Mini-review

# The use of hydrocolloids in physical modelling of complex biological matrices

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# 1 introduction

## 1.1 Scope

Life on earth is water-based, and nature has evolved complex water-based matrix materials, biological hydrogels, that are highly adapted to support cellular functions(Baranova, Attili, Wolny, & Richter, 2011). Found within cells and tissues(Chirila & Hong, 1998; Frantz, Stewart, & Weaver, 2010; Lieleg & Ribbeck, 2011; Oyen, 2014), between cells and the external environment(Corfield, Carroll, Myerscough, & Probert, 2001; Flemming & Wingender, 2010; Fudge, Levy, Chiu, & Gosline, 2005; Lillehoj & Kim, 2002) or at cell surfaces(Authimoolam & Dziubla, 2016; Baranova, Attili, Wolny, & Richter, 2011; Button, et al., 2012; Hattrup & Gendler, 2008) (see figure 1), these matrices are highly diverse both in composition and functions but both their highly hydrated nature, and the functional importance of their physical properties are fundamental shared features. Historically, these materials were considered primarily as inert filler materials but the complexity of their functions is becoming ever clearer(Lutolf, 2009; Tatko, 2008), and with this the challenge of understanding these materials and their molecular components grows. As with many complex systems the use of models to aid their study is widespread, and hydrocolloids, with physical properties similar to natural tissue, are frequent components of such models(Lutolf, 2009).

This mini review does not aim to provide a comprehensive overview of the structure and function biological hydrogels or hydrocolloid based models of these materials, but rather to illustrate the challenges they pose to scientific study and, through the use of specific examples, demonstrate how hydrocolloids can contribute to the modelling, and hence better understanding, of these systems.

## 1.2 Examples of biological hydrogels

A number of biological hydrogels, their central molecular components and functions are presented in table 1. Many classes of biomolecules are represented in these hydrogels but typically the long range order in the hydrogel matrix is primarily provided by polysaccharides, fibrous proteins, proteoglycans and high molecular weight glycoproteins or a combination of these, with other smaller biomolecules contributing both physically and biochemically to the total functionality of the material. Some biological hydrogels, such as the cumulus cell-oocyte complex matrix(Camaioni, Salustri, Yanagishita, & Hascall, 1996) or the membrane bound mucin layer(Hattrup & Gendler, 2008), are surface bound and in this case the cell membrane has a central structural role to play in maintaining matrix architecture. Cell surface structures and membrane bound molecules also interact with secreted hydrogel matrices with both anchoring and signalling functions(Flemming & Wingender, 2010; Frantz, Stewart, & Weaver, 2010; Hattrup & Gendler, 2008; Migliorini, et al., 2014) and thus may contribute significantly to the overall properties of the material. This intimate relationship between the cell and the environment adds further complexity to already complex systems. Within these cell plus matrix systems the cells may exist as a structured part of an organised tissue(Frantz, Stewart, & Weaver, 2010), or as individual cells acting in a cooperative way as for example in a bacterial biofilm(Flemming & Wingender, 2010; Parsek & Fuqua, 2004). Figure 1 presents a graphic representation of A, the secreted mucus matrix and membrane bound mucins of the glycocalyx that protect mucosal epithelia from the external environment, B, extracellular matrix between cell types within a tissue and C, the cells and extracellular polymeric substance of a bacterial biofilm, illustrating these various situations.

## 1.3 studying biological hydrogels

There are many motivations to study biological hydrogels; to investigate how they function and what molecular elements are important for their function, to study how we can control, improve or disrupt their function (eg medical applications), to understand how they contribute to a 'bigger picture' in nature (eg biofilms in ecosystems and whole organ function), or to copy their functionality in synthetic or semi synthetic systems (biomimetics).

