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Adhesive strength of marine mussel extracts on porcine skin ☆

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Abstract

The adhesive characteristics of marine mussel adhesive extracts were examined. Adhesive protein extracted from mussels (*Mytilus edulis*) was used to bond porcine skin in an end-to-end joint cured in controlled environments, without the use of chemical cross-linking reagents. The two curing conditions were similar to common surgical environments—"dry" (25°C and 40% relative humidity) and "humid" (37°C and 80% relative humidity). The first condition is similar to that of an external incision while the second is similar to conditions for internal incisions that are not exposed to significant flow of body fluids. Results were compared with performance of the commercial adhesive fibrin. Cyanoacrylate was also examined to validate the testing procedure. The tissue joint strength was ~1 MPa for mussel extract joints cured for 24 h under "humid" conditions. Under both conditions, joints bonded with mussel extract showed adhesive strengths similar to those bonded with fibrin, for cure times between 12 and 24 h. For shorter cure times (<12 h) the mussel adhesive bond was weaker than the fibrin bond under both conditions. The presence of moisture seemed to have a significant effect on the performance of both adhesives, especially mussel extracts. These results indicate that tissue joints formed using mussel extract adhesives have comparable strengths to those formed using fibrin ($P = 0.38$), albeit with a slower curing rate. Further investigation of curing agents for the mussel adhesive extract is warranted.

Keywords: Mussel adhesive; Adhesive strength; Surgical adhesives; Skin; Tissue joining

1. Introduction

Surgical reconnection of severed tissues is essential for restoration of their structure and function. The most widely used methods for joining tissues focus on mechanical fasteners such as sutures and staples. A useful fastener should hold the joined tissues in close proximity to promote adequate healing and arrest the leakage of biological fluids. The joint must also be able to resist tensile loads. Mechanical fasteners such as sutures have significant limitations. First, these devices cannot independently prevent fluid leaks from hollow structures like blood vessels. Secondly, application of sutures is inherently traumatic to the surrounding tissue. Finally, the quality of the mechanical union may be compromised if the surgeon is working in a confined region of the body.

Surgical adhesives provide attractive alternatives in situations where mechanical fastening is undesirable. Glues have been employed in clinical studies for a variety of soft tissue repairs [1–13], for sealing to prevent loss of fluids [14–18] and for carrying drugs [19]. Although surgical adhesives have performed satisfactorily in many instances, each material has limitations. An ideal adhesive will adhere to the tissue substrate, providing adequate strength in the presence of physiological fluids. The adhesive should enable wound healing by maintaining close apposition of tissue for sufficient time, should not elicit an immune response, and should be biodegradable with no tissue toxicity. Additionally, the material should be easy to handle, cure rapidly, and be affordable [20]. At present, there is no surgical adhesive that rigorously fulfills all these criteria. For example, cyanoacryalate adhesives, which cure rapidly and form strong tissue joints, are often toxic to tissue

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[21] and elicit dose-dependent carcinogenicity [22]. Although commercially available fibrin has been used in many surgical situations, it is inadequate for supporting tissue joints with significant tensile loads [23]. Presently, fibrin is made from human serum and is dependent on the donation of blood [24]. The development of an ideal adhesive is an exciting challenge for many clinical investigators, as indicated by the increasing number of experimental adhesive formulations reported in research journals [24–37].

Our investigation focuses on proteinaceous adhesive extracts from marine mussels (*Mytilus edulis*), first studied for their adhesive potential by Waite et al. [25–26]. This extract is one of the several adhesive formulations examined by subsequent studies in the adhesive community, primarily because of the ability to cure rapidly in underwater conditions [38–43]. When it has cured, the adhesive bonds the mussels strongly to underwater structures such as rocks and ship hulls. During the past three decades, significant information has been gathered regarding the nature of these adhesives. Specific procedures for extraction of the precursor proteins have been published [44–46]. Some of these formulations have been patented for commercial use in cell immobilization and sealing of tissue perforations such as full thickness corneal holes [47–48]. A few investigators have also studied the biocompatibility of these protein products and have reported that they elicit minimal immune response and cytotoxicity [48–52].

Although these reports are encouraging, more extensive use of this material as a surgical adhesive has been hindered by the lack of understanding of the exact processes employed by the mussels to rapidly cure the precursor proteins into a strong biological adhesive. In addition, a literature search located only a relatively small amount of data on the strength of tissue joints formed using these proteins. As mentioned previously, adhesive strength is one of the most important attributes of any successful surgical adhesive. In the past, investigators have examined two types of joints using mussel adhesive proteins—the lap joint, which involves shear forces as would be experienced by grafts, and the end-to-end joint, which involves tensile forces and simulates an excision wound. Benedict and Picciano [48] reported an average strength of mussel extract with catechol oxidase of 0.015 ± 0.007 MPa on bovine corneas tested in a lap joint configuration in vitro (20 min of curing). Variations of the test are reported in the associated patent [47]. Most of these specimens were kept moist during curing by overlaying them with a dialysis bag filled with water. Schnurrer and Lehr [53] tested the use of mussel adhesive proteins as mucoadhesives. When the adhesive was applied between two layers of intestinal mucosa tissue and pulled apart, it was found to be at least as strong as polycarbophil, which is the best available adhesive that adheres to mucus. The mean adhesive strength was ~ 0.00008 MPa (cure time = 1 min, physiological solution at 37°C). Chivers and Wolowacz [54] used mussel adhesive on porcine skin in an end-to-end specimen configuration. They cured the specimens in saline solution and reported that the mussel adhesive did not adhere well to the skin samples, even after curing for 22 h. The estimated strength was approximately 0.0003 MPa. These studies cannot be compared directly with each other because there were differences in curing conditions, cure times, types of joint and tissue substrates. However, they indicate that the bonds formed between these adhesive formulations and tissue are relatively weak.

