed to adhere to a glass plate and were air dried. The tubules on glass plates were removed by peeling, leaving the layer of adhesive, and potentially other components of the tubule wall as a print on the glass (DeMoor et al., 2003). This material was collected by removal with a sharp razor blade and was then extracted in electrophoresis sample buffer, containing 2-mercaptoethanol. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was based on the method of Laemmli (1970) using Invitrogen NuPAGE Novex 4-14% Bis-Tris Gel with MES running gel buffer, at 180V for 60 min. Molecular weights were determined by comparison to globular protein standards (BioRad) using BioRad Quantity One v.4.4.0 software. For protein identification, gels were stained by Coomassie Blue R-250. Samples that had not been dried completely, but only sufficient to remove excess liquid, appeared to give samples that contained less insoluble material, although the yield of adhesive proteins was less.

2.5 Amino acid analysis

Protein extracts were separated by SDS-PAGE, followed by transfer of the protein bands to PVDF membrane using Invitrogen NuPAGE Transfer buffer (NP0006-1). Amino acid analysis of PVDF membrane pieces used vapour-phase hydrolysis (5.8 M HCl at 108 °C for 18 h), followed by precolumn derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (Cohen & DeAntonis, 1994). Derivatives were separated and quantified by reversed phase (C18 Waters AccQTag) HPLC at 37 °C (Cohen, 2001) (Australian Proteome Analysis Facility), using a Waters Alliance 2695 Separation Module, a Waters 474 Fluorescence Detector and a Waters 2487 Dual λ Absorbance Detector in series.

2.6 Adhesion properties

Adhesion properties were measured using a 90 Degree Adhesive Peel Strength Test (Dimas et al., 2000), adapted to the rapid evaluation of tubules from a single animal under different experimental conditions. Individual expelled tubules, as above, were transferred to a wash solution in a plastic trough containing a wash solution determined by the particular test (see below). The numbers of samples tested in a given experiment are given in the results Tables; in each case, tubules from a minimum of 3 separate animals were used. After 60 ± 2 sec, the tubule was removed from the wash solution and allowed to drain for 5 sec. It was then laid across the width of a 25 mm wide strip of substratum, selected for the particular test. The tubule was allowed to adhere to the test substratum under its own weight for 60 ± 2 sec. This differs from previous studies where a load was applied during adhesion (Flammang et al., 2002). During the adhesion period the tubule was trimmed to leave <10 mm overhanging one side of the substratum and about 50 mm on the other side. The flat width of the tubule was measured and also recorded photographically, with a ruler placed adjacent, for subsequent verification. At the end of the 60 sec adhesion period, each tubule-substratum assembly was then transferred to a frame that allowed the substratum to be held horizontally with the adhered tubule on the underside, i.e. with the free *c*. 50 mm length of tubule hanging below. The load was then increased stepwise (2.5 g/5 sec) to the overhang of the tubule until the tubule-substratum adhesion failed by peeling. The total load at failure was recorded. The maximum force tested was 0.2 N (approximately 20 g load) which equated to about 0.05 N/mm for an average tubule, because higher loads typically took too long to add and the tubule could have begun to desiccate at that stage, potentially changing the adhesive strength. A minimum of six determinations was made for each test condition. Data are presented as the total force at failure (N) divided by tubule width (mm). Although we did not test values above 0.05 N/mm, our conservative approach did not hinder examination of conditions that led to reduction of adhesive strength.

Experiments to test adhesion to different substrata used a wash solution of simulated sea-water comprising 3.5% NaCl, 10 mM sodium phosphate buffer, pH 7.6. Various substrata were tested, including clean glass (microscope slide), aluminium, polyvinyl chloride, chitin (from crab), polycarbonate, poly(methyl methacrylate) (PMMA) and polytetrafluoroethylene (PTFE), all cut to a similar size. As the chitin substratum lacked stiffness, the samples were first glued with cyanoacrylate onto a glass microscope slide. The chitin sample also had an irregular surface and was not uniform like the other materials.

The effects on tubule-glass adhesion of various chloride or sodium salts (50 mM) were examined by supplementing the 3.5% NaCl, 10 mM sodium phosphate buffer, pH 7.6, before washing the tubules. The effect of NaCl concentration on tubule-glass adhesion was examined using different NaCl concentrations in 10 mM sodium phosphate buffer, at final pH 7.6. The effect of pH on tubule-glass adhesion was examined using solutions prepared using three salts: Tris/chloride, sodium citrate and sodium acetate, each at 50 mM in 3.5% NaCl, 10 mM sodium phosphate. Similarly, the effect of urea on tubule-glass adhesion was examined using different urea concentrations in 3.5% NaCl, 10 mM sodium phosphate at a final pH of 7.6. Glass was used as the standard substratum as it was readily available in uniform quality, and had previously been shown to be an excellent material for adhesion of *H. forskåli* tubules (Flammang et al., 2002).

