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On the mechanical properties of bovine serum albumin (BSA) adhesives

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Abstract Biological adhesives, natural and synthetic, are of current active interest. These adhesives offer significant advantages over traditional sealant techniques, in particular, they are easier to use, and can play an integral part in the healing mechanism of tissue. Thus, biological adhesives can play a major role in medical applications if they possess adequate mechanical behavior and stability over time. In this work, we report on the method of preparation of bovine serum albumin (BSA) into a biological adhesive. We present quantitative measurements that show the effect of BSA concentration and cross-linker content on the bonding strength of BSA adhesive to wood. A comparison is then made with synthetic poly(glycidyl methacrylate) (PGMA) adhesive, and a commercial cyanoacrylate glue, which was used as a control adhesive. In addition, BSA samples were prepared and characterized for their water content, tensile strength, and elasticity. We show that on dry surface, BSA adhesive exhibits a high bonding strength that is comparable with non-biological commercial cyanoacrylate glues, and synthetic PGMA adhesive. Tensile testing on wet wood showed a slight increase in the bonding strength of BSA adhesive, a considerable decrease in the bonding strength of cyanoacrylate glue, and negligible adhesion of PGMA. Tests performed on BSA samples demonstrate that initial BSA concentration and final water content have a significant effect on the stress–strain behavior of the samples.

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1 Introduction

Natural and synthetic biological adhesives are of current interest. These adhesives offer numerous advantages over traditional sealant techniques, in particular, they are easier to use, non-invasive (or minimally invasive), offer controllable mechanical resistance and elasticity, and can play an integral part in the healing mechanism of tissue. They have several applications that range from tissue adhesion, hemostasis, wound healing, to sealing of body fluids in surgery. Biological adhesives may prove to have a great future if they show stability over time, controllable adhesion, elasticity, and mechanical resistance.

Although biological adhesives have been employed in a number of fields with reported advances [1–3], experience with their use in the medical field is limited. Widely used adhesives in biological applications include: cyanoacrylate, fibrin, gelatin, and bovine serum albumin (BSA) adhesives. Several variations of biocompatible FDA approved cyanoacrylate adhesives have been tested for different applications such as wound healing and tissue adhesion. Octyl-2-cyanoacrylate adhesive was shown [2] to be an excellent alternative to suture closure in upper blepharoplasty. Ethyl-2-cyanoacrylate (Medglue) was tested on rat skin and proved to be a viable alternative for suture treated skin incisions [1]. *N*-butyl-2-cyanoacrylate was tested for

resistance and stability for bonding biological materials in cardiac bioprostheses [4]. The adhesive maintained a high degree of elasticity while subjected to tensile testing, but did not confer sufficient resistance to replace sutures in the placement of bioprostheses [4]. Similarly, gelatin-based adhesives have been investigated and used, such as gelatin resorcinol formalin (GRF) which has been used in cardiovascular surgery as an adjunct to operative procedures where tissue integrity is poor and hemostasis is a challenge. However, it was found that the use of this adhesive in cardiovascular surgery must be carefully considered due to late time complications [5–7].

Bioglue, an albumin-based adhesive, is a commercially available sealant manufactured by Cryolife as a hemostatic adjunct for cardiac and vascular surgery. This sealant has evolved conceptually from the GRF glue to act as an agent to strengthen friable tissues, particularly in acute aortic dissections. Previous work performed by Sidle et al. [8] has shown that Bioglue remained stable for more than 12 months. Despite the potential of BSA-based adhesives such as Bioglue in medical applications, little work has been done to characterize its mechanical properties. Garcia Paez et al. [9] evaluated the mechanical resistance and elasticity of calf pericardium samples bonded with Bioglue. The objective of the study was to investigate the possibility of using biological adhesives in cardiac bioprostheses. Traditional suture techniques have limited elasticity and produce a tearing effect on the pericardium [10–13]. Despite the added elasticity due to the use of Bioglue, the mechanical resistance was found to be poor for bonding valve leaflets in cardiac bioprostheses [9].

In this work, we study the mechanical behavior of BSA adhesive cross-linked with glutaraldehyde. The effect of BSA and glutaraldehyde content on the adhesive characteristics was investigated by varying their concentrations. The experiments consisted of tensile testing on wood samples glued by overlapping an area of 2 cm^2. In addition, BSA samples were prepared and characterized for their water content, tensile strength, and elasticity.

