Material- and size-dependent effects of submicron and nanoplastics after oral uptake *in vitro*

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INTRODUCTION

Microplastics (Ø < 5 mm) have become one of the most intensively discussed topics in human consumer protection research, due to constantly rising production, usage and waste of plastic products. As a broad mixture of polymer particles with different sizes and material, it can reach the human body via oral uptake. Only plastic particles smaller than 1.5 µm might be able to be systematically distributed after oral uptake. This provides evidence for microplastics to have a limited bioavailability. With regard to smaller submicro- (Ø 1000-100 nm) and nanoplastic (Ø < 100 nm) particles, a scarce data availability on toxicological endpoints and gastrointestinal absorption exists.

Many *in vitro* models based on the cell line Caco-2 exist to simulate the human intestinal barrier. After 21 days of differentiation, Caco-2 cells represent a functional intestinal monolayer with microvilli and tight junctions. By using Transwell® systems, the uptake and transport across the intestinal barrier can be investigated (Figure 1). Caco-2-based cocultures with M-cells (stimulated by Raji B) or mucus-secreting goblet cells (HT29-MTX) represent different aspects of the intestinal monolayer (Figure 1). Furthermore, cellular effects can be examined on gene expression and protein level, using qPCR and fluorescence-based kits.

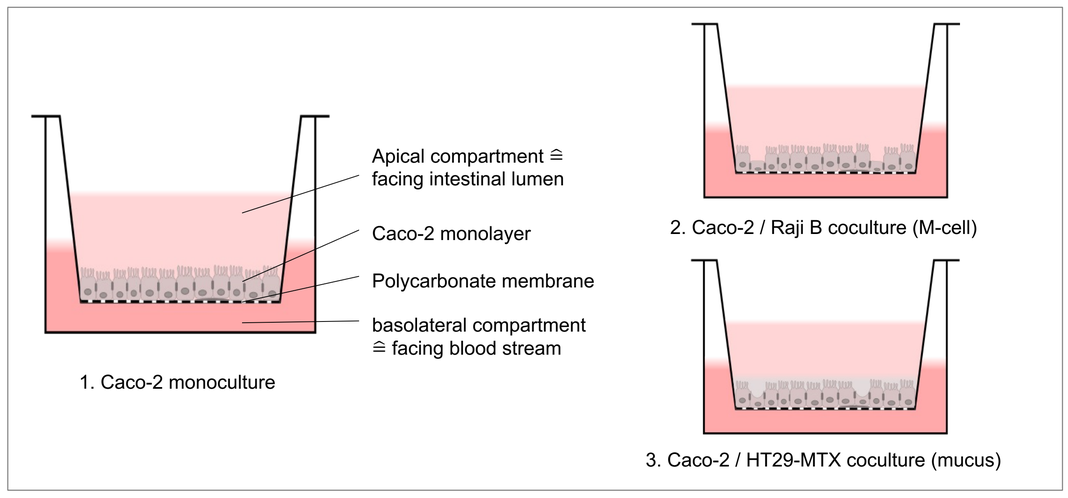


Figure 1: Different *In vitro* Transwell® models of the human intestinal barrier with differentiated Caco-2 cells

