Supplementary Material (ESI) for Soft Matter

Developing synthetic mucosa-mimetic hydrogels to replace animal experimentation in characterisation of mucoadhesive drug delivery systems

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Experimental Details

Materials

Acetonitrile, acryloyl chloride, ammonium persulfate, ethanol, D-glucosamine hydrochloride, 2-hydroxyethyl methacrylate, 2-hydroxyethyl acrylate, N,N'-methylenebisacrylamide, N,N,N',N'-tetramethylethylenediamine and N-vinyl pyrrolidone were used as received from Sigma-Aldrich (UK). Potassium carbonate was used as received from Fisher Scientific (UK). Sorbitol methacrylate was purchased from ABCR (Germany) and used without further purification.

Synthesis of N-acryloyl-D-glucosamine

N-acryloyl-D-glucosamine was synthesised following the method described by Matsuda et al.[1] D-Glucosamine hydrochloride (8.60 g, 40 mmol) and $NaNO_2$ (0.14 g, 2.5 mmol) were dissolved in a stirred 2M aqueous solution of K_2CO_3 (20 mL). The reaction mixture was cooled to 0 ^oC in a salt and ice bath and acryloyl chloride (4.00 g, 73 mmol) added drop wise, with vigorous stirring while maintaining a reaction temperature below 5 ^oC. After 3 h, the reaction was slowly warmed to room temperature over 24 h with stirring, then poured into 200 mL of absolute ethanol, refrigerated overnight and the precipitated salts filtered off. The solution was concentrated under vacuum and the product was purified by recrystallisation with methanol/ethyl acetate/diethyl ether. The product yield

was 69 %. The structure of the resulting monomer was confirmed by ^{1}H NMR spectroscopy giving signals in agreement with the literature data.[1]

Synthesis of hydrogels

Ammonium persulfate (APS), water, N,N,N',N'-tetramethylethylenediamine (TMEDA), monomer(s) and N,N'-methylenebisacrylamide (MBA) were added to glass vials in the quantities listed in Table S1. The mixtures were vortexed until complete dissolution of all ingredients. Ethanol was then added before being mixed again and the mixtures were bubbled for 5 minutes with nitrogen. The vials were then sealed and placed in a preheated water bath at 60 ^{o}C to initiate polymerisation. Polymerisation was terminated by cooling the reaction mixture down with cold water after 3 hours. The hydrogels were purified by immersing samples in deionised water, which was changed daily, for two weeks to remove any unreacted chemicals.

Table S1. Composition of feed mixtures for synthesis of hydrogels

Sample	Co-monomer(s)	HEMA/co-monomer(s) ratio, mol %	MBA content, mol %	HEMA, g	Co-monomers(s), g	APS, g	TMEDA, g	MBA, g	Ethanol, mL	Water, mL
1	-	100:0	0.01	4.3337	-	0.0380	0.0116	0.0005	5.25	5.25
2	-	100:0	0.1	4.3337	-	0.0380	0.0116	0.0052	5.25	5.25
3	-	100:0	0.5	4.3337	-	0.0380	0.0116	0.0257	5.25	5.25
4	-	100:0	1.0	4.3337	-	0.0380	0.0116	0.0513	5.25	5.25
5	NVP	70:30	0.1	3.0323	1.1114	0.0380	0.0116	0.0052	5.25	5.25
6	NVP	90:10	0.01	3.9050	0.3668	0.0380	0.0116	0.0005	5.25	5.25
7	NVP	50:50	0.1	2.1668	1.8505	0.0380	0.0116	0.0052	5.25	5.25
8	HEA	70:30	0.1	3.0323	1.1612	0.0380	0.0116	0.0052	5.25	5.25
9	HEA	90:10	0.1	3.9050	0.3832	0.0380	0.0116	0.0052	5.25	5.25
10	SMA	95:5	0.01	4.1124	0.4016	0.0380	0.0116	0.0005	5.25	5.25
11	SMA	90:10	0.1	3.9050	0.7874	0.0380	0.0116	0.0052	5.25	5.25
12	SMA	80:20	0.1	3.4711	1.5591	0.0380	0.0116	0.0052	5.25	5.25
13	SMA	70:30	0.1	3.0323	2.3622	0.0380	0.0116	0.0052	5.25	5.25
14	NVP:SMA	60:20:20	1	2.6028	0.7335/1.5591	0.0380	0.0116	0.0513	5.25	5.25
15	AGA	90:10	0.1	3.9050	0.7695	0.0380	0.0116	0.0052	5.25	5.25
16	AGA	85:15	0.1	3.6830	1.1661	0.0380	0.0116	0.0052	5.25	5.25
17	AGA	80:20	0.1	3.4711	1.5393	0.0380	0.0116	0.0052	5.25	5.25
18	AGA	70:30	0.1	3.0323	2.3322	0.0380	0.0116	0.0052	5.25	5.25
19	NVP:AGA	80:10:10	0.1	3.4711	0.3668/0.7696	0.0380	0.0116	0.0052	5.25	5.25