2 Materials and methods

2.1 Materials

The BSA used had an Mw of 66 kDa and was 96% pure. Glycidyl methacrylate (GMA), tetrahydrofuran (THF), and 2,2′-azobisisobutyronitrile (AIBN) were used to prepare poly(glycidyl methacrylate) (PGMA) polymer adhesive. In addition to these chemicals, glutaraldehyde, formaldehyde, and other miscellaneous items used in this research were purchased from Sigma–Aldrich. Commonly available cyanoacrylate glue (Krazy Glue) was used as a control adhesive.

2.2 Method of preparation

Two types of samples were prepared to characterize BSA adhesive cross-linked with glutaraldehyde. Pinewood strips glued with BSA adhesive by overlapping an area of 2 cm^2 were prepared for tensile testing. The aim of these experiments was to investigate the effect of BSA concentration, cross-linker concentration, and wood water content on bonding strength. Comparison was made with PGMA synthetic adhesive, while cyanoacrylate glue applied in a similar manner was used as a control adhesive. Bond failure can either occur at the adhesive substrate interface (adhesive failure), within the adhesive itself (cohesive failure), or in the substrate (substrate failure). To investigate the cohesive strength and elasticity of glutaraldehyde cross-linked BSA material, BSA samples were prepared and characterized for their water content, tensile strength, and elasticity.

2.2.1 PGMA preparation

About 100 ml GMA was polymerized in 500 ml THF as a solvent. Prior to reaction, nitrogen was bubbled through the reaction mixture in an enclosed vessel for about 1 h to remove oxygen. The temperature was increased to 50°C and 0.5 g of the polymerization catalyst, AIBN, was added to generate free radicals for the polymerization to begin. The reaction was then stirred in an enclosed vessel for about 5 h with a nitrogen blanket. Afterwards the clear colorless reaction mixture was left to cool. Free radical polymerization of GMA was performed with 0.1% w/v AIBN as an initiator at 50°C [14, 15]. Initial removal of impurities and solvent was performed by slow dripping of the reaction mixture into a large beaker of 600 ml stirred water acidified with drops of hydrogen chloride. Upon contact with the water, the polymer solidified into strands, eventually resulting in a white emulsion of polymer in water. The excess solvent remained in the liquid phase. The resulting solid stands were filtered and then redissolved in acetone, and the process was repeated until no trace of THF remained. The polymer was then dried in a vacuum oven for 24 h to allow evaporation of acetone at a low temperature. The structure of PGMA was confirmed using nuclear magnetic resonance and Fourier transform infrared.

2.2.2 Adhesive-wood samples

The BSA-wood samples were prepared from commercially available pinewood cut into identical pieces: 0.6 cm thick, 2 cm wide, and 5.5 cm long. BSA and glutaraldehyde solutions, having known concentrations, were prepared and

Table 1 Preparation of BSA adhesive

BSA solution concentration (%)	Glutaraldehyde solution concentration (%)	$V_{BSA\ sol.}/V_{glutaraldehyde\ sol.}$	BSA concentration (%)	Glutaraldehyde concentration (%)	$m_{BSA}/m_{glutaraldehyde}$	Hardening time (s)
10.5	25	19.9	10	1.25	8.0	–
21.1	25	18.3	20	1.33	15.0	174
22.2	25	9.1	20	2.50	8.0	52
25.0	25	4.1	20	5.00	4.0	23
33.3	25	1.5	20	10.00	2.0	13
39.5	25	4.9	33	4.12	8.0	–

stored separately and then loaded into two different syringes at a predetermined ratio to achieve the desired BSA and cross-linker concentrations (Table 1). The two syringes were connected through polyvinyl chloride piping, and the BSA solution was thoroughly mixed with the glutaraldehyde by moving the solutions between the two syringes (Fig. 1). A 0.5 ml of the mixed BSA/glutaraldehyde solution was then applied to one surface of the substrate to form a contact area of 2 cm^2 (Fig. 2). In Table 1, the BSA solution concentration is defined as the weight percentage of BSA to the total weight of the BSA solution, and the glutaraldehyde solution concentration is defined as the weight percentage of glutaraldehyde to the total weight of the glutaraldehyde solution. Alternatively, the BSA and glutaraldehyde concentrations (also in Table 1) are defined as the weight percentage of BSA and glutaraldehyde, respectively, to the total weight of the adhesive at the time of preparation. In addition to glutaraldehyde, formaldehyde was tested as a cross-linker for BSA.

