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Ultrasound-induced Piezoelectric Nanoparticles for anticancer treatment

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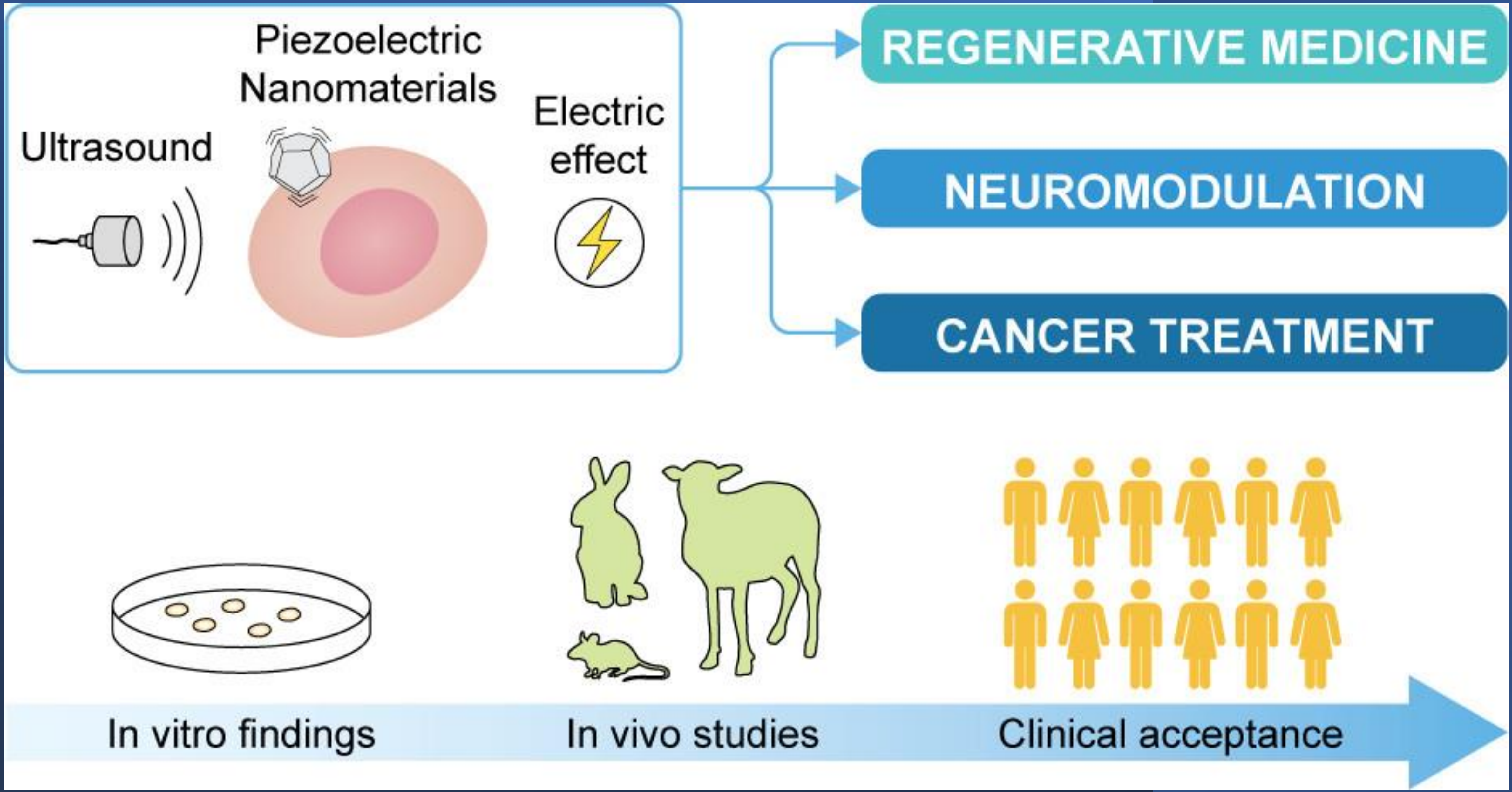
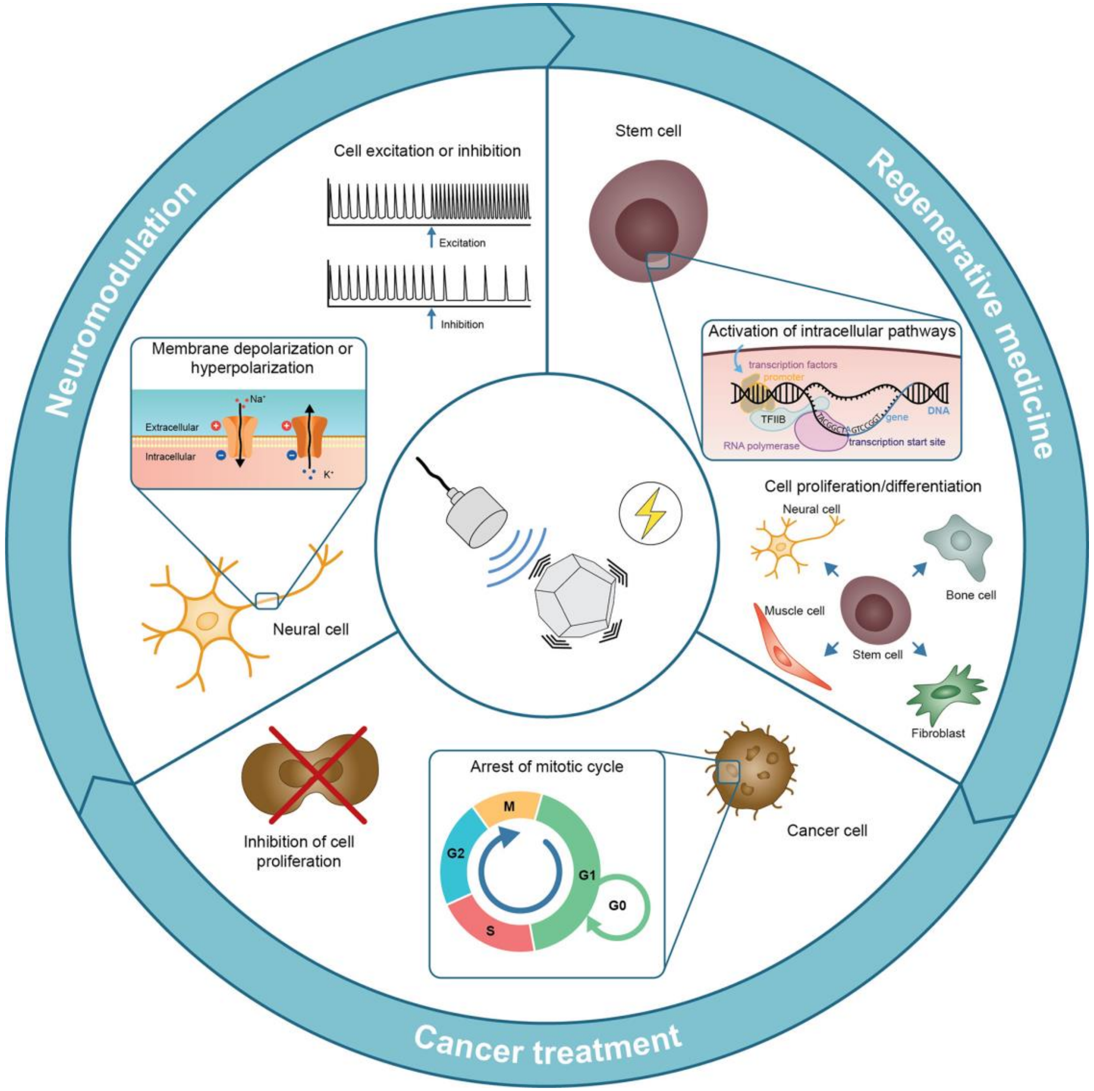
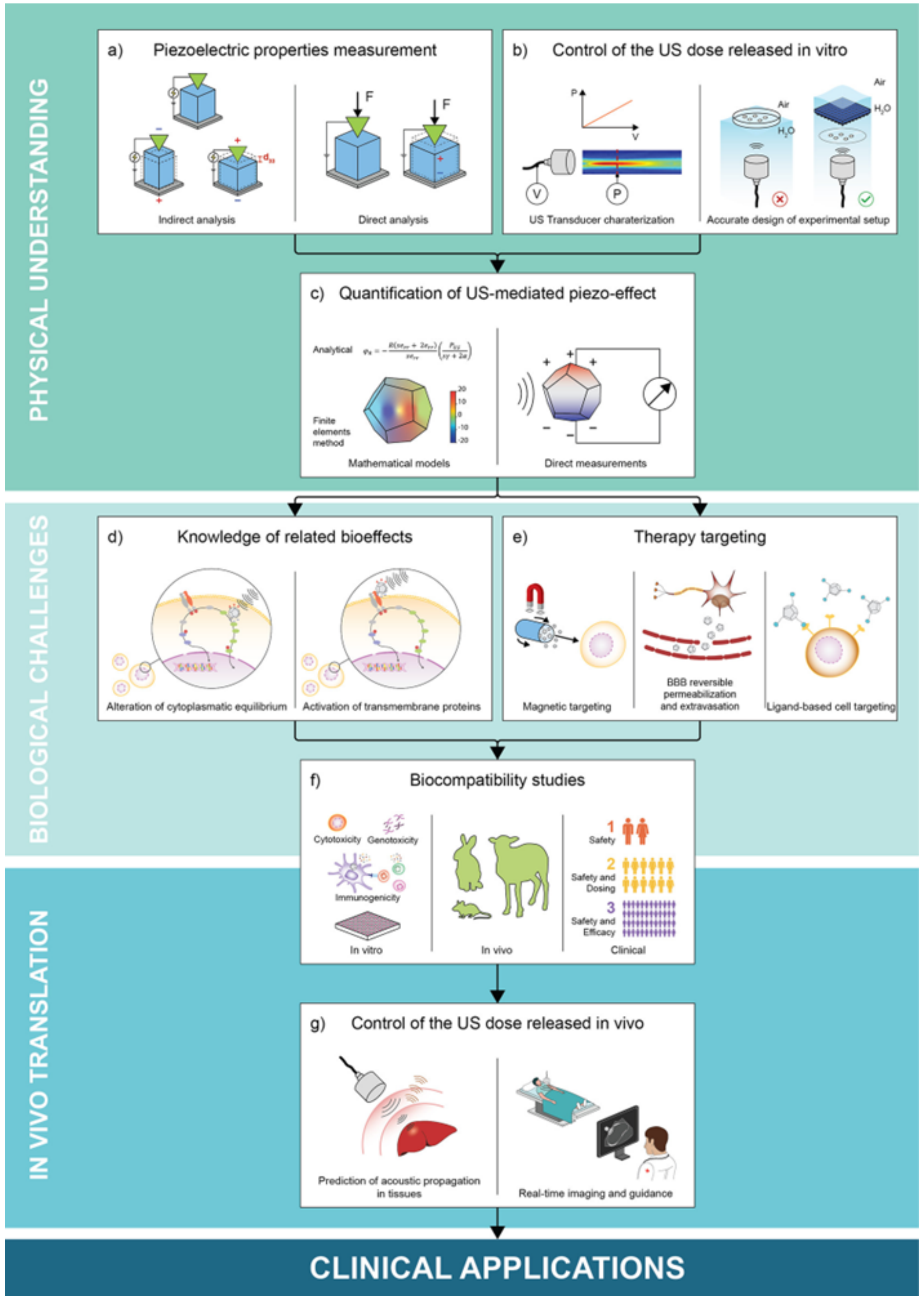
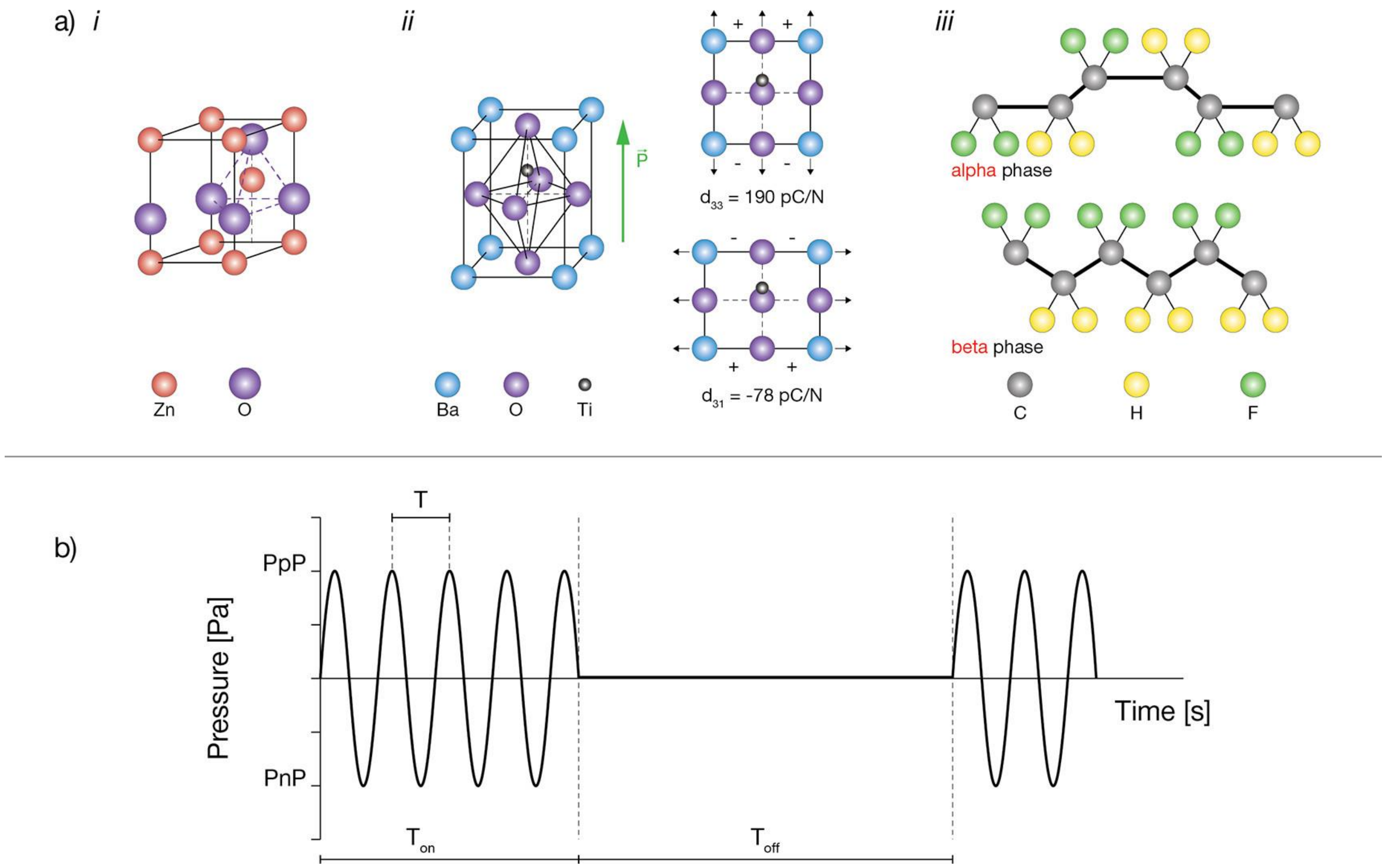

