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Adhesive strength and curing rate of marine mussel protein extracts on porcine small intestinal submucosa*

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Abstract

An adhesive protein extracted from marine mussel (*Mytilus edulis*) was used to bond strips of connective tissue for the purpose of evaluating the use of curing agents to improve adhesive curing. Specifically, mussel adhesive protein solution (MAPS, 0.5 mM dihydroxyphenylalanine) was applied, with or without the curing agents, to the ends of two overlapping strips of porcine small intestinal submucosa. The bond strength of this lap joint was determined after curing for 1 h at room temperature (25°C). The strength of joints formed using only MAPS or with only the ethyl, butyl or octyl cyanoacrylate adhesives were determined. Although joints bonded using ethyl cyanoacrylate were strongest, those using MAPS were stronger than those using butyl and octyl cyanoacrylates. The addition of 25 mM solutions of the transition metal ions V⁵⁺, Fe³⁺ and Cr⁶⁺, which are all oxidants, increased the bond strength of the MAPS joints. The V⁵⁺ gave the strongest bonds and the Fe³⁺ the second strongest. In subsequent tests with V⁵⁺ and Fe³⁺ solutions, the bond strength increased with V⁵⁺ concentration, but it did not increase with Fe³⁺ concentration. Addition of 250 mM V⁵⁺ gave a very strong bond.

Keywords

Tissue adhesive; Porcine tissue; Small intestinal submucosa; mussel adhesive protein; Metal ion; cross-linking

1. Introduction

Surgical adhesives are increasingly being used in soft tissue repair as fasteners and sealants because they can be applied easily without physically injuring the tissue [1-20]. An ideal adhesive would adhere rapidly to the tissue substrate and provide adequate strength to the joint under physiological conditions while enabling the wound to heal. In addition, it would be biocompatible, affordable, elicit minimal immune response and be easy to handle [21]. At the

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present time there is no surgical adhesive that fulfills all these characteristics. The need has stimulated research on the use of biomaterials with adhesive properties that could be adapted for use as a surgical adhesive.

One of the experimental adhesives that has received considerable attention is a family of proteins produced by the blue mussel (*Mytilus edulis*), which has the ability to cure underwater. The potential of these proteins was first investigated by Waite et al. [22,23]. Research during the past three decades has increased the scientific community's understanding of the adhesive's molecular composition and structure, and inspired many simpler synthetic analogs [24-37]. However, only a few investigators have studied the use of this exotic adhesive for surgical applications. Benedict and Picciano [38], Schnurrer and Lehr [39], and Chivers and Wolowacz [40] reported that the mussel adhesive formed weak bonds. These investigators used distinctly different curing conditions and joint configurations, which makes direct comparison cumbersome. In a previous paper Ninan et al. [41] demonstrated that extracts of mussel adhesive protein formed strong bonds with porcine skin. However, the time required to achieve a strong bond was between 12 and 16 h when the adhesive was incubated in a humid environment at 37°C. Obviously, this curing technique would not be suitable for use in surgery. This paper describes efforts to increase the curing rate of mussel protein extracts.

Although several recent studies have increased the scientific community's understanding of how blue mussels cure their adhesive precursors [26-30,42-51], the mechanism is not yet completely understood. It appears that the precursor proteins are cross-linked by a complex interaction of dihydroxyphenylalanine (DOPA) motifs in the protein, facilitated by enzymes and oxidative reagents such as metal ions. Our previous studies using spectroscopic techniques [50] and quantitative material tests [51,52] indicated that curing agents that are simultaneously transition metal ions and oxidants induce curing of protein extracts. The present work examines the use of these reagents with mussel extracts in experiments with a clinically relevant substrate. The reagents selected for use were those that performed best in previous investigations [51, 52], and the adhesive test substrate used was small intestinal submucosa (SIS), a biomaterial that is increasingly being used in a variety of soft tissue reconstruction applications [53-55].

The objectives of this study were to test the strength of the adhesive bonds formed when known amounts of mussel adhesive protein solution (MAPS) were applied to two strips of SIS that formed a lap shear joint and to evaluate the effect of various curing agents on the MAPS bond strength. In addition, the strength of the MAPS bond was compared to the strengths of bonds formed using three cyanoacrylates. The lap shear joint was used because the SIS is relatively thin and flexible and well suited for testing in this configuration. In addition, this configuration is relevant to clinical applications where the biomaterial is used as a bridging scaffold between healthy tissue, such as vascular grafts, reconnection of severed nerves or body wall repair. The tensile strength of the bonded joints was determined after the bond was cured for 1 h at ambient laboratory conditions. The strips of SIS were cut from a four-layer lyophilized laminate, which is one of the clinically approved forms of the biomaterial.

