

Mucoadhesion and mucosa-mimetic materials – a mini-review

Michael T. Cook^{a*} and Vitaliy V. Khutoryanskiy^{b**}

5 ^aDepartment of Pharmacy, University of Hertfordshire, Hatfield, AL10 9AB, U.K.;

^bSchool of Pharmacy, University of Reading, Reading, RG6 6AD, U.K.;

*E-mail: M.Cook5@Herts.ac.uk

Tel: +44 (0)1707 283439

**E-mail V.Khutoryanskiy@Reading.ac.uk

10 Tel: +44 (0) 118 378 6119

Abstract

Mucoadhesion describes an attractive interaction between dosage form and mucosal membrane.

15 The evaluation of mucoadhesive excipients often requires the use of *ex vivo* mucosal tissues taken from laboratory animals. These can be difficult to source, highly heterogeneous, and require the use of animal products. Thus, from both a user-convenience and ethical point-of-view, it is desirable to produce a synthetic alternative to these tissues – a mucosa-mimetic material. In this mini-review, the use of alternative materials to test the performance of mucoadhesives is reviewed and
20 discussed. There is a surprising prevalence of the use of mucosa-mimics in the literature, which hitherto has not been compiled and compared.

Keywords

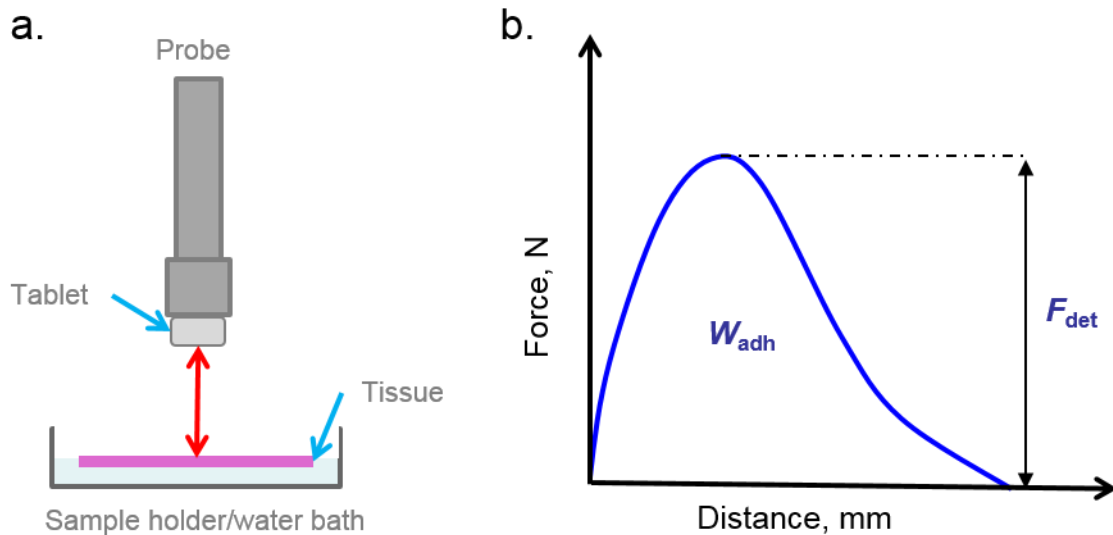
Mucoadhesive, mucus-like, mucous, topical drug delivery, biomimetic, biomimicry

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1. Introduction

In order for drugs to be effectively absorbed by the body they will often have to pass through a mucosal surface in the body. These surfaces cover the eyes, GI tract, and nose, as well as parts of the reproductive organs. The rate of diffusion of drugs may be low across these surfaces, which can lower the bioavailability of an administered medicine. Additionally, retention on the mucosal surface may be very low, as in the eye, where many drugs are quickly eliminated via the lacrimal gland (Urtti, 2006). To address this, a mucoadhesive may be used. Mucoadhesive materials have a strong affinity for mucosal surfaces and adhere to the surface of these tissues. Drugs may be physically or chemically bound to these mucoadhesives in order to increase their residence time at a specific location in the body. Retaining a drug at a mucosal surface allows for improved absorption of the drug at the intended site of delivery, due to the longer times over which uptake through the tissue may occur. Additionally, the mucoadhesive effect allows for site-specific delivery of drugs to the mucosa. Mucoadhesive materials may take many forms, such as: tablets for buccal delivery (Nafee et al., 2004), *in situ* gelling systems for ocular drug delivery (Ludwig, 2005), microgels for intravesicular administration (Cook et al., 2015), or nanoparticles targeting the GI tract (Sakuma et al., 1999).

In order to assess mucoadhesion, many different techniques have been developed. The difficulty in the assessment of mucoadhesion is that there are various different dosage forms which require different experimental conditions for testing which are usually only comparative between their own samples, thus there appears to be a degree of heterogeneity between techniques. Solid dosage forms, such as tablets, will often have their mucoadhesive properties assessed by measurement of the force needed to remove the tablet from an *ex vivo* mucosal surface (Hall et al., 2011). This gives a force-displacement curve from which the maximum force of detachment and work of adhesion (area under curve) can be determined (Figure 1).



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Figure 1: A probe, usually from a texture analyser, with a tablet attached is pressed against *ex vivo* mucosa and detached (a). This gives a force-displacement curve (b), from which the maximum force of detachment (F_{det}) and work of adhesion (W_{adh}) can be determined. The detachment profile is also characteristic for each substrate (Khutoryanskaya et al., 2010).