The PGMA adhesive in powdered form, prepared in our laboratory, was dissolved in acetone to make a 33% w/w solution. A 0.05 ml of adhesive was applied to each surface of the substrate and 0.05 ml of hydrogen peroxide (curing agent) was applied to the adhesive prior to joining the surfaces. Commercial cyanoacrylate glue was used as purchased, and applied as recommended by the manufacturer to one surface of the substrate. A load of 200 g/cm^2 was then applied while the samples were left overnight for the adhesive to cure. A total of seven BSA-wood samples,

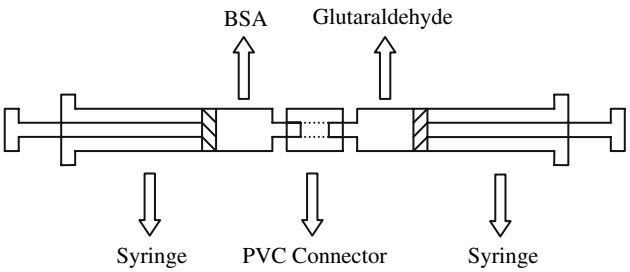

Fig. 1 Schematic of BSA adhesive preparation set-up

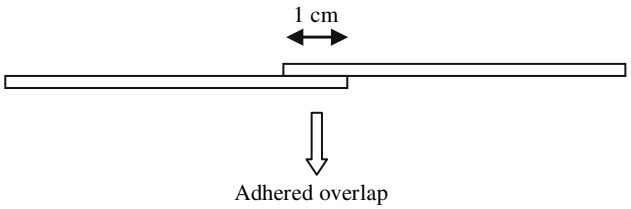

Fig. 2 Schematic of adhesive-wood samples

two cyanoacrylate-wood samples, and two PGMA-wood samples were prepared (Table 2). Tests were performed 24 h after curing. The BSA and cross-linker concentrations in Table 2 are defined as the weight percentage of BSA and cross-linker, respectively, to the total weight of the adhesive at the time of preparation.

2.2.3 BSA samples

Separate BSA and glutaraldehyde solutions were prepared and loaded into syringes as explained in the previous section (Fig. 1). After being mixed in the syringe set-up, the solutions were loaded into a mold to form BSA samples (Fig. 3). For BSA samples to be tested at low water content, the samples were dried at 35°C in a hot-air oven for 8 h. A total of four BSA samples were prepared (Table 3). The BSA concentration in Table 3 is defined as the weight percentage of BSA to the total weight of the sample at the time of preparation, while the final water content is defined as the weight percentage of water to the total weight of the sample at the time of testing.

2.3 Characterization

2.3.1 Evaluation of BSA sample water content

The target application of BSA adhesive significantly affects its water content. For example, if the adhesive is intended for use in surgery, its water content will be high.

Table 2 Adhesive-wood samples

Sample number	Adhesive type	Adhesive concentration (%)	Cross-linker	Cross-linker concentration (%)	Wood water content (%)	Shear strength (MPa)
1	BSA	20	Glutaraldehyde	1.33	8	5.84
2	BSA	20	Glutaraldehyde	2.50	8	6.11
3	BSA	20	Glutaraldehyde	5.00	8	6.25
4	BSA	10	Glutaraldehyde	1.25	8	5.11
5	BSA	33	Glutaraldehyde	4.12	8	6.41
6	BSA	33	Glutaraldehyde	4.12	80	6.74
7	BSA	20	Formaldehyde	2.50	8	0.11
8	PGMA	–	Hydrogen Peroxide	–	8	6.11
9	PGMA	–	Hydrogen Peroxide	–	80	–
10	Cyanoacrylate	–	–	–	8	8.11
11	Cyanoacrylate	–	–	–	80	4.43