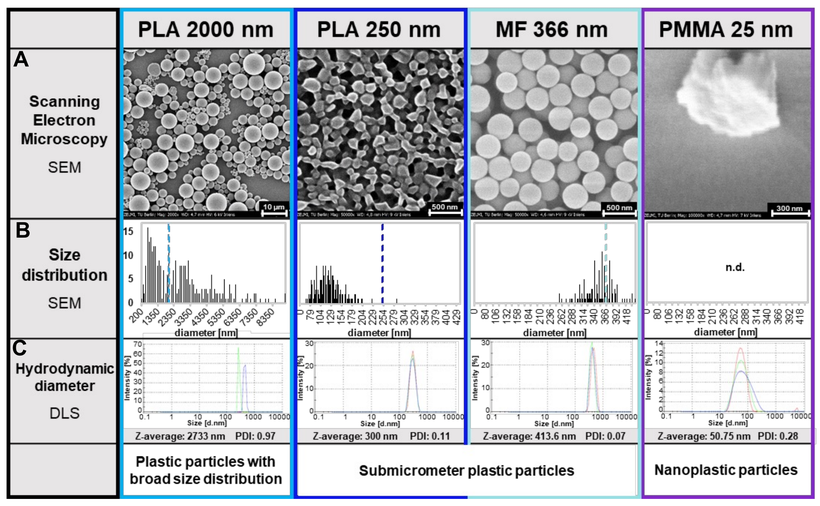


Figure 2: Characterization of particles in stock dispersion A: SEM images of PLA2000, PLA250, MF366 and PMMA25. PMMA25 formed agglomerates after SEM preparation. B: Size distribution analysis of SEM images. Size distribution of PMMA25 was not evaluable. C: DLS analysis of particles (n=3).
PLA2000 was characterized as microplastic particles with broad size distribution and round shape. PLA250 and MF366 were characterized as round, monodisperse submicrometer particles. PLA250 particles tend to agglomerate. PMMA25 were characterized as nanoplastic particles.

MATERIAL AND METHODS

Fluorescent plastic particles polylactic acid (PLA2000, Ø 2 µm and PLA250 Ø 250 nm), melamine formaldehyde resin (MF366, Ø 366 nm) and polymethylmetacrylate (PMMA25, Ø 25 nm) were used. The particles were characterized using scanning electron microscopy (SEM), dynamic light scattering (DLS) and MTT cytotoxicity assay. The human intestinal cell line Caco-2 was differentiated and incubated for 24h with different concentrations of plastic particles testing the cellular metabolic activity. Uptake and transport of micro- and nanoplastic particles was measured and characterized using Caco-2 cells in mono- and cocultures. Cells were grown on a Transwell® and cellular interaction and transport across the intestinal barrier was measured after 24h using a plate reader, confocal microscopy and side scatter analysis. Octanol-water distribution was quantified in a plate reader. Potential emergence of oxidative stress was quantified in a flow cytometer with ROS Brite™ 670 dye after 24h. Non-cytotoxic concentrations of each particle type were used.

RESULTS

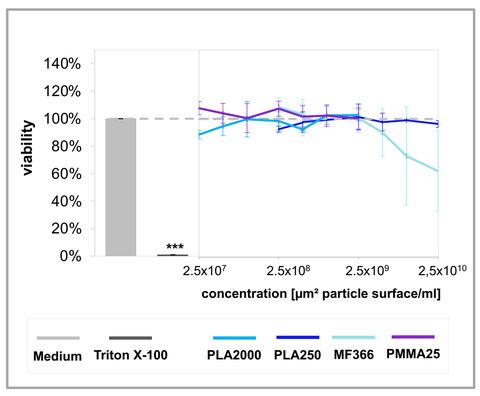


Figure 3: MTT cytotoxicity assay. Caco-2 cells were differentiated and incubated for 24h with PLA2000, PLA250, MF366 and PMMA25. Different concentrations ranging from $2.5x10^7$ to $2.5x10^{10}$ µm² particle surface/ml were used. Triton X-100 (0.01 %) served as positive control and medium as negative control. Viability of Caco-2 decreases only for MF366 concentrations higher than $2.5x10^9$ µm² particle surface/ml due to particle overload. Data are presented as means ± standard deviation. The significance was calculated by performing one-way ANOVA Dunnett's test (*** = p<0.001, n=3).