Piezoelectric Nanomaterials
Ultrasound
Electric effect
REGENERATIVE MEDICINE
NEUROMODULATION
CANCER TREATMENT
In vitro findings
In vivo studies
Clinical acceptance


Neuromodulation
Regenerative medicine
Cancer treatment
Cell excitation or inhibition
Excitation
Inhibition
Membrane depolarization or hyperpolarization
Extracellular
Intracellular
Neural cell
Stem cell
Activation of intracellular pathways
transcription factors
TFIIB
RNA polymerase
DNA
gene
transcription start site
Cell proliferation/differentiation
Neural cell
Bone cell
Muscle cell
Stem cell
Fibroblast
Cancer cell
Arrest of mitotic cycle
M
G1
G0
S
G2
Inhibition of cell proliferation


PHYSICAL UNDERSTANDING
a) Piezoelectric properties measurement
Indirect analysis
Direct analysis
b) Control of the US dose released in vitro
US Transducer charaterization
Accurate design of experimental setup
c) Quantification of US-mediated piezo-effect
Analytical
Finite elements method
Mathematical models
Direct measurements
BIOLOGICAL CHALLENGES
d) Knowledge of related bioeffects
Alteration of cytoplasmatic equilibrium
Activation of transmembrane proteins
e) Therapy targeting
Magnetic targeting
BBB reversible permeabilization and extravasation
Ligand-based cell targeting
f) Biocompatibility studies
Cytotoxicity
Genotoxicity
Immunogenicity
In vitro
In vivo
Safety
Safety and Dosing
Safety and Efficacy
Clinical
IN VIVO TRANSLATION
g) Control of the US dose released in vivo
Prediction of acoustic propagation in tissues
Real-time imaging and guidance
CLINICAL APPLICATIONS

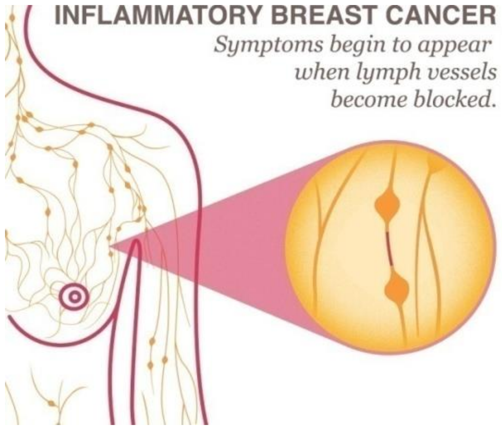


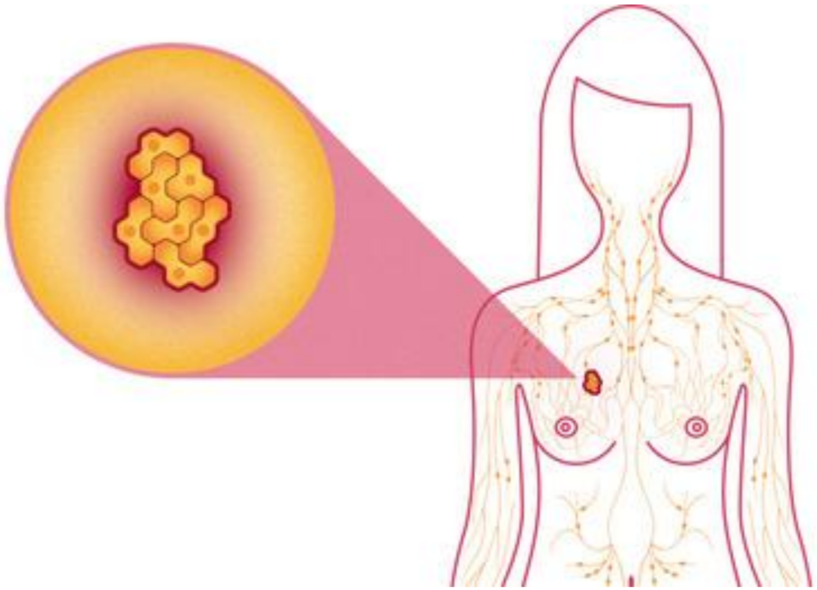
The piezoelectricity arises due to a relative displacement of ionic species, while a repositioning of molecular dipoles in organic materials occurs

