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Tuneable Denture Adhesives using Biomimetic Principles for Enhanced Tissue Adhesion in Moist Environments

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Abstract

Nature provides many interesting examples of adhesive strategies. Of particular note, the protein glue secreted by marine mussels delivers high adhesion in wet and dynamic environments owing to existence of catechol moieties. As such, this study focuses on denture fixatives, where a non-zinc-containing commercial-based formulation has been judiciously modified by a biomimetic catechol-inspired polymer, poly(3,4-dihydroxystyrene/styrene-*alt*-maleic acid) in a quest to modulate adhesive performance. *In vitro* studies, in a lap-shear configuration, revealed that the catechol-modified components were able to enhance adhesion to both the denture base and hydrated, functional oral tissue mimic, with the resulting mode of failure prominently being adhesive rather than

cohesive. These characteristics are desirable in prosthodontic fixative applications, for which temporary adhesion must be maintained, with ultimately an adhesive failure from the mucosal tissue surface preferred. These insights provide an experimental platform in the design of future biomimetic adhesive systems.

Keywords- catechol, biomimetic, adhesive, denture, poly(3,4-dihydroxystyrene)

1. Introduction

Tissue adhesives are a family of biomaterials that find widespread use in various aspects of medical device technology including wound care, ostomy devices, dermal delivery systems and bioelectrodes [1]. A commonality between these and indeed most soft tissue adhesives is that they need to deliver temporary adhesion and be removed or degraded with ease after their adhesive role is carried out [2]. Tissue surfaces present a variable range of substrates amongst which the oral environment is particularly challenging because it is constantly moist. The field of dental prosthetics or prosthodontics, has seen, over a period of several decades the development of adhesives that not only function in the moist oral cavity but also make active use of saliva in the achievement of their adhesive properties. Importantly, the use of denture fixatives continues to increase as does the life expectancy of the world population and the consequent incidence of partially or completely edentulous patients [3]

The active ingredients mainly responsible for the adhesive properties of early formulations were natural materials such as karaya gum. In subsequent decades adhesive behavior was improved by the use of synthetic polymers and the incorporation of divalent cations such as calcium or zinc salts [4]. However, zinc has now been recognized to contribute to neurologic

disease when ingested excessively and therefore the current trend for developing denture adhesives is focused on zinc-free products [5-8]. Current adhesives are predominantly based on carboxymethylcellulose (CMC) and poly methylvinylether -maleic acid (PMVE-MA) copolymers with non-active ingredients, such as petrolatum and mineral oil being added as binding materials [7, 9, 10]. Commercial denture adhesives exist in a number of forms; powders, pastes, strips, cushions or pads [7, 11]. This study focuses on the most commonly used paste form with a control formulation based on a commercial non-zinc-containing denture adhesive [8, 12, 13].

The desired function of the temporary adhesive is to decrease lateral and vertical movement of dentures and to increase incisal bite force. The shear strength of denture adhesives is an important property that is typically overlooked in *in vitro* studies, with more focus placed on tensile bond strengths. However, shear bond strength is the most widely accepted technique of quantifying adhesion and standard deviations are recognized to be larger in the tensile or peel configurations [14, 15]. Both tensile and shear detachment forces will take place during chewing and removal of the dentures.

The adhesive and cohesive strength capabilities will dictate the mode of failure of adhesives; adhesive failure occurs when the bond between the substrate and adhesive fails, whereas cohesive failure occurs within the bulk of the adhesive rather than at either interface [16]. In this application, adequate temporary adhesion (12 to 18 hours) to both the denture, typically fabricated from poly(methyl methacrylate) (PMMA) [17] and oral mucosal tissue is required, with an adhesive failure occurring ultimately at the tissue interface [18-22]. This is primarily due to current commercial adhesives being challenging to remove from the oral mucosa after use [10, 23, 24]. Food is known to migrate in between the denture and

denture-bearing mucosa, however, the use of an adhesive with better retentive (temporary) properties against the oral tissue can reduce the extent of this occurring [25].

PMMA is a moderate surface energy non-hydrated rigid substrate (modulus > 1 GPa) whereas the tissue surface is the more complex, hydrated, higher surface energy stratified squamous epithelium of the oral mucosa (modulus < 9 MPa [26]). The two most challenging features of this particular tissue adhesive are the need to modulate adhesion to these two very different surfaces and need to do so in the presence of an aqueous biological fluid – saliva. Saliva plays an important role in the fixative function; as the adhesive hydrates, it swells by some 50-150 % in volume. This reduces the levels of friction and irritation to the oral tissue [7, 9, 13, 27]. However, even though the products rely on the surrounding aqueous conditions to activate their adhesive capability, their optimum performance will fall within a specific range of hydration.

Currently, denture adhesives are typically assessed *in vitro* using PMMA:PMMA interfaces,[13, 28-32] or, in one case, PMMA:wet cotton fabric [33]. Herein, we have created an interface that more closely models the oral environment by combining a purpose-synthesized wet tissue analogue in a shear adhesion configuration with a PMMA substrate. This approach has been combined with the use of natural models in the synthesis of a biomimetic adhesive for use in the oral environment.

The natural adhesive systems of marine organisms are promising candidates for the design of biomimetic adhesives, particularly the adhesive proteins secreted from mussel species, such as Mytilus edulis, which have been characterized extensively [34]. Mussel adhesive proteins are known to contain the amino acid 3,4-dihydroxyphenylalanine (DOPA), which

has pendent catechol functionality [15, 35-37]. This chemical entity contributes significantly to interfacial adhesion to both inorganic and organic surfaces in aqueous environments [38, 39]. DOPA originates from tyrosine, which following hydroxylation mediated by tyrosine hydroxylase, yields the catechol moiety [34, 40, 41]. The structural simplicity of the catechol group has previously been exploited as an analogous reactive group to DOPA in many polymer-based adhesive strategies, resulting in enhanced adhesion against a wide range of both high energy and low energy substrates [15, 42]. In many cases, 3,4-dihydroxystyrene (DHS) is distributed within a polymer backbone as a way of mimicking the catecholic substituent of DOPA [35, 37, 43, 44] and maintain the ideal geometric, steric and electronic properties that are associated with the adhesive properties of catechol-based systems.

We have harnessed the chemistry of marine mussel adhesive proteins in the synthesis of a modified poly(styrene-*alt*-maleic acid), P(S-*alt*-MA), which has then been introduced into a non-zinc-based commercial denture adhesive formulation, in place of the conventional PMVE-*alt*-MA component. The objectives of this study were to: (1) design and synthesize a novel biomimetic catechol-inspired denture adhesive; and (2) evaluate and compare the adhesive performance of the novel systems to the control in a series of purpose-designed *in vitro* experiments in the context of the challenging features of the oral environment already highlighted.

