Bionanoteam
Nanobiology & Nanotoxicology Research
Biotechnical Faculty | University of Ljubljana

ODDELEK ZA BIOLOGIJO
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Cytotoxic and genotoxic effects of ZnO nanoparticles, ZnO microparticles and $ZnCl_2$ on MDCK kidney cell line

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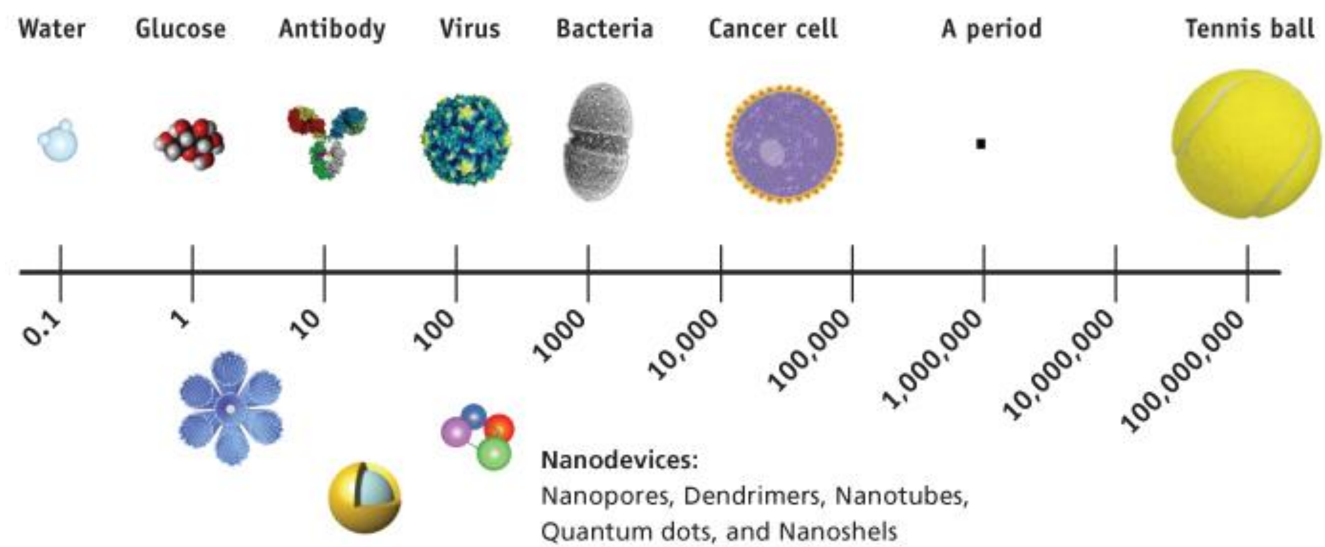
CUTTING EDGE 2015

Nanoparticles (NPs)

- NPs = particles < 100 nm



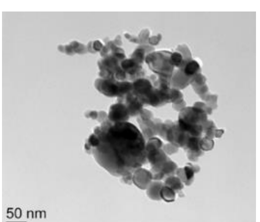
Nanoparticles (NPs)