However there are multiple challenges in studying biological hydrogels. They are highly dynamic systems both in terms of molecular turnover and in terms of environmental responsiveness(Baranova, Attili, Wolny, & Richter, 2011), and are highly susceptible to both enzyme and bacterial induced degradation, so for example the quality of *ex vivo* matrices tends to decline quite rapidly limiting the opportunities for study. It is often challenging to understand structure function relationships in biological hydrogels. Firstly, a single matrix often both contains multiple components and performs several functions, making it difficult to clearly assign a functional role to a single component. Secondly, structurally differing matrices may perform very similar functions, suggesting the structural requirements for such functions are broad(Lieleg & Ribbeck, 2011),. Finally, for classes of materials (such as extracellular matrix, mucus or bacterial biofilms) that are produced by multiple cell types or tissues the specific physical, topological and biochemical composition of the matrix is both source specific and heterogeneous,(Authimoolam & Dziubla, 2016; Flemming & Wingender, 2010; Frantz, Stewart, & Weaver, 2010) further complicating the elucidation of structure function relationships. Indeed, simply identifying and quantifying to biomolecules present in these complex matrices may be extremely challenging with analysis tools performing less well in mixed, non-dilute systems and extraction procedures resulting in damage, selective loss or concentration of component molecules even in well understood systems such as the alginate matrix of seaweeds(Hernandez-Carmona, McHugh, Arvizu-Higuera, & Rodriguez-Montesinos, 1998; Vauchel, Kaas, Arhaliass, Baron, & Legrand, 2008).

Given the inherent complexity of the native materials, there is a clear role for model matrices to enable and assist research efforts. When the hydrogel is formed from surface anchored biopolymers it can be modelled by grafting the appropriate constituents onto solid supports(Authimoolam & Dziubla, 2016; Migliorini, Thakar, Sadir, Pleiner, Baleux, Lortat-Jacob, Coche-Guerente, & Richter, 2014) but this approach is not sufficient for reproducing bulk hydrogel materials. As previously stated, hydrocolloids are frequently used in models of biological hydrogels due to their physical similarities to native tissue(Lutolf, 2009). In some cases the hydrocolloids used in models are native constituents of the matrix being modelled, such as alginate based models of bacterial biofilms(Schmid, Messmer, Yeo, Zhang, & Zenobi, 2008), in other cases hydrocolloids may be added as additional support to other functional matrix molecules(Boegh, Baldursdottir, Mullertz, & Nielsen, 2014; Taylor, Pearson, Draget, Dettmar, & Smidsrod, 2005), alternatively, studies may exploit the similarities between hydrocolloid gels and the native biological hydrogel to replicate a single properties such as viscosity(Hasan, Lange, & King, 2010) or water binding(Covington, Gardner, Hamilton, Pearce, & Tan, 2007) to investigate the influence of biological hydrogels on other functions. Model hydrogels can be highly controlled in terms of composition and behaviour and therefore be used to provide highly reproducible material for high throughput screening studies(Groo & Lagarce, 2014), or to investigate the influence of individual components on bulk properties(Stewart, Ganesan, Younger, & Solomon, 2015). This level of control also makes model

hydrogels useful for validating tools that may be of use for studying native biological hydrogels(Ntarlagiannis & Ferguson, 2009). The use of hydrocolloids in the modelling of biological hydrogels will now be explored for two biological hydrogel matrices, mucus and bacterial biofilms, by considering the nature of the native hydrogel, the specific challenges associated with their study and exploring examples of hydrocolloid use in modelling these systems.