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Liquid dosage forms, however, are clearly not suited to this technique, and mucoadhesion can be measured by alternative techniques, such as rheology (Hassan and Gallo, 1990), or using a flow-through system with HPLC analysis (Friedl et al., 2013) or by fluorescently labelling the mucoadhesive (Irmukhametova et al., 2011; Withers et al., 2013). A schematic of the flow-through method is shown in Figure 2. Generally, this experiment is set up with a channel, onto which *ex vivo* mucosal tissue is placed (Figure 2a). A liquid or semi-solid preparation may then be placed onto the mucosa (Figure 2b), which may or may not contain a drug. A simulated biological solution is then washed over the surface using a suitable pumping system (Figure 2c), which is collected and analysed to quantify the concentration of mucoadhesive or drug, using standard analytical techniques such as HPLC. Alternatively, the mucoadhesive may be dyed and images taken to show retention of solution on the mucosa directly.

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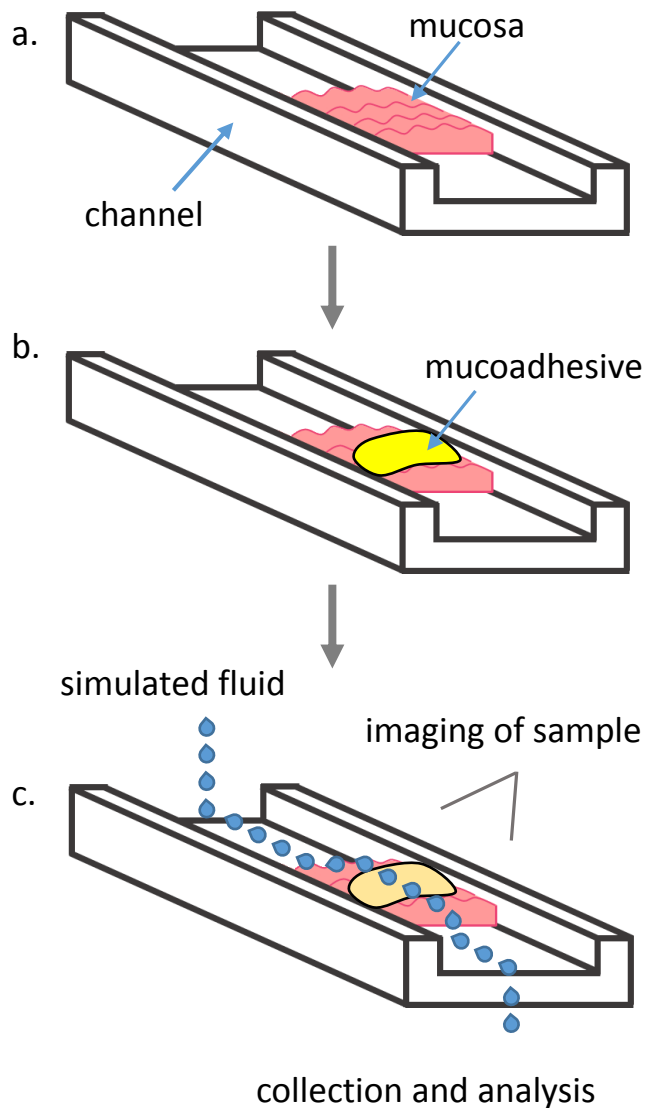


Figure 2. An example flow-through experiment for assessing mucoadhesion.

Common throughout the methods used for assessment of mucoadhesion is the use of *ex vivo* tissues. Many of these tissues come from lab animals specifically slaughtered for their mucosa. Thus, in the interest of animal ethics and the three R's (Hobson-West, 2009), there is a need to reduce the number of animals killed for this tissue. One possible approach for achieving this is the production of a synthetic material to simulate the *ex vivo* mucosal tissues used. There has recently been the

75 emergence of research in this area of 'mucosa-mimetic' materials, and the research conducted to date will be evaluated within this mini-review.

In order to discuss the latest advances in mucoadhesion and mucosa-mimetic materials, it is important to have a good understanding of the structure of mucosal surfaces, and an understanding of the interactions that mediate mucoadhesion. Thus, this review will first of all discuss mucosal membranes, with a focus on mucins, the glycoproteins which cover mucosal surfaces. The latest 80 understanding on how mucoadhesive interactions occur will then be discussed. Finally, the research conducted to date on mucosa-mimetic materials will be summarised and discussed. Previous reviews have described the variety of mucoadhesive materials discovered (Bernkop-Schnürch and Greimel, 2005; Khutoryanskiy, 2011; Sosnik et al., 2014), so this will not be covered herein.

2. The structure of mucins and mucus layers

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In order to study mucoadhesion, it is vital to have a good knowledge of the structure of mucosal surfaces in the body. Almost all of these surfaces have some mucus component, the nature and thickness of which depends on the location in the body. The layer of mucus plays an important role as a diffusion barrier for various nutrients, drugs and parasites, and binds to bacteria, slowing the 90 invasion of pathogens (Moncada et al., 2003). This mucus layer is generally comprised of water (up to 95 % w/w), mucin (< 5 % w/w), salts (~ 1 % w/w), carbohydrates and lipids (Peppas and Huang, 2004). The mucin found within this layer represents the primary organic component of the gel. Mucins are a group of glycoproteins coded for by *MUC* genes, after which the proteins are named (Dekker et al., 2002). It is important to note that these mucins can be either membrane-bound or 95 secreted; the latter make up the viscoelastic, shear-thinning, gel on epithelial surfaces. There is a large heterogeneity within the mucin family of proteins. For instance, MUC1 is a trans-membrane protein expressed by epithelial cells lining reproductive tracts (Brayman et al., 2004), whereas MUC2

is secreted by goblet cells in the colon and small intestine (Allen et al., 1998). MUC2 is the most abundant secreted mucin in the intestines. MUC4 is a tracheobronchial mucin (of the airways), along
100 with MUC5(AC and B) (Campbell, 1999). MUC5AC and MUC7 make up the major salivary mucins (Thomsson et al., 2002), whilst the stomach is primarily lined with MUC6 (Campbell, 1999). This diversity in secreted mucins adds an additional complexity to the understanding of mucoadhesion.

