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A Method for Measuring the Adhesion Strength of Marine Mussels Jeremy R. Burkett^a; Jessica L. Wojtas^a; Joshua L. Cloud^a; Jonathan J. Wilker^a ^a Department of Chemistry, Purdue University, West Lafayette, Indiana, USA

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A Method for Measuring the Adhesion Strength of Marine Mussels

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Marine mussels produce a byssal adhesive assembly for attachment to surfaces in the marine environment. The byssus is characterized by an array of adhesive plaques, each attached to threads that are anchored inside the animal. Here we describe a rapid method for determining detachment force, area, and overall adhesion of mussel plaques. Adhesion forces for mussels attached to glass, aluminum, acrylic, polyvinyl chloride (PVC), and Silastic[®] T2 are reported. This method may aid in the development of new adhesive materials and antifouling surfaces.

Keywords: Adhesion; Bioadhesion; Biological adhesion; Biological material; Biomaterial; Marine; Mussel

I. CONGRATULATIONS

We are happy to join the chorus of friends and colleagues in congratulating Herb Waite on his well-deserved receipt of the 2009 Adhesion Society Award for Excellence in Adhesion Science, Sponsored by 3M. This special journal issue (one of several) is a testament to the impact he has made in this field. From his early studies on mussel adhesive [1] to more recent work on worm jaws [2] and squid beaks [3], Herb has brought great visibility and interest to biological materials, with a particular emphasis on adhesion. His work has

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One of a Collection of papers honoring J. Herbert Waite, the recipient in February 2009 of *The Adhesion Society Award for Excellence in Adhesion Science, Sponsored by 3M.*

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provided a basis for other researchers to engage in studies on biological adhesives. With this contributed paper, we hope to provide a method for bridging studies of biological and synthetic adhesives.

II. INTRODUCTION

A. Biological Adhesion

The oceans are home to an amazing array of organisms utilizing different forms of adhesion for their survival. Starfish employ adhesive bonding for locomotion [4]. Mussels [5], barnacles [6,7], and ovsters create adhesive materials for surface attachment and stability. Marine worms use an adhesive composite to generate the protective shells of their dwellings [8]. From studies of these systems we may gain knowledge useful in the design of new synthetic materials as well as ideas for the development of antifouling strategies. We also wish to place the performance of these biological materials in a context relative to well-known, commercially available synthetic materials such as the white polyvinyl acetate (PVA) and cyanoacrylate "super glues." Both the understanding of adhesive-surface interactions and the development of antifouling strategies require new means of assessing the performance of biological adhesives. Such an ability to characterize bioadhesives will permit quantitative comparisons of adhesion upon different surfaces.

B. Mussel Adhesive

The common blue mussel, *Mytilus edulis*, produces a discrete adhesive system that lends itself well to quantifying surface adhesion in a controlled laboratory setting (Fig. 1). Adhesive formation begins with extension of the animal's foot from the shell and contact with the surface. After depositing a protein-rich adhesive plaque (or pad), the foot retreats back to the interior of the shell, leaving a thread that secures the animal to the freshly deposited plaque. Many repetitions of this process result in a strong attachment assembly typically consisting of between ~ 10 and 40 plaques.

Generally speaking, total adhesion of a system is a function of both the force required to break the bonding between two surfaces and the overlap (or contact) area of the materials. Adhesion data are typically reported in Pascals, or Newtons of force to bring about detachment divided by the overlap area in square meters (Pa = N/m^2). Alternatively, adhesion data may be quantified in pounds of force per square inch of overlap (PSI). In order to obtain accurate



FIGURE 1 A marine mussel adhering to a sheet of glass.

adhesion measurements, we need methods to quantify both maximum detachment force as well as the contact area between the material and surface. With bulk synthetic materials, detachment force measurements are often performed by lap shear methods such as the ASTM D1002 standard [9]. However, these procedures do not lend themselves well to studies of biological systems owing to the small quantities of material generally available and the irregular shapes. In the case of mussels, the arrangement of the byssal assembly, threads, and plaques further complicates collection of adhesion data.

