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Adhesive designed by nature (and tested at Redstone Arsenal)

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Abstract Many adhesives are not particularly environmentally friendly. Montana Biotech has been working on an affordable, water-based adhesive produced from a renewable resource. The high molecular weight polysaccharide is non-cytotoxic, biodegradable and has a melting point of 225 °C. This "green" adhesive has good tensile strength approximately 6.2 mPa on bare aluminum and is especially useful on epoxy glass and manufactured woods. Cured adhesive maintained full strength in an environmental chamber during a week-long 85% humidity, temperature-cycling program.

Introduction

In 2001, the US used 2.5 billion kg of adhesive (Nick 2002) but there are significant environmental issues associated with adhesives. Many are derived from petrochemicals. Sixteen percent of adhesives include toxic solvents (Nick 2002) such as toluene, methyl ethyl ketone and trichloroethane.

Researchers are increasingly turning to biomimicry. This is the science of problem solving using biological systems as the source of environmentally friendly solutions. Perhaps the best known biomimetic invention is Velcro, inspired by the barbs on weed seeds. The path forward for translating natural adhesives into commercial success hasn't been easy. Decades ago, scientists began studying adhesives produced by barnacles and mussels. Materials were found with excellent adhesive properties, particularly for the difficult job of under water adhesion. So why isn't mollusk glue available in all hardware stores today? It takes 10,000 mussels to make 1 g of adhesive (Roberto 2000, Waite and Tanzer 1981). Although progress has been made, scale up of the complex, multi-part system has proven to be cumbersome.

It turns out mollusks aren't the only natural source of adhesives. Microorganisms such as bacteria and algae, are often found firmly glued to surfaces ranging from rocks in fast flowing streams to the inside of water distribution pipes. These microbial life forms produce extracellular polymeric substances (EPS) and it is the EPS that anchor organisms in place (Allison and Sutherland 1987, Pringle and Fletcher 1986, Suci et al. 1995, Whitfield 1988). In natural settings, sometimes so much EPS is produced that it is readily visible to the naked eye. If you doubt the strength of EPS adhesion, try scraping the slime off rocks in a stream.

The big advantage of microorganisms over mollusks is that microbial systems are far less complex than the higher life forms, and the methods for producing microorganisms in large volume use standard technology.

A number of polymers from bacteria are commercially available. Some have long been exploited as emulsifiers, thickeners, stabilizers and gelling agents. For example, xanthan gum, produced by *Xanthomonas campestris*, produces highly viscous solutions at low concentrations. It is used by the food industry in everything from salad dressing to ice cream. Interestingly, none of these bacterial polymers are in widespread, commercial use as adhesives.

Against this background and with support from the Strategic Environmental Research and Development Program (SERDP), Montana Biotech screened polymers produced by microorganisms, for adhesive properties.

Methods

Production of adhesive

Montana Biotech has a substantial in-house collection of microorganisms from a range of habitats. A total of 350 organisms were chosen from this collection for use in this project. A process was worked out to determine which organisms were producing materials with adhesive properties. Each organism was grown in a 500-ml minifermenter for 2–4 days. Components of the sterilized medium were 9 g/l yeast extract, 15 g/l peptone and

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10 g/l dextrose. The pH was adjusted to within 0.5 units of the original isolation pH and the temperature to within 10 °C of the habitat temperature. Fermenters were aerated at a rate of 1 ml air/ml medium/min. Following growth, 150 ml spent medium was mixed with 300 ml cold isopropyl alcohol and allowed to stand at −20 °C for 2 days. The solution was centrifuged at 1,500*g* for 10 min, the supernatant was discarded and the precipitated polymer was used as the adhesive without further purification.