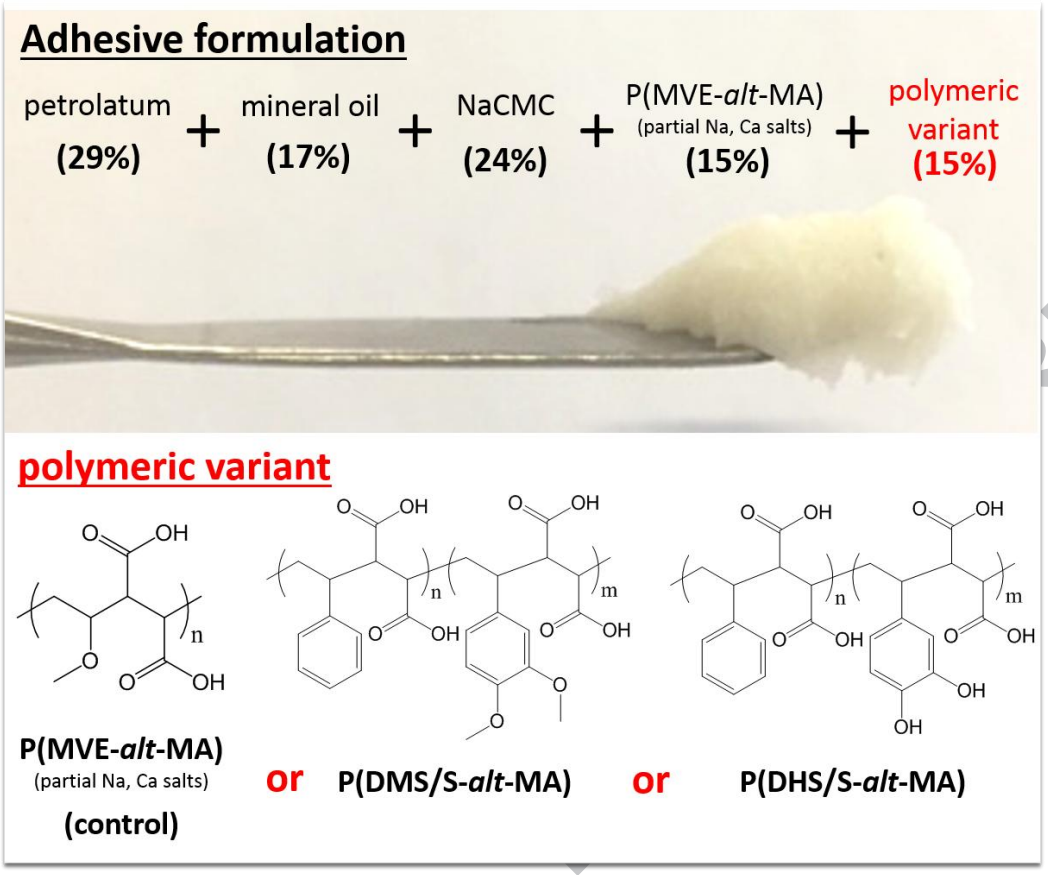


Figure 1. Adhesive formulation by weight percentage and chemical structures of the polymeric variants used in this study.

2. Materials and methods

2.1 Materials

3,4-Dimethoxystyrene (DMS, technical grade, 99 %), styrene (S), maleic anhydride (MA), boron tribromide (BBr_3, >99.99 %), sodium hydroxide (NaOH) and mucin, type III, partially purified powder were purchased from Sigma-Aldrich. P(MVE-MA) (Gantrez MS-955) and NaCMC were purchased from Ashland Chemical. Petrolatum and mineral oil were purchased from Sonneborn. Toluene, ethyl acetate, dichloromethane (DCM), methanol (Fisher Scientific, Laboratory grade), Irgacure 2959 (CIBA Additives), benzoyl peroxide (BPO), 2-hydroxypropyl methacrylate (HPMA) (BDH), *N,N*-methylenebisacrylamide (MBA), *N*-

(hydroxymethyl)-acrylamide (HMAA, 48 wt % solution in water), *N*-hydroxyethyl acrylamide (HEA, 97%) (Sigma Aldrich), were all used as received.

2.2.1 Synthesis of poly(3,4-dimethoxystyrene/styrene-alt-maleic anhydride), P(DMS/S-alt-MAn)

3,4-dimethoxystyrene and styrene were extracted with 10% aqueous NaOH solution prior to polymerization in order to remove the hydroquinone inhibitor. The following example describes the synthesis of a poly(3,4-dimethoxystyrene/styrene-*alt*-maleic anhydride) (0.5:0:5:1 monomer feed molar ratio, respectively) precursor; this protocol is representative of all polymerizations (with varying styrenic molar ratios, as shown in Table 1). In a typical polymerization, 40 mL of toluene and 10 mL of ethyl acetate were added to a three-neck round bottom flask equipped with a reflux condenser, thermometer, nitrogen gas inlet system and a magnetic stirrer bar. 2 g of maleic anhydride, 1.2 mL styrene and 1.5 mL 3,4-dimethoxystyrene were added before the flask was sealed with a rubber septum and purged with nitrogen at 70 °C. After 15 minutes, 40 mg of benzoyl peroxide (dispersed in 1 mL toluene) was added drop wise *via* a syringe. The reaction mixture was left to polymerize for 6 hours, before washing with methanol, resulting in a pale yellow colored powder, which was then filtered and dried under vacuum at room temperature. Typical yields were between 78-87 %.

2.2.2 Synthesis of poly(3,4-dihydroxystyrene/styrene-alt-maleic anhydride), P(DHS/S-alt-MAn)

Hydrolysis of the dimethoxy groups was carried out according to previous methods [15, 42]. In short, 2 g P(DMS/S-*alt*-MAn) were dissolved in 50 mL of DCM in a round bottom flask, equipped with a magnetic stirrer bar. A rubber septum was used to seal the flask and the

mixture was purged with nitrogen for 15 minutes at room temperature. The nitrogen inlet was then turned off and the reaction was cooled to -20 °C for 10 minutes under constant stirring, before 2 mL of BBr_3 were added drop-wise *via* a syringe through the rubber septum. After 10 minutes, the solution was stirred for 18 hours room temperature. The resultant dark brown solution was cooled to -20 °C and 30 mL of deionized (DI) water was added to the flask before the resultant dark grey precipitate was filtered and washed with three rounds of DI water and DCM and then finally collected and dried under vacuum. Typical yields were between 85-91 %.

2.2.3 Synthesis of poly(3,4-dimethoxystyrene/styrene-alt-maleic acid), P(DMS/S-alt-MA), and poly(3,4-dihydroxystyrene/styrene-alt-maleic acid), P(DHS/S-alt-MA)

The maleic anhydride component of the polymer products was hydrolysed in DI water at 80 °C, with the progressive addition of 1 M NaOH until pH 11 was reached. Subsequently, the resulting solutions were freeze-dried, resulting in either a pale brown [P(DMS/S-*alt*-MA)] or purple powder [P(DHS/S-*alt*-MA)] as the final product.