C. Measuring Biological Adhesion

Prior studies on the attachment strength of mussels have employed various methods. A spring scale force gauge was used to remove entire animals from surfaces, recording the maximum pulling force required to effect detachment [10–12]. Regulated water jets have been used to dislodge submerged mussels, thereby indicating total detachment force [13]. An Instron[®] materials testing system was also used to observe the breaking of individual threads and players [14,15].

When working in the field, whole animal detachment is likely the best approach to take. However, pulling an entire animal off a given surface involves some compromises. Multiple material failure modes may be occurring simultaneously, thereby complicating data analysis. The threads have two distinct regions, an elastic, proximal portion and a crystalline, harder, distal section [16]. These threads may break at each region at different times or forces, prior to adhesive plaque failure ("thread breakage"). The thread-plaque junction can break apart ("thread-plaque failure"). "Cohesive failure" within the plaque is possible, when the plaque is torn apart. Separation of an entire, intact plaque from a surface constitutes "adhesive failure". The root junction, where the threads join inside the animal, can break apart and release threads. The entire root structure, where the threads are anchored, may also fail.

A further complication with whole animal adhesion quantification is the pull angle. A 90° pull, with respect to the surface and force direction, is required for true tensile measurements. Given the splayed array of adhesive produced by mussels (Fig. 1), we cannot pull a whole animal up from a surface and have all plaques pulled at 90° . Each plaque is pulled at a different angle. After taking all these factors into account, we concluded that the optimal force measurement will require analysis of individual plaques, rather than whole animals.

Complete adhesion measurement methods must include obtaining data on the plaque-surface contact area. Mussel plaques are not perfect circles or ovals. The small size ($\sim 2 \text{ mm}$ diameter) and irregular shape complicate obtaining accurate areas. Thus, the use of rulers or calipers is not viable for this application.

We have observed that plaques removed from seawater change mechanical properties quite rapidly, within 10 minutes after removal from a tank (unpublished results). The threads also change properties upon dehydration [17]. Furthermore, we will be measuring the adhesion of numerous plaques from many animals in order to obtain reliable data with good statistical significance. We also wish to study the adhesion of mussels on different surfaces to note trends in strength. Consequently, it is desirable for the methods of quantifying both detachment forces and contact area to be performed quickly.

Here we describe a rapid method for determining both mussel plaque detachment force and plaque surface area. Each measurement is performed on individual plaques. This process employs an Instron materials testing system for collecting tensile force data and digital imaging for area measurements. The resulting measurements provide standard adhesion data that can be compared directly with other biological or synthetic adhesives. We also include data on synthetic adhesives, for just such a comparison. Having the ability to quantify and compare the adhesion of mussels may facilitate a better understanding of how marine organisms attach to surfaces, aid in the development of novel antifouling strategies, and provide insights for the development of biomimetic materials.

III. EXPERIMENTAL

A. Cultivating Mussels

Adult mussels (*Mytilus edulis*) of \sim 5–7 cm in length were collected from intertidal locations in New Hampshire and Maine or purchased from mussel farms, also located in Maine. The mussels were grown in a home-built, ~ 350 gallon (1325 liter) aquarium system in our laboratory. Artificial seawater was made by dissolving Aqua Craft[®] Marine Environment Reef Formula (Hayward, CA, USA) in reverse osmosis-purified water. All water was aerated vigorously for at least 1 week prior to use in the aquarium system. Two 150 gallon (\sim 568 liter) insulated tanks were linked through a sump system. Each tank was aerated vigorously and contained a bed of ~ 6 inches (~ 15 cm) of crushed coral. Water flowed from the main 150 gallon tank to the second 150 gallon surge tank via a siphon system. Gravity drained water from the surge tank into the 30 gallon (\sim 114 liter) sump. A pump then carried water back to the main tank at a rate of ~ 20 gallons (\sim 76 liter) per minute. A chiller circulated water in and out of the main tank at ~ 30 gallons (~ 114 liter) per minute. This 1.5 horsepower Aqua Logic (San Diego, CA, USA) chiller maintained the temperature at 4°C. A protein skimmer helped clean the water by circulating in and out of the sump.