Structurally, mucins are a family of glycoproteins, with molecular weights from 0.2 to >50 MDa (Berry et al., 1996; Sigurdsson et al., 2013). These proteins contain a central region with an
105 abundance of proline, threonine and serine amino acids (the 'PTS region'), of which, threonine and serine are O-glycosylated, giving the PTS region an extended and stiff, "bottle-brush", conformation (Johansson et al., 2011). Some mucins contain non-PTS regions flanking this core, some of which contain cysteine residues (Dekker et al., 2002), and therefore possess thiol groups. These unglycosylated blocks of protein, may be responsible for intermolecular association of mucins, and
110 concomitant gel formation, through the formation of disulphide bonds (Ensign et al., 2012). Due to the unglycosylated regions of mucins being hydrophobic, it has been suggested that these blocks form segregated domains (Peppas and Huang, 2004). Transmembrane mucins contain hydrophobic domains to assist in their tethering to cell surfaces (Campbell, 1999). MUC2, MUC5AC, MUC5C, and MUC6 are the most common secreted mucins, and show significant homology in their non-PTS
115 domains (Dekker et al., 2002). The O-linked oligosaccharide chains adorning the PTS region are from 2-12 sugars long (Bansil et al., 1995), and are typically composed of galactose, fucose, acetyl-D-glucosamine, acetyl-D-galactosamine, and sialic acid. These oligosaccharides dominate the structure of mucin, comprising 50-80 % of the dry weight (Campbell, 1999). There is also the presence of some N-linked oligosaccharides in the non-PTS region, though these are less abundant (Campbell, 1999).
120 An overview of the structure of two mucins is shown in Figure 3.

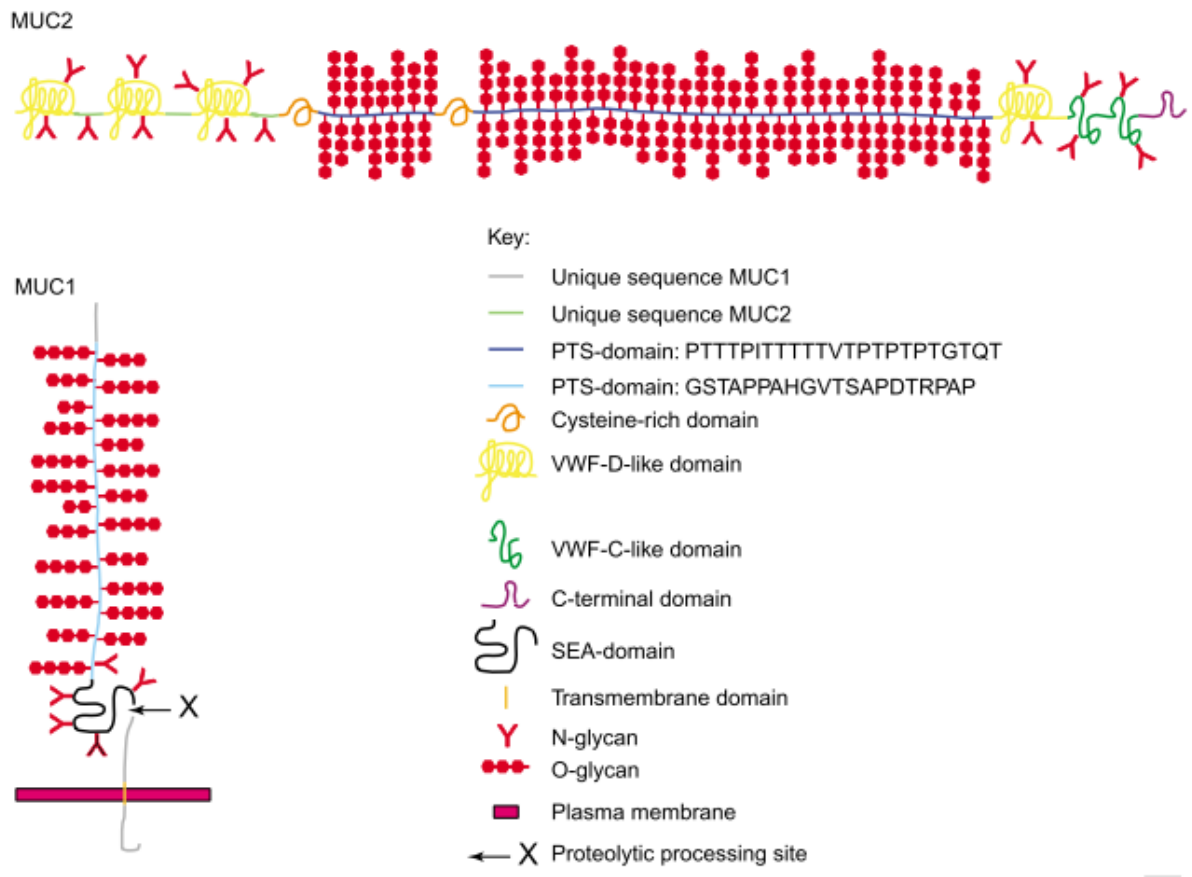


Figure 3. An overview of the structure of a secretory mucin (MUC2) and a transmembrane mucin (MUC1). These images demonstrate the structural heterogeneity between different mucins. Note the presence of cysteine-rich domains in MUC2, and large regions of O-glycosylation on the PTS region of each protein. Figure reprinted from Dekker et al. (2002) with permission from Elsevier (license number: 3683131023038)

From a mucoadhesion standpoint, these oligosaccharides provide potential for hydrogen bonding, and electrostatic interaction with the carboxylic functionality of the sialic acid. Additionally, some sulfated sugars have been identified at the terminus of the oligosaccharides in mucins, primarily those mucins in the colon and respiratory tract, which provides another possible site of electrostatic interaction (Thomsson et al., 2002). The pK_a of the acidic groups in mucin is between 1 and 2.6 (Khutoryanskiy, 2011), and as a result, mucin carries a net negative charge at pHs above this (Bansil and Turner, 2006). It is important to note, therefore, that the degree of ionisation of the mucin

oligosaccharides will greatly decrease as the pH approaches pH 1, as in the stomach. This loss of
135 ionisation will likely have a bearing on the interaction of materials with mucin.