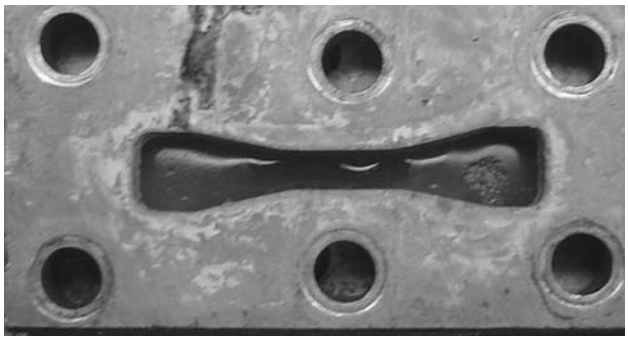

Fig. 3 Mold used to prepare BSA samples

Table 3 BSA samples

Sample number	BSA concentration (%)	Final water content (%)	Ultimate tensile strength (MPa)
1	10	87	0.11
2	10	46	3.61
3	33	61	0.71
4	33	23	13.95

Alternatively, if it is intended for use for wound healing, then the adhesive will dry and the water content will be low. This necessitated the testing of BSA samples having different water content. To evaluate water content, the samples were weighed and then dried at 50°C for 12 h. Afterwards, the samples were cooled in a desiccator to room temperature and weighed again. The weight difference was reported as water content.

2.3.2 Wood water content

To test BSA adhesive bonding strength in wet environment, wood pieces were immersed in water for specified time intervals. The dry wood was stored in a controlled environment having a temperature of 25°C, and 60% humidity. To evaluate wood water content, wood strips (dry and wet) were weighed and then dried at 60–65°C for 12 h. The strips were then cooled in a dessicator to room temperature and weighed again. The weight difference was reported as water content.

2.3.3 Tensile strength and elasticity

The bonding strength of adhesive-wood samples was measured by an Instron 4411 Universal Testing Machine (Fig. 4) at an extension rate of 1.27 mm/min (0.05 in./min). This test method is adapted from ASTM International Standard D2339–98 "Standard Test Method for Strength Properties of Adhesives in Two-Ply Wood Construction in Shear by Tension Loading." The separation force was divided by the contact area (2 cm^2) to yield the shear strength. In addition, the tensile strength and elasticity of

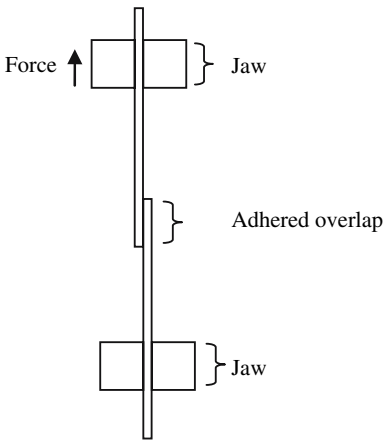

Fig. 4 Schematic of Instron force testing machine

BSA samples were measured using the same tensile testing system at an extension rate of 0.05 in./min. The test method for BSA samples is adapted from ASTM standard E 8M-01 "Standard Test Methods for Tension Testing of Metallic Materials." The separation force recorded by the Instron machine was divided by the cross-sectional area to yield the tensile strength, and the elongation was divided by the initial length to yield the strain.

2.3.4 Measurement of hardening time

Hardening of aqueous BSA solutions was performed by cross-linker addition at room temperature during stirring with a magnetic stirring bar. In particular, a known amount of glutaraldehyde solution (25% w/w), was added to a known amount of BSA solution with a varying concentration according to Table 1. The time period required for the magnetic bar to stop stirring because of cross-linker addition was used as a measure of the hardening time for the BSA adhesive.

3 Results and discussion

3.1 Tensile strength and elasticity

Figure 5 shows the effect of glutaraldehyde concentration on bonding strength of BSA adhesive while BSA concentration was maintained at 20%. Glutaraldehyde (which contains two aldehyde groups) is a bifunctional compound that links covalently to the amine groups of lysine in what is called a condensation reaction [16, 17]. BSA molecules contain 59 lysine amino acids [18], and thus with the bifunctional glutaraldehyde as the bridge, the BSA molecules are cross-linked together to form the adhesive. From inspection of Fig. 5, it is evident that an increase in glutaraldehyde concentration from 1.33 to 5.0% has little effect on the adhesive strength (7% increase in strength, Table 2). This suggests that at 1.33% glutaraldehyde concentration (for 20% BSA concentration, $m_{\mathrm{BSA}}/m_{\mathrm{glutaraldehyde}} = 15$), most of the reactive sites available in the adhesive are cross-linked, and further increase in cross-linker concentration does not lead to a significant improvement. We comment that the level of glutaraldehyde used to cross-link BSA adhesive for medical applications should be minimized due to the toxic effects of unmixed glutaraldehyde [19]. In this work, the ratio $m_{\mathrm{BSA}}/m_{\mathrm{glutaraldehyde}} = 8$ was used for further testing of the BSA adhesive.

