Enhanced biostability and biocompatibility of zinc oxide nanocrystals shielded by a phospholipid bilayer

TNH Lab

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MOTIVATION

Zinc oxide nanocrystals (ZnO NCs), thanks to their unique properties such as intrinsic cytotoxicity and easy synthesis and functionalization, represent an interesting diagnostic and therapeutic tool for cancer treatment. In order to enable their use in clinical applications, a better control of their chemical and colloidal stability in the biological environment and of the material's biocompatibility is required.
In this study we propose to modify the surface of pristine ZnO NCs with a biomimetic phospholipidic shell, constituted by self-assembled liposomes or cell-derived extracellular vesicles, in order to promote the stability and biocompatibility of the nanocrystals in the physiological environment.

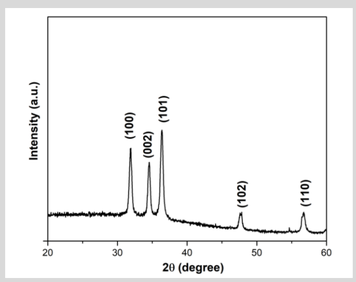
SYNTHESIS and CHARACTERIZATION

MICROWAVE-ASSISTED SYNTHESIS

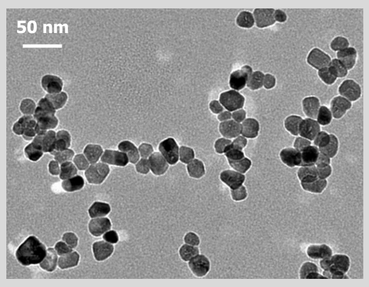
Sol 1: 0.1 M Zinc Acetate di-hydrate in Methanol
+480 µL H_2O bd (**nucleation promoter**)
+**Sol 2**: 0.2 M KOH in Methanol

- Synthesis mixture in microwaves oven at 60°C for 30 minutes
- Two washing steps (centrifugation at 3046 rcf for 10 min and redispersion in Ethanol)

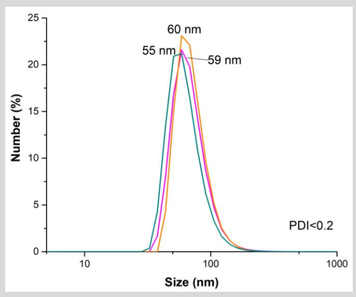
X-Ray Diffractogram



Transmission Electron Microscopy (TEM)



DLS measurements in Ethanol

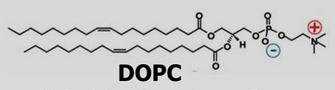


ZnO NCs

- Monocrystalline particles of about 20 nm.
- Homogeneous and monodisperse size distributions.

LIPID-SHELL FUNCTIONALIZATION with SYNTHETIC LIPIDS

SOLVENT EXCHANGE METHOD



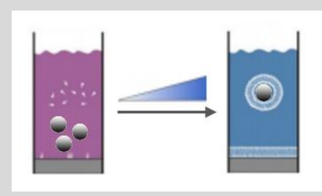
DOPC
(1,2-dioleoyl-sn-glycero-3-phosphocholine)

ZnO NCs

10 mL of lipid solution (40 vol% EtOH-60 vol% H_2O bd with C_{DOPC}=2.5 mg/ml)

+

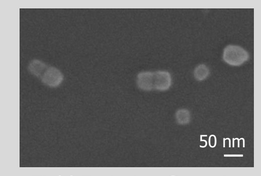
100 mg of ZnO NCs (centrifuged to remove Ethanol)



Addition of 70 mL of H_2O bd to form the supported lipid bilayer

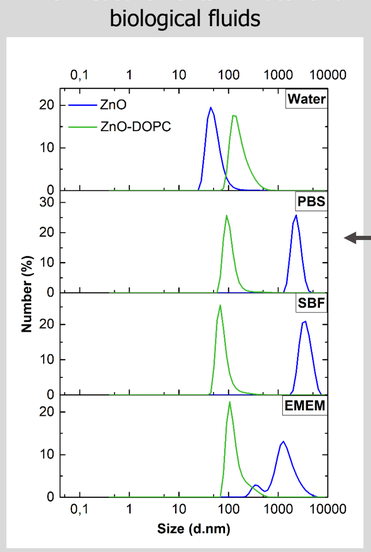
ZnO-DOPC NCs

	Z-Pot in water
ZnO NCs	26 mV
DOPC	-15 mV
ZnO-DOPC NCs	1.3 mV



Field Emission Scanning Electron Microscopy (FESEM)

