Supporting Information:

The Role of Human Intestinal Mucus in the Prevention of Microplastic Uptake and Cell Damage

Ellen W. van Wijngaarden[a,b], Sandra L. Arias[c], Matthew Rhee[c], Meredith N. Silberstein[a,b], Ilana L. Brito[b,c,]*

[a] Sibley School of Mechanical and Aerospace Engineering, Cornell University, 124 Hoy Rd, Ithaca, NY 14850, USA.

[b] Engineered Living Materials Institute, Cornell University, Ithaca, NY 14850, USA.

[c]Meinig School of Biomedical Engineering, Cornell University, 237 Tower Rd, Ithaca, NY 14850, USA.

CONTENTS

1. SUPPORTING FIGURES

1.1 Analysis of MUC2/5 Mucus Composition using qPCR

A SYBR-based qPCR was performed in triplicate to quantify the amount of Muc2, Muc5B, and Muc5AC expressed by the HT-29-MTX cell line. 20 µl reactions were performed according to the New England Biolabs Protocol[1]. GAPDH was used as a reference gene to understand relative expression levels for each MUC gene through comparison of mean cycle threshold (CT) values from three independent tests. Primer sequences for each MUC gene and GAPDH gene were taken from [2]. Figure S1 shows a higher amount of MUC2 (CT = 28.6) in comparison to MUC5AC (CT =33.4) and MUC5B (CT = 32.3), suggesting the HT-29-MTX cell line produces mucus similar to that of healthy person, whereas a mucus with a higher MUC5AC and MUC5B expression profile than MUC2, would suggest mucus more similar to a CRC patient.[3] Previous studies have measured higher expression of MUC5 compared to MUC2 in HT-29-MTX[4,5]. However, Elzinga et al. demonstrated that the ratio of MUC2 to MUC5 could be altered, leading to higher MUC2 expression than MUC5, through cell culture conditions[5]. These results indicate that changes in culture conditions affect epithelial cell glycoprotein expression and demonstrate the need for verifying mucosal composition for differing studies. Previous work from our lab also indicates that the mucus model matches the mechanical properties (storage and loss moduli) of mucus samples from healthy patients providing further support that the composition provides a suitable model for the human mucus layer.[6]

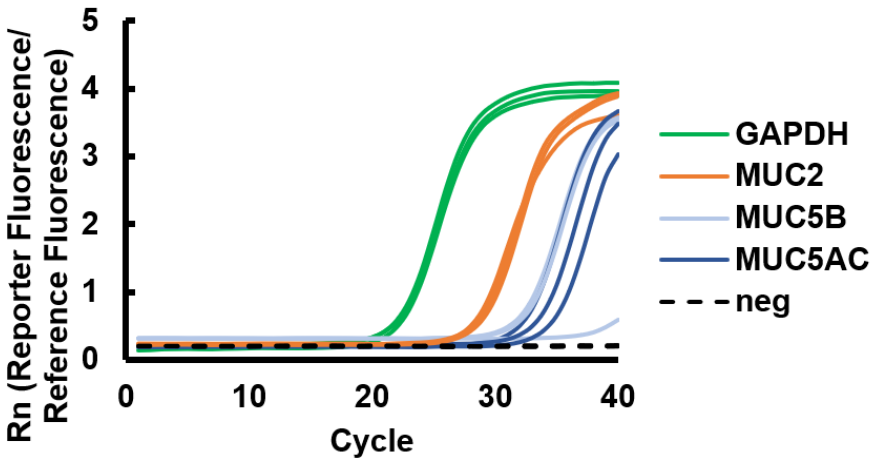

Figure S1. qPCR results demonstrating a higher level of MUC2 than MUC5B and MUC5AC in HT-29-MTX cells used in this study for the described culture conditions (Materials and Methods Section 2.1.)

1.2 Theoretical and Experimental Comparison of Diffusivities

To compare the microrheology technique with theoretical tests, the diffusivity values for PS-COOH particles were measured in PBS solution. The theoretical and PBS values have a p-value < 0.0001 for each size primarily due to the high sample size for each group. The percentage difference for 500, 200, and 100 nm are 10%, -17% and -13% respectively indicating that the measurement technique does not skew the values in a particular direction. This variation is likely due to the assumption that particles move independently from one another. The charge on PS-COOH particles and particle aggregation may contribute to particles not moving independently of one another.