2. Methods and Materials

2.1. Preparation of lap shear joints

Strips of four-layer lyophilized SIS (henceforth referred to as SIS-4) were cut from sheets obtained from Cook Biotech Inc., West Lafayette, IN. Figure 1 shows the manner in which the porcine intestine sheet (the SIS-4) was cut to form the SIS-4 strips (70 mm long in the intestine's circumferential direction by 5 mm wide in the intestine's longitudinal direction). Each strip was divided into two halves which were 35 mm long and would overlap by 10 mm when the bond was formed. The 5 mm × 10 mm areas of overlap were marked off lightly on the dry SIS-4 using a pencil.

2.2. Preparation of the MAPS

Excised *M. edulis* feet were obtained from Northeast Transport (Waldoboro, ME) and stored at -80 °C. The extraction of mussel adhesive protein ("mussel extract") from *M. edulis* was based on a literature procedure [30] with minor modifications, and is consistent with extraction procedures followed by other researchers working with mussel adhesives. DOPA to protein ratios of the extract vary during the calendar year, with higher ratios occurring during the winter. Therefore, all the proteins used in this study were extracted from mussel feet collected over a 1-3 day period in the winter, ensuring consistency of the ratio.

Extraction procedures 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 mass of perchloric acid used was approximately ten 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 $31,000 \times g$ for 30 min. The supernatant (S1) was collected and acidified with concentrated sulfuric acid (volume = $S1 \times 0.0168$). While stirring, the protein was precipitated out of solution via the dropwise addition of acetone (volume = $S1 \times 2$). The protein precipitate was formed into a pellet via centrifugation ($31,000 \times g$, 30 min). After draining, these tan-colored pellets had a thick, paste-like texture. The pellets were collected and stored in high-purity water at 4 °C until they were needed for preparation of the MAP solution.

Prior to the tests, stored pellets were placed in a tissue grinder (one pellet at a time) and approximately 1 ml of water was added to each pellet. The pellet and water were ground gently for no more than five 'grinds' or 'pushes'. The supernatant of the resulting mixture was pipetted into a 1.5 ml plastic centrifuge tube and centrifuged at $15,000 g$ for 5 min, and the MAPS decanted. The following paragraph describes the method used to determine the total protein content and concentration of active DOPA in a typical MAPS sample.

Total protein content was determined by absorption at 280 nm. From the reported procedure, extractions for the DOPA-containing proteins can be estimated to be ~80% Mefp-1 protein and ~20% Mefp-2 [30]. The extinction coefficient was calculated from the sequences of Mefp-1 [56] and Mefp-2 [57] using standard methods [58,59]. Estimates of the coefficients were $223,500 \text{ M}^{-1} \text{ cm}^{-1}$ for Mefp-1 and $19,140 \text{ M}^{-1} \text{ cm}^{-1}$ for Mefp-2, and these were combined to reflect an 80/20 mixture giving a composite value of $182,600 \text{ M}^{-1} \text{ cm}^{-1}$. For a typical extraction, protein absorption, $\text{Abs}_{280 \text{ nm}} = 2.93$, indicated that the total protein concentration was $16 \mu\text{M}$. The active DOPA content of this protein solution was determined using a colorimetric assay (Arnow's method) [60] and found to be $350 \mu\text{M}$. Thus each protein contained approximately 22 active DOPA residues.

2.3. Shear strength of various adhesives

The performance of the following adhesives was determined by forming a lap joint from two strips of SIS-4, as shown in Fig. 2: ethyl cyanoacrylate (EAN), butyl cyanoacrylate (Vetbond®, BAN), octyl cyanoacrylate (Nexaderm®, OAN) and MAPS, with a DOPA concentration of 0.5 mM. Lap shear joints were also formed using a control solution of purified water (resistance 18 MΩ). All cyanoacrylate adhesives were purchased from World Precision Instruments (WPI, Sarasota, FL). EAN was chosen for comparison due to its high bond strength and rapid curing rate. It forms a rigid bond and contains potentially harmful chemicals and therefore cannot be used in surgery. BAN and OAN were chosen because they are currently being used to a limited extent in veterinary and human tissue repair, respectively.