3. Results and discussion

3.1 Tubule structure and microscopy

Dissection of euthanised *H. dofleinii* showed that the body cavity contained a large number, several dozen, Cuvierian tubules in their compressed form (Figure 1A). In their compressed form the tubules were not sticky, but they rapidly became sticky on mechanical extension, even from the dead animals. The compressed, individual tubules showed a corrugated and folded surface (Figure 1B), which would allow extension when required, like a concertina bellows. In cross-section (Figure 1C) a three-lobed channel could be seen that would allow fluid insertion for expansion of the tubules. When expelled and fully extended *in vivo*, these tubules became instantly sticky and changed from the 25-35 mm compressed length up to around 350-400 mm. The fully extended tubules (Figure 1D) were typically about 4mm flat width when fully inflated, and still showed some patterning from the folding that was present in the compressed state. Individual animals contained several dozen non-inflated tubules, but when the animals were stimulated only a small number were expelled, normally around 8-12.
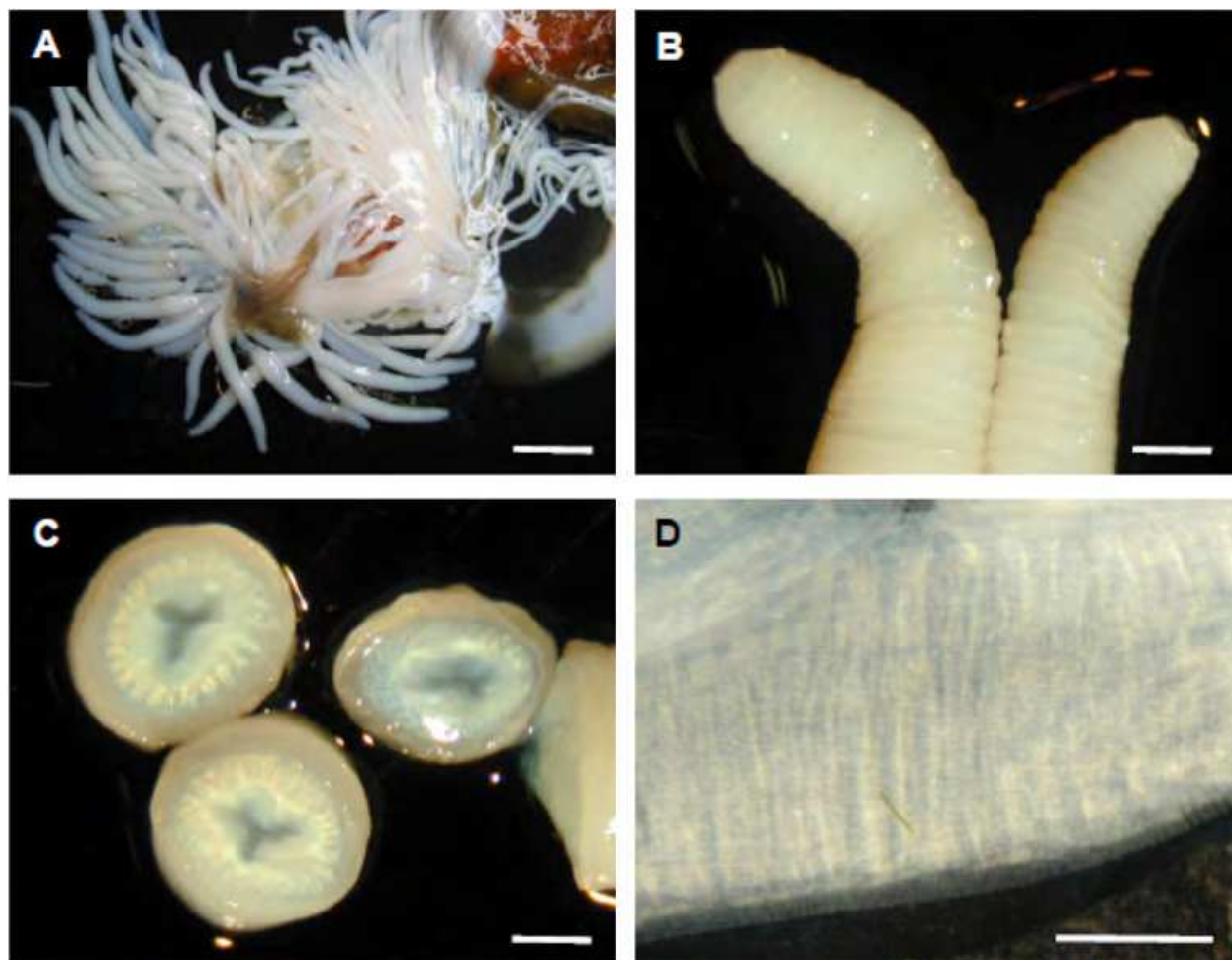

Fig. 1. Cuvierian tubules for *H. dofleinii*. (A, B, C) After dissection of a euthanised animal, showing, (A) the total mass of tubules, (B) the tips of compressed tubules, and (C) the cross-section of compressed tubules. (D) The surface of an *in vivo* expelled tubule. Bar (A) = 10 mm, Bars (B, C, D) = 1 mm.