Breast cancer

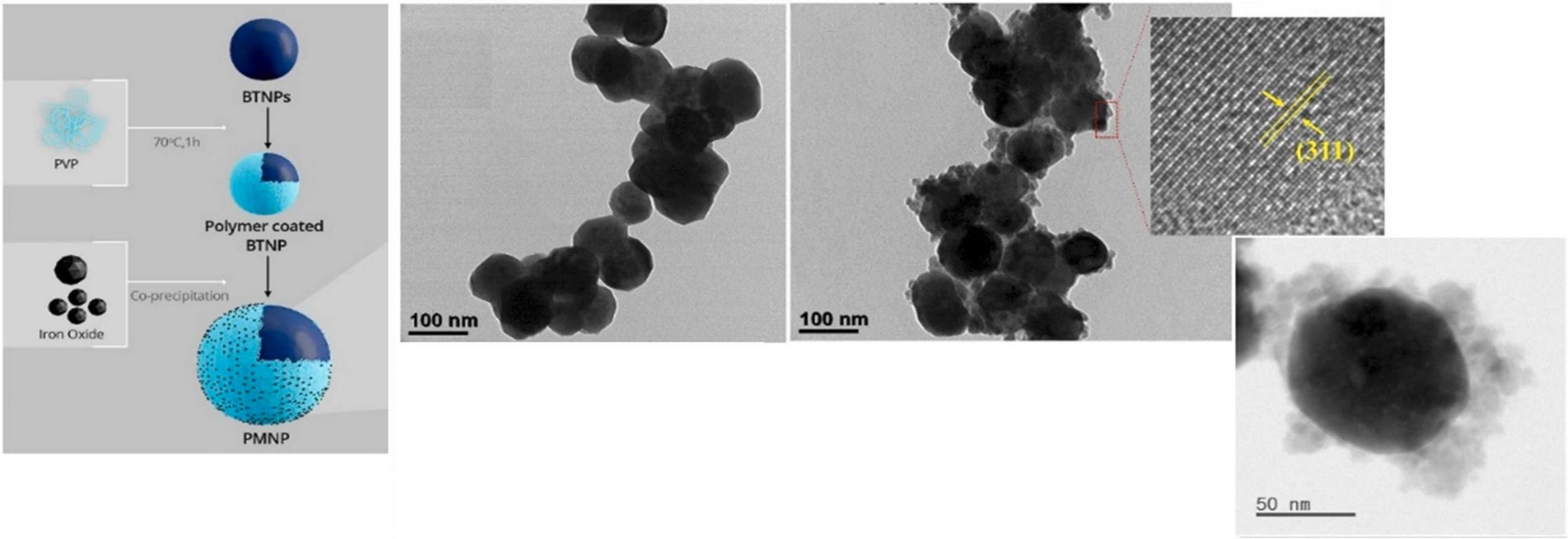
- Breast cancer is one of the most common invasive cancer in women worldwide. It affects about 12% of cancer cases in women worldwide.

- Triple-negative breast cancer (TNBC), as defined by the absence of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 expression, is a challenging disease with the poorest prognosis of all breast cancer subtypes.





Physicochemical characterization of NPs



PMNPs synthesis

- **PMNPs were synthesised by a two-step reaction. In the first step, synthesis of tetragonal BTNPs by a low temperature solvothermal method by using BaCO3 as barium precursor described elsewhere followed by a high temperature annealing process.**

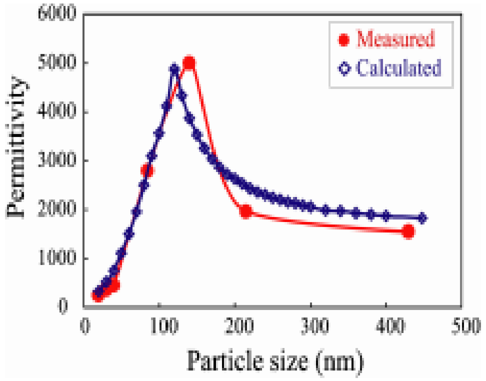
- **Piezoelectric materials are a subset of inorganic and organic dielectric compounds characterized by their ability to become electrically polarized when they are mechanically stimulated, and vice versa, they strain when they are subject to electric fields.**

- **Piezoelectric analysis of PMNPs: Frequency dependent hysteresis polarization (P)-electric filed (E) loops at room temperature,**

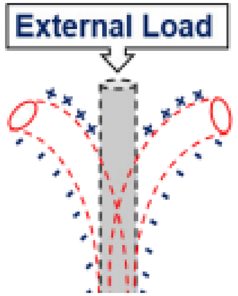
- **Switching polarity test of PMNPs device up on a shaft load (2 kg) periodically operated with the accelaration of 1 m/s^2 and the inset shows the device located perpendiculaly to the shaft load (2 kg) of a linear motor. The output voltage response of PMNPs device upon the shaft load operated with various accelations (0.1 and 1 m/s^2).**

Piezoelectric particles: candidates

	biocompatibility/ biodegradability	piezoelectric properties
$BaTiO_3$	*Non-degradable, but biocompatible [1]*	*The d33 coefficient is maximal for the tetragonal phase. Piezoelectric properties of $BaTiO_3$ particles depend on particles size and disappear < 20-30 nm.*
Zn nanowires	*Biodegradable [2]*	*Zinc oxide shows piezoelectricity in Wurtzite phase and is maximal for nanowires. Piezoelectrical properties depend mainly on the aspect ratio of nanowires.*
PNPs	*Relatively new material; not widely investigated yet, some work about biocompatibility of KNN [3]* *High piezoelectric coefficients*	*All crystallographic phases of KNN-LTS and KNN have similar piezoelectric properties and for particles > 20 nm only a weak size dependence.*



DOI: 10.1063/1.4990046



Doi: 10.1088/0022-3727/47/34/345102

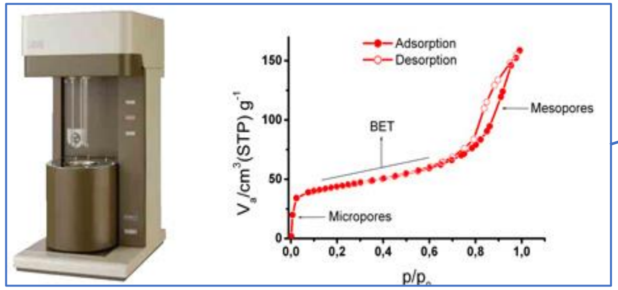
Target sizes for piezoelectric nanoparticles

- **Barium titanate particles:**
  particle size of 100 nm, 200 nm, 500 nm, 1 μm and 2 μm

- **Zinc oxide nanowires:**
  pillar size: 200 nm, 500 nm and 1 μm;
  aspect ratios: 5, 25 and 50

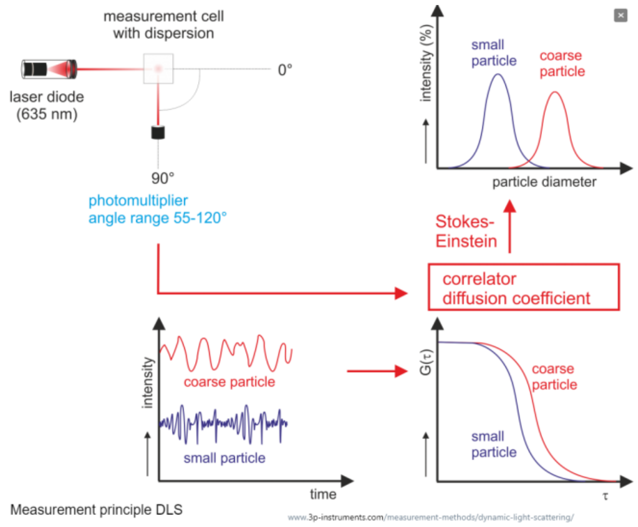
Piezoelectric particles: characterization

BET method (Brunauer–Emmett–Teller theory) → SSA / indirect APS



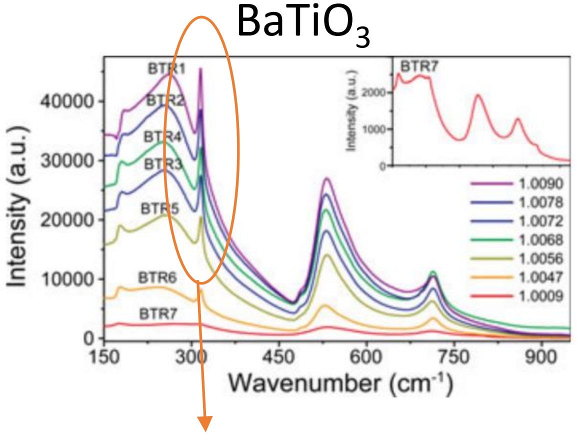
→ Specific surface area (SSA), m^2/g → $d=6000/(SSA*\rho)$ for spherical particles
$d=4000/(SSA*\rho)$ for nanowires,
where density $\rho = 6{,}02\ g/cm^3$ ($BaTiO_3$), $\rho = 5{,}61\ g/cm^3$ (ZnO) and $\rho = 4{,}3\ g/cm^3$ (KNN) → Average particle size (APS) according to BET

DLS (dynamic light scattering) method
→ hydrodynamic APS

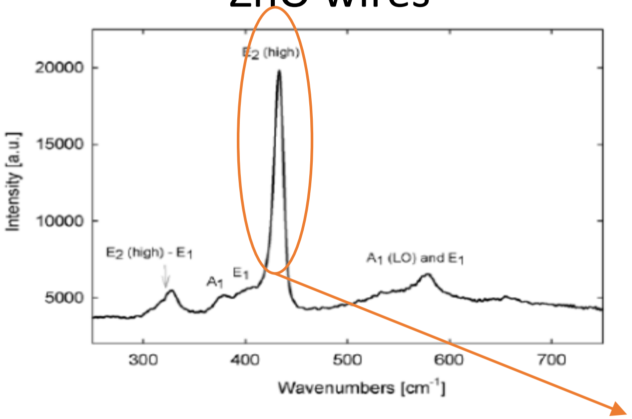


Raman Spectroscopy → crystal properties

$BaTiO_3$



ZnO wires

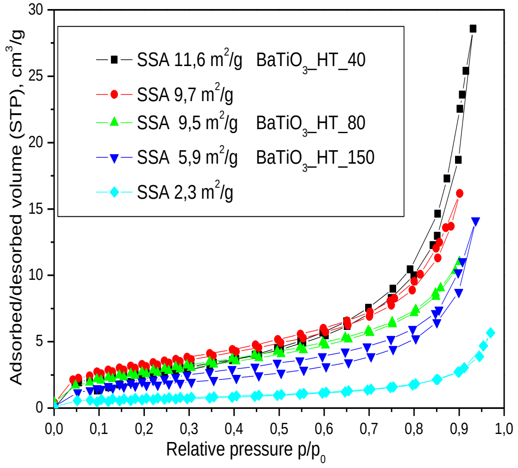


Peak at 305 cm^{-1} is good evidence of the $BaTiO_3$ tetragonality and peak at 430-433 cm^{-1} is an attribute of the ZnO nanowire's morphology