The presence of some moisture is essential for optimal performance of the cyanoacrylate adhesives [61]. Therefore, when these adhesives were tested, a thin film of high-purity water was applied to the bonding surfaces of the strips. The presence or absence of moisture did not

play a significant role in the bond strength of joints formed with ethyl cyanoacrylate, most of which failed at the tabs rather than at the lap joint. However, joints formed using BAN and OAN without the application of a thin film of moisture lost considerable strength and were even weaker than control joints formed using only high-purity water to bond the SIS-4. The majority of these non-moistened BAN and OAN joints debonded by the time 1 h of ambient curing had been completed (unpublished observations).

After the two pieces of moistened SIS-4 were weighed, 25 μ l of each adhesive solution was applied to one of the two strips being joined, and the bonding region of the second half was gently laid on top of the bonding region of the first half, which held the solution. The joint was rolled gently with a Teflon® cylinder (diameter ~50 mm). This compressed the sample, squeezing out the excess solution, and ensured that the joint bonding area was consistently 50 mm². Following this, the bonded strips were carefully weighed. The difference in weight of the bonded strips and the dry SIS-4 strips from which the bond was formed was recorded, and was used as an estimate of the weight of solution added to each joint. The joints were kept in the laboratory at ambient conditions and allowed to cure. After 1 h, silicone rubber tabs were attached to the specimens (Fig. 2) using Krazy Glue® (Elmer's Products Inc., Columbus, OH). The specimens were then tested in an MTS/Sintech computerized testing machine (MTS Corporation, Eden Prairie, MN) using a crosshead speed of 10 mm min⁻¹. The maximum load sustained by the joint was divided by the joint bonding area to give the shear stress at which the joint failed.

2.4. Effect of curing agents on adhesive strength of MAPS

A second set of experiments was conducted using lap shear joints formed with an adhesive mixture of 25 μ l of MAPS and 25 μ l of curing agent solution. The curing agents used were aqueous solutions of KMnO₄ (Mn⁷⁺), Mn[CH₃COO]₃ (Mn³⁺), K₂Cr₂O₇ (Cr⁶⁺), NaVO₃ (V⁵⁺) or Fe(NO₃)₃ (Fe³⁺). Each had a metal ion concentration of 50 mM. These reagents were chosen on the basis of results from our previous work [51,52]. All specimens were weighed before the addition of the adhesive solutions. MAPS and the metal ion solutions were added sequentially to the 10 mm overlap region on one of the two SIS-4 specimens to be joined. The two solutions were mixed using a pipette tip. The corresponding bonding area of the second half was gently laid on top of the first half's bonding area, which held the adhesive and curing agent. Excess adhesive solution was removed by rolling the Teflon® cylinder across the joint, as described in Section 2.3. Control specimens used an adhesive mixture composed of 25 μ l of MAPS and 25 μ l of high-purity water (18 M Ω). These specimens were prepared in the same manner as the specimens that used MAPS and the curing agents. The final MAPS concentration in all the adhesive formulations was 0.25 mM DOPA and that of the metal ions (where applicable) was 25 mM. After the excess solution was squeezed from the joint using the cylinder, all specimens were weighed again, and the difference in weights was assumed to be the weight of adhesive mixture retained by the SIS-4 joint. The specimens were cured for 1 h at room temperature and then the silicone rubber tabs were attached using Krazy Glue®. Following this, they were tested to failure using the same procedure described in Section 2.3, and the maximum shear strength of each joint was calculated from the maximum loads sustained by the joint.

2.5. Effect of concentration of curing agent on adhesive strength of MAPS

In the third set of tests, the effect of the concentration of the curing agent on the adhesive properties of MAPS joints was determined. The tests were similar to those used in the previous comparisons, except that the concentrations of NaVO₃ (V⁵⁺) and Fe(NO₃)₃ (Fe³⁺), the two curing agents that were determined to be most effective in the second set of experiments, were varied. The concentrations used were 5, 50 and 500 mM. Twenty-five microliters of each solution was used with 25 μ l of MAPS having a DOPA concentration of 0.5 mM. As in the

previous sets of experiments, the final DOPA concentration in all MAPS formulations was 0.25 mM and the concentrations of the metal ions were 2.5, 25 and 250 mM. After 1 h of curing at room temperature, the rubber tabs were attached to the specimens, the specimens were tested to failure and the maximum shear strengths were determined as explained previously.