The surfaces of naturally extended tubules showed strong binding of FITC-DSA (Figure 2A) and FITC-LEA lectins to the tubule surface, with a series of bands that resemble the original folding of the un-extended tubule. FITC-ConA lectin binding was weak, suggesting that the DSA and LEA binding was specific for carbohydrate or glycosylated protein on the surface, rather than non-specific binding. These 2 lectins recognise very similar carbohydrate entities; (N-acetyl glucosamine)$_2$ by DSA and (N-acetyl glucosamine)$_3$ by LEA, which are distinct from the α-mannose or α-glucose recognised by ConA. SEM of the surface of expelled tubules (Figure 2B) showed that the surface had fibrous-like structures, suggesting aggregates of the adhesive material overlaying a further fibrous, collagenous layer of the wall of the tubule.
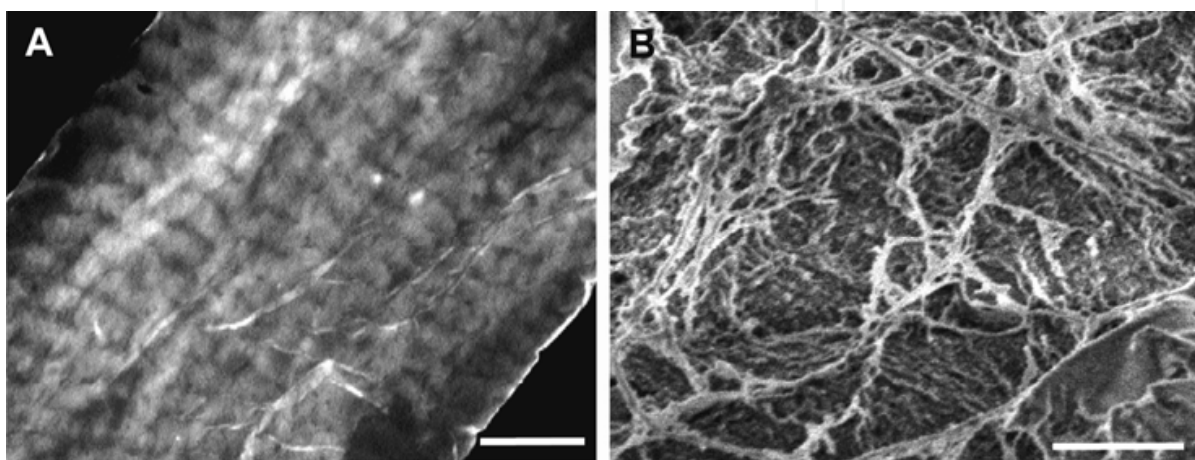

Fig. 2. Cuvierian tubules from *H. dofleinii*. (A) Fluorescence microscopy of FITC-labelled DSA bound to expelled Cuvierian tubules. Bar = 0.5 mm. (B) SEM of expelled Cuvierian tubules. Bar = 2.5 μm

When freshly expelled tubules were briefly immersed in 3.5% NaCl containing Bio-Gel P2 particles, and then washed in 3.5% NaCl, particles bound to the tubule (Figure 3). These data showed that the surface of the tubule was generally adhesive, and there was no specific localisation of the particles, for example to the fibrous-like patches seen by SEM.
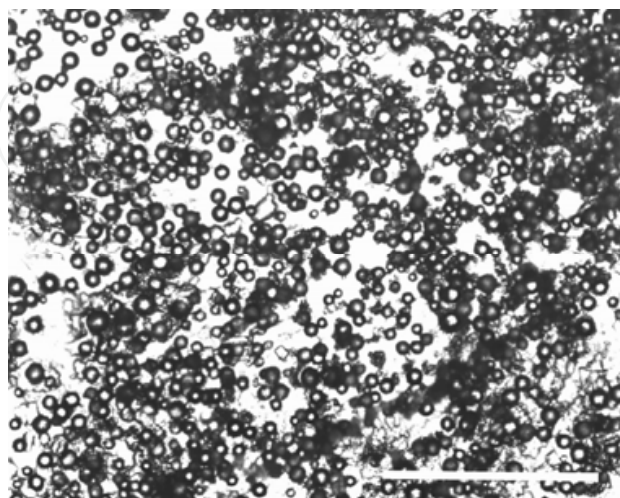

Fig. 3. Cuvierian tubules from *H. dofleinii*. Adhesion of Bio-Gel P2 beads to freshly expelled Cuvierian tubules from *H. dofleinii*. Bar = 0.5 mm