Tensile strength testing identified 15 organisms that produced polymer with adhesive properties. The one making the largest amount of adhesive was selected for production of sufficient polysaccharide for all the tests reported herein. The medium was optimized as follows (g/l): 0.04 $K_2HPO_4 \cdot 3H_2O$, 0.1 $MgSO_4 \cdot 7H_2O$, 0.001 $FeSO_4 \cdot 7H_2O$, 0.2 $(NH_4)_2SO_4$, 0.5 citric acid monohydrate, and 21.0 sucrose. Organisms were grown for 2 days at 35 °C at pH 6.4. Spent medium was centrifuged to remove the biomass. One part of clarified medium was mixed with two parts of alcohol and the entire solution was cooled to −20 °C. The cooled solution was centrifuged at 1,500*g* for 10 min, the supernatant was discarded and the precipitate was used as the adhesive.

To demonstrate the feasibility of producing this natural adhesive on a large scale, Montana Biotech partnered with Shanghai Cathay Biotechnology Co., Ltd. to produce polymer in their 5,000-l reactors. This company produces a number of other polymers by fermentation and has expertise in scale up of microbial polymers from the laboratory to full production volume. It is material produced at this facility that was used for the environmental test chamber work and much of the other work reported in this paper.

Tensile strength testing

Specimens for the tensile testing were prepared in accordance with ASTM D2094-00. The testing itself was done in accordance with ASTM D2095-96. All screening utilized tensile strength tests on 2024 aluminum adherends. These adherends were machined so that the surface area of the ends was 6.45 cm^2. Approximately half the adherends were bare aluminum, while the other half were anodized. The surface coating did make a difference in adhesive strength, so care was taken to record the surface coating and to make sure that all samples to be compared used the same coating. Adhesive was spread on one face of a pair of adherends. The pair was immediately placed in a device designed specifically for holding these adherends called a constant force fixture (CFF). This device consisted of a pair of aluminum holders mounted on tracks which forced alignment of two opposing adherends. Holders were spring loaded and 0.014 mPa exerted on the adherends. Adherends were held in the CFF for 1 h, providing ample time for the adhesive to set up prior to being handled. Adherends then were allowed to cure for 1–2 weeks at 35 °C.

Following curing, tensile tests were read on a manually operated Mark 10, Model BGI, a force measuring instrument. The tensile strength was generally determined on five pairs of adherends and the average reported.

Coatings and surface preparation for shear testing

Aluminum coupons measuring 2.54 cm by 10.16 cm were used for shear testing. Coupons were divided into four groups as follows:

1. Anodized
2. Anodized and epoxy coated
3. Anodized, epoxy undercoat and urethane topcoat
4. Anodized, epoxy undercoat and CARC (chemical agent resistant coating) topcoat

Specifications were as follows:

1. Anodized: AMS 2471 sulfuric acid. A film of aluminum oxide is built up on the aluminum substrate, and then sealed with hot water.
2. Epoxy: MIL-P-23377. This is a standard epoxy primer, used under the outer coating on many military vehicles.
3. Urethane: MIL-PRF-85285. Urethane provides a tough but slick topcoat.
4. CARC: MIL-C-53039 or MIL-C-46168. CARC is the topcoat seen on most military vehicles. It comes in many colors from olive drab to tan to black.

Barry Avenue Plating (Los Angeles, Calif.) applied all coatings. Urethane was roughed using three passes with sandpaper in one direction, turning the coupon 90°, and subjecting the surface to three more passes with sandpaper. All surfaces were wiped with ethanol or the green solvent EnSolv (*n*-propyl bromide, Enviro Tech, Melrose Park, Ill.) immediately prior to application of the adhesive. Adhesive was applied to a 2.54-cm by 1.27-cm area of one coupon and the second coupon overlapped that area. Coupons were clamped together for 1 h to allow adhesive set up. They were cured for 10 days at 35 °C.

Shear strength testing

Shear strength testing was conducted on samples prepared at Montana Biotech and sent to the US Army Aviation and Missile Command (AMCOM) Materials Lab at Redstone Arsenal. The samples were divided into two sets. The initial set was lap shear tested in accordance with ASTM Specification D1002-99 (1999), using an Instron model TTC mechanical tester. Of the second set, some samples were environmentally conditioned using a humidity chamber; others were tested in an "as is" state for controls. The second set of lap shear tests were conducted, in accordance with ASTM D1002-99 (1999), using an Instron model 1321 hydraulic tester.