2.6. Statistical Analysis

Standard deviations were calculated for the average failure loads (mean $\pm$ one standard deviation). A two-sided, unpaired Student's *t*-test was used for one-to-one comparisons of mean bond strengths of SIS joints formed using various adhesive treatments. The underlying assumption was that for each set of samples the data (failure loads) being compared were distributed normally about their mean values with equal variances. The parameter *t'* was calculated as follows:

$$t' = \frac{\bar{x} - \bar{y} - \Delta_0}{\sqrt{\left(\frac{s^2}{m}\right) + \left(\frac{s^2}{n}\right)}}$$

where $\bar{x}$ and $\bar{y}$ are the sample means and Δ_0 is the assumed difference in means, which in this case is 0. The variable *s* denotes the sample variance, and *m* and *n* are the number of data points in the two groups being compared. Microsoft Excel® software was used for statistical comparison of means. The significance level for rejecting the null hypothesis (no difference in the failure strength) was $P < 0.05$. ANOVA was used to evaluate the statistical significance of bond strength data in studies investigating the effect of the concentration of the curing agent on the bond strength of SIS-4 and MAPS joints. The level of significance for rejecting the null hypothesis was also $P < 0.05$.

3. Results

3.1 Comparison of various surgical adhesives

The performance of SIS-4 lap shear joints formed using different surgical adhesives is summarized in Table 1. The EAN joints were the strongest. For these specimens, the SIS-4 failed near the grips and not at the joint. Therefore, their actual average joint strength was greater than 379 kPa. Joints formed using BAN and OAN adhesives were, in general, very weak. Three out of the eight BAN joints debonded by the end of 1 h of curing and before the shear test was initiated, and there was partial debonding in three of the remaining samples. Only two specimens appeared to bond properly. Seven out of the eight OAN joints failed during the shear test and the remaining specimen had debonded at the end of 1 h of curing. Visual inspection revealed that there were obvious flaws in the bonds of two out of the remaining seven joints, such as a partial peeling away of one strip from the other or a slight curvature indicative of residual stresses. All specimens that debonded prior to testing were assigned joint strengths of 0 kPa before averages were computed. The average strengths of the joints formed using adhesives other than EAN ranged from 57 ± 70 kPa for the BAN to 233 ± 58 kPa for the MAPS. All of the MAPS joints failed due to shear at the joint. Only weak bonds were formed by the deionized (DI) water control. The average strength of these bonds was only 43 ± 41 kPa and all the specimens failed during the shear test.

3.2 Effect of curing agents on adhesive strength of MAPS

Table 2 summarizes the results of tests performed on SIS-4 samples bonded together using a mixture of MAPS and one of the metal ion curing agents. All joints except those formed using Fe^{3+} were fabricated using nearly the same amount of adhesive solution, and the differences in the mean weights of these solutions with respect to the control solution (MAPS + DI water)

were not statistically significant. Joints bonded using a mixture of MAPS and Fe^{3+} contained a lesser amount of solution, and the difference was statistically significant compared to the control specimens. A careful review of procedures and observations revealed nothing that could explain this variation.

In general, the bonds formed using MAPS solutions and the metal ions were stronger than the control joints formed using high-purity deionized water (DI, 18 M Ω). The greatest average joint strength (284 ± 47 kPa) was achieved when V^{5+} was added, and bond strengths for the other curing agents were, in order of decreasing strength, Fe^{3+} , Cr^{6+} , Mn^{3+} and Mn^{7+} . The average strength of the control specimens, bonded using a mixture of MAPS and DI water, was 184 ± 35 kPa. This is noticeably lower than the 233 ± 58 kPa reported for tests in Section 2.3, where only 25 μl of MAPS was used. It is possible that the additional 25 μl of DI water weakened the bond.