3.2 Gel electrophoresis analysis

The adhesion print isolated from glass showed a range of proteins of well-defined molecular weights when analysed under reducing conditions by SDS-PAGE (Figure 4). A similar pattern, except as noted below, was observed from >10 separate samples from different animals. Seven bands, H2 at 89 kDa, H3 at 70 kDa, H4 at 61 kDa, H6 at 44 kDa, H7 at 37 kDa, H8 26 kDa and H9 at 17 kDa, were consistently present in all tubule samples that were examined (n > 12). Band H4 has been examined as a single entity, but in some gels (Figure 4) it appeared that it may comprise 2 components. For all other bands, although each appeared to be a single component, it is also possible that more than one component could be present, migrating similarly. In many samples, but not all, an additional band, H5 at 53 kDa, was present. As it was not consistently present it was assumed that it may not be a key component of the adhesive system and hence was not examined further. In a few samples, an additional band H1 was observed at 150-170 kDa. This band seemed to be more prevalent in samples where tubule fragments were present in the adhesion print, and could possibly be related to the collagen that is the main structural component of the tubule (Watson and Silvester, 1959). The collected material sometimes contained a proportion of material that remained insoluble in the sample buffer. Examination of the adhesive prints under a microscope suggested that the samples that subsequently contained more insoluble material also contained more fibrous material that could be from the collagenous wall of the tubule. Samples with little wall material typically had little, if any insoluble material.
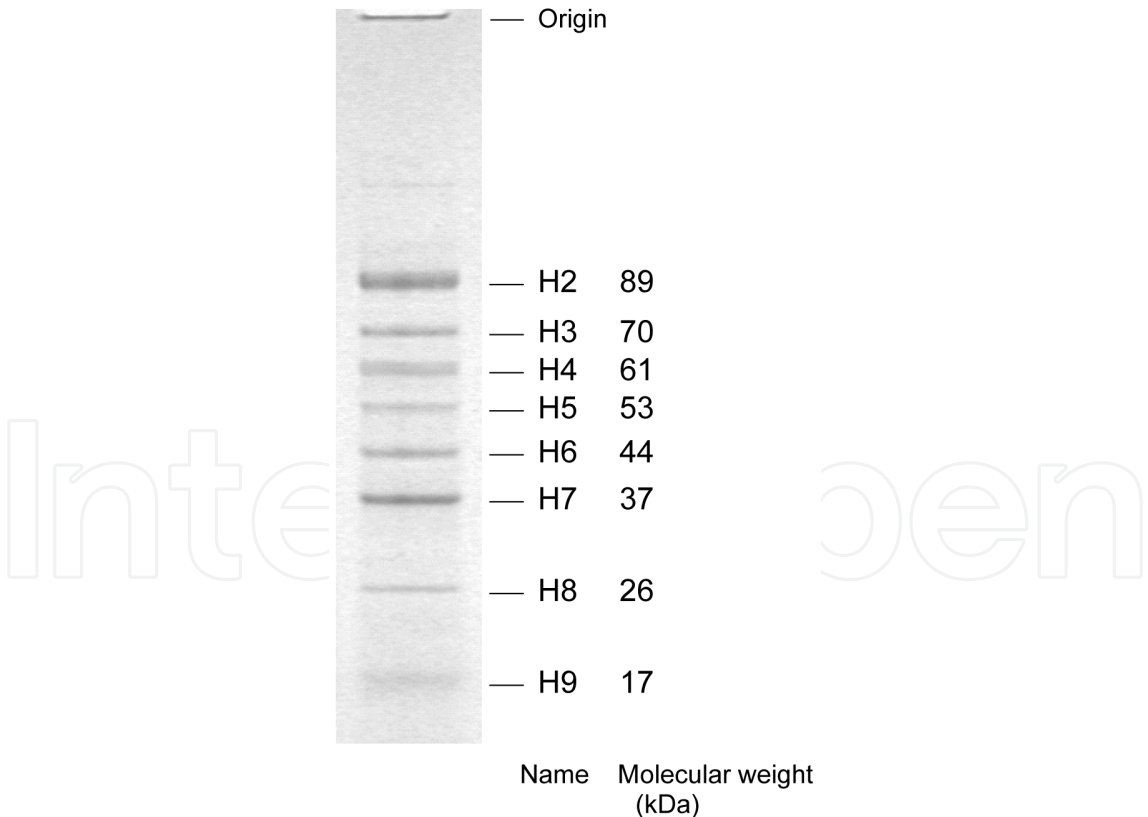

Fig. 4. SDS-PAGE of the reduced proteins from the surface of a freshly expelled Cuvierian tubule from *H. dofleinii*. The gel was stained with Coomassie Blue R-250. Key bands are labelled and their estimated molecular weights, interpolated from a standard curve using globular protein standards (BioRad), are given.

Previously, Flammang and colleagues (DeMoor et al., 2003) have shown a gel electrophoresis pattern for the tubule print from *H. forskali* samples. In this case, a high background staining was present, and the bands were generally less well defined and more poorly resolved. In some cases the apparent *H. forskali* bands had comparable molecular weights to those observed in the present study. Thus the sharp bands at 95 kDa and 45 kDa may be similar to the H2 (89 kDa) and H6 (44 kDa) bands, while the diffuse bands at 63 kDa and 33 kDa may be similar to the H4 (63 kDa) and the H7 (37 kDa) bands, respectively.

3.3 Amino acid analysis

The amino acid compositions determined from amino acid analyses of the 6 principal bands are given in Table 1. No data were collected for Band H4 as it appeared to be a doublet (Figure 3). Deamidation during acid hydrolysis means that Asn cannot be distinguished from Asp, nor can Gln be distinguished from Glu; the two pairs are given as Asx and Glx respectively. This prevents an estimation of pI for each of these proteins. Hydroxyproline was not observed in any of the 6 principal bands.

Comparison of the analyses for the various bands did not show signature features for any particular band, and the compositions were broadly similar for all bands. All of the bands had high contents of Gly (7.8-16.8 mol%) and Glx (11.6-16.2 mol%) relative to the average for eukaryotic proteins (6.9 and 9.7 mol%, respectively) (Doolittle, 1986); similarly, DeMoor et al. (2003) observed high Gly contents (16-22 mol%) for the proteins extracted from *H. forskåli.* As noted above, the SDS-PAGE molecular weight data suggests that it is possible that some bands could be related between the species - H2 and 95 kDa, H6 and 45 kDa and H7 and 33 kDa. Comparison of the amino acid composition data, however, did not show strong similarities. However, it has been suggested (Flammang and Jangoux, 2004), that the protein components present in the adhesives differ between species.

