Supporting Information

In vitro reconstitution of an intestinal mucus layer

shows that cations and pH control the pore structure

that regulates its permeability and barrier function

Abhinav Sharma¹, Jun-Goo Kwak¹, Kristopher W. Kolewe¹, Jessica D. Schiffman^{1,3}, Neil

Forbes^{1,2,3}*, Jungwoo Lee^{1,2,3}*

1. Department of Chemical Engineering, 2. Molecular and Cellular Biology Graduate Program,

3. Institute for Applied Life Sciences, University of Massachusetts, Amherst, MA 01003.

* Co-corresponding authors

Correspondence to:

Neil Forbes (forbes@umass.edu) Tel.: +1 413 577 0132

Jungwoo Lee (jungwoo@umass.edu) Tel.: +1 413 545 6290

N525 Life Sciences Laboratories

Department of Chemical Engineering

University of Massachusetts Amherst

240 Thatcher Rd

Amherst MA 01003-9364

S1

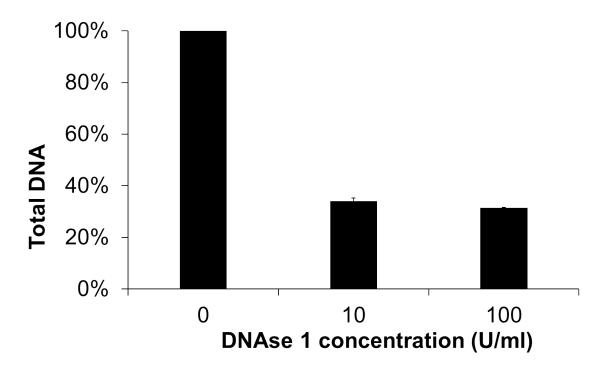


Figure S1. Change in total DNA after DNAse treatment of PSIM at room temperature for 10 hours compared to the untreated PSIM.

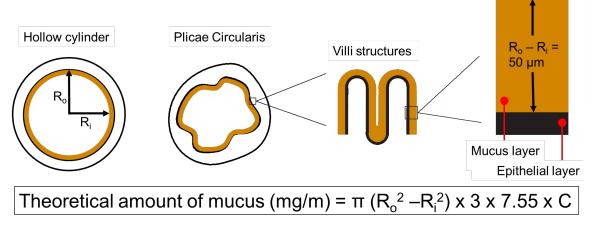


Figure S2. Schematic representation of gross small intestinal (SI) morphology and microstructure showing the components that contribute to increase in the surface area of the small intestine. In the equation for mucus amount, $\pi (R_o^2 - R_i^2)$ is the cross-sectional surface area of a hollow cylinder, and plicae circularis and villi structures increased the cross-sectional area by factors of 3 and 7.55, respectively. The theoretical amount of mucus per meter length was based on an outer radius (R_o) of 12.5 mm, an inner radius (R_i) of 12.45 mm, and pig small intestinal mucus concentration (C) of 20 mg/ml.

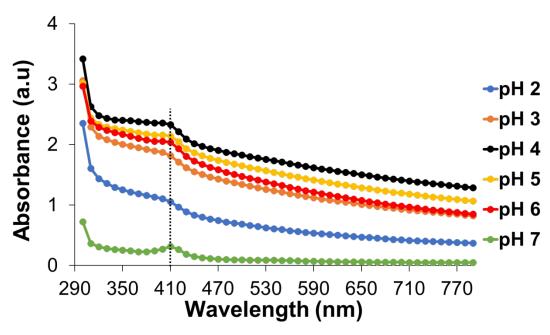


Figure S3. Absorbance spectra of 2 % (w/v) PSIM in the pH range 2-7 (Data plotted as an average of 3 independent samples).

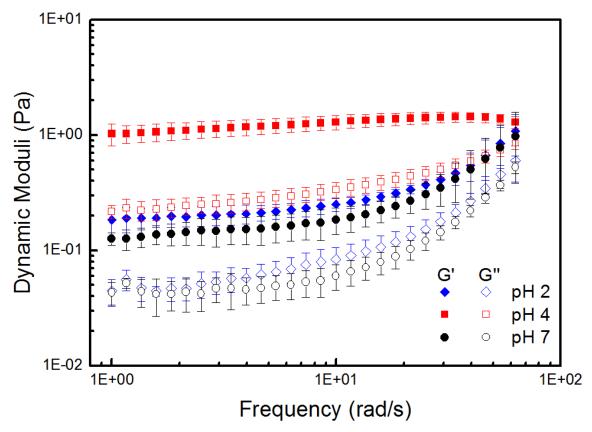


Figure S4. Rheological data showing the change in dynamic moduli (G' and G'') of PSIM (2% w/v) in the frequency range 1-68.3rad/s at 37°C (N = 3 PSI).

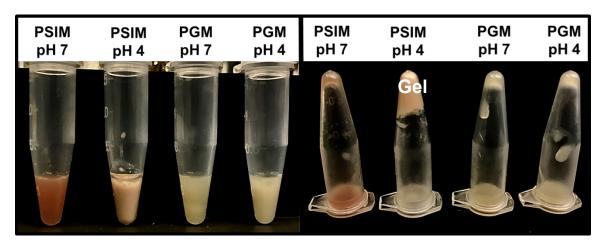
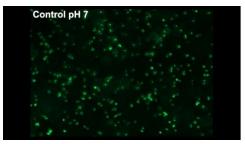
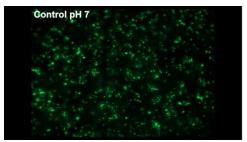


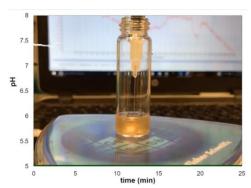
Figure S5. Gross image of PSIM and Porcine gastric mucin (Type II, Sigma Aldrich), 'solubilized' in 20mM HEPES solution at a concentration of 8% (w/v), and pH 7 and 4.



Movie S1. Bacterial motility in PSIM with pH change (GFP tagged *E. coli*).



Movie S2. Bacterial motility in PSIM with pH change (GFP tagged Salmonella).



Movie S3. Reconstituted PSIM reversible bulk property changes with pH.



Movie S4. Ca²⁺ and EDTA dependent reversible viscoelastic property change in PSIM.