3.3 Effect of concentration of curing agent on adhesive strength of MAPS

Table 3 summarizes the results of lap shear tests performed on SIS-4 joints bonded using MAPS along with solutions having varying concentrations of Fe^{3+} and V^{5+} . In the case of joints bonded using MAPS and Fe^{3+} solutions, the strongest bonds were formed using 25 mM Fe^{3+} . An increase in bond strength with increasing Fe^{3+} concentration was not observed. However, the strength of joints bonded using MAPS and V^{5+} increased with increasing concentration of V^{5+} . For a given concentration of curing agent, bonds formed using V^{5+} were stronger than those formed using Fe^{3+} . These results are analyzed in the following section.

4. Discussion

The goals of the present study were to compare the strength of bonds formed from two strips of tissue substrate joined using mussel extract (MAPS) as the adhesive with those formed using three cyanoacrylates, and to investigate the effects of curing agents on the MAPS bond strength. There were significant differences in the strengths of the bonds formed by the three cyanoacrylates. As noted previously, the maximum strength of the joints formed with the EAN could not be determined because all specimens failed when the SIS-4 ruptured near the clamp as a result of stress concentrations. The lap shear joints remained intact. Joints formed using SIS-4 and either OAN or BAN were weaker than the EAN joints. However, the OAN joints were stronger than the controls formed using DI water, and the difference in strength was statistically significant ($P = 0.02$). Although the OAN joints appeared to be stronger than those formed with BAN, the difference was not statistically significant ($P = 0.12$). The BAN bond strength was slightly greater than that of the bonds formed using the DI control solution. However, the difference was not statistically significant ($P = 0.65$). As noted previously, three out of the eight BAN joints and one out of the eight OAN joints debonded during curing. As a result of these inconsistencies, there was a large standard deviation in the strengths of the BAN and OAN bonds.

Bonds formed using similar amounts of MAPS (without any curing agents) were stronger than those formed using BAN, OAN and the control DI solution. The average strength of the MAPS bonds was 233 ± 58 kPa, and the difference in strength with respect to the OAN, BAN and control bonds was statistically significant ($P < 0.01$). The bond strengths of both the EAN and MAPS were relatively consistent, as demonstrated by their relatively small coefficients of variation, which were both approximately 13%. In contrast to this, the coefficients of variation for the BAN and OAN were 122% and 63%, respectively. Only joints bonded using EAN were stronger than the MAPS joints. These results were encouraging and suggest that a reasonably strong bond could be achieved if a method of rapidly curing the MAPS in a clinical environment could be developed.

In nature, the blue mussel accumulates transition metal ions, and tissue concentrations of these ions are ~5 orders of magnitude greater than those in the open marine environment [50]. Based on prior research [49-51], the authors of this paper hypothesized that transition metal ions that are also strongly oxidative may cause the MAP protein chains to cross-link among themselves. Those investigations demonstrated that metal-induced cross-linking is a combination of both chelation of the metal ions by the protein and oxidation of the protein by the metal [50], and that increasing concentration of oxidative transition metals increased cross-linking of MAP protein chains [51,52]. One goal of the present work was to determine whether these observations could be extended to the formation of bonds with a collagenous substrate such as SIS-4. In general, the bonds formed by applying 25 μ l of solutions containing the transition metal ions Mn^{3+} , Mn^{7+} , V^{5+} , Fe^{3+} and Cr^{6+} were stronger than the bonds formed using MAPS and 25 μ l of distilled water (Table 2). These metal ions also performed well in the previous studies. However, only the increases in strength for the V^{5+} , Fe^{3+} and Cr^{6+} were statistically significant when compared to the strength of bonds formed using MAPS with DI water only ($P < 0.01$, $P = 0.02$ and $P = 0.04$, respectively). These are similar to the patterns found in previous tests using mussel adhesive extract pellets cured using transition metal ions [51,52]. In that study, the reagents that increased the degree of cross-linking of the mussel adhesive pellets were, in the order of most effective to least effective, Cr^{6+} , V^{5+} , Mn^{7+} , Mn^{3+} and Fe^{3+} . However, in this study the joints formed using Mn^{3+} and Mn^{7+} were no stronger than the control joints, where DI water was used with the MAPS adhesive. It should be noted that the previous investigation focused on cohesive strength, the ability of these reagents to crosslink protein chains of mussel extracts among themselves. The results reported in this paper suggest that the effects of some of the ions on cohesion can be different from their effects on adhesion to a substrate, at least for the SIS substrate.