The objective of our study was to evaluate the adhesive potential of mussel extracts on porcine skin under conditions that are conducive to "natural" (dehydration) curing. The approach used in the present investigation differs from approaches taken in earlier investigations in two ways—(1) the extract is not a pure precursor protein, but a pelletized form (described in the materials section), and (2) no external curing agents (cross linkers) were used. By eliminating the curing agent we were able to separate adhesive strength from finding a suitable curing agent. This was not done in previous studies. Simulating an incision repair, we joined the skin specimens in an end-to-end configuration using the adhesive extract, controlling for quantity of adhesive and using curing conditions that were similar to those found in two surgical applications: external incision and an internal incision with negligible fluid flow. These data were compared to the adhesive properties of the commercially available adhesive fibrin (Tisseel™). Cyanoacrylate (ethyl cyanoacrylate, Loctite−45404) was also tested to validate the procedures used. We show that mussel adhesive extract forms strong bonds between pieces of porcine skin under certain curing conditions.

2. Materials and methods

2.1. Skin sample preparation

Porcine hide was used in this study because it has physical properties similar to those of human tissue [55]. Hides of white pigs weighing approximately 100 kg were procured from a local slaughter house. Dorsal sections (approximately 30×30 cm) were cut from the hide and scrubbed thoroughly with warm water. A razor was used to remove hair. Prior to testing, the tissue was stored at 4°C in Krebs solution treated with gentamycin antibiotic (12.5 ml/l of Krebs solution). Unlike the conventional Krebs solution [56], this formulation was devoid of L-ascorbic acid (10 mM), as it is a known inhibitor of cross-linking of some polymers [57]. The maximum storage time for the tissue was 15 days.

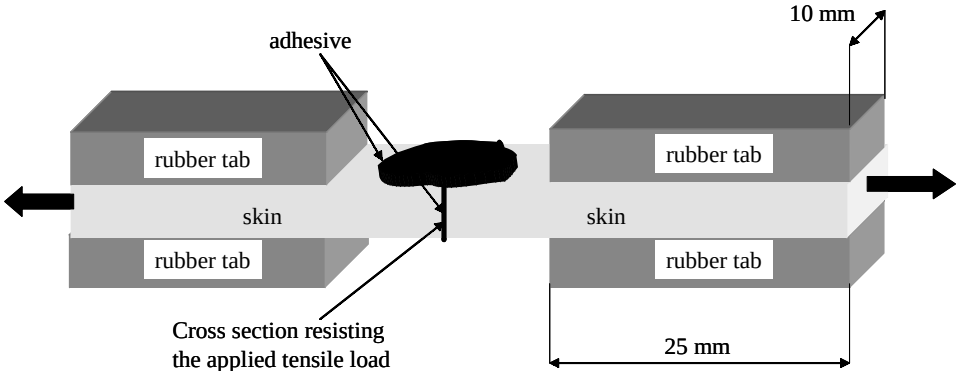

Fig. 1. Schematic of the end-to-end joint formed between samples of porcine skin. Positioning of the rubber tabs, used to facilitate gripping of the sample, is also shown.

Thin strips were cut from the stored tissue and the subcutaneous layers were removed using a surgical scalpel. These strips of skin were rectangular in shape with lengths of 35 mm and widths of 10 mm. The average thickness of the porcine skin samples was ~3.5 mm. The configuration used for these tests is shown in Fig. 1. Silicone rubber tabs 25 mm long and 10 mm wide were fastened to one end of each strip using commercially available Krazy Glue® (Elmer's Products Inc., Columbus, OH). This configuration left ~10 mm of each piece exposed to the ambient air. The length of exposed tissue was small in order to reduce dehydration during the curing process. The site of application was blotted with paper towels and adhesive was applied to the tissue (Fig. 1).

2.2. Preparation of the mussel extract

Excised *M. edulis* feet were obtained from Northeast Transport (Waldoboro, Maine) and stored at −80°C. The extraction of mussel adhesive protein ("mussel extract") from *M. edulis* was based on a literature procedure [44] with minor modifications. All manipulations were carried out at 4°C. Briefly, 30–60 g of mussel feet were blended in 0.6% (w/v) perchloric acid for 60 s using an Osterizer blender. The volume of perchloric acid was 10 times the mass of the mussel feet. After blending, the suspension was centrifuged (Beckman J2-21M/E centrifuge with fixed angle JA-20 rotor) at 31,000 *g* for 30 min. The supernatant (*S*1) was collected and acidified with concentrated sulfuric acid (volume = $S1 \times 0.0168$). While stirring, the protein was precipitated out of solution via the drop wise addition of acetone (volume = $S1 \times 2$). The protein precipitate was formed into a pellet via centrifugation (31,000 *g*, 30 min). After draining, these tan pellets had a thick, paste-like texture with a thin, outer "skin". The collected pellets were stored under 5% acetic acid at 4°C prior to use in adhesion tests.

