Food-Associated Stimuli Enhance Barrier Properties of Mucus

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Statement of Purpose: Mucus is a complex network consisting of mucin glycoproteins, lipids, salts, and cellular serum macromolecules provides a barrier through which nutrients and orally delivered drugs must penetrate before entering the circulatory system. Mucus provides a significant, yet poorly characterized barrier to particulate, pathogen, and small molecule transport to epithelial surfaces. It is significantly important to understand the role of mucus barrier in modulating pathogen invasion, drug delivery, and nutrient/toxin absorption. While great strides have been made, the barrier properties of mucus, and how they change with different physiological states and disease, are generally poorly defined. This project describes the impact of physicochemical changes occurring upon food arrival in gastrointestinal mucus barrier properties.

Methods: Particle transport across porcine intestinal mucus from pig jejunum was investigated. 200 nm carboxylate-, PEG modified particles were used to study the influence of surface chemistry. 20-, 40-, 100-, 200-, and 500 nm carboxylate-modified particles were used to examine the size dependence of particle transport through mucus. Spherical particles were diluted in maleate buffer, bile salts (NaTDC) and simulated fed intestinal contents ("FED state") including maleate buffer, bile salts/phospholipids (lecithin) and a lipid mixture containing soybean oil and monoglycerol for a particle concentration of 0.0025 wt.-%. Calcium ion composition was adjusted by changing $CaCl_2$ levels in maleate buffer, and medium pH was controlled by adjusting NaOH concentration. Particle diffusion was measured by tracking the positions of diluted microspheres using real-time multiple particle tracking technique (MPT). Olympus IX51 was used to detect particles at 40 X magnification and videomicroscopy. Positions of particle centroids were used to calculate time-averaged mean squared displacements (MSD) and effective diffusivities (D_{eff}): $MSD = [x(t+\tau)-x(t)]^2 + [y(t+\tau)-y(t)]^2$ and $D_{eff} = MSD/(4\tau)$ where x and y are positional data and τ is the time scale. Particle effective diffusion coefficients were then used to estimate the fraction of nanoparticles expected to penetrate an intestinal mucus layer with a given thickness using a numerical integration of Fick's second law : $dC/dt = D_{eff}\ d^2C/dx^2$ where C is the concentration of particles, t is time and x is position. To further address the effect of food-associated stimuli on barrier properties of mucus lectin staining was performed. 80 µl of mucus was stained with 4 µl of 10 µg/ml lectin from Ulex europaeus agglutinin (UEA-1) conjugated with TRITC. After 20 min incubation in a dark humid chamber, 4 µl of yellow-green fluorescently labeled carboxylate-modified particles (diameter: 0.2 µm) diluted in medium was added as a model particulate system and allowed to diffuse across the mucus barrier for 2 h. Macro-scale change on mucus structure was observed by taking images for each type of preparation.

Results:

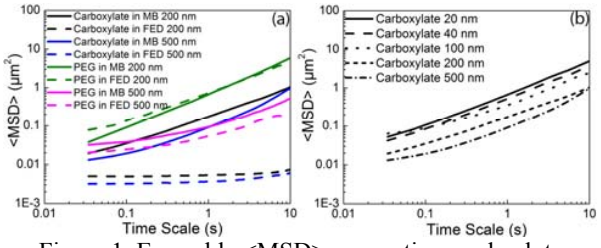

Figure 1. Ensemble <MSD> versus time scale plots

Fed state intestinal contents containing lipids significantly retarded model drug carrier diffusion rates, resulting in a 140 –fold reduction in the transport rate of 200 nm carboxylate modified microspheres. Influence of surface chemistry is significant. PEGylation greatly increased particle transport rates, as is evident by the 135-, and 15-fold higher ensemble MSDs (1s) of 200-, and 500-nm PEGylate particles compared with corresponding carboxylate- modified particles of the same size in FED State. This is likely PEGylation (nearly at neutral surface charge) reduce particle interactions with GI mucus diminishing the effect of FED State. While there is a reduction in transport with size, it doesn't appear to be as significant as with exposure to stimuli, likely due to heterogeneity of mucus structure.

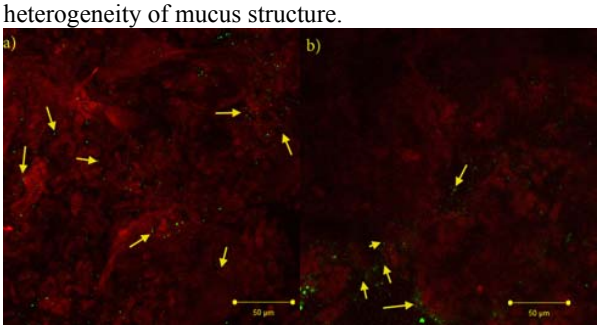

Figure 2. Confocal imaging reveals particle penetration across mucus barrier. Upon mucus exposure to a) Maleate buffer, particles were dispersed homogeneously; b) Fed state (lipids along with phospholipids and bile salts) particles (arrows) were hindered from penetrating an aggregated, cloudy mucus structure. Bars = 50 µm.

Macroscopic visual observation and micro-scale lectin staining patterns indicate mucus gel structural changes, specifically clumping into segments impenetrable by model drug carries correlate with transport properties.

Conclusions: Taken together, the observed exploration of stimuli associated with eating altered GI mucus barriers and potentially dosing of lipid-based delivery systems. Understanding the impact of these stimuli will enable us to improve the effective transport of drug carriers for oral delivery.

References:

Suh, J., Dawson, M., and Hanes, J., *Real-time multiple-particle tracking: Applications to drug and gene delivery.* Adv. Drug Deliv. Rev., 2005(57): p. 63-78.