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Review

Physicochemical properties of mucus and their impact on transmucosal drug delivery

Jasmim Leal, Hugh D.C. Smyth, Debadyuti Ghosh*

Division of Molecular Pharmaceutics and Drug Delivery, College of Pharmacy, The University of Texas at Austin, 2409 University Ave., Austin, TX 78712, USA

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ABSTRACT

Mucus is a selective barrier to particles and molecules, preventing penetration to the epithelial surface of mucosal tissues. Significant advances in transmucosal drug delivery have recently been made and have emphasized that an understanding of the basic structure, viscoelastic properties, and interactions of mucus is of great value in the design of efficient drug delivery systems. Mucins, the primary non-aqueous component of mucus, are polymers carrying a complex and heterogeneous structure with domains that undergo a variety of molecular interactions, such as hydrophilic/hydrophobic, hydrogen bonds and electrostatic interactions. These properties are directly relevant to the numerous mucin-associated diseases, as well as delivering drugs across the mucus barrier. Therefore, in this review we discuss regional differences in mucus composition, mucus physicochemical properties, such as pore size, viscoelasticity, pH, and ionic strength. These factors are also discussed with respect to changes in mucus properties as a function of disease state. Collectively, the review seeks to provide a state of the art roadmap for researchers who must contend with this critical barrier to drug delivery.

1. Introduction

One of the major challenges to transmucosal drug delivery is the presence of a complex mucus barrier lining the mucosal epithelium of tissues. Mucus is a complex hydrogel biopolymer barrier located in the airways, gastrointestinal tract, reproductive tract, and the eyes (Bansil and Turner, 2006; Lieleg and Ribbeck, 2011). It is continuously produced, secreted, and finally digested, recycled, or discarded (Lai et al., 2009d) and its main functions include lubrication of the epithelia, maintenance of a hydrated layer, exchange of gases and nutrients with the underlying epithelium, as well as acting as a barrier to pathogens and foreign substances (Bansil and Turner, 2006; Lai et al., 2009d). Moreover, mucus is involved in various disease states like asthma, bronchitis, cystic fibrosis, and cancer (Bansil and Turner, 2006; Hollingsworth and Swanson, 2004; Khanvilkar et al., 2001; Rose and Voynow, 2006; Thornton et al., 2008; Williams et al., 2006). Mucus is a barrier which impedes transport of drugs and other molecules, and its physicochemical properties such as pore size, viscoelasticity, pH, ionic strength, and charge can impact the eventual fate and delivery of drug delivery systems in mucosal tissues (Bansil and Turner, 2006; Cone, 2009).

During homeostasis, the airway epithelium contains a protective continuous mucus layer, with a thickness of 5–15 μm in the nasal cavity (Beule, 2010; Ugwoke et al., 2005), 10–30 μm in the trachea and 2–5 μm in the bronchi (Patton, 1996; Sanders et al., 2009; Wine, 1999). The gastrointestinal tract not only allows the digestion and absorption of nutrients, electrolytes, and fluids but also acts as a barrier against environmental threats. The gastrointestinal epithelium has a protective continuous mucus layer preventing direct access by microorganisms to the intestinal mucosa (Ensign et al., 2012a; Goldberg and Gomez-Orellana, 2003; Macpherson and Harris, 2004), with a mean thickness of 100–300 μm in the stomachs of rats and 100–900 μm in the intestine (Atuma et al., 2001; Jordan et al., 1998). The stomach and colon are comprised of two mucus layers, a loosely adherent mucus layer and an underlying firmly adherent mucus layer attached to the mucosa; the small intestine contains a single mucus layer. The gastrointestinal mucus hinders the diffusion of bacteria (Macpherson and Harris, 2004) and macromolecules (Schenk and Mueller, 2008), decreasing permeation across this barrier. In the vagina, its surface is lined and protected by cervical mucus (thickness ~50 μm (McKinley et al., 2014)), which changes in rate of production and viscoelastic properties throughout the menstrual cycle. During ovulation, cervical mucus is less viscoelastic, resulting in higher permeability of molecules (Wolf et al., 1978). In the eye, the conjunctiva is a mucosal epithelium that has a mucus layer secreted by goblet cells lined with the cornea to form a precorneal mucin gel with a reported thickness of 3 μm to more than 30 μm depending on the measurement method used. The conjunctiva acts as a lubricant and a stabilizer of the tear film (Ellingham et al., 1999;

* Corresponding author.
E-mail address: dghosh@austin.utexas.edu (D. Ghosh).

