Biomimetic hybrid nanoconstructs for cancer therapy

TNH Lab

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MOTIVATION

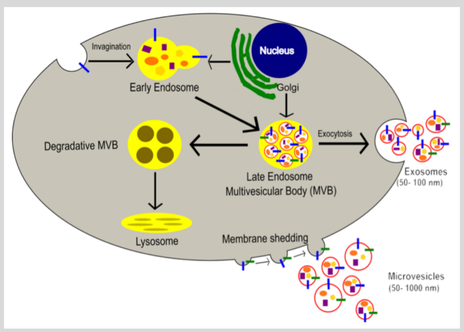
Cancer is a group of genetic diseases that involved uncontrolled cell division, replicative immortality and resistance to cell death. The current treatments have severe side effects and sometimes they are uneffective. One of the major cause of death related to cancer is the lack of selective delivery of anti-cancer drugs to the cancerous tissues. Thus an improvement of targeted delivery is extremely important to overcome the current limitations in cancer therapy. Nanomedicine is the design and development of therapeutics and diagnostic tools distinguished by the nanoscopic scale of its delivery vehicles and diagnostic agents. Recent developments in nanotechnologies are going to improve the drug delivery, increasing the therapeutic efficiency and at the same time reducing the toxic side effects.

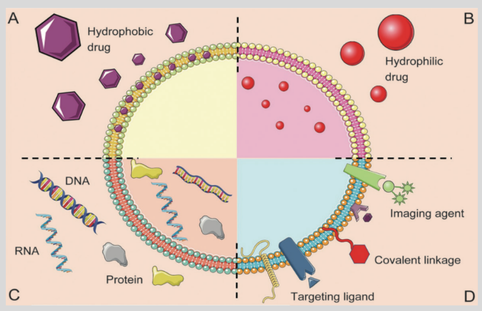
EXOSOMES

BIOLOGICAL FUNCTIONS:

EXOSOMES
- Diameter 30-120 nm
- Phospholipid bilayer
- Secreted by all cells, especially by tumors
- Contain lipids, proteins, RNAs, DNA

They are involved in many biological function:
- Intercellular communication
- Immune response
- Influence proteins activity
- Spreading cancer and neurodegenerative disease





EXOSOMES FOR THERAPEUTICS:

Exosomes could be loaded with small molecules or used as vehicles for drug delivery.

ADVANTAGES::
- Better stability in the physiologic and pathologic conditions
- Cross biological barriers
- Intrinsic ability of targeting
- If autologous, invisible to the immune system

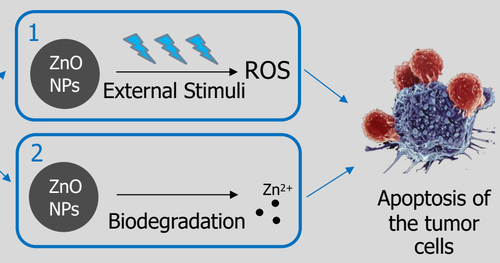
ZINC OXIDE NANOPARTICLES

ZINC OXIDE:

Zinc is the second most abundant trace metal in the human body, it has important biological functions, including a crucial role in the defence from cancer since it maintains the activity of the tumor suppressor gene p53.

ZINC OXIDE NANOPARTICLES (ZnO NPs)
- High interaction with biological compartments, thanks to their nanometric dimensions
- Non toxic, biosafe, biocompatible
- If in excess, are cytotoxic
- Preferential cytotoxicity towards cancerous cells, in particular on rapidly dividing cancer cells

MECHANISMS OF CYTOTOXICITY



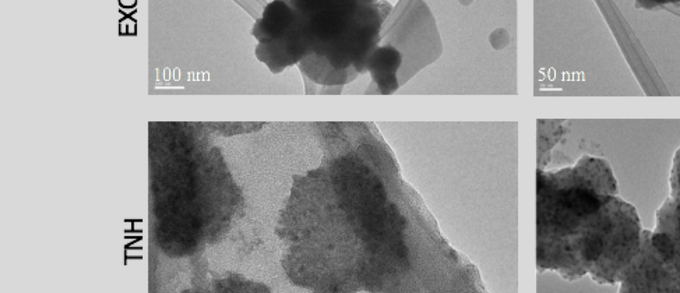
1) ZnO direct interaction with cells
- Direct damaging of cell structure
- Interference with mythocondrial electron transport chain
- ROS production

2) Zn^{2+} release
- Mythocondrial toxicity
- Protein activity disequilibrium
- Disregulation of cell signaling

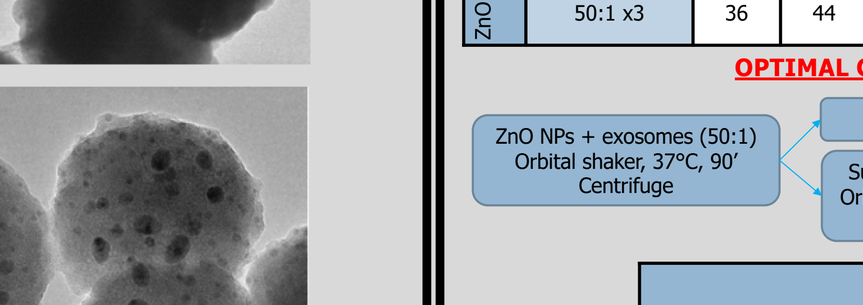
MATERIAL PREPARATION

1) Exosomes extraction

Cells are cultured for 48 hours in extracellular vesicles (EVs) free medium.
Then exosomes are extracted from culture media with an ultracentrifuge following the sterile differential ultracentrifugation protocol shown below.