## 2 Use of hydrocolloids in mucus models

### 2.1 Mucus

Mucus is the collective name given to viscoelastic secretions composed primarily of highly hydrated networks of polymeric mucin molecules(Corfield, Carroll, Myerscough, & Probert, 2001; Lillehoj & Kim, 2002). Mucins are high molecular weight glycoproteins with a protein backbone that is heavily O-glycosylated forming a bottlebrush structure where up to 80% of the molecules weight may be carbohydrate. Mucins can be membrane bound or secreted, and the secreted mucins can be further divided into those which are polymeric and those which are secreted as monomers(Corfield, Carroll, Myerscough, & Probert, 2001; Hattrup & Gendler, 2008; Lillehoj & Kim, 2002). Mucus secretions are widespread in nature being found in organisms as diverse as mammals, snails and corals, but in a mammalian context mucus forms a protective barrier at epithelial surfaces of the body exposed to the external environment, such as the gastrointestinal, respiratory and genitourinary tracts(Authimoolam & Dziubla, 2016; Corfield, Carroll, Myerscough, & Probert, 2001; Lieleg & Ribbeck, 2011; Lillehoj & Kim, 2002). The secreted mucus has diverse roles including lubrication(Authimoolam & Dziubla, 2016; Corfield, Carroll, Myerscough, & Probert, 2001; Taylor, Draget, Pearson, & Smidsrod, 2005) (for example in the GI and female reproductive tracts), an integral role in mucociliary transport systems(Button, Cai, Ehre, Kesimer, Hill, Sheehan, Boucher, & Rubinstein, 2012; Lillehoj & Kim, 2002) (for example in the lungs), and providing a robust unstirred layer to allow the maintenance of a pH gradient at the gastric mucosal surface. The barrier function of mucus is ubiquitous, helping to reduce the contact of damaging agents, including bacteria and viruses, with the epithelial cell surface. In this context it is worth noting that the membrane bound mucins of the cell glycocalyx provide an even tighter steric barrier than the overlying secreted mucus layer(Button, Cai, Ehre, Kesimer, Hill, Sheehan, Boucher, & Rubinstein, 2012; Hattrup & Gendler, 2008). When we consider the functions performed by secreted mucus it is clear that the physical (viscoelastic) properties are critical to effective function, and that the ideal viscoelastic properties for maintaining the pH gradient in the stomach differ from those needed as part of the mucocilliary clearance system in the airways. Unfortunately there is no clear universal link between mucin structure, either in terms of gene product or glycosylation and the viscoelastic properties of the mucus matrix.

### 2.2 Modelling mucus in drug delivery research

Interest in mucus as a barrier in the context of drug delivery has increased significantly over the last decade(Groo & Lagarce, 2014). This increased interest has been driven primarily by an increased interest in nanomedicine and nanoscale drug delivery systems. These drugs have length scales similar to those associated with the bacteria or viruses, entities that mucus has evolved to act as a barrier to. As a result of this, whilst small molecule drugs typically experience a few fold reduction in diffusion in mucus as opposed to water(Larhed, Artursson, & Bjork, 1998) nanoscale drug delivery systems may be effectively immobilised(Groo & Lagarce, 2014) so they are prevented from contact