Formaldehyde (which contains one aldehyde group) was also tested as a cross-linker for BSA. It is evident from Fig. 5 that formaldehyde is less effective as a hardening agent than glutaraldehyde. The bonding strength of the formaldehyde cross-linked adhesive is less than 2% of that cross-linked with glutaraldehyde having the same concentration (Table 2). This low bonding strength can be attributed to the chemical reaction between formaldehyde and BSA which is detailed in Rott et al. [20].

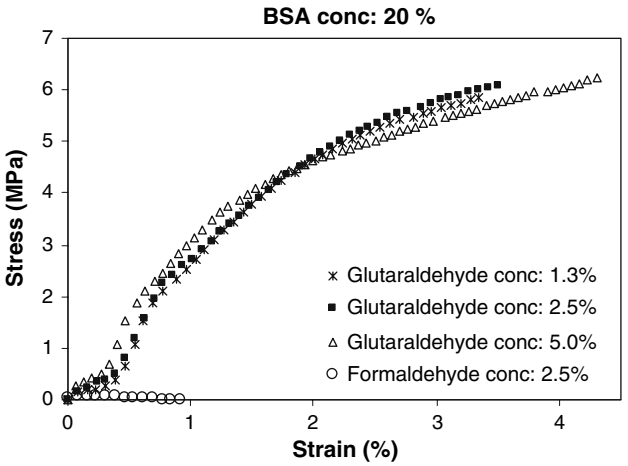

Fig. 5 Effect of cross-linker concentration on bonding strength of BSA adhesive

Figure 6 shows the effect of BSA concentration on bonding strength. To eliminate the effect of cross-linker concentration, a constant ratio of BSA to glutaraldehyde by weight was maintained ($m_{\mathrm{BSA}}/m_{\mathrm{glutaraldehyde}} = 8$). By inspection of Fig. 6 and Table 2, it is clear that the bonding strength increases from 5.11 to 6.41 MPa (25% increase in strength) as the BSA concentration is increased from 10 to 33%. It is believed that an increase in BSA concentration results in an increase in the reactive sites within the adhesive which leads to additional cross-linking throughout the matrix. A more heavily polymerized matrix yields higher strength. As a consequence, the effectiveness of the

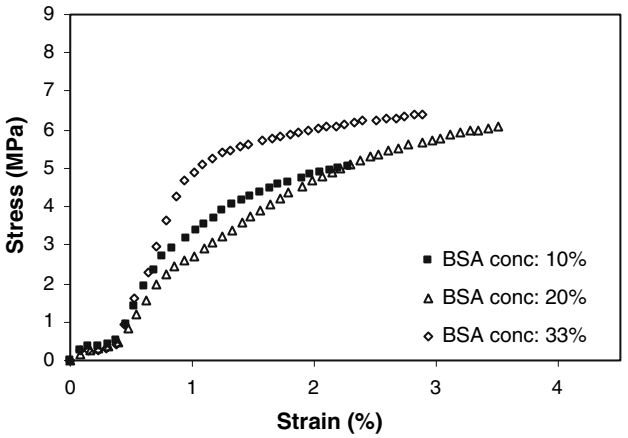

Fig. 6 Effect of BSA concentration on bonding strength of BSA adhesive

adhesive can be further improved by additional increase in its BSA concentration, but the limiting factors are BSA solubility limit and viscosity of the BSA solution. Future experiments will be conducted to determine the additional increase in strength that can be achieved without solubility and viscosity problems taking effect. However, it is important to note that the BSA solution used in this work had a maximum concentration of 39.5% (w/w) and a viscosity of about 50 cP [21] (about 50 times the viscosity of water). At this concentration, the adhesive was prepared without any viscosity-related problems, which indicates that more viscous solutions can be prepared using the existing set-up. In addition, the solubility limit of BSA in water was determined by Kozinski and Lightfoot [22] to be equal to 58.5%, and thus more concentrated BSA adhesives can be achieved.

