ヒト関連モデルに基づくマイクロプラスティックの体内動態の解明と評価基盤の構築

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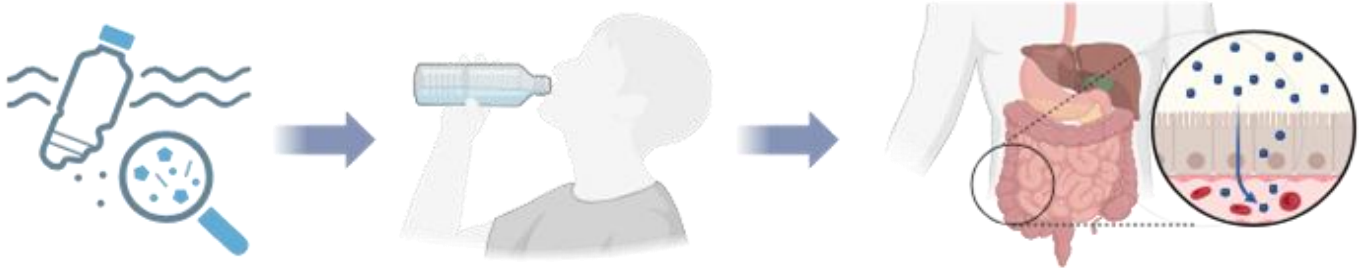
Human Exposure to Micro/Nanoplastics

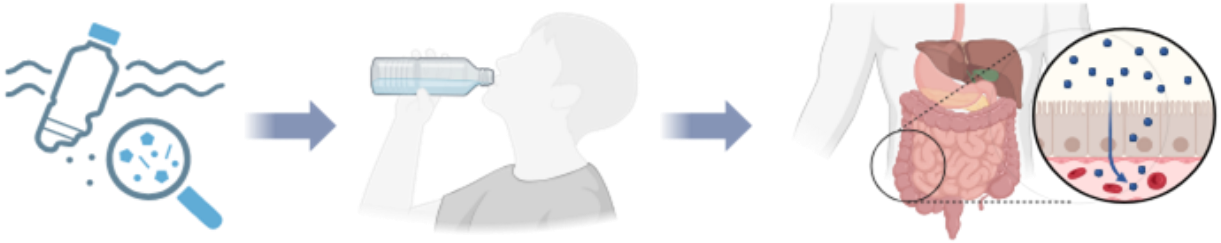


- Microplastics (MPs, <5 mm) and nanoplastics (NPs, <1 μm) have become pervasive contaminants due to extensive plastic production, use, and environmental degradation.
- Humans are continuously exposed to MNPs through contaminated food, drinking water, and airborne particles, making the **gastrointestinal (GI)** tract one of the major routes of exposure [1].
- Previous studies observed the accumulation of plastic particles **up to 20 μm** in human blood, placenta, and other tissues, suggesting that ingested particles may cross biological barriers [2].

Understanding how particle properties and intestinal microenvironments influence MNP transport is critical for human health risk assessment.

[1] Vethaak & Legler, (2021); [2] Wright & Kelly, 2017).

Background: Human Exposure to Micro/Nanoplastics



- Microplastics (<5 mm) and nanoplastics (<1 μm) have become pervasive contaminants due to extensive plastic production and environmental degradation.
- Humans are continuously exposed through food, drinking water, and inhalation.
- **Gastrointestinal (GI)** tract is one of the major routes of Micro/Nanoplastic (MNP) exposure [1].
- **Up to 20 μm** particles have been detected in human blood, placenta, and other tissues, suggesting indicating systemic distribution [2].

Understanding how particle properties and intestinal microenvironments influence MNP transport is critical for human health risk assessment.

[1] Vethaak & Legler, (2021); [2] Wright & Kelly, (2017).

Background: Current MNP Risk Assessment Research

1. *In Vivo* & Human Studies

Research Focus

- Detection of MNPs in tissues and organs
- Biodistribution and accumulation after exposure

Limitations

- Possible overestimation due to analytical contamination
- Limited mechanistic understanding of intestinal translocation

2. Particle Characterization

Research Focus

- Particle size, shape and polymer type
- Surface chemistry and aging
- Physicochemical characterization alone

Limitations

- No interaction with biological systems

3. Computational Modeling

Research Focus

- Exposure assessment
- Environmental fate
- Long-term risk prediction

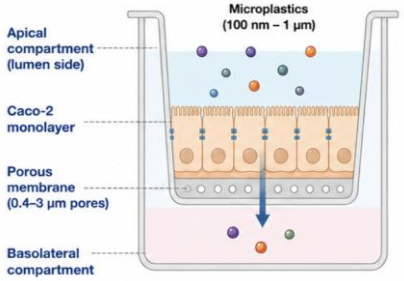
Limitations

- Strongly dependent on model assumptions
- Lack of experimental validation for human transport

A physiologically relevant *in vitro* model is necessary to bridge particle properties and human exposure mechanisms.

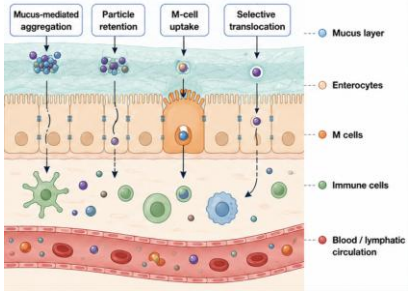
Background: Limitations of Existing Intestinal Models

Current Approach	Advantages	Limitations for Microplastic Research
In vivo Animal Model	Physiologically complex (includes mucus, immune cells, architecture).	Species differences limit extrapolation to humans; difficult to identify specific mechanisms.
Conventional *In vitro* Caco-2 Transwell Model	Simple, reproducible, and widely used for permeability testing.	Designed for drugs; pore size imposes a structural ceiling causing mechanical exclusion for larger MPs.
Existing *In vitro* Intestinal Tri-culture Models	Improved physiological relevance with specialized epithelial cells.	HT29-derived mucus drastically thinner; lack of physiological controllability.



