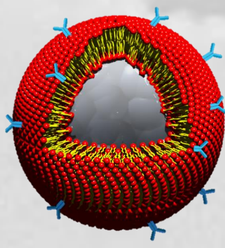
Investigation of cytotoxic effects of different ZnO nanostructures on living cancer cells



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INTRODUCTION

Thanks to its intrinsic and unique physico-chemical properties, in recent years the use of zinc oxide (ZnO) has increased for applications in different fields, from biosensing to cancer therapy. Besides, ZnO is known to be a versatile material, easy to synthesize in different shapes and sizes, as nanowires and nanoparticles.

In this study the cytotoxic behavior of different ZnO nanostructures was *in vitro* evaluated analyzing the long-term responses (until 72 hours) of the tumor KB cell line (human oral carcinoma). Cells were cultured with different concentrations of bare ZnO nanoparticles (ZnO NPs) and nanowires (ZnO NWs), and amino-propyl functionalized ZnO nanoparticles (ZnO-NH_2 NPs). Then ZnO-NH_2 NPs and ZnO NWs cytotoxic effects were directly monitored through transmission electron microscopy (TEM).

SYNTHESIS AND CHARACTERIZATION

Sol. 1
- Zinc Acetate di-hydrate 818 mg (3,73 mmol)
- Methanol 42 mL
- H_2O b.d. 318 μL

Sol. 2
- NaOH 288,8 mg (7,22 mmol)
- H_2O b.d. 23 mL

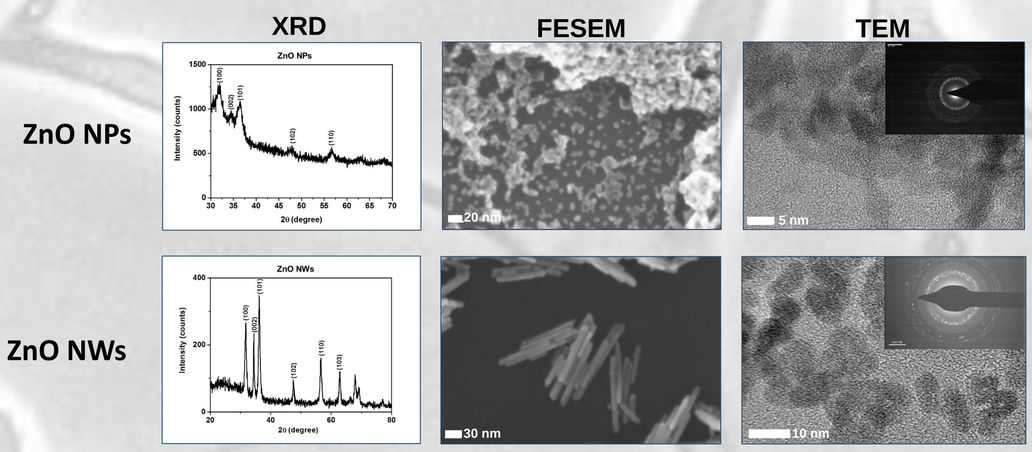
ZnO NPs synthesis:

-Dropwise addition of sol.2 in sol.1 for 15 min at 60°C under stirring
-Stir at 60°C for 5 hours
-Centrifugation at 5000 rpm for 10 min
-Wash with Ethanol twice
-Final dispersion in Methanol and then in the culture medium at desired concentration

ZnO NWs synthesis:

-Dropwise addition under N_2 atmosphere
-Stir under reflux at 60°C and N_2 for 2 hours
-Evaporation in rotovapor at 55°C until the volume is reduced to 10 mL
-Reflux under stirring for 48 hours at 60°C
-Centrifugation at 5000 rpm for 10 min
-Wash with Ethanol twice
-Final dispersion in Methanol and then in the culture medium at desired concentration