Figure 7 shows a comparison between BSA (33 wt%), cyanoacrylate and PGMA adhesives as the wood water content is varied. The results are summarized in Table 2. On a relatively dry surface (8% water content), BSA adhesive exhibits considerably lower strength than the cyanoacrylate glue (6.25 vs 8.11 MPa), and marginally higher strength than PGMA (6.25 vs 6.11 MPa). However, as the wood water content is increased to 80%, the BSA adhesion increases to 6.74 MPa, while cyanoacrylate adhesion drops to 4.43 MPa, and PGMA adhesion becomes negligible. It is hypothesized that the hydrophilic BSA adhesive diffuses into the wet wood and then forms a strong bond as it hardens inside the wood. These results demonstrate the viability of using BSA adhesive on wet surfaces such as in medical applications.

To investigate the cohesive strength and elasticity of glutaraldehyde cross-linked BSA material, BSA samples were prepared and characterized as mentioned previously. The separation force recorded by the Instron machine was divided by the cross-sectional area to yield the tensile strength, and the elongation was divided by the initial length to yield the strain. Figure 8 shows the stress–strain relationship of BSA samples prepared at different BSA concentrations and tested at different water content. The results are summarized in Table 3. Inspection of Fig. 8 shows that both parameters (BSA concentration and final water content) have a significant effect on the stress–strain behavior of the BSA material. An increase in the BSA concentration is accompanied by an increase in the sample tensile strength and a decrease in its elasticity. A decrease in water content results in a significant increase in the material tensile strength, without having a major effect on elasticity. For example, according to Fig. 8 a decrease in the water content of the 10% BSA material from 87 to 46% leads to an increase in the ultimate tensile strength from 0.11 to 3.61 MPa (3181.2% increase in strength). Similarly, a decrease in the water content of the 33% BSA material from 61 to 23% results in an increase in the ultimate tensile strength from 0.71 to 13.95 MPa (1,864.8% increase in strength). We comment that the highest tensile strength achieved by BSA samples (13.95 MPa) is about 17% of the strength achieved by epoxy polymer structural material (80 MPa). The ability to control the mechanical characteristics of BSA adhesive permits its optimization for different medical applications.

The function of valve leaflets of implanted cardiac bioprostheses subjects them to a mechanical stress of less than 0.25 MPa [23]. At this level of stress, the deformation of the suture that maintains the shape of the bioprosthetic valve leaflet made of calf pericardium is barely perceptible while the pericardium itself may have suffered an elongation of up to 8% with respect to its original length [13]. In this work, we have demonstrated that the BSA adhesive can be designed to achieve strength of 6.74 MPa on hydrated substrate (Fig. 7), and up to 13% strain (Fig. 8),