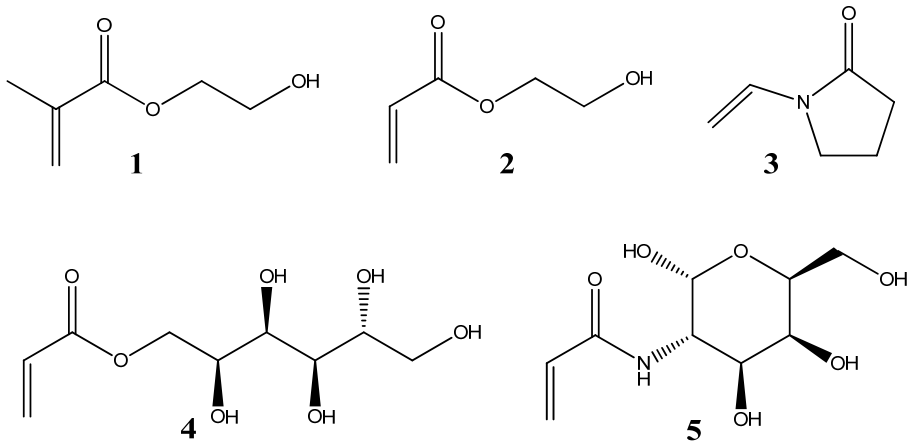


Figure S1. Monomers used to synthesise hydrogels: HEMA (1), HEA (2), NVP (3), SMA (4) and AGA (5).