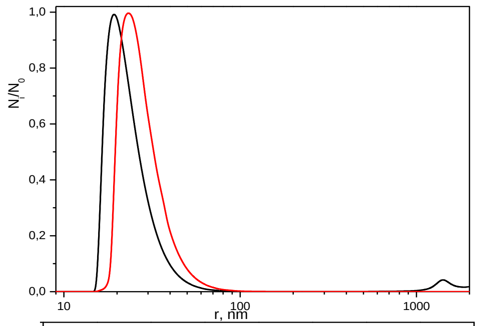
$BaTiO_3$ preparation: hydrothermal method

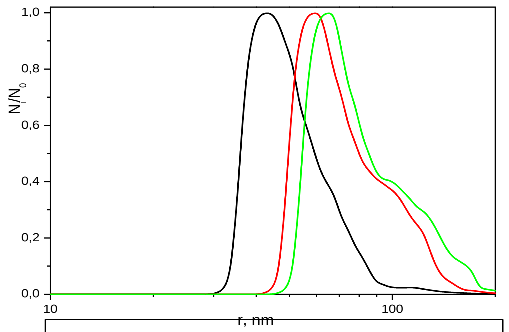
SSA:	11,6 m²/g	9,5 m²/g	5,9 m²/g
Size BET:	86 nm	105 nm	169 nm
Size DLS:	40-70 nm	80-110 nm	150-220 nm
Tetragonality:	+	+++	++

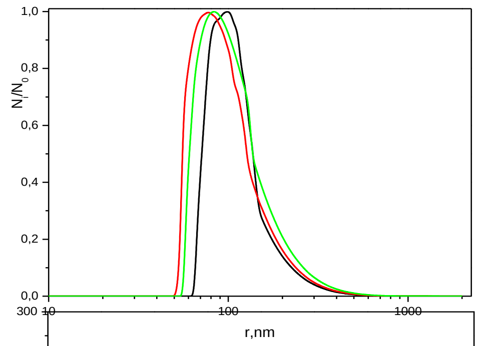
BET



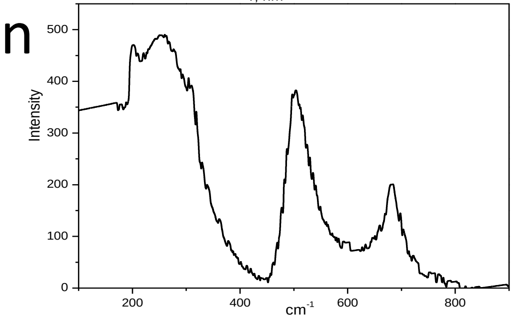
DLS

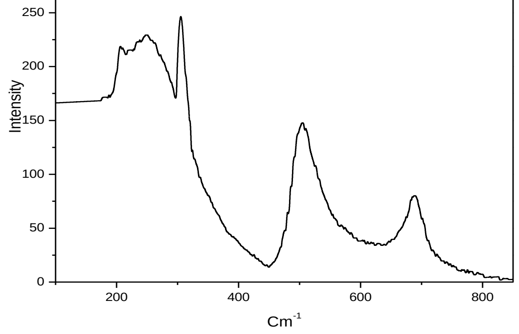


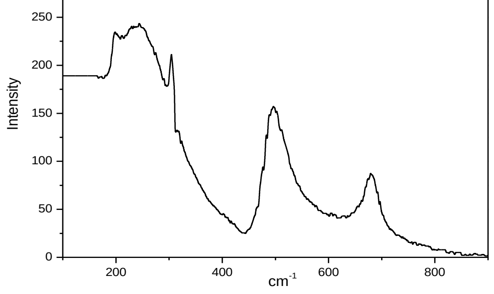




Raman





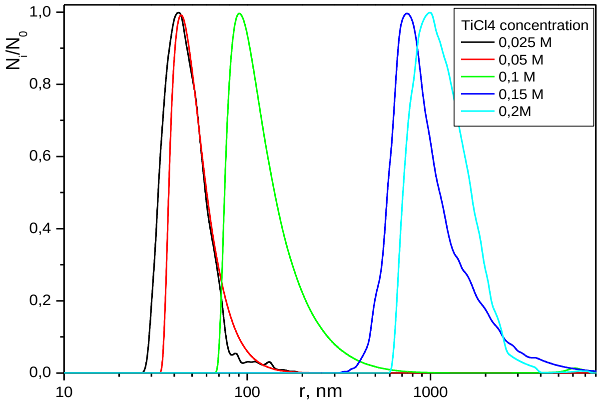


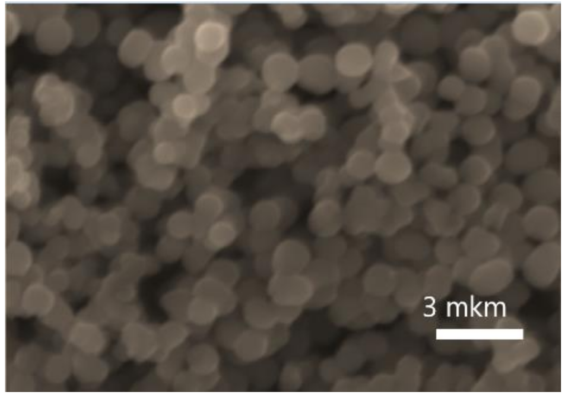
BaTiO3_HT-40 BaTiO3_HT-80 BaTiO3_HT-150

Converting spherical TiO_2 particles to $BaTiO_3$

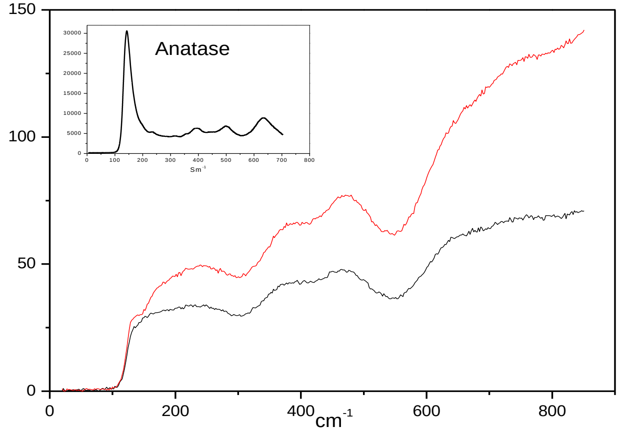
TiO_2 spherical particles → spherical **$BaTiO_3$** particles

DLS and SEM of the initial TiO_2





Raman spectra of initial TiO_2 converted to $BaTiO_3$



New name

BaTiO3_CONV_1000

SSA: --

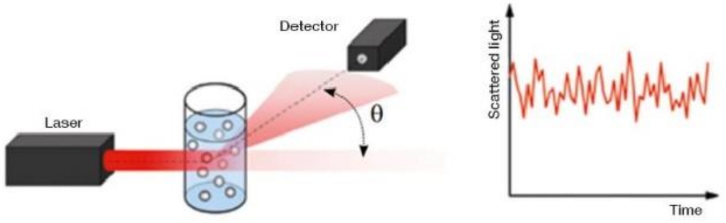
Size BET: --

Size DLS: 1-2 µm

Tetragonality: ???

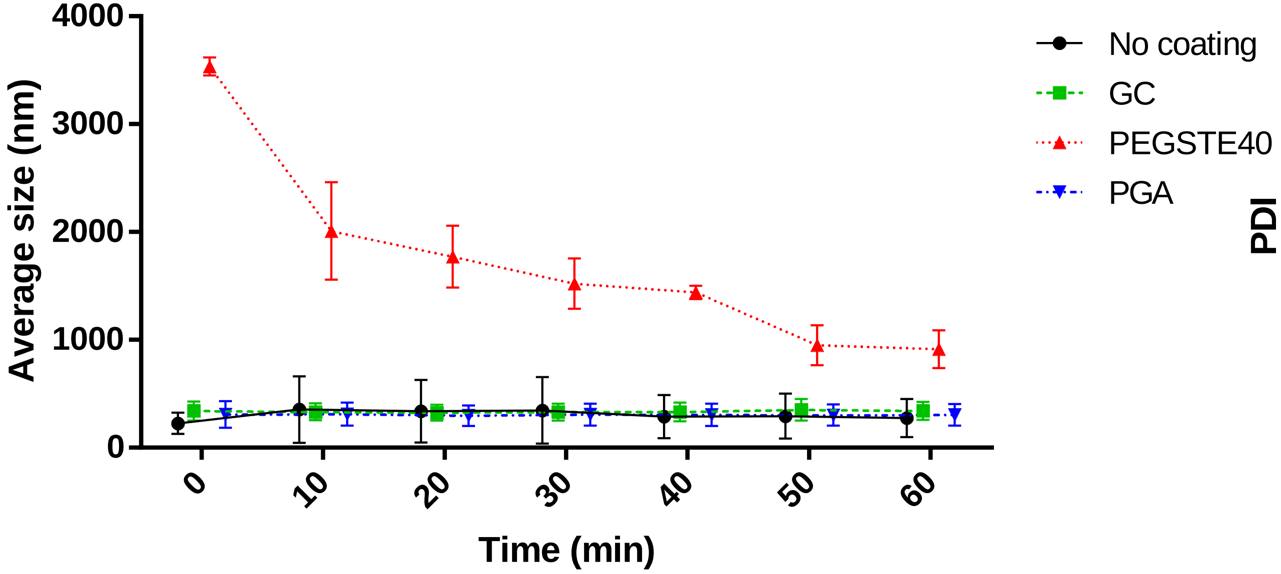
Both, initial and converted particles can be visualized by optical microscope.
The morphology of particles did not change, but there was no clear evidence of tetragonality.