Conventional *In Vitro* Model

Missing Physiological Complexity



Human Intestinal Environment

Current models may not accurately represent the behavior of MNPs in the human intestine, leading to misidentification of the transport potential.

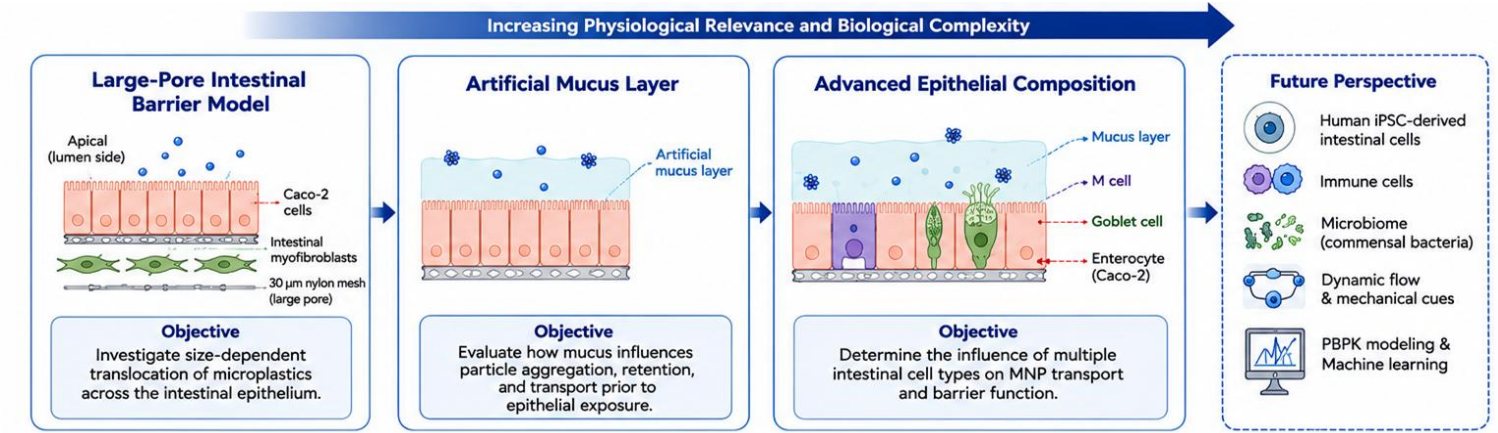
Research Questions & Objectives

Overarching Goal:

- **Investigate the physiological translocation of MNPs by creating more advanced, human-relevant *in vitro* intestinal barrier models.**
- **Understand what governs the fate and transport of MNPs in the intestine.**

Research Questions

- How do particle properties influence intestinal translocation?
- How does mucus alter particle fate prior to epithelial exposure?
- How can advanced intestinal models improve microplastic exposure assessment?



Research Aim 1: Large-Pore Intestinal Barrier Model

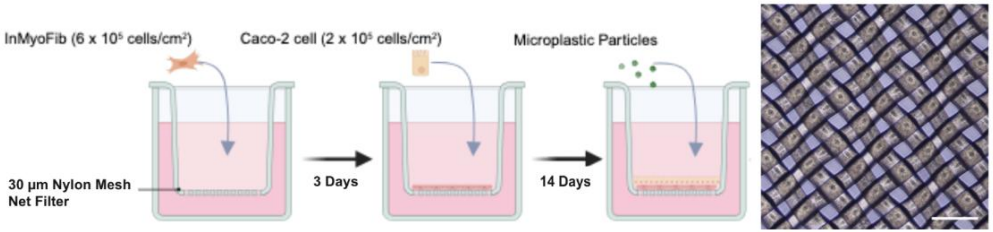
Size-Dependent Restriction of Microplastic Translocation in an *In Vitro* Large-Pore Intestinal Co-Culture Model

Background:

- Previous published studies from Sakai-Nishikawa Lab demonstrated that epithelial composition (enterocytes, goblet cells, and M cells) plays a critical role in nanoplastic translocation.
- However, the transport behavior of environmentally relevant larger microplastics (>1 μm) remains largely unexplored due to limitations of conventional in vitro models.

Objectives:

1. To develop an ***in vitro*** **intestinal co-culture model** that enables the evaluation of large MP particle permeation - **Before MP Exposure (Integrity of cell layer)**
2. To evaluate the **translocation of large microplastics** with **3 different sizes** using the novel intestinal co-culture model - **After MP Exposure (48 Hrs)**



Research Aim 1: Large-Pore Intestinal Barrier Model

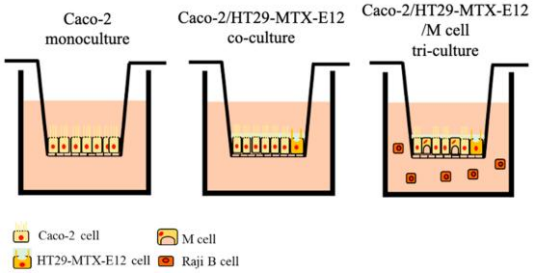
Size-Dependent Restriction of Microplastic Translocation in an *In Vitro* Large-Pore Intestinal Co-Culture Model

Background:

- **Previous studies (Sakai–Nishikawa Lab):** epithelial composition (enterocytes, goblet cells, and M cells) regulates nanoplastic translocation [1].
- **Knowledge gap:** Translocation of microplastics (>1 μm, <5 mm) remains largely unexplored.
- **Research gap:** Conventional membrane impose a physical barrier that prevents MP evaluation

Size-Dependent Internalization of Microplastics and Nanoplastics Using In Vitro Model of the Human Intestine—Contribution of Each Cell in the Tri-Culture Models

by Hyunjin Choi *, Shohei Kaneko, Yusei Suzuki, Kosuke Inamura, Masaki Nishikawa and Yasuyuki Sakai



Objectives:

1. **Before MP Exposure:**
   Develop an ***in vitro*** **intestinal co-culture model** to evaluate large MP particle permeation.
1. **After MP Exposure:**
   Translocation of large microplastics with **3 different sizes** using this advanced model.

