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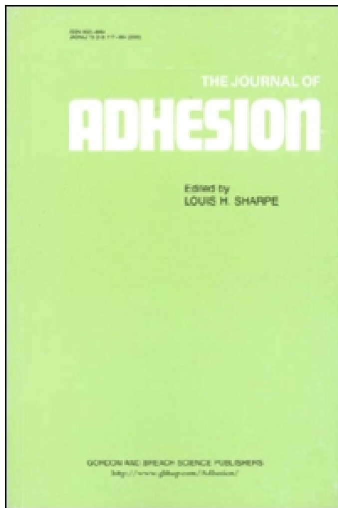

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Mussel Adhesion: Finding the Tricks Worth Mimicking

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Mussel Adhesion: Finding the Tricks Worth Mimicking

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The byssus is a holdfast structure that allows the marine mussel (Mytilus) to adopt a sessile mode of life even in the most wave-swept habitats. The success of byssus as an adaptation for attachment is at least in part responsible for the fouling caused by these organisms, but it has also provided inspiration for the design of underwater adhesives and coatings. A valuable bio-inspired concept emerging from mussel adhesion is that of polymers with catecholic and phosphate functionalities for robust underwater surface coupling. Prepolymer processing by complex coacervation for good spreading and functional gradients is also likely to find applications.

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Keywords: Mussel; Mytilus; Adhesion; Byssus; Biomimetics; Dopa

INTRODUCTION

Biomimetics is a research initiative that seeks to identify and replicate adaptive biological attributes with potential technological applications. Although this pursuit can strike a poetic chord in many, it is fraught with difficulty and controversy [1, 2]. At issue is that biological attributes are evolved by trial and error over a very long time to a

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specific and changing set of challenges. Given the unique life histories of organisms, few adaptations are likely to lend themselves to human technology without substantial revision. The adhesive attachment by mussels as well as other aquatic organisms to hard surfaces in their habitat has been the target of biomimetic investigations. The aim of this report is to examine the rationale for these endeavors and the lessons that have been learned.

Biomimetics is not the first research initiative to scrutinize mussel adhesion. Spat attachment is crucial for normal mussel development and growth in mussel mariculture [3]. Mussel fouling of offshore platforms [4], water-cooled power plants [5], and the hulls of ships [6] has driven an antifouling initiative for at least 25 years. Antifouling treatments such as tributyl tin, although very effective, have been banned in most countries because of their toxicity to nontarget species [6, 7]. Equally effective but specific antifouling strategies await a better fundamental understanding of marine adhesion [8, 9]. It is frequently pointed out that an emphasis on fundamental science has the potential to do more than lead to development of a specific "green" antifoulant; it may also inspire a new generation of water resistant adhesive polymers [10–12].

Clearly the value of a fundamental understanding of mussel adhesion is not in question, but is it possible to translate this knowledge into a profitable glue? Three attributes of mussel adhesion argue that technological potential may be limited: (1) even the best bond strengths of byssal adhesion (1–5 MPa, [13]) pale in comparison to the best high performance synthetics (50 MPa for polyimides, [14]); (2) byssal adhesion is based on proteins that are readily digested by common enzymes (bad for polymer stability, good for biodegradability) [15], and (3) byssal adhesion requires the presence of moisture to work properly [16] (bad for aircraft and space). In support of biomimetics are the following: (1) byssal adhesion is extremely versatile. Mussels stick to virtually any hard surface in their habitat [13]; (2) the presence of water/ moisture is business as usual for mussel byssal adhesion [17], all stages of the bonding process occur rapidly under ambient and wet conditions; and (3) byssal adhesive structure appears to be smart in the sense that it exhibits compliance or modulus matching [18]. In the following sections, we expand on three aspects of mussel byssus that appear to be generally relevant to making improvements in adhesive technology: structure, chemistry, and processing.

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Mussel Byssus

Mussel byssus, particularly from marine mussels belonging to the genus *Mytilus*, has been reviewed many times and from different

perspectives [e.g., 17, 19]. For the purposes of this report, only a brief description of the structure is necessary. A typical mussel 4–5 cm long has a byssus, essentially a taut bundle of 50 to 100 threads, attached proximally at the foot base from which it can be jettisoned, and distally to a hard foreign surface (Fig. 1A). The threads perhaps 3–4 cm in length emerge from the ventral gap in the underside of the shell in the manner of spokes on a bicycle wheel, with attachments that extend in many different directions (Fig. 1). Only the distal half of each thread is normally exposed, the remainder being shielded within the shell where the threads merge onto a stem-like structure at the base of the foot. Each thread is distally tipped by an adhesive plaque, which, with a diameter of 1–2 mm, is attached to the underlying substratum (Fig. 1B). This picture is a static one. In reality, more threads are always being made to enhance the tenacity of the mussel and to replace broken threads. The mussel is perched upon its byssus like a child clutching to the center of a trampoline. Figure 2 presents a typical thread schematically with two descriptive nomenclatures, one traditionally morphological (*top*) and the other (*bottom*), wishfully biomimetic with technologically relevant domains such as a surface coupling layer, bulk adhesive, coating, and fibrous core.