Across the gastrointestinal (GI) tract, there is an abundance of surfaces bearing secreted mucus
layers. These layers have been separated into 'firmly' and 'loosely' adherent layers, the thickness of
which varies throughout the GI tract (Ensign et al., 2012). The thickest secreted mucus layers in
humans are found in the stomach and colon, at 40-450 μm and 110-160 μm , respectively. Depending
140 on the location in the gastrointestinal tract, the pH of the digestive milieu will vary heavily, from pH
1-2 in the stomach, up to around pH 7 in the colon. The pH of the digestive fluid will almost certainly
affect the interaction of dosage forms with mucosal surfaces (Zhu et al., 2004), so should be
accounted for when determining mucoadhesion.

3. Why are some materials mucoadhesive? 145

Mucoadhesion is a complex phenomenon, which is governed by many types of interaction
(Khutoryanskiy, 2011, 2014). These may be specific chemical interactions, such as hydrogen-
bonding, or rheological effects, as you may expect for any adhesive. Thus, there is some variation in
the specificity of mucoadhesion. Several different theories have been suggested that may explain
150 mucoadhesive interactions, most of which are not mutually exclusive. These have been summarised
in previous reviews (Andrews et al., 2009; Khutoryanskiy, 2011; Peppas and Huang, 2004; Smart,
2005), but are briefly outlined below.

In terms of general adhesive interactions, there have been some key effects identified (Smart, 2005).
Spreading of liquid dosage forms across mucosal tissue will be related to the liquids affinity to the
155 tissue, which is related to its contact angle. The roughness of mucosal surfaces will affect the
adhesion of liquid dosage forms after drying, due to interlocking of the tissue and material. Finally,
for solid dosage forms it has been suggested that the movement of water from the hydrated mucus
into the dosage form will assist in its adhesion to mucosal surfaces.

Several chemical interactions appear to play a part in mucoadhesion. This type of interaction will be
160 important for large dosage forms, such as tablets, but be much more crucial for particulates and
liquid dosage forms, as there is not the same propensity for swelling and wetting. These chemical
interactions are proposed to occur after a period of physical entanglement of the mucus and
polymer, which has been supported by rheological studies (Mortazavi, 1995). Peppas and Huang
(2004) have outlined the key interactions governing the mucoadhesion at the nanoscale, which will
165 hold for many liquid dosage forms based on polymers. These are: hydrogen bonding, electrostatic
interactions, hydrophobic interactions, and polymer chain interdiffusion. In addition to these quite
general interactions, there have been reports of materials which utilised specific chemical reactions
to form covalent linkages to mucins.

Hydrogen bonding to mucins is a well-researched area of mucoadhesion. There are various sites on
170 the mucin which are potentially hydrogen-bonding active, such as the hydroxyl groups on the
oligosaccharides covering the PTS region. These hydrogen-bonding sites will be associated with
water in solution, however, the ability to hydrogen-bond will be screened unless the hydrogen-
bonding interaction with a mucoadhesive is sufficiently strong. It has been suggested that many of
these hydrogen bonding effects are actually ion-dipole interactions between hydrogen bond donors
175 and ionic species (Peppas and Huang, 2004). This is due to the greater strength of this type of
interaction, relative to straightforward hydrogen-bonding (Mortazavi, 1995; Teague and Davis,
1999). It has been found that some materials, such as poly(acrylic acid) (PAA), have pH-dependent
mucoadhesion. This was attributed to hydrogen bonding at pHs below the pK_a of poly(acrylic acid)
(4.5), which was overcome by electrostatic repulsion as the carboxylic groups ionised at pHs higher
180 than 4-5 (Park and Robinson, 1987).

The presence of acidic saccharides and sulphate esters in the glycosylated regions of the mucin
appear to have an effect on the adhesion of polymers to the surface of mucus. As previously
mentioned, above approximately pH 2.6 the mucin carries a net negative charge, and given the

strength of electrostatic interactions, positively charged species can interact with the mucin. One
185 example is chitosan, which has shown to be mucoadhesive through a combination of effects, with
electrostatics identified as an important interaction (Sogias et al., 2008). Additionally, Bogataj et al.
(2003) found that there was a decrease in mucoadhesion as the zeta-potential of 3 polyelectrolytes
became increasingly negative. This result suggests that electrostatic repulsion may reduce
mucoadhesive effects.

190 Hydrophobicity plays a role in mucoadhesion. It has been shown that mucins can adsorb onto
hydrophobic surfaces, indicating interaction (Peppas and Huang, 2004). The so-called 'hydrophobic
effect' has been theorised to play a part in mucoadhesion. Briefly, this effect is caused by the loss of
entropy of water molecules as they associate to a hydrophobic macromolecule in solution.
Interaction between water molecules and the macromolecule will occur due to van der Waals forces,
195 however the enthalpic gain is not sufficient to make the free energy of the process favourable. This
causes nonpolar regions of macromolecules to aggregate, lowering the surface area accessible to
water. This effect has been seen experimentally when studying the interaction of chitosan and
mucin by isothermal titration calorimetry (Menchicchi et al., 2014). In addition to hydrophobic
effects between mucin and mucoadhesive, the lyophilicity of mucoadhesives affects their affinity for
200 aqueous solutions, so colloids with relatively poor colloidal stability may be driven onto the mucosa
more readily. This effect was identified as key in the mucoadhesion of two milk proteins to *ex vivo*
mucosal tissue (Withers et al., 2013).

