Cytotoxic effects of polystyrene particles on murine cells [334154]

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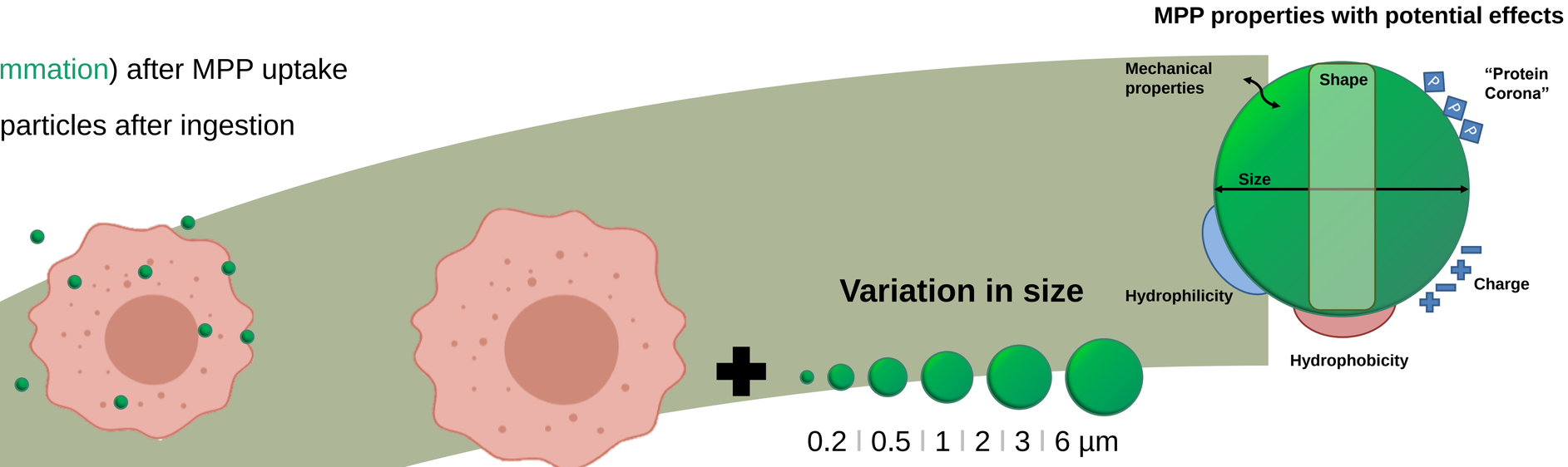
What kind of biological effects are induced by microplastic particles (MPP) in mammalian cells?

- Uptake and distribution
- Cellular effects (toxicity, inflammation) after MPP uptake
- Accumulation or excretion of particles after ingestion
- Long-term effects on cells



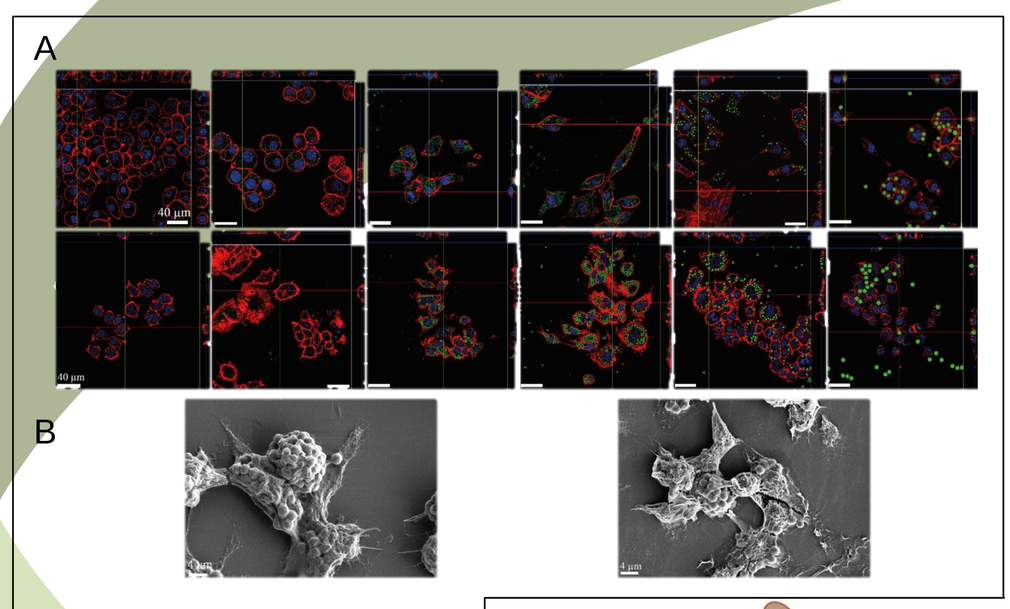
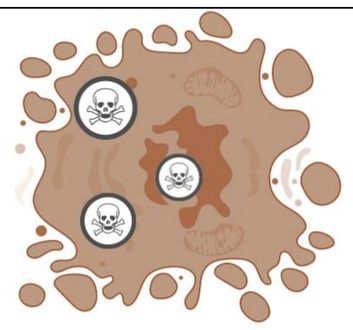