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Biomimetic Theme: Structural Concepts

Adhesive Foam

In relying on a byssus for attachment mussels diverge from other invertebrates such as oysters, serpulid worms and barnacles that cement their calcareous shells to rocks. The byssus mediates attachment of the soft mushy body of the mussel to a very stiff and hard substratum. Were they to be joined directly, there would be a significant mismatch in the stiffness of the two, for example, E_i (retractor muscle stiffness) = 0.2 MPa and E_i (rock) = 25 GPa, resulting in contact deformation of the softer one. Contact deformation is defined as the damage inflicted on the softer of two joined materials and is caused by residual stresses that arise at their common interface [20]. Mussel byssus circumvents this in some elegant ways that are only gradually emerging. In the attachment plaque, the structural adhesive appears to be a solid foam [21–23]. Although the process by which this foam is made is still unclear, we do know that foams are not uncommon among marine and freshwater invertebrate adhesives [24–26]. Figure 3 shows the structure of a plaque from *Mytilus californianus* to be a continuous, partially open cell network in which the pore size gradually increases from the interface towards the cuticle. Several important features are apparent: contact at the interface with the underlying surface is

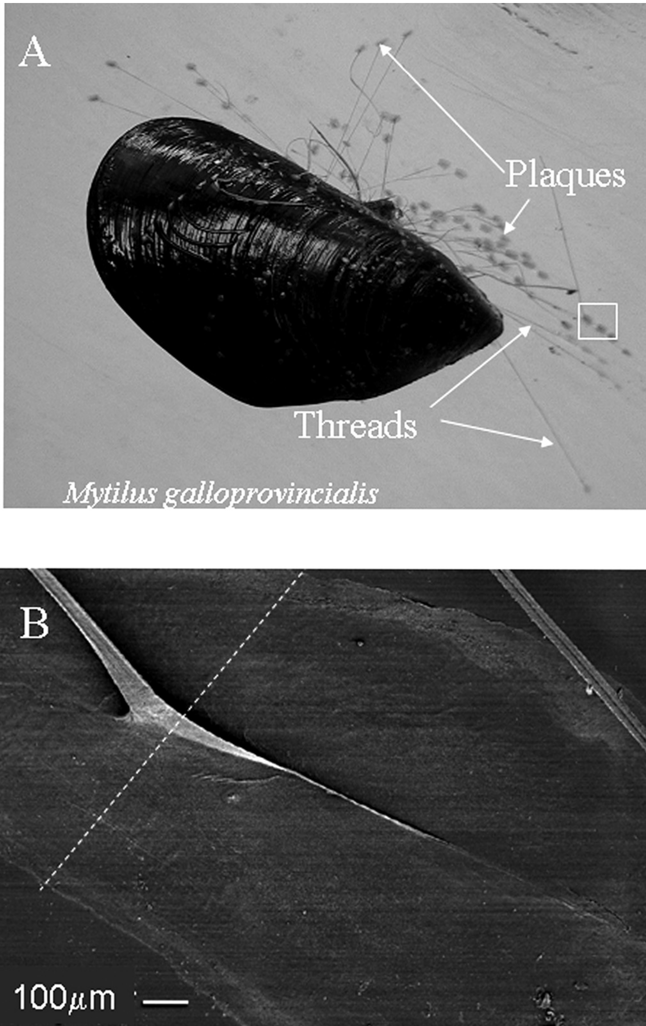

FIGURE 1 Mussel (*Mytilus galloprovincialis*), about 4 cm long, suspended from numerous byssal threads tipped by adhesive plaques (A) The substratum with several attached plaques (white box) was examined by scanning electron microscopy. An SEM micrograph of an adhesive plaque (length 2 mm) is shown in B. The white line indicates the cleavage plane for the section in Figure 4. The fibrous stem-like portion in the upper left-hand corner is the distal portion of the thread.

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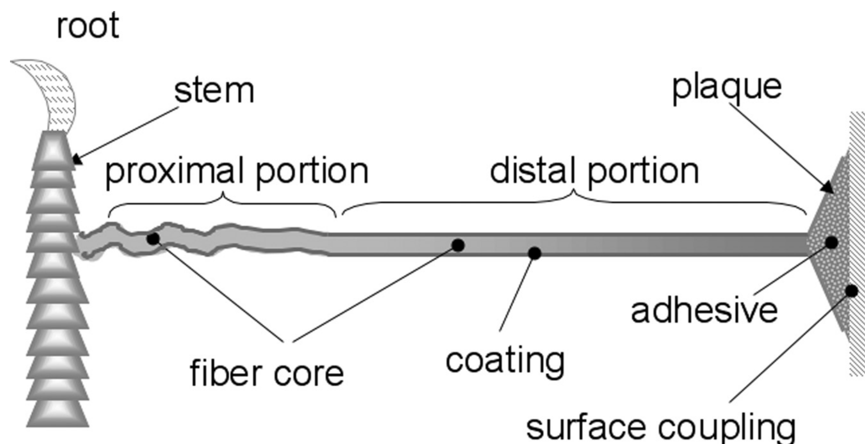

FIGURE 2 Schematic drawing of a single byssal thread extending from the stem structure, which is rooted in living tissue, to the distal adhesive plaque. Descriptions over the thread denote the classical terminology used to describe the morphology of byssus; below the thread are technologically based descriptors.

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continuous; the differential in distal to proximal pore diameter is about 3-fold; finally, the microfibrils descending from the thread extend into the foam like tree roots [Jewhurst, unpublished]. There are thus 2 gradients to mitigate possible damage by contact deformation: a porosity gradient, from nonporous near the interface with the hard substratum to mostly porous in the center of the adhesive, reverting gradually again to small pores as the foam approaches the protective outer coating of the plaque (Fig. 3B). The other gradient is more akin to joining techniques such as dovetailing and mortise and tenon used in carpentry. Like these, small projections of one material (rooted collagen fibrils) extend into the other (foam), thus increasing the contact area between the two. The advantages of solid structural foams include economy, compliance, crack-stopping behavior, and low density and are already widely recognized in industry [27]. Porosity gradients, however, are a more recent development and largely limited to high tech ceramics [28].