	H2	H3	H6	H7	H8	H9
Asx	9.7	8.7	12.1	13.1	9.7	15.0
Ser	9.8	11.6	7.0	6.5	10.2	8.0
Glx	16.2	13.3	13.2	12.7	15.0	11.6
Gly	16.1	16.8	7.8	8.1	13.6	10.5
His	1.1	1.0	1.3	ND	0.7	1.3
Arg	3.6	5.2	4.9	4.1	5.5	4.4
Thr	3.9	5.2	5.1	5.0	4.9	4.6
Ala	7.8	7.0	9.2	9.4	6.8	8.0
Pro	3.2	4.9	3.9	3.2	6.3	3.1
Tyr	1.8	2.5	2.4	1.9	3.0	2.4
Val	4.6	5.5	6.4	6.4	5.5	5.7
Met	ND	0.4	3.2	3.8	0.4	5.0
Lys	8.8	4.9	8.3	9.4	5.0	7.0
Ile	4.1	3.8	5.6	5.3	3.5	4.8
Leu	7.0	7.0	7.3	8.3	7.5	5.8
Phe	2.4	2.4	2.5	3.0	2.5	2.6

Table 1. Amino acid analysis of individual protein bands after separation by SDS-PAGE. Results are given as Mol %. ND = Not detected. Trp was not determined.

3.4 Adhesion characteristics

In the present study, a 90 Degree Peel Test was used to evaluate the adhesion of freshly expelled Cuvierian tubules. This method was chosen as we had encountered problems when tensile testing the *H. dofleinii* tubules following the approach used by Flammang and colleagues (Flammang et al., 2002). Specifically, when *H. dofleinii* tubules were sandwiched between two materials to which there was good adhesion, e.g. glass or metals, testing could lead to strength values which reflected the structural failure of the Cuvierian tubule rather than the failure of the adhesive, especially if some drying had occurred (data not shown). This method would only allow the determination of a minimum value for the adhesive strength as the latter exceeded the break strength of the tubule material itself.

The present test was suitable for rapidly examining numerous, freshly expelled samples, thus allowing ready comparison between the effects of various treatment solutions. The various treatments (i.e., incubations of tubules in the appropriate wash solutions) prior to adhesive testing were rapid (1 min) as it appeared that the adhesion could decline if tubules were left soaking for lengthy periods (data not shown). With *H. forskåli*, a lag period of about 60 min at 16 °C was recorded before adhesion started to decline, decreasing to about 15 min at 26 °C (Müller et al., 1972). In another study (Flammang et al., 2002) a longer lag phase was observed, and an initial increase in adhesive strength was reported. Yet others have reported adhesive strength to fall after 20 min (Zahn et al., 1973). The present approach, therefore, used short incubations in order to minimise time-based variations and to mimic the timescale over which tubules would be required to act in the natural environment.

Previous studies (Flammang et al., 2002) have shown that a compressive force of 2–10 N during adhesion led to a 6- to 8-fold increase in the resulting bond strength. In the present case, no compressive load was added so as to better simulate the natural process of ensnaring a predator.

Tubule widths showed little variation between individual samples, the average size being 4.0 mm. Tubules that were not fully expelled, and which therefore had a lesser diameter, were discarded. The observed width is larger than that found for *H. forskåli* (Flammang et al., 2002; Zahn et al., 1973) and *H. leucospilota* (Flammang et al., 2002), the species previously studied in detail, and also larger than for *H. impatiens* and *H. maculosa;* these other species generally have tubule diameters of 1–2 mm (Flammang et al., 2002). Although there are many potential tubules within the body cavity (Figure 1A) *H. dofleinii* expels only a few, typically 8 - 12 for organisms stimulated in the holding tanks compared with the more numerous thin tubules expelled by *H. leucospilota* or *H. forskali* (Flammang et al., 2002).

Adhesive strength was also found to vary when different substrata were examined, all after washing the tubules in 3.5% NaCl 10 mM Na/PO$_4$, pH 7.6. There was a trend for strongest adhesion to be observed with hydrophilic substrata, glass and aluminium (Table 2). Adhesion to polycarbonate, PMMA, and PTFE was very poor; indeed, for PMMA and PTFE, the load required for peel was barely more than the weight of the 50 mm of tubule overhang. Intermediate adhesion values were observed with polyvinyl chloride and crab chitin surfaces (Table 2). The chitin samples were unusual in having a textured surface rather than a smooth one. Previously, Zahn, Flammang and colleagues had shown strong adhesion to hydrophilic surfaces such as glass and stainless steel, and poor adhesion to hydrophobic ones such as paraffin wax, polystyrene and polyethylene (Zhan et al., 1973; Flammang et al, 2002). In general our results are consistent with this trend: the best adhesion was observed with glass whilst the poorest was observed with PTFE.

