Measuring Microplastic Migration Through Human Intestinal Mucus

CNF Project Number: 3058-23
Principal Investigator(s): Meredith Silberstein
User(s): Max Tepermeister, Ellen van Wijngaarden

Affiliation(s): Sibley School of Mechanical and Aerospace Engineering, Cornell University

Primary Source(s) of Research Funding: College of Engineering Sprout Award

Contact: meredith.silberstein@cornell.edu, mt845@cornell.edu, ewv8@cornell.edu

Research Group Website: silbersteinlab.com

Primary CNF Tools Used: Harrick Plasma Generator, Malvern Nano ZS Zetasizer

Abstract:

Microplastic accumulation in the body has become a growing concern due to widespread harmful physiological effects. This research is focused on understanding how the intestinal mucus layer prevents the migration of ingested microplastics. We studied how size and surface charge of microplastics of different compositions, functionalization and size alter particle movement through mucus. Previous studies have used alternative mucus models such as porcine gastric mucus and have focused primarily on one plastic composition. Our study uses mucus derived from a human colonic cancer cell line, HT29MTX, and tests a broad range of plastic compositions and sizes. Results highlight the importance of the mucus layer in hindering the migration of particles > 200 nm and identify compositions diffuse more easily through mucus.

Summary of Research:

Over 400 million tons of plastic are produced every year [1]. Many of these plastics end up in the environment, gradually breaking down into microplastics. Microplastics are defined as plastic particles under 5 mm and can be found in the water soil and air. Ingestion of these particles has been linked to negative health impacts including metabolic disorders, neurotoxicity and intestinal inflammation [2]. The mucus layer within the intestine acts as a protective barrier against harmful substances however, studies have demonstrated that microplastics often travel through intestinal mucus and end up in other organs [3]. The goal of this research project is to characterize microplastic migration through intestinal mucus to identify key factors contributing to microplastic migration and understand the role of the mucus layer. Previous work has looked at particle migration in various mucus models such as porcine gastric mucus [4], hydrogels, human lungs, human cervix and various animal sources [4,5]. Most studies focus on polystyrene microplastics however a broad array of particle compositions are often ingested [2]. We used particle characterization techniques and microrheology to study the migration of different compositions, surface functionalizations and sizes in intestinal mucus produced by HT29MTX human colorectal adenocarcinoma cells.

HT-29MTX cells were chosen due to their high mucus production and common use in human intestinal studies. Cells were grown as a monolayer in growth media for 21 days for a mature mucus layer. Zeta potential was used to measure the effective surface charge of particles in solution. Zeta potential was measured using a Malvern Nano Zs Zetasizer (Malvern Pananalytical, Malvern, U.K.). A total of three measurements were taken for each particle type. Each particle composition and functionalization was tested with values ranging from -43 mV to 14 mV, as shown in Figure 1.

Cells were cultured in 6-well plates on circular cover glasses for microrheology to determine particle diffusivity. The particle of interest was added to the culture media at a concentration of 1 μl/ml and left to equilibrate for 15 minutes. The diffusion of particles through the mucus layer was imaged on an Elyra Super Resolution Microscope. A time series was collected with a step of 0.05 s with at least three biological replicates and at different locations over the cell monolayer. Images were analyzed using ImageJ Trackmate and Matlab to determine mean squared displacements and diffusion coefficients [6].

Diffusivity was determined to compare the effects of size, composition and surface functionalization. Diffusivity increased as microplastic size decreased, as shown in Figure 2. Polyethylene and polystyrene particles had the highest diffusivity out of all particle compositions tested, while silica particles had the lowest diffusivity. The diffusivity of 500 nm particles could not be determined