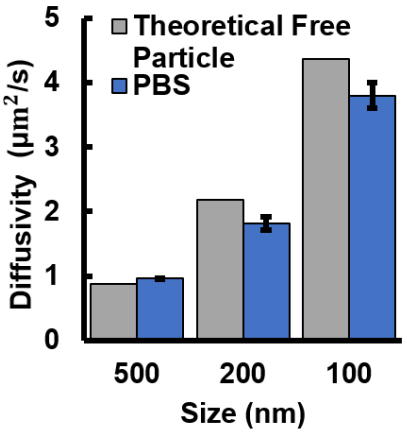

Figure S2. Comparison of microrheology results with diffusivity of theoretically free particles according to the Stokes-Einstein equation demonstrating a close match with our experimental results for PS-COOH particles of different sizes. (n = 1281, 1030, 8569)

1.3 T-Test Analysis for Particle Sizes, Compositions, and Surface Functionalizations

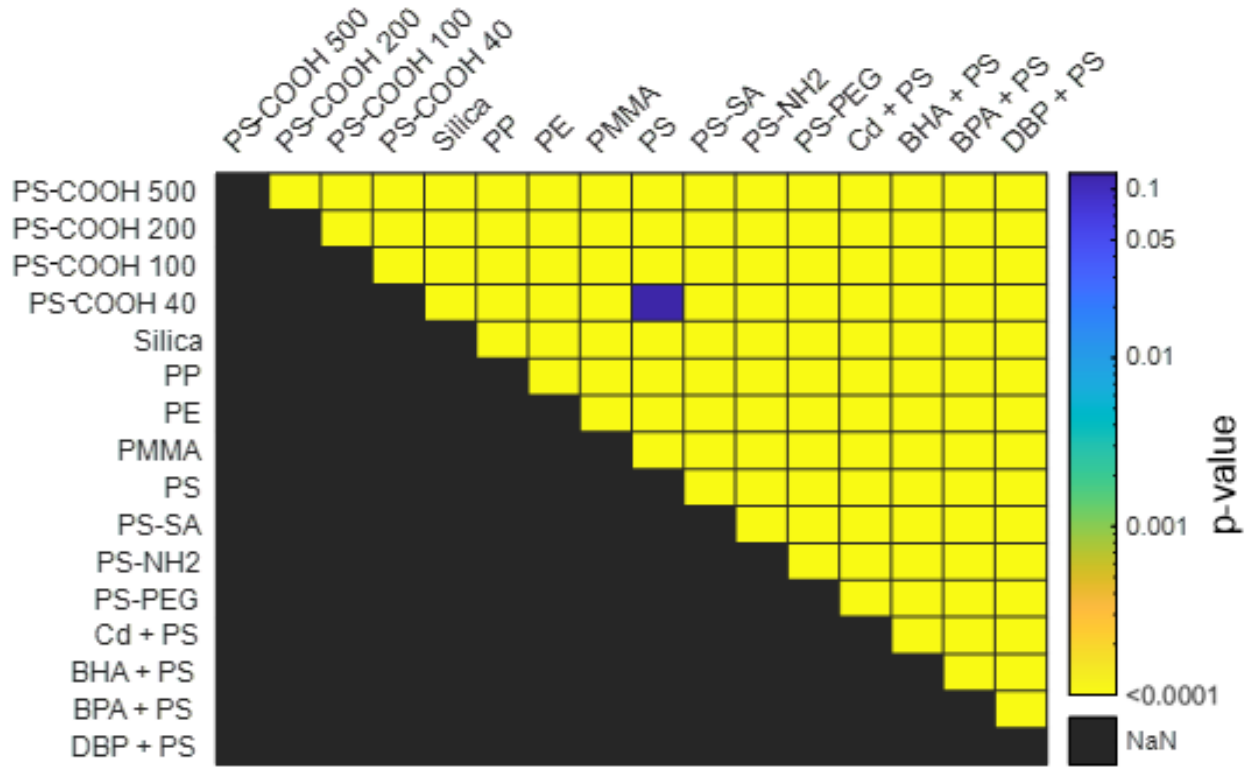

Figure S3. Two tailed student t-tests for determination of statistical significance between particle migration in mucus based on size, functionalization, composition and chemical exposure.