2.3. Application of adhesives to tissue samples

2.3.1. Mussel adhesive extract

Prior to use, pellets were removed from the 5% acetic acid, blotted dry with paper towels and then rinsed in deionized water. The washed pellets were again blotted dry and weighed. Deionized water was added in a 2:1 pellet:water ratio by weight. The pellet and water were placed in a Duall glass tissue grinder (Size 21, Kontes Glass Co., Vineland, NJ) and homogenized into a paste. This paste, 300 mg total (200 mg pellet and 100 mg water), was then applied to the end-to-end skin joint using a spatula. The amount of adhesive used was determined by weighing the applicator before and after the adhesive was placed on the joint (Table 1). The adhesive does not cross link immediately and application to the joint is easy.

2.3.2. Fibrin adhesive

Tisseel®, a commercially available fibrin adhesive, was procured from Baxter Corporation in 1-ml containers. The adhesive is marketed as a two part system: one part fibrinogen and one part bovine thrombin. The adhesive was prepared according to instructions included with the kit. Fibrinogen was mixed with aprotonin solution. Thrombin was combined with a calcium choride solution. The two mixtures were warmed in a water bath to 37°C and loaded into a set of duplex syringes. As they were dispensed, the two components were mixed at the outlet of the syringe system where they cross-linked to form fibrin adhesive. The amount of fibrin used in each sample, ~200 mg, was determined from the weight of the syringe before and after application of the adhesive. Application of fibrin to the tissue joint was cumbersome because of its propensity to polymerize within the applicator needle. As the standard deviations in mass of applied adhesive imply (Table 2), it was difficult to dispense the same amount of adhesive consistently to each tissue joint.

Table 1
Mechanical properties of end-to-end joints formed using mussel extract to bond porcine skin and cured in "dry" and "humid" environments

Cure time (h)	Curing conditions	No. of samples	No. of samples tested to joint failure	Cross-sectional area (mm^2)	Quantity of adhesive (mg)	Maximum strength (MPa)	Average strength (MPa)
3	Dry	10	10	34.7 (2.5)	300 (10)	0.07	0.04 (0.02)
6	Dry	10	10	35.8 (1.8)	303 (12)	0.22	0.12 (0.06)
12	Dry	10	10	33.0 (1.7)	300 (11)	0.43	0.15 (0.14)
24	Dry	10	10	34.3 (2.9)	306 (14)	0.60	0.33 (0.17)
48	Dry	10	2	37.0 (1.8)	304 (13)	0.23	0.13 (0.13)
3	Humid	10	10	38.1 (4.7)	306 (8)	0.01	0.01 (0.00)
6	Humid	10	10	37.9 (4.0)	300 (7)	0.01	0.00 (0.00)
12	Humid	10	10	29.8 (2.4)	303 (4)	0.03	0.01 (0.01)
24	Humid	10	10	30.2 (3.5)	302 (6)	1.44	0.93 (0.32)
48	Humid	10	10	38.8 (2.3)	299 (6)	1.18	0.95 (0.19)

Standard deviations are shown in parentheses.

Table 2
Mechanical properties of end-to-end joints formed using fibrin to bond porcine skin and cured in "dry" and "humid" environments

Cure time (h)	Curing conditions	No. of samples	No. of samples tested to joint failure	Cross-sectional area (mm^2)	Quantity of adhesive (mg)	Maximum strength (MPa)	Average strength (MPa)
3	Dry	8	8	31.0 (4.2)	202 (41)	0.06	0.04 (0.02)
6	Dry	7	7	33.4 (3.4)	211 (6)	0.50	0.25 (0.14)
12	Dry	7	7	32.5 (1.6)	196 (18)	0.62	0.38 (0.16)
24	Dry	6	6	30.6 (2.5)	203 (20)	1.16	0.54 (0.39)
48	Dry	6	6	31.9 (3.3)	205 (14)	0.89	0.43 (0.28)
3	Humid	7	7	37.2 (2.8)	224 (29)	0.01	0.01 (0.00)
6	Humid	7	7	34.1 (2.1)	238 (59)	0.11	0.02 (0.04)
12	Humid	7	7	38.9 (4.2)	195 (33)	0.19	0.13 (0.05)
24	Humid	7	7	29.8 (3.0)	210 (30)	0.67	0.43 (0.19)
48	Humid	7	7	32.1 (2.1)	201 (14)	1.29	1.04 (0.23)

Standard deviations are shown in parentheses.

2.3.3. Cyanoacrylates

Cyanoacrylate adhesive from Loctite Corporation, Rocky Hill, CT (45404 ethyl cyanoacrylate) was used. This adhesive has a high viscosity which is desirable when the material is applied to the end-to-end joint. When a lower viscosity cyanoacrylate was used for preliminary tests, it flowed out of the joint. This flowing precluded control of the amount of adhesive added. The adhesive mass used was determined by weighing the dispensing tube before and after application to each joint. Approximately 200 mg of cyanoacrylate was applied to all samples (see Table 3). Uniform application of the adhesive to all parts of the tissue interface was difficult due to its rapid curing characteristics. The adhesive began curing immediately and it was difficult to manipulate, forming "strings" between the joint and applicator.

2.3.4. Control specimens

Control specimens without any adhesive were treated in the same manner as the adhesive samples. A syringe was used to apply 200 mg of Krebs solution, in place of adhesive, to each joint.

2.4. Testing conditions

Two curing environments were used. A "dry" curing condition was achieved by placing adhesive laden specimens in air adjusted to 25°C and 40% relative humidity. For the "humid" curing environment, the air was adjusted to 37°C and 80% relative humidity. The air conditions were achieved by placing the specimens in a humidity chamber (Percival Scientific, Perry, IA). Similar conditions were used in several previous studies on other adhesives [53,55,57–58].