Graded Block CoPolymers

We have investigated two other structural components of the byssus: the fibrous threads and the protective cuticle covering them. The threads extend distally from the plaque to the stem, a structure, which mediates their fusion and insertion into the living tissue at the base of

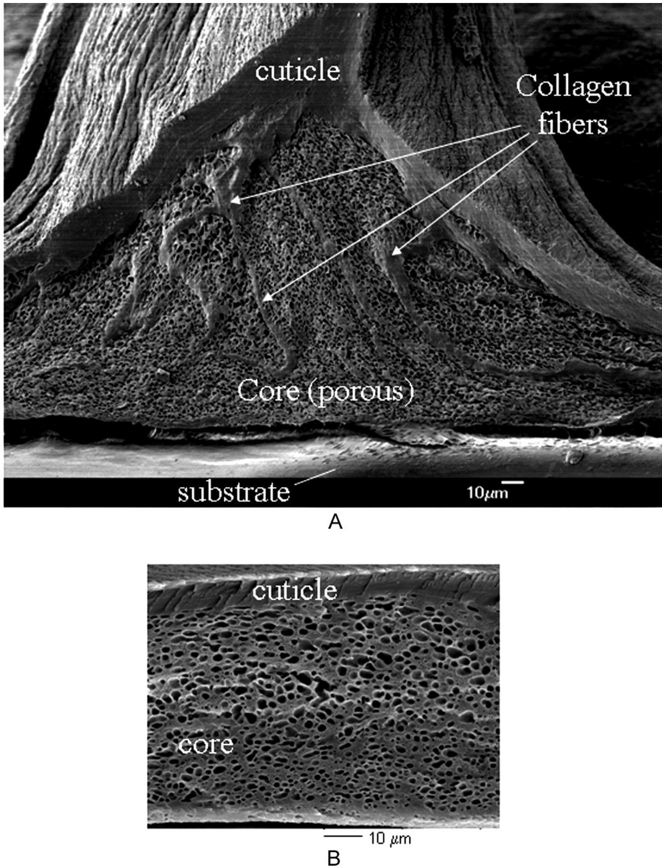

FIGURE 3 Solid foam structure of a byssal adhesive plaque. A. Section made by freeze fracture according to orientation given in Figure 1B. The delamination from substrate surface occurred during freeze drying. B. enlargement of adhesive-substratum interface. Note paucity of pores near the interface (bottom of section).

the foot. The stiffness gradient in byssal threads as has been discussed in detail elsewhere [18], ranges from a high distal modulus of 500 MPa to a low proximal value of 50 MPa in *M. galloprovincialis*. The basic building block of the collagenous microfibrils in the plaque and thread is the preCOL, a kinked collagen core element with three different types of flanking core domains—silk (very stiff), elastin (soft), and amorphous (intermediate) (Fig. 4). In order to achieve the ten-fold decrease in stiffness going from the plaque to the stem, mussels appear to distribute their preCOLs as follows: preCOL-NGs with the

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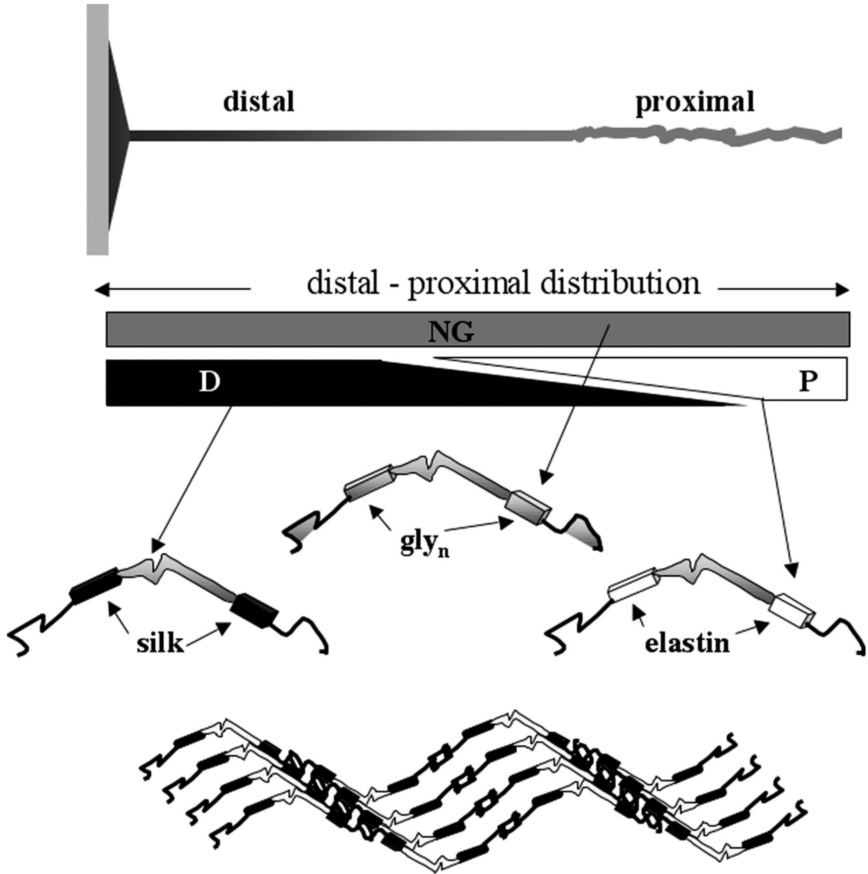

FIGURE 4 Molecular gradients in the thread. The basic tensile unit in the thread is a bent core collagen (preCOL) of which 3 types are shown: preCOL-D with silk-like flanking domains, preCOL-NG with polyglycine rich flanking domains, and preCOL-P with elastin-like flanking domains. A molecular gradient with mechanical consequences is constructed by holding the concentration of preCOL-NG uniform while gradually replacing preCOL-D with preCOL-P along the distal to proximal axis.

collagen and flanking amorphous domains are uniformly present; preCOL-Ds with the silk-like flanking domains prevail distally and gradually, beginning in the proximal half or third, give way to preCOL-Ps, which contain the core collagen flanked by elastin-like domains (Fig. 4). Such a molecular gradient has mechanical consequences primarily because the flanking domains in preCOL-D and preCOL-P are estimated to have very different stiffnesses, e.g.,

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10 GPa in the silk-like domains and 2 MPa in the elastin like domains in preCOL-P [18].