Mucoadhesive tablet preparation

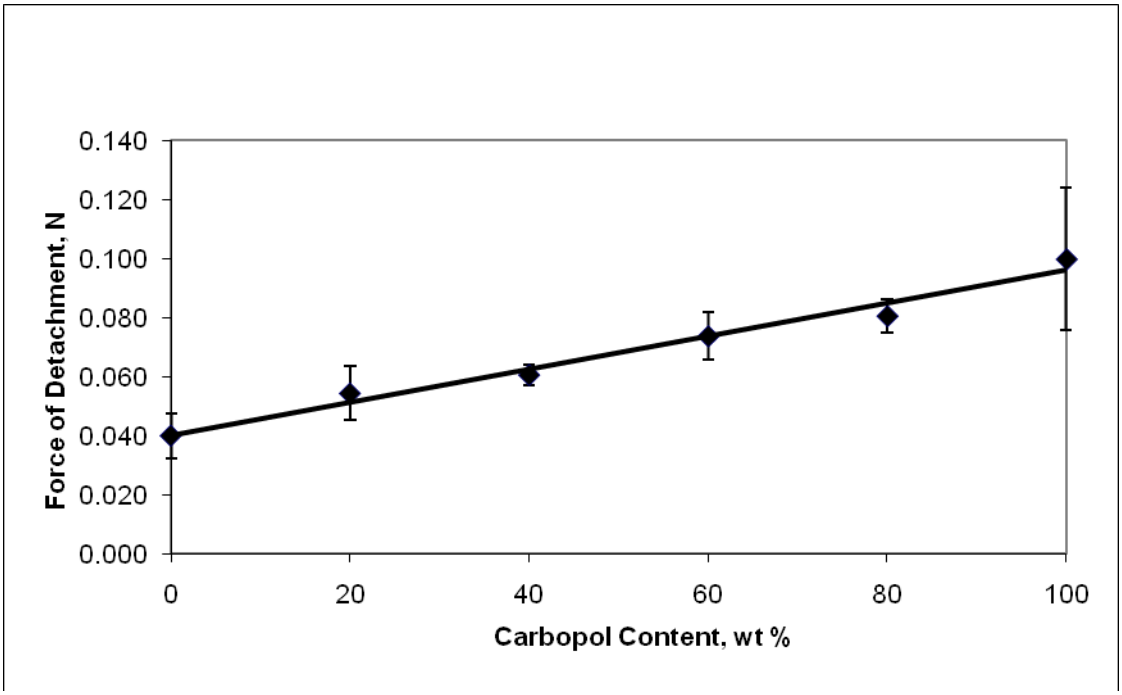
Model mucoadhesive tablets were prepared by direct compression of powder mixtures. Blends of Carbopol® 940, HPMC and 2.5% w/w magnesium stearate (used as a lubricant) were mixed together in a Willy A. Bachofen AG Maschinenfabrik mixer (Switzerland) and then compressed using a Riva SA Minipress MII single punch tablet press (Argentina). The hardness of the tablets was assessed using a 6D tablet tester, Copley Scientific (UK) and their dimensions measured with a Fowler IP 54 digital micrometer (USA).The tablets had the following characteristics: 44.2 ± 1.1 mg in weight, diameter of 6.00 ± 0.01 mm, thickness of 2.8 ± 0.1mm and hardness of 29.0 ± 3.6 N.

Adhesion testing

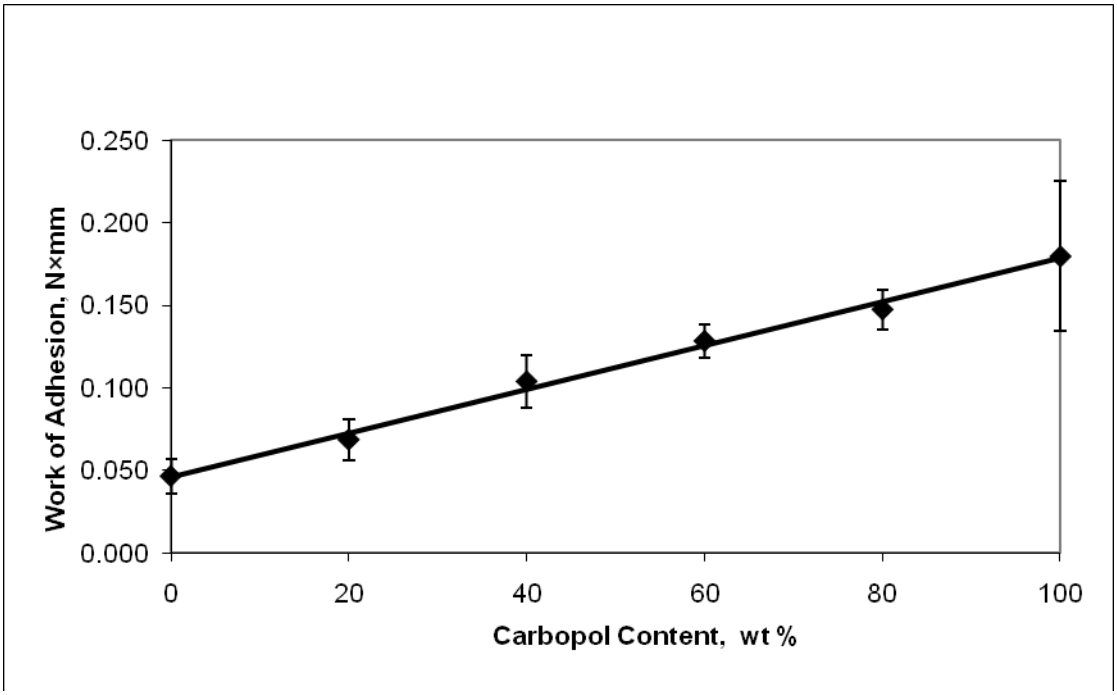
The adhesive properties of the tablets to the hydrogels were assessed using a TA.XT Plus texture analyser (Stable Micro Systems, UK). The hydrated hydrogels were clamped into a circular holder, immersed in deionised water covering to a depth of 1-2 mm above the hydrogel surface and