1.4 Particle Uptake Analysis Using Microscopy

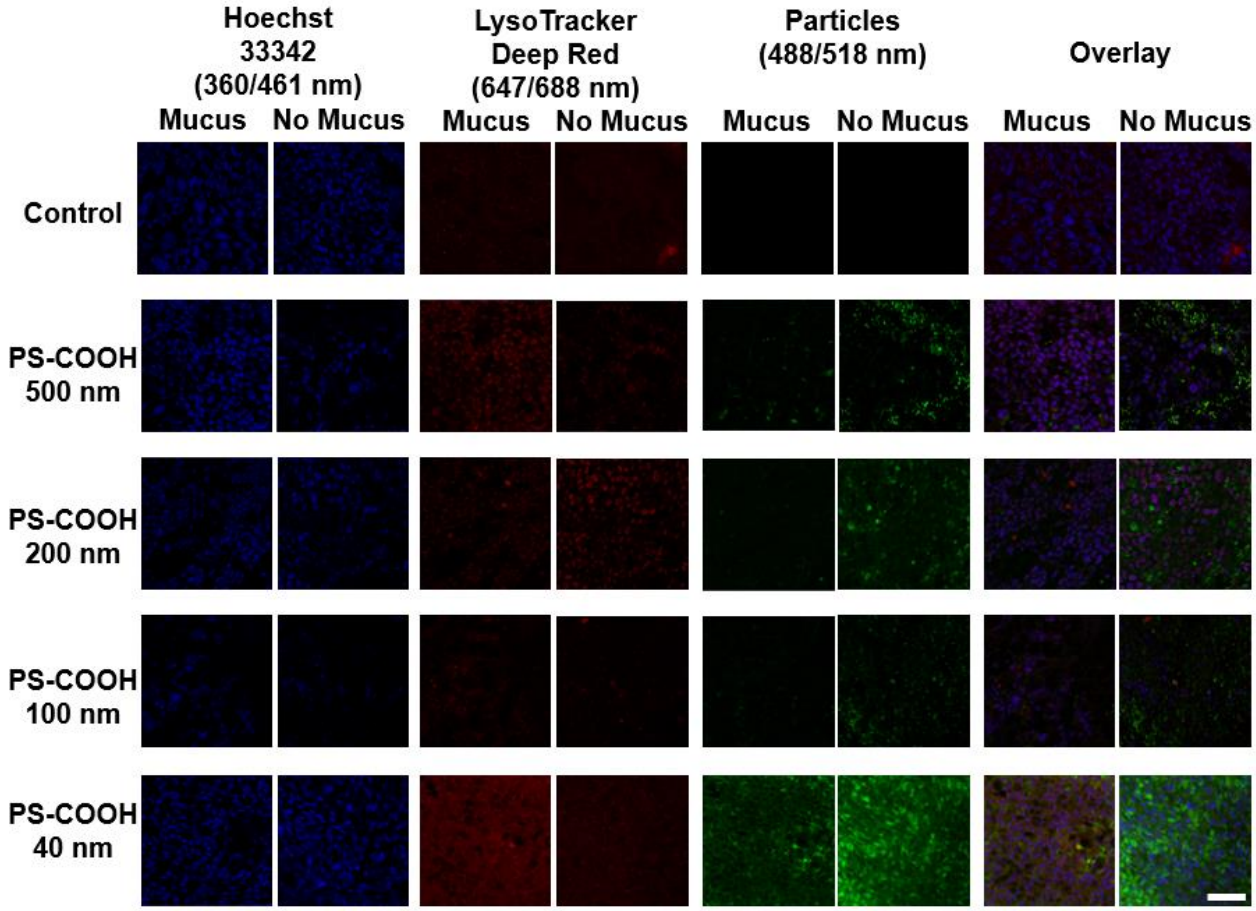

Figure S4. Microscopy results for microplastics of difference sizes demonstrating decreased cell viability, increased membrane destabilization and increased particle uptake for cells with no mucus layer for each composition and particle size tested. Cells with no mucus layer show decreased Hoechst 33342 fluorescence indicating decreased viability. Cells with no mucus layer show decreased red fluorescence when stained with LysoTracker Deep Red indicating membrane destabilization of the acidic vacuolar compartment. Cells with a mucus layer show intact lysosomes where as cells without a mucus layer show fewer red dots indicating damage to late endosomes and lysosomes. Cells without a mucus layer show diffuse green cytosolic fluorescence showing increased uptake of particles. Scale bar = 50 μm.

