# Toxicity and mechanisms of uptake and translocation of ingested polystyrene micro-nanoplastics in advanced in vitro small intestinal models

*Glen M. DeLoid, Davood Kharaghani, Zhenning Yang, Lila Bazina, Ping He, Ben Swenor, Trung Huu Bui, Nubia Zuverza-Mena, Carlos Tamez, Craig Musante, Michael Verzi, Jason C. White, and Philip Demokritou*

Nanoscience & Advanced Material Center (NAMC) | RUTGERS HEALTH Environmental and Occupational Health Sciences Institute | RUTGERS NEW BRUNSWICK | CAES The Connecticut Agricultural Experiment Station — Putting Science to Work for Society since 1875

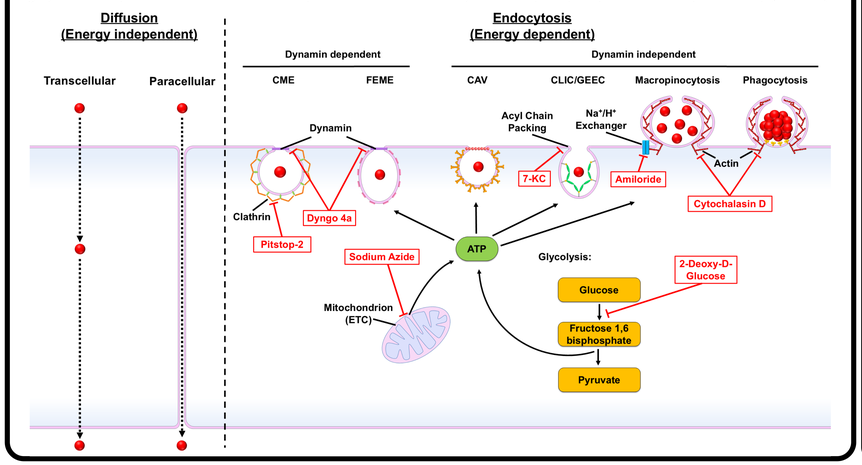
## Abstract

Micro and nanoplastics (MNPs) are now ubiquitous contaminants of food and water. Cellular and animal studies have shown that ingested MNPs can breach the intestinal barrier to reach the circulation. To date however, the mechanisms involved in intestinal absorption of MNPs have not been investigated with physiologically relevant models, and thus remain unknown. We employed both an immortalized human cell line-based triculture transwell small intestinal epithelium (SIE) model and a microfluidic primary human intestinal epithelial organoid-based intestine-on-a-chip (IOC) model, combined with in vitro simulated 3-phase digestion, and a panel of ATP synthesis and endocytosis pathway inhibitors, to assess the toxicological impacts and mechanisms effectuating uptake and translocation of nanoscale polystyrene MNPs in the small intestine. In the triculture model, 24 h exposure to digestas of 26 nm carboxylated polystyrene (PS26C), at an effective oral concentration (EOC) of 125 µg/mL, caused no significant toxicity. Inhibition of ATP synthesis reduced PS26C translocation by only 35%. Translocation was also significantly decreased by inhibition of dynamin and clathrin, suggesting involvement of clathrin mediated endocytosis (CME) and fast endophilin-mediated endocytosis (FEME). Inhibition of actin polymerization also significantly reduced translocation, suggesting involvement of macropinocytosis or phagocytosis. However, inhibition of the Na+/H+ exchanger had no effect on translocation, ruling out macropinocytosis. Together these results suggest uptake by passive diffusion as well as by active phagocytosis, CME, and FEME pathways. In the IOC model, with organoids from both healthy human donors and patients with Crohn's disease, 24 h exposure to digestas of 25 nm polystyrene shell-gold core (AuPS25) tracer MNPs at an EOC of 60 µg/mL caused minimal toxicity. RNAseq analysis of epithelial cell lysates from studies in the healthy donor IOC model identified 9 dysregulated genes, notably including downregulation of IFI6 (interferon alpha-induced protein 6), which has antiviral and immunosuppressive functions in the intestine. Inhibitor studies in the healthy donor IOC model revealed that AuPS25 uptake occurred by both passive and active mechanisms, including phagocytosis and/or macropinocytosis, and CME and/or FEME, consistent with our findings in the triculture SIE model. Together these results indicate that uptake of nanoscale PS MNPs occurs by both passive diffusion and multiple active endocytic pathways, including phagocytosis, CME, and FEME. Further studies are needed to assess uptake mechanisms for other environmentally relevant MNPs as a function of polymer, surface chemistry, and size.