Our previous investigation also indicated a concentration effect of transition metal ions on cross-linking. We hypothesized that there could be a similar dependence in the interaction of MAPS with clinically relevant substrates like SIS-4. Two curing agents that performed best in our experiments, Fe^{3+} and V^{5+} , were used to investigate the concentration effect.

The bonds formed with MAPS and solutions containing 2.5 and 250 mM Fe^{3+} were weaker than those formed using MAPS and 25 mM Fe^{3+} . However, an ANOVA of the bond strength data across the full range of concentrations of Fe^{3+} (2.5, 25, 250 mM) showed that these apparent differences related to concentration were not statistically significant ($P = 0.08$). Comparison of bond strengths of joints using 2.5 and 250 mM Fe^{3+} indicated that the slight differences in strength compared to the strength of the control joints formed using DI water and MAPS were not statistically significant ($P > 0.05$). However, the difference in strength between the MAPS bond formed using 25 mM Fe^{3+} and the DI control was statistically significant ($P = 0.02$).

When solutions with increasing concentrations of V^{5+} were used along with MAPS, the bond strength was concentration dependent ($P < 0.001$). Although the differences in bond strength for 2.5 vs. 25 mM V^{5+} was not statistically significant, the difference in bond strengths for a solution with a concentration of 250 mM of V^{5+} was statistically significant. In fact, the average strength at this concentration of V^{5+} was comparable to the strength of joints formed using EAN.

The effects of increasing concentration of curing agents in this study were, in general, similar to trends observed in previous investigations [51,52], especially in the case of V^{5+} . In those studies, as the concentration of V^{5+} in pellets of marine mussel extracts increased, the resistance to compression increased about 40 times (0.45 mM vs. 45 mM of V^{5+}). That increase in compressive resistance of pellets of mussel adhesive treated with curing agents was attributed to the increase in the formation of cross-links between peptide chains. Compressive tests in

those studies also indicated an increase in compressive resistance with the addition of Fe^{3+} . However, the increase was only by a factor of 6, which is considerably less than the increase achieved with V^{5+} .

The effect of curing agent concentration on the bond strength of SIS-4 joints in the present analysis is related to cross-linking not only among protein chains of MAP but also between MAP and SIS-4. In a recent study [62], scanning electron microscopy (SEM) of hydrogels of marine mussel adhesive protein, with and without the addition of iron, confirmed that Fe^{3+} promotes cross-linking of the MAP. The presence of Fe^{3+} transformed the normally porous hydrogel structure to a compact matrix. Accompanying rheological studies indicated that the Fe^{3+} increased the stiffness of the MAP hydrogel. The data presented in that paper suggested that the cross-linking was partial and imperfect and the authors concluded that partial cross-linking may be beneficial in the use of mussel adhesive extracts as an adhesive with desirable elastic modulus. However, to be an effective adhesive, there must also be a balance between cross-linking within the adhesive (which would affect the elastic modulus of the joint) and cross-linking between the adhesive and the substrate (which would affect bond strength). If there were only cross-linking between adhesive and substrate, the prospective adhesive would form a thin layer on the substrate surface but it would not interact with the rest of the adhesive material. If there were only cross-linking within the bulk adhesive, the prospective adhesive would form a solid that would not adhere to the substrate. There must be a balance. It is possible that the V^{5+} promotes cross-linking within the MAP and between the SIS-4 and the MAP and that both types of cross-linking increase with concentration, whereas the Fe^{3+} promotes both types of cross-linking only at the intermediate concentration.

The concentration dependence of the effectiveness of curing agents such as Fe^{3+} and V^{5+} means that the curing agent could be chosen on the basis of factors such as desired bond strength or biocompatibility. As mentioned earlier, metal-induced cross-linking is a combination of both chelation of the metal ions by the mussel adhesive protein as well as oxidation of the protein by the metal. The relatively high concentration of the curing agent (2.5–250 mM) with respect to the DOPA concentration (0.25 mM), and the increase in cohesive and adhesive cross-linking observed at higher concentrations of V^{5+} , could mean that excess V^{5+} relative to DOPA promotes oxidation and improves protein adhesion. The wide range in effective concentration may permit the V^{5+} concentration to be adjusted so that the bond strength and tissue strength are nearly equal, thereby reducing the likelihood of stress concentrations developing or the bond distorting. On the other hand, at higher concentrations, some transition metals may have low biocompatibility. Therefore, when biocompatibility is a major concern, the curing agent could be chosen to achieve the greatest strength at the highest biocompatible concentration. Studies of these and other factors are planned.