DLS measurements in water and biological fluids



Phosphate Buffered Saline (PBS) – Composition: Na_2HPO_4, KH_2PO_4, KCl, NaCl

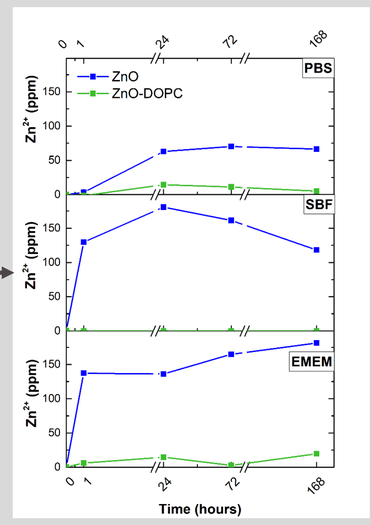
Simulated Body Fluid (SBF) – Composition: NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, Na_2SO_4, HCl and $NH_2C(CH_2OH)_3$

Cell culture medium + 10% FBS – Composition: $CaCl_2$, $MgSO_4$, KCl, $NaHCO_3$, NaCl, NaH_2PO_4, aminoacids, vitamins, glucose, plasma proteins, hormones

Lipid-shell efficiently prevents:

- NPs aggregation
- NPs dissolution into Zn^{2+} → improves the **BIOSTABILITY**

ICP/MS Measurements



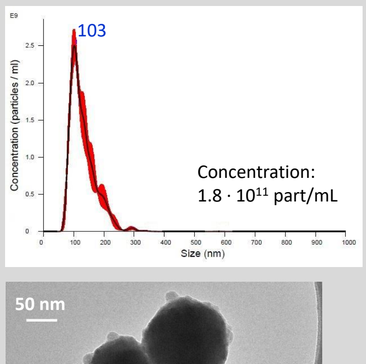
AUTOLOGOUS EXTRA-CELLULAR VESICLES to improve the BIOCOMPATIBILITY

EXTRA-CELLULAR VESICLES (EVs)

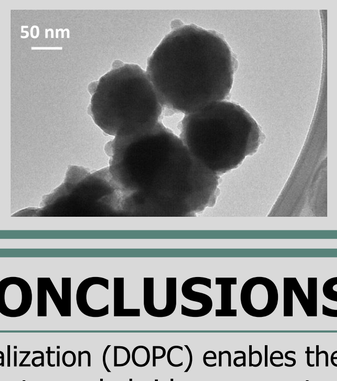
Heterogeneous group of cell-derived membranous structures (comprising exosomes and microvescicles) present in many biological fluids.

- ✓ Autologous -> low immunogenicity
- ✓ Natural stability in blood
- ✓ Biocompatible and non-toxic
- ✓ Small size -> ability to cross biological barriers
- ✓ Intrinsic targeting ability

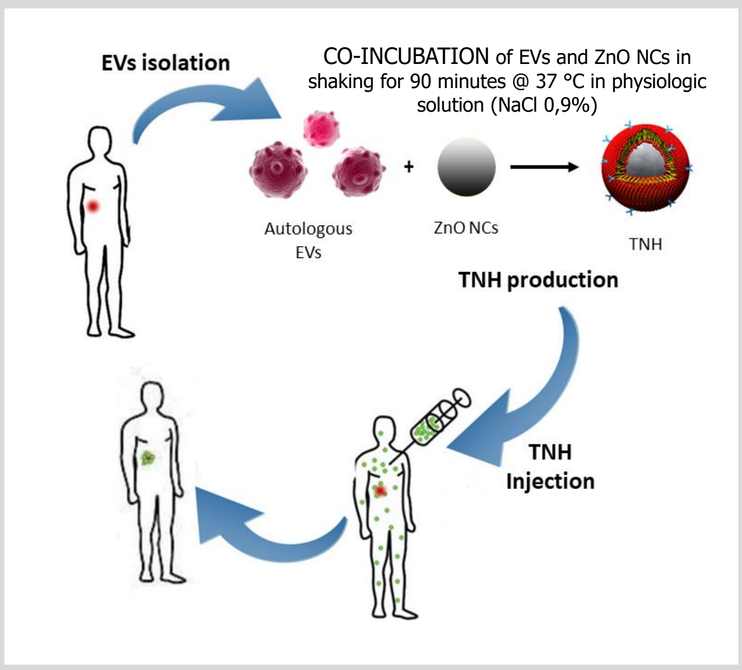
Nanoparticle Tracking Analysis (NTA)