Fig. 1: Cells (3 x 10^4) were incubated with particles for 24h. After incubation, cells were fixed, nucleus and actin filament were immunostained for confocal microscopy (A) (Actin filament, nucleus, MPP)

Dehydrated samples (B) were analysed via Scanning electron microscopy.

The influence of **plain PS-MPP with varying size** on murine macrophages (J774A.1 / ImKC) and epithelial cells (STC-1 / BNL CL.2) was analysed.
The **uptake** of MPP could be shown by confocal microscopy. Macrophages showed a high uptake frequency for all MPP-sizes (Figure 1). For epithelial cells (not shown) a lower and size dependent uptake frequency was found.
A **standard cytotoxicity assay (MTT)** showed, even for highest concentrations, no effects for epithelial cells. Very high concentrations (> 100 µg/ml) showed a slight cytotoxicity for macrophages (Figure 2).
Reactive oxygen species (ROS) increased in a concentration dependent manner in ImKC cells. For the remaining cell lines no significant increase was found (Figure 3).



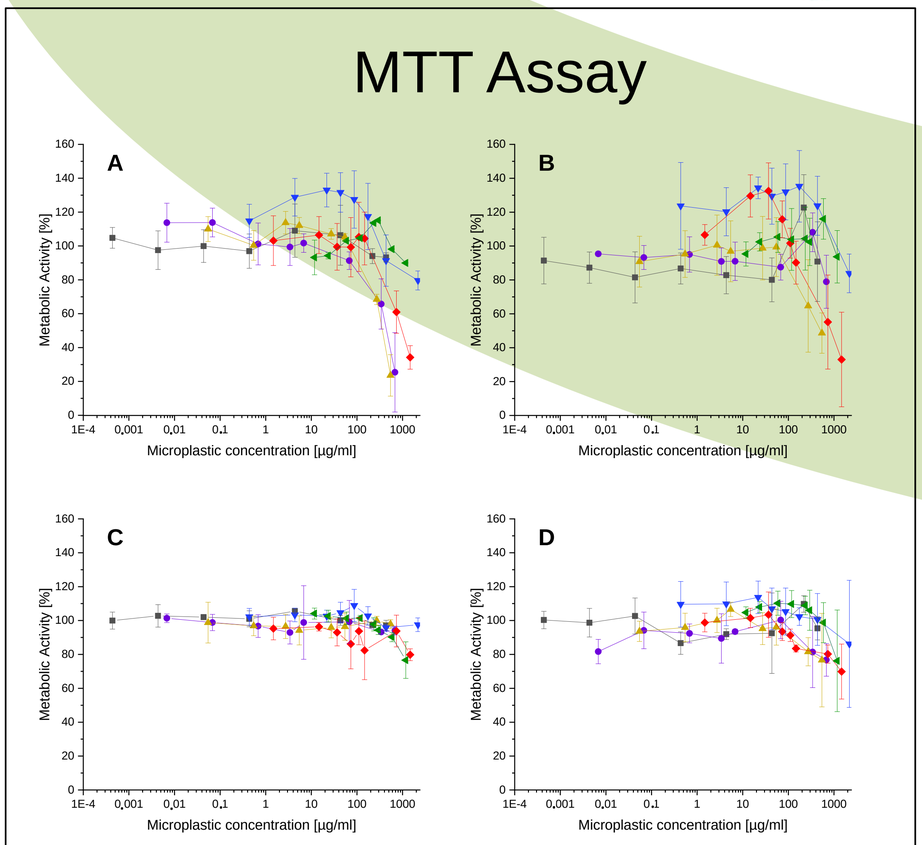


Fig. 2: 1x10^4 cells/well (J774A.1 (A), ImKC (B), BNL CL.2 (C)) / 2.5 x 10^4 cells/well (STC-1 (D)) were incubated with MPP of varying sizes (0.2, 0.5, 1, 2, 3, 6 µm) and increasing concentrations in 100 µl growth media (96-well) with 