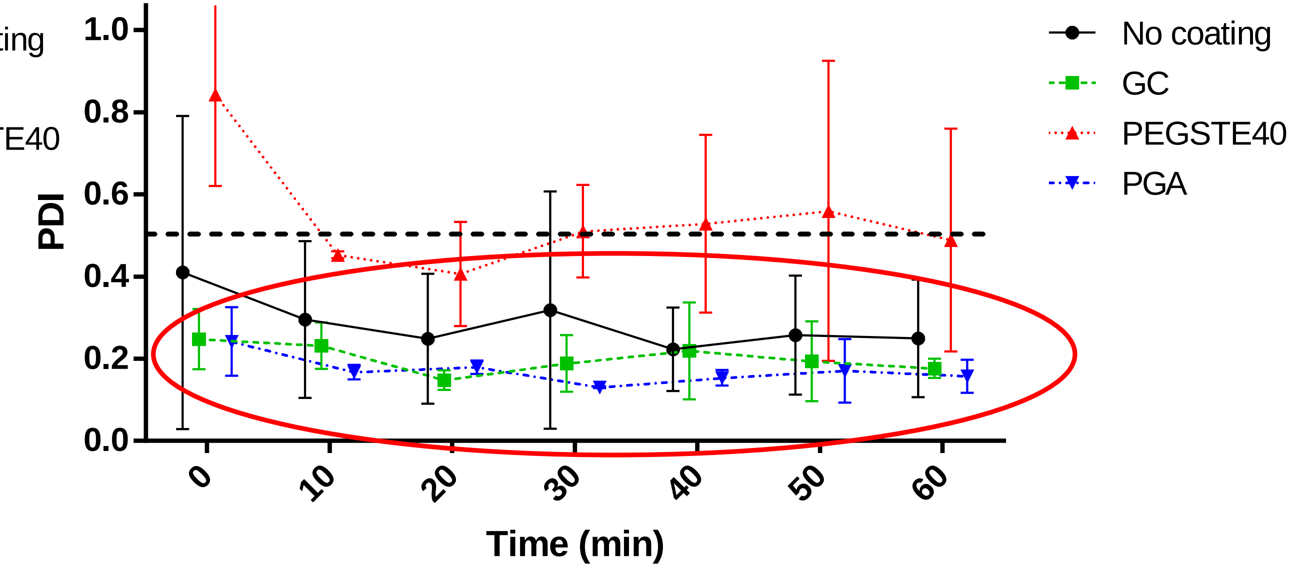
Analysis of nanomaterials stability with Direct Light Scattering



Confirmed hydrodynamic diameter

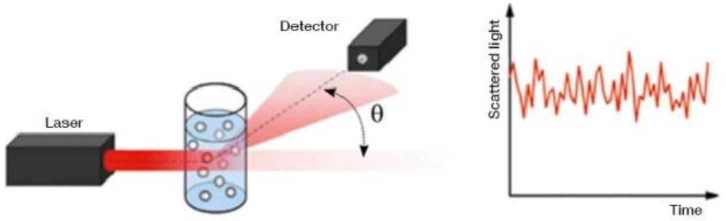
- *Average size stable over time*
- *Polydispersivity index (PDI) lower than 0.5 [1]*





Hansson, Annasara, et al. "Preparation and evaluation of nanoparticles for directed tissue engineering." International journal of pharmaceutics 439.1-2 (2012): 73-80

Analysis of nanomaterials stability with Direct Light Scattering

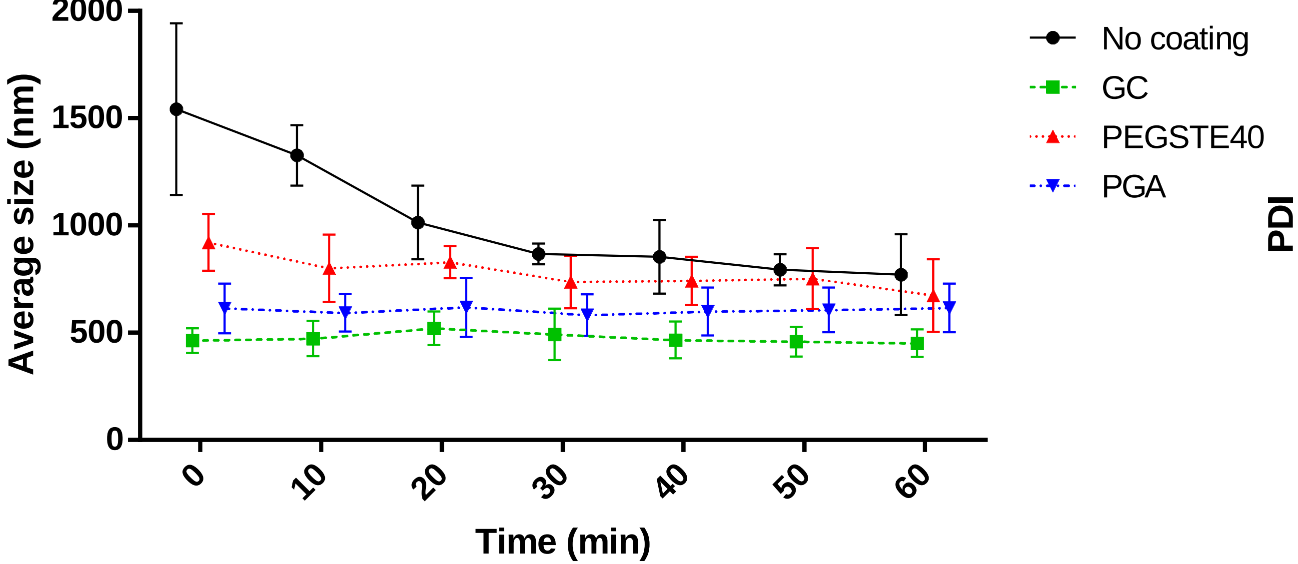


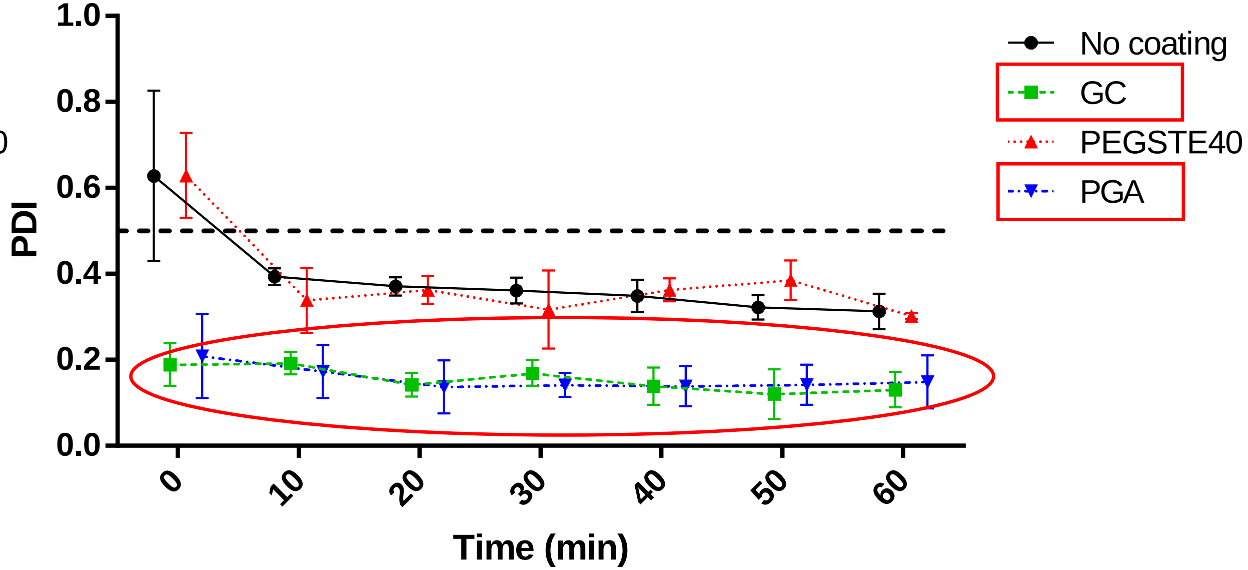
- *Average size stable over time*
- *Polydispersivity index (PDI) lower than 0.5 [1]*

BaTiO3

ICS > 19 > 19.120
ISO 22412:2017
Particle size analysis — Dynamic light scattering (DLS)

Confirmed hydrodynamic diameter





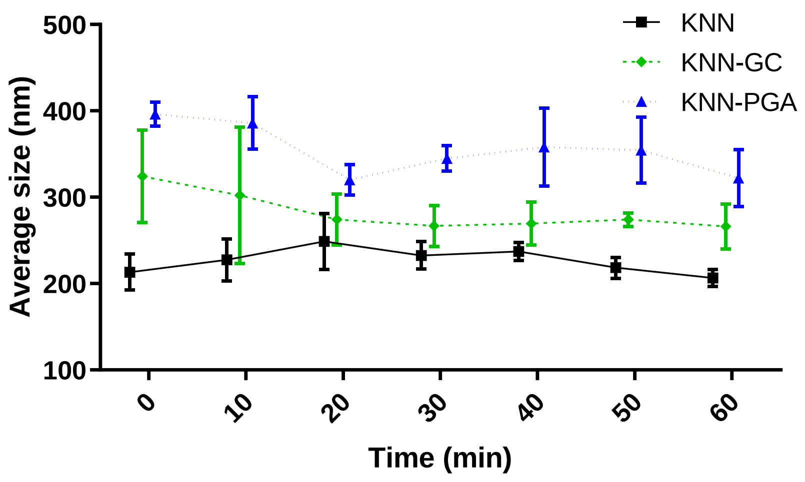
GC and PGA showed the best behavior in terms of nanoparticle dispersion stability