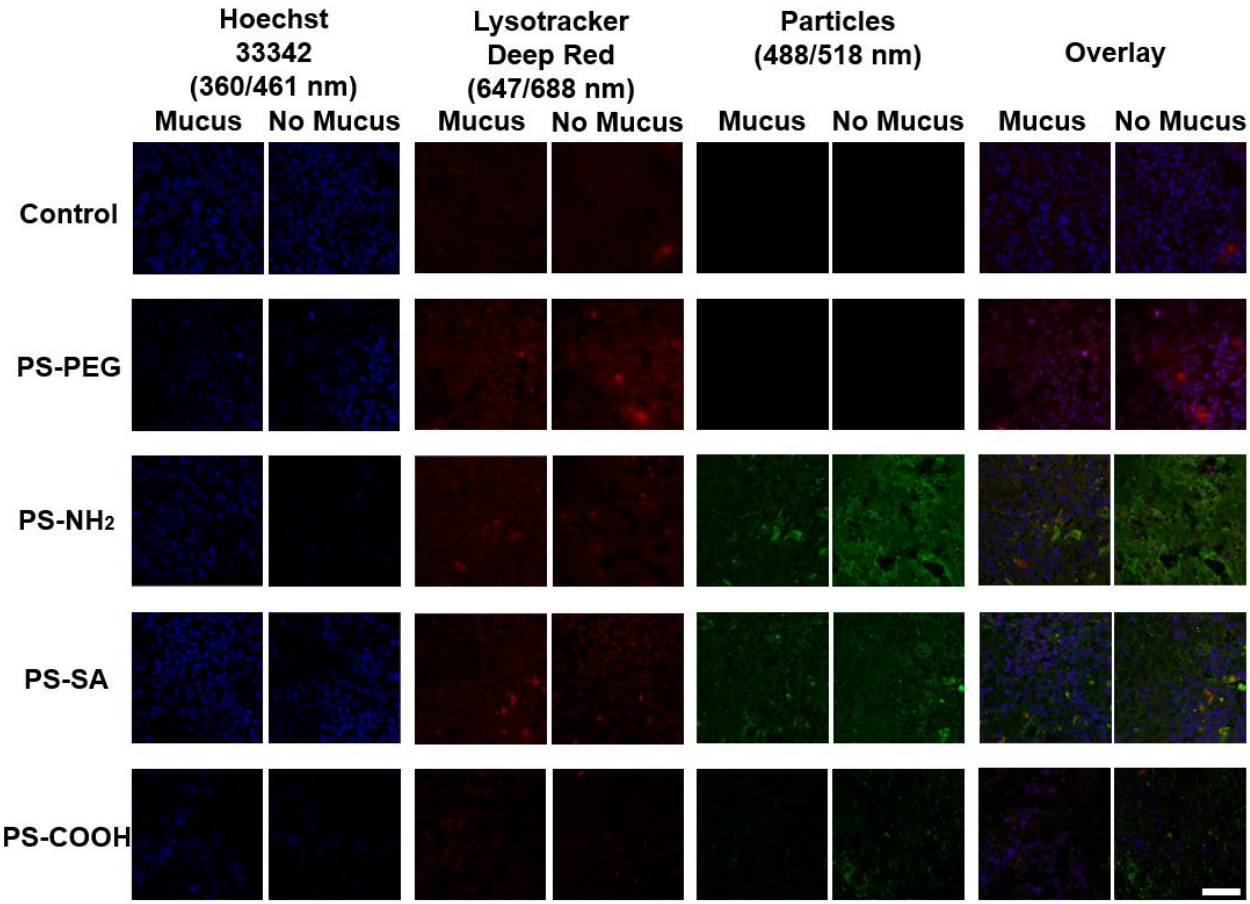

Figure S5. Microscopy results for PS particles with various surface functionalizations demonstrating general decreased cell viability, increased membrane destabilization and increased particle uptake for cells with no mucus layer for each composition and particle size tested. Scale bar = 50 μm.