with mucosal cells and drug uptake is not achieved. It is therefore highly important to be able to study the fate of nanomedicines in mucus, in terms of nanoparticle stability and mobility in mucus matrices and nanoparticle uptake in *in vitro* models that include a mucus component(Boegh, Baldursdottir, Mullertz, & Nielsen, 2014), in order to engineer the most functional drug delivery systems. Such comparative studies require mucus matrices which are sufficiently homogeneous and reproducible in their functionality, and available in sufficient quantity to allow conclusions about the relative effectiveness of different drug delivery systems to be drawn. Although small intestinal mucus is available in reasonable quantities from pigs at slaughter, there is significant inter and intra individual variation in the material properties(Boegh, Baldursdottir, Mullertz, & Nielsen, 2014; Groo & Lagarce, 2014) and *ex vivo* small intestinal mucus samples have a tendency to degrade rather rapidly at room temperature due to the high load of digestive enzymes and bacteria within the samples further complicating the picture. For these reasons mucus models have the potential to make a significant contribution in this research area, however such a contribution is dependent on both the quality of the model in its ability to replicate the functions of the native mucus and a clear understanding of the limitations of the model so that the appropriate controls and comparisons to the native mucus are undertaken. In 2014 Groo and Lagarce published a review of mucus models to evaluate nanomedicines for diffusion. In this article they describe a number of different mucus models that have been developed and highlight some of the challenges, with perhaps the most significant being the limited number of mucin preparations available commercially (porcine gastric or bovine submaxillary) and the fact that the commercial extraction process damages the polymeric structure of the mucins reducing their ability to form a viscoelastic network and thereby their ability to replicate the mucus barrier. In an attempt to overcome this problem Boegh and co-workers have developed a cell compatible mucus model, which is like many others based on commercially available porcine gastric mucin with the addition of other mucus components but uniquely includes the addition of the hydrocolloid polyacrylic acid to add long range support to the matrix and better replicate the viscoelastic properties of the native secretion(Boegh, Baldursdottir, Mullertz, & Nielsen, 2014). Whilst polyacrylic acid is not a component of native mucus, diverse mucous systems have be shown to be capable of incorporating other polymers within their matrix, to provide this same kind of long range support, without dramatically altering other functional properties of the material (Bocker, Ruhs, Boni, Fischer, & Kuster, 2016; Taylor, Draget, Pearson, & Smidsrod, 2005; Taylor, Pearson, Draget, Dettmar, & Smidsrod, 2005) so this strategy certainly has a sound background. However, at the time of writing there is no universally accepted mucus model for use in intestinal drug delivery research, and a similar situation applies to mucus models for other mucosal surface.

### 2.3 Modelling mucus in lung clearance systems

Mucus has a protective function in the airways as part of the mucociliary clearance system. Turbulent airflow promotes the deposition of inhaled particular matter, bacteria and viruses on to the mucus surfaces of the conducting airways, where they become entrapped in the sticky mucus blanket that covers the epithelium and is propelled upwards and out of the lungs by the beating cilia. In this manner the air reaching the delicate gas exchange surfaces of the alveoli is relatively cleaner than the inhaled air(Lillehoj & Kim, 2002). The lungs also have a secondary clearance mechanism, cough, which comes into play when the baseline mucociliary clearance is overwhelmed either through increased mucus production or viscosity as a result of infection or underlying disease, or as

a first line response to inhalation of a high concentration of particulate matter such as dust(Hasan, Lange, & King, 2010; Lillehoj & Kim, 2002).

Baseline mucociliary clearance is often compromised in patients undergoing artificial ventilation but physical interventions to improve mucus clearance often have a poor evidence base to establish how, and under what circumstances they improve mucus clearance. Tatkov and Pack(Tatkov & Pack, 2011) have taken one such intervention, symmetrical waveform high frequency oscillation (HFO), the effectiveness of which is debated in the literature, and designed a study which combines *ex vivo* mammalian tracheal tissue with its endogenous mucus and the addition of larger volumes of a hydrocolloid gel comprising of polyethylene oxide in phosphate buffer to simulate pathological mucus secretions found in lung diseases such as COPH. By subjecting their *ex vivo* tissue to HFO they were able generate data suggesting that whilst the clearance of the endogenous mucus in the trachea was unchanged by this intervention the clearance of the simulated pathological mucus model improved. In this case the use of a mucus model which was rheologically matched to pathological sputum but homogeneous and stable in its properties allowed conclusions to be drawn using far fewer *ex vivo* trachea than would be required to produce statistically significant data using highly variable and inhomogeneous *ex vivo* sputum samples.

Looking at mucus and cough clearance, Hasan and co-workers (Hasan, Lange, & King, 2010) have considered the disease transmission angle and investigated the influence of artificial mucus on the characteristics of airborne bioaerosol droplets generated during simulated coughing. They used locus bean gum, sodium tetraborate and sodium dodecyl sulphate to produce mucus preparations were the viscoelasticity and the surface tension could be controlled by altering the hydrocolloid and surfactant components respectively. They conclude that viscoelastic properties of the mucus but not surface tension significantly influence droplet production, and propose, rather interestingly, that pharmacological interventions to alter mucus rheology (and thus reduce droplet formation) could be given simultaneously with treatment for infectious respiratory diseases to limit transmission of such diseases within the population.