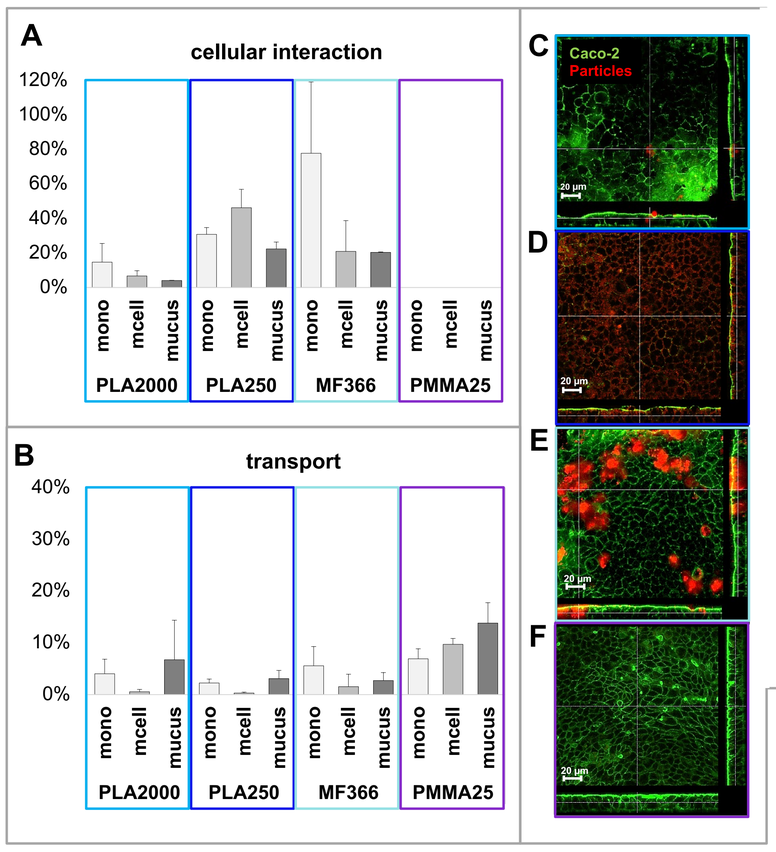


Figure 4: *In vitro* cellular interaction and transport in Caco-2 monoculture, mcell and mucus coculture Transwell® model. A: Percentage of particles on top or inside the Caco-2 cells. Highest cellular interaction was quantified for submicrometer particles. B: Percental transport of particles across the Caco-2 monolayer ranging from 1-12 %. Highest amount of transport was detected for PMMA25 in the mucus model. C-F: Representative confocal images of plastic particle interaction with the Caco-2 cell monolayer. Green colour represents Caco-2 cells and red colour particles. C: PLA2000. D: PLA250. E: MF366. F: PMMA25. Data are presented as means ± standard deviation (n=3).

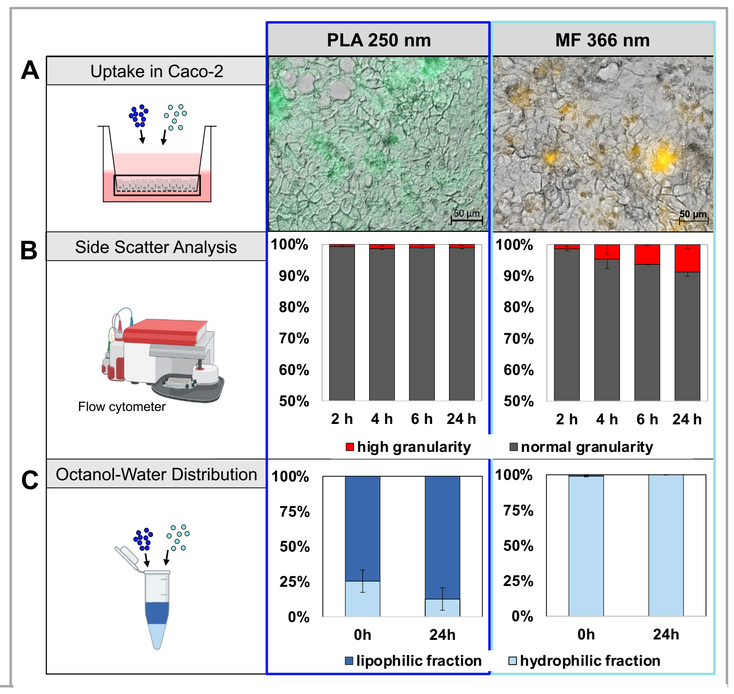


Figure 5: Characterization of uptake mechanisms of PLA250 and MF366 in Caco-2 cells. A: Representative fluorescent microscopic images of uptake in Caco-2 cells. Images indicate different uptake mechanisms, because particles show different accumulation in cells. B: Flow-cytometric side scatter analysis. A time-dependent increase of granularity in Caco-2 cells after MF366 incubation was quantified. C: Octanol-water distribution quantified after 0h and 24h. PLA250 congregated in the organic phase and MF366 in the aqueous phase. Data are presented as means ± standard deviation (n=3).