- Unintentionally produced NPs

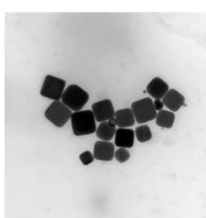




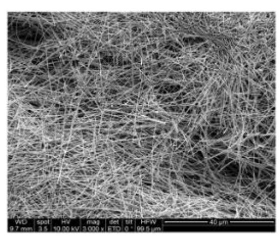
- Engineered NPs: Nanotechnology



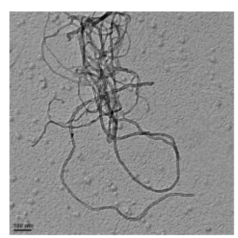
CuO NPs



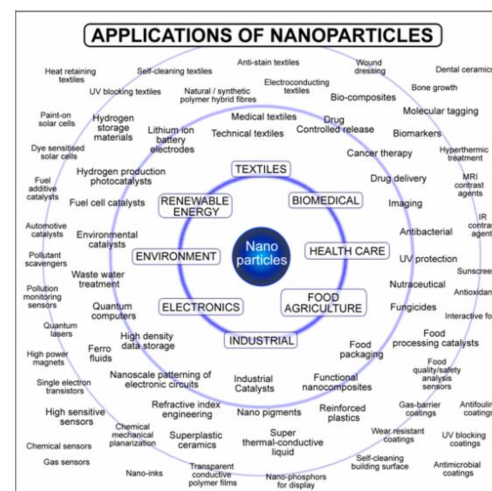
Ag nanocubes



Ag nanowires

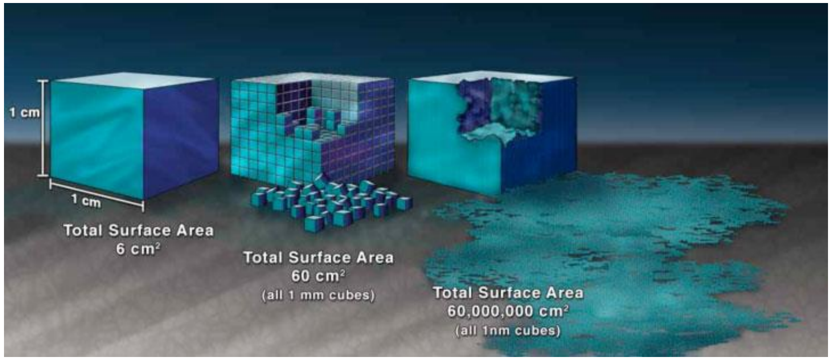


Carbon nanotubes

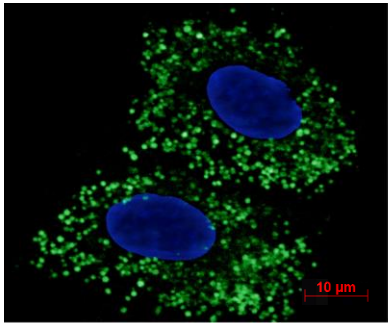


What's so special about nanoparticles?

- Small size → high relative surface area → high reactivity



- Small size → easier penetration into organism and cells

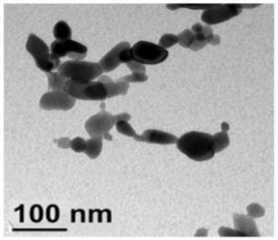


Cytotoxicity
DNA damage
Immunotoxicity
Oxidative stress
...

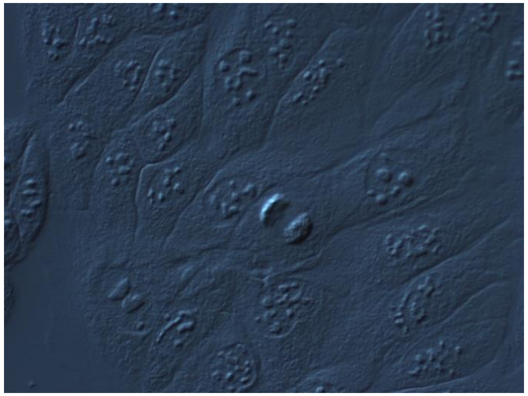
Picture from www.nano.gov

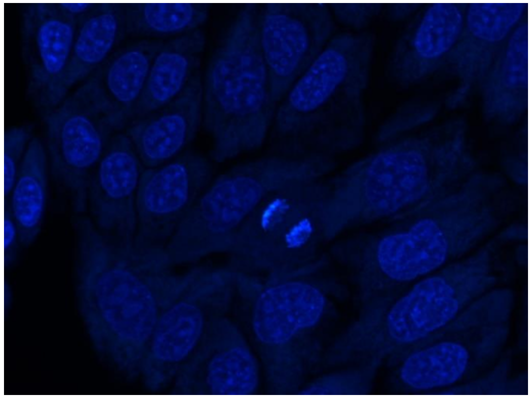
Our study

- **ZnO NPs**: mass production (>1,2×10^6 tons per year), wide applicability



- **MDCK cells**: *in vivo* studies have shown that ZnO NPs can be retained in the kidney (Wang et al., 2012; Li et al., 2012), but NP-impact on the excretory system is still poorly understood