Table 3
Mechanical properties of end-to-end joints formed using cyanoacrylate to bond porcine skin and cured in "dry" and "humid" environments

Cure time (h)	Curing conditions	No. of samples	No. of samples tested to joint failure	Cross-sectional area (mm^2)	Quantity of adhesive (mg)	Maximum strength (MPa)	Average strength (MPa)
3	Dry	10	10	30.6 (1.6)	200 (6)	1.53	0.99 (0.27)
6	Dry	10	9	28.5 (1.0)	199 (11)	NA[a]	NA[a]
12	Dry	10	10	31.0 (2.6)	202 (17)	2.13	1.39 (0.39)
24	Dry	10	10	29.2 (1.9)	209 (18)	2.97	2.01 (0.58)
48	Dry	10	3	37.0 (2.4)	207 (18)	NA[a]	NA[a]
3	Humid	10	2	34.6 (2.2)	210 (9)	NA[a]	NA[a]
6	Humid	10	6	37.3 (2.8)	201 (4)	NA[a]	NA[a]
12	Humid	10	10	30.6 (2.0)	206 (4)	1.56	1.07 (0.29)
24	Humid	10	10	36.6 (3.1)	220 (34)	1.35	0.96 (0.24)
48	Humid	10	10	32.4 (3.0)	205 (7)	1.75	1.27 (0.27)

Standard deviations are shown in parentheses.
[a] Not all specimens could be tested to failure because the sample slipped from the clamps at very high tensile loads. The values of average strength indicate the maximum average stress sustained by the joint and not the average failure stresses.

Fig. 2. Test fixture holding a cured skin specimen readied for testing to failure.

The specimens were cured for 3, 6, 12, 24, and 48 h. The tensile strengths of multiple specimens were averaged for each cure time ($n = 10$ for cyanoacrylate and mussel extract, and $n = 7$ for fibrin). The fixture used to load the cured specimens is shown in Fig. 2. It was mounted on a Sintech computerized materials testing machine (MTS Corporation, Eden Prairie, MN) and the force applied to the fixture was measured with a 45 N (maximum capacity) load cell. Specimens were clamped at the rubber tabs and stretched at a rate of 10 mm/min, similar to experiments reported in the literature [28,34]. The maximum load sustained by the joint is reported as the failure load of the specimen. Failure stresses, expressed in MPa, were calculated by dividing the failure load by the cross-sectional area of the skin specimens (typically 35 mm^2).

2.5. Statistical analysis

A two-sided, unpaired Student's *t*-test was used to compare the mean values of the failure loads at each time and curing condition. The significance level for rejecting the null hypothesis (no difference in the failure strength) was $P < 0.05$ for the *t*-tests. Averages are expressed as mean ± standard error. In addition, a multivariate analysis of variance (ANOVA) was performed over the entire time period. The strength of the bonded joints was considered as the dependent variable. Time of cure (3, 6, 12, 24, and 48 h), type of adhesive (fibrin and mussel extract) and conditions of cure ("dry" and "humid") were the independent variables. As with the *t*-tests, a significance level of $P < 0.05$ was used.

3. Results and discussion

Figs. 3 and 4 give a graphical comparison of the bond strengths for the tests on fibrin and mussel extract for conditioning with "dry" and "humid" air, respectively. Differences between the two adhesives for each condition and time of cure that were statistically significant at the 0.05 level are indicated with '*'. The following sections give detailed results for these tests along with tests conducted with cyanoacrylate. Cyanoacrylate was not included in the graphs because it is much stronger

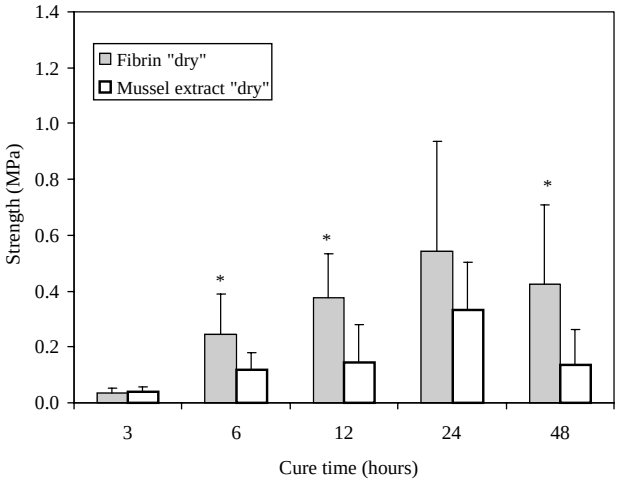

Fig. 3. Comparison of strengths of mussel extract and fibrin end-to-end bonds of porcine skin samples after curing under "dry" conditions. For those comparisons designated by '*', the difference was statistically significant ($P<0.05$).

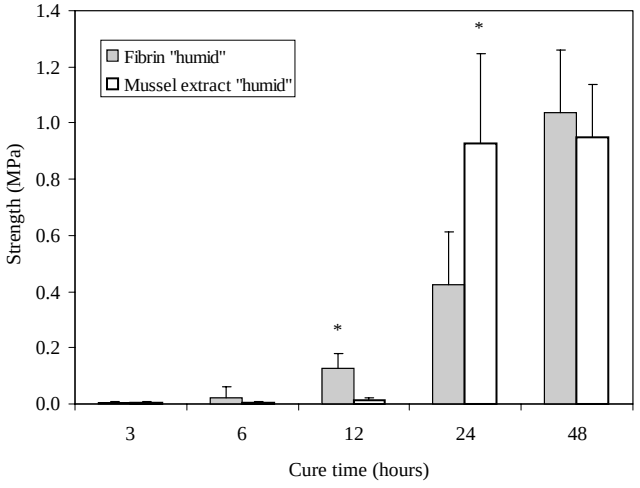

Fig. 4. Comparison of strengths of mussel extract and fibrin end-to-end bonds of porcine skin samples after curing under "humid" conditions. For those comparisons designated by '*', the difference was statistically significant ($P<0.05$).

and its average failure strength could not be determined for all cure times tested using the present experimental setup (Table 3).