2.3 Gel Permeation Chromatography

Number-average molecular mass (M_n) and dispersity (M_w/M_n, $Đ$) were measured using gel permeation chromatography (GPC) (flow rate 0.8 mL/min) through two PL gel 5 µm mixed-C 300 x 7.5 mm columns using degassed DMF eluent system containing 1 g/L LiBr. The system operated at 50 °C and was calibrated with poly(methyl methacrylate) standards.

2.4 Ultraviolet and visible light spectroscopy (UV-Vis)

Ultraviolet and visible (UV-vis) absorbance spectra were obtained using a SpectraMax M2 system in the wavelength range 250 - 750 nm, using DI water as a solvent at appropriate concentrations.

2.5 Preparation of tissue mimic (TM) substrates

In a typical experiment HEA, HMAA, HPMA, Irgacure 2959 (10 mg/100 µL in ethanol), MBA and DI water were mixed in a ratio of 20:20:10:1:1:48 (by mass), respectively and injected into a mold of two glass plates (held together by spring clips) each covered with a Melinex® sheet and separated by two polyethylene gaskets (0.1 mm thick, cavity 5.5 x 5.5 cm)[45]. The mold was placed horizontally on a conveyor belt and passed five times at a speed of 5 m/min under a UV lamp (GEW Ultraviolet Lamp 100W/cm^2). The hydrogel was carefully removed from the mold and placed in DI water for one week (changing the water daily). Following removal from the DI water, the gel was cut into 2.3 x 1.5 cm pieces and the surfaces blotted with filter paper to remove excess surface water before being super-glued onto a Melinex® strip (2.3 x 7.5 cm) to provide the substrate for the lap shear test. The substrate was then placed in DI water until testing, where it was blotted again with filter paper prior to testing.

2.6 Adhesive formulations

The control formulation was made according to published patents [46, 47] and compositions of non-zinc-containing commercial formulations [8, 12, 13]. Firstly, 29 g of petrolatum was mixed with 17 g of mineral oil using a speed mixer, Synergy device for 2 minutes. 30 g of P(MVE-*alt*-MA) calcium/sodium partial salts and 24 g of NaCMC were then added and mixed again for 2 minutes. The formulation was then coarsely stirred with a spatula in order to

allow any excess powder particles to be dispersed within the mixture before mixing again for 4 minutes. For the advance formulations, batches were made by replacing 50 % of the P(MVE-*alt*-MA) calcium/sodium partial salts with either P(DMS/S-*alt*-MA) or P(DHS/S-*alt*-MA) as shown in Figure 1. The amount of NaCMC, petrolatum and mineral oil was always kept constant.

2.7 Adhesion studies – lap shear strength tests

For adhesion studies, PMMA substrates for lap shear adhesion testing were provided by GSK (Weybride, UK) with dimensions of 2.3 x 7.5 x 0.3 cm. The formulations were mixed with either DI water or mucin-rich solution (1 g /20 mL DI water) at varying ratios (precise wt% ratios for each experiment are indicated in the corresponding figure captions) using a spatula and the adhesive joint was made by placing the hydrated adhesive onto a substrate (PMMA), followed by a clean substrate (PMMA or TM) and placed on top with an overlap of 1.5 cm (See Figure 3). A 200 g weight was placed on top of the overlap for 5 seconds to allow constant pressure to be exerted upon each sample. When placed in the tensometer grips, DI water (0.18 mL) was sprayed once onto the strips before the test was conducted. Each sample was tested at room temperature and as close to 30 seconds (+/- 5 secs) as possible after mixing. An Instron Hounsfield tensometer equipped with a 10 N load cell was used to conduct the lap shear adhesion measurements. The samples were pulled apart at a rate of 10 mm/min and the final adhesive strength was obtained from the force at break (Newtons) divided by the interfacial surface area (mm^2). The locus of failure (cohesive or adhesive) for each sample was identified by visual observation. Three samples were measured for each adhesive variant and the average adhesion strength of these values is reported. The error bars shown indicate ± standard deviation.

2.8 *Statistical analysis*

SPSS software was used to carry out the statistical analysis. A Shapiro-Wilk's test ($p > 0.05$), visual inspection of histograms, normal Q-Q- plots and box plots were carried out to verify that the lap shear test method to assess the dependent variable (adhesion strength) as a function of the independent variable (adhesive formulations) are approximately normally distributed. One way-ANOVA in conjunction with Tukey's HSD test was performed to evaluate the statistical significance of adhesion strength amongst the adhesive formulations and a value $p < 0.05$ were considered to be statistically significant.

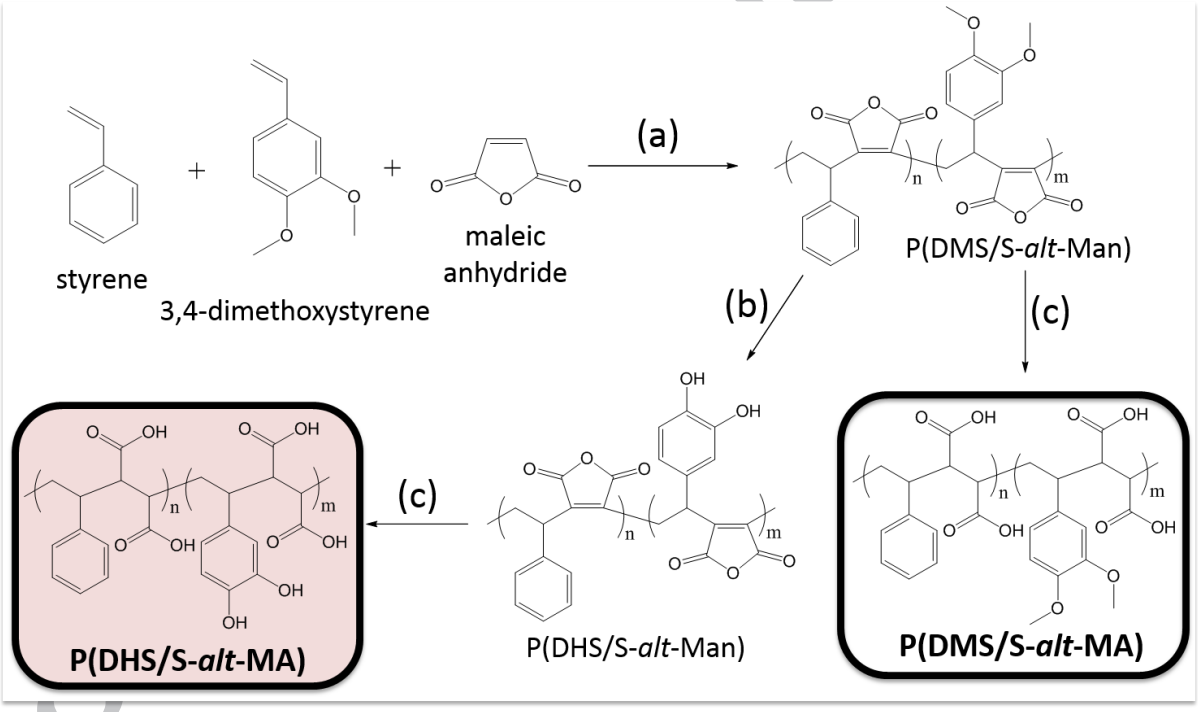
2.9 Rheology

The rheological character of the adhesives either hydrated (1:1, adhesive: water) or dehydrated was assessed using a Bohlin CVO Rheometer, recording the storage (*G'*) and loss modulus (*G"*) at 37 °C to mimic body temperature. Samples were subjected to a frequency sweep between 0.5 and 10 Hz under oscillating conditions at constant strain (0.002). Parallel plate (10 mm) geometry was used with a 3 mm gap. Each adhesive sample was performed in triplicate and results are plotted as an average.