Wang L, Wang L, Ding W, Zhang F. Acute toxicity of ferric oxide and zinc oxide nanoparticles in rats. J Nanosci Nanotechnol. 2010;10(12): 8617-8624
Li CH, Shen CC, Cheng YW, Huang SH, Wu CC, Kao CC, Liao JW, Kang JJ. Organ biodistribution, clearance, and genotoxicity of orally administered zinc oxide nanoparticles in mice. Nanotoxicology. 2012;6(7):746-56
Kumar A., et al., 2012. Microorganisms: A Versatile Model for Toxicity Assessment of Engineered Nanoparticles. Nano-Antimicrobials

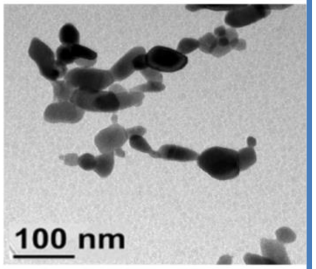
Our study

Question 1:

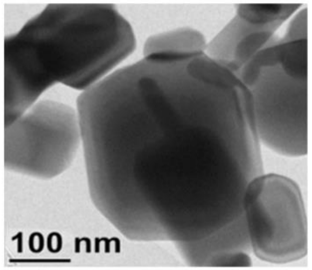
Are toxic effects of ZnO dependant on particle size and ion dissolution?

Treatment of cells with:

ZnO nanoparticles (20-100 nm)
NPs

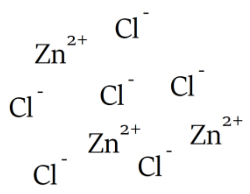


ZnO microparticles (100-500 nm)
MPs



Zinc salt

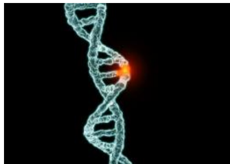
$ZnCl_2$



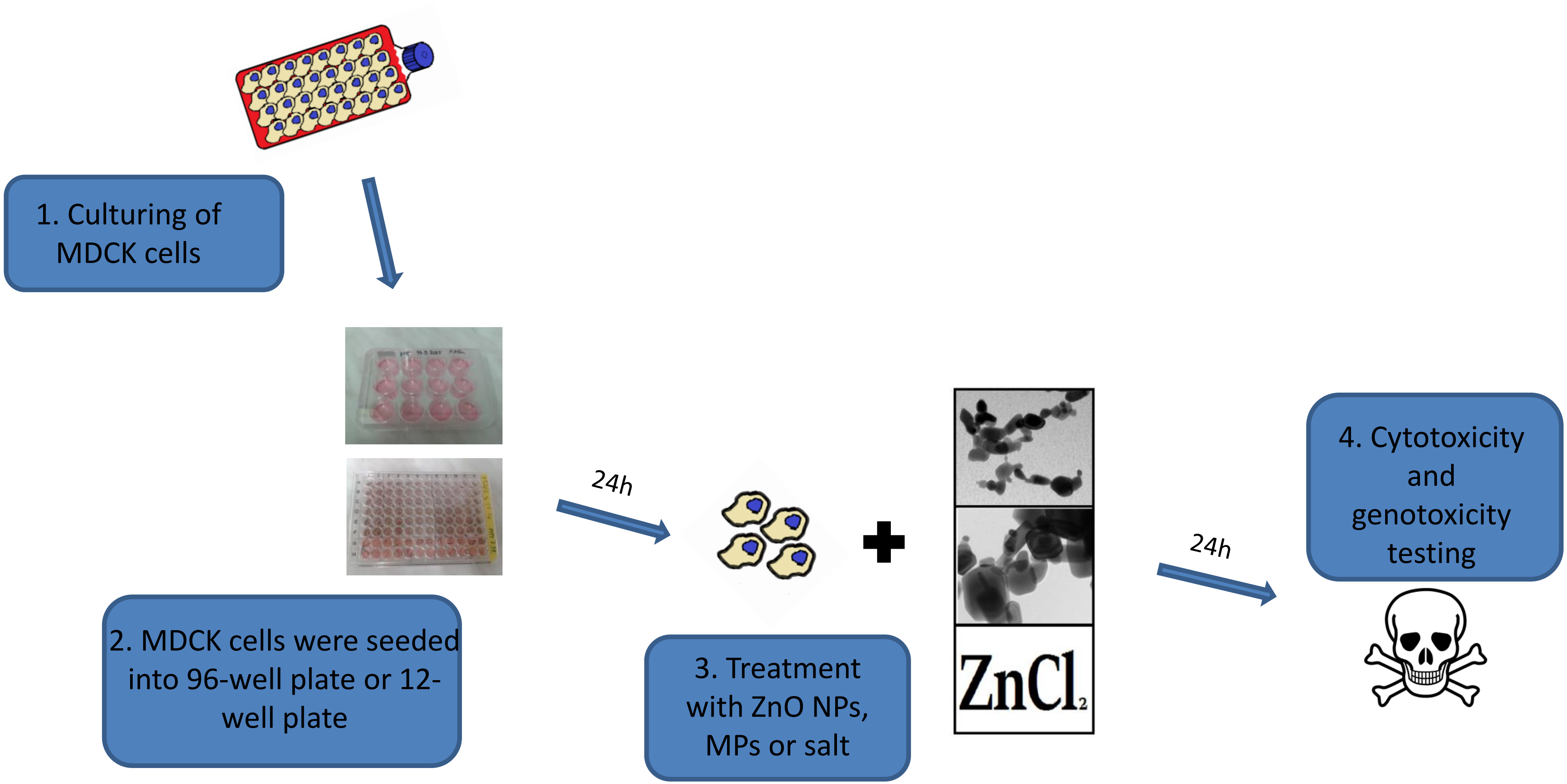
Question 2:

Can sub-cytotoxic concentration of ZnO NPs induce DNA damage?

Determine cytotoxic concentrations > use of sub-cytotoxic concentrations in genotoxicity experiments.



Design of experiments



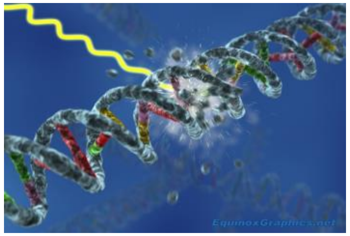
Cytotoxicity testing

1. MTT assay

2. Neutrale red uptake assay

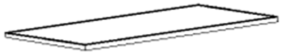
3. Trypan blue exclusion assay

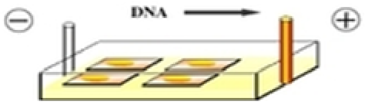
Genotoxicity testing

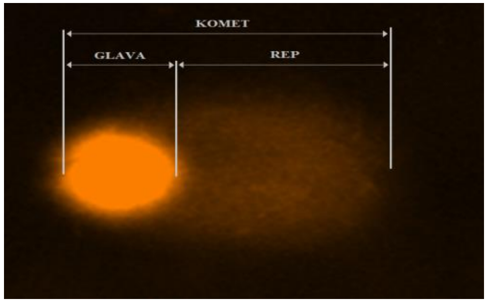


1. <u>Comet assay (detection of single- and double-stranded DNA breaks)</u>

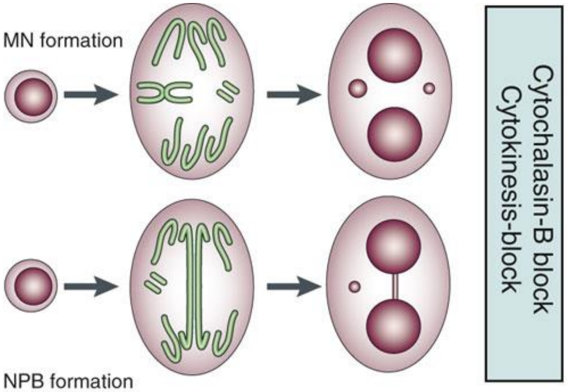
Cells are embedded in agarose gel > lysis > DNA unwinding > electrophoresis > microscopic evaluation