Solution	Force/width	S.D.	n

	(N/mm)		
Glass	> 0.050	-	10
Aluminium	> 0.050	-	6
Polyvinyl chloride	0.024	0.006	11
Chitin	0.021	0.005	8
Polycarbonate	0.010	0.002	8
PMMA	0.009	0.003	7
PTFE	0.008	0.002	6

Table 2. Force required to peel Cuvierian tubules off various substrata to determine adhesive strength.
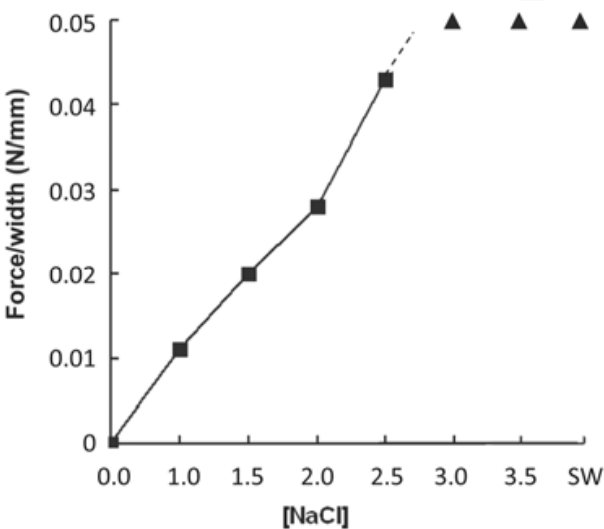
Fig. 5. The effect of different washing solutions on the adhesiveness of *H. dofleinii* Cuvierian tubules for glass. The effect of NaCl concentration; where ▲ indicates conditions where the force per unit width exceeded 0.05 N/mm. SW is natural sea water.

Adhesive strength decreased with decreasing NaCl concentration (Figure 5). At ≥3% NaCl the adhesion exceeded 0.05 N/mm. Reducing the NaCl concentration incrementally from 2.5% to 1.0% NaCl led to a steady decline in adhesive strength (Figure 5). The adhesive strength at 1% NaCl, which is comparable in concentration to physiological saline, was significantly weaker than in 3.5% NaCl simulated seawater. This is consistent with the previous observations on *H. forskåli* tubules (Flammang et al., 2002). It suggests that hydrophobic interactions may be important in the adhesive mechanism.

The effects on tubule-glass adhesion of other chloride or sodium salts (50 mM) (Table 3) showed that in all cases there was a loss of adhesive strength. For chloride salts, the loss was smaller when Tris rather than ammonium was the cation (Table 3). The other salts examined were all sodium salts of carboxylic acids, for whose action no simple mechanism could be proposed. Thus while formate (a monocarboxylate) and oxalate (a dicarboxylate) both showed similar adhesion, that observed with acetate (another monocaboxylate) was ~35% below the value observed for formate. However, the values presented in Table 3 show only a trend as the errors in measurement are such that the different systems are not necessarily distinguishable. Supplementation with EDTA (a tetracarboxylate) was the most effective at disrupting bond strength, and essentially led to complete loss of adhesion (Table 3). It is not

clear whether this is due to the multiple carboxyl groups of this salt, to its strong metal ion chelating capability, or to some other property. However, certain other marine adhesives, such as that from *Mytilus*, do require metal activity (Hwang et al., 2010). A previous study which tested 15 different amino acids at 0.5% w/v solutions on adhesion by *H. forskåli* tubules (Müller et al., 1972) showed that most had little, if any, effect. The exceptions were the hydrophobic amino acids leucine (20% loss) and phenylalanine (57% loss). For phenylalanine, the loss was slow to develop (taking several minutes) and could not be reversed by washing (Zahn et al., 1973).

Solution	Force/width (N/mm)	S.D.	n
3.5% NaCl	> 0.050		>8
Tris/chloride	0.050	0.008	6
Sodium formate	0.047	0.012	6
Sodium oxalate	0.046	0.011	8
Ammonium chloride	0.036	0.008	8
Sodium citrate	0.035	0.009	7
Sodium acetate	0.030	0.006	8
Sodium EDTA	<0.003		8

Table 3. Comparison of the effects of different salt solutions on adhesion of Cuvierian tubules onto glass. All salts were 50mM in 3.5% NaCl, 10 mM sodium phosphate, pH 7.6