10% (v/v) foetal calf serum for 24 h. After incubation, 1 mg/ml MTT reagent was added to measure the metabolic activity. The produced formazan crystals were diluted in isopropanol and the Abs_{570nm} was measured. Non-treated cells (growth media without MPP) were used as 100% control. Mean ± SD, n = 3.

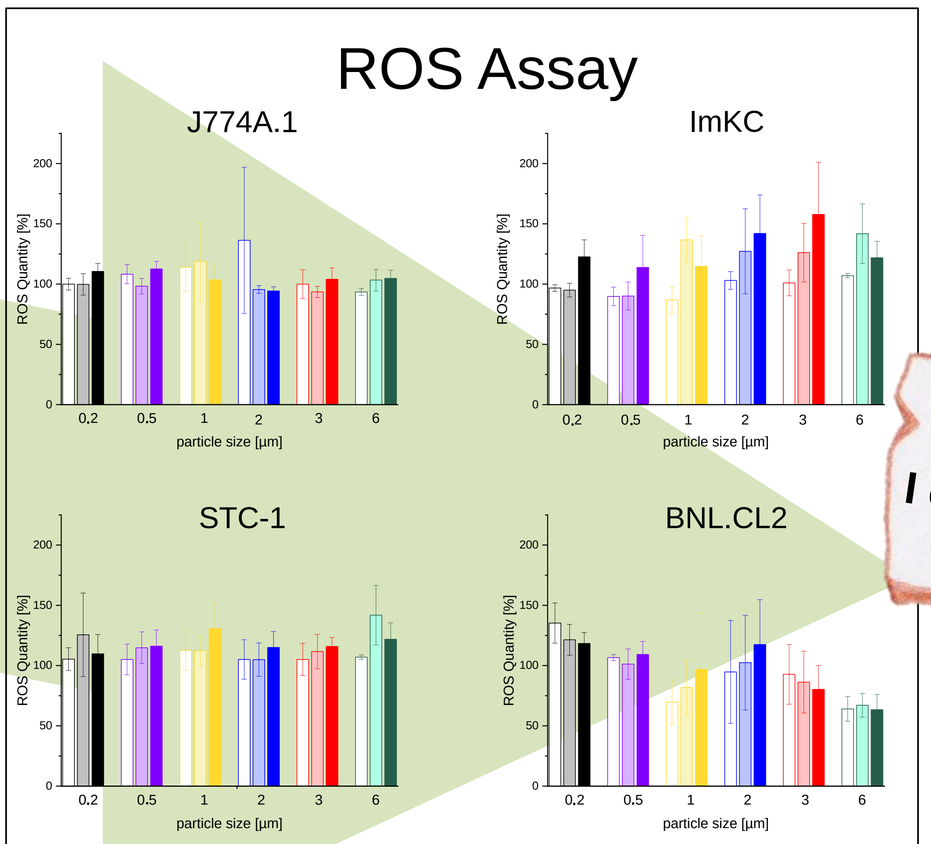


Fig. 3: 1x10^5 cells/well were incubated with MPP of varying size (0.2, 0.5, 1, 2, 3, 6 µm) and increasing concentrations (Table 1) in 1 ml growth media (12-well) with 10 % (v/v) foetal calf serum for 24 h. After incubation, DCFDA was added for 24 h to the cells to measure the ROS production by flow cytometry. Non-treated cells (growth media without MPP) were used as 100 % control. Mean ± SD, n = 3.

Table 1: Particle concentrations for ROS

	Particle size [µm]	0.2	0.5	1	2	3	6
concentration [µg/ml]	bright	4,35*10^{-3}	6,80*10^{-2}	0,544	4,35	14,7	23,5
	light	0,435	3,40	5,44	43,5	73,5	118
	dark	43,5	68,0	54,4	174	147	294

* Cartoons generated with Biorender.com

Summary: For untreated PS-MPP no significant short-time cytotoxicity on murine cell lines could be determined. In following project steps, single cell analysis and long-term investigations will be extensively analysed.

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