Our experience has shown that mussels seem to produce more adhesive in turbulent waters. Consequently, we created turbulence in the surge tank using a siphon system. Water was pumped up into an elevated barrel at a rate of ~15 gallons (~57 liters) per minute. Upon filling, the siphon released the water back down into the surge tank. Approximately 35 gallons (~132 liters) of water flowed from the barrel into the surge tank over 30 seconds. After 2 minutes of filling the barrel, another flow began.

Approximately 500 mussels are typically maintained in this system although the capacity is up to ~1,000. Mussels (~500) were fed twice weekly using DT's Premium Reef Blend (Sycamore, IL, USA) of phytoplankton. Each feeding used ~220 mL total, divided between the two tanks. The protein skimmer was not used for 8 hours after a feeding. Light (11 hours) and dark (13 hours) cycles were maintained in the aquarium room using six 100-watt halogen lights. This schedule simulated coastal Maine in February.

The water was checked for pH, calcium, copper, alkalinity, phosphate, nitrate, nitrite, and ammonia levels weekly, using test kits (Nutrafin, Hageu Co., Montreal, Canada; API, American Pharmaceuticals, Cheafout, PA, USA; Tetratest, Tetra Co., Blacksburg, VA, USA). Water changes provided additional maintenance with ${\sim}25\%$ of the water changed monthly and ${\sim}75\%$ changed twice per year. The coral beds were vacuumed and the tank sides were scrubbed at each monthly water change.

B. Surface Preparation

Plates $(4 \times 4 \text{ in.})$ (~10 × 10 cm) of surfaces (acrylic, PVC, glass, and aluminum) were cleaned by washing with soap and rinsing with distilled water prior to contact angle measurements and placement in the surge tank. Silastic[®] T-2 from Dow Corning (Midland, IN, USA) was used for a fifth surface. This silicone-based antifouling coating was brushed onto clean glass plates followed by a 24-hour cure. The resulting T-2 layers were ~2–3 mm thick.

C. Contact Angle Measurements

Water contact angle measurements were taken for all five clean surfaces examined in this study. A Tantec (Lunderskov, Denmark) contact angle meter was used. Data were calculated using the half-angle method. Reported values are averaged from 20 measurements and show one standard deviation.

D. Mussel Adhesive Production

Mussels were placed in the surge tank for adhesive deposition experiments. Individual animals were rubber-banded to the 4×4 in. plates. Banding was required to prevent the animals from moving and adhering to each other in clusters. Individual mussels remained tethered to a given plate for 3 days prior to removal for adhesion measurements. Typically each animal produced 10–20 plaques during this 3-day period. For these studies, 12 mussels were attached to plates of each surface. Individual mussels could be used for \sim 3 deposition cycles before adhesive production decreased and that animal was no longer used. After removal of the animals from the tank for data collection, the rubber bands were cut away. The threads were then cut at the animal's shell line with a razor blade, thereby maintaining the threads and adhesive plaques attached to the surface.

Test plates with attached plaques and threads were photographed for adhesive area calculations. A Nikon (Melville, NY, USA) D80 digital camera fitted with a Nikon 50 mm f-1.8 lens and a Kenko 12 mm extension tube was held on a small tripod with the lens oriented directly onto the plate below. The lens was \sim 18 cm above the plates being photographed. Typical exposure settings included f=3.5, 1/15 second shutter speed, and 200 ISO. Ambient light was sufficient to capture the images. Photographs were taken in JPEG format at maximum resolution (~3.3 MB per image). Individual plaques were numbered using a marker prior to taking the photograph so that the numbers were included in the image. These labels allowed correlation of specific plaques to tensile strength data. At the time of photographing the plaques, area standards were also photographed. Coins provided convenient standards. Photographing a plate took ~30 seconds, thereby allowing immediate collection of tensile strength data. The digital images of the plates were revisited later for calculating surface area.