Results

3.1 Polymer synthesis and characterization

Scheme 1 shows the synthetic route employed to obtain the P(DMS/S-*alt*-MA) and P(DHS/S-*alt*-MA) sets of target polymers. In total, six polymers (three of each set) were prepared where the level of DMS or DHS *versus* styrene was altered, whilst maintaining the overall molecular mass approximately constant and ratio of the styrenics: maleic acid at 1:1.



Scheme 1. Synthetic route for the preparation of P(DMS/S-*alt*-MA) and P(DHS/S-*alt*-MA). Polymers are depicted as perfectly alternating polymers for clarity purposes. (a) BPO, 70 °C, $C_6H_5CH_3$, $CH_3COOC_2H_5$ (b) BBr_3, CH_2Cl_2, -20 °C to RT, CH_3OH, H_2O (c) H_2O, 1M NaOH, 80 °C.

Table 1. Molecular characterization data for P(DMS/S-*alt*-MAn) polymers.

polymer name	monomer feed (target molar ratio)			calculated polymer ratio (^{1}H NMR)			GPC data	
	styrene	3,4-dimethoxystyrene	maleic anhydride	styrene	3,4-dimethoxystyrene	maleic anhydride	M_n	$Đ$ (M_w/M_n)
P(DMS/S-*alt*-MA)1	0.75	0.25	1	0.77	0.38	1	51,000	2.75
P(DMS/S-*alt*-MA)2	0.5	0.5	1	0.39	0.55	1	42,000	2.4
P(DMS/S-*alt*-MA)3	0.25	0.75	1	0.37	0.76	1	44,000	2.6

The molar ratio of 3,4-dimethoxystyrene to styrene was varied (at constant maleic acid molar ratio) to produce a series of poly(3,4-dihydroxystyrene/styrene-alt-maleic acid), P(DHS/S-*alt*-MA) variants. The actual ratio of monomers in each polymer was calculated by ^{1}H NMR spectroscopy and is presented alongside the molecular mass data obtained *via* GPC in Table 1. Overall, the 3,4-dimethoxystyrene content of the polymers correlated with the amount that was initially placed in the feed and was closer in the higher catechol targeted derivatives. The molecular mass profiles of the polymers were very similar to one another [number-average molecular mass (M_n) = 42,000-51,000 g/mol, $Đ$ = 2.4 – 2.75] as shown in the GPC traces (Figure S1, Supplementary data). Subsequent deprotection of the dimethoxy groups with BBr_3 and hydrolysis with DI water, followed by further hydrolysis of the maleic anhydride, yielded the P(DHS/S-*alt*-MA) products depicted in Scheme 1. ^{1}H NMR and FTIR spectra before and after hydrolysis with BBr_3, indicated successful conversion to the desired products (provided in the Supporting Information in Figures S2 and S3). The P(DMS/S-*alt*-MA) series of polymers were made by carrying out the maleic anhydride hydrolysis after the free-radical polymerization of P(DMS/S-*alt*-MAn) without hydrolyzing the methoxy groups.

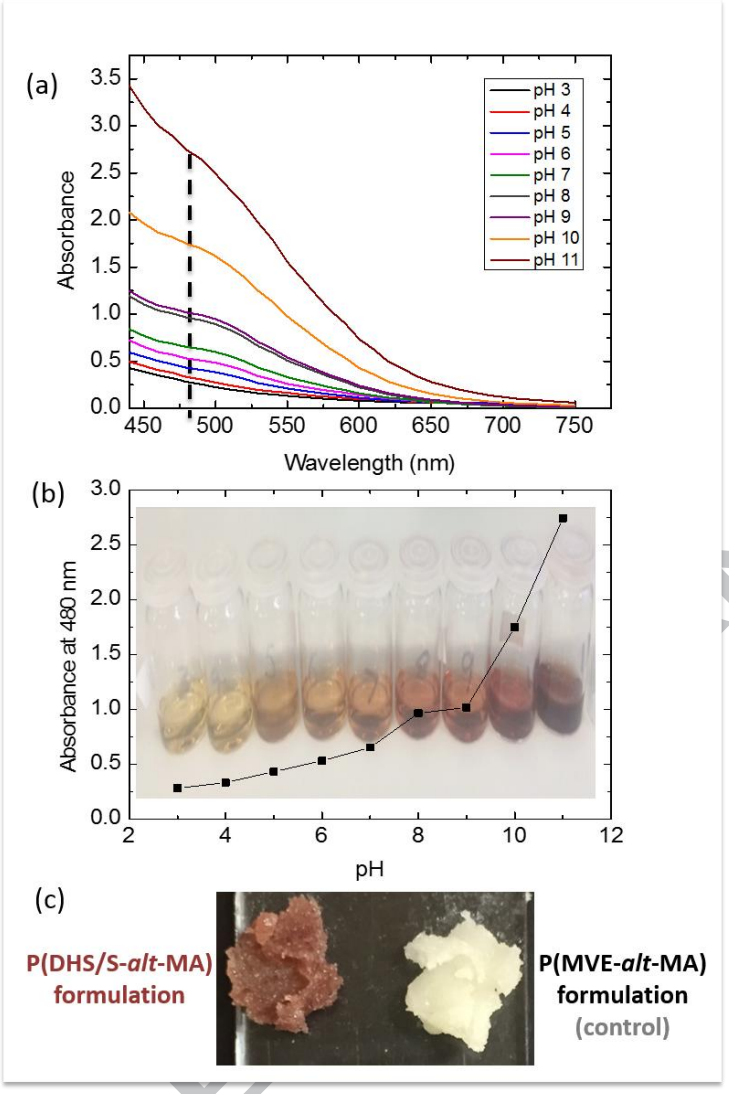


Figure 2. (a) UV-vis absorption spectra taken at progressive pH intervals during the hydrolysis of the maleic anhydride component in P(DHS/S-*alt*-MAn)2 to give P(DHS/S-*alt*-MA)2 . (b) UV-vis absorbance of P(DHS/S-*alt*-MA)2 at 480 nm against pH. (c) Images of the P(DHS/S-*alt*-MA)2 and control final adhesive formulations, showing the purple formulation in contrast to the white control adhesive, containing solely P(MVE-*alt*-MA) as the polymeric variant.