[1] Choi et al., (2024)

Research Aim 1: Materials & Methods

1. Experimental Model

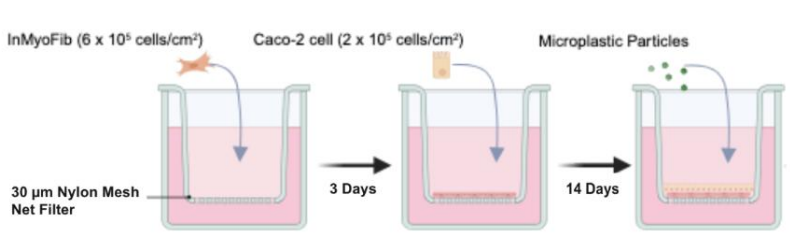
Intestinal co-culture model

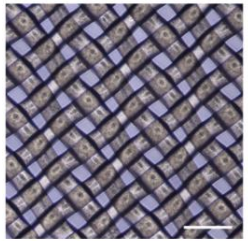
- Caco-2 epithelial cells
- Intestinal myofibroblasts (IMF)
- 30 µm nylon mesh support
- Conventional 3 µm pore size Transwell insert as control

2. Microplastic Exposure

Fluorescent polystyrene microplastics

- 3 Different sizes: 1 µm, 12 µm, 22 µm
- Exposure concentration: 0.2 mg/mL
- 48 hrs apical exposure time





Scale bar = 100 µm

3. Transport Evaluation

Experimental Analysis

Barrier Integrity
TEER measurement

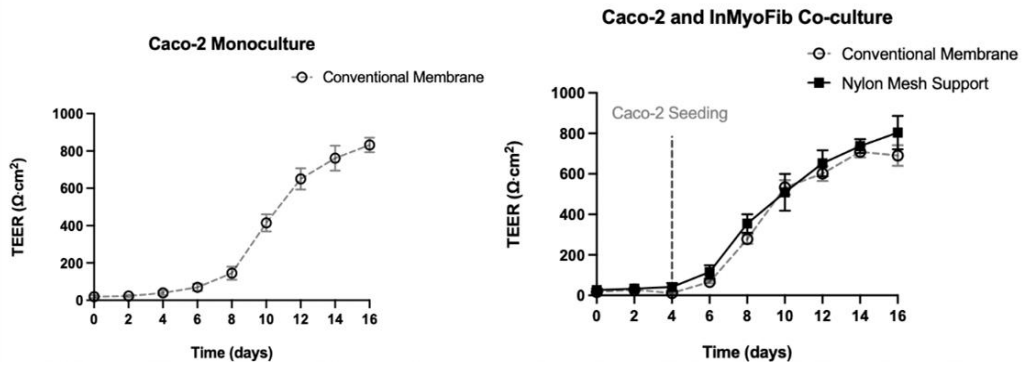
Paracellular Permeability
FITC-dextran (4 kDa) permeability assay

Confocal Imaging
Localization of microplastics and epithelial structure (ZO-1, F-actin, DAPI)

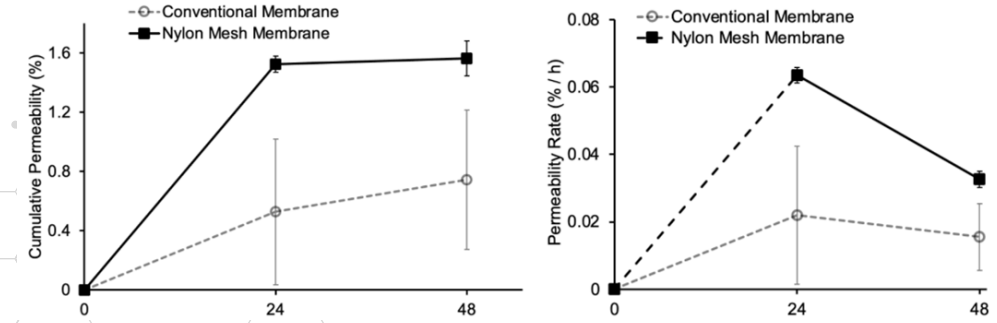
Fluorescence Quantification
Basolateral fluorescence measurement

Research Aim 1: Results Before MP Exposure

TEER Values of Caco-2 & InMyoFib Co-culture & Caco-2 Monoculture



4 kDa FITC-Dextran of CaCO-2 & InMyoFib co-culture



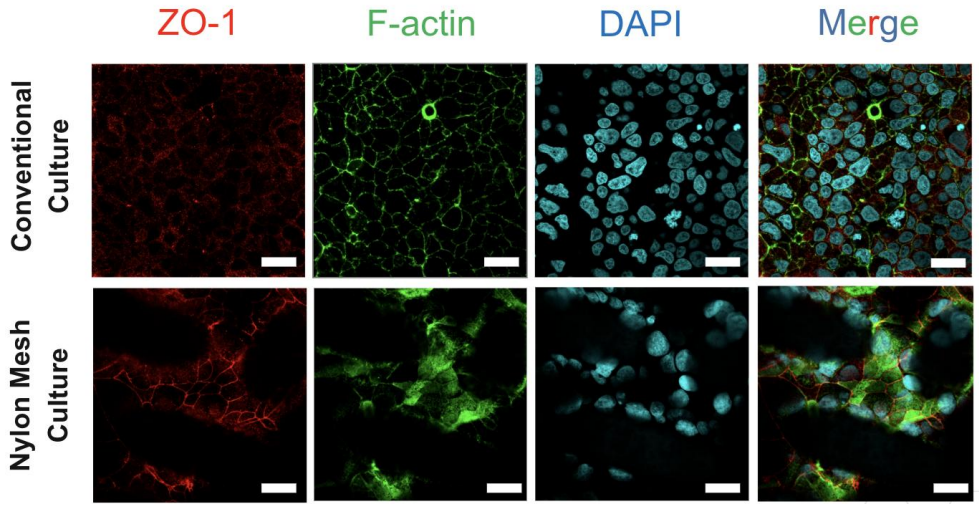
Barrier Characterization Before MP Exposure

- Validate epithelial barrier formation on the 30 μm nylon mesh support
- TEER increased after Caco-2 seeding and reached a stable plateau by Day 14 in both models.
- 4 kDa FITC-dextran permeability rate remained low in 48 hours (<0.1 %/h), indicating intact barrier function.