E. Measuring Detachment Force

In order to measure detachment forces, plates bearing plaques and threads were clamped to the base of an Instron[®] 5544 (Norwood, MA, USA) materials testing system. A custom designed stainless steel base was fabricated for these experiments. The base was a circular platform 15 cm in diameter with a post and pinhole on the bottom, allowing bolting into the bottom mount of the Instron. Test plates with adhesive were fastened to this base with two Quick-Grip Handi Clamps (Wilmington, OH, USA) holding down opposite corners. The Instron was fitted with one screw-action grip (part 2710-004) on the top and a 5N load cell. Detachment force measurements began by lowering the grips directly over an individual plaque. The thread was lifted by hand and placed in between the grips. The grips were then tightened over the thread. The thread and clamps were oriented such that the upward pull would be perpendicular to the surface and provide a tensile measurement. Grips were positioned as close to the surface as possible, in order to cover the entire thread. Pulling up on the entire thread minimized thread breakage, thereby eliminating a failure mechanism that would otherwise complicate data analysis. Having the entire thread covered by the clamps meant that we could not see the threads and measure the exact angle of upward force, relative to the surface. As much as possible, the thread was arranged between the clamps to approximate a 90° angle of pull.

Force data were obtained by pulling up on a given thread at a rate of 10 mm/min. In the resulting extension-*versus*-load plots, rising force was noted until the maximum point, at which time the material failed and the force dropped rapidly. Figure 2 shows a typical example of such a plot for a single adhesive plaque and thread. The maximum load, in Newtons, was then recorded. Typical values for plaque maximum loads were between ~0.15 and ~0.45 N. With all the steps



FIGURE 2 Typical extension-versus-load plot for an individual adhesive plaque and thread.

described here, one test plate bearing \sim 10–20 plaques could be photographed and each plaque detachment force measured within a total of \sim 7–10 minutes after the plate was removed from the tank.

During force measurements, the failure modes were noted for each plaque. Options for failure included plaque adhesive, plaque cohesive, plaque-thread, and thread breakage. Table 1, shows the distribution of failure modes found upon each surface. Thread breakage was rare (<1%) and not included in analyses. Force data for the other three failure modes were combined to provide total adhesion on a given surface. As will be seen, some surfaces displayed a predominance of adhesive failure and others induced a majority of plaque-thread detachment. Consequently, we cannot calculate direct comparisons of, say, adhesive failure for each surface. Nonetheless, our main goal here is to provide a means for assessing the overall binding of mussels to varied surfaces.

	Adhesive failure (%)	Cohesive failure (%)	Plaque-thread failure (%)
PVC	98.1	1.9	
Acrylic	94.3	5.7	
T-2	100		
Aluminum		46.3	53.7
Glass		8.7	91.3

TABLE 1 Observed Modes of Failure for Mussel

 Adhesive on Different Surfaces

F. Tensile Adhesion of Synthetic Adhesives

Elmer's white glue (PVA) (Columbus, OH, USA) and Krazy Glue[®] (ethylcyanoacrylate) (Columbus, OH, USA) were chosen for adhesive comparisons with the mussel glue. Cylindrical aluminum adherends measuring 15 mm in diameter by 75 mm in length were machined, polished, and glued together by applying an even layer of ~ 15 mg of adhesive to the polished circular face of the adherend. The adherends were held together tightly for 30 seconds. The adherends were then allowed to stand for 18 hours at ambient temperature for curing. Instron tensile testing was used to collect the detachment force data. The overlap area was determined from the adherend interface. Adhesion values were averaged from 20 replicates of each material and reported with one standard deviation.

G. Plaque Area and Adhesive Calculations

Surface areas of the plaques were determined from the digital images taken of each plate prior to obtaining detachment force data. Using Photoshop Elements 4.0 (San Jose, CA, USA) software, an outline of each plaque perimeter was drawn manually. The number of pixels contained within each plaque was obtained from the image histogram. Digital photographs were also taken, under identical conditions, of circular objects to provide area standards. For example, an image taken of a coin was traced to determine the area in pixels. Pixels were then converted to area using the known size of the standards. Final adhesive performance of each plaque was then determined by dividing the failure force (in Newtons) by the area (in m^2) to yield values in Pascals ($Pa = N/m^2$) for each plaque. Reported average values of force, area, and adhesion in Table 2 are taken from individual plaques and reported with one standard deviation. Digital image analysis has been used previously to trace the outline of plaques [18] and for determining plaque areas [19].