An interesting set of colour changes took place during the hydrolysis of P(DHS/S-*alt*-MAn) to P(DHS/S-*alt*-MA) as shown in Figure 2. On the other hand, the dimethoxy derivative showed no such change. Figure 2b shows UV-vis absorbance plots of the P(DHS/S-*alt*-MA)2 solutions in Figure 2a at 480 nm against pH. 480 nm is a characteristic absorption band for PDHS [42] and the absorption at this wavelength increased as a function of increasing pH, significantly so under basic conditions. The hydrolysis reactions yielding P(DMS/S-*alt*-MA) and P(DHS/S-*alt*-MA) were all terminated at pH 11 and subsequently freeze-dried before being added into the overall adhesive formulation.

3.2 Adhesion studies

The nature of the adhesive formulations is illustrated in Figure 1 and Scheme 1; bonding performance was assessed in a lap shear configuration against either (i) two PMMA plates and (ii) a PMMA plate and the TM substrate, post-mixing with either water or mucin-rich solution as described in section 2.7 (Figure 3).

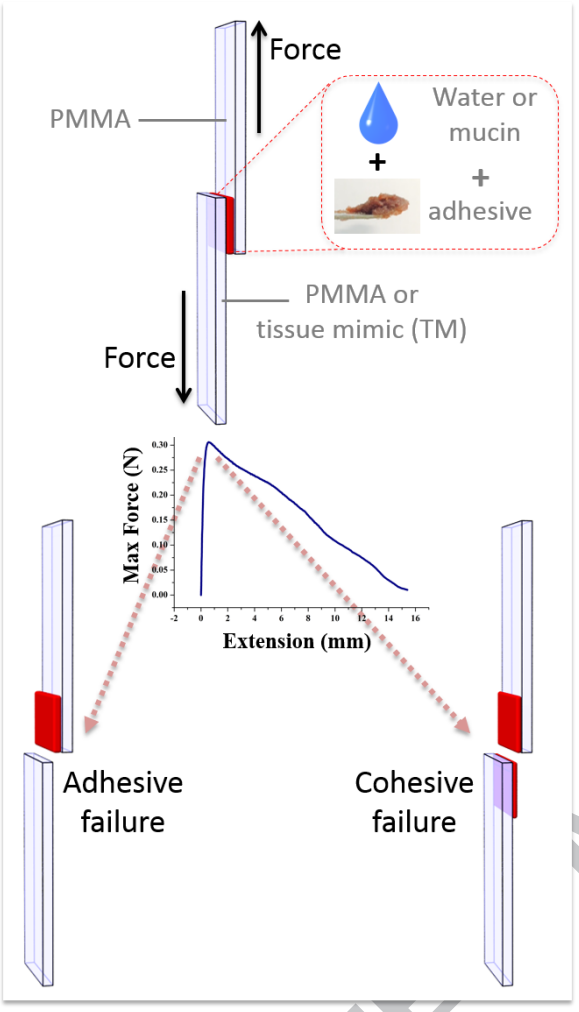


Figure 3. Lap shear bond test setup used to assess adhesion strength and locus of failure of adhesive formulations.

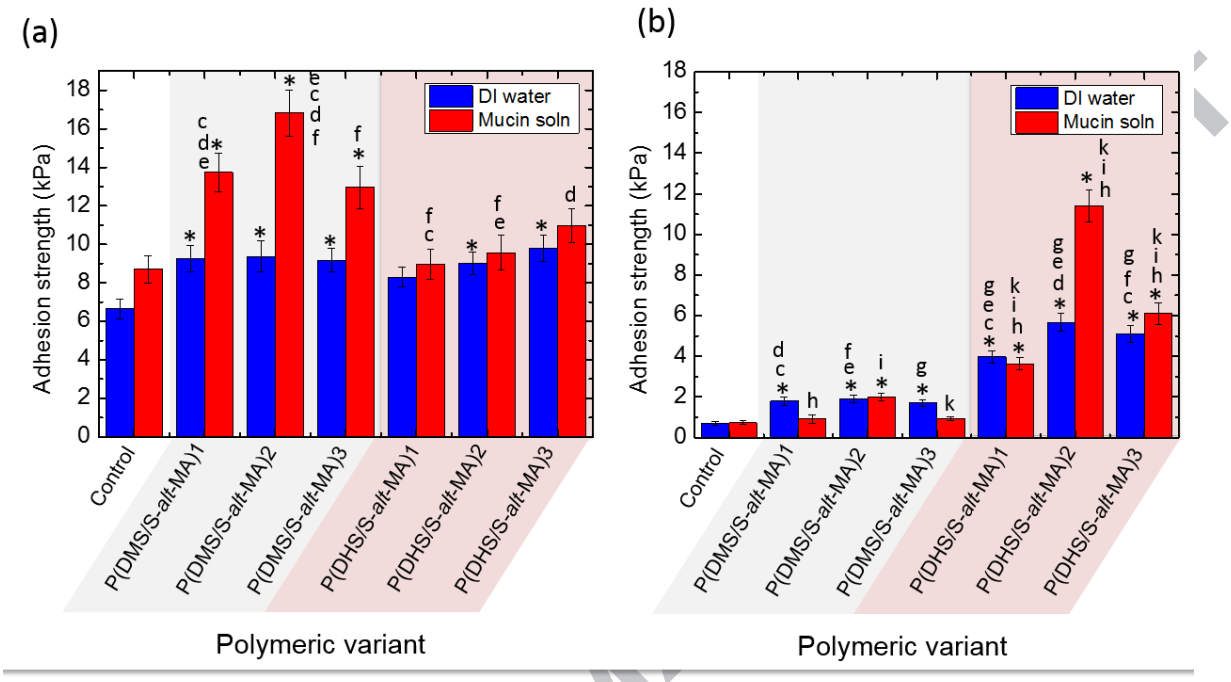


Figure 4. (a) Maximum adhesion strength against PMMA or (b) PMMA and TM substrates as a function of the polymeric variant with 50 wt% DI water or mucin solution. The asterisk (*) is used to identify data that show statistically significant differences from the control ($p < 0.05$). Data identified with the same letter are significantly different from each other ($p < 0.05$).

Fig 4a shows that P(DHS/S-*alt*-MA)2 and 3 adhesives gave higher adhesion (against PMMA/PMMA) compared to the control when mixed with DI water ($p < 0.05$) whereas none of the PDHS-based adhesives produced significantly higher adhesion to that of the control when mixed with mucin solution ($p < 0.05$). The P(DMS/S-*alt*-MA) adhesive systems, however outperformed the control across all of the PDMS loading contents ($p < 0.05$), in both aqueous phases. There were significant differences between all of the PDMS and the PDHS-based adhesives with the exception between P(DMS/S-*alt*-MA)3 AND P(DHS/S-*alt*-

MA)3 when mixed with mucin solution however no significant differences were seen between the PDMS and PDHS-containing variants when mixed with DI water ($p > 0.05$).