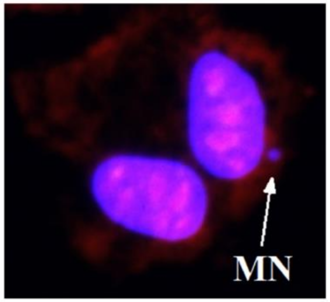


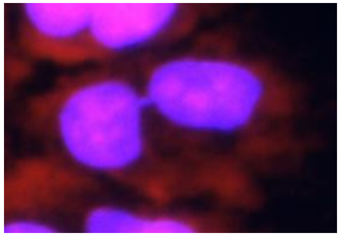




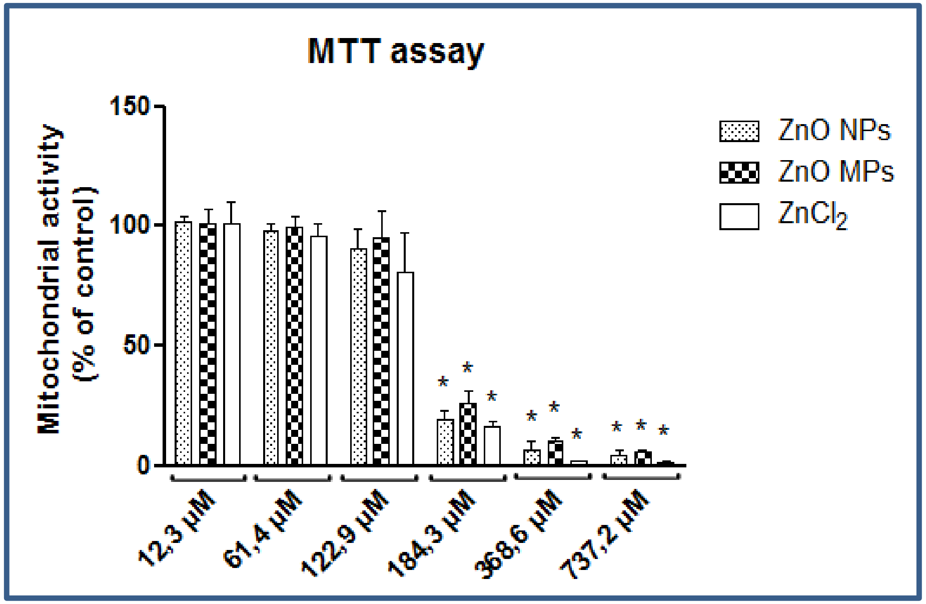
1. <u>Micronucleus assay (detection of DNA damage at chromosome level)</u>