equilibrated at 37 ±1 °C for 0.5 h. The Carbopol®940/HPMC tablets were attached to a mobile probe (cylindrical, P/6) using double sided adhesive tape. The probe was lowered at a speed of 1 mm/s until it reached the hydrogel surface, where it was left in contact for 1 min with a contact force of 0.1 N applied. The probe was then withdrawn at a rate of 0.05 mm/s until complete detachment of the tablet from the hydrogel was observed. The maximum force of detachment and the work of adhesion (the area under the force/distance curve) were determined using Texture Exponent 32 software (Stable Micro Systems, UK). All measurements were performed at least 3 times and the adhesion parameters calculated as mean values ± standard deviation.

Adhesion of tablets to animal mucosal tissues were studied using porcine buccal mucosa taken from female Great White pigs weighing 65–75 kg, which were obtained from MutchMeats Ltd (UK). These tissues were collected immediately after the slaughter of animals and were stored frozen at -20 ºC. Before testing, the mucosal tissues were defrosted in water at 35–37 ºC. The detachment characteristics of mucoadhesive tablets from porcine buccal mucosa are summarised in Figure S2.



(a)



(b)

Figure S2. Force of detachment (a) and total work of adhesion (b) for mucoadhesive tablets from porcine buccal mucosa vs Carbopol®940 content in the tablets.