The assembly of this molecular gradient is made possible by several important adaptations some of which are further developed below. First, the preCOLs are not assembled one by one, but are prefabricated in the foot as smectic liquid crystals having diameters of about 1–2 μm [29–30]. Second, the preCOLs and therefore the liquid crystals made from them have interlocking molecular connectors like LEGO™ toys. At least in vitro, these are based on histidine metal binding and are pH triggered [18]. Finally, the mussel foot takes a "microfluidics" approach to thread formation. That is, liquid crystals that are titrated with different amounts of preCOL-D and preCOL-P have prearranged gradients of a distal to proximal orientation along the length of the foot [31]. At intervals of a few microns, these liquid crystals are released locally into the ventral groove where they coalesce and become cross-linked.

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Durable Coating

The exposed rocky intertidal habitat of mussels is not a place for creatures without protective armor. The surf that washes mussels has a high particulate content and this, combined with a wave velocity of between 5 and 15 m/sec, amounts to cyclic sandblasting [32]. The effects are evident in the shells, which are stripped clean of their organic outer layer (periostracum). Many mussels survive this abrasion of their shells by thickening from the inside. However, as predominantly organic structures that extend as far as 5 cm away from the animal, the threads would seem particularly vulnerable to damage by abrasion. The standard industrial strategy for improving resistance to abrasion is to coat with something having a high hardness to stiffness ratio. This is challenging on an organic fiber with strain ranging from 60 and 200% [33]. The coating on the distal or exposed portion of byssal threads is shown in Figure 5. By scanning electron microscopy (SEM), it appears as a surface studded with bumps or knobs having a diameter of 1 μm. In thin sections examined by transmission (TEM), the knobs reveal an intricately layered internal structure [34]. Scanning probe microscopy in our lab has shown the granules to be stacked 4–5μ deep dispersed in a homogeneous organic matrix over the fibrous core (Fig. 5B). Preliminary elemental analyses have detected iron, silicon, aluminum and bromine in the coating but not in the fibrous core. Whether these elements comprise a mineral, and how they are distributed with respect to the granules vis-à-vis the matrix, remain to be resolved. It is tempting to speculate that the cuticle consists of hard mineralized inclusions in a malleable matrix but nanomechanical analyses of byssal cuticle have

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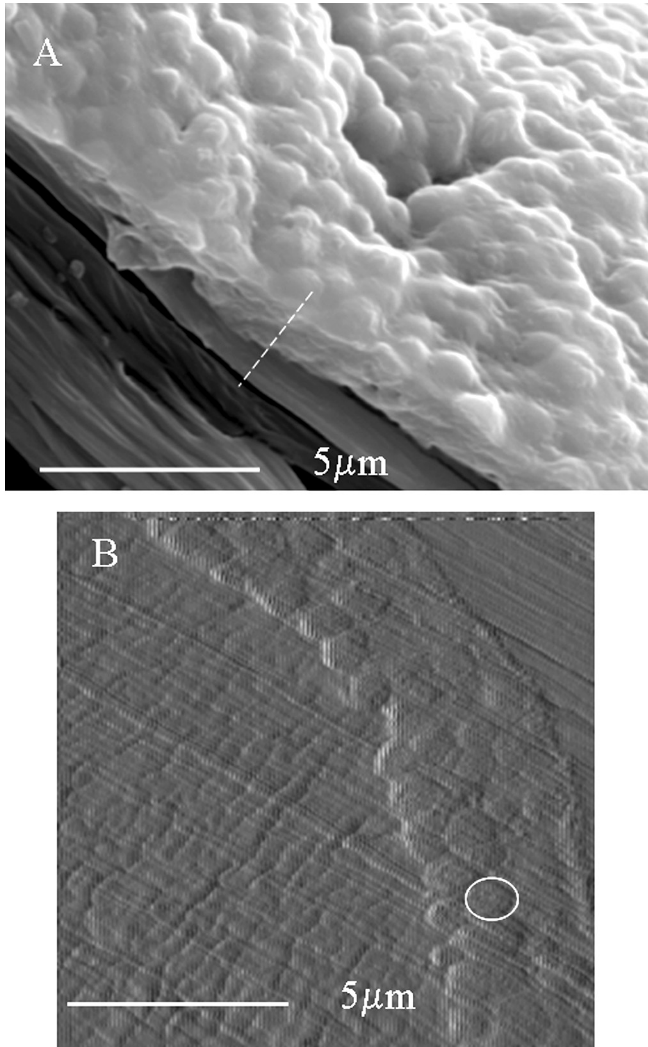

FIGURE 5 The protective coating (cuticle) of a byssal thread. A. SEM of distal thread portion showing a granular surface contrasted by the underlying fibrous core exposed by a tear (lower left). Average diameter of knobs is 1 μm; B. Thin section through coating imaged by scanning probe microscopy, in which sections through granules appear as slightly elevated circles with 1 μm diameter. A white circle outlines a section of one granule.

only recently commenced and must, to be relevant, ultimately be carried out with hydrated threads [Holten-Andersen, unpublished].

