European Journal of Pharmaceutics and Biopharmaceutics 95 (2015) 144–150

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Dynamic responses in small intestinal mucus: Relevance for the maintenance of an intact barrier

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ARTICLE INFO

Article history:
Received 15 October 2014
Accepted in revised form 26 January 2015
Available online 2 February 2015

Keywords:
Mucus rheology
Stress strain hardening
Biological hydrogel
Matrix dynamics
Mucin dynamics
Small intestine

ABSTRACT

Mucus in the small intestine acts as both a lubricant and a barrier to reduce mucosal exposure to damaging agents, and must perform these functions in a highly mechanically dynamic environment, both in terms of luminal shear and gut wall contractility. Here we investigate the dynamic responses of *ex vivo* pig small intestinal mucus to applied shear of different magnitudes over different timescales. At low levels of applied stress of 1 Pa, which is of the order of magnitude of that induced by gut wall contractility, the mucus demonstrates stress hardening responses to the applied deformation. This hardening behavior is lost at higher levels of applied stress. Such hardening behavior is likely to contribute to the ability of small intestinal mucus to maintain a barrier at the mucosal surface in the mechanically dynamic gut environment.

1. Introduction

1.1. Mucus in the GI tract

The mucus in the small intestine has a central role in mucosal protection, both by acting as a barrier to prevent digestive enzymes accessing the mucosa, leading to auto-digestion, and by acting as a lubricant, which helps prevent mechanical damage to the mucosa by attenuating the shear stresses associated with the mechanical processes of digestion [1–4]. Such barrier and lubricative functions are a common feature of mucus secretions, both in the gastrointestinal tract and elsewhere [5]. However, the small intestinal mucosa is primarily an absorptive surface for the uptake of nutrients [4], so one is left with the rather dichotomous situation of a barrier covering an absorptive surface. It is therefore not surprising that the mucus layer of the small intestine is both thinner and less rigid than the mucus layer found at other anatomical locations in the gastrointestinal tract [2].

1.2. Structure of the mucus barrier

The gastrointestinal mucus barrier consists of the membrane bound mucins of the epithelial cell glycocalyx, a firmly adherent secreted mucus gel adjacent to the mucosa and a more viscous secreted mucus gel at the luminal side, with the firmly adherent gel being minimal in the small intestine [2,6]. Secreted mucus is a highly hydrated (>95% water) gel of polymeric mucin molecules, which are high molecular weight glycoproteins related to each other and to Von Willebrand factor. Mucin subunits consist of a protein backbone with C-terminal cysteine knot domains and a central tandem repeat region rich in serine and threonine that is heavily O-glycosylated. This heavily glycosylated region, with a central peptide backbone and oligosaccharide side chains is known as the bottle brush region. To form mucin polymers, mucin subunits are first dimerized through disulfide bridging between C-terminal regions, and these dimers then form multimers through disulfide bridging of the N-terminal regions [7]. The predominant mucin gene product in both the firmly adherent and more viscous mucus gels of the small intestine is MUC2 [8–10]. It has been proposed that multimerization of the MUC2 gene product produces two dimensional nets or sheets of mucins as a result of trimerization at the N-terminal [10] as opposed to linear or branched polymers [11]. Regardless of the supramolecular structure of the mucin polymer, it is clear that gel formation is a result of multiple non-covalent interactions between mucin polymers forming a three dimensional polymeric matrix of the physical gel type, rather than covalent bonding resulting in chemical gel or a purely entangled system resulting in a pseudo-gel [5,12–14]. The resultant material is, like the majority of mucus secretions, a viscoelastic gel with predominantly elastic (solid like) behavior with a significant viscous (liquid like) component [12].

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http://dx.doi.org/10.1016/j.ejpb.2015.01.024
0939-6411/

1.3. Barrier properties of small intestinal mucus

In its simplest form the mucus can be considered as a maintained unstirred water layer adjacent to the mucosa across which a concentration gradient develops, in which small molecule nutrients, and indeed many drugs, diffuse across relatively unhindered [15,16]. For larger molecules and nanoscale particulates (of food or pharmaceutical origin) the situation is more complex and mucus can provide both a steric and an interactive barrier substantially reducing the absorption of these components. It has been demonstrated that for particles smaller than around 500 nm in diameter it is the interactive barrier, that is particles becoming entrapped by non-covalent interactions with the mucin matrix, that is most relevant for reduced diffusion in the mucus layer [17–20] and consequently reduced absorption. It is clear that mucus in the small intestine functions as selective barrier, both in terms of size and in terms of potential intermolecular interactions, which allows physiological absorption of nutrients but reduces the accessibility of the mucosa to potentially damaging entities. It is interesting to consider the mucus barrier in the context of its gel properties, particularly the ability of the gel to provide a functional barrier despite a significant flow component to its behavior. While this flow character is of central importance in allowing the secreted mucus to flow to cover the epithelial surface, to adapt to deformations in that surface and re-anneal to cover any defects in the layer [2,5,21], it also gives rise to the possibility of 'with mucus' as opposed to 'through mucus' diffusion. In the case of 'with mucus' diffusion a nanoparticle could interact strongly with mucin molecules to the extent that it is functionally entrapped yet the inherent flow character of mucus could lead to movement of the mucin–nanoparticle complex within the bulk matrix.