Mixing the adhesives with mucin-rich solution compared to DI water did not effect adhesion strength for the catechol-containing systems with the PMMA substrates ($p > 0.05$). Conversely increased adhesion as a result of changing the aqueous phase was seen for the control ($p < 0.05$), however this effect was much more pronounced in the DMS systems, with P(DMS/S-*alt*-MA)2 reaching 16.8 (±1.2) kPa ($p < 0.05$ for all PDMS systems mixed with mucin compared to corresponding PDMS systems mixed with DI water). Replacing a PMMA substrate with the TM substrate on one side of the lap shear joint configuration brought about a different trend in adhesion. Fig 4 (b) shows that the control, all P(DMS/S-*alt*-MA) and P(DHS/S-*alt*-MA)1 and 3 variants were unable to deliver significant adhesion; adhesion strength decreased dramatically compared to the corresponding formulations tested in the PMMA/PMMA system ($p < 0.05$). For the PDHS-based systems, the adhesive strength was reduced in the "more realistic" PMMA/TM tests, but to a lesser extent than the other systems studied. Interestingly, P(DHS/S-*alt*-MA)2 gave increased adhesion when mixed with mucin solution in the PMMA/TM tests compared to the PMMA/PMMA setup ($p < 0.05$).

Table 2. Summary of adhesion strength (kPa) of all polymeric variant samples when mixed with DI water and mucin solution against PMMA and functionalised hydrated tissue mimic (TM) substrates.

Polymer	Substrates	Mean (± SD)		AF/CF[a,b]	
		DI water	Mucin soln	DI water	Mucin soln
P(MVE-*alt*-MA)	PMMA/PMMA	6.6 (0.55)	8.7 (0.64)	AF	AF
(control)	PMMA/TM	0.7 (0.12)	0.7 (0.1)	AF	AF
P(DMS/S-*alt*-MA)1	PMMA/PMMA	9.3 (0.58)	13.7 (1.0)	CF	CF
	PMMA/TM	1.8 (0.1)	0.9 (0.2)	AF	AF
P(DMS/S-*alt*-MA)2	PMMA/PMMA	9.4 (0.8)	16.8 (1.2)	CF	CF
	PMMA/TM	1.9 (0.21)	2 (0.2)	AF	AF
P(DMS/S-*alt*-MA)3	PMMA/PMMA	9.2 (0.6)	13 (1.1)	CF	CF
	PMMA/TM	1.7 (0.19)	0.9 (0.1)	AF	AF
P(DHS/S-*alt*-MA)1	PMMA/PMMA	8.3 (0.56)	9 (0.81)	CF	AF
	PMMA/TM	4 (0.32)	3.6 (0.3)	AF	AF
P(DHS/S-*alt*-MA)2	PMMA/PMMA	9 (0.75)	9.6 (0.97)	CF	AF
	PMMA/TM	5.7 (0.46)	11.4 (0.81)	AF	AF
P(DHS/S-*alt*-MA)3	PMMA/PMMA	9.8 (0.66)	11 (0.88)	CF	AF
	PMMA/TM	5.1 (0.4)	6.1 (0.67)	AF	AF

a) CF = cohesive failure, AF = adhesive failure.
b) For the PMMA/TM systems, adhesive failures occurred at the tissue mimic interface in all cases.

The data in Table 2 confirm that the adhesive failure mode varies with chemical composition of the polymers. All P(DMS/S-*alt*-MA)-containing formulations produced 100 % cohesive failures when tested between two PMMA substrates. In contrast, the occurrence of adhesive failures was observed for all catechol-bearing systems when mixed with mucin solution, independent of the substrates used. One common trend observed amongst all the samples tested was that they produced adhesive failures from the tissue mimic side.

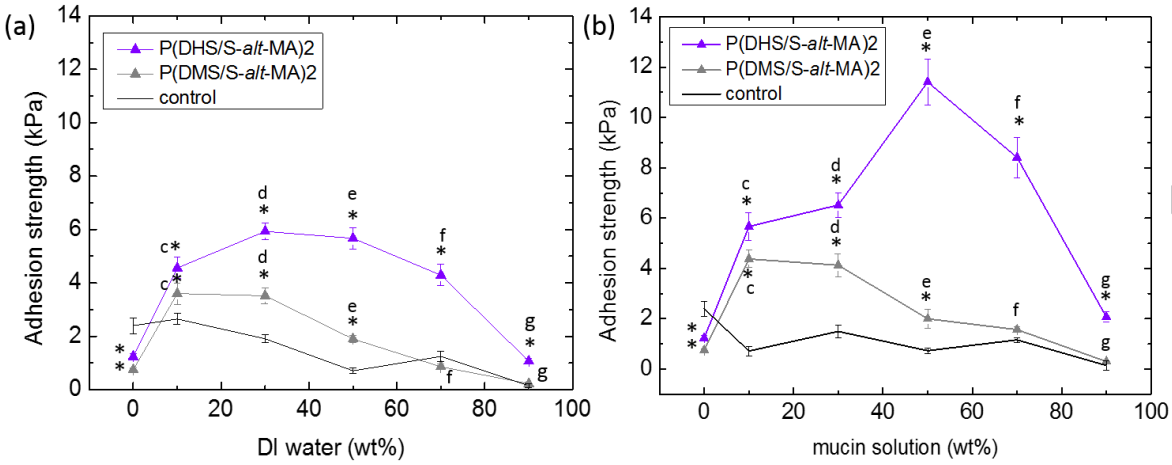


Figure 5. Maximum adhesion strength for each formulation between PMMA and TM substrates with increasing (a) DI water and (b) mucin-rich solution contents. The asterisk (*) is used to identify data that show statistically significant differences from the control ($p < 0.05$). Data identified with the same letter are significantly different from each other ($p < 0.05$).

To understand the performance of our formulations in varying conditions, the adhesive properties were assessed in the *in vitro* PMMA/TM system as a function of hydration of the adhesive (Figure 5a and b). P(DMS/S-*alt*-MA)2 and P(DHS/S-*alt*-MA)2 were chosen for these sets of lap shear tests and benchmarked against the control as these formulations previously outperformed the corresponding polymer variants with different comonomer ratios. The control formulation scored higher than P(DMS/S-*alt*-MA)2 and P(DHS/S-*alt*-MA)2 ($p < 0.05$) when no aqueous phase was introduced into the system. In contrast, when an aqueous phase is introduced, the catechol-containing adhesive showed significantly higher adhesion than P(DMS/S-*alt*-MA)2 and the control adhesive for all levels of hydration, to a even bigger extent when mixed with mucin solution ($p < 0.05$). Generally, as the ratio of aqeuous phase increased, the adhesion of the P(DMS/S-*alt*-MA)2 would diminish, particularly from 30% hydration and upwards ($p < 0.05$). The control did not show as much

sensitivity to the changing hydration levels, but maintained low adhesion throughout all ratios of hydration ($p > 0.05$). However, the catechol-based formulation maintained adhesion up to 50 % hydration ($p < 0.05$) in both aqeuous systems. Adhesive failures from the tissue mimic side (desired *in vivo* outcome) were seen with the P(DMS/S-*alt*-MA)2 and control systems for 10-50 % hydration and cohesive failures occurred at 70-90 % hydration. The catechol-harnessed adhesives gave adhesive failures up until 70 % hydration.