ZnO NPs and ZnO NWs characterization

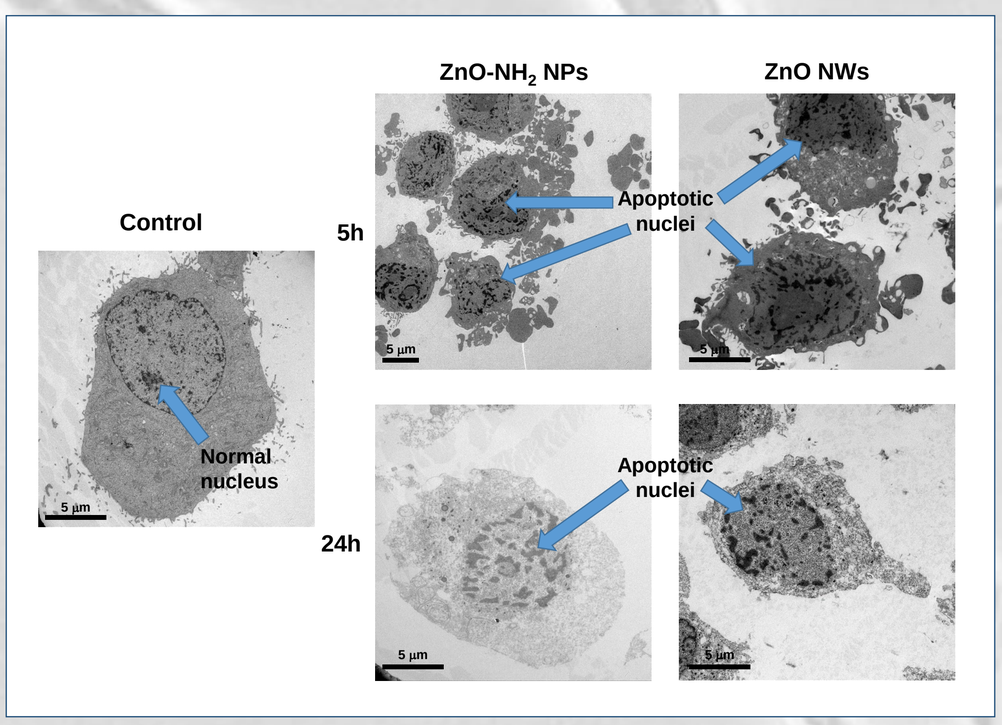


$ZnO-NH_2$ NPs synthesis:

Chemical functionalization with aminopropyl groups was carried out with 100 mg (1,22 mmol) of ZnO NPs in a glass round flask in their original medium (Methanol) with 0,122 mmol of aminopropyltrimethoxysilane (APTMS: 21,9 mg), corresponding to 10 mol% of the ZnO molar amount. The solution was refluxed for 6 h under a nitrogen atmosphere. The functionalized NPs (ZnO-NH_2 NPs) were washed with Methanol to remove unbound molecules and then redispersed in clean Methanol.

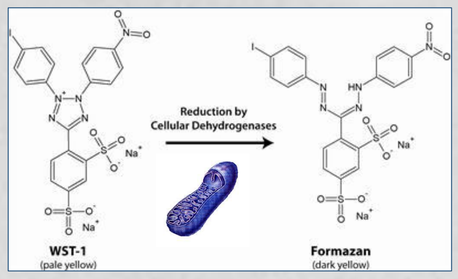
TEM ANALYSIS

Control cells look healthy and regular in shape, while cells incubated with ZnO-NH_2 NPs or ZnO NWs begin to show intense vesciculation activity. There are many vacuoles in the citosol and nuclei are in apoptotic stage.



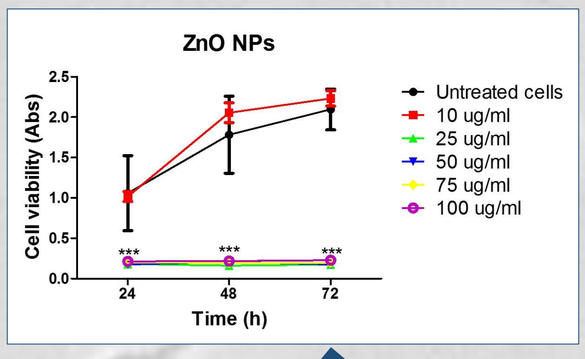
$ZnO-NH_2$ NPs and ZnO NWs can both induce apoptosis in cancer cells at 5 and 24 hours