The effect of pH on the adhesive strength of the *H. dofleinii* adhesive showed that for Tris/chloride buffer, the best observed strength of those tested was at pH 7.6, and that the observed strength decreased at both lower and higher pH values (Figure 6). For citrate and acetate buffers, adhesive strength declined progressively as the pH was lowered from pH 7.6, with little adhesion remaining at pH 5.0 (Figure 6). A loss of adhesive strength at acidic pH values was also observed by Müller et al. (1972), who used paraffin wax as a (poor) substratum for tubule adhesion.
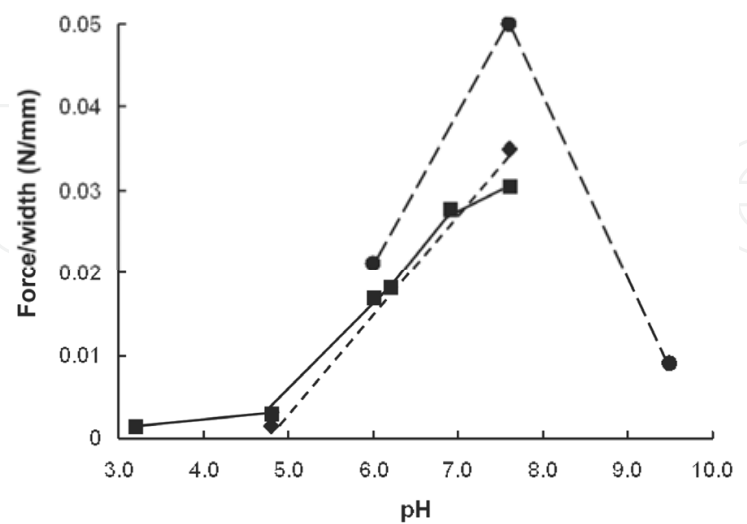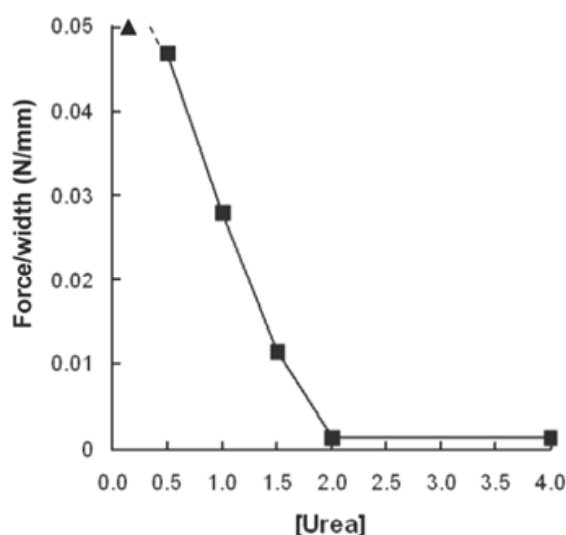

Fig. 6. The effect of different washing solutions on the adhesiveness of *H. dofleinii* Cuvierian tubules for glass. The effect of changes in pH, where ■ indicates acetate buffers, ♦ indicates citrate buffers and ● indicates Tris/Chloride buffers.

Some reports suggest that proteins may play an important role in the adhesion of Cuvierian tubules from *H. forskåli* (DeMoor et al., 2003; Flammang & Jangoux, 2004; Müller et al., 1972). For example, the adhesive residue left when tubules are peeled from a surface consists mainly of protein (DeMoor et al., 2003), and the treatment of tubules with proteases causes loss of adhesion (Müller et al., 1972). However, it has been reported that the proteins most likely differ between species (Flamman & Jangoux, 2004), making further comparative biochemical surveys important for elucidating the mechanism.

The effect of urea on tubule-glass adhesion showed that bond strength decreased progressively with increasing urea concentration until it was completely lost at 2 M urea (Figure 7). However, if tubules that had been incubated for about 60 sec in 2 M urea were then rinsed for about 60 sec in simulated sea water, some adhesion was restored, although the extent was rather variable. Urea disrupts hydrogen bonding, and its effect on adhesion may reflect some partially reversible protein unfolding (Zahn et al., 1972). The rapidity and partial reversibility of the effect indicates that there is not a complete urea-mediated release of proteins from the tubule surface.

Fig. 7. The effect of different washing solutions on the adhesiveness of *H. dofleinii* Cuvierian tubules for glass. (C) The effect of urea concentration, where ▲ indicates conditions where the force per unit width exceeded 0.05 N/mm.

4. Conclusion

The distinct features of the Cuvierian tubule adhesion mechanism, especially its rapid action under water, are unique. If the mechanism can be understood, then it may be possible to design a synthetic system with analogous properties. An adhesive that provided instant grip in an aqueous environment would be very valuable, especially in medical applications, as the majority of existing adhesives bind well only to dry surfaces.

It appears that although the tubules of *H.dofleinii* are distinct from those of other species, especially in their size and the number expelled, the adhesive properties of these Cuvierian tubules (including preferences for hydrophilic surfaces, pH optima, etc.) are similar to those found in other species, even if mechanistic details may differ between species as has been proposed previously (Flammang and Jangoux, 2004). It is thought that during expulsion and tubule elongation, granular cells that are internal in the pre-release tubule become located

on the tubule surface and release their contents on contact with a surface (VandenSpiegel and Jangoux, 1987) leading (in whole or in part) to the observed adhesion. Histology has shown that these granules contain protein and lipid, but lack polysaccharide (VandenSpiegel and Jangoux, 1987). Biochemical studies have indicated that the granules contain a protein of around 10 kDa, and it has been suggested that polymers of this protein account for the higher molecular weight proteins that are seen in the adhesive prints (Flammang and Jangoux, 2004), but this seems highly unlikely in *H. dofleinii* as the protein bands are very well resolved by gel electrophoresis and the calculated molecular weights of these bands do not conform to such a regular series of increases.

Our present study emphasises that the adhesives of natural systems are optimised for the specific environments in which they have evolved, such as the present marine environment. An analogue intended for medical use would need to be optimised to yield maximum adhesion in the physiological conditions that prevail in mammalian tissues. In the present case, the adhesion works better at higher NaCl concentrations that found in medical applications so understanding more about the mechanism and the protein structures and properties will be needed in order to adapt this system for applications where lower NaCl concentrations are present.