Polymer chain interdiffusion and subsequent entanglement can aid mucoadhesion. This was first
introduced to the field by Peppas and coworkers (Jabbari et al., 1993; Peppas and Huang, 2004), who
205 also demonstrated that the incorporation of free polymer chains into a hydrogel film can promote its
mucoadhesion by movement of free polymer chains from hydrogel to mucosa (Sahlin and Peppas,
1997).

Specific chemical reactions on mucins may also be used to enhance mucoadhesion. Reaction with the thiol groups found in the cysteine residues of mucin has been utilised, due to the high reactivity of these groups (Bonengel and Bernkop-Schnürch, 2014; Iqbal et al., 2012). This sort of covalent attachment has been demonstrated as a very effective method of improving the retention of dosage forms. Most commonly polymers containing thiol groups, named 'thiomers' are used. The majority of this work has been conducted by Andreas Bernkop-Schnürch and coworkers. For instance, (Hornof et al., 2003) have reported that a thiolated polymer ocular insert was able to adhere to the human eye through the formation of disulphide linkages. This 'thiolation' is applicable to many polymers, using simple amide-coupling chemistry, which is possible in aqueous solvents (Bernkop-Schnürch and Greimel, 2005). These materials are very effective mucoadhesives, but are prone to oxidation, which has led to the development of 'preactivated' thiomers, which usually use a labile nicotinic acid moiety to protect the thiol groups from oxidation (Iqbal et al., 2012). Thiomers have recently started to make their way onto the market, with the first thiomers product expected to reach market shortly (Bonengel and Bernkop-Schnürch, 2014).

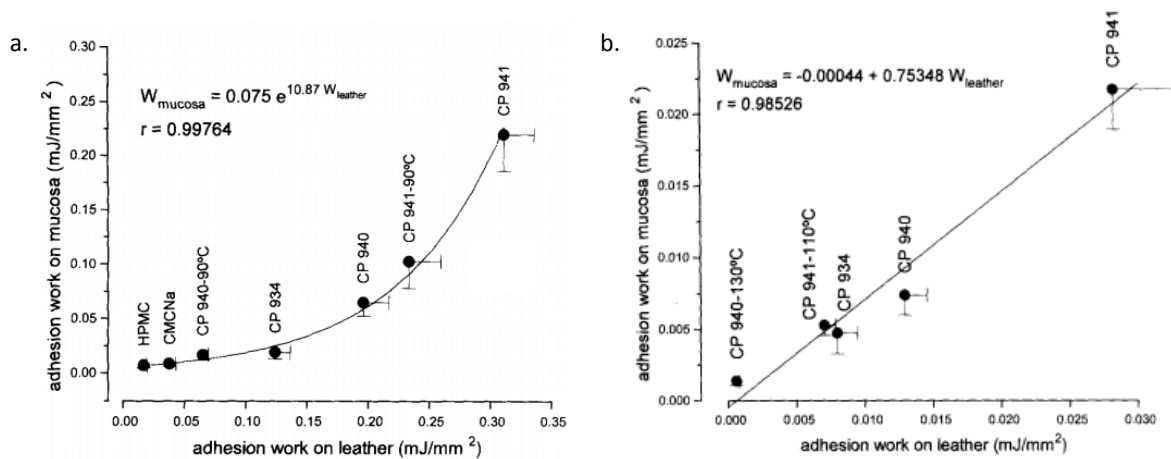
4. Mucosa-mimetic materials

In order to test mucoadhesive formulations, *ex vivo* mucosal tissues are usually used for detachment or flow-through type testing, depending on whether the dosage form is a solid, or liquid respectively. For at least the last 25 years there have been reports in the literature of researchers attempting to use substrates other than mucosa in these experiments, and comparing the performance of this substrate to tissue. The nature of mucosal membranes depends highly on their type and origin, so it is likely that materials which mimic mucosa are specific to the tissue tested against. Sources of heterogeneity between mucosal tissues include the presence or absence of secretory mucus, the pH of the biological fluids present, the topology of the tissue, and the cellular nature of the tissue itself. Mucosa-mimetic materials validated against tissues bearing no secreted mucus will need to mimic

the cell surfaces of the columnar epithelial lining. The sophistication of mucosa-mimetic materials
235 has increased with time, so the key publications in this area are presented in approximate
chronological order.

4.1. Mucosa-mimics from animal and plant sources

240 Takayama et al. (1990) used a lyophilised porcine dermis as a model of mucosa. After the dermis has
been rehydrated, the adhesion of tablets to this tissue was determined and compared to rabbit
peritoneal membrane. They found a good correlation between the dermis and peritoneal
membrane, however this membrane is not mucosal so validation of the dermis model is not
complete. Additionally, this mucosa-mimetic still required animal tissue, so offered only limited
245 benefits over *ex vivo* mucosa. At a similar time, Blanco-Fuente et al. (1996) conducted a larger study
of the adhesion of a carbopol tablets to tanned leather as a model for sublingual mucosa. They
found that the force of adhesion of tablets to tanned leather had an “adequate correlation” with
those for sublingual mucosa (Figure 4). However, the values were not directly comparable, and had a
nonlinear relationship with each other unless tested whilst immersed in liquid. Tanned leather is an
250 animal product, so whilst this study starts to demonstrate the concepts of mucosa-mimetics, and
would provide an easily-accessible, homogeneous, substrate for testing adhesives, it is still an animal
product. Nakamura et al. (1996) investigated the adhesion of inhalable polymer particles using agar
as a mimic. Briefly, the particles were placed onto an agar plate which was inclined by 30°, and the
distance travelled by the particles measured which gives a tribological-type measurement, more
255 representative of the *in vivo* behaviour of particles in the nasal cavity. This method gave the same
rank order of adhesiveness as measured in rabbits. Agar has also been used as a model mucosa by
McInnes et al (2007) to conduct tribological measurements of mucoadhesive nasal formulations. The
agar was not validated against mucosa in this study.