The results demonstrate successful formation of an intact epithelial barrier on the large-pore nylon mesh.

(n = 3, 3 biological replicates)

Research Aim 1: - Results (Before MP Exposure)



Morphological Validation Before MP Exposure

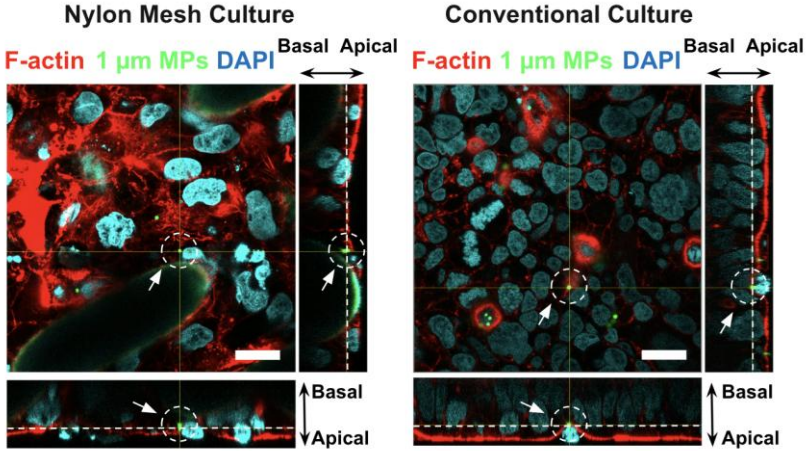
- Verify epithelial morphology and tight junction formation on the nylon mesh support via confocal microscopy.
- ZO-1 staining confirmed continuous tight junction formation.
- F-actin revealed an organized epithelial cytoskeleton.
- Comparable staining patterns were observed on both conventional and nylon mesh supports.

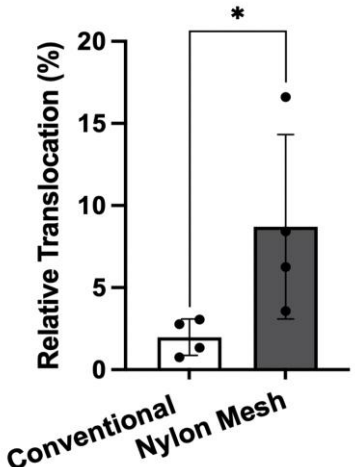
Morphological integrity was maintained on the large-pore support.

100x Magnification Scale bar = 20 µm

Research Aim 1: Results (After MP Exposure)

1 µm Microplastic Localization & Quantitative Analysis





Confocal Localization

- 1 µm MPs detected within the epithelial layer in both models under confocal microscopy.
- Particles observed beneath the epithelium in the model, indicating apical-to-basal particle transport.

100x Magnification Scale bar = 20 µm

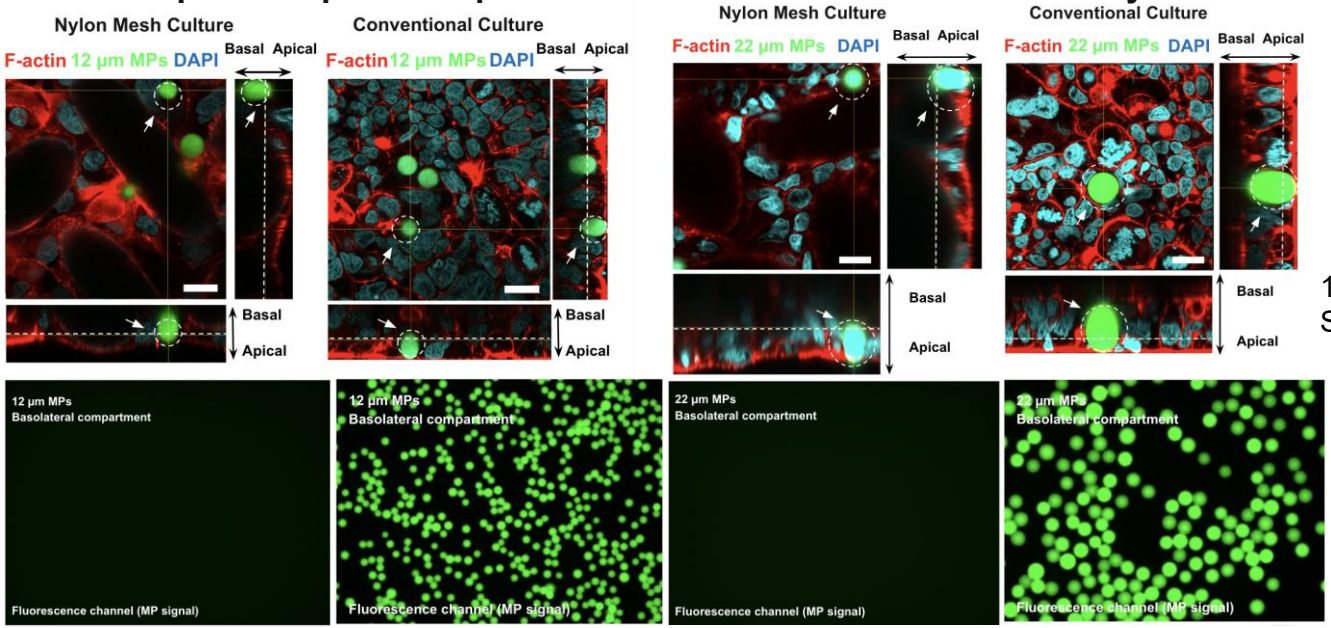
Basolateral Fluorescence Quantification

- Basolateral recovery measured by fluorescence intensity (NanoDrop).
- Significantly higher relative translocation in the nylon mesh model (Welch's t-test, $p = 0.0243$).
- Difference is support-dependent rather than barrier integrity-dependent.