## 2.4 Modelling mucus as a functional element of organ systems

The role of mucus in the effective functioning of major organs such as the stomach is well understood but this is not the case for all structures within the body. Dollinger and co-workers have considered the case of the larynx(Dollinger, Grohn, Berry, Eysholdt, & Luegmair, 2014) where the influence of mucus on phonation, that is sound production, is poorly understood despite widespread consensus that mucus plays an important role in voice performance. In their preliminary study the authors used *ex vivo* human larynges and investigated the influence of two hydrocolloid mucus models, linear polystyrene sulfonate and the same polystyrene sulfonate crosslinked with the ionic porphyrin TAPP to form nanoscale networks. A central element of phonation is the mechanical vibration of the vocal folds, which is defined by both the mechanical properties of the tissue and the transfer of energy from the airstream to the tissue. It is reasonable to assume that the boundary layer of mucins/mucus on the vocal folds will affect this transfer, and this preliminary study supported that conclusion suggesting that not only the presence of mucus, but also the structure of the mucus can influence the mechanics of vocal fold vibrations and thus phonation.

Gardner and co-workers (Gardner, Covington, Tan, & Pearce, 2007) have taken a biomimetic approach and made use of a polymer systems mimicking mucus, not to understand an *in vivo* organ

system but rather to improve the function of an electronic 'nose'. Taking inspiration from studies demonstrating that the mucus layer that coats the nasal epithelium has partitioning properties similar to gas chromatography contributing to the coding of olfactory information, they have engineered an artificial nose that incorporates a retentive polymer coating in addition to chemical sensors, improving its functionality. In this case the 'model mucus' used (Polymonochloroparaxylene C) bares little similarity to the native material and rather falls outside the scope of this article, however it is included here to illustrate the shear breadth of applications where models for biological hydrogels can potentially be utilised.

## 3 Use of hydrocolloids in bacterial biofilm models

### 3.1 Bacterial biofilms

Bacteria have historically studied as pure cultures of dispersed planktonic single cells, but in the environment they are most often found accumulated at surfaces as polymicrobial aggregates with bacterial cells embedded in a matrix of different types of biopolymers collectively referred to as extracellular polymeric substances (EPS)(Battin, et al., 2007; Flemming & Wingender, 2010; Parsek & Fuqua, 2004), and as such it is important to gain an understanding of bacteria within these matrix colonies. These bacterial biofilms pose the same kind of challenges to research as the hydrogel matrices originating from eukaryote cells; they are highly variable and inhomogeneous, isolation of matrix components is difficult, and understanding of the precise role and interactions of biofilm matrix polymers in limited. In fact EPS have been called "the dark matter of biofilms" as a result of the difficulty in analysing them (Flemming & Wingender, 2010). Again, the case for using hydrogel models to increase our understanding of these materials is strong, aided by the fact than some of the EPS polysaccharides such as alginate, gellan and xanthan can be generated by bacteria in bioreactors providing a better source of molecular constituents than is available for many of the eukaryote matrix polymers. Indeed Hellriegel and co-workers have performed a detailed study of gellan, obtained from *Sphingomonas elodea*, gelled under differing polymer and mono and divalent cation concentrations and evaluated it as a physiochemical biofilm model(Hellriegel, et al., 2014).