Biomimetic Theme: Processing Concepts

Each new byssal thread including the plaque takes the mussel five minutes or less to make. Many new threads can be made in succession. Adhesion of the plaque is spontaneous and necessarily so for each new thread of the byssus is immediately recruited into resisting drag and lift forces. Adhesive processing involves the foot particularly the groove and distal depression on the ventral surface. These resemble molds in foam manufacture using reaction injection molding. For example, the distal depression of the foot is pressed against a hard surface and, by using suction, creates a small conical space under negative pressure [17]. The negative pressure may be necessary to draw in the glue. All precursors of the thread including the glue for the plaque are produced and stockpiled in specific locations of the foot. A large gland called the "phenol gland" sits on top of the distal depression and is connected to it by conducting tubules and pores. Up to ten different fps or "foot proteins" are produced in the phenol gland. But they are not likely to be stockpiled together within the same cells of the phenol gland. Investigations to localize fp-1, 2, and 3 in the foot conclude that they have different though slightly overlapping distributions [35–36]. There is probably a cue or protocol for secretion with fp-3 and 5 among the first to be deposited on the substratum, followed by fp-2 and 4 [37].

When mussels synthesize and formulate the proteins that they use for adhesion, they rely on a cellular pathway called regulated protein secretion. Protein secretion has been intensively studied in the last ten years and shows a high degree of uniformity among many different secretory cell types [38]. Briefly the process is as follows for an exported protein: Translation of the messenger RNA starts on ribosomes in the cytosol of the secretory cell. Appearance of a signal peptide sequence in the first 25 amino acids of the initiated translation directs the mRNA-ribosome complex to an enclosed membrane-bound tubular network called the endoplasmic reticulum (ER), where the resumption of translation is directed into the lumen of the ER. Many enzymes that covalently modify certain residues in the protein also reside in the ER, where they can act on the protein during or after translation. The list includes the folding enzymes (chaperonins), protein disulfide isomerases, and prolyl- and lysyl hydroxylases.

As protein synthesis is completed, nascent protein is sequestered to regions of the endoplasmic reticulum that bud off into membrane-bound vesicles. These migrate to and merge with another tubular network, the Golgi apparatus, where proteins are sorted according to their fate, e.g., regulated secretion, constitutive secretion, lysosomes,

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membranes, etc. Proteins (such as the byssal adhesives) to be secreted by regulated pathways, bud off the trans portion of the Golgi, again as vesicles, and fuse with one another to form large vacuoles. Vacuoles serve as reservoirs for accumulating protein and as a place where protein condensation can begin. This is a precarious step for the nascent protein. To be properly processed it must remain in a concentrated fluid state often akin to a liquid crystal. Precipitation or crystallization prior to release could lead to protein degradation or cell death. Condensation occurs at a pH of about 5.5 and a protein concentration as high as 30% w/v. Ca^{+2} concentrations are about 10 mM [38] and other divalent metals such as zinc may also be present [39].

Some of the most crucial processing steps for adhesive proteins are thought to occur while secretory granules are undergoing condensation in the cell. Despite the many years of intense research on protein secretion, the conditions leading to condensation are poorly understood. Research on the proteins destined for the byssal plaque of *Mytilus* has been extensive, but some critical components are still lacking. The following processing model has been inspired by for the adhesive proteins of sandcastle worms (*Sabellariidae*), which build large mound-like structures by gluing together countless grains of sand [26]. We take the liberty of comparing tubeworm cement with mussel byssus because several key details are similar e.g., both contain very basic proteins with about 10 mol% Dopa and form solid foam structures. The tubeworm glue is a mixture of two types of extremely charged proteins: a strongly anionic phosphoserine rich protein (pI 2.5) and a pair of cationic proteins rich in lysine and arginine (pI $\sim$ 10) [40]. The maturation of the adhesive containing secretory granules has been investigated by light and transmission electron microscopy [41–42]. One or both of the basic proteins may be processed together in the same granule with the phosphoserine-rich protein.

When two or more polyelectrolytes such as proteins in solution combine to form soluble aggregates it is called complex coacervation. Bungenberg de Jong [43] was the first to systematize the conditions for complex coacervation, and this was provided a theoretical basis by Voorn and Overbeek [44]. Complex coacervation has the following properties: it is (1) pH dependent in that it is driven by the neutralization of two or more polyions in solution; (2) involves a liquid/liquid phase separation—a depleted equilibrium phase and a denser concentrated coacervate; and (3) typically exhibits a very low interfacial tension. More recent studies with model biomolecular coacervates have revealed other unusual properties: the viscosity of the coacervate is up to 5-fold lower than the noncoacervated solution assuming additivity in the contribution of each polyion [43, 45], and molecules of

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roughly equal mass can have diffusivities differing by as much as 10 times in the coacervate [46]. The coacervate concept was the first to explain how cells might concentrate proteins in a manageable fluid state in their secretory granules. The benefits of protein coacervates, however, go well beyond concentration: (a) the coacervated adhesive is phase separated from water, thus will not readily dissolve in seawater; (b) most complex coacervates have low interfacial tensions in water ($\sim$0.0005 dynes/cm) and exhibit $\sim 0^\circ$ contact angles [43]. They thus tend to surround any particulates added to water including carbon black, vaseline, glass, carmine, immiscible organics, and pollen. De Jong [44] reported only one particle of the many tested that resisted being surrounded (*amylum* starch particles). (c) Finally, with the right choice of proteins, it is possible to trigger gelling or cross-linking of the coacervate by temperature (e.g., gelatin) or pH (e.g., casein plus calcium) [47].

In the cement gland, secretory granules filled with concentrated coacervated adhesive proteins await a signal transduction to release their contents (Fig. 6). They are stored in a Ca^{+2}/Mg^{+2} rich medium at pH 5 at which they roughly neutralize one another's charges [42, 43]. At the appropriate time, granule contents are released onto a hard surface over which they readily flow. Probably the condensed state of the protein helps dehydrate the surface. The high pH of seawater (pH 8.2) will drastically change the solubility product of Mg^{+2}/Ca^{+2} with phosphorylated serines, and in phosphoproteins such as casein a gelling ensues. Finally, Dopa residues will be oxidized to quinones leading to cross-link formation and solidification [48–49].