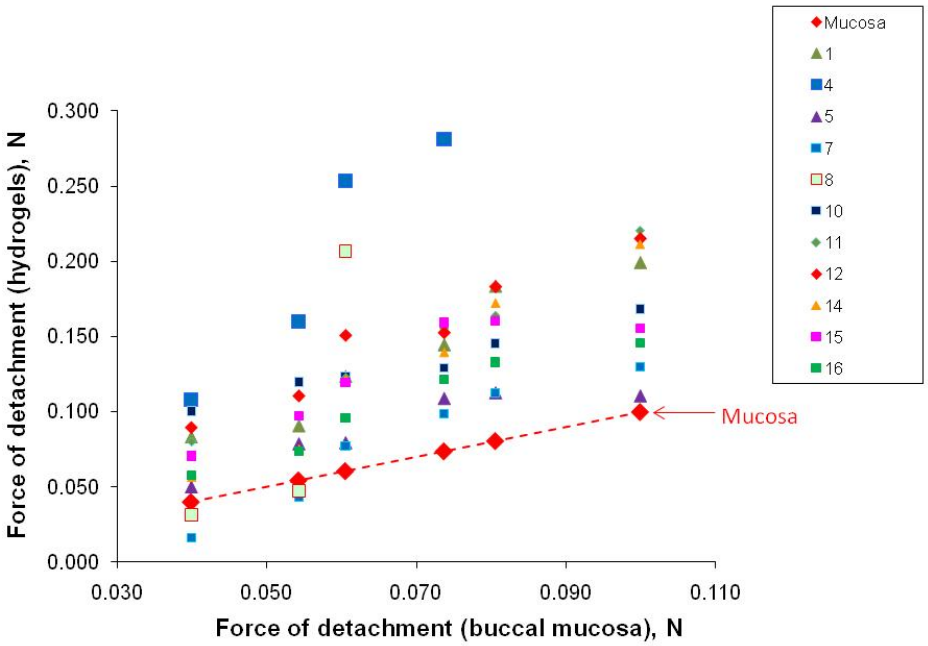


Figure S3. Correlation between F_{det} determined for detaching mucoadhesive tablets from synthetic hydrogels and from buccal mucosa (samples not included in Figure 1).

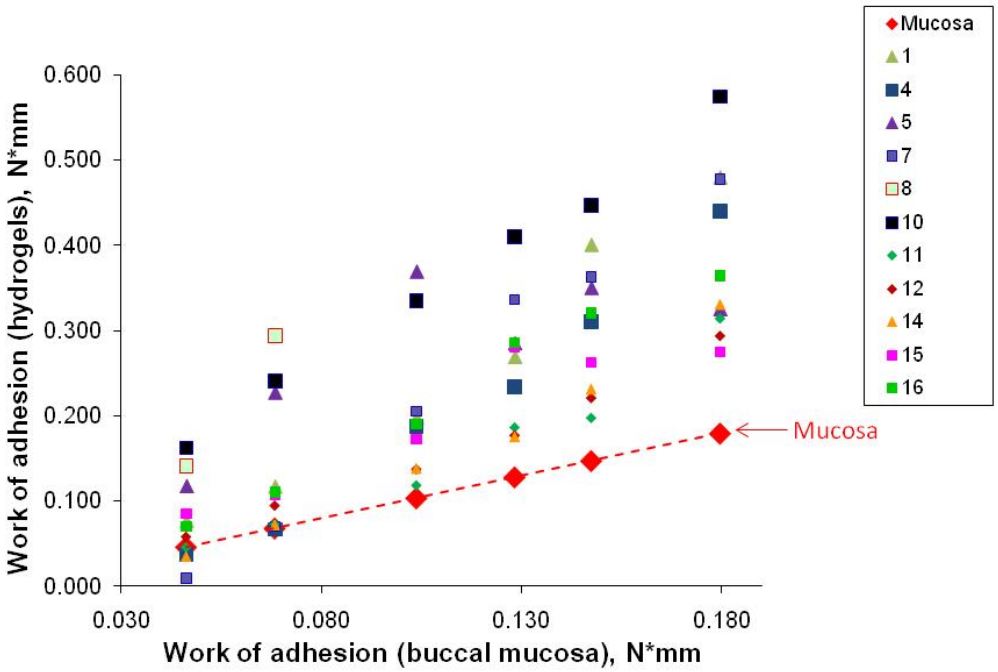


Figure S4. Correlation between W_{adh} determined for detaching mucoadhesive tablets from synthetic hydrogels and buccal mucosa (samples not included in Figure 2).

Analysis of hydrogel water content

The equilibrium swelling degrees (ESDs) of hydrogels were determined by weighing the swollen samples with subsequent freeze-drying to obtain the weight of dry polymer. Each experiment on characterisation of swelling properties was repeated at least 3 times for every hydrogel and the ESDs were calculated as mean values ± standard deviation using the following formula:

$$ESD = (W_s - W_d)/W_d,$$

where W_s and W_d are the weights of the swollen and dry sample, respectively.

Ability of hydrogels with different degree of swelling to mimic mucosa

To establish the effect of hydrogels' swelling degree on their ability to mimic biological mucosa the work of adhesion difference (ΔW_{adh}) was calculated according to the following formula:

$$\Delta W_{adh} = W_{adh}\,(\text{hydrogel}) - W_{adh}\,(\text{mucosa})$$

This difference can be used as an indication of the ability of a hydrogel to mimic biological mucosa. Perfect mimicking properties are expected when $\Delta W_{adh} = 0$. The dependence of ΔW_{adh} on hydrogels' ESD values is shown in Figure S5.