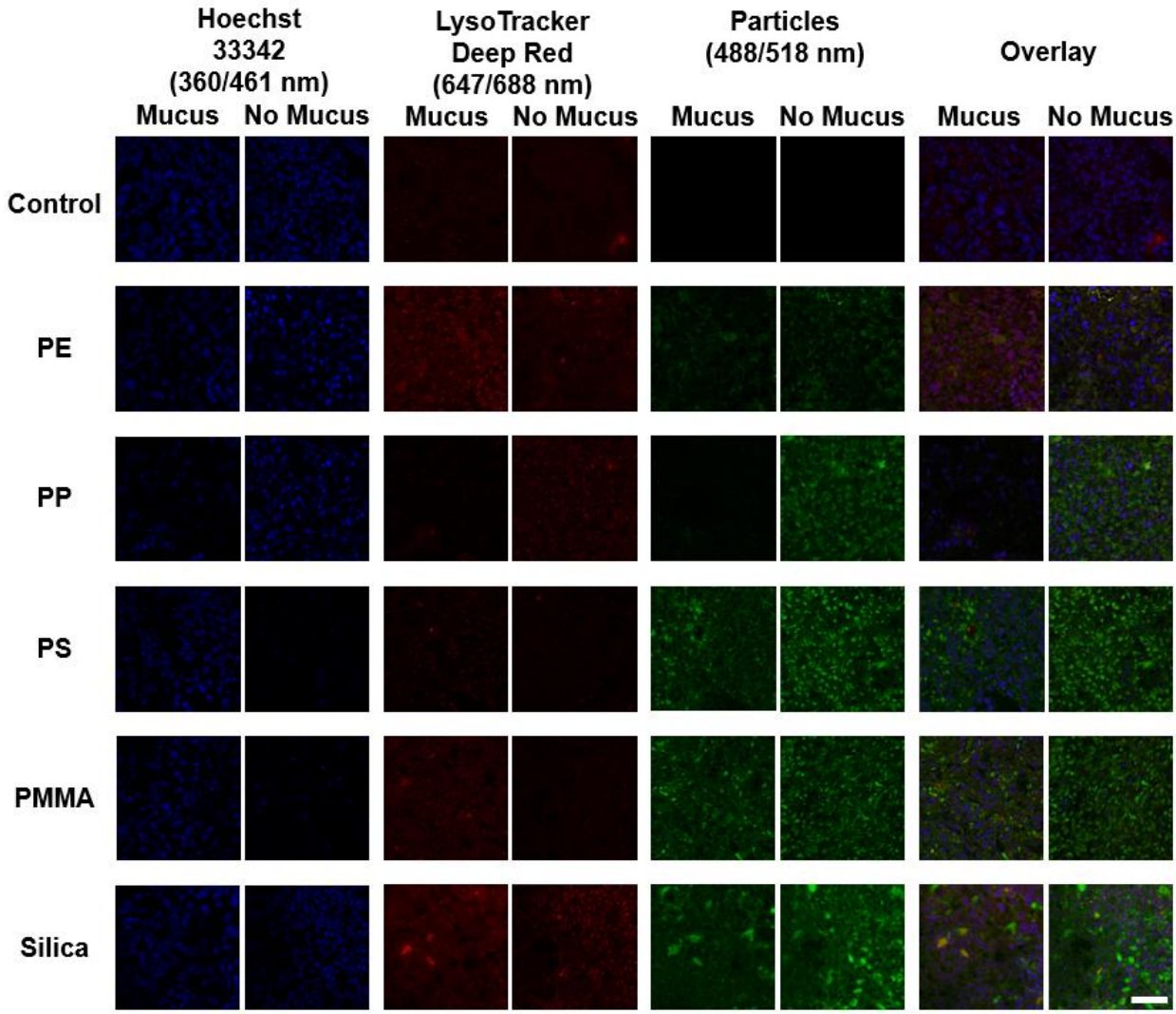

Figure S6. Microscopy results for particles of different compositions demonstrating general decreased cell viability, increased membrane destabilization and increased particle uptake for cells with no mucus layer for each composition and particle size tested. Scale bar = 50 μm.

