SOT Inhalation and Respiratory Specialty Section: 
*Macrophage and Epithelial response to microplastics*

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The ingestion and inhalation of microplastics – an immunological cause for concern.

**MICROPLASTIC PROBLEM**

- Industrial microplastic particles (MP) have infiltrated aquatic and terrestrial ecosystems.

- MP alter microbial communities (aquatic and terrestrial).

- Ingestion of MP in laboratory animals leads to dysbiosis.

- MP have been found in human stool samples.

- MP have been found in **lungs**, livers, spleen and kidneys of donated human cadavers.

- **Human consumption of MP 39,000 to 142,000 particles annually.**

- Increases to 211,000 particles when inhalation is considered.

- **Inhalation intake can also contribute to gut exposure through contaminated mucus ingestion.**

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**Global average weekly plastic consumption**

On average people swallow this number of plastic particles each week from the following foods/drinks that have the highest plastic levels

- Plastic particles (0-1mm)
  - **Total plastic ingested = 5g**
  - 1,769 Tap/bottled water
  - 10 Beer
  - 11 Salt
  - 182 Shellfish

**The equivalent of one credit card**

K. Seathirias, T. Palanisami, University of Newcastle, How much microplastics are we ingesting? Estimation of the mass of microplastics ingested. Report for WWF Singapore, Mar 2019

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Microplastics in our water source – tap and bottled.

MP have been found in tap water, bottled water and food sources such as fish.
Macrophages
- Mononuclear phagocytes
- **Highly present in the lung and gut.**
- Captures, degrades and clears microbes.
- Can presents antigens to T and B cells.
- **Can dictate the response (TH1, TH2, TH17)**
- Clears inhaled particles and apoptotic cells.

Epithelial cells
- **Majority of cells in lung and gut**
- Barrier function
- Surface protection
- Immune defense

Specifically, we will show how MP affect cellular metabolism.

At steady-state, both alveolar and intestinal macrophages as well as epithelial cells rely heavily on mitochondrial metabolism such as FAO and OXPHOS.
Microplastic engulfment leads to macrophage activation

Macrophages take up 10μm PS MP

PS MP uptake activates CD80/CD86 expression

A

B

C

D
Can MP modulate cellular metabolism?

**Metabolic Reprogramming**

**Experimental Design**

**Seahorse XF Glycolytic Rate Assay Profile**

Litvak et al., Science 362, 1017 (2018)
Microplastics modulate macrophage metabolism.

**Metabolic Kinetics**

- **Total Proton Efflux Rate (PER)**
  - Basal
  - MP

- **GlycoPER (pmol H+/min)**
  - Basal
  - MP
  - LPS
  - MP/LPS

- **MitoOCR/glycoPER**
  - Basal
  - MP
  - LPS
  - MP/LPS

- **% of GlycoPER**
  - Basal
  - MP
  - LPS
  - MP/LPS

- **Compensatory PER**
  - Basal
  - MP
  - LPS
  - MP/LPS
Metabolomics show PS MP alter macrophage metabolism

Metabolomic Profiling

Increase in lactic acid
Microplastics perturb mitochondrial membrane potential

Mitochondrial assessment

Cellular Metabolism

Decreased mitochondrial membrane potential

MitoTracker Red CMXRos

Basal 31.3%  PS MP 58.3%

MitoTracker Green

MitoTracker Green+ BMM

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MP engulfment by macrophages enhances glycolysis

The link between a glycolytic state and inflammation

- **Glucose**
  - Hexokinase 1 (HK1) (A)
  - Fructose-6-phosphate
  - Phosphoglucone isomerase

- **Fructose-6-phosphate**
  - Fructose-2,6-biphosphate
  - Phosphofructokinase-1 (PFKFB3) (B)

- **Glyceraldehyde-3-P dehydrogenase (GAPDH)**
  - 1,3-Bisphosphoglycerate (x2)
  - Phosphoglycerate kinase
  - 3-Phosphoglycerate (x2)
  - Phosphoglyceromutase
  - 2-Phosphoglycerate (x2)
  - Enolase (ENO1)
  - Phosphoenolpyruvate (x2)
  - Pyruvate kinase (PKM2) (C)

- **Pyruvate**
  - Lactate dehydrogenase (LDH)
  - Pyruvate dehydrogenase (PDH)
  - Acetyl-CoA

- **PDH fuels itaconate production from citrate** (F)