## Introduction

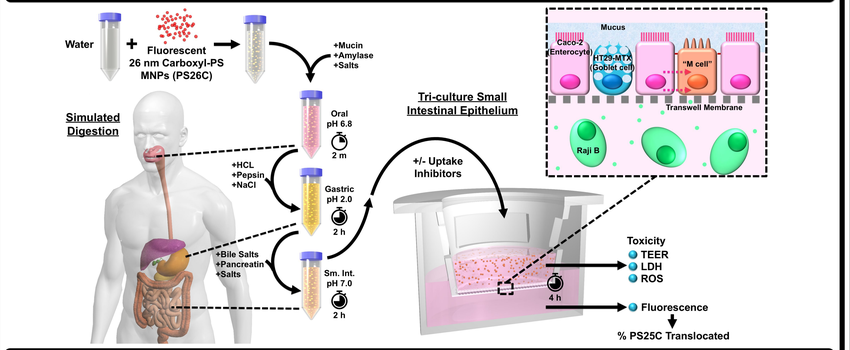
Plastics production increased from 2 million to 400 million metric tons between 1950 and 2022, generating a total of 6.3 billion metric tons of plastic waste as of 2015. Only 9% of waste plastic is recycled, while 12% is incinerated and 79% is disposed in landfills. As a result of mechanical, thermal, and photo-oxidative degradation of this waste plastic, our environment and food web have become widely contaminated with micron to nanometer scale plastic fragments (micro-nanoplastics or MNPs). Due to the widespread use of plastics in agricultural systems and processes, soil and irrigation water have been contaminated with MNPs. MNPs have also been found in surface and groundwater samples. Soil and water MNPs are readily taken up by edible plants and crops, which are directly consumed by livestock and humans. MNPs have been found in many foods, including meats, dairy products, grains, and seafood. Significant human ingestion exposure to MNPs is therefore clearly ongoing and likely to increase. Despite the growing evidence of widespread heavy MNP contamination of foods and water, very little is known about the fate, biointeractions, or health implications of environmentally relevant ingested MNPs. However, a number of *in vitro* and *in vivo* studies have reported significant toxic effects. Animal studies have demonstrated that ingested MNPs are readily absorbed in the gastrointestinal tract (GIT), and bypass biological barriers to accumulate in many organs and tissues. It is therefore not surprising that recent human biomonitoring studies have found MNPs of multiple polymers in human placenta, feces, colon, blood, and brain.

A fundamental question that has not been fully addressed is "what cellular mechanisms are involved in MNP uptake by and translocation across the gastrointestinal epithelium?" These could include either passive diffusion (energy-independent), or one or more of six active endocytic pathways (energy-dependent), summarized in the figure below: (1) clathrin-coated pit-mediated endocytosis (CME); (2) fast endophilin-mediated endocytosis (FEME); (3) clathrin-independent carrier glycosyl-phosphatidylinositol-anchored protein enriched early endocytic compartment (CLIC/GEEC); (4) macropinocytosis; (5) phagocytosis; and (6) caveolae mediated endocytosis (CAV). In the studies described herein, we employed two advanced *in vitro* models of the small intestinal epithelium: (1) a transwell triculture model developed from immortalized cell lines (Caco-2, HT29-MTX, and Raji B), and (2) a two-channel (endothelial and epithelial) microfluidic intestine-on-a-chip (IOC) model developed using human small intestinal epithelial organoids and primary human intestinal microvascular endothelial cells, combined with simulated digestion of model polystyrene (PS) MNPs (to reproduce physicochemical transformations that occur during digestion), and a panel of inhibitors of ATP synthesis and specific uptake pathways (indicated in red in the figure below) to assess the roles of energy and each uptake pathway on the uptake and translocation of PS MNPs. In the triculture model, we employed red fluorescent carboxylated 26 nm PS MNP (PS26C), which allowed fluorescence-based quantification of translocation. In the IOC model, a 25 nm gold core – PS shell MNP was used to allow highly accurate quantification of the PS MNP uptake and translocation by inductively-coupled plasma mass spectrometry (ICP-MS).