Contact angle (°)	Number of plaques	Average detachment force (N)	Average area (mm ²)	Average adhesion (kPa)
112.0 ± 1.5	72	0.26 ± 0.09	1.91 ± 0.51	144 ± 55
98.6 ± 3.3	44	0.34 ± 0.14	2.58 ± 0.82	133 ± 34
85.5 ± 2.7	21	0.29 ± 0.10	3.11 ± 0.58	94 ± 36
42.8 ± 2.1	55	0.49 ± 0.19	1.78 ± 0.57	288 ± 110
32.9 ± 2.8	36	0.42 ± 0.19	2.52 ± 0.82	171 ± 65
	Contact angle (°) 112.0 ± 1.5 98.6 ± 3.3 85.5 ± 2.7 42.8 ± 2.1 32.9 ± 2.8	Contact angle (°)Number of plaques112.0±1.57298.6±3.34485.5±2.72142.8±2.15532.9±2.836	$\begin{array}{c} \mbox{Contact} \\ \mbox{angle (°)} \end{array} \begin{tabular}{lllllllllllllllllllllllllllllllllll$	$\begin{array}{c c} Average \\ \hline Contact \\ angle (^{\circ}) \end{array} \begin{array}{c} Number \\ of plaques \end{array} \begin{array}{c} Average \\ detachment \\ force (N) \end{array} \begin{array}{c} Average \\ area \ (mm^2) \end{array} \end{array}$

TABLE 2 Mussel Adhesion Data on Different Substrates

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IV. RESULTS AND DISCUSSION

A. Adhesion Data in Newtons and Pascals

Mussel adhesion studies were performed on five different substrates: acrylic, PVC, T-2, aluminum, and glass. All surfaces were handled according to the same procedure. We will describe experiments on acrylic in detail and then summarize data for the other four surfaces. Twelve mussels were tethered to 12 separate 4×4 in. acrylic plates for 3 days. These mussels produced a total of 44 plaques. The average maximum load of these plaques was 0.34 ± 0.14 N.

For adhesion in general, force is related to overlap area, with greater area yielding higher binding forces in Newtons [20]. Figure 3 shows an area-*versus*-force plot for each plaque on acrylic. A positive correlation was observed, with larger plaques yielding greater detachment force. Each surface examined here displayed this same trend. On acrylic, plaque areas were in a range of $(\sim 1-4) \times 10^{-6} \text{ m}^2$, or $\sim 1-4 \text{ mm}^2$. The average surface area of plaques on acrylic was $(2.6 \pm 0.82) \times 10^{-6} \text{ m}^2$. For each plaque both the area and detachment force were measured to yield adhesion in kPa. The kPa values of individual plaques were combined to provide an average adhesive strength of 133 ± 34 kPa on acrylic.

Table 2 summarizes data for mussel adhesion to acrylic as well as for the other surfaces examined. The columns of detachment force, area, and adhesion were each averaged from individual plaques. Note that average adhesion varies by nearly a factor of 3, depending upon



FIGURE 3 Plot of area-versus-tensile load for mussel plaques on acrylic. A linear fit to the data is shown (R = 0.77).

the surface. The water contact angle was measured for each surface, prior to placement in the tanks, and these data are also shown in Table 2. The data provide insight on the relative surface energy of each substrate. When looking at the energies of the test materials, surfaces with lower energies (acrylic, PVC, T-2) demonstrated lower average detachment forces than the surfaces with higher energies (glass, aluminum). When detachment force was converted to adhesion in Pa, this correlation with surface energy persisted. In general, lower energy surfaces (acrylic, PVC, T-2) minimized the animal's ability to adhere and the higher energy surfaces (glass, aluminum) maximized adhesion.