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Biomimetic Theme: Interfacial Chemistry

For biomimetics, the single most alluring aspect about mussel adhesion is that bonding to metal and mineral surfaces takes place rapidly in a wet saline environment at ambient temperature. This allure is not limited to mussels but extends to any sessile aquatic organism—barnacles [50], tubeworms [40], sea cucumbers [51], limpets [52] that attaches to wet surfaces. One reason that man-made adhesively bonded structures cannot be made underwater is that synthetic adhesives cannot displace surface water. The limiting effects of moisture, however, extend even to adhesive bonds engineered under clean-room conditions and then immersed in water. Brockmann [53] states: "The most important factor in the long term properties of metal/polymer composites is the stability of interfacial adhesion against humidity (p. 265)." The subversive effects of water on

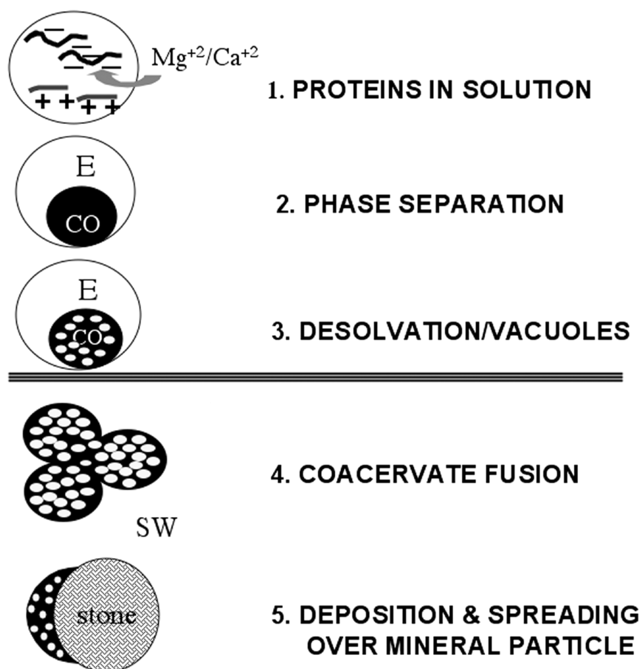

FIGURE 6 Model of adhesive processing. Complex coacervation for marine adhesive proteins. Steps: (1) Accumulation of high (+) and low pI (−) proteins at pH $\sim$ 5; (2) Phase separation into coacervate (CO) and equilibrium solution (E) by charge neutralization; (3) Vacuolation caused by trapped equilibrium phase during condensation and desolvation. This could be a mechanism of solid foam formation; (4) Secretion and coalescence of coacervates in seawater (SW); (5) Spreading and gelation over stone substratum. Gelation may involve either insoluble salt bridging, e.g., Ca^{+2} and protein phosphate groups or eventually, cross-linking, e.g., Dopa oxidation. The line between steps 3 and 4 represents the cell membrane of the adhesive secreting cells.

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manufactured adhesive bonds were further articulated by Comyn [54] as follows: (a) water is a weak boundary layer at the interface; (b) water wicks or crazes into interfaces; (c) water induces hydrolysis or erosion of adhesive; and (d) water swells or plasticizes the adhesive.

Mussels not only stick and remain stuck to wet surfaces, they synthesize, modify, mix, apply and cure their glue under ambient wet conditions. Many of these features are worthy of scrutiny, but the goal of highest priority at this time is how, once applied, the adhesive sticks strongly to the wet surface. If adhesion is reduced to terms of noncovalent interactions along an interface and their energies, a generalized

relationship emerges [55]:

$$\text{Interaction energy} = \left[Q_1^a Q_2^b\right] / \left\{k(4\pi\varepsilon_0\,\varepsilon)\ ^d r^f\right\}$$

where $Q_{1,2}$ reflects charge or tendency for electropolarization in each type of interacting functionality, ε_0 = permittivity of space, ε is the dielectric constant, r is the interatomic distance, a, b, d, and f are exponents whose magnitude is defined by the type of interaction, and k is a constant also dependent on the type of interaction. In charge-charge (Coulombic) interactions, for example, k, a to d and f all go to 1. The problem with water arises principally in ε. In a vacuum at 20°C, interaction energies can be high because the dielectric constant of vacuum is one; however, in nonpolar liquids (e.g., oils), it is 2–3, and in water it is 80. With water comprising an average of 70% of the weight of living tissues, the prospect for strong adhesion in living matter would seem to be a hopeless one. Biology has, however, fundamentally overcome the challenge by multiple strategies. The most common strategy about which little will be said is based on the use of specific recognition patterns that are coupled to protein conformation [56]. This works well for folded proteins and their ligands, in which the ligand-binding site is concealed in a nonpolar, solvent-inaccessible crevice that with a small conformational change can admit a ligand when present. Once admitted, ligand binding becomes robust because the nonpolar crevice helps to desolvate the ligand, and, once desolvated, the ligand is free to engage in noncovalent interactions uncompromised by the presence of water. This is not how mussels appear to stick. The surfaces that mussels stick to are mostly minerals and metals, in other words, very polar and hydrophilic. Similarly, the adhesive proteins they use are very polar and hydrophilic.