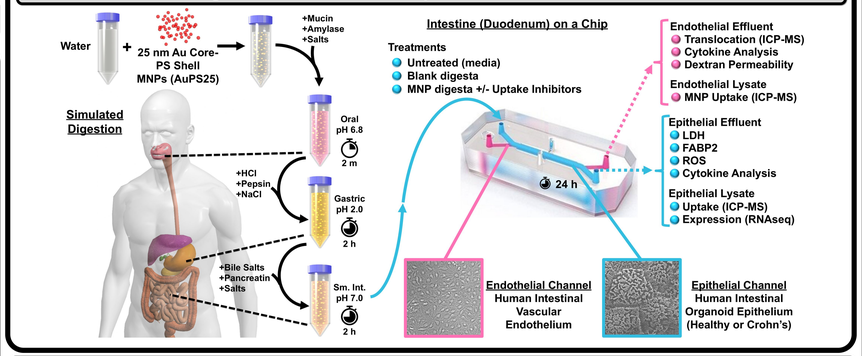



## Materials and Methods

### Studies in a triculture small intestinal epithelium model:



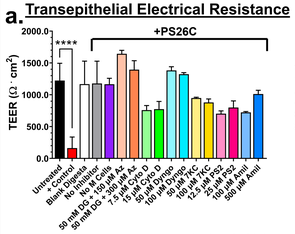

### Studies in a human organoid intestine-on-a-chip:



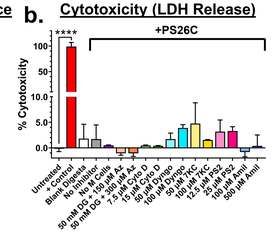

## Results

### Studies in a triculture small intestinal epithelium model:

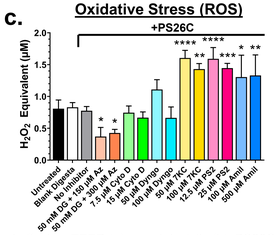
**a. Transepithelial Electrical Resistance**



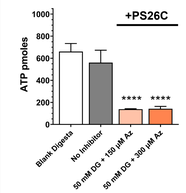

**b. Cytotoxicity (LDH Release)**



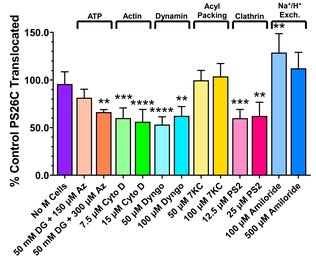

**c. Oxidative Stress (ROS)**




Treatment for 4 h with digestas of PS26C (IOC 125 µg/mL) alone or with inhibitors had no significant effect on TEER (a), or cytotoxicity (b). PS26C digesta treatment alone had no effect on ROS production, but ROS was significantly increased by treatment with PS26C plus inhibitors 7-ketocholesterol (7KC), Pitstop-2 (PS2), and Amiloride (Amil).



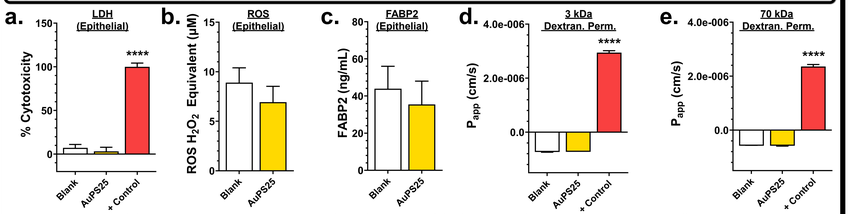

50 mm 2-Deoxy Glucose (DG) with 150 or 300 µM sodium azide (Az) decreased cellular ATP by ~75%




Inhibition of ATP synthesis reduced translocation by only 35%, suggesting uptake by both active endocytic pathways and passive diffusion. Translocation was also significantly decreased by inhibition of dynamin and clathrin, suggesting involvement of clathrin mediated endocytosis (CME) and fast endophilin-mediated endocytosis (FEME). Inhibition of actin polymerization also significantly reduced translocation, suggesting involvement of macropinocytosis or phagocytosis. However, inhibition of the Na+-H+ exchanger had no effect on translocation, thus ruling out macropinocytosis. Inhibition of Acyl Packing had no effect on translocation, ruling out the CLIC/GEEC pathway.

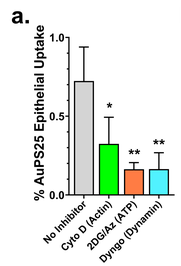
## Results

### Studies in a human organoid intestine-on-a-chip:

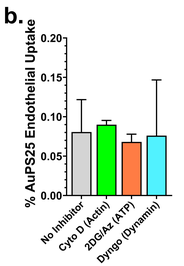



In the healthy donor IOC model, Treatment with digestas of AuPS25 at an EOC of 60 µg/mL for 24 h had no significant effect on epithelial general cytotoxicity (LDH release, (a)), oxidative stress (ROS, (b)), or enterocyte cytotoxicity (FABP2 release, (c)), or on barrier integrity (3 kDa and 70 kDa dextran permeability, (d) and (e)). The same treatment also had no effects on toxicity in the Crohn's donor IOC model (data not shown).