1.4. Dynamics, shear flow and peristalsis

The gastrointestinal tract is a mechanically dynamic environment, with luminal flow and mixing of liquid, solid and semi-solid digesta and various forms of contractile activity in the different sections of the gut [4]. Contractile activity in the gut wall is a complex process [22] that initiates mechanical breakdown of the food bolus, mixing of the gut contents with digestive secretions and propulsion of the digesta longitudinally through the GI tract. In the small intestine both mixing and propulsion are important results of contractile activity [4] and the physical properties of the digesta influence contractile activity [23]. As a result there is a large variation in the shear forces that develop within the small intestine, in terms of both their magnitude and their duration; however, Jeffrey et al. have measured shear stresses of around 1 Pa in the guinea pig ileum [24], which gives an indication as to the order of magnitude of the forces at play.

1.5. Lubrication and maintaining an intact barrier

Lubrication and the attenuation of shear forces at the mucosal surface are central function of mucus secretions in the gastrointestinal tract where the mucus layer is exposed to both luminal shear forces and forces resulting from the contractility of the gut wall. Mucus is a shear thinning thixotropic material, which will transit from solid like to flow behavior under increasing shear and regain solid like behavior as the applied shear is removed [5,25] and this behavior underpins the lubricative function of mucus; as the shear increases the mucus begins to flow thus preventing the forces being transmitted to the mucosa below, and as the shear reduces the mucus re-gels so it functions as a multi-use lubricant, which reduces the requirement for the secretion of new mucus. It has been proposed that the secreted mucus bilayer of the GI tract is central to the lubricative function, where the luminal, less rigid, mucus gel flows more easily protecting the more ridged mucus layer below which can maintain a protective barrier undisturbed. This is particularly important in the stomach, where the rigid mucus layer protects the mucosa from the acidic gastric secretions [1] and in the colon where it forms a barrier preventing bacterial access to the mucosa [26,27]. The nature of the mucus gel, particularly the fact that it is maintained by non-covalent interactions, which are able to rupture and reform without damage to the molecular structures, underpins this rheological reversibility of mucus, which is so important to its physiological function [5,14,28]. It has been proposed that when sheared the mucus does not weaken equally throughout its bulk, but rather the breakdown of gel forming interactions is limited to a certain depth within the gel and a slippage plane develops [5]. The glycosylated bottlebrush structures of the mucin molecule function well as boundary lubricants and facilitate the sliding of mucin coated surfaces [29]. It is known that within viscoelastic layers, such as mucus, subjected to shear stress the distribution of stress at different depths will vary, a phenomenon known as shear banding [30,31], which would support the formation of a slippage plane. It has also been suggested that two dimensional laminar arrays of mucins could enhance the slippage plane effect [10]. The slippage plane model, regardless of mucin polymeric structure, contributes to the ability of mucus to both flow and maintain a barrier. While it is well established that mucus will flow in response to increasing shear, it is also clear that under large deformations mucus, from various sources, demonstrates dynamic stress/strain hardening [25,28], but there is no unified theory behind these hardening behaviors, which have been identified in different secretions, under different stress/strain regimes, and over differing timescales (frequencies).

Here, we present evidence of stress hardening behavior in *ex vivo* porcine small intestinal mucus when subjected to repeated creep compliance testing, which may indicate that deformations resulting from gut contractility may induce gel strengthening in the mucus layer and thus contribute to the maintenance of an intact barrier.

2. Materials and methods

2.1. Mucus samples

Small intestines from recently slaughtered pigs were obtained from the local abattoir. The intestines were opened longitudinally with scissors and very gently rinsed to remove luminal chyme. The mucus gel was then gently scraped from the mucosa using a round edged spatula, pooled into aliquots of approximately 10 g, which were stirred and then frozen at −20 °C.

2.2. Rheological studies

Before performing rheological studies, aliquots of small intestinal mucus were removed from the freezer and allowed to thaw overnight at 4 °C, to allow for complete recovery of gel structure while maintaining a low temperature throughout the matrix to limit enzymatic and bacterial degradation. Rheological studies were performed using a Rheological StressTech rheometer fitted with a cone and plate geometry with a diameter of 40 mm and an angle of 4° having gap volume of 1.172 cm^3. Approximately 1.2 g of mucus was weighed out, allowed to equilibrate to room temperature and loaded onto the rheometer plates. Loading speed below 3 mm was limited to 0.001 mm/s and the gel was given 30 min equilibrium time before measurements were initiated. Samples were surrounded with low viscosity silicone oil to prevent evaporation and experiments were carried out at 25 °C as opposed to 37 °C to limit enzymatic and bacterial degradation of the