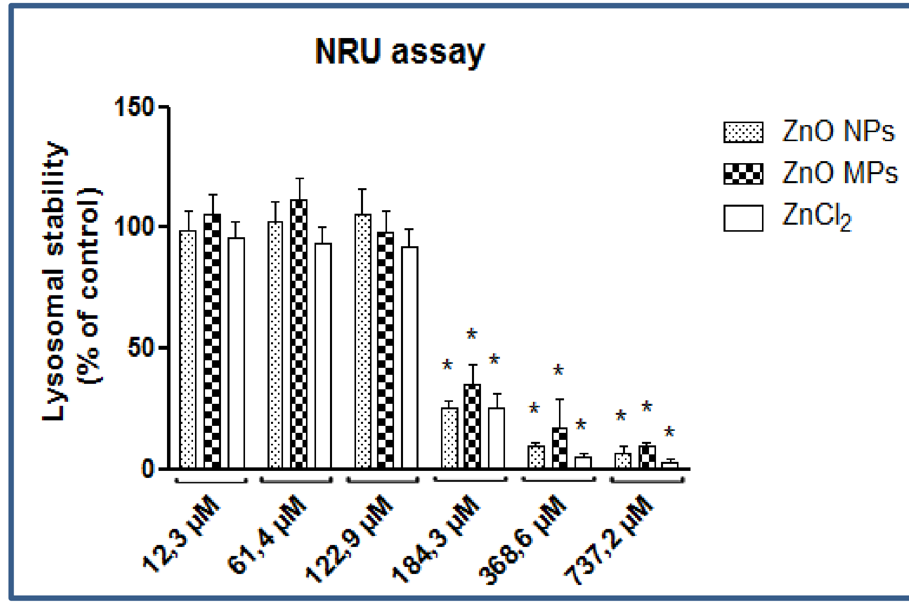


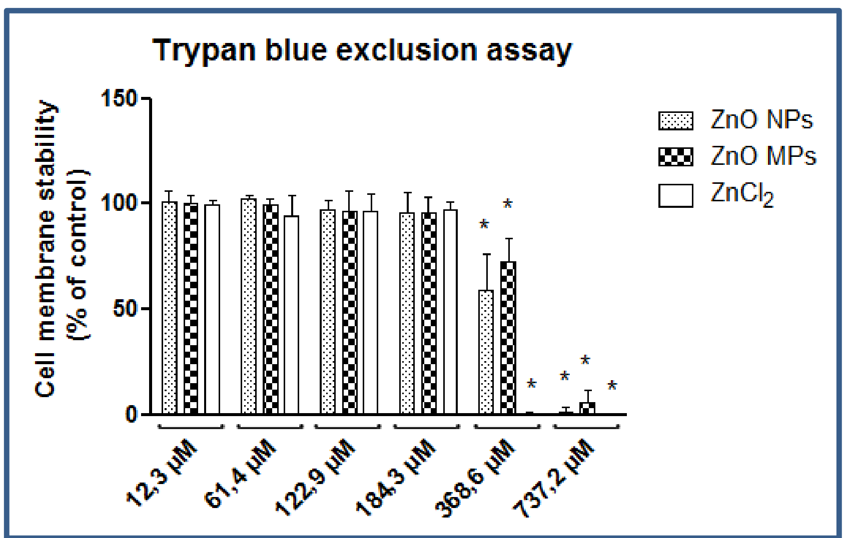




Cytotoxicity results