The trend correlating surface energies and adhesion was not perfect and variations may be resulting from differences in surface moduli (*i.e.*, compliance or hardness) as well as surface chemistry changes. The T-2 surface has a particularly low modulus in relation to the other substrates examined here [21]. Generally speaking, more compliant surfaces decrease adhesion [21-23]. Thus, the weaker adhesion found for mussels on T-2 may be a modulus effect. By contrast, the particularly high adhesion on aluminum may be influenced by chemistry of both the surface and the adhesive itself. Metal oxide surfaces such as aluminum tend to have a slightly anionic character (*i.e.*, negative charge) [24,25]. The proteins known to constitute mussel adhesive harbor a somewhat cationic (*i.e.*, positive) charge [26]. Such an ion-cation interactions could contribute to enhanced adhesive bonding between the plaques and surface. Other chemical contributions to strong adhesion on aluminum may include hydrogen bonding between the proteins and the metal oxide surface as well as chelation of surface aluminum ions by the 3,4dihydroxyphenylalanine (DOPA) amino acids of the mussel proteins [27–29]. A glass surface could also exhibit analogous hydrogen bonding and anion-cation interactions. The plastics (PVC, acrylic) and T-2 are less likely to have such bonding modes available, thereby contributing to the decreased adhesive forces measured here.

B. Comparisons with Literature Data

To date, literature data for the tensile strength of mussel plaques on various surfaces have focused on force measurements. A variety of units have been used to report these data (*e.g.*, N, kg). Comparison of our work with some of these studies provides correlations. Ackerman *et al.* performed whole animal studies with a spring scale and showed the detachment force of mussels on aluminum to be 0.46 ± 0.05 N [30]. Our results here, on aluminum, show a very similar average value of 0.49 ± 0.19 N.

Price used a spring scale and reported the required detachment force of *Mytilus edulis* from rocks to vary between $\sim 1.2-2.4$ kg over the course of a year in their natural habitat [10]. With 1 kg = 9.8 Nand mussels producing between 10–40 plaques, a rough conversion of these data to Newtons provides a force range of $\sim 0.5-1.2$ N per plaque. On glass substrates, Dolmer and Svane reported detachment forces of individual threads to be just under 300 g, measured using a spring scale [12]. This detachment force converts to ~ 2.9 N. Crisp et al. described adhesion of mussels on glass, among other substrates, to be in a range of 320-750 kPa [31]. However, the methods used for determining force and area were not described in detail [31]. Presumably these authors used the same techniques outlined in a separate paper in which adhesion to glass was reported at 310 kPa [16]. These experiments started by using a commercial cyanoacrylate adhesive for bonding a metal wire to the thread [16]. Consequently, these prior data [16] are not directly comparable with our current studies in which we are examining detachment of the natural plaque and thread assembly.

Mussel tenacity is another means by which researchers have quantified mussel attachment. Bell and Gosline utilized a spring scale to record the detachment force (in Newtons) of mussels from granitic rock in southern British Columbia [11]. Measurements were then taken of the shell height and width of the animals to determine the shell planform area (A_{pl}). Detachment forces were then divided by A_{pl} to yield data reported as tenacity. We may take the reported range of tenacity data in N/m², multiply by the range of planform areas found in m², and then divide these forces by the range of plaques produced per animal. The resulting estimate yields 0.041–26 N per plaque. Although our current average forces per plaque of 0.26–0.49 N, depending upon the surface, do fall into this range, we must be careful about drawing any close parallels. Tenacity and adhesion, although related in this context, are not directly comparable properties.

C. Modes of Material Failure

The distribution of material failure modes, shown in Table 1, differed upon each surface. For acrylic, a lower energy surface, nearly all plaques (\sim 94%) exhibited adhesive failure. Only a small fraction (\sim 6%) underwent cohesive failure. Generally speaking, adhesives tend not to bond well to low energy surfaces [32]. The PVC and T-2 surfaces are also of somewhat lower energy and exhibited a predominance of adhesive failures (Table 1). For glass and aluminum, both higher energy surfaces, a significantly higher proportion of failures were due to thread breakage at the plaque-thread junction (Table 1). Some cohesive failure was noted, more so for aluminum than glass. These trends are consistent with stronger adhesive interactions with higher energy surfaces, thereby inducing the alternative failure modes of cohesive failure and thread breakage.