260 Figure 4: Blanco-Fuente et al. (1996) demonstrating an early mucosa-mimic. The article demonstrates a correlation between the work required to remove tablets from tanned leather (a) and sublingual mucosa. Immersing the leather in water improved the similarity of the mimic (b), as indicated by a move towards a linear relationship. Figure reprinted from Blanco-Fuente et al. (1996) with permission from Elsevier (license number: 3683131395233).

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4.2. Cell culture methods of mimicking mucosa

Another alternative to *ex vivo* tissue for mucoadhesion testing is the use of cell culture. Some cell lines can be grown in such a way that they express mucus on their surface, which can subsequently be used for mucoadhesion testing. For instance, Keely et al (Keely et al., 2005) have used the HT29-MTX-E12 cell line to grow a monolayer of mucus-secreting goblet cells. After the secretion of a mucus layer on the surface of the cells, the monolayer was exposed to a fluorescent polymer solution. The quantity of polymer adhering to the mucus after one hour could be calculated by measurement of the fluorescence intensity of the free polymer. This was compared to the polymers' adhesion to rat intestinal sacs, showing a similar rank order of adhesiveness. This method also allowed mucus to be removed by mucolytic agent, which could confirm that mucoadhesion to the mucus layer was seen, rather than bioadhesion to the monolayer surface. A method for measuring

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bioadhesion has also been described previously (Park and Robinson, 1984), which used cells labelled with pyrene to measure polymer adsorption onto a monolayer. Pyrene gives information on
280 membrane viscosity, which is altered in the presence of bound polymers.

4.2. Synthetic mucosa-mimics

To our knowledge, the first description of a completely synthetic mucosa-mimic was reported by
285 Mortazavi and Smart (1995), who reported the adhesion of tablets to rat intestinal mucosa and PVC strips. A correlation was found between the work of adhesion measured on mucosa and PVC, and it was suggested that the PVC provides a substrate on which to measure general adhesive properties, which appeared to dominate mucoadhesion in this case. At a similar time, Maggi et al. (1994) compared the adhesion of hydrated tablets on plexiglass (poly(methyl methacrylate)) to bovine
290 buccal mucosa. They found that the same rank order could be found when testing against each substrate. Whilst this is a useful for their application, it is likely that the same effect is seen as in Mortazavi and Smart's article, i.e. that the mucoadhesion process is a general adhesion process, which will not be the case for every mucoadhesive formulation. Jacques and Buri (1997) then used plexiglass and reconstituted porcine gastric mucin for measurement of the mucoadhesive
295 interaction of PAA hydrogels. Some correlation was seen between interfacial forces between PAA and tablet, as measured on a tensile tester, and the force of detachment of tablets from the mucin gel. However, rehydrated porcine gastric mucin has a different composition and rheology than native mucus (Boegh and Nielsen, 2015), so this substrate requires additional validation. This study allowed the authors to confirm that at least part of the adhesion phenomenon was a result of some
300 physical processes, rather than chemical interactions. Larhed et al. (1998) compared the diffusion of drugs through porcine intestinal mucus and an artificial mucus containing porcine gastric mucin. They found that the artificial mucus was not able to satisfactorily mimic that taken from the pig, and

highlighted a loss of gel-forming ability in the rehydrated mucin samples. This study exemplifies the importance of validation of mucosa-mimics against *ex vivo* mucus, rather than rehydrated mucin solutions. The gel-forming ability of mucin was mimicked by Hamed and Fiegel (2014) who used glutaraldehyde to cross-link porcine gastric mucin to form gels. These were able to mimic the rheological properties of mucus from the trachea, by validation against literature values of storage and loss moduli, and showed the same viscoelastic properties under various different conditions. Shojaei et al. (2000) compared the adhesion of a novel mucoadhesive to wet glass with *ex vivo* mucosa, finding that the glass was a poor mimic of mucosa, and demonstrating that the surface properties of a substrate are crucial in their ability to mimic mucosal tissue. Choi et al. (1999) investigated the adhesive properties of polyacrylic acid, complexed with PEG, on polypropylene, but this was not validated against mucosa. An *in vitro* retention test with a dialysis membrane as a model mucosa was used to evaluate the mucoadhesion of some self-assembling peptides by Tang et al (Tang et al., 2014). The 'elastic and soft' mechanical properties of the membrane made in mucosa-like when wet. However, *ex vivo* mucosa was not used for comparative purposes.

4.3. Novel mucosa-mimetic materials

Whilst the early mucosa-mimics used readily-available substrates, recently there have been some examples of novel synthetic materials designed in a semi-rational manner to be mucosa-mimics. For the evaluation of solid dosage forms, Khutoryanskaya et al. (2010) used hydrogen-bonded interpolymer complexes formed from poly(acrylic acid) and methylcellulose on a glass slide to test mucoadhesive tablets. Whilst these materials were not a perfect mimic of mucus, it was demonstrated that modulating the properties of the hydrogel the detachment force of the mucoadhesive tablets could be adjusted. It was also suggested that the force-displacement profile of the tablets should be used to confirm whether materials are mucus-like, as mucosa-mimics can show similar maximum detachment forces whilst having different force-displacement curves. In a

continuation of this research, Hall et al. (2011) synthesised a library of hydrogels for use as mucosa-
330 mimics. The detachment of a series of mucoadhesive tablets from these hydrogels was compared to
porcine buccal mucosa (Figure 5).