Comparisons were made with a commercially available adhesive. The metric was a 3M gasket adhesive (3M 4799). The 3M product is not designed for use on all substrates used in this project but served as a constant and readily available reference needed since some changes were made in parameters used in production of the Montana Biotech adhesive over the course of the project.

A Cincinnati Sub-Zero programmable humidity chamber was used to condition the samples. The conditioning requirements were tailored from the Department of Defense (DoD) standard for environmental tests, MIL-STD-

810E, DoD Test Methods Standard for Environmental Engineering Considerations and Laboratory Tests, Method 507.3, Humidity, using a modified aggravated humidity cycle.

1. The temperature was cycled between 30 °C and 60 °C; the humidity was held (relatively) steady at 85% RH; two cycles per day for 7 days gave 14 cycles.
2. Humidity chamber program:

Temperature (°C)	Humidity (%)	Time (h)	Explanation of step
30	85	1	Bring chamber to initial conditions
60	85	2	Ramp chamber to high temperature
60	85	4	Hold chamber at high temperature
30	85	2	Ramp chamber to low temperature
30	85	4	Hold chamber at low temperature

Loop to 60 °C ramp up; repeat 14 times.

3. After completion of conditioning, the samples were removed from the chamber and returned to dry storage at ambient temperature.

After environmental conditioning, the samples were tested in accordance with ASTM D1002-99 (1999).

Results

Comparison with other natural polymers

The question arose as to whether the polymer developed at Montana Biotech was unusual in its adhesive properties, or if other microbial polymers were equally as adhesive, but had simply not been used for this purpose before. Comparisons of tensile strength were made between the selected microbial adhesive and other natural polymers. An attempt was made to dilute all preparations to 25% solids. However, for materials traditionally used as thickeners, it was not possible to use the materials at such a high percent solids. After sufficient dilution to make a spreadable paste, preparations were allowed to stand for 24 h to complete solubilization. All adhesive materials were applied to bare aluminum adherends, cured for 10 days at 35 °C and then tested for tensile strength on the Mark 10. Solutions of Montana Biotech's polymer had an inherently low viscosity so it was possible to prepare solutions with 50% solids that could be readily spread on adherends. However, in this case, more was not better. It was found that solutions of 25% solids had maximum tensile strength on bare aluminum. Ten sets of coupons were adhered with the polymers indicated. Following curing, the tensile strength was determined and the averages of the ten tests are reported in Table 1. For aluminum adherends, the Montana Biotech polymer did have greater tensile strength than other commonly available polymers. Dextran, is used in some specialty applications such as cigarette pasting and certain machine labeling procedures and its adhesiveness is reflected on the aluminum as tested here.

Table 1. Comparison of Montana Biotech adhesive with commercially available natural polymers

Polymer	% solids	Tensile strength (mPa)
Montana Biotech adhesive	25	>6.83
Montana Biotech adhesive	50	5.90
Corn starch, cooked	25	4.76
Dextran	25	3.30
CMC, sodium salt	17	1.33
Guar gum	8	0.43
Xanthan gum	17	0.23
Alginic acid, sodium salt	17	0.14

Adhesive strength tests at Redstone Arsenal

Initial testing

All shear testing was done under provisions of a Cooperative Research and Development Agreement (CRDA) at Redstone Arsenal. Five sets of aluminum coupons were adhered with either the Montana Biotech adhesive or a commercially available metric. After curing, the shear strength was determined and the averages are reported in Table 2. Results reported are the average of five tests.

Environmental testing

The adhesive failure mode may be classified as adhesive or cohesive. Adhesive failure occurs at the substrate/adhesive bondline. This is an indication that the bond between the adhesive and the substrate is weaker than either the adhesive itself or the substrate material. Cohesive failure is usually seen as a failure within the body of the adhesive. When both the adhesive and the bond between the adhesive and the substrate are very strong, the substrate material may fail.