Transmission Electron Microscopy (TEM)

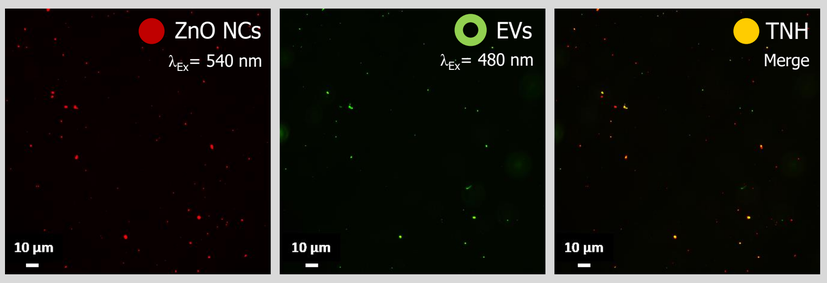


COUPLING and THERAPEUTIC STRATEGY

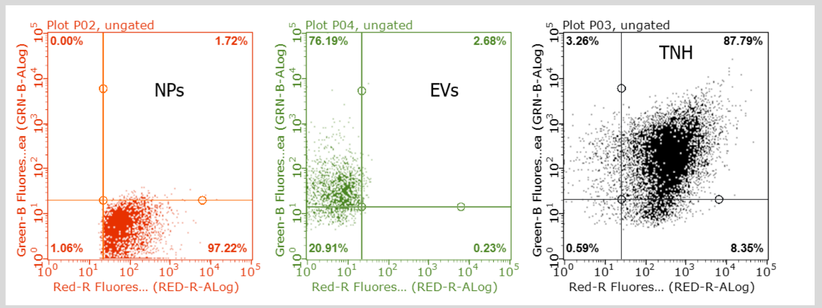


TNH CHARACTERIZATION

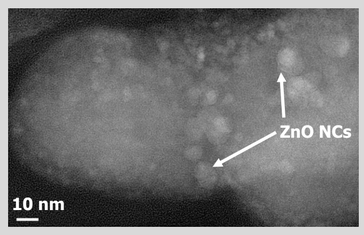
Fluorescence Microscopy (Immersion oil-60X magnification)



Flow Cytometry Analysis



Scanning Transmission Electron Microscopy (STEM)



CONCLUSIONS AND FUTURE OUTLOOK

- Lipid-shell functionalization (DOPC) enables the stabilization of ZnO NCs in biological media and prevents their degradation.
- It is possible to create an hybrid nanoconstruct (TNH) formed by ZnO NCs and cell-derived vesicles (EVs) that, thanks to their biological nature, can provide an enhancement of the biocompatibility.
- Optimisation of TNH production protocol and further characterization of its morphology, bio-stability and bio-compatibility.
- Detailed analysis of therapeutic and imaging capabilities of TNH.

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[2] T. Moore, L. Rodriguez-Lorenzo, V. Hirsch, S. Balog, D. Urban, C. Jud, B. Rothen-Rutishauser, M. Lattuada, and A. Petri-Fink, *Chem. Soc. Rev.* **2015**, 44, 6287-6305.
[3] B. Dumontel, M. Canta, H. Engelke, A. Chiodoni, L. Racca, A. Ancona, T. Limongi, G. Canavese, and V. Cauda, *J. Mater. Chem. B* **2017**, 5, 8799-8813.

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