3.1. Mussel extract pellets

The results for "dry" curing conditions of the skin samples bonded with mussel extract are shown in Table 1. The adhesive strength of the mussel extract joints increased to its maximum average value of 0.33 ± 0.17 MPa after 24 h of cure. However, the average cure strength decreased when specimens were cured for 48 h. Only two out of the 10 specimens that were cured for 48 h could be tested to failure. All other specimens failed at the joint during fixture alignment and clamping. The two specimens that were tested to failure sustained maximum stresses of 0.23 and 0.04 MPa.

Under "humid" curing conditions (Table 1) the joints were very weak for cure times less than 12 h. However, after 12 h there was a significant increase in adhesive strength. The average strengths for cure times of 24 and 48 h, 0.93 and 0.95 MPa, respectively, approach the strengths of cyanoacrylate adhesives cited in the literature [54]. There was considerably less shrinkage and distortion of these specimens compared to those cured under "dry" conditions. The specimens cured in "dry" conditions were stronger than their "humid" counterparts ($P<0.05$) for cure time $\leqslant 12$ h. The reverse was true for cure times greater than 12 h. All tissue joints using mussel extract adhesive appeared to fail by cohesive failure of the adhesive layer in the region of the joint (Fig. 1).

3.2. Fibrin (Tisseel)

Under "dry" curing conditions, the strength of the fibrin skin joints increased with cure time to a maximum of 0.54 ± 0.39 MPa after 24 h of curing (Table 2). After 48 h, strength decreased to 0.43 ± 0.28 MPa. Under "humid" curing conditions, the strength of fibrin joints increased steadily to a maximum of 1.04 ± 0.23 MPa (48 h). Beyond 12 h of curing, specimen distortion increased with curing time.

As was observed for mussel extracts, the samples cured in "dry" conditions were stronger than their "humid" counterparts ($P<0.05$) for curing times $\leqslant 12$ h. At 24 h, fibrin performed nearly the same under "dry" and "humid" conditions. The fibrin samples, like the mussel extract specimens, exhibited cohesive failure at the skin–skin junction for all curing conditions and times.

3.3. Cyanoacrylate

Cyanoacrylate joints behaved differently from fibrin and mussel extract joints. The curing environment also had a different effect. These joints cured rapidly and had significantly higher failure loads than their fibrin or mussel extract counterparts (Table 3) for both curing conditions. As shown in Table 3, the adhesive joints reached strengths ~ 1 MPa within 3 h for both curing conditions. After 3 h, the samples cured under "humid" conditions were stronger than those cured under "dry" conditions. For cure times of 6 h, the average strength was 1–2 MPa and the adhesive performed nearly the same for both cure conditions. The dry samples were stronger than their wet counterparts for cure times exceeding 6 h.

The textures of the cyanoacrylate joints were also very different and there was significant hardening of the

tissue-adhesive composite. The rapidly curing cyanoacrylate formed a stiff layer over the skin. For shorter cure times ($\leqslant 12$ h), the samples that failed did so at the interface between the cyanoacrylate layer and the substrate, indicating an adhesive failure. For longer cure times, when the modulus of the skin in contact with the cyanoacrylate had increased, the samples that failed exhibited a cohesive failure of the adhesive crust manifested by fracture at the skin–skin joint line. As explained in the footnote to Table 3, many specimens did not fail at the joint. Instead they slipped out of the rigid clamp. The cyanoacrylate strength values are reported only for specimens that failed at the joint.

3.4. Control specimens

Most of the control specimens (Krebs solution, no adhesive) subjected to "dry" curing conditions failed before a tensile test could be performed. Of the 10 specimens cured for 3 h, eight failed without any adhesion, while two withstood a stress averaging 0.0007 MPa. Among the 10 specimens cured for 6 h, nine failed without any adhesion, while one sustained a stress of 0.0012 MPa. For specimens cured for 12 h, seven failed without adhesion, while the average strength of the remaining three samples was 0.04 MPa and the maximum strength was 0.16 MPa. The only specimen that showed adhesion after curing for 24 h withstood a maximum stress of 0.38 MPa. All specimens cured for 48 h lost considerable amounts of moisture causing the skin to shrink and pull away from the joint. All control specimens cured under "humid" conditions failed prior to tensile testing. The specimens that were cured for shorter times failed due to lack of adhesion, while those subjected to 24 and 48 h of cure failed when the skin shrank and the joint separated.

3.5. Statistical analysis

The Student's *t*-test revealed significant differences in the performance of fibrin and mussel extract joints for each cure time and condition. For "dry" curing conditions, the fibrin joints were stronger than the mussel extract joints for 6, 12, and 48 h of cure and this difference was statistically significant. The differences were insignificant for cure times of 3 and 24 h. For "humid" curing conditions, the two types of joints showed no significant difference in bond strength for cure times of 3, 6 and 48 h. After 12 and 24 h of curing, the differences were significant, with fibrin being stronger at 12 h and mussel extract being stronger at 24 h.

Multifactor ANOVA indicated that the type of adhesive (fibrin or mussel extract) did not affect the bond strength of the tissue joint ($P = 0.97$). However, curing conditions ($P = 0.02$) and time of cure were significant ($P < 0.0001$).