(n = 3, 3 biological replicates)

Research Aim 1: Results After MP Exposure

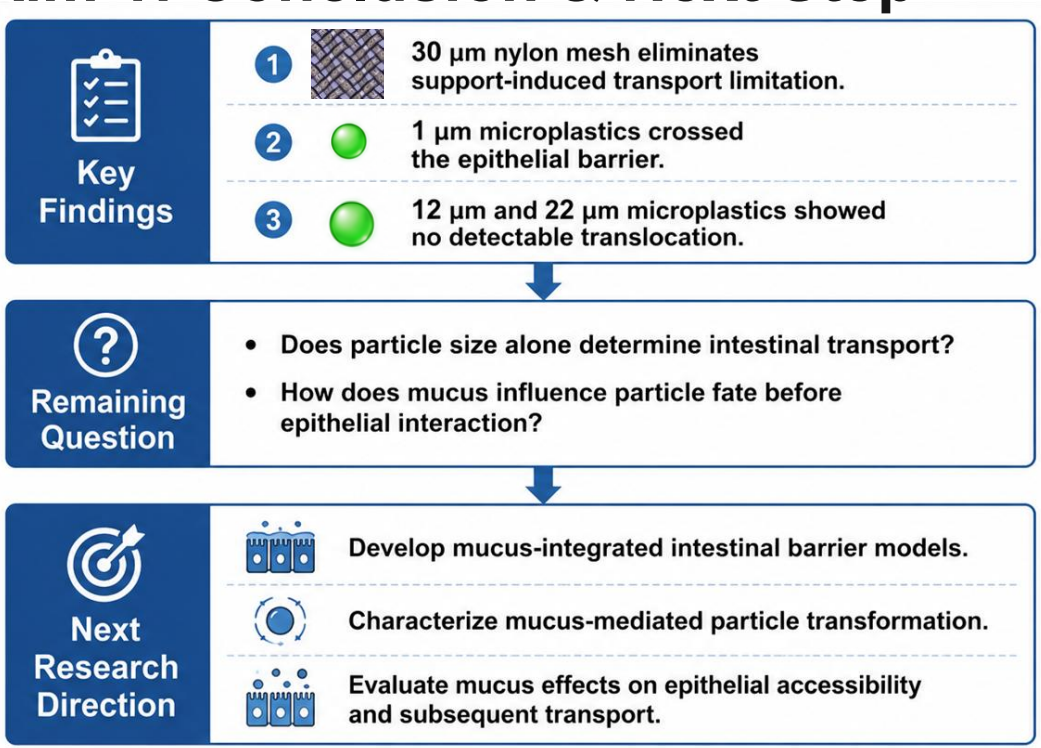
12 µm & 22 µm Microplastic Localization & Quantitative Analysis



100x Magnification
Scale bar = 20 µm

- Confocal imaging after 48 h exposure: the localization of 12 µm and 22 µm MPs in both models.
- Particles remained on the apical side of the epithelial barrier.
- Basolateral compartment was screened by fluorescence microscopy.
- No fluorescent particles were detected after exposure to either 12 µm or 22 µm particles.

Research Aim 1: Conclusion & Next Step



Wei Y., Kaneko S., Choi H. J., Katsuda T., Nishikawa M., Sakai Y.
Size-Dependent Restriction of Microplastic Translocation in an In Vitro Large-Pore Intestinal Co-Culture Model
AATEX (Under Review) Submitted: April 2026 Minor revision received: July 2026

Research Aim 2: Research Background & Objectives

Background:

- Particles <1 μm are the most relevant size range for intestinal translocation based on previous studies by Choi et al. and Chapter 2 findings [1].
- Artificial mucus developed by Cao et al. provides a controllable platform to investigate particle behavior before epithelial contact [2].
- How mucus alters particle physicochemical properties remains largely unknown.

Objectives: Characterize mucus-mediated particle transformation

1. Evaluate aggregation and size changes of MNPs in artificial mucus.
2. Determine particle penetration through the mucus layer.
3. Identify physicochemical changes before epithelial interaction.

[1] Choi et al., (2024); [2] Cao et al, (2025).

Research Aim 2: Materials & Methods

Micro/Nanoplastic Particles

Fluorescent polystyrene microplastics

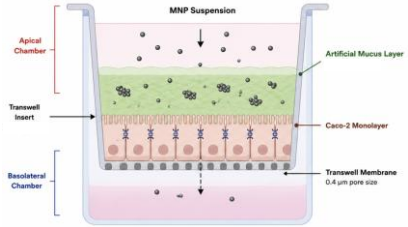
- Carboxylate-modified surface: negatively charged surface comparable to weathered environmental MPs
- 3 Different Sizes: 100 nm, 500 nm, 1 µm
- Exposure concentration: 0.1 mg/mL
- Exposure time: 48 hours

100 nm 500 nm 1 µm

Artificial Mucus Model

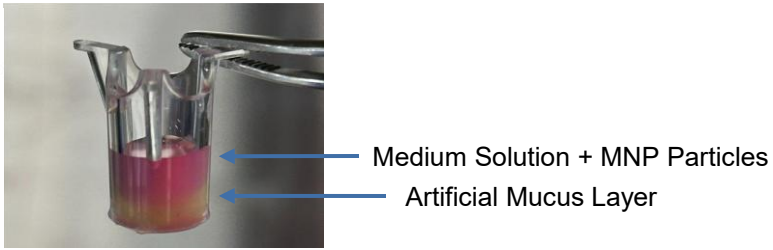
- Artificial mucus with a defined formulation: 5% mucin, 0.9% polyacrylic acid (PAA), and 3.1% BSA [1].
- Mucus Layer Volume & Thickness: 50 µm & 1 mm
- Exposure time: 48 hours.

