Impact of Food-associated Stimuli on Barrier Properties of Gastrointestinal Mucus to Bacteria Transport

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Introduction:

Mucus is a highly viscoelastic medium composed of glycoproteins known as mucins, lipids, cellular and serum macromolecules, electrolytes, cells and cellular debris.[1-2] This high viscoelasticity and adhesivity of mucus provides a defensive barrier across many epithelial surfaces including gastrointestinal (GI) tracts. GI tract mucus functions as a robust barrier to trap and immobilize hazardous particulates including bacteria and viruses. In the mean time it allows the passage of orally delivered drugs and nutrients. These two opposite properties are crucially important in the small intestine where the mucus layer is thinnest.[3] However the mechanism of this selective barrier function is poorly characterized, especially in relation to transport of particulate systems. Particulates transport derived from foods ranging from bacteria to partially digested food is not fully understood. Particles in the GI tract including cellular material, bacteria, fat are mostly exposed to physiological surfactants that can change the physical properties of surface and modulates mucus particle interactions.[4] These include phospholipids as well as bile salts which involves on particle transport in mixed micelles. In this project, we have investigated the mechanisms of effects of physiologically relevant stimuli: Lipids, pH and [Ca^{+2}] on bacteria transport through the gastrointestinal mucus barrier. All these parameters are contributed with gastrointestinal changes regarding food intake and they can also be easily controlled.

Experimental Method

Native porcine intestinal mucus was collected from porcine jejunum within 2 h of slaughter. ATCC strain 700926 Escherichia coli (Fig.1) from frozen stocks (-70 °C) was streaked onto agar plates three times to obtain single colonies to inoculate into 10 ml Luria-Bertani (LB) medium. Single colonies were grown at 37 °C for ~12 h with vigorous shaking (300 rpm). E. coli was dosed into test media: Fed state levels of maleate buffer, bile salts-maleate buffer and fed state lipids in digested states. All these media were prepared at a relevant ph (3.5, 6.5), physiological [Ca^{+2}] concentration (10 mM). 5 µl of cells in LB was diluted in 25 µl of test medium and the diluted cell solution was added to 200 µl of native mucus solution. Bacteria diffusion was measured by tracking the positions of cells using an inverted fluorescence microscope at 75 X magnification and videomicroscopy. Three videos were taken from each individual preparation and the coordinates of cells were used to calculate time-averaged mean squared displacements: $MSD=[x(t+r)-x(t)]^2+[y(t+r)-y(t)]^2$, where t is the time scale and x, y are the coordinates. Time-independent diffusion coefficients (D_0) were determined by plotting MSD versus time scale to

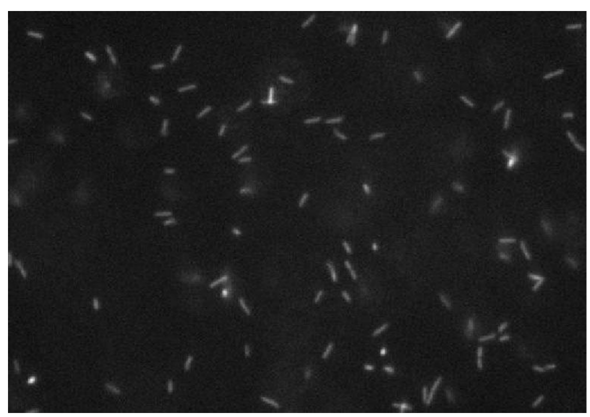

Figure 1. ATCC strain 700926 Escherichia coli

the anomalous subdiffusive transport relation: MSD=$4D_0T^{\alpha}$, where α is the anomalous exponent, indicates the degree of particle transport obstruction. (α = 1 for unobstructed Brownian diffusion)

Results and Discussion

Fed state intestinal contents (buffer+model bile+lipid mixture) significantly decreased bacteria transport rate through intestinal mucus compared to buffer. The constant MSD values in Fig. 2a show that the bacteria were unable to diffuse across the mucus barrier. The transport rate of E Coli was reduced 6 fold lower in FED State compared to buffer solution. Similar behavior was observed in the presence of bile salt. Presence of Calcium ion also reduced particle transport rate (Fig.2b) as did reduction in pH (Fig.2c).

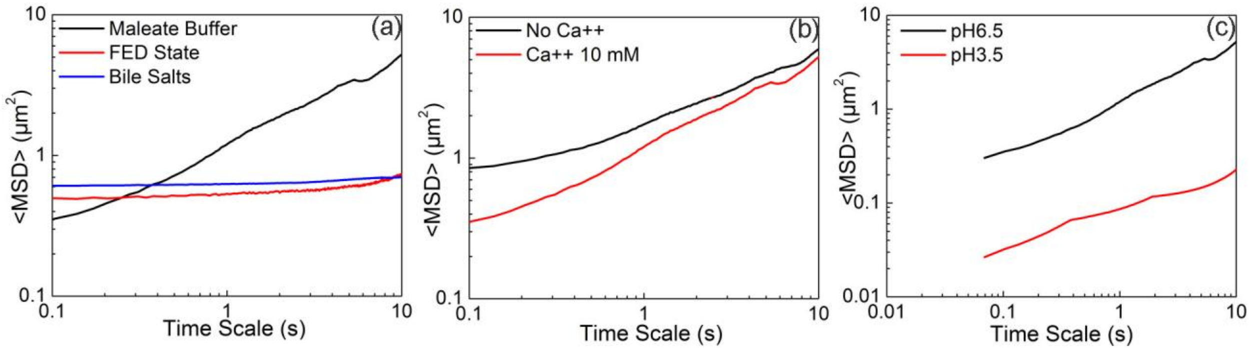

Figure 2. Ensemble <MSD> versus time scale plots for E. Coli a) Effects of the presence of Bile Salts and Lipids on bacteria diffusion in mucus b) Crosslinking Ca^{+2} ions affect the transport rate of the bacteria through gastrointestinal mucus barrier c) Elevation of medium pH affects mucus viscoelasticity, which leads to less hindered bacteria transport

Ca++ could be forming inter protein bonds between mucus fibers to form agglomerated multimeric structures that increased viscosity and leads hindered transport of particle diffusion. pH elevation induces decrease in viscoelastic moduli of the mucus structure which leads to less hindered tranport mobility. Most likely E. coli achieves motility by altering the rheological properties due to pH increment. These findings support the significance of food associated stimuli on mucus barrier properties, and also the naturally changing factors (lipids, calcium and pH) in the GI tract to pathogen transport.

References

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