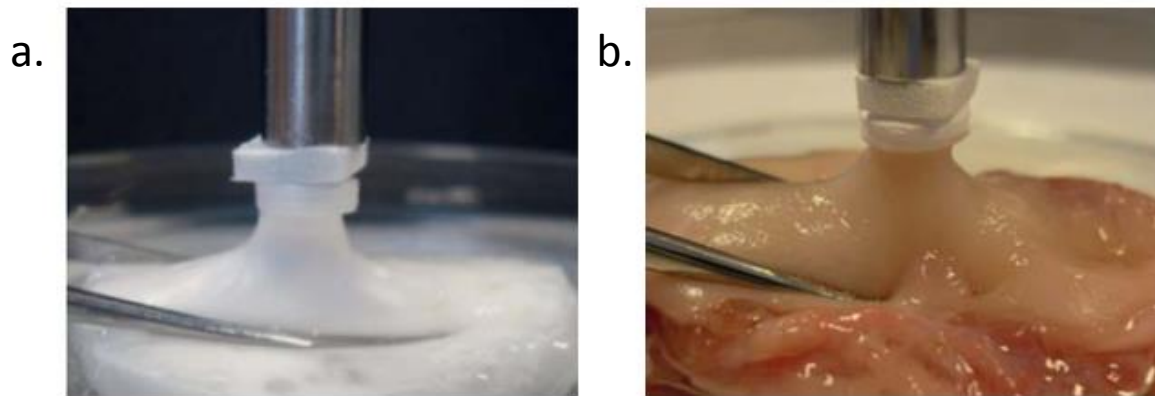
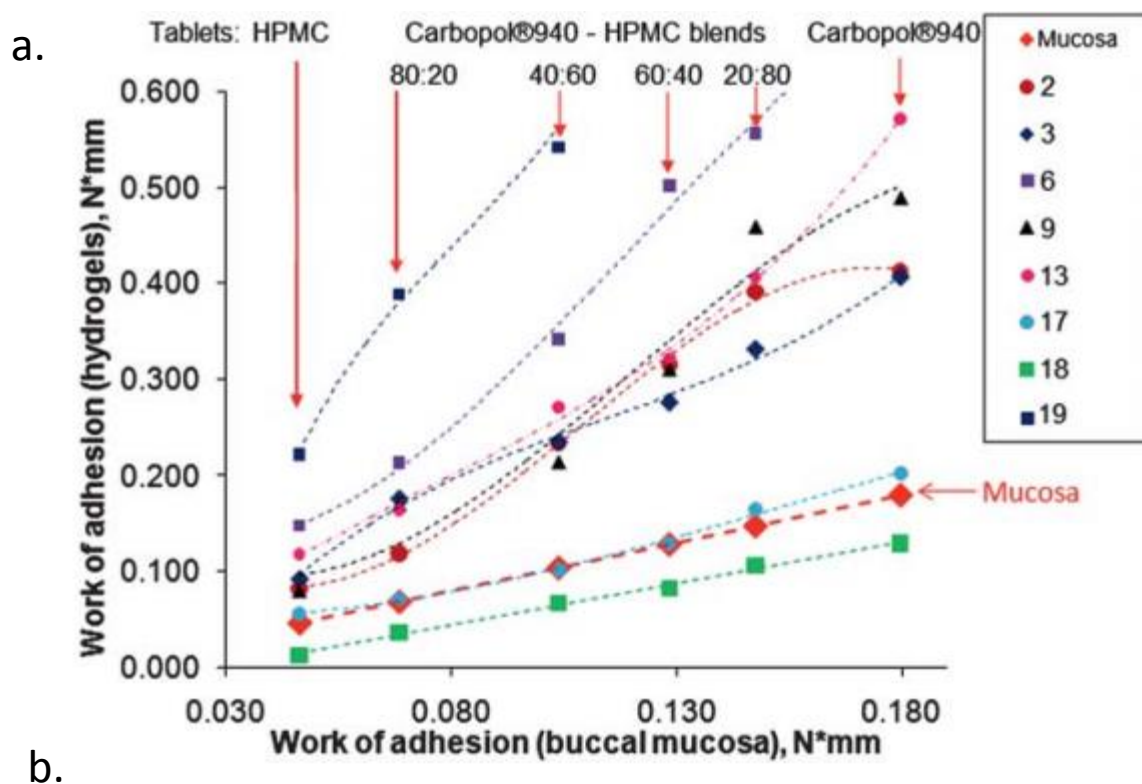


Figure 5: A synthetic hydrogel (a) is able to mimic porcine buccal mucosa (b) by the tensile method of assessing mucoadhesion.

335 Two hydrogels were identified as good mucosa-mimics, having comparable work of adhesion, force of detachment, and force-displacement curves (Figure 6). These two materials contained N-acryloyl glucosamine (AGA) and 2-hydroxyethyl methacrylate (HEMA) to form glycopolymer hydrogels. Glycopolymers bear pendant sugar moieties, which it was hypothesised made these materials similar to the oligosaccharide side-chains found in mucin glycoproteins, resulting in the comparable results
340 for detachment experiments. In addition to having comparable calculated values for mucoadhesion, the tensile method used also demonstrated that the synthetic hydrogels had similar shapes to their force-displacement curves, described as an important factor in the design of mucosa-mimetics by Khutoryanskaya et al. (2010). This demonstrates that the synthetic materials showed similar deformation behaviour to the buccal mucosa during tablet removal. It is logical that the force-
345 displacement behaviour will be governed by both the strength of binding at the interface between tablet and substrate, and the deformation of the substrate itself (assuming that the tablet has far greater tensile strength). Thus, for future materials used as mucosa-mimetics for testing solid dosage

forms, it would be interesting to investigate the mechanical properties of the mucosa and mucosa-mimic to see if they bore similarity.



b.