1.5 Cell Damage and Uptake Microscopy Analysis

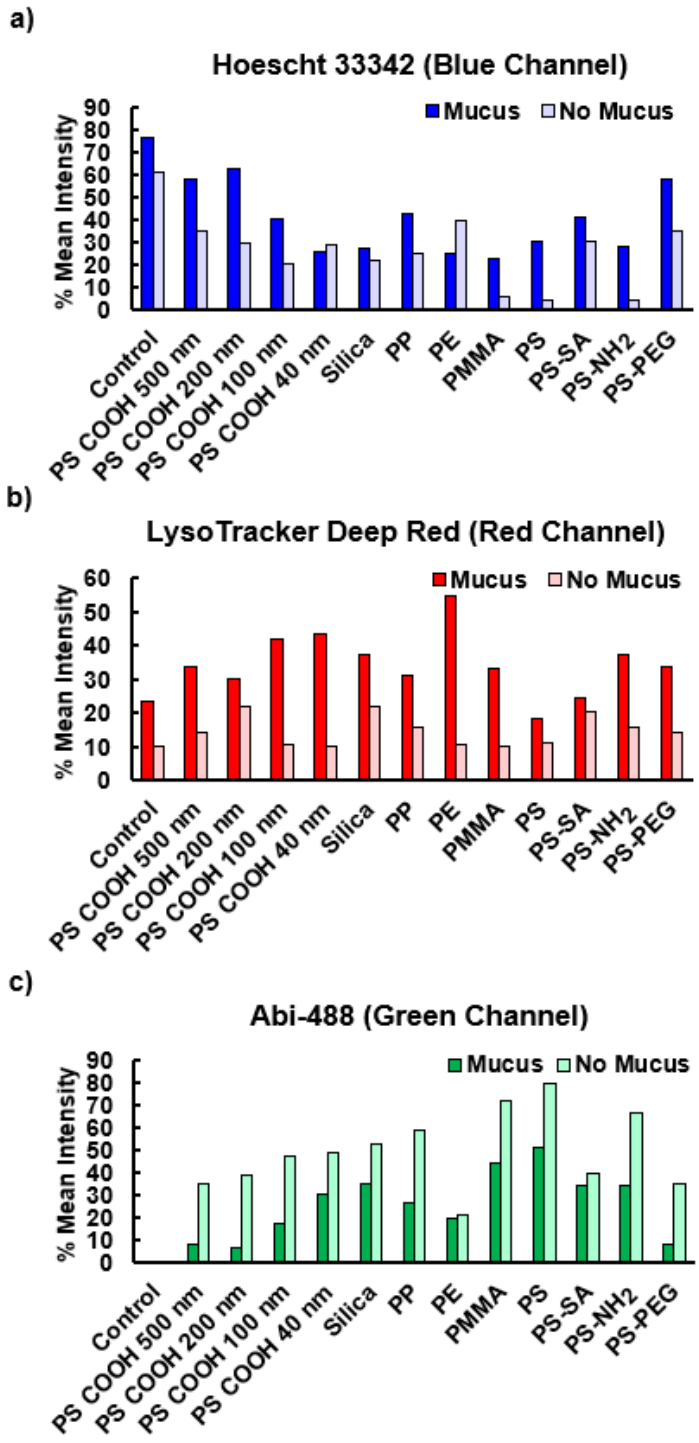

Figure S7. Percentage mean intensity for microscopy images for each channel for a) Hoescht 33342 (blue) b) LysoTracker Deep Red (Red) and c) Green Fluorescent Protein (Green).

1.6 Particle Uptake Analysis Using Flow Cytometry

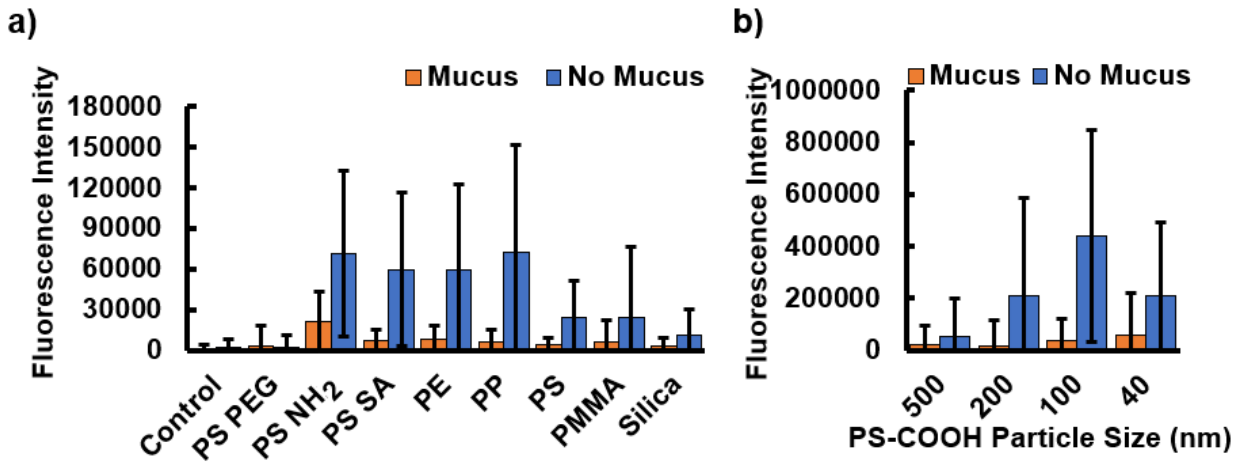

Figure S8. Absolute fluorescence values for particle uptake analyzed using flow cytometry for a) various particle compositions and b) particle sizes for PS-COOH.