At least 10 proteins are present in the byssal adhesive plaques of *Mytilus*. Only two of these are implicated as interfacial by matrix-assisted laser desorption ionization spectrometric analysis of plaque footprints: these are mefp-3 and mefp-5 [57]. Mefp-3 and -5 are extremely polar, and they are among the most modified of all byssal proteins. In mefp-3, 42% of the amino acids are modified, whereas in mefp-5, 37% are modified. The primary modification in both proteins is tyrosine hydroxylation to Dopa. Other modifications are O-phosphoserine and 4-hydroxyarginine [58]. The presence of Dopa and phosphoserine is suggestive of the mussel's bioadhesive strategy. Both can engage in interactions with mineral and metal surfaces that exceed the noncovalent possibilities in water. The *o*-dihydroxyphenolic moiety of Dopa has been implicated in strong H-bonding to hydroxyapatite [59] and coordinate complexes with the oxides of iron [60], zinc

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[61], titanium [62], and aluminum (gibbsite) [63], as well as gold [64] surfaces (Fig. 7). In the case of zinc, titanium and iron it was possible to detect a charge transfer complex between the metal and the ligand. More to the point, the necessity of Dopa for metal binding was demonstrated using the unmodified tyrosine containing peptides, which did not bind [64, 65].

The first attempt to quantify mussel adhesion with respect to surface chemistry was made by Young and Crisp [13], who reported the not unexpected finding that the load to failure of byssal plaques in *M. edulis* was directly correlated to critical surface energy with highest loads (1–5 MPa) occurring on slate surfaces. Another mussel, *Limnoperna*, showed similar tendencies, and careful analysis suggested hydrogen bonding to be the primary interaction when strong adhesion occurs [66, 67]. As Dopa containing byssal adhesive proteins have become increasingly available, however, more and more studies have investigated the surface behavior of byssal proteins particularly fp-1. Fp-1 (e.g. mefp-1 from *M. edulis*) is a large (100 kD) basic protein with about 80 tandem repeats of {AlaLysHypSerTyrHyp*HypThrDopaLys} in which Hyp denotes *trans*-4-hydroxy-proline, and Hyp* *trans*-2,3-*cis*-3,

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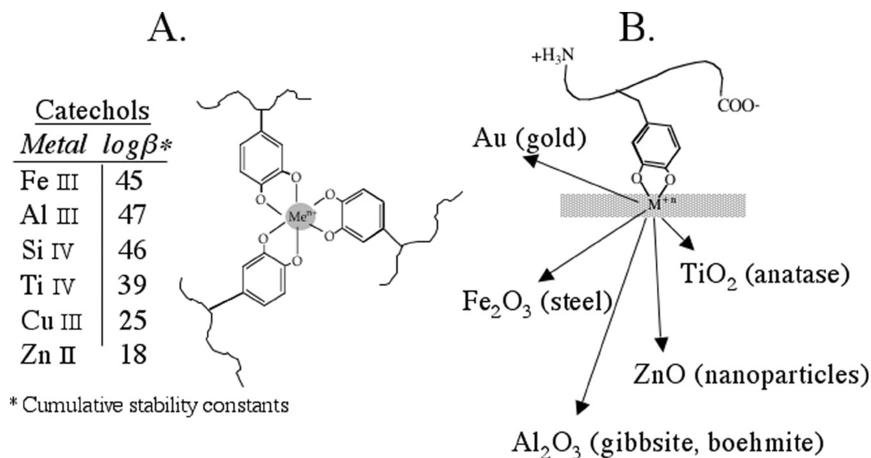

FIGURE 7 Catechol complexations in solution (A) and on solid surfaces (B) where catechol represents the o-dihydroxyphenyl functionality of Dopa. The stability constants (logβ) for solution complexes with Fe^{+3}, Al^{+3}, Ti^{+4}, Cu^{+2}, Zn^{+2}, are from [95] for standard conditions of temperature and ionic strength. Although more constrained and more difficult to quantify, a similar chemistry is known to occur on mineral or metal oxide surfaces as indicated. See text for references. The complex with gold is more likely to be via π bonds.

4-dihydroxyproline [68]. Fp-1 has an open extended conformation in solution [69] that may arise from a polyproline II helix with bends [70–71].

Using the quartz crystal microbalance and surface plasmon resonance (SPR), mefp-1 was shown to reach asymptotic adsorption levels within minutes and adsorb irreversibly to nonpolar (gold modified with methyl terminated SAMs) and polar (silica) surfaces [72, 73]. Greater film thickness (20 nm) on the former was attributed to higher hydration and entrapped water than on silica. The film thickness, however, could be reduced to 5 nm by chelated metals such as Cu^{+2} or by Dopa oxidation using periodate. The effect of various ions on the work of adhesion to silica using AFM showed that all polyvalent ions increased adhesion [74]. This, too, might be due to binding by Dopa given the near absence of acidic residues in the protein.

On germanium crystal surfaces, attenuated total internal reflectance FTIR spectroscopy was used to show that, although fp-1 and fp-2 have similar adsorption kinetics, when added together fp-1

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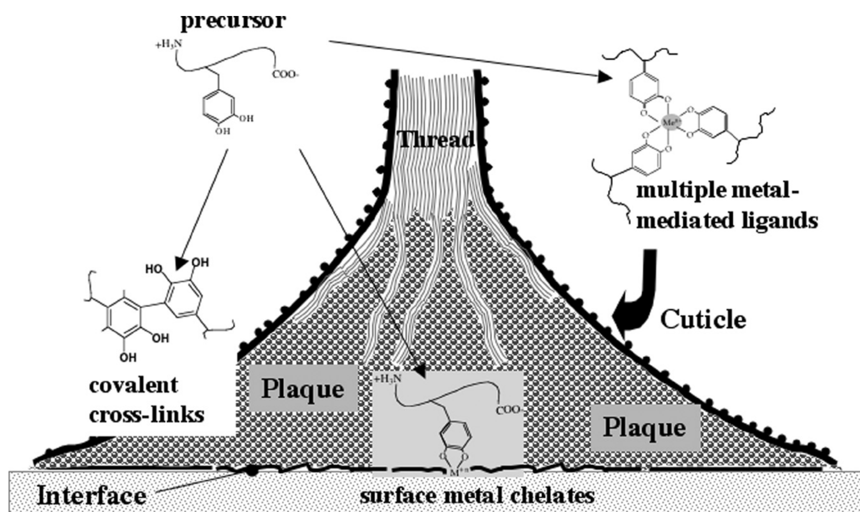