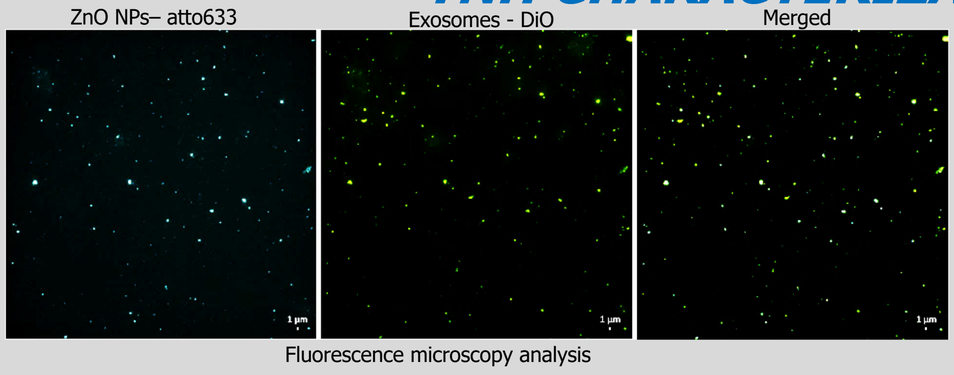
2) Exosomes coupling with ZnO NPs



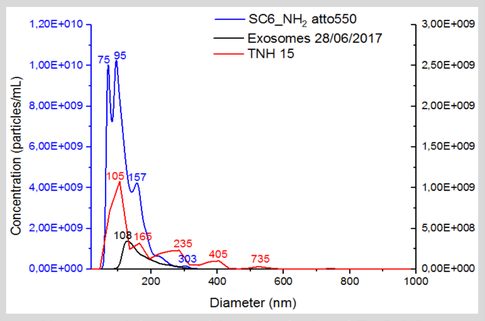
The coupling is carried out analysing different parameters such as:
- Temperature
- Mixing method
- Volume
- Time
- Amount of ZnO nanoparticles
- Sonication
- Media of suspension

The sample is labelled with fluorescent dyes for the characterization with fluorescence microscpy.

TNH CHARACTERIZATION



Fluorescence microscopy analysis



Nanoparticles Tracking Analysis of ZnO NPs, exosomes and TNH

Transmission Electron Microscopy (TEM)

RESULTS

PHYSICAL PARAMETERS		%ZnO	%Exo	%TNH
TEMPERATURE	4°C	20	27	14
	RT	21	28	13
	37 °C	42	32	22
MIXING METHOD	Tube rotator	11	22	6
	Orbital shaker	26	22	13
TIME	90 minutes	47	25	18
	8 hours	31	16	12
	24 hours	38	8	7
ZnO NPs : EXO RATIO	50:1	47	25	18
	500:1 at t0	11	18	7
	50:1 x3	36	44	24

MEDIA OF SUSPENSION	%ZnO	%Exo	%TNH
PBS	47	25	18
PHYSIOLOGIC SOLUTION	50	25	21

Physiologic solution is better because:

SAMPLE	Z-POTENTIAL
EXOSOMES	- 9,53
ZnO in PBS	-15,5
ZnO in PHYSIOLOGIC SOLUTION	12,3

ELECTROSTATIC INTERACTION

OPTIMAL COUPLING PROTOCOL

ZnO NPs + exosomes (50:1) Orbital shaker, 37°C, 90' Centrifuge
→ Pellet=TNH run 1
→ Supernatant+ZnO NPs Orbital shaker, 37°C, 90' Centrifuge
→ Pellet=TNH run 2
→ Supernatant+ZnO NPs Orbital shaker, 37°C, 90' Centrifuge

	%ZnO	%Exo	%TNH
OPTIMAL PROTOCOL	**68**	**40**	**34**

CONCLUSIONS AND FUTURE OUTLOOK

In the last decade ZnO NPs has acquired an increasing interest by the researcher thanks to its ability to exert a toxic activity preferably against cancerous cells, saving the healthy ones. The exosomes coating increases their stability in physiological environment and drives them towards their target facilitating also the internalization in cancerous cells. Exosomes also avoid the aggregation of the ZnO NPs in the bloodstream and the attack by the immune system.
TNH seems to be a very promising nanoconstruct for cancer treatment. However there are some aspects that need to be deepen, i.e. the stability over time of the TNH and their cytotoxity, the mechanisms of uptake by cells, the possibility of targeting on the exosomes coating and the conservation of this nanoconstruct.

MERCK & ELSEVIER Young Chemists Symposium

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Acknowledgment:

European Research Council – ERC-Starting Grant «TROJANANOHORSE» – N° 678151
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