3.6. Discussion

Mussel extract joints exhibited lower average strengths than fibrin joints for all durations of "dry" curing (Tables 1 and 2, Fig. 3). However, the differences at 3 and 24 h of cure were not statistically significant ($P = 0.72$ and 0.17 respectively). For curing times of 12 h or less under "humid" conditions, the fibrin was again stronger, although the difference was only statistically significant after 12 h of cure (Tables 1 and 2, Fig. 4). For more than 12 h of "humid" curing, a rapid increase in strength was observed in mussel extract joints. At 24 h, the average joint strength was 0.93 MPa compared to fibrin's 0.43 MPa and the difference was statistically significant ($P = 0.002$). At 48 h, both fibrin and mussel extract had similar strengths (1.04 vs. 0.95 MPa, $P = 0.4$).

These results point to three important conclusions regarding the adhesive properties of mussel extract: (1) mussel extract is capable of forming strong tissue joints (~ 1 MPa) in the end-to-end configuration, given adequate curing time; (2) the time required for mussel extract to reach the maximum adhesive strength on porcine connective tissue substrate is between 12 and 24 h; and (3) mussel extract joints are similar in strength to fibrin joints ($P = 0.97$ for the multifactor ANOVA), although they cure more slowly than fibrin.

Although ANOVA indicated that the type of adhesive had no significant effect on joint strength, it revealed that curing conditions ("dry" and "humid") and the curing time (3–48 h) were significant. Fibrin and mussel adhesive were influenced similarly by the curing conditions. The effect of moisture is noteworthy. Both mussel extract and fibrin joints were stronger for "dry" curing conditions than for corresponding "humid" curing conditions at cure times up to 12 h ($P < 0.01$ for 3, 6 and 12 h). Moisture effects may have been responsible for the very low bond strength reported by Wolowacz and Chivers [54] who cured the mussel extract in very moist conditions. A second ANOVA analysis was performed using time and curing conditions as independent variables, and either fibrin or mussel extract bond strength as the dependent variable. This analysis indicated that moisture had more influence on the strength of the mussel extract joint ($P < 0.01$) than on the fibrin joint ($P = 0.70$). The effect of curing time was similar for both types of joints ($P < 0.0001$). These results indicate that mussel extract curing kinetics are very sensitive to moisture.

In our opinion, dehydration of the skin tissue prevented us from getting reliable results at long durations of cure (> 24 h). As mentioned earlier, increased distortion of the skin samples was observed

at longer cure times, especially under "dry" curing conditions. The stresses induced by distortion weakened the joint and introduced unknown errors in the measured values of joint strength, especially after 48 h of curing. This observation could explain the reduction in average strengths of not only mussel extract but also fibrin joints after 48 h of "dry" curing.

We mentioned previously lap shear tests in which bond strength was very weak. Benedict and Picciano [48] found much greater strength after only 20 min of curing in the presence of various cross linkers. We believe that difficulty in removing moisture from the mussel extract when the interface was not exposed to air, a characteristic of the lap joint configuration, delayed the cure of the adhesive. The same factor may have been responsible for the relatively low strengths reported by Wolowacz and Chivers [54]. The addition of special chemical cross linkers could enable mussel extract protein (or its derivative formulations) to be used as a surgical adhesive.

4. Conclusions

This present study was performed to determine the maximum possible adhesive strength of a soft connective tissue joint using mussel adhesive extract. To the best of our knowledge, this report is the first to characterize the adhesive potential of the mussel extract without the use of a curing agent. The adhesive performance was compared to that of commercially available fibrin adhesive. The mussel adhesive extract joint reached its maximum strength between 12 and 24 h of curing in an environment of 80% relative humidity and physiological temperature. Moisture appears to have a significant negative impact on the curing characteristics of mussel extract. The extract may have significant surgical applications if the rate of cure can be accelerated and if it is shown to be biocompatible. The effect of various curing agents on the present formulation of mussel extract and tests for their biocompatibility are currently under investigation.

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References

[1] Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Djordjevic M, Kenney EB. The use of bovine porous bone mineral in combination with enamel matrix proteins or with an autologous fibrinogen/fibronectin system in the treatment of intrabony periodontal defects in humans. J Periodontol 2001;72(9):1157–63.

[2] Bernard L, Doyle J, Friedlander SF, Eichenfield LF, Gibbs NF, Cunningham BB. A prospective comparison of octyl cyanoacrylate tissue adhesive (dermabond) and suture for the closure of excisional wounds in children and adolescents. Arch Dermatol 2001;137(9):1177–80.

[3] Shelper TR, Seiff SR. Use of isobutyl cyanoacrylate tissue adhesive to stabilize external eyelid weights in temporary treatment of facial palsies. Ophthalmic Plast Reconstr Surg 2001; 17(3):169–73.

[4] Cintron JR, Park JJ, Orsay CP, Pearl RK, Nelson RL, Sone JH, Song R, Abcarian H. Repair of fistulas-in-ano using fibrin adhesive: long-term follow-up. Dis Colon Rectum 2000;43(7): 944–9.

[5] Perez M, Fernandez I, Marquez D, Bretana RM. Use of *N*-butyl-2-cyanoacrylate in oral surgery: biological and clinical evaluation. Artif Organs 2000;24(3):241–3.

[6] Gallenmore RP, Green J, Shorr N, Goldberg RA. Use of isobutyl cyanoacrylate tissue adhesive to stabilize mucous membrane grafts in total socket reconstruction. Ophthalmic Plast Reconstr Surg 1999;15(3):210–2.