### 3.2Hydrocolloids models for studying diffusion in biofilms

Diffusion in biofilms is of significant interest as the bacteria in biofilms are less susceptible to anti-microbial treatment. Biofilm formation can provide a significant challenge to controlling microorganisms in diverse situations from implanted medical devices to microbial induced clogging and corrosion in industrial processes(Hu, Miyanaga, & Tanji, 2012). By utilizing an agarose gel biofilm model with and without the addition of *Pseudomonas aeruginosa* and a chlorine microelectrode to monitor chlorine concentration Chen and Stewart have elegantly demonstrated that the reaction rate of chlorine with cellular biomass is sufficiently fast that diffusion of chlorine into the biofilm is rate-limiting, providing an explanation for the poor efficacy of chlorine as a disinfectant when used against microorganisms in biofilms(Chen & Stewart, 1996). Hu and co-workers have also utilized agar as a model biofilm to study the diffusion of a bacteriophage. Using *Escherichia coli* as host cells they found that dead host cells significantly slowed phage diffusion, but the addition of the phage to the biofilm model when the host cells were in an exponential growth phase significantly increased phage diffusion(Hu, Miyanaga, & Tanji, 2012). Thus model biofilms show promise in increasing our understanding of the efficacy (or lack of efficacy) of antimicrobial strategies against bacteria in biofilms.

### 3.3 Hydrocolloid models of biofilms for understanding matrix interactions

Another strategy for biofilm control is to gain an understanding of cell matrix interactions in biofilm assembly and disassembly. Stewart and co-workers have used native *Staphylococcus epidermidis* biofilms and biofilm models of *S. epidermidis* with chitosan to study the assembly, disassembly and viscoelastic properties of biofilms(Stewart, Ganesan, Younger, & Solomon, 2015). They conclude that thermodynamic phase instability of the extracellular polymeric substances (EPS) drives colloidal self-assembly of biofilms and that pH induced solubilisation of the EPS matrix drives disassembly of the biofilm.

### 3.4 hydrocolloid models for validating use of characterisation techniques in biofilms

There is clearly interest in *in situ* characterising the matrix of biofilms and the content and structures of the molecular components, and confocal laser scanning microscopy (CLSM) combined with fluorescent dyes or fluorescently labelled lectins and antibodies is a widespread tool(Flemming & Wingender, 2010). However labelling techniques carry an associated risk of introducing artefacts, making label-free chemical characterisation of nanostructures in biological systems, including biofilms, particularly attractive. Schmid and co-workers have used well defined alginate gels to test the feasibility of tip enhanced Raman spectroscopy as a tool for identifying nanostructures in biofilms and concluded that this method shows some promise(Schmid, Messmer, Yeo, Zhang, & Zenobi, 2008). At a rather different length scale Ntarlagiannis and Ferguson have addressed the problem of identifying environmental biofilms in the subsurface by geophysical methods. They used a controlled alginate gel within a packed column system to mimic a biofilm in the subsurface and evaluate the ability of the spectral induced polarization method to detect the biofilm, concluding the method has potential for use in the field(Ntarlagiannis & Ferguson, 2009).

## 4 Summary

Hydrocolloid gels have shown their usefulness in modelling biological hydrogels in a wide range of contexts. However, it is important to emphasise that the limitations of each individual systems must be considered when drawing general conclusions, and these model systems should be seen as a supplement to rather than a replacement for studies on native matrices.

## 5Future prospects

Whilst our understanding of biological hydrogels is improving, aided by the use of model systems, it is generally difficult to model the spacial complexity and variability of matrix structures. However, the rise in 3D printing and tissue engineering is giving rise to exciting developments in terms of producing complex biological structures and microenvironments (Hinton, et al., 2015; Zhang, et al., 2016), which could lead to a paradigm shift in the way we model and study biological hydrogels.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