[1] Cao et al, (2025).



Characterization Methods

- Dynamic Light Scattering (DLS): Hydrodynamic diameter & particle aggregation
- Zeta Potential: Surface charge & colloidal stability
- Confocal Microscopy: Particle localization & mucus penetration



Research Aim 2: Ongoing Research & Current Results

Zeta Potential: Evaluates particle surface charge and stability.
(Higher absolute values = more stable dispersions; Lower absolute values = greater tendency to aggregate.)

Particle Size	Mili Q	Complete Medium	10x Mili Q Diluted Artificial Mucus
100 nm	-56.4 mV	-9.79 mV	-31.5 mV
500 nm	-44.9 mV	-9.28 mV	-29.6 mV
1 µm	-52.5 mV	-9.54 mV	-31.9 mV

Particles were incubated (20 µg/mL) in different solvents before zeta potential measurement for 48 hours.

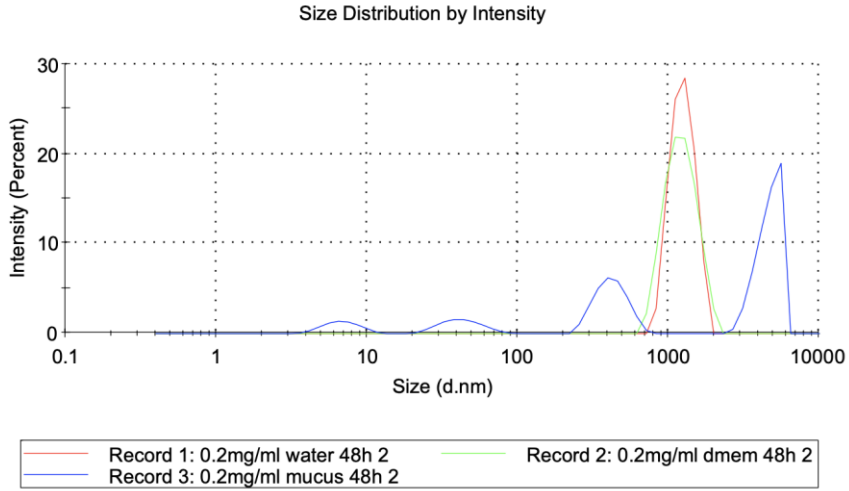
- **Milli-Q:** Highly negative zeta potentials, reflecting the intrinsic surface charge of the particles.
- **Complete medium:** Rapid adsorption of serum proteins and dissolved ions onto the particle surface, forming a **biological corona** that partially masks the original particle charge.
- **Artificial mucus:** Formation of a distinct mucus–particle interface.

Current interpretation:

- **Artificial mucus modifies particle surface properties differently from cell culture medium.**
- **Additional DLS and confocal analyses are required to determine whether these surface changes alter particle aggregation and transport.**

Research Aim 2: Ongoing Research & Current Results

Dynamic Light Scattering (DLS) Test: Measures hydrodynamic diameter and particle size distribution in suspension. (An increase in hydrodynamic size may indicate particle aggregation or adsorption of surrounding biomolecules.)



1 µm particles were incubated (0.1 mg/mL) in different solvents before DLS analysis for 48 hours.

- **Milli-Q water & complete medium (Red & Green Curve):** dominant peak remained close to the actual particle size, indicating relatively stable dispersions.
- **Artificial mucus:** broader particle size distribution with a increased hydrodynamic diameter.
- Absence of a 1000 nm peak suggests that the observed shift is not explained by simple particle aggregation alone.

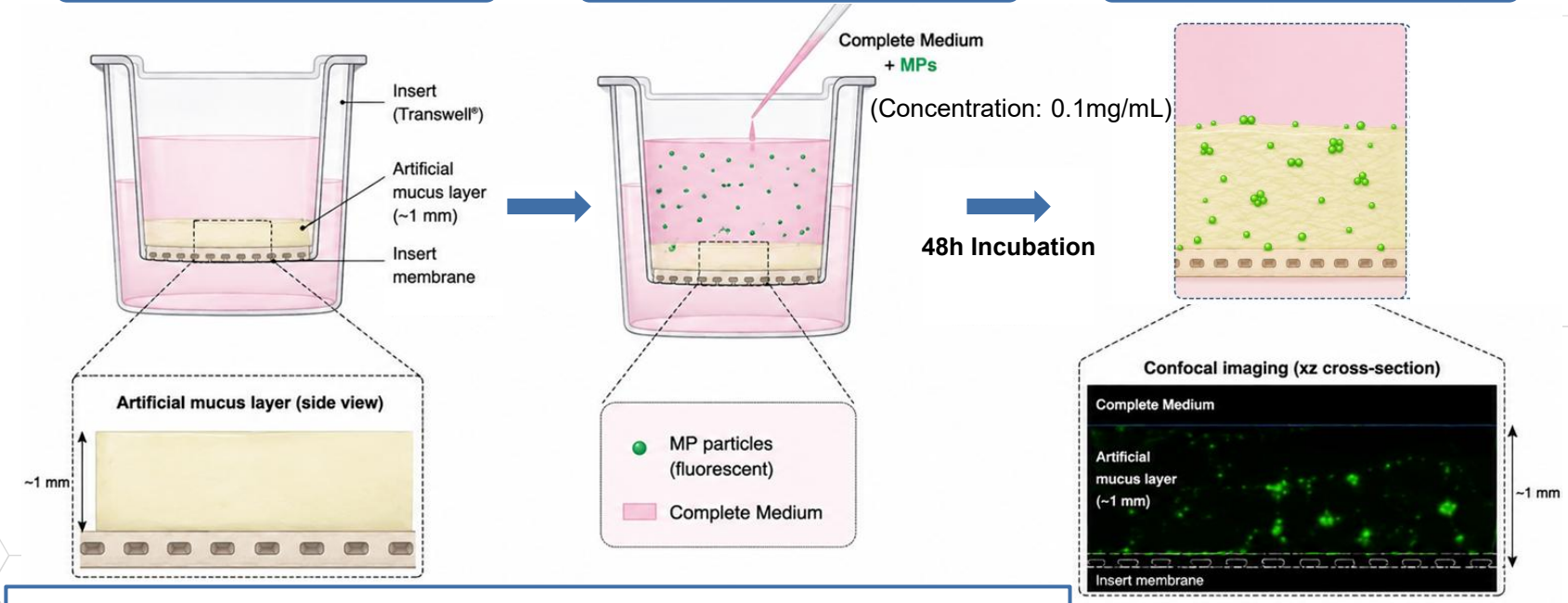
The altered DLS profile suggests changes in particle organization, but the underlying mechanism cannot be resolved by DLS alone.