[7] Greenhalgh DG, Gamelli RL, Lee M, Delavari M, Lynch JB, Hansbrough JF, Achauer BM, Miller SF, MacPhee M, Bray GL. Multicenter trial to evaluate the safety and potential efficacy of pooled human fibrin sealant for the treatment of burn wounds. J Trauma—Injury Infect Crit Care 1999;46(3):433–40.

[8] Ota K, Shirai Z, Masuzaki T, Tanaka K, Higashihara H, Okazaki M, Arakawa M. Endoscopic injection sclerotherapy with n-butyl-2-cyanoacrylate for rupture duodenal varices. Journal Gastroenterol 1998;33(4):550–5.

[9] Hartnett ME, Hirose T. Cyanoacrylate glue in the repair of retinal detachment associated with posterior retinal breaks in infants and children. Retina 1998;18(2):125–9.

[10] Mommaerts MY, Beirne JC, Jacobs WI, Abeloos JS, De Clercq CA, Neyt LF. Use of fibrin glue in lower blepharoplasties. J Cranio-Maxillofac Surg 1996;24(2):78–82.

[11] Robicsek F, Rielly JP, Marroum MC. The use of cyanoacrylate adhesive (Krazy glue) in cardiac surgery. J Cardiac Surg 1994; 9(3):353–6.

[12] Kacker A, Huo J. Reinforcement of and end-to-end tracheal resection anastomosis with fibrin glue: a case report. Ear Nose Throat J 2001;80(4):234–6.

[13] Sasajima T, Yamazaki K, Sugimoto H, Hirata S, Yatsuyanagi E. Successful repair of tracheal defect using gelation–resorcin–formaldehyde–glue-reinforced fascia patch. Thorac Cardiovasc Surgeon 2000;48(3):159–61.

[14] Pescatore P, Verbeke C, Harle M, Manegold BC. Fibrin sealing in peptic ulcer bleeding: the fate of the clot. Endoscopy 1998;30(6): 519–23.

[15] Kjaergard HK, Fairbrother JE. Controlled clinical studies of fibrin sealants in cardiothoracic surgery—a review. Eur J Cardio-Thorac Surg 1996;10(9):727–33.

[16] Harmanli OH, Wapner RJ, Lontz JF. Efficacy of fibrin glue for in vitro sealing of human chorioamniotic membranes. J Reprod Med 1998;43(11):986–90.

[17] Larrazabal R, Pelz D, Findlay JM. Endovascular treatment of lenticulostriate artery aneurysm with *N*–butyl cyanoacrylate. Can J Neurol Sci 2001;28(3):256–9.

[18] Felipetto R, Vigano L, Cecchi M, Florentini L, Minerini R. Use of fibrin sealant in the treatment of prostatic cutaneous fistula in a