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Greaves and Wilson, 1993; Ludwig, 2005; Prydal and Campbell, 1992).

Mucus is composed of water, mucins, globular proteins, salts, DNA, lipids, cells and cellular debris (Bansil and Turner, 2006; Button et al., 2013; Carlstedt and Sheehan, 1989; Cone, 2005; Thornton and Sheehan, 2004), wherein the homeostasis of these several components is complex and highly interdependent. Minor changes within mucus constituents can significantly alter the physicochemical properties and affect disease states. For instance, the mucus layer hydration state is directly related to ions, salts and water concentrations in the lungs (Button and Button, 2013) and is critical for the mucociliary clearance process, which rapidly removes particles that remain deposited on the ciliated cells (Boucher, 2007b; Knowles and Boucher, 2002). Indeed, there is a negative correlation between airway surface mucus concentration (i.e. percentage solids and total mucins concentration) and mucociliary clearance in patients with chronic bronchitis (Anderson et al., 2015). Additionally, there is evidence that mucociliary clearance in the airways can be regulated by luminal ATP concentrations as a feedback system in response to changes in the hydration status of the mucus layer to maintain rheological properties that ensure efficient mucus clearance (Button et al., 2013). Mucus concentration is also a dominant variable affecting mucus viscosity (Button et al., 2016). It has been proposed that ionic strength and pH also modulate the mucus hydration and viscoelasticity (Celli et al., 2005; Georgiades et al., 2014; Tam and Verdugo, 1981). Specifically, there is evidence suggesting that not only chloride ions, but calcium and bicarbonate ions play an important role in the expansion of polymeric mucins after their secretion, a fundamental process in mucus formation and transport (Cooper et al., 2013; Thornton et al., 2008). Moreover, understanding mucus viscoelasticity is critical to elucidate mucus physiological processes and disease states, relevant for the design of mucosal drug delivery systems (Khanvilkar et al., 2001; Lai et al., 2009d).

In this review, we discuss physicochemical properties of mucus in different tissues and how the mucus barrier impacts on drug delivery systems to mucosal surfaces. The gastrointestinal tract, nose, lungs, vagina, and eyes are sites with mucosal epithelia that are accessible for mucosal drug delivery systems.

2. Mucus composition

In general, mucus is mainly composed of water (~95% w/w), mucins (~0.2 to 5.0% w/v), globular proteins (~0.5% w/v), salts (~0.5 to 1.0% w/w), lipids (1–2% w/w), DNA, cells, and cellular debris (Allen et al., 1993; Bansil and Turner, 2006; Boegh et al., 2013; Button et al., 2013; Carlstedt and Sheehan, 1989; Cone, 2005; Fahy et al., 1993; Ghani and Soothill, 1997; Khanvilkar et al., 2001; Kilbourn, 1978; Lai et al., 2009d; Lopata et al., 1974; Matthews et al., 1963; Thornton and Sheehan, 2004) and forms a dense, viscoelastic layer over epithelial cells to serve as a selective barrier to drugs and other molecules. The mucus layer has a high number of physical entanglements stabilized by covalent and noncovalent interactions, including hydrophobic, electrostatic, hydrogen bonds, or other specific binding interactions that contribute to the mucus viscoelasticity, creating a mesh network filter that decreases penetration of molecules and particles and their diffusion rates (Lieleg and Ribbeck, 2011; Sanders et al., 2009).