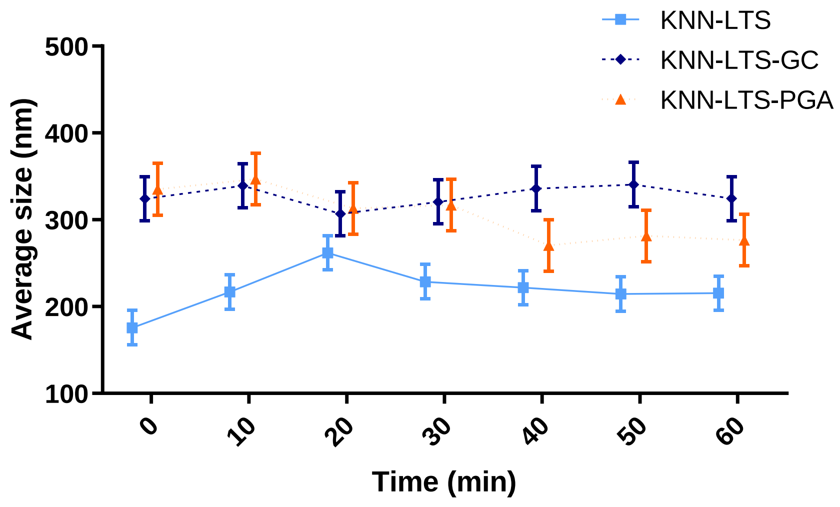
Colloidal stability of piezoelectric nanoparticles with Zeta Potential and DLS

The piezoelectric charge coefficient, d33, of dense KNN can reach a value of 80 pC/N. The piezoelectricity for KNN can be also enhanced as Li is added on it.

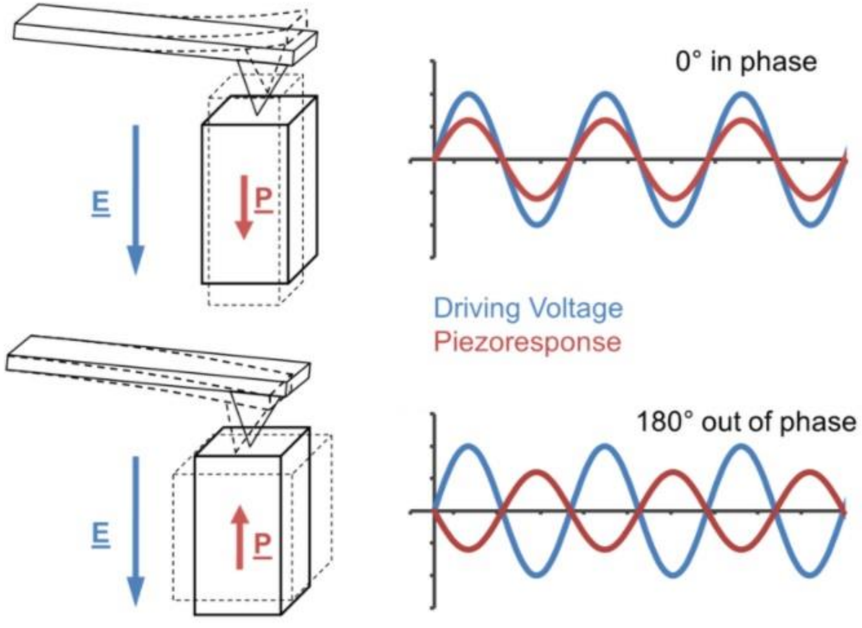
Zeta potential analysis of KNN and KNN-LTS, with and without surfactants

	No coating	GC	PGA
KNN	-48.53 ± 2.51	-1.84 ± 0.17	-12.8 ± 0.3
KNN-LTS	-42.03 ± 3.40	-4.82 ± 0.23	-17.4 ± 0.78

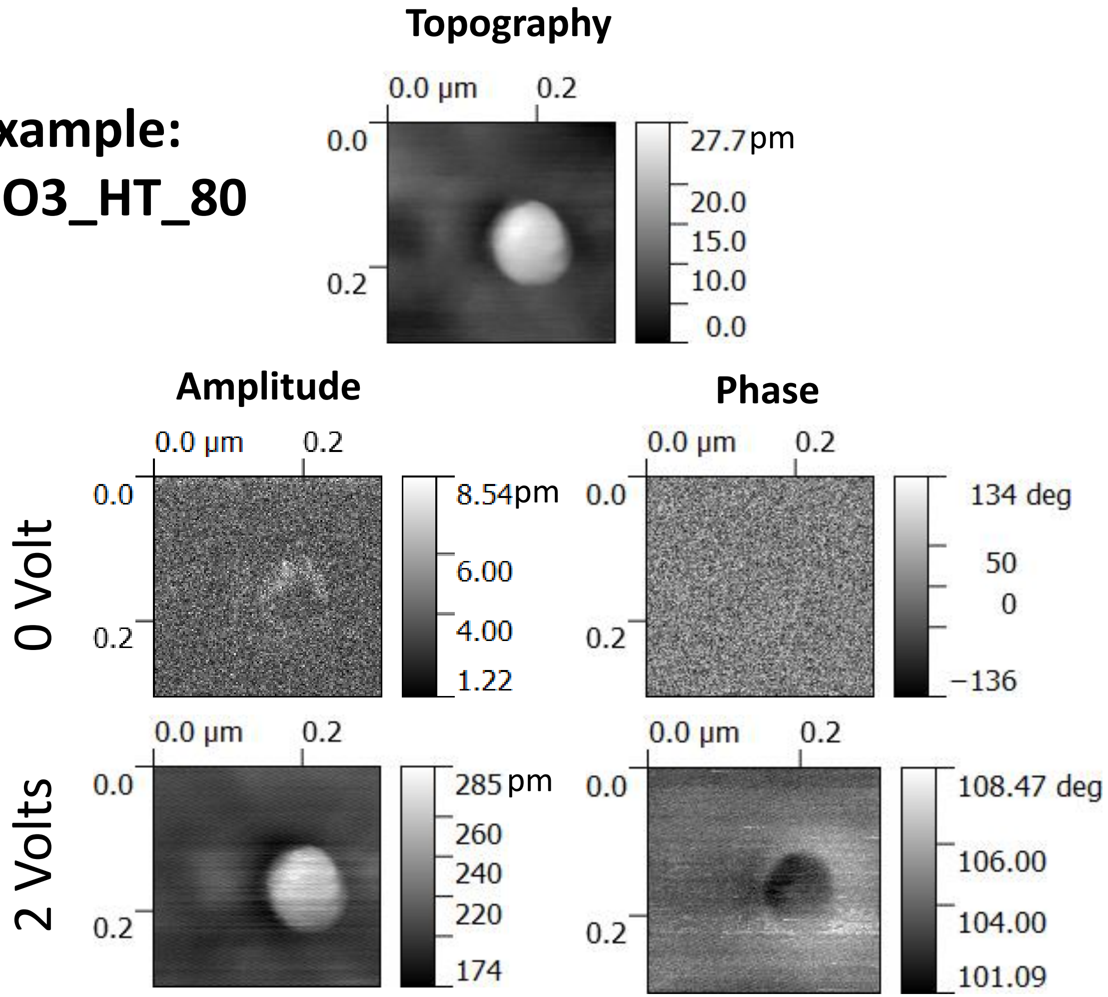




Piezoelectric analysis through Piezoforce response microscopy (PFM)



Example:
BaTiO3_HT_80



- High resolution at the nanometer scale
- Simultaneous acquisition of topography and piezoresponse
- Non-destructive
- Limited force