case of *Pseudomonas porstatitis*. Int Urol Nephrol 1995;27(5): 563–5.
[19] Kitajiri S, Tabuchi K, Hiraumi H, Kaetsu H. Relief of post-tonsillectomy pain by release of lidocaine from fibrin glue. Laryngoscope 2001;111(4, Part 1):642–4.
[20] Spotnitz WD. History of tissue adhesives. In: Sierra DH, Saltz R, editors. Surgical adhesive and sealants—current technology and applications. Lancaster, PA: Technomic Publishing; 1996. p. 3–12.
[21] Papatheofanis FJ. Cytotoxicity of alkyl-2-cyanoacrylate adhesives. J Biomed Mater Res 1989;23:661–8.
[22] Samson D, Marshall D. Carcinogenic potential of Isobutyl-2-cyanoacrylate. Neurosurgery 1986;65:571–2.
[23] Strausberg RL, Link RP. Protein-based medical adhesives. Trends Biotechnol 1990;8:53–7.
[24] Sekine T, Nakamura T, Shimizu Y, Ueda H, Matsumoto K, Takimoto Y, Kiyotani T. A new type of surgical adhesive made from porcine collagen and polyglutamic acid. J Biomed Mater Res 2001;54:305–10.
[25] Waite JH. Polyphenolic substance of *Mytilus edulis*: novel adhesive containing L-Dopa and hydroxyproline. Science 1981; 212:1038–40.
[26] Waite JH. Nature's underwater adhesive specialist. Int J Adhesion Adhesives 1987;7(1):9–14.
[27] Tatehata H, Mochizuki A, Kawashima T, Yamashita S, Yamamoto H. Model polypeptide of mussel adhesive protein. I. Synthesis and adhesive studies of sequential polypeptides (X-Tyr-Lys)n and (Y-Lys)n. J Appl Polym Sci 2000;76:929–37.
[28] Iwata H, Matsuda S, Mitsuhashi K, Itoh E, Ikada Y. A novel surgical glue composed of gelation and *N*-hydroxysuccinimide activated poly(L-glutamic acid): Part 1. Synthesis of activated poly(L-glutamic acid) and its gelation with gelatin. Biomaterials 1998;19:1869–76.
[29] Matsuda T, Itoh T, Yamaguchi T, Iwata H, Hayashi K, Uemura S, Ando T, Adachi S, Nakajima N. A novel elastomeric surgical adhesive. Trans Am Soc Artif Intern Organs 1986;32:151–8.
[30] Hwang JJ, Stupp SI. Poly(amino acid) bioadhesives for tissue repair. J Biomater Sci Polym Edn 2000;11(10):1023–38.
[31] Basu S, Marini CP, Bauman FG, Shirazian D, Damiani P, Robertazzi R, Jacobowitz IJ, Acinapura A, Cunningham Jr JN. Comparative study of biological glues: cryoprecipitate glue, two-component fibrin sealant, and French glue. Ann Thorac Surg 1995;60:1255–62.
[32] Hall WW, Wrye SW, Banducci DR, Ehrlich P. Microvascular anastomosis using 2-octyl cyanoacrylate in the rat femoral artery. Ann Plast Surg 2000;44:508–11.
[33] Szomor ZL, Appleyard R, Tyler MJ, Murrell GAC. Meniscal Repair with a new biologic glue. 47th Annual Meeting, Orthopaedic Research Society, 2001, p. 47.
[34] Otani Y, Tabata Y, Ikada Y. Effect of additives on gelation and tissue adhesion of gelatin and poly (L-glutamic acid) mixture. Biomaterials 1998;19:2167–73.
[35] Linden Jr CL, Shalaby SW. Performance of modified cyanoacrylate composition as tissue adhesives for soft and hard tissues. J Biomed Mater Res (Appl Biomater) 1997;38:348–55.
[36] Sung HW, Huang DM, Chang WH, Huang RN, Hsu JC. Evaluation of gelatin hydrogel crosslinked with various cross-linking agents as bioadhesives: in vitro study. J Biomed Mater Res 1999;46:520–30.
[37] Nakayama Y, Matsuda T. Photocurable surgical tissue adhesive glues composed of photoreactive gelatin and poly(ethylene glycol) diacrylate. J Biomed Mater Res (Appl Biomater) 1999;48:511–21.
[38] Rzepecki LM, Chin S-S, Waite JH, Lavin MF. Molecular diversity of marine glues: polyphenolic proteins from five mussel species. Mol Marine Biol Biotech 1991;1:78–88.
[39] Rzepecki LM, Waite JH. DOPA proteins: versatile varnishes and adhesives from marine fauna. In: Scheuer PJ, editor. Bioorganic marine chemistry, vol. 4. New York: Springer; 1991. p. 120–48.
[40] Rzepecki LM, Waite JH. Wresting the muscle from mussel beards: research and applications. Mol Mar Biol Biotech 1995;4:313–22.
[41] Waite JH. The phylogeny and chemical diversity of quinone-tanned glues and varnishes. Comp Biochem Physiol 1990;97B: 19–29.
[42] Deming TJ. Mussel byssus and biomolecular materials. Curr Opin Chem Biol 1999;3:100–5.
[43] Vreeland V, Waite JH, Epstein L. Polyphenols and oxidases in substratum adhesion by marine algae and mussels. J Phycol 1998; 34:1–8.
[44] Waite JH. Precursors of quinone tanning: DOPA-containing proteins. Methods Enzymol 1995;258:1–19.
[45] Waite JH. Process for purifying, stabilizing catechol-containing proteins and materials obtained thereby. US Patent No. 4,496,397, 1985.
[46] Waite JH. Decapeptides produced from bioadhesive polyphenolic proteins. US Patent No. 4,585,585, 1986.
[47] Benedict CV, Picciano PT. Adhesives derived from bioadhesive polyphenolic proteins. US Patent No. 5,015,677, 1988.
[48] Benedict CV, Picciano PT. Adhesives from marine mussels. In: Hemingway RW, Conner AH, Branham SJ. editors. Adhesives from renewable resources. No. 385. Washington, DC: American Chemical Society; 1989. p. 465–83.
[49] Grande DA, Pitman MI. The use of adhesives in chondrocyte transplantation surgery: preliminary studies. Bull Hospital Jt Dis Orthop Inst 1988;48(2):140–8.
[50] Fulkerson JP, Norton LA, Gronowicz G, Picciano P, Massicotte JM, Nissen CW. Attachment of epiphyseal cartilage cells and 17/28 rat osteosarcoma osteoblasts using mussel adhesive protein. J Orthop Res 1990;8(6):793–8.
[51] Kossovsky N, Gelman A, Hnatyszyn HJ, Rajguru S, Garrell RL, Torbati S, Freitas SSF, Chow G. Surface-modified diamond nanoparticles as antigen delivery vehicles. Bioconjugate Chem 1995;6(5):507–11.
[52] Pitman MI, Menche D, Song E, Ben-Yishay A, Gilbert D, Grande D. The use of adhesives in chondrocyte transplantation surgery: in-vivo studies. Bull Hospital Jt Dis Orthop Inst 1989;49(2):213–20.
[53] Schnurrer J, Lehr C-M. Mucoadhesive properties of the mussel adhesive protein. Int J Pharma 1996;141:251–6.
[54] Chivers RA, Wolowacz RG. The strength of adhesive bonded tissue joints. Int J Adhesion Adhesives 1997;17:127–32.
[55] Sierra DH, Feldman DS. A method to determine shear adhesive strength of fibrin sealants. J Appl Biomater 1992;3:147–51.
[56] Shi R, Pryor JD. Temperature dependence of membrane sealing following transaction in mammalian spinal cord axons. Neuroscience 2000;98(1):157–66.
[57] Albes JM, Krettek C, Hausen B, Rohde R, Haverich A, Borst H-G. Biophysical properties of the gelatin–resorcin–formaledehyde/glutaraldehyde adhesive. Ann Thorac Surg 1993;56:910–5.
[58] Sierra DH, O'Grady K, Toriumi DM, Foresman PA, Rodeheaver GT, Eberhardt A, Feldman DS, Lemons JE. Modulation of mechanical properties in multiple-component tissue adhesives. J Biomed Mater Res 2000;52:534–42.