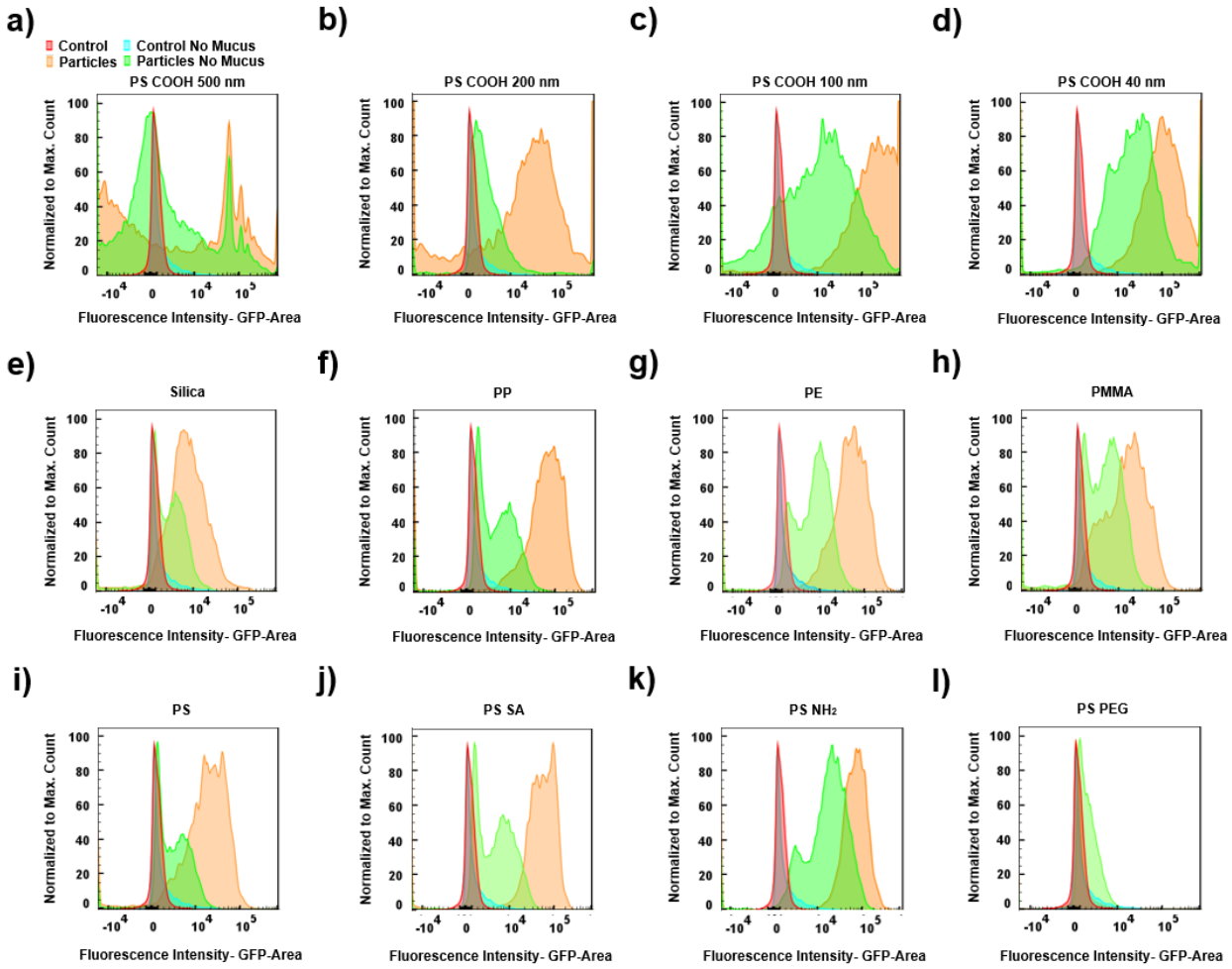

Figure S9. Histograms a) through l) for each particle composition and size tested showing an increased mean fluorescence indicating higher cell particle uptake when the mucus layer is absent (orange) compared to when a mucus layer is present (green) for most conditions. The control with mucus (red) shows no difference compared to the control without mucus (blue), indicating no fluorescence shift due to the removal of mucus.