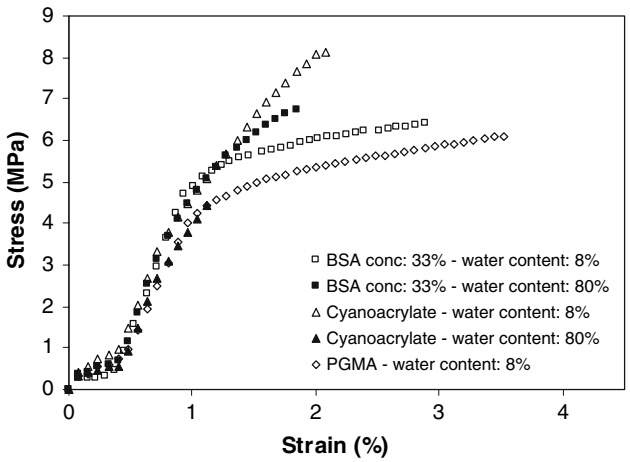

Fig. 7 Effect of wood water content on bonding strength of adhesives

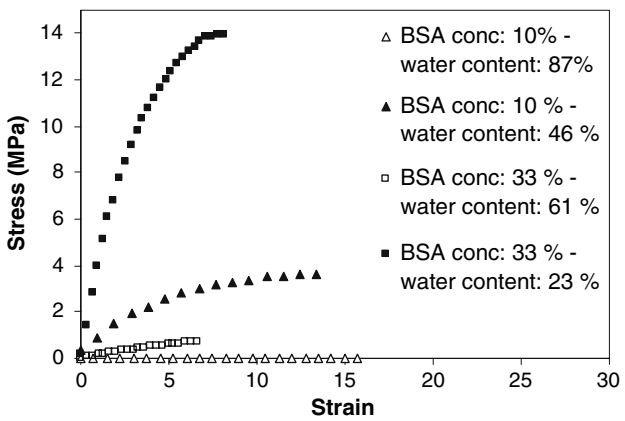

Fig. 8 Stress versus strain relation for BSA samples prepared at different BSA concentration and tested at different water content

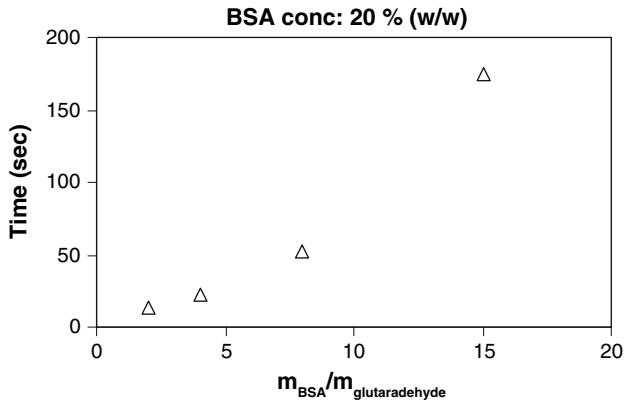

Fig. 9 Effect of glutaraldehyde concentration on hardening time of BSA adhesive

which indicates that BSA adhesive has great potential in cardiac bioprostheses. However, testing in physiologically relevant models needs to be performed.

3.1.1 Hardening time

The potential application of BSA adhesive is dependent on its hardening time which is determined by cross-linker concentration [16]. Figure 9 shows the effect of glutaraldehyde concentration on hardening time while maintaining a constant BSA concentration. From inspection of the figure, it is evident that an increase in cross-linker concentration (decrease in $m_{BSA}/m_{glutaraldehyde}$) results in an initial sharp decrease in the hardening time which then levels off. BSA adhesive having a glutaraldehyde concentration of 1.33% ($m_{BSA}/m_{glutaraldehyde} = 15$) hardened in 174 s, while BSA adhesive having a glutaraldehyde concentration of 10% ($m_{BSA}/m_{glutaraldehyde} = 2$) hardened in 13 s (Fig. 9, Table 1). Thus, BSA adhesives can be tailored for different applications, but considering the toxic effect of unmixed glutaraldehyde [19], a balance needs to be achieved between the desired adhesive strength, hardening time, and toxicity.

4 Conclusions

The mechanical behavior of BSA adhesive was evaluated in this study. It was shown that an increase in glutaraldehyde concentration beyond 1.33% (for adhesive with 20% BSA concentration; $m_{BSA}/m_{glutaraldehyde} = 15$) did not have a significant effect on the bonding strength. Alternatively, an increase in BSA concentration resulted in a considerable increase in bonding strength. Comparison with other adhesives revealed that on relatively dry surfaces (8% water content), BSA adhesive exhibited lower strength than commercial cyanoacrylate glue. However, as the wood water content was increased (80% water content), the BSA exhibited higher strength than cyanoacrylate. Tests performed to investigate the cohesive strength and elasticity of glutaraldehyde cross-linked BSA material demonstrated that initial BSA concentration and final water content have a significant effect on the stress–strain behavior of the samples. Cardiac bioprostheses is one of the most mechanically demanding applications for biological adhesives, where the valve leaflets are subjected to a mechanical stress of $\sim$0.25 MPa and an elongation of up to 8%. Thus, the high adhesive (6.25 on dry wood and 6.74 MPa on wet wood) and cohesive (13.95 MPa) strengths that can be achieved with BSA biological glue coupled with up to 13% of strain, indicate that this adhesive has great potential in a number of biomedical applications that range from bioprostheses, hemostasis, wound healing, to sealing of body fluids in surgery. Future work will be focused on testing in clinically relevant models.

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