D. Surface Preferences

The same number of mussels were used here for plaque deposition onto each surface. However, the animals produced differing quantities of material. Although the number of plaques varied with each surface, we did not observe any trend correlating with surface energy, shown in Table 2. On this point, the literature has been mixed. One study showed mussels to lay more plaques onto materials of lower surface energy [19]. Other reports, however, described an opposite trend [31,33,34]. In light of our not observing a trend and the contradictory literature, we may conclude that surface energy, alone, is not a predominant factor in determining the propensity of mussels to deposit adhesive. Differences in surface chemistries and moduli may be playing a role here.

E. Plaque Areas

Similar to a lack of trending found between surface energy and the number of plaques, we also found no strong influence of the surface upon plaque areas. The data in Table 2 show that water contact angles and average plaque areas do not appear to relate. Here, too, the literature presents a somewhat mixed story. Older reports noted that plaque areas increased when changing from high energy surfaces, such as glass, to low energy surfaces, such as paraffin wax [16,31]. More recently, a study using controlled surfaces prepared from self-assembled monolayers (SAMs) of various thiols on gold found that larger plaques resulted when the wettability or surface energy was increased [19]. Note that although plaque areas may vary from amongst different surfaces, the total volume of adhesive deposited by the animal appears to remain constant [14,16,31]. In other words, plaques with high coverage areas tend to be thinner and low area plaques are thicker [14,16,31]. Given these seemingly contradictory findings, surface coverage area of plaques may not be solely dependent upon surface energy. Again, variations of surface moduli and schemistries may also be influential.

F. Comparisons with Synthetic Adhesives

We found that the tensile strengths of synthetic adhesives were stronger than those of the mussel plaques. White polyvinyl acetate (PVA) glue on aluminum exhibited adhesion at 890 ± 260 kPa, roughly three times as great as mussel plaques on aluminum. The cyanoacrylate-based Krazy Glue yielded a lower limit of adhesion at ~8,000 kPa, with several trials exceeding the maximum tensile load of our instrument. These data show that this commercial adhesive is significantly stronger than the mussel glue. Although weaker than these synthetic materials, mussel adhesive does exhibit unique properties such as the ability to set well in a wet environment.

V. CONCLUSION

Here we have presented methods to measure both the adhesion force and area of mussel plaques. Data are collected rapidly, to both permit obtaining many replicates and to minimize changes in the material during measurements. We have quantified adhesion strength and the failure modes of this biological material on a series of different surfaces. Data on individual mussel plaque adhesion is reported in Pascals, thereby factoring in area. Consequently, we can now place the performance of this biological material into the familiar context of man-made synthetic adhesives. These procedures may now aid the future development of biomimetic materials as well as antifouling surfaces.

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REFERENCES

- [1] Waite, J. H. and Tanzer, M. L., Science 212, 1038-1040 (1981).
- [2] Lichtenegger, H. C., Schoberl, T., Bartl, M. H., and Waite, J. H., Science 298, 389–392 (2002).
- [3] Miserez, A., Schneberk, T., Sun, C. J., Zok, F. W., and Waite, J. H., Science 319, 1816–1819 (2008).
- [4] Hennebert, E., Viville, P., Lazzaroni, R., and Flammang, P., J. Struc. Biol. 164, 108–118 (2008).
- [5] Waite, J. H., Integr. Comp. Biol. 42, 1172-1180 (2002).
- [6] Naldrett, M. J. and Kaplan, D. L., Marine Biol. 127, 629-635 (1997).