1.7 MPs as Vectors for Environmental Pollutants: Mucus Protection

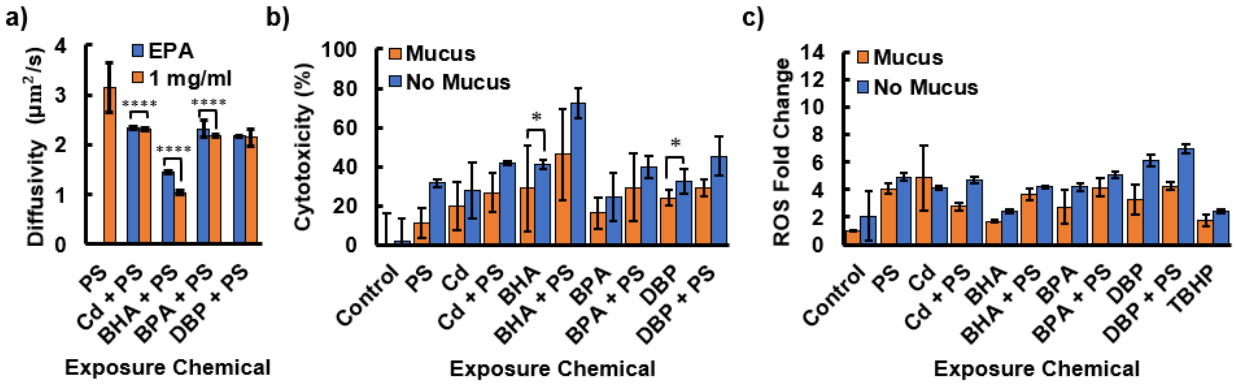

Figure S10. a) Diffusivity of PS particles in HT-29-MTX cell mucus following exposure to various environmental toxins at concentrations of 1 mg/ml. b) Cytotoxicity with and without the mucus layer for environmental toxins (1 mg/ml) alone and toxins with PS particles. c) Reactive oxygen species fold change for environmental pollutants (1 mg/ml) with and without the mucus layer.

REFERENCES

(1) *Luna® Universal qPCR Master Mix Protocol (#M3003) | NEB*. https://www.neb.com/en-us/protocols/2016/11/08/luna-universal-qpcr-master-mix-protocol-m3003 (accessed 2024-10-27).

(2) Huang, X.; Gao, Y.; Li, S.; Wu, C.; Wang, J.; Zheng, N. Modulation of Mucin (MUC2, MUC5AC and MUC5B) mRNA Expression and Protein Production and Secretion in Caco-2/HT29-MTX Co-Cultures Following Exposure to Individual and Combined Aflatoxin M1 and Ochratoxin A. *Toxins* **2019**, *11* (2), 132. https://doi.org/10.3390/toxins11020132.

(3) Bu, X.-D.; Li, N.; Tian, X.-Q.; Li, L.; Wang, J.-S.; Yu, X.-J.; Huang, P.-L. Altered Expression of MUC2 and MUC5AC in Progression of Colorectal Carcinoma. *World Journal of Gastroenterology : WJG* **2010**, *16* (32), 4089. https://doi.org/10.3748/wjg.v16.i32.4089.

(4) Lesuffleur, T.; Porchet, N.; Aubert, J.-P.; Swallow, D.; Gum, J. R.; Kim, Y. S.; Real, F. X. Differential Expression of the Human Mucin Genes MUC1 to MUC5 in Relation to Growth and Differentiation of Different Mucus-Secreting HT-29 Cell Subpopulations. *Journal of Cell Science* **1993**, *106* (3), 771–783. https://doi.org/10.1242/jcs.106.3.771.

(5) Elzinga, J.; Lugt, B. van der; Belzer, C.; Steegenga, W. T. Characterization of Increased Mucus Production of HT29-MTX-E12 Cells Grown under Semi-Wet Interface with Mechanical Stimulation. *PLoS ONE* **2021**, *16* (12), e0261191. https://doi.org/10.1371/journal.pone.0261191.

(6) Arias, S. L.; van Wijngaarden, E.; Balint, D.; Jones, J.; Silberstein, M. N.; Brito, I. L. Commensal Gut Bacteria Modify the Physical Properties of Mucus. In *Biomedical Engineering Society 2024 Annual Meeting*; Biomedical Engineering Society: Baltimore, MD, 2024.