(n=3, 3 biological replicates)

Research Aim 2: Ongoing Research & Current Results

Confocal Microscopy: Physicochemical Characterization of Microplastics in Artificial Mucus

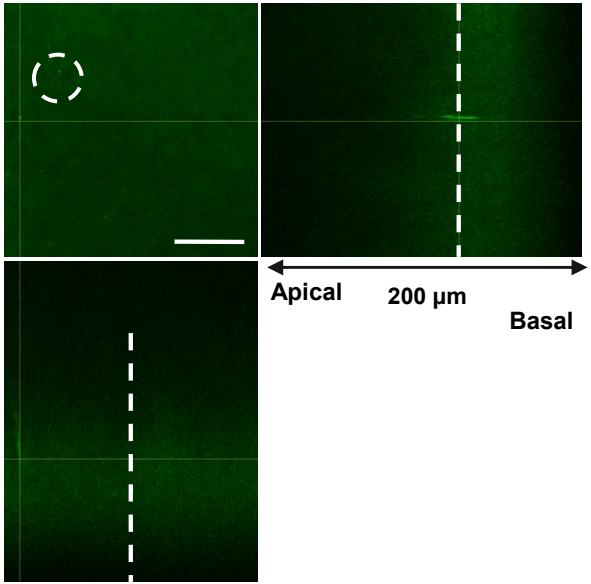
1. Insert with mucus layer → **2. Complete medium + MPs** → **3. Confocal Microscopy**



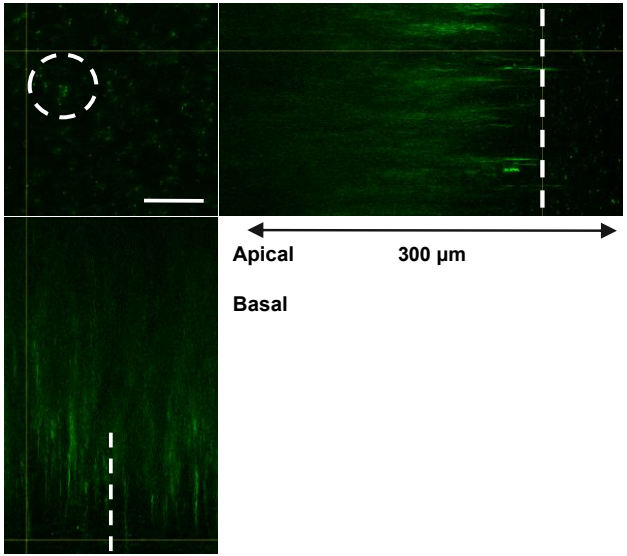
Main Research Question: Can microplastics penetrate a physiologically relevant mucus barrier before reaching the epithelial surface?

Research Aim 2: Ongoing Research & Current Results

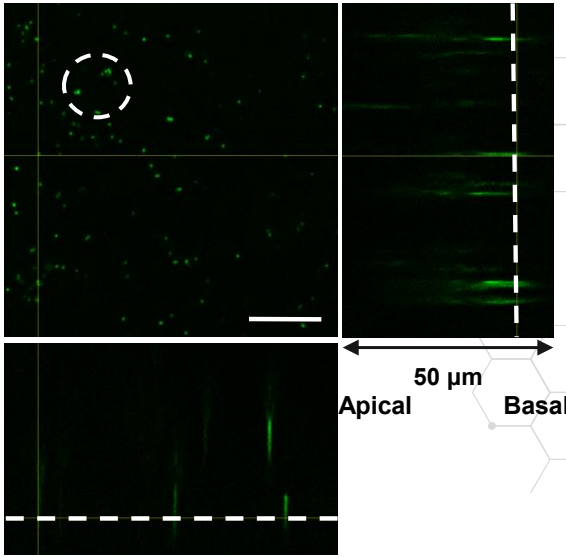
100 nm Microplastic Localization



500 nm Microplastic Localization



1 µm Microplastic Localization



- **White dash line:** The location of the insert membrane.
- Particles of all three sizes were detected throughout the mucus layer, indicating the MP penetration.
- Localized **particle clusters** were observed within the mucus, suggesting mucus-associated aggregation.
- Both dispersed and aggregated MP populations were observed in all sizes.

Scale bar = 20 µm

Research Aim 3: Research Background & Objectives

Background:

- Particles reaching the epithelial surface still face multiple biological barriers.
- Conventional Caco-2 models lack important physiological components, including mucus and specialized epithelial cells (e.g., M cells).
- More human-relevant models are required to evaluate realistic intestinal transport.