5. Acknowledgments

6. References

Cohen, S.A. (2001). Amino acid analysis using precolumn derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate. *Methods in Molecular Biology*, Vol.159, pp. 39-47, ISSN 1064-3745

Cohen, S.A. & DeAntonis, K.M. (1994). Applications of amino acid analysis derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate: Analysis of feed grains, intravenous solutions and glycoproteins. *Journal of Chromatography,* Vol.661, No.1-2, (February), pp. 25-34, ISSN 0021-9673

DeMoor, S.; Waite, J.H.; Jangoux, M. & Flammang, P. (2003). Characterization of the adhesive from cuvierian tubules of the sea cucumber *Holothuria forskali* (Echinodermata, Holothuroidea). *Marine Biotechnology,* Vol.5, No.1, (January), pp. 45-57, ISSN 1436-2228

Dimas, D.A.; Dallas, P.P.; Rekkas, D.D. & Choulis, N.H. (2000) Effect of several factors on the mechanical properties of pressure-sensitive adhesives used in transdermal therapeutic systems. *AAPS PharmSciTech*, Vol.1, No.2, (June), pp. 80-87, ISSN 1530-9932

Doolittle, R.F. (1986). *Of URFs and ORFs: a primer on how to analyse derived amino acid sequences,* University Science Books, ISBN 0-935702-54-7, Mill Valley, CA, USA

Flammang, P. & Jangoux, M. (2004). *Final Report ONR Grant* N00014-99-1-0853

Flammang, P.; Ribesse, J. & Jangoux, M. (2002) Biomechanics of adhesion in sea cucumber Cuvierian tubules (Echinodermata, Holothuroidea). *Integrative and Comparative Biology,* Vol.42, No.6, (December), pp. 1107-1115, ISSN 1540-7063

Hamel, J.-F. & Mercier, A. (2000). Cuvierian tubules in tropical holothurians; usefulness and efficiency as a defence mechanism. *Marine and Freshwater Behaviour and Physiology,* Vol.33, pp. 115-139, ISSN 1023-6244

Hwang, D.S.; Zeng, H.; Masic, A.; Harrington, M.J.; Israelachvili, J.N. & Waite, J.H. (2010). Protein- and metal-dependent interactions of a prominent protein in mussel adhesive plaques. *Journal of Biological Chemistry,* Vol.285, No.33, (August), pp. 25850-25858, ISSN 0021-9258

Jones, I.; Lindberg, C.; Jakobsson, S.; Hellqvist, A.; Hellman, U.; Borg, B. & Olsson, P.E. (2001). Molecular cloning and characterization of spiggin. *Journal of Biological Chemistry,* Vol.276, No.21, (May), pp. 17857-17863, ISSN 0021-9258

Kamino, K. (2008). Underwater adhesive of marine organisms as the vital link between biological science and material science. *Marine Biotechnology,* Vol.10. No.2, (March), pp. 111-121, ISSN 1436-2228

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* Vol.227, No.5259, (August), pp. 680-685, ISSN 0028-0836

Lawrence, J.M. (2001). Function of eponymous structures in echinoderms; a review. *Canadian Journal of Zoology,* Vol.79, No.7, (July), pp. 1251-1264, ISSN 0008-4301

Lin, Q.; Gourdon, D.; Sun, C.; Holten-Andersen, N.; Anderson, T.H.; Waite, J.H. & Israelachvili, J.N. (2007). Adhesion mechanisms of the mussel foot proteins mfp-1 and mfp-3. *Proceeding of the National Academy of Sciences of the United States of America,* Vol.104, No.10, (March), pp. 3782-3786, ISSN 0027-8424

Mancuso-Nichols, C.A.; Nairn, K.M.; Glattauer, V.; Blackburn, S.I.; Ramshaw, J.A.M. & Graham, L.D. (2009) Screening microalgal cultures in search of microbial exopolysaccharides with potential as adhesives *Journal of Adhesion,* Vol.85, No.2-3, pp. 97-125, ISSN 0021-8464

Müller, W.E.G.; Zahn, R.K. & Schmid, K. (1972). The adhesive behaviour in Cuvierian tubules of *Holothuria forskåli.* Biochemical and biophysical investigations. *Cytobiology,* Vol.5, No.3, pp. 335-351, ISSN 0070-2463

Nakano, M.; Shen, J.R. & Kamino, K. (2007). Self-assembling peptide inspired by a barnacle underwater adhesive protein. *Biomacromolecules,* Vol.8, No.6, (June), pp. 1830-1835, ISSN 1525-7797

VandenSpiegel, D. & Jangoux, M. (1987). Cuvierian tubules of the holothuroid *Holothuria forskali* (Echinodermata): a morphofunctional study. *Marine Biology,* Vol.96, No.2, pp. 263-275, ISSN 0025-3162

VandenSpiegel, D.; Jangoux, M. & Flammang, P. (2000). Maintaining the line of defence: regeneration of Cuvierian tubules in the sea cucumber *Holothuria forskali* (Echinodermata, Holothuroidea). *Biological Bulletin,* Vol.198, No.1, (February), pp. 34-49, ISSN 0006-3185

Watson, M.R. & Silvester, N.R. (1959). Studies of invertebrate collagen preparations. *Biochemical Journal,* Vol.71, No.3, (March), pp. 578-584, ISSN 0264-6021

Zahn, R.K.; Müller, W.E.G. & Michaelis, M. (1973). Sticking mechanisms in adhesive organs from a *Holothuria. Research in Molecular Biology,* Vol.2, pp. 47-88, ISSN 0340-5400

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