| Biological hydrogel | Typical constituents | Functions | ref |
|---|---|---|---|
| **Extracellular matrix** | Fibrous proteins (collagens, elastin, fibronectin, tenasin, laminin), proteoglycans and glycosaminoglycans (hyaluronan, aggrecan, perlecan) | Physical scaffolding of tissues, biochemical and biomechanical signalling, selective diffusion barrier | (Frantz, Stewart, & Weaver, 2010) |
| **Respiratory mucus** | Secreted mucin (MUC5AC, MUC5B), shed membrane bound mucins (MUC1, MUC4, MUC16), lipids (e.g. cholesterol, phospholipids), proteins (e.g. IgA, anti-microbial peptides), cell debris (DNA etc) | Mucocilliary clearance, epithelial protection, selective diffusion barrier | (Hattrup & Gendler, 2008; Lillehoj & Kim, 2002) |
| **Intestinal mucus** | Secreted mucin (MUC2), shed membrane bound mucins (MUC1, MUC3, MUC4), lipids (e.g. phospholipids, ceramides), proteins (e.g. serum proteins, IgA, defensins, trefoil peptides), cell debris (DNA etc) | Epithelial protection, lubrication, selective diffusion barrier | (Corfield, Carroll, Myerscough, & Probert, 2001; Larhed, Artursson, & Bjork, 1998) |
| **Cumulus cell-oocyte complex matrix** | Hyaluronan, inter-α-trypsin inhibitor, dermatan sulphate proteoglycans | Cell adhesion and fertilization | (Baranova, Attili, Wolny, & Richter, 2011; Camaioni, Salustri, Yanagishita, & Hascall, 1996) |
| **Vitreous humour** | Hyaluronan, versican, collagens, albumin, IgG | Light penetration, mechanical support, diffusion of metabolic solutes | (Chirila & Hong, 1998) |
| **Bacterial biofilm** | EPS (extracellular polymeric substances) including neutral and charged polysaccharides, proteins, nucleic acids and lipids | Scaffold for biofilm architecture, surface adhesion, cohesion, signalling | (Flemming & Wingender, 2010) |
| **Hagfish slime** | Mucins, protein slime threads | Defence (produced when stressed or provoked) | (Bocker, Ruhs, Boni, Fischer, & Kuster, 2016; Fudge, Levy, Chiu, & Gosline, 2005) |

303 Table 1 examples of biological hydrogels, their components and functions

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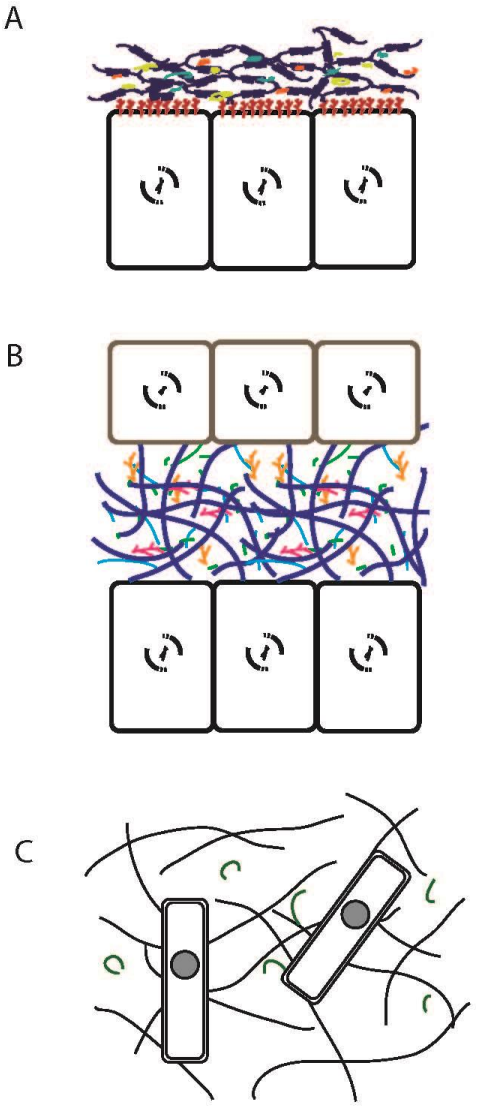


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307 Figure 1 graphic representation of A, the secreted mucus matrix and membrane bound mucins of
308 the glycocalyx that protect mucosal epithelia from the external environment, B, extracellular matrix
309 between cell types within a tissue containing fibrous proteins and proteoglycans, and C, the cells and
310 extracellular polymeric substance (polysaccharides and proteins) of a bacterial biofilm

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