NEST
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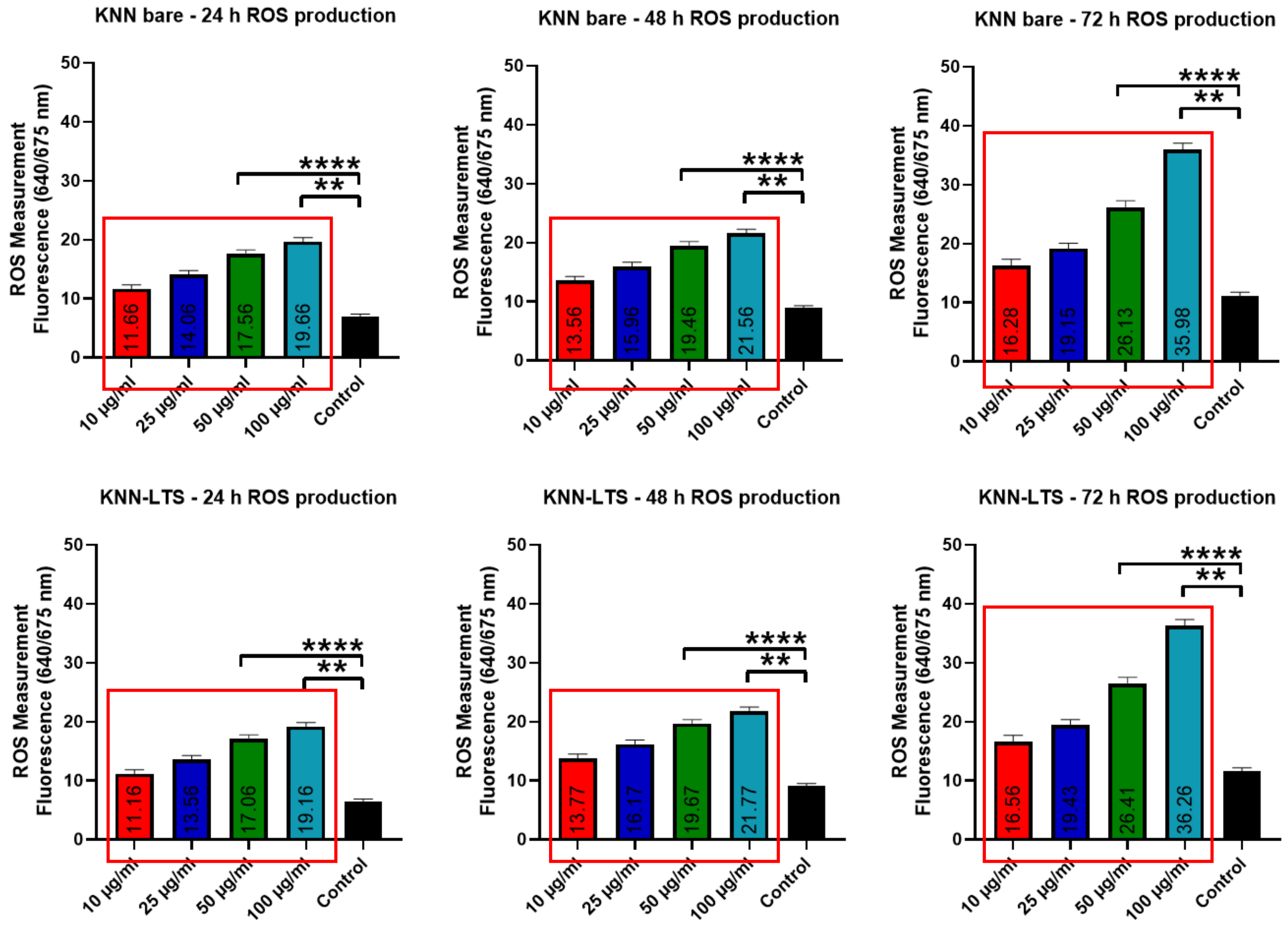
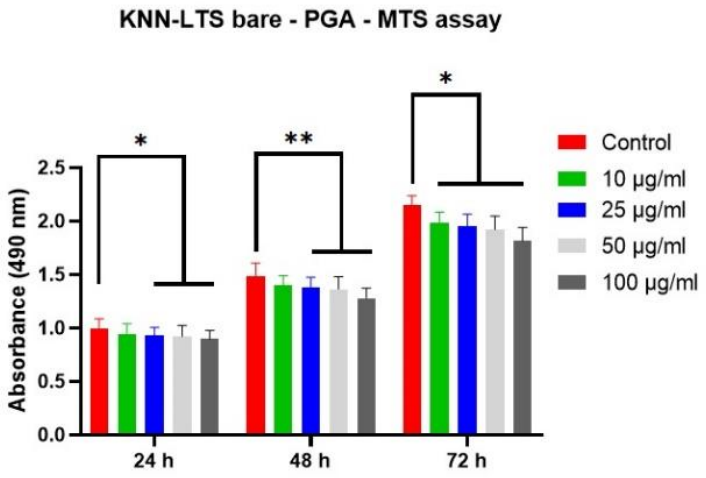
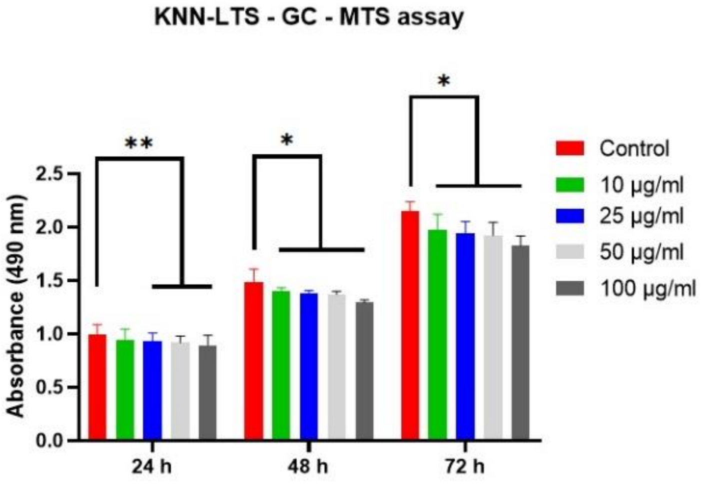
ROS production

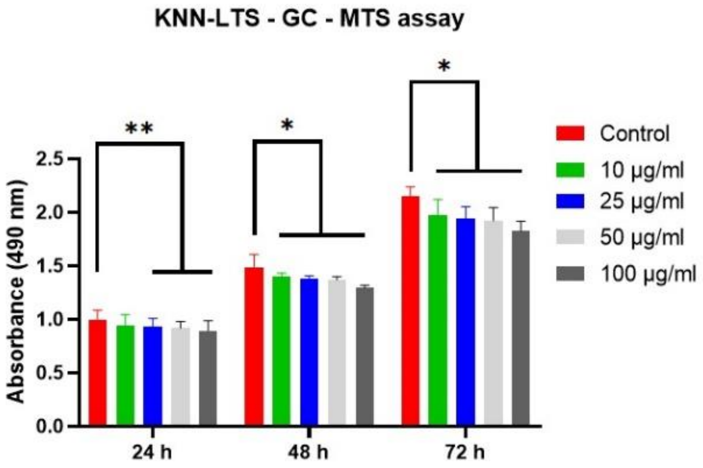


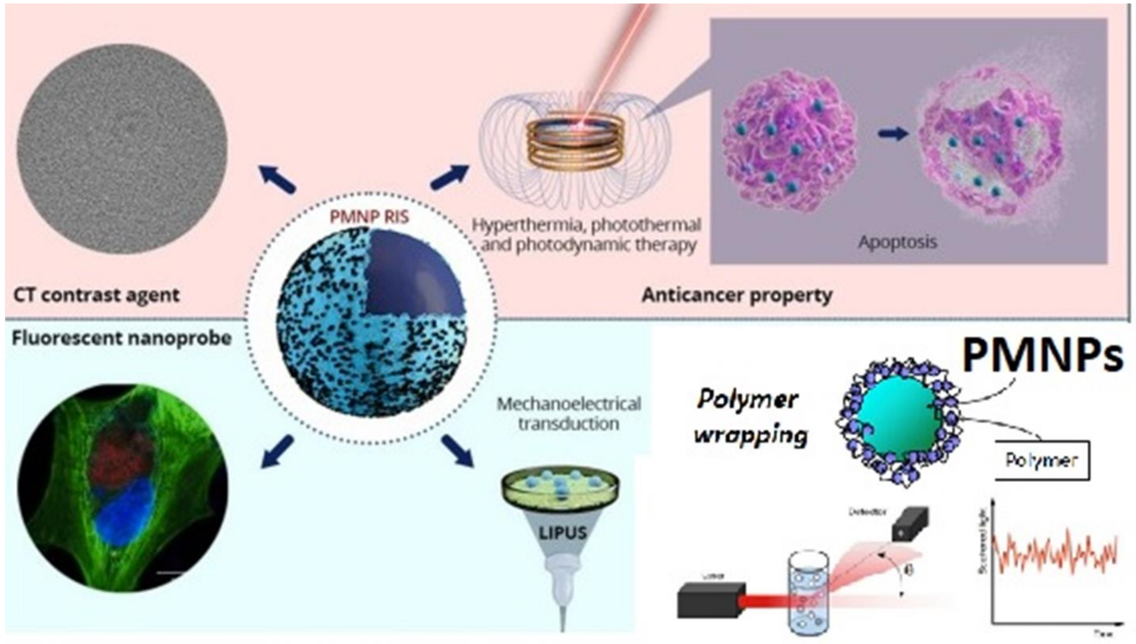
Cytocompatibility evaluation of KNN-LTS nanoparticles combined with GC and PGA with MTS assay

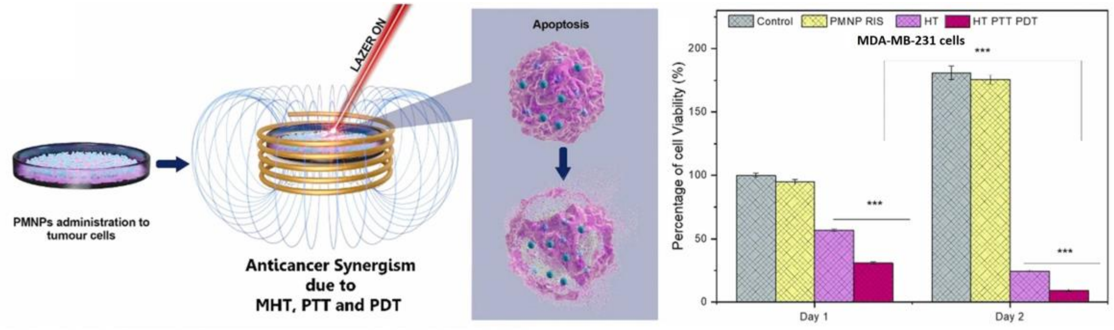
- ✓ **Both KNN-LTS with GC and PGA showed cytocompatibility compared to control on MCF-10 cells**



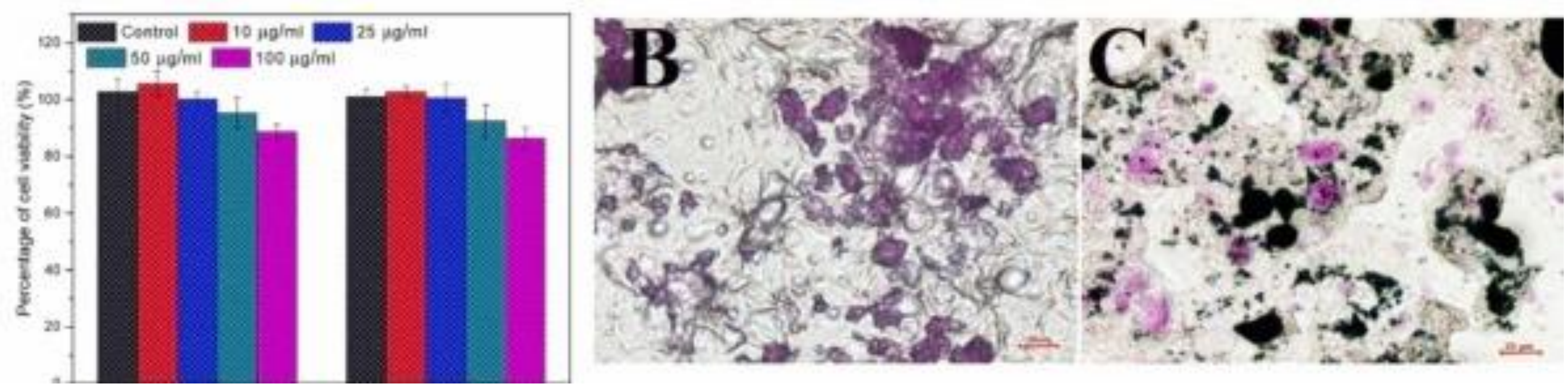





CT contrast agent
PMNP RIS
Hyperthermia, photothermal
and photodynamic therapy
Apoptosis
Anticancer property
Fluorescent nanoprobe
Mechanoelectrical
transduction
LIPUS
PMNPs
Polymer
wrapping
Polymer