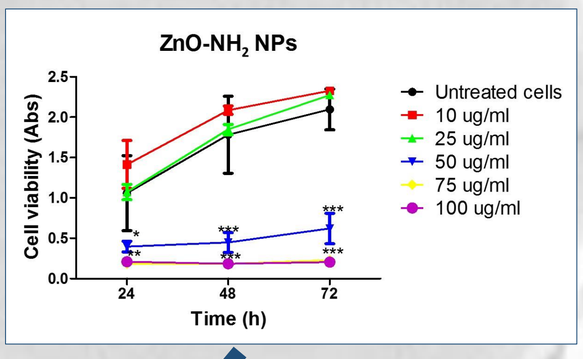
CYTOTOXICITY



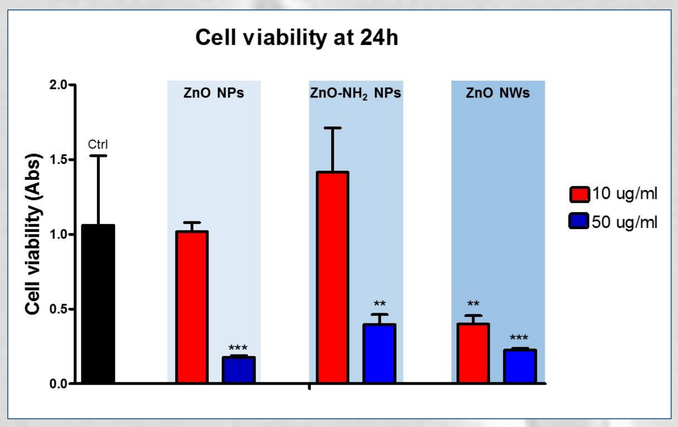
WST-1 colorimetric assay

The stable tetrazolium salt WST-1 is cleaved to a soluble formazan by cellular mitochondrial dehydrogenases. Expansion in the number of viable cells results in an increase in the activity of the mitochondrial dehydrogenases, which in turn leads to increase in the amount of formazan dye formed. The formazan dye produced by viable cells can be quantified by measuring the absorbance (Abs) at λ=440 nm.



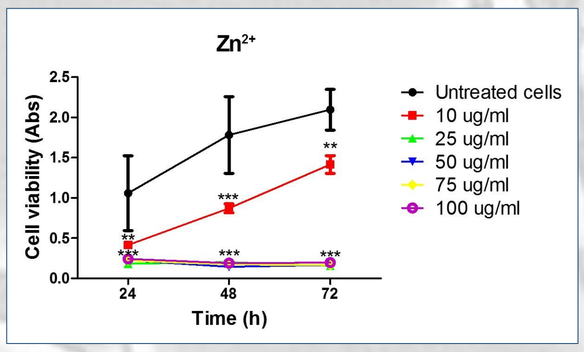


ZnO NPs are more toxic than ZnO-NH_2 NPs



ZnO NWs affect more cell viability

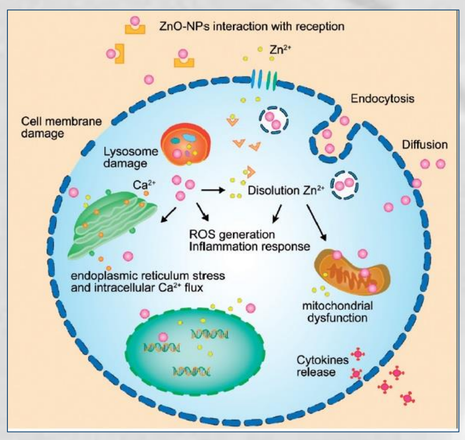
Possible mechanism?



Cell viability upon incubation with different concentrations of $ZnCl_2$ (source of Zn^{2+} ions) shows the same trend

The release of Zn^{2+} ions from ZnO nanoparticles or nanowires can lead to cell death

CONCLUSIONS AND FUTURE OUTLOOKS



- ✓ Shape and functionalization are important to control the cytotoxity
- ✓ ZnO nanoparticles and nanowires induce principally apoptosis in cancer cells
- ✓ Nanoparticles internalization and mechanism?
- ✓ Study the responses in different cell lines
- ✓ Improve targeting: functionalize nanoparticles and nanowires with antibodies or peptides
- ✓ Prevent cytotoxicity: create a functional shell with polymers, lipid bilayers from liposomes and exosomes

Bibliography

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