FIGURE 8 Dopa reactivity with respect to location in the byssus. Different Dopa containing precursors are stockpiled in the foot. Some such as fp-3 and fp-5 are deposited at the interface with a solid substratum and remain with their Dopa intact (*surface metal chelates*). Others such as fp-2 and -4 are secreted as bulk adhesive where they become cured by the formation of oxidized diDopa crosslinks (*covalent crosslinks*). The third group (fp-1) is thought to be cosecreted with Fe^{+3} in the thread coating where bis- and tris-iron(III) catecholates are formed (*multiple metal mediated ligands*).

appears to exclude fp-2 from adsorption until the Dopa in fp-1 has been oxidized to quinone [75]. The mode of fp-1 adsorption—mono- or multilayer—also depends critically on whether Dopa oxidation has occurred prior to adsorption. At low concentrations, oxidation tends to compact proteins prior to adsorption, whereas at higher concentrations, oxidized fp-1 adsorbs as multichain aggregates [76]. No multilayer adsorption occurs without prior oxidation [76]. It is difficult to fathom what these studies reveal about byssal adhesion à la moule, except to suggest that what happens to Dopa and when it happens critically affects the outcome of adhesion.

It would be misleading to represent the contribution of Dopa to mussel adhesion as a simple but effective surface coupling reaction. Equally plausible data suggest it may mediate formation of covalent diDopa cross-links [49, 77], of bis- and triscatecholato-Fe^{III} complexes with mefp-1 [78] (Fig. 7), and even triscatecholato-Fe^{III} complexes that serve as vehicles for diDopa cross-link formation by a one-electron redox exchange [79]. All of these are possible by proper control of the local microenvironment. Figure 8 illustrates how the chemical destiny of Dopa may differ as a function of location in the byssus.

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CONCLUSION

"There are agents in Nature able to make the particles of bodies stick together with very strong attraction and it is the business of experimental philosophy to find them out." [80]. This was Sir Isaac Newton's rumination at the beginning of the 18th century. Applied to the adhesive agents of spiders, tubeworms, barnacles or mussels, the tools for "finding them out" did not become available to experimental philosophy until the 21st century. Recombinant DNA and cloning have the power to provide sequence and milligram to gram quantities of even the rarest adhesive molecules. By careful and systematic mutation analysis of transgenic model organisms, molecular genetics offers razor sharp tools for dissecting the structure-function relationships of any adhesive in situ [81]. Such have been the promises at any rate.

Such promises often fall short of the mark when protein function is dependent on two additional steps: chemical and physical processing. Chemical processing entails enzymatically catalyzed posttranslational modification of the protein sequence encoded by the DNA sequence of the gene. There are many possible modifications including protein backbone truncation, side-chain transformation and addition of new functionalities. From a mechanistic perspective, the best-studied relationship between adhesion and chemical

processing involves selectins—adhesive proteins located on the outer surface of leucocytes that are strategically glycosylated on threonine residues and sulfated on tyrosines. Highly sulfated and glycosylated selectins make leucocytes sticky whereas the unglycosylated, unsulfated selectins do not. More importantly, stickiness can be tuned to whatever level is physiologically necessary by carefully controlling the degree of sulfation and glycosylation in the leucocyte selectins [82].

All of the adhesive proteins of marine mussels and tube-building worms contain posttranslational modifications. Dopa is the best known, but there are other examples involving proline, arginine and serine whose contribution to adhesion is not yet clear [58]. Unlike leucocyte adhesion, byssus adhesion is final and permanent. Notwithstanding this, however, chemical processing of mussel adhesive proteins may still play an important role in optimizing adhesion. It is possible that in mussels, "tuning" of the degree (0–100%) to which tyrosine is modified to Dopa, for example, might be an adaptation for sticking better to different kinds of surfaces encountered, and this remains under very active investigation.

The chemical processing exercised by the mussel in the maturation of adhesive proteins is not generally honored when these are expressed as recombinant proteins in host bacteria or yeast [83–84]. To the chagrin of the many initiatives on mussel adhesion in biotechnology, unmodified recombinant mussel adhesive proteins have few if any useful properties. In contrast, solid phase synthesis has succeeded in replicating adhesive consensus sequences with a few [85], most [64, 86–88], or all [89] modifications in place, but these are still limited to low molecular weights in the closest analogs. Dopa is crucial for adhesive properties, but a recent study suggests that Dopa plus its local primary sequence in mfp-1 adheres better than tethered Dopa per se [64]. An efficient enzymatic approach to hydroxylating tyrosine residues to Dopa in peptides has been reported, but this has yet to be optimized for large recombinant proteins [90].

Fundamental investigations of mussel byssus have lead to bioinspired applications and will continue to do so. First and foremost, the dopa or catecholic functionality, has been adopted as a robust anchor to improve the strength, durability of adhesive polymer bonding to wood, metals, minerals and mucosa [85, 91–94]. Biomimetics is a valuable but sobering endeavor because few of nature's lessons translate directly into instant technological hits. When the chemistry is properly controlled, the Dopa/catechol group is definitely a hit. Of the other two lessons discussed, complex coacervation is already widely practiced in the technology of microencapsulation [45], but not yet in adhesion.

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Making gradient structures between different materials, in contrast, is very appealing from an engineering perspective but it is limited at present by a dearth of affordable processing strategies.

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