Elucidating modes of interaction of redox-active nanomaterials with biological systems exposed to microgravity

Final presentation

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Smart Bio-Interfaces

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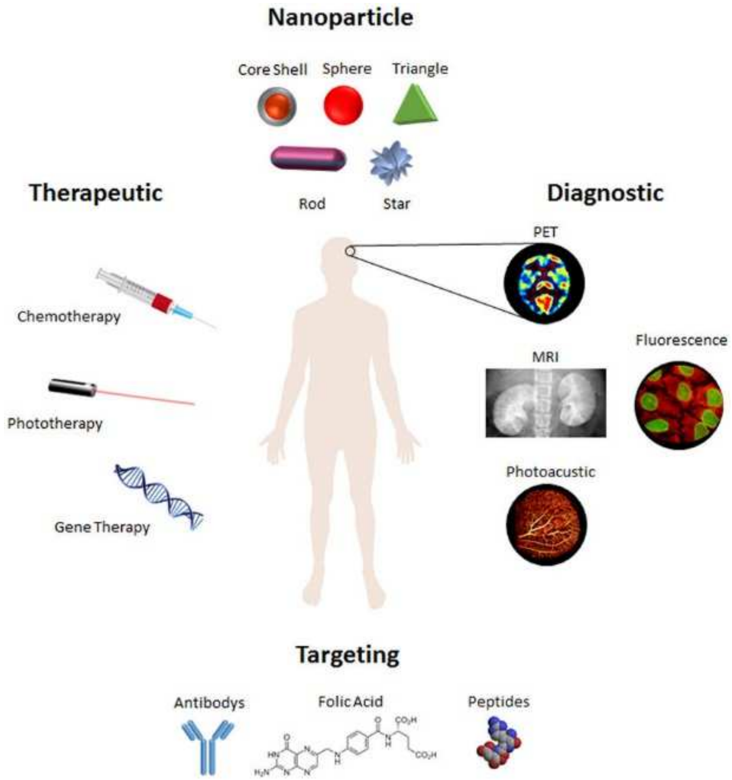
Background

Permanence in microgravity conditions such those on low-Earth orbit has detrimental effects on biological systems and living organisms, limiting human space operations and exploration, both over short and long term periods, as **it promotes/accelerates some degenerative processes associated to aging and often to pathology onset on Earth**.

→ *Exposure to real and simulated microgravity provides a useful means to identifying therapeutic approaches useful on Earth and in space*

Nanomaterials are proposed for many different biomedical applications on Earth, ranging from diagnosis to therapeutics, with different degree of success depending on many variables including **material chemistry, administration routes, modes of interaction at tissue/cell level and clearance**.



Pedrosa P. et al. 2015 doi: 10.3390/nano5041853.

Research questions

Crucial questions that this project aims at addressing are:

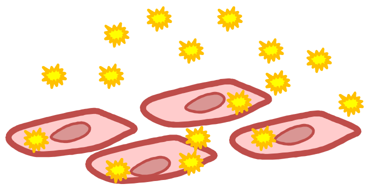
1. Are nanomaterials internalized by cells when delivered under mechanical unloading conditions? If yes, to what extent and by which route?

2. In case of modest internalization, can nanomaterials have biological effects by acting only in the extracellular environment?

3. Are antioxidant nanomaterials effective at decreasing microgravity-associated oxidative stress?

4. What is the time scale of action of antioxidant nanomaterials under mechanical unloading?

Purpose: Identification of the main intracellular signaling cascade triggered by the nanoparticle themselves and by the redox milieu.



Project activities

Tasks:

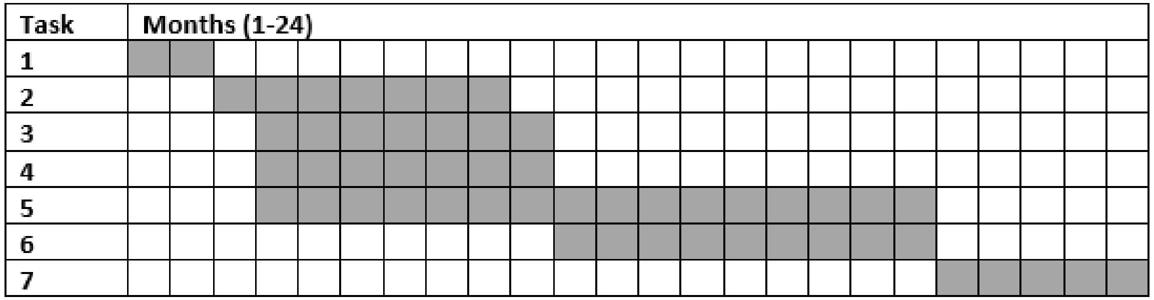
- T1: Bibliographic research and cell culture set up development
- T2: Performance of altered gravity experiments with random positioning machine

→ 1st achievement: achievement of a full panel of cultures at different proliferation and differentiation stages exposed to both nanoparticles and microgravity

- T3: Imaging by transmission electron and confocal microscopy
- T4: Performance of inductively coupled plasma mass spectrometry
- T5: Validation of putative signaling pathways
- T6: Data analysis

→ 2nd achievement: identification of nanoparticle entry routes and intracellular transduction pathways

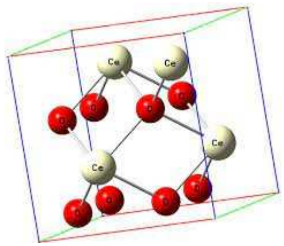
- T7: Result publication and dissemination



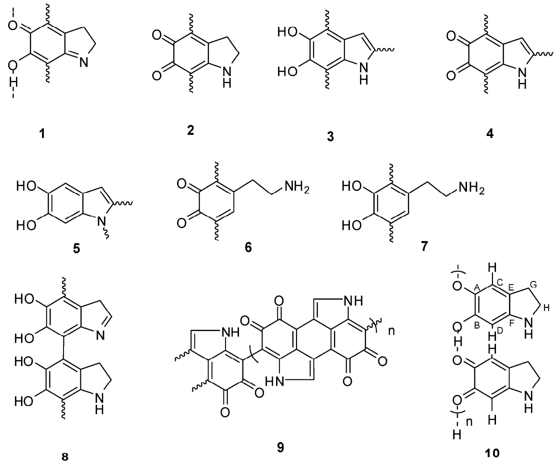
Project challenges

- Identification of the most suitable cellular target of experiments under simulated microgravity (animal *vs*. human model).

Inorganic: CeO_2