Samples were prepared for testing in an environmental chamber at Redstone Arsenal as described in Methods. Most often, the failure was largely cohesive. The urethane coating showed 50/50 cohesive/adhesive failures. The CARC trials produced mixed results. A summary of tests in the environmental chamber is presented in Table 3. Four to ten sets of aluminum coupons were adhered with either the Montana Biotech adhesive or a commercially available metric. After curing, the shear strength was determined and the averages reported here. 50/50 refers to

Table 2. Shear strength on coated aluminum coupons in mPa

Surface coating	Montana Biotech adhesive	3M 4799
Anodized	5.65	1.18
CARC	4.49	1.28
Epoxy	3.97	1.01

Table 3. Environmental chamber test results

Adhesive	Treatment	Failure mode	Shear strength (mPa)	Number of tests (t); number broken in transit (b); number failed in chamber (f)
Anodized				
Microbial adhesive	Control	Cohesive	2.70	t=5
Microbial adhesive	Chamber	Cohesive	4.54	t=7, f=3
3M 4799	Control	Cohesive	1.18	t=5
3M 4799	Chamber	Cohesive	0.55	t=4, f=1
CARC				
Microbial adhesive	Control	50/50	1.74	t=4, b=1
Microbial adhesive	Chamber	Cohesive	1.90	t=7, b=2, f=1
3M 4799	Control	Cohesive	1.28	t=5
3M 4799	Chamber	Cohesive	0.65	t=5
Epoxy				
Microbial adhesive	Control	Cohesive	2.48	t=1, b=5
Microbial adhesive	Chamber	Cohesive	3.11	t=2, b=2
3M 4799	Control	Cohesive	1.01	t=5
3M 4799	Chamber	Cohesive	0.72	t=5
Urethane				
Microbial adhesive	Control	50/50	2.34	t=2, b=9
Microbial adhesive	Chamber	50/50	2.60	t=4
3M 4799	Control	Cohesive	1.28	t=5
3M 4799	Chamber	Cohesive	0.75	t=5

the failure being attributed equally to cohesive and adhesive modes.

Plastics and wood

Some plastics are fairly difficult to glue together, so a screening test was done using half a dozen different plastics. Epoxy glass (an epoxy resin/fiberglass composite), DAP (diallyl phthalate) and ABS (acrylonitrile butadiene styrene) were the only plastics that exhibited shear strength above 0.69 mPa. Four selected plastics were tested in the environmental chamber. Of these four, epoxy glass was the only plastic that could be reliably adhered with the microbial adhesive. It had an average shear strength of 1.68 mPa. Good surface wetting and good cohesion were observed and none of the test samples separated in the environmental chamber.

A few shear tests were done on manufactured wood and the soft wood, fir. Bond shear strength was greater than the substrate for medium density fiberboard, particle board and fir.

Toxicology

Two toxicology tests were performed on the parent compound. It was found to be non-cytotoxic, meeting the requirements of the Agar Diffusion Test, ISO 10993. Endotoxin was found to be present in the preparation. Dilutions were done out to 1:256 and the corrected Endotoxin Units/ml was reported as greater than 615. By way of comparison, xanthan gum, a commercially available microbially produced polysaccharide used as a food additive, was also tested and found to have greater than 1280 Endotoxin Units/ml.

Heat stability

For a biological material, the polymer was found to be unusually heat stable. The melting point and the glass transition temperatures were determined by differential scanning calorimetry (DSC). The scan was started at 25–150 °C at 10 °C/min, cooled to room temp, and then rescanned from 25 to 250 °C at 10 °C/min. The first scan was performed to volatilize residual water. The T_g (glass transition temperature) was at 138 °C. The polymer melted with decomposition at 225 °C.

Summary

Extracellular polymeric substances from bacteria were found to have good adhesive properties. A selected bacterium was used to produce an affordable "green" adhesive from sugar. The high molecular weight, water-based polysaccharide is non-cytotoxic and environmentally friendly. Tensile testing demonstrated greater adhesive strength than other commercially available natural polymers. It also compared favorably with a commercial adhesive when used to join aluminum coupons with various surface coatings.

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