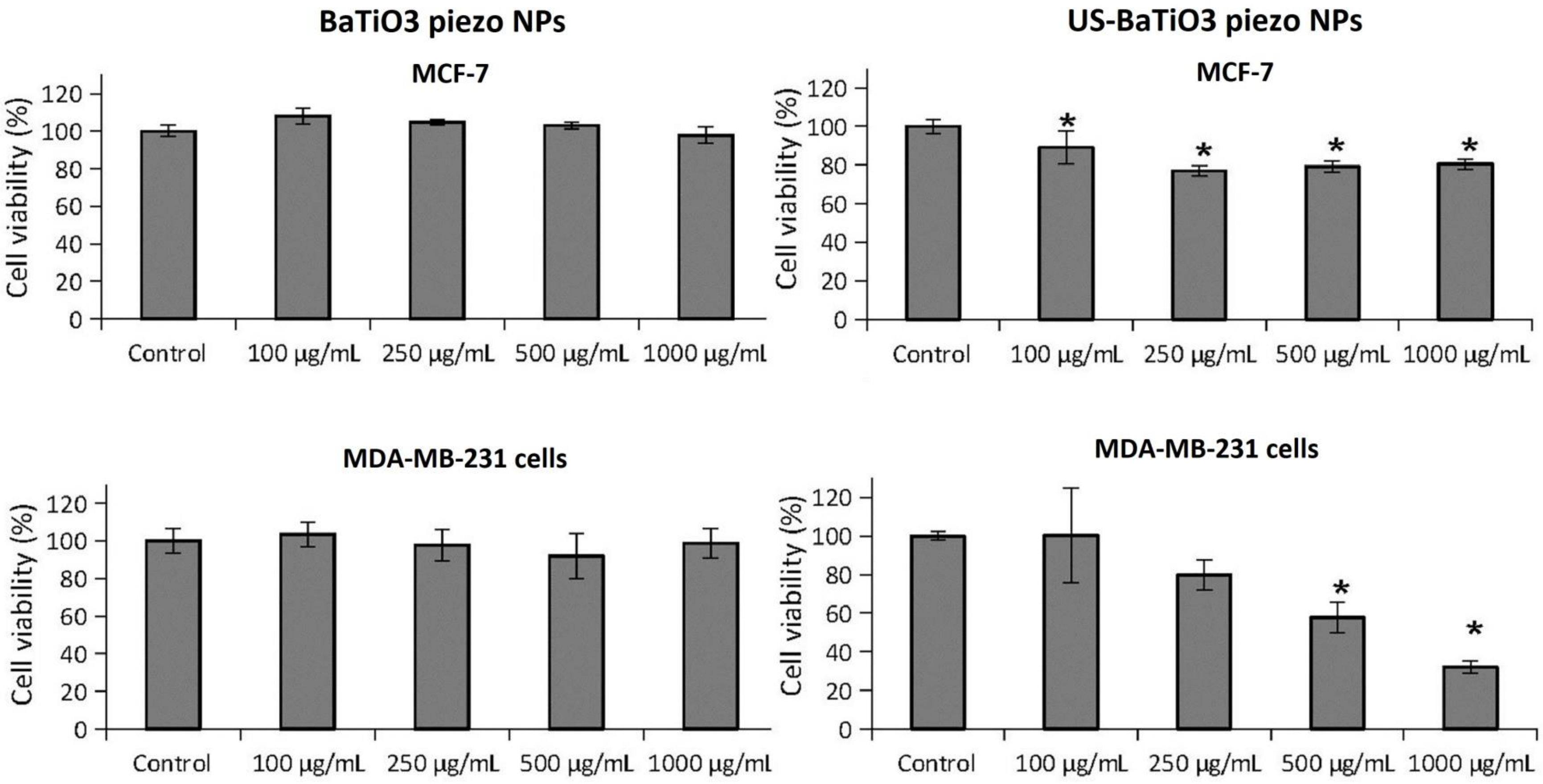

PMNPs administration to
tumour cells
LAZER ON
Anticancer Synergism
due to
MHT, PTT and PDT
Apoptosis
Control
PMNP RIS
HT
HT PTT PDT
MDA-MB-231 cells
Percentage of cell Viability (%)
200
150
100
50
0
***
***
***
Day 1
Day 2

Anticancer effect of PMNPs

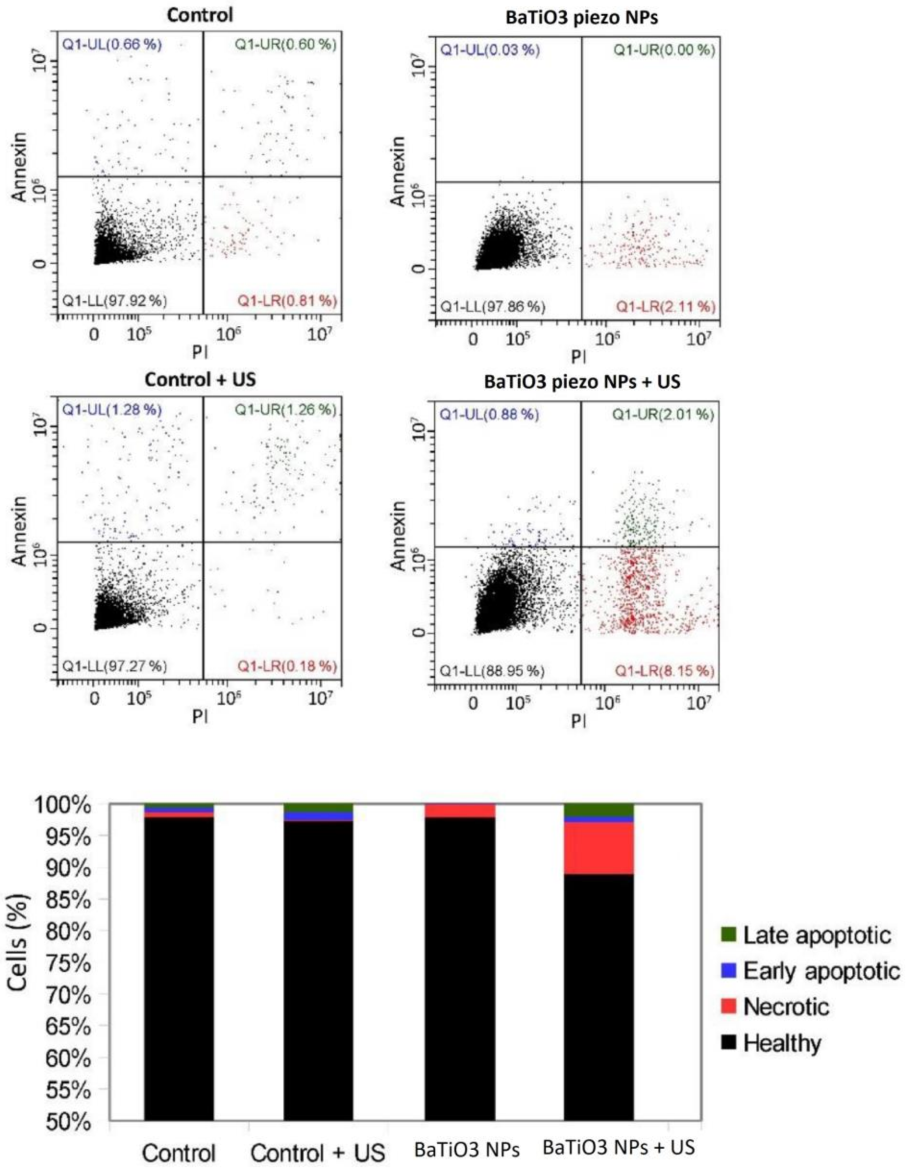


The anticancer efficacy of PMNPs was studied on human MDA-MB-231 cancer cell lines. The cells were first incubated for 3 h with PMNPs (50 µg/ml) to taken up by the cells and the anticancer effects were studied under magnetic hyperthermia and magnetophotothermia.

Piezoelectric nanoparticles effects on breast cancer cells



Apoptosis and necrosis analysis by flow cytometry in MDA-MB-231 breast cancer cells



US-activated piezoelectric nanoparticle stimulation” paradigm would constitute a promising tool to solve such an undesired effect

A mild electrical stimulation is effective in inhibiting cancer cell proliferation, as an alternative to other more traditional approaches.

Low-intensity electrical cues are indeed able not only to affect cancer cell proliferation without the use of any drugs/chemicals but also to reduce multidrug resistance phenomena. Furthermore, cells originated from abnormal mitosis events due to the chronic electric stimulations result unable to proliferate.

So far the anticancer effects have been proven on osteosarcoma cells, colorectal cancer cells, breast cancer cells, and glioblastoma multiforme cells

US-BTNPs combination can generate toxic reactive oxygen species (ROS), thus down-regulating the Ki-67 proliferative marker and showing a piezo catalytic tumor eradication potential

Results

- Recent findings reported the successful remote stimulation of different cancer cell types by the synergic exploitation of inorganic piezoelectric nanoparticles combined with US.

- In vitro studies with BTNPs showed that chronic piezoelectric stimulation arrests cancer cell cycle in G_0/G_1 phases by interfering with Ca^{2+} homeostasis and up-regulating the expression of the gene encoding for Kir3.2 inward rectifier K^+ channels, and by affecting the organization of mitotic spindles during mitosis.

- Moreover, the organization of cytoskeletal elements mediating cell mitosis is affected. Anticancer effects have been proven both on ER+/PR+ and triple-negative in vitro model of breast cancer cells

Acknowledgements

Thanks for your kind attention