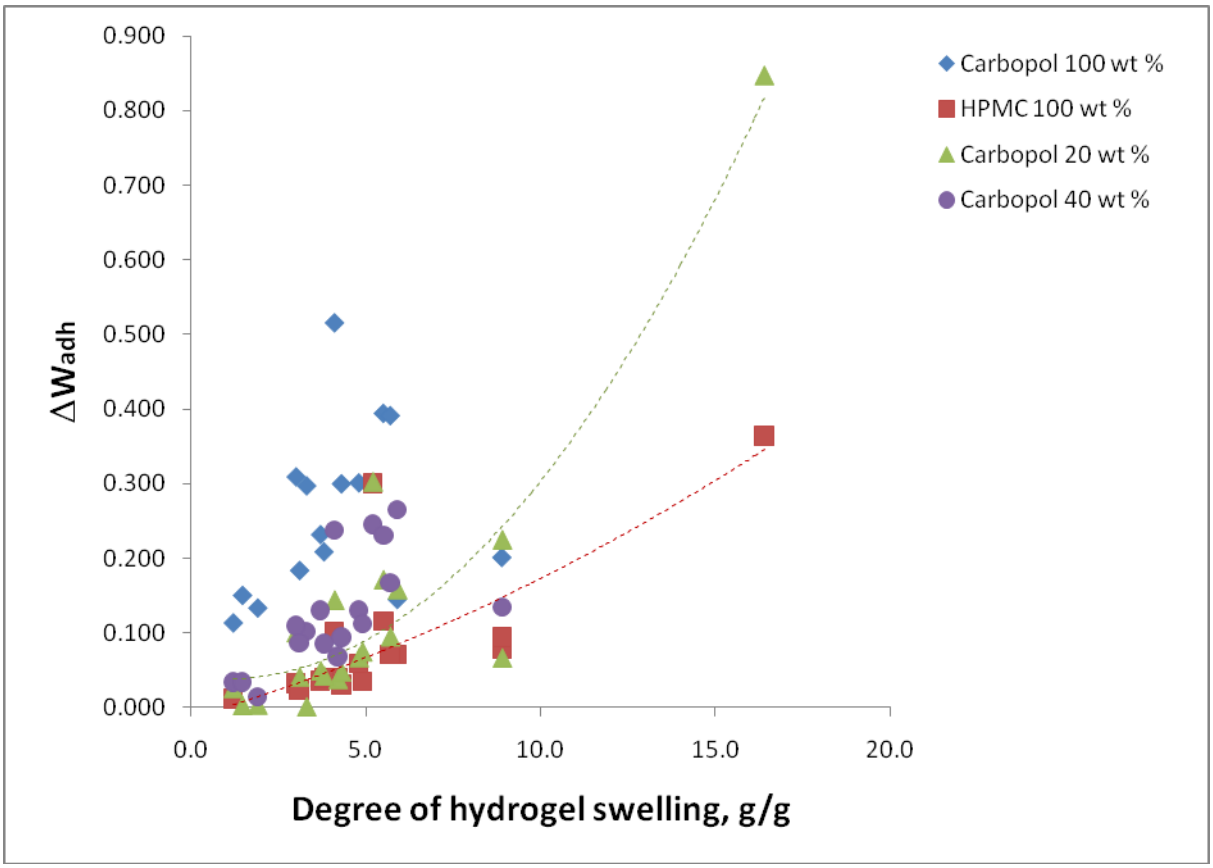


Figure S5. Work of adhesion difference (ΔW_{adh}) as a function of hydrogels' equilibrium swelling degree.

References

1. T. Matsuda, T. Sugawara, *Macromolecules*, 1996, **29**, 5375.