Mucins are very high molecular weight (10–40 MDa) polymeric gel-forming glycoproteins (Sheehan et al., 1986) secreted by epithelial goblet cells and submucosal glands (Carlstedt and Sheehan, 1989; Thornton and Sheehan, 2004). Mucin fibers are filamentous O-linked glycoproteins with 'PTS' (proline, threonine, and serine) repeated domains, which are highly glycosylated with a carbohydrate density of more than 70% (Fig. 1) (Lamblin et al., 1991; Thornton et al., 2008). Glycosylation involves primarily *N*-acetylgalactosamine (GalNac), *N*-acetylglucosamine (GlcNac), fucose, galactose (Gal), and sialic acid and low amounts of mannose and sulfate (Bansil and Turner, 2006). Due to their dense glycosylation, mucins are arranged in a brush-like structure (Lieleg and Ribbeck, 2011). Inside the secretory glands, high

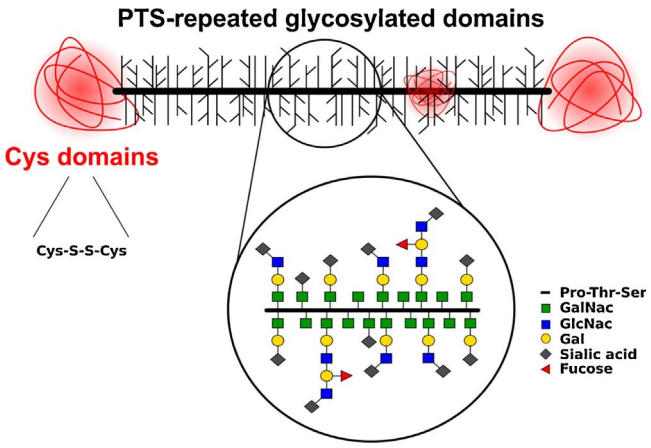

Fig. 1. Mucin O-linked glycoproteins. A mucin protein backbone typically consists of 'PTS' (proline, threonine, and serine) repeated domains, and interspersed with cysteine domains stabilized by internal disulfide bonds. Various O-glycans are linked to threonine or serine residues in the 'PTS' repeated domains. N-terminus to C-terminus from left to right, respectively. GalNac – N-acetylgalactosamine, GlcNac – N-acetylglucosamine, and Gal – galactose, not drawn to scale.

concentrations of calcium ions aid mucin condensation by shielding negatively charged sulfate and sialic acid groups. After secretion, mucins undergo dramatic swelling, with over 500-fold expansion in volume (Cone, 2009; Verdugo, 1990). Additionally, steric interactions of O-linked GalNac residues with the protein core contribute to the expanded mucin structure (Shogren et al., 1989). Furthermore, the PTS-domains are interspersed with hydrophobic globular regions with a high proportion of cysteine, which form intradisulfide bonds, and the subsequent polymerization forms long linear oligomers that provide the adhesive and swellable properties of the mucus layer (Bansil and Turner, 2006; Sheehan et al., 1986). High sialic acid and sulfate content in most mucin glycoproteins confer a strongly net-negative surface charge which increase the stiffness via charge repulsion (Shogren et al., 1989). At acidic pH, mucins change conformation from random coil to extended conformation and form a gel phase in mucus (Cao et al., 1999). These conformational changes were proposed to facilitate cross-links among mucin macromolecules through hydrophobic interactions at a low pH, leading to a sol-gel transition state (Cao et al., 1999). Additionally, changes in ionic strength may play a role in the formation of a gel phase in mucus, demonstrated by a study that calcium ions might promote assembly of mucins into large linear or branched aggregates (Raynal et al., 2003). Thus, physicochemical characteristics like composition, pH, ionic strength, conformation are important in the formation, function and rheological properties of mucus.

Currently, there are 21 mucin genes (MUC) identified in humans according to the HUGO gene nomenclature committee (http://www.genenames.org/, accessed 05.04.17). There are two types of mucins: membrane-bound mucins and secreted mucins. Membrane-bound mucins are related to cellular adhesion, pathogen binding, and signal transduction functions, while secreted mucins are highly related to the viscoelastic properties of mucus (Hollingsworth and Swanson, 2004; Williams et al., 2006).

Although the PTS-repeated domains are common to all mucins, their glycosylation, their specific sequence and number of tandem repeats are variable, and different collections of mucin genes are expressed in different tissues, suggesting that individual mucins have evolved to perform specific roles where they are expressed. Specific organs and correspondent secreted mucins are described in Table 1.

2.1. Mucus in airways

In the airways, MUC5AC and MUC5B are the major polymeric mucins present (Davies et al., 1999; Hovenberg et al., 1996b; Kirkham