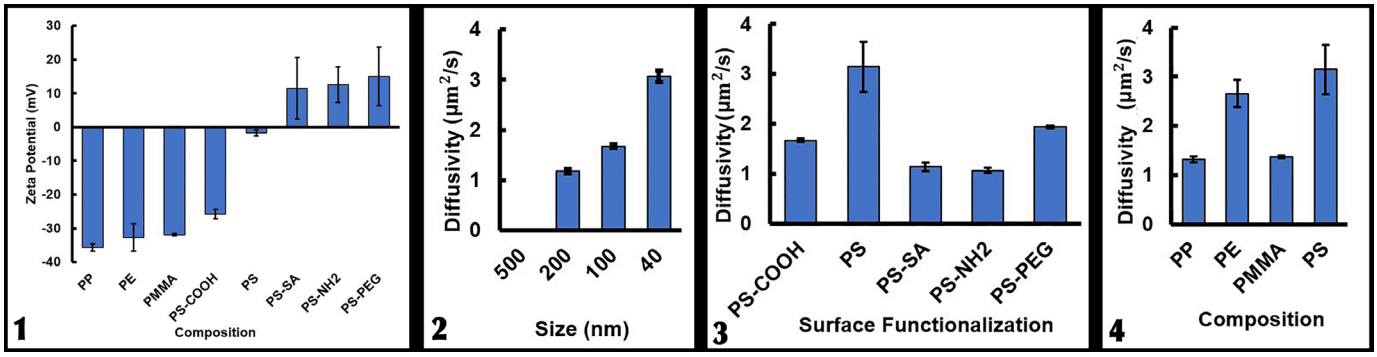

Figure 1: Zeta potential for each particle composition and surface functionalization.

Figure 2: Diffusivity for each particle size.

Figure 3: Diffusivity for each particle composition at 100 nm for PS, PP, PE, and PMMA.

Figure 4: Diffusivity for each surface functionalization of for PS 100 nm particles.

as these particles did not migrate through the mucus. Mucus has a pore size of approximately 200 nm, which prevented these particles from passing through [7]. In contrast, 40 nm particles were able to diffuse much more easily through the mucus layer. Figures 3 and 4 indicate that unfunctionalized polystyrene had the highest diffusivity. These results highlight the role of the mucus layer in preventing the migration of particles > 200 nm and indicate that certain compositions present a greater health threat. Further study is needed to understand how diffusivity might be related to negative health effects such as inflammation and cell death.

Conclusions and Future Steps:

The tools and technical expertise provided at the CNF was essential to the rapid testing necessary for biological samples. Future work will investigate how the mucus layer structure and mechanical properties alter cell uptake of particles through the design of microfluidic chips for cell growth and imaging. We will also expand on our preliminary tests to include particles carrying other environmental pollutants which may alter surface properties and change particles migration through the mucus.

References:

[1] Y. Li, L. Tao, Q. Wang, F. Wang, G. Li, M. Song, Environ. Health 2023, 1, 249.

[2] G. Visalli, A. Facciolà, M. Pruiti Ciarello, G. De Marco, M. Maisano, A. Di Pietro, Int J Environ Res Public Health 2021, 18, 5833.

[3] M. M. Garcia, A. S. Romero, S. D. Merkley, J. L. Meyer-Hagen, C. Forbes, E. E. Hayek, D. P. Sciezka, R. Templeton, J. Gonzalez-Estrella, Y. Jin, H. Gu, A. Benavidez, R. P. Hunter, S. Lucas, G. Herbert, K. J. Kim, J. Y. Cui, R. R. Gullapalli, J. G. In, M. J. Campen, E. F. Castillo, Environmental Health Perspectives 2024, 132, 047005.

[4] P. Georgiades, P. D. A. Pudney, D. J. Thornton, T. A. Waigh, Biopolymers 2014, 101, 366.

[5] A. Cobarrubia, J. Tall, A. Crispin-Smith, A. Luque, Frontiers in Physics 2021, 9.

[6] J.-Y. Tinevez, N. Perry, J. Schindelin, G. M. Hoopes, G. D. Reynolds, E. Laplantine, S. Y. Bednarek, S. L. Shorte, K. W. Eliceiri, Methods 2017, 115, 80.

[7] J. McCright, A. Sinha, K. Maisel, Cell Mol Bioeng 2022, 15, 479.