**a.**



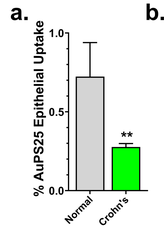

**b.**



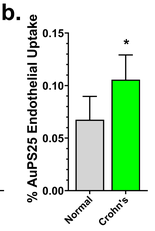

In the healthy donor IOC model, depletion of ATP by 2-deoxy D glucose (2DG) and azide (Az) resulted in a 77.5% reduction of epithelial AuPS25 uptake (p<0.01), indicating involvement of both energy-dependent uptake mechanisms and passive diffusion (a). Inhibition of actin polymerization by treatment with cytochalasin D resulted in a 55% reduction of epithelial AuPS25 uptake (p<0.05), suggesting involvement of phagocytosis and/or macropinocytosis (a). Inhibition of dynamin with by Dyngo-4a resulted in a 77.2% (p<0.01) reduction in epithelial AuPS25 uptake, suggesting involvement of either CME or FEME pathways (or both).

In the endothelial channel, neither ATP depletion nor inhibition of either actin polymerization or dynamin activity had any significant impact on uptake of AuPS25. This suggests that uptake in the endothelium occurs primarily by passive diffusion.

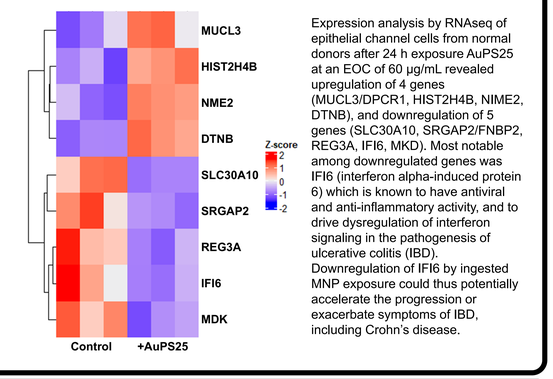
**a.**




**b.**




Uptake of AuPS25 by the Crohn's epithelium was significantly lower (by 62%, p<0.01) than that in the healthy epithelium (a). In contrast, uptake in the endothelial layer was significantly greater in Crohn's than healthy donor chips (by 30%, p<0.05) (b).




Expression analysis by RNAseq of epithelial channel cells from normal donors after 24 h exposure AuPS25 at an EOC of 60 µg/mL revealed upregulation of 4 genes (MUCL3/DPCR1, HIST2H4B, NIME2, DTNB), and downregulation of 5 genes (SLC30A10, SRGAP2/FNBP2, REG3A, IFI6, MKD). Most notable among downregulated genes was IFI6 (interferon alpha-induced protein 6) which is known to have antiviral and anti-inflammatory activity, and to drive dysregulation of interferon signaling in the pathogenesis of ulcerative colitis (IBD). Downregulation of IFI6 by ingested MNP exposure could thus potentially accelerate the progression or exacerbate symptoms of IBD, including Crohn's disease.

## Conclusions

- **In the triculture model, 4 h exposure to PS26C at an EOC of 125 µg/mL causes no significant toxicity.**
- **Likewise, in the IOC model, 24 h exposure to AuPS25 at an EOC of 60 µg/mL causes no significant toxicity.**
- **24 h exposure to AuPS25 in the IOC model causes significant downregulation of IFI6. Because IFI6 has important antiviral and immunosuppressive functions in the intestine, its downregulation suggests impairment of innate immune function, which could have adverse health consequences.**
- **Inhibitor studies in the triculture model revealed that uptake and translocation of PS26C MNPs occurs by both passive diffusion and active endocytic pathways. Those pathways include phagocytosis, and either CME or FEME, or both, but not macropinocytosis or CLIC/CEEG.**
- **Results of inhibitor studies in the IOC (with AuPS25) confirmed findings in the triculture model: uptake of nanoscale PS MNPs occurs by both passive diffusion and active endocytic pathways, including phagocytosis, and CME and/or FEME.**

## References and Acknowledgements

1. DeLoid GM, Yang Z, Bazina L, Kharaghani D, Sadrieh F, Demokritou P (2024). Mechanisms of ingested polystyrene micro-nanoplastics (MNPs) uptake and translocation in an in vitro tri-culture small intestinal epithelium. *J. Hazard. Mater.* 473

2. Kharaghani D, DeLoid GM, He P, Swenor B, Bui TH, Zuverza-Mena N, Tamez C, Musante C, Verzi M, White JC, Demokritou P (2025). Toxicity and absorption of polystyrene micro-nanoplastics in healthy and Crohn's disease human duodenum-chip models. *J Hazard. Mater.* 490

Support for this work was provided by Rutgers NIEHS Center for Environmental Exposure and Diseases (CEED) (grant # P30 ES005022), NIFA/USDA grant # 2023-67017-39267, and NIH/NIEHS R01 grant #R01DK126446