- [7] Kamino, K., Inoue, K., Maruyama, T., Takamatsu, N., Harayama, S., and Shizuri, Y., Biol. Chem. 275, 27360–27365 (2000).
- [8] Jensen, R. A. and Morse, D. E., J. Comp. Physiol. B 158, 317-324 (2004).
- [9] ASTM D1002-05 (American Society for Testing and Materials International, West Conshocken, PA, 2005).
- [10] Price, H. A., Mar. Biol. Ass. UK 60, 1035-1037 (1980).
- [11] Bell, E. C. and Gosline, J. M., Mar. Ecol. Prog. Ser. 159, 197–208 (1997).
- [12] Dolmer, P. and Svane, I., Ophelia 40, 63-74 (1994).
- [13] Dormon, J. M., Coish, C., Cottrell, C., Allen, D. G., and Spelt, J. K., J. Environ. Engineering 123, 933–938 (1997).
- [14] Allen, J. A., Cook, M., Jackson, D. J., Preston, S., and Worth, E. M., J. Moll. Stud. 42, 279–289 (1976).
- [15] Smeathers, J. E. and Vincent, J. F., J. Moll. Stud. 45, 219-230 (1979).
- [16] Young, G. A. and Crisp, D. J., Adhesion 6, 19–39 (1982).
- [17] Aldred, N., Willis, T., Williams, D. N., and Clare, A. S., J. R. Soc. Interface 4, 1159– 1167 (2007).
- [18] Zhao, H., Robertson, N. B., Jewhurst, S. A., and Waite, J. H., J. Biol. Chem. 281, 11090–11096 (2006).
- [19] Aldred, N., Ista, L. K., Callow, J. A., Lopez, G. P., and Clare, A. S., J. R. Soc. Interface 3, 37–43 (2006).
- [20] Wang, T. T., Ryan, F. W., and Schonhorn, H., J. Appl. Polymer Sci. 16, 1901–1909 (1972).
- [21] Brady, R. F. and Singer, I. L., *Biofouling* 15, 73-81 (2000).
- [22] Irwin, E. F., Saha, K., Rosenbluth, M., Gamble, L. J., Castner, D. G., and Healy, K. E., J. Biomaterials Sci. Pol. Ed. 19, 1363–1382 (2008).
- [23] Sun, Y., Guo, S., Walker, G., Kavanagh, C. J., and Swain, G. W., *Biofouling* 20, 279–289 (2004).
- [24] Ostomel, T. A., Shi, Q., Stoimenov, P. K., and Stucky, G. A., *Langmuir* 23, 11233– 11238 (2007).
- [25] Dobson, K. D., Connor, P. A., and McQuillan, A. J., Langmuir 13, 2614–2616 (1997).
- [26] Rzepecki, L. M., Chin, S. S., and Waite, J. H., Molec. Mar. Biol. and Biotech. 1, 78–88 (1991).
- [27] Taylor, S. W., Luther, III, G. W., and Waite, J. H., Inorg. Chem. 33, 5819–5824 (1994).
- [28] Taylor, S. W., Chase, D. B., Emptage, M. H., Nelson, M. J., and Waite, J. H., *Inorg. Chem.* 35, 7572–7577 (1996).
- [29] Sever, M. J., Weisser, J. T., Monahan, J., Srinivasan, S., and Wilker, J. J., Angew. Chem. Int. Ed. 43, 448–450 (2004).
- [30] Ackerman, J. D., Cottrell, C. M., Ethier, C. R., Allen D. G., and Spelt, J. K., J. Environ. Engineering 122, 141–148 (1996).
- [31] Crisp, D. J., Walker, G., Young, G. A., and Yule, A. B., *Colloid. Interface Sci.* 104, 40–50 (1985).
- [32] Thurston, R. M., Clay, J. D., and Schulte, M. D., J. Plastic Film and Sheeting 23, 63–78 (2007).
- [33] Yamamoto, H., Ogawa, T., and Nishida, A., J. Marine Biotech. 5, 133-136 (1997).
- [34] Ohkawa, K., Ayako, A., Honma, R., Matsui, Y., Nagaya, K., Yuasa, A., and Yamamoto, H., *Biofouling* 13, 337–350 (1999).