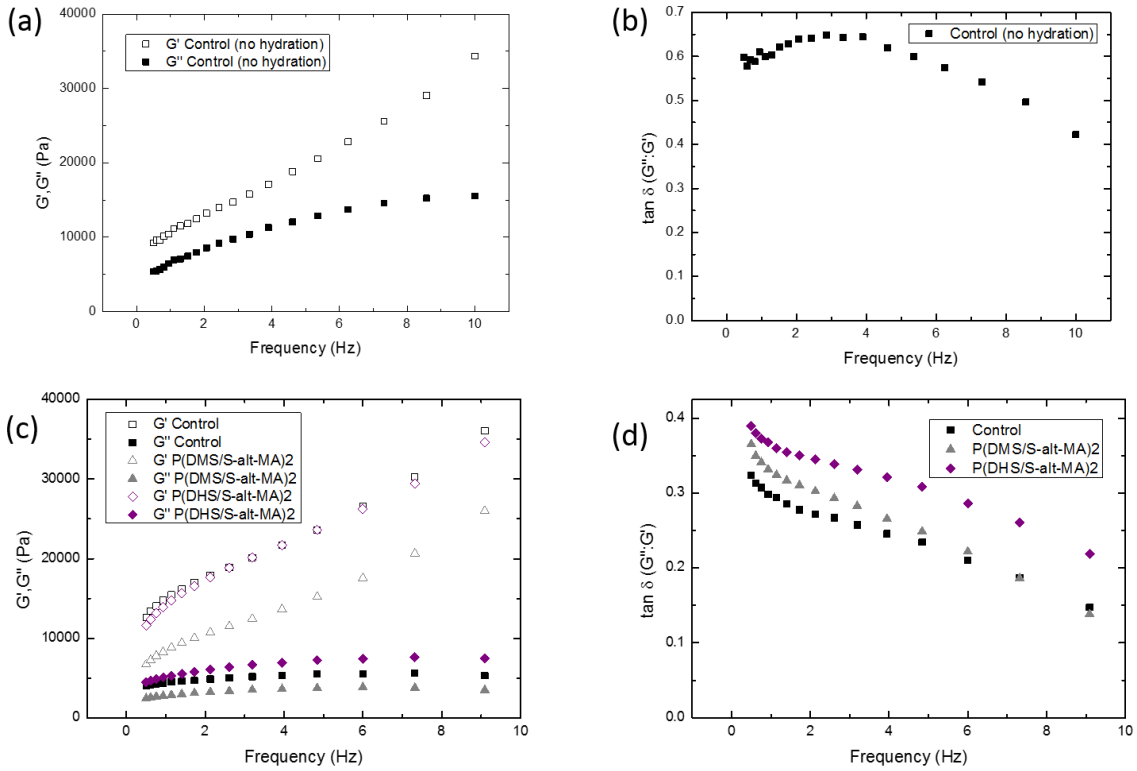


Figure 6. Elastic (G′) and viscous modulus (G″) as a function of frequency of (a) control with no hydration and (c) control, P(DMS/S-*alt*-MA)2 and P(DHS/S-MA)2 hydrated. Tan δ as a function of frequency of (b) control with no hydration and (d) control, P(DMS/S-*alt*-MA)2 and P(DHS/S-MA)2 hydrated.

The rheological properties (viscosity and flow) of denture adhesives have been studied and optimized for patient use, convenience and comfort [3, 32, 48, 49]. Figure 6 shows examples of the rheological behavior of this family of adhesive materials. The elastic (G′) and viscous (G″) components of the non-hydrated control sample are shown as a function of oscillation frequency in Figure 6a. Figure 6b shows the corresponding values of tan delta (G″/G′), and gives a useful indication of the relative dominance of the two components over a frequency range relevant to dental applications. Figure 6c and d show test data for the hydrated control material under the same conditions and additionally for the experimental P(DMS/S-*alt*-MA)2 and P(DHS/S-*alt*-MA)2 adhesives. The spectrum of results in Figure 6 clearly illustrates very similar rheological profiles of the test and control samples. Throughout the frequency range, values of G′ > exceed those of G″, and values of the phase angle (tan δ) are consistently below unity. Examination of the G′ and G″ values for the non-hydrated and hydrated samples neatly demonstrates important underlying trends. The addition of water reduces the viscosity (by around 50% at 4 Hz) in the value of G″ whilst in contrast, the elastic (G′) component is somewhat enhanced, reflecting the bridging ability associated with the strongly hydrogen bonded water phase.

Discussion

Denture fixatives are required to form temporary adhesive bonds between the denture and the denture-bearing oral mucosa in a saliva-rich environment [33, 50]. The over-riding aim of this study was to harness the adhesive capabilities of the catechol group, which has been shown to be the key component driving the function of sub-aquatic mussel adhesive proteins. It was important to assess differential adhesion of the formulations against both hydrated (TM) and non-hydrated (PMMA) interfaces.

Catechol groups were introduced to a composition based on a non-zinc commerical denture adhesive formulation by use of poly(styrene-*alt*-maleic acid) copolymers in which the styrene moiety was progressively replaced by the dimethoxystyrene (DMS) intermediate that was, in turn, converted to the dihydroxy (catechol) form (DHS). The adhesive capabilies of these variants were tested after hydration to mimic their behaviour in the oral environment where these products rely on physiochemical interactions with saliva to activate their tack properties. When used with PMMA/PMMA substrates, the DMS-containing samples showed higher adhesion strength, even more so when mixed with mucin-solution. These materials gave cohesive failures with both aqueous phases as highlighted in Table 2, which is attributed to the more hydrophobic character of P(DMS/S-*alt*-MA) and hydrophobic interactions at the PMMA interface.

Although the detailed biochemistry of the oral mucosal surfaces is extremely complex, very useful partial synthetic analogues can be produced. The aim here was that they should mimic the highly hydrated nature of the natural tissue, contain a significant concentration of amide and hydroxyl groups, reflecting the protein and carbohydrate content of tissue surfaces and, importantly, possess adequate mechanical strength to enable lap-shear adhesion measurements to be carried out. Hydrated functionalized hydrogel tissue models were prepared by UV polymerization of HEA and HMAA monomers in sheet form. HPMA was also included, to modulate the equilibrium water content and surface hydropobicity of the hydrogel with respect to wettability values of the oral tissue reported in the literature [51, 52]. When one of the PMMA substrates was replaced with the tissue mimic (TM) the effect of the catechol moiety providing adhesive sites in the significantly hydrated

environment was evident and lead to the biomimetic adhesives showing higher adhesion strength than the other polymeric variants.