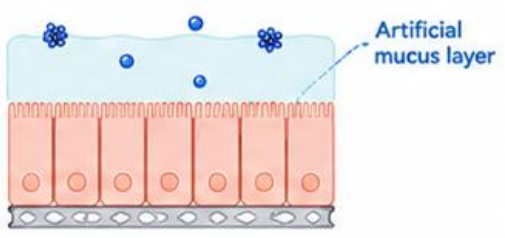
Objectives: Characterize mucus-mediated particle transformation

1. Incorporate artificial mucus into Caco-2 models.
2. Establish mucus-containing co-culture models with specialized epithelial cells.
3. Evaluate how epithelial composition influences MNP transport.

Research Aim 3: Materials & Methods

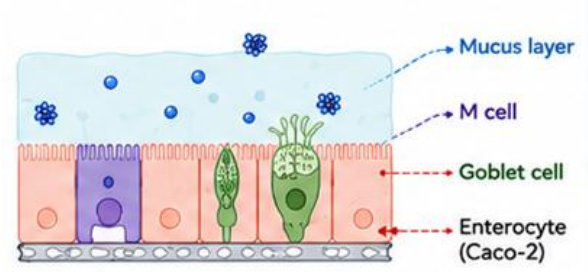
Current Model (Ongoing)

- **Caco-2 monolayer** cultured on Transwell inserts.
- Artificial mucus layer (1 mm) applied to mimic the intestinal mucus barrier.
- Carboxylate-modified fluorescent PS-MPs in 3 different sizes: 100 nm, 500 nm, 1 µm.
- Transport and particle behavior were evaluated.



Physiologically Relevant Model

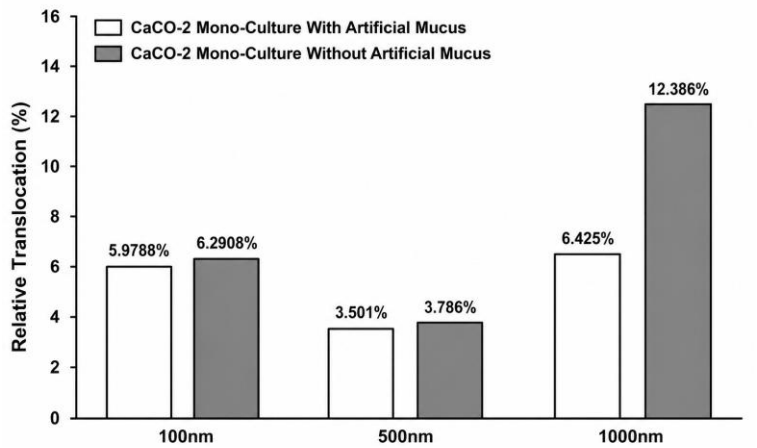
- Physiologically relevant **co-culture/tri-culture model**.
- The combined effects of mucus, epithelial composition, and M-cell-mediated transport will be evaluated [1].



[1] Choi et al., (2024)

Research Aim 3: Ongoing Research & Current Results

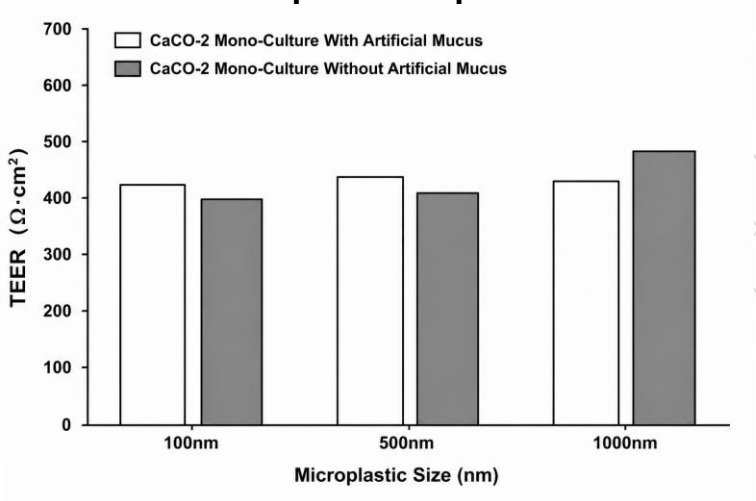
Preliminary Assessment of Microplastic Translocation Across the Caco-2 Monoculture



- Artificial mucus decreased MNP transport across the epithelial barrier.
- Greatest reduction observed for 1 μm MPs.
- These observations suggest a potential barrier effect of artificial mucus on microplastic transport.

Artificial mucus influences MNP transport while maintaining epithelial barrier integrity.

TEER Measurements After 48-hr Microplastic Exposure



- TEER values remained within a comparable range after exposure to all tested particle sizes.
- No evidence of mucus-induced epithelial damage.
- The exposure conditions did not induce substantial disruption of the epithelial monolayer.

(n=3, 1 biological replicate)

Progress Summary & Next Steps

COMPLETED

Background & Introduction

Literature review & research rationale

Research Aim #1

- Large-pore intestinal co-culture model
- Size-dependent MP transport
- Manuscript submitted (Apr 2026)
- Minor revision received (Jul 2026)

ONGOING

Research Aim #2

- Preliminary characterization completed
  - Zeta potential
  - DLS
  - Confocal imaging

Current focus

- Optimize protocols
- Improve visualization of particle aggregation
- Elucidate mucus-mediated particle transformation

Research Aim #3

Current model (completed)

- Caco-2 monolayer ± artificial mucus

Current focus

- Verify reproducibility
- Quantify transport changes

Next

- Add Goblet cells
- Add M cells
- More complex, physiologically relevant model

Jul 2026 to Sep 2026

Research Aim #2
The 1st Part of Research Aim #3

- Protocol optimization
- The evaluation of current model.

Oct 2026 to Jan 2027

The 2nd Part of Research Aim #3

- Build the advanced intestinal model with more complexity.

Feb 2027 to May 2027

Experiments and Data Analysis

- Manuscript preparation and submission.

Jun 2027 to Jul 2027

Future perspective

- Long-term human risk assessment of MNPs

Thank You for Listening!

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