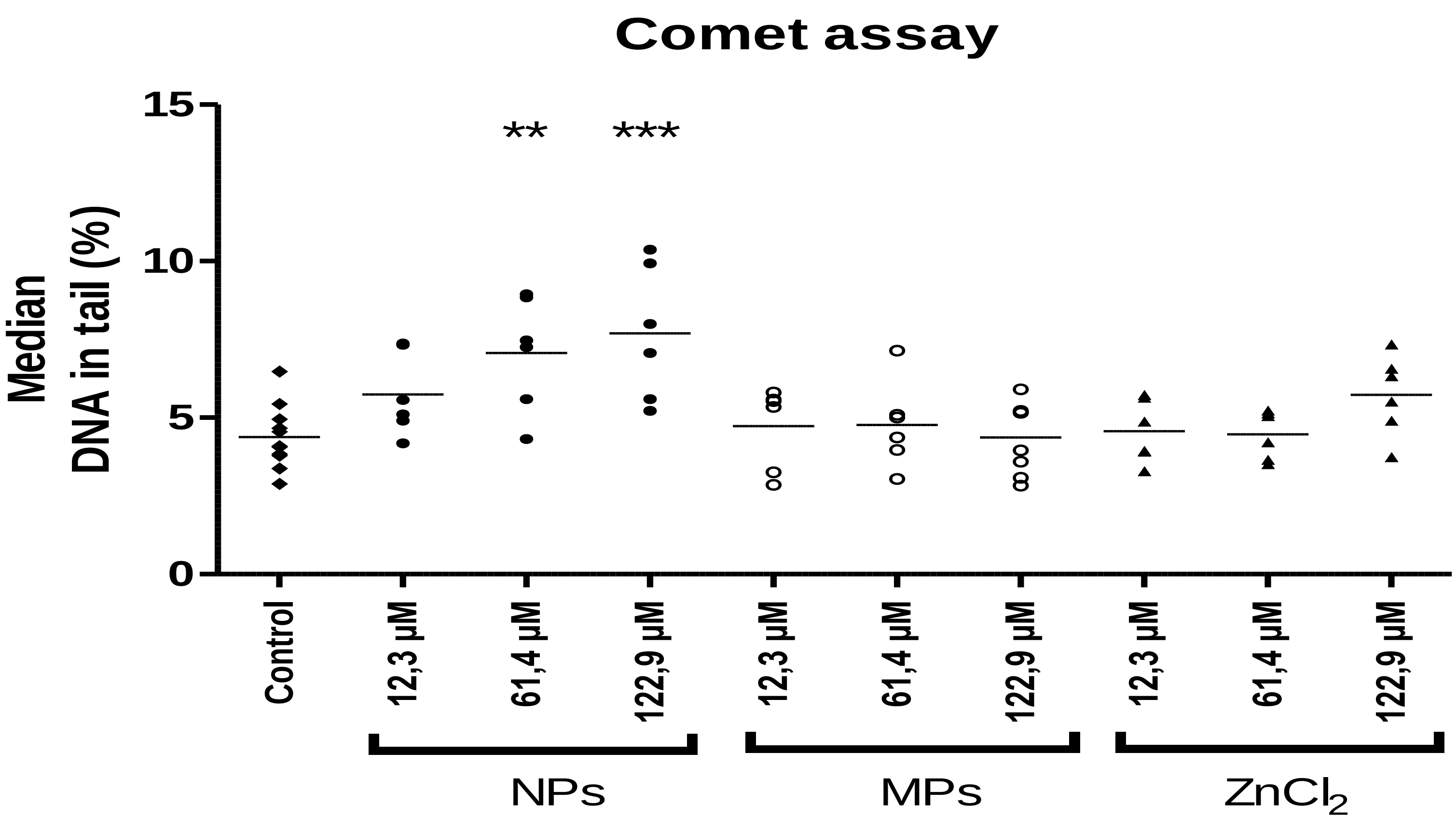
Sub-cytotoxic concentrations for genotoxicity studies:

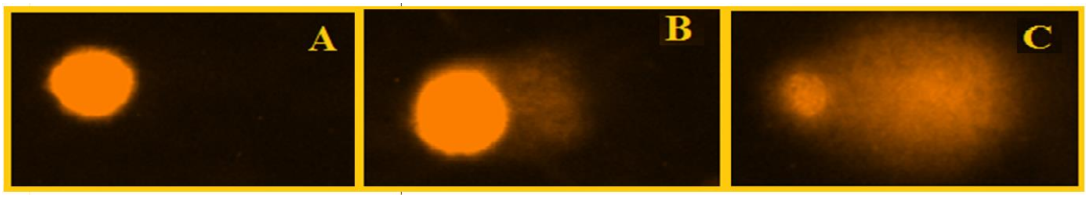
-122.9 µM

- 61,4 µM

- 12,3 µM

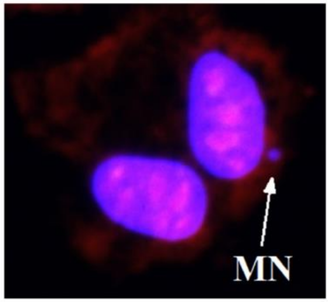
Genotoxicity results

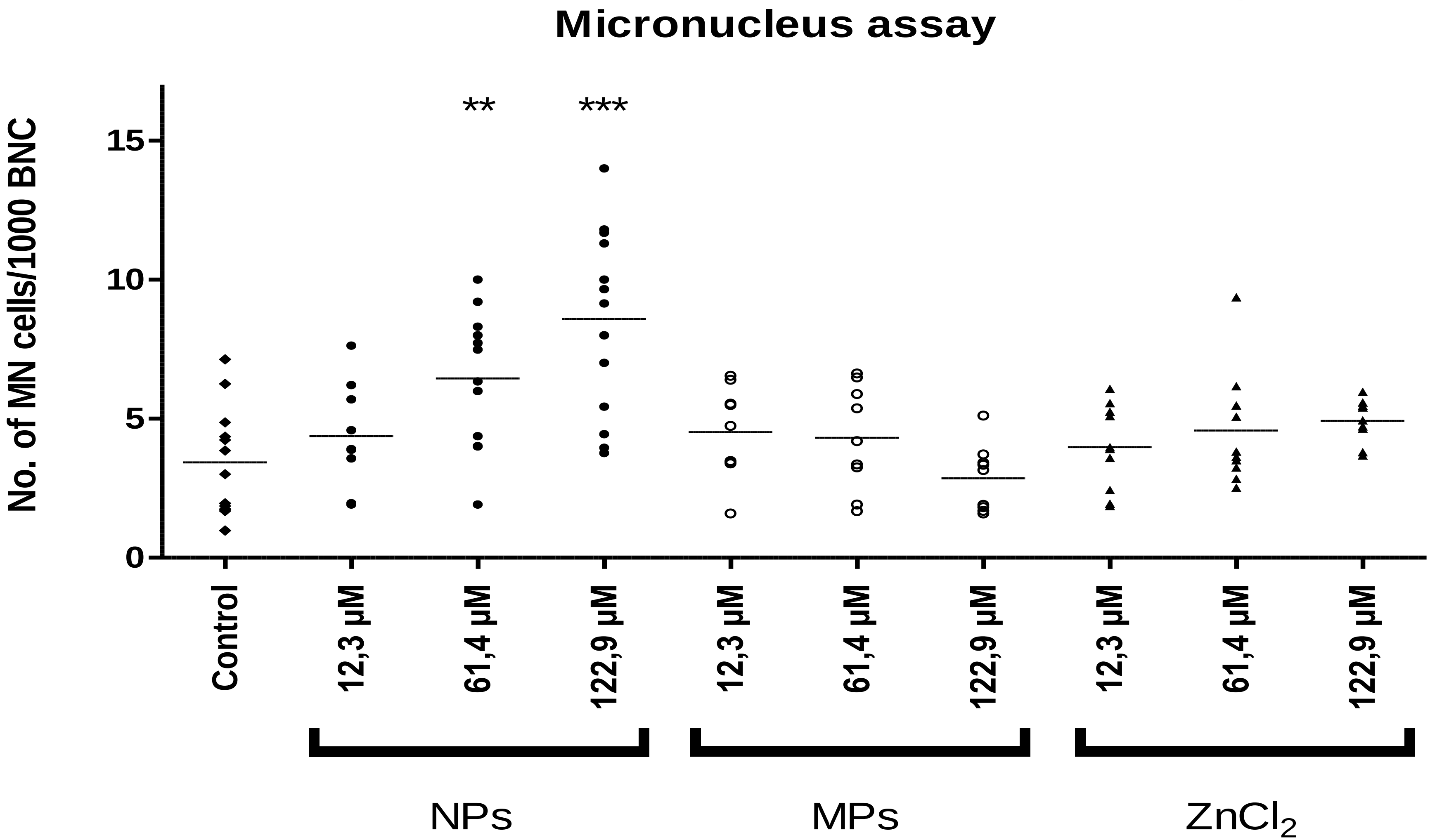




Low DNA damage Medium damage High damage

Genotoxicity results





Conclusions

- MDCK cells are prone to cytotoxic and genotoxic effect of ZnO NPs.
- Cytotoxicity concentration range of ZnO NPs, ZnO MPs and $ZnCl_2$ was very similar (starts at 184,3 µM).
- MDCK cells were more sensitive to mitochondrial and lysosomal disruption than to cell membrane permeabilization.
- Only ZnO NPs induced significant DNA damage at non-cytotoxic concentrations.
- Since equimolar concentrations of ionic zinc did not cause genotoxic effect, genotoxicity of ZnO NPs cannot be fully explained just by NP ion dissolution.
- ZnO particle size is important factor of ZnO genotoxicity.

Thank you!