It is logical to expect that in parallel with variations in natural systems, the balance between PDHS and the more hydrophobic styrene will be a significant factor in influencing adhesion behavior. Amongst the variants studied here, P(DHS/S-*alt*-MA)2 appears to represent a favorable balance in the DHS:S ratio; the adhesive strength of this material (11.4 ± 0.8 kPa) when hydrated with mucin-rich medium in the *in vitro* setup was much greater than the control and P(DMS/S-*alt*-MA) variants ($p < 0.05$). All catechol-bearing systems when mixed with mucin-rich solution produced adhesive failures, regardless of the nature of the substrates used and this observation could be linked to the cohesive interaction of catechol moieties and mucin molecules. The DOPA-mucin interaction force has been shown to be twice that between a mucin peptide fragment and its corresponding antibody [53]. All adhesive variants produced failures at the tissue mimic interface, which is the preferred outcome for *in vivo* applications.

The presence (and amount) of saliva will directly impact upon the bond strength of the adhesives. This factor is important at the initial application stage of the prosthesis to the oral mucosa as a sufficent layer of saliva is required to “activate” the denture adhesive and the initial tack will influence the overall performance throughout the day. However, the adhesives are also prone to dilution and this factor will effect their retentive properties [29, 54]. The quality and quantity of saliva vary amongst the denture-wearing population, particularly amongst those that suffer from Xerostomia, Sjogren’s syndrome or Sialorrhoea [55, 56]. The results in Figure 5 highlight that out of all the samples tested, only the catechol-bearing adhesive could deliver substantial adhesion at higher levels of hydration.

The PDHS-based materials revealed a set of colour changes during the hydrolysis of the maleic anhyride component as shown in Figure 2b. The polymer behaves in a similar way and is structurally reminiscent of phenolphthalein which is colorless in acidic solution and pink under basic conditions. This observation is associated with the catechol moiety in the PDHS-maleic copolymer over a pH range corresponding to proton removal from the acidic hydroxyl groups leading to enhanced conjugation. As a direct conseqeunce of this pH-dependent color change of PDHS, the biomimetic adhesives were ultimately purple whereas the control and P(DMS/S-*alt*-MA)-modified adhesives were off-white, as illustrated in Figure 2c. Interestingly, the color (that is to say the intensity of pink-purple) of the final adhesive formulation can be controlled by controlling the pH when hydrolysing the maleic anhydride (Figure 2b). This phenomenon is potentially advantageous for denture adhesive applications, which conventially contain colorants, to render them more aesthetically pleasing to the user.

The sensory effects associated with the use of denture adhesives are also an important factor to consider. The rheological properties undergo a number of physical transitions during use and have been evolved to optimize patient acceptance. Generally, products are supplied as a low viscosity pastes to facilitate application to the denture [32, 57]. As the polymers interact with and absorb saliva, the adhesive swells to provide a cushioning effect, decreasing mechanical trauma to the mucosal tissue which is commonly thinned by age [58]. The behavior of the biomimetic adhesives showed similar rheological profiles to that of the control sample. In all cases the viscous component was lower in magnitude than the elastic component but significantly the change from the non-hydrated to hydrated state of the control led to a decrease in viscous (G”) component but an increase in elastic (G’)

behavior. This corresponds to the desirable characteristics of adhesives, from easy initial application to the elastic, cushion-like feel after equilibration in the saliva-rich environment. It is an important target feature of any biomimetic adhesive that it should match the rheological behavior of established adhesives.

3. Conclusions

The unique chemistry of mussel adhesive proteins has been exploited in a polymeric system that is able to modulate adhesion to that of a control formulation in a specialized denture adhesive under wet conditions that reflect those in the oral cavity. A systematic approach was employed to prepare a family of bio-inspired polymers, which were then incorporated in an adhesive formulation based on non-zinc containing current commercial denture adhesive products. Whilst the P(DMS/S-*alt*-MA) variants were able to deliver high bonding strength when placed between two PMMA plates, the biomimetic P(DHS/S-*alt*-MA) family was the only material able to deliver significant adhesion when a hydrated tissue mimic was used as one of the substrates. The P(DHS/S-*alt*-MA)-based system was also the better adhesive candidate across all moisture levels. The catechol-containing adhesives also produced more adhesive failures when hydrated with mucin-rich solution (in comparison to DI water). This study has provided an experimental platform towards the design and development of biomimetic prosthodontic tissue adhesives for the oral environment.

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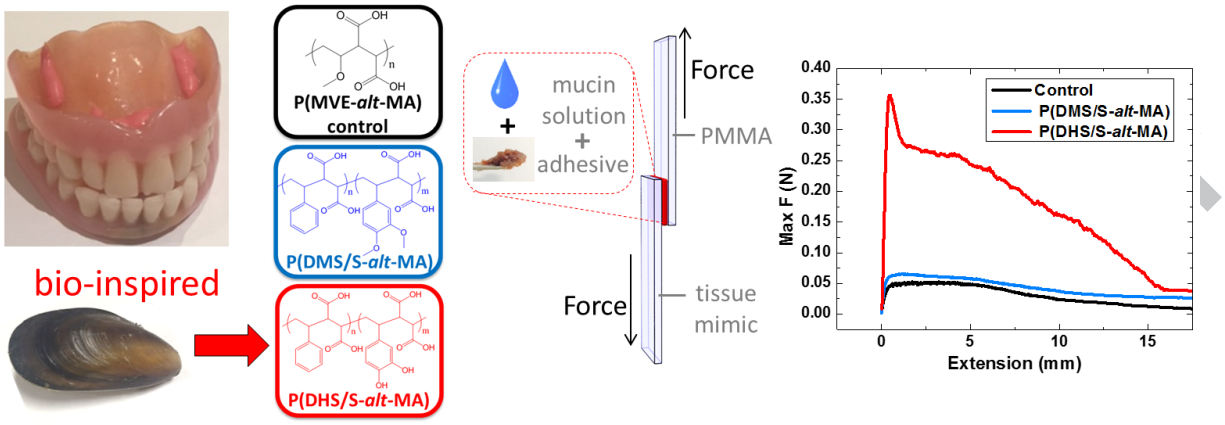

Statement of Significance

Mussel adhesive proteins have proven to be promising biomimetic adhesive candidates for soft tissues and here for the first time we have adapted marine adhesive technology into a denture fixative application. Importantly, we have incorporated a soft tissue mimic in our *in vitro* adhesion technique that more closely resembles the oral mucosa than previously studied substrates. The novel biomimetic-modified adhesives showed the ability to score the highest adhesive bonding out of all the formulations included in this study, across all moisture levels.

This paper will be of major interest to the Acta Biomaterialia readership since the study has illustrated the potential of biomimetic principles in the design of effective prosthodontic tissue adhesives in a series of purpose-designed *in vitro* experiments in the context of the challenging features of the oral environment.