Sample	Co-monomer(s)	HEMA/co-monomer ratio (mol%)	Cross-linker concentration (MBA, mol%)
2	-	100:0	0.1
3	-	100:0	0.5
6	N-vinyl pyrrolidone (NVP)	90:10	0.01
9	2-hydroxyethyl acrylate	90:10	0.1
13	Sorbitol methacrylate	70:30	0.1
17	AGA	80:20	0.1
18	AGA	70:30	0.1
19	NVP:AGA	80:10:10	0.1

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Figure 6. A synthetic glycopolymer hydrogel is able to mimic the detachment profile of mucoadhesive tablets to *ex vivo* mucosa. From a library of candidate materials, this hydrogel (17) shows comparable work of adhesion to buccal mucosa (red line, where x and y values are identical) when tested against a series of mucoadhesive tablets (c). The composition of the other hydrogels is provided (d). Reproduced and adapted from (Hall et al., 2011) with permission from The Royal Society of Chemistry.

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Having already studied the mucoadhesion of a solid dosage form (tablets), this combination of monomers was subsequently covalently bound to glass by Cook et al. (2015) and the retention of liquid mucoadhesives determined. This was conducted using a flow-through method, coupled with fluorescence microscopy to determine the amount of mucoadhesive remaining on the mucosal tissue. These retention profiles were then compared to those on porcine gastric mucosa and bovine cornea. It was found that the same 1:4 molar ratio of AGA to HEMA in the feed mixture shown to be effective in the previous publication (Hall et al., 2011) gave glycopolymer hydrogels that were able to mimic the retention of three liquid formulations on porcine gastric mucosa (Figure 7). It was postulated that specific interactions with the sugar components, and the network structure of the gel may be important in explaining this phenomenon due to the mediation of mucoadhesion by both chemical interactions and physical entanglement. In total, three different polymers were tested against the hydrogels, in three different eluents. In all cases the 20 mol% AGA hydrogel was able to successfully mimic porcine gastric mucosa, however, a mimic for bovine cornea was not found. This is possibly due to the thick secretory mucus layer that coats the gastric mucosa, whilst the cornea only has a very thin mucus layer, making it less similar to a hydrogel.

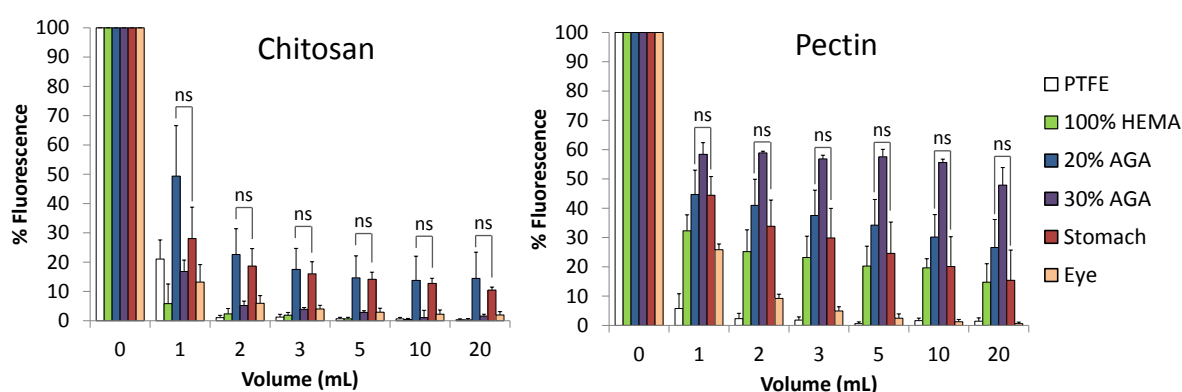


Figure 7: The retention of the liquid mucoadhesives chitosan (left) and pectin (right) on glass-bound hydrogels and *ex vivo* mucosal tissue, during washing with phosphate buffered saline. No significant

difference was seen between 20 mol% AGA and porcine gastric mucosa. (Cook et al., 2015) -

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Finally, Mahalingam et al. (2011) have designed copolymers of phenylboronic acid and

salicylhydroxamic acid with 2-hydroxypropyl methacrylamide as a mucus-like material to inhibit HIV

380 transmission. This material shared several properties of mucin, i.e. transient network formation at low pH, bioadhesiveness and the ability to inhibit the transport of HIV. However, this material was designed as a method of stopping HIV transmission, rather than to completely mimic the mucus.

5. Concluding remarks

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Mucosa-mimics have gradually been gaining attention from researchers in the area of

mucoadhesion. This is due to the inconvenience of procuring fresh mucosa, and ethical issues with the use of animal products in research. Whilst there are a very limited number of publications

directly concerning mucosa-mimicry, the use of alternative substrates in the literature is surprisingly

390 common. Early mimics, typically made from animal products or readily-available substrates, were able to give correct rank-order prediction of mucoadhesiveness. However, there is little evidence

that the magnitude of mucoadhesion could be correctly predicted. Recent advances in the area have

used fully-synthetic materials which have shown promise as correct predictors of mucoadhesives'

performance. These materials will certainly require validation against a great number of conditions

395 before they are fully-accepted as alternatives to mucosal tissue in preliminary testing. We believe

that the future of this work lies in the development of rationally designed mucosa-mimics, possibly

from glycopolymers, which provide researchers in the area of mucoadhesion a material which is

reliable, homogenous, and easily-available, without the need for laboratory animals.

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