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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 mussels (*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 (SIS).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 V^{5+}, Fe^{3+} and Cr^{6+}, which are all oxidants, increased the bond strength of the MAPS joints. The V^{5+} gave the strongest bonds and the Fe^{3+} the second strongest. In subsequent tests with V^{5+} and Fe^{3+} solutions, the bond strength increased with V^{5+} concentration, but it did not increase with Fe^{3+} concentration. Addition of 250 mM V^{5+} gave a very strong bond.

Keywords: Tissue adhesive; Porcine tissue; Small intestinal submucosa; Mussel adhesive protein; Metal ion

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 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

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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. Fig. 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.

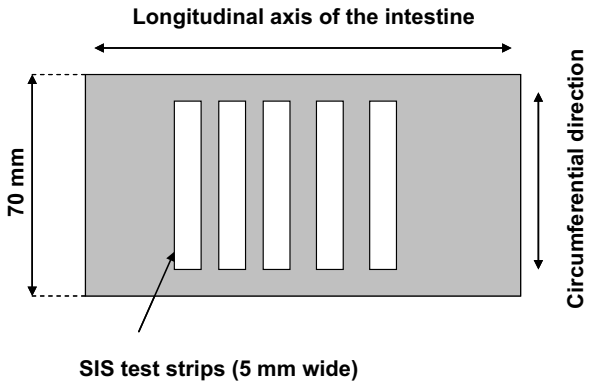

Fig. 1. A sketch showing the orientation of the strips cut for the lap shear joint tests with respect to the orientation of the SIS-4 biomaterial. Strips were cut from pieces of four-layer lyophilized SIS that were 70 mM wide and 200 mM long. The orientation of the sheet with respect to the length of the porcine intestine is indicated in the sketch.

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*g* for 30 min. The supernatant (S1) was collected and acidified with concentrated sulfuric acid (volume = S1 × 0.0168). While stirring, the protein was precipitated out of solution via dropwise addition of acetone (volume = S1 × 2). The protein precipitate was formed into a pellet via centrifugation (31,000*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