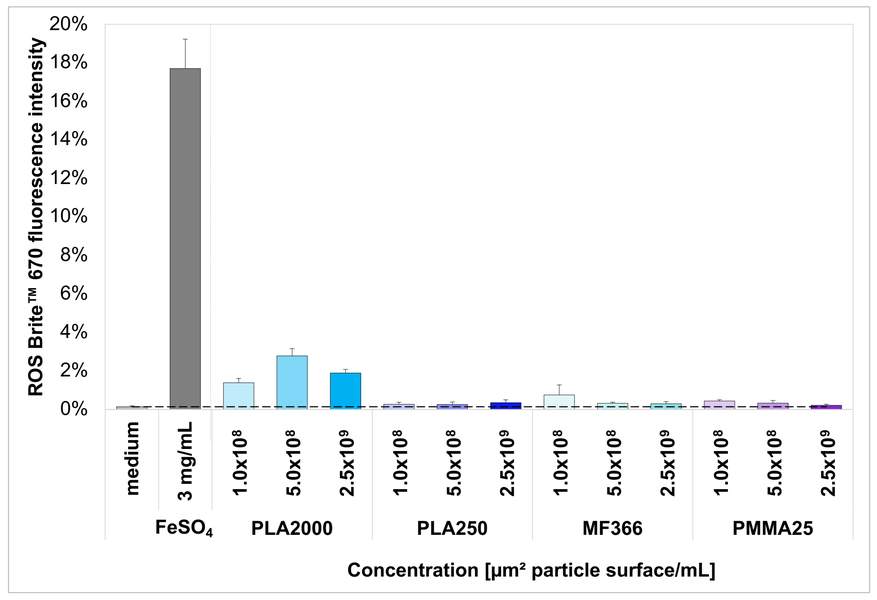
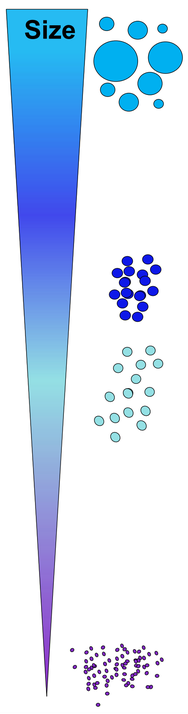


Figure 6: Quantification of oxidative Stress in Caco-2 cells after 24h. Three different particle concentrations of PLA2000, PLA250, MF366 and PMMA25 ranging from $1.0x10^8$ to $2.5x10^9$ µm² particle surface/ml were used. $FeSO_4$ served as positive control and medium as negative control. A slight increase of fluorescence was only quantified for PLA2000. Additional fluorescence microscopy showed unspecific reaction of the dye with particles, but not within the cells (data not shown). Data are presented as means ± standard deviation (n=3).

CONCLUSION – Uptake, transport and effects in Caco-2 cells



PLA Ø 2000 nm
- Non-cytotoxic
- Minor uptake of smaller particles
- No oxidative stress
- Transport of ~ 2-5 %

PMMA Ø 25 nm
- Non-cytotoxic
- No cellular interaction
- No oxidative stress
- Transport of ~ 6-12%

PLA Ø 250 nm
- Non-cytotoxic
- Incorporation of many particles in cell membranes or between cells
- Distribution of particles all over the cells
- No oxidative stress
- Transport of ~ 2-4 %

COMPARABLE PARTICLE SIZE
⇩
DIFFERENT UPTAKE BEHAVIOR
⇧
DIFFERENCES IN HYDROPHOBICITY

MF Ø 366 nm
- Cytotoxic in highest concentrations
- Partial uptake of agglomerated particles
- Accumulation in part of the cells
- No oxidative stress
- Transport of ~ 4-6 %

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