- **NLRP3 in inflammasome activation** + IL-1β

- **PKF3 drives glycolysis** + Anti-viral response

- **GAPDH dissociation from TNF mRNA** + TNF

- **Expressed on cell-surface of inflammatory Mφ** + TNF IL-1α/β IFN-γ

- **Forms complex with HIF1α**
  - Phosphorylates STAT3
  - Activates NLRP3

- **IL-1β, IL-6** + Anti-microbial activity

Van den Bossche et al. 2017 Trends Imm.
Epithelial cell response to microplastics

**Epithelial Metabolism**

- C2 colonocyte
  - Fiber
  - Microbiota
  - Butyrate
  - Tight junction proteins
  - Fatty acids
  - Oxidation
  - D-glucose
  - Inflammation

- C1 colonocyte
  - Nitrite (NO)
  - Oxygen (O2)
  - Lactate
  - Pyruvate
  - Inos (INOS)
  - D-glucose
  - D-glucose

**Wound Healing**

- A: Injury
  - Basal membrane
  - TGF-β
  - TFF
  - TGF-α

- B: Loss of cell polarity
  - Production of growth factors by IECs
  - Epithelial cell migration

- C: Proliferation
  - Stimulation of proliferation induced by growth factors, cytokines or bacterial / viral components
  - NF-κB
  - STAT1
  - STAT3

- D: Differentiation
  - villus tip
  - Fibroblasts
  - Macrophages
  - Lymphocytes
  - Wnt
  - YAP
  - Notch

Martini et al. 2017 Cell Mol Gastro Hepatology; Litvak et al., 2017 Science
Alteration of PS MP via UV exposure

Environmental exposure enhances the internalization of microplastic particles into cells


<table>
<thead>
<tr>
<th></th>
<th>C-O-H %</th>
<th>C=O %</th>
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<tbody>
<tr>
<td>1μm new</td>
<td>51.55</td>
<td>48.45</td>
</tr>
<tr>
<td>1μm aged</td>
<td>52.92</td>
<td>47.08</td>
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<tr>
<td>5μm new</td>
<td>40.75</td>
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<tr>
<td>5μm aged</td>
<td>58.41</td>
<td>41.59</td>
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</table>

High resolution XPS spectra of carbon regions
Changes in functional groups on PS MP
PS MP modulate human lung epithelial cellular metabolism

SEM – PS MP (Fresh and Aged)

PS MP increases glycolysis in a549 cell line
PS MP impede wound healing in human lung epithelial cells

Electric cell-substrate impedance sensing (ECIS) assay

Automated Cell Migration Assay

ECIS on a549 cell line – fresh PS MP

Martini et al. 2017 Cell Mol Gastro Hepatology
UV-aged PS MP impede wound healing in human lung epithelial cells

Electric cell-substrate impedance sensing (ECIS) assay

ECIS on a549 cell line – UV-aged PS MP

Martini et al. 2017 Cell Mol Gastro Hepatology
PS MP modulates the intestinal epithelial cell barrier

**PS MP increases glycolysis in Caco2 cells**

- Basal 
- LPS 
- PS MP

**MP impede wound healing in Caco2 cells**

- Wounding

**PS MP alters intestinal permeability in organoids**

Utilization of human intestinal organoids

**PS MP increases permeability in organoids**

- CTRL
- PS MP

Relative Epithelial Resistance (%)
The ingestion of microplastics leads to changes in the gut microbiota of mice. PS 1mg/wk (8wks) was administered, followed by 16S rRNA sequencing using Illumina MiSeq on stool samples.

**CTRL** and **PS MP** groups were compared for changes in microbial composition.

- **Firmicutes**: Reduced in PS MP compared to CTRL.*
- **Bacteroidetes**: No significant difference between groups.
- **Actinobacteria**: Increased in PS MP.
- **Other**: No significant change.

Actinobacteria - highly aerotolerant bacteria that are either aerobes or facultative anaerobes.

*Litvak et al., Science 362, 1017 (2018)*
PS MP induce a low-level of intestinal inflammation

Colonic gene expression and fecal LCN2 analysis

Intestinal Inflammation: Lipocalin-2

Intestinal Inflammation: Cytokines
What does this mean for the IRSS?

Environmental signals
- Antibiotics
- Microplastics
- Pollution

Diet
- Dietary fiber
- Animal-derived saturated lipids
- Dietary cholesterol

Metabolism

Gut microbiota signaling metabolites allow communication with organs and tissues in the host.

- LPS/endotoxin
- Bile acids
- SCFAs
- Trimethylamine

Adipose tissue
Liver
Brain
Cardiovascular system
Lung
What does this mean for Gastrointestinal and Respiratory health?

Cellular metabolism is crucial for Tight junction function, macrophage function, epithelial O₂ consumption.
Inhalation and Respiratory Specialty Section  
Society of Toxicology

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