5. Conclusions

The adhesive strength of MAPS, a natural adhesive harvested from blue mussels, was tested using SIS-4, which is currently being used for surgical repairs of soft tissues. SIS-4 strips were joined in a lap shear configuration. Commercially available cyanoacrylate adhesives were also tested. The joints formed using MAPS were stronger than those formed using the two commercial cyanoacrylates (butyl and octyl cyanoacrylates), while the bonds formed by ethyl cyanoacrylate were the strongest. The addition of oxidizing transition metal ions, such as V^{5+} , Fe^{3+} and Cr^{6+} , increased the strength of the MAPS bonds. In studies on the relationship of bond strength to the concentration of the first curing agent, Fe^{3+} , only joints using MAPS and 25 mM Fe^{3+} were stronger ($P = 0.02$) than the control solutions, while those formed with 2.5 and 250 mM Fe^{3+} were not. However, there was a concentration dependence for the second curing agent, V^{5+} , for which there was a 60% increase in bond strength at the highest concentration of V^{5+} (250 mM), compared to the joints using the lowest concentration (2.5

mM). Biocompatibility and optimization studies on the use of MAPS with oxidative metal curing agents are planned.

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Figure 1.

A sketch showing the orientation of the strips cut for the lap shear joint tests with respect to the orientation of the SIS-4 biomaterial. Strips were cut from pieces of four-layer lyophilized SIS that were 70 mm wide and 200 mm long. The orientation of the sheet with respect to the length of the porcine intestine is indicated in the sketch.

Figure 2.

A sketch of an SIS-4 lap shear joint used to measure adhesive strength.

Table 1

A comparison of the shear strength of joints formed by bonding strips of four-layer lyophilized SIS using various surgical adhesives. The numbers in parentheses indicate standard deviations. Sample size for each adhesive solution was $n = 8$. For each sample set, $25 \mu\text{l}$ of adhesive solution was applied.

Adhesive solution	n	Adhesive weight (mg)	Strength (kPa)
EAN	4	13 (2)	>379 (50)
BAN	8	16 (1)	57 (70)
OAN	8	14 (3)	115 (73)
MAPS (0.5 mM DOPA)	8	10 (2)	233 (58)
Controls (DI water)	8	11 (3)	43 (41)

Table 2

The results of lap shear tests of joints formed by bonding strips of four-layer lyophilized SIS using MAPS adhesive along with various metal ion solutions as curing agents. The numbers in parentheses indicate standard deviations. The sample size for each adhesive solution was $n = 8$. The final concentrations of all metal ions in their respective solutions were 25 mM and the DOPA concentration in the MAPS was 0.25 mM.

Adhesive solution	Adhesive weight (mg)	Strength (kPa)
MAPS + DI water (controls)	17 (2)	184 (35)
MAPS + Mn ⁷⁺	18 (3)	190 (28)
MAPS + Cr ⁶⁺	18 (3)	233 (51)
MAPS + Mn ³⁺	18 (3)	199 (47)
MAPS + V ⁵⁺	19 (3)	284 (47)
MAPS + Fe ³⁺	14 (3)	241 (52)

Table 3

Results of the lap shear tests of joints formed by bonding strips of four-layer lyophilized SIS using MAPS along with Fe^{3+} or V^{5+} metal ion solutions of different concentrations as curing agents. Numbers in parentheses in column 1 indicate the final concentration of the curing agent in the adhesive solution. Numbers in parentheses in columns 3 and 4 indicate the standard deviations.

Curing agent used with MAPS	n	Adhesive weight (mg)	Strength (kPa)
DI water (0 mM)	8	17 (2)	184 (35)
Fe^{3+} (2.5 mM)	5	15 (1)	190 (30)
Fe^{3+} (25 mM)	8	14 (3)	241 (52)
Fe^{3+} (250 mM)	8	14 (1)	190 (49)
V^{5+} (2.5 mM)	5	19 (2)	240 (27)
V^{5+} (25 mM)	8	19 (3)	284 (47)
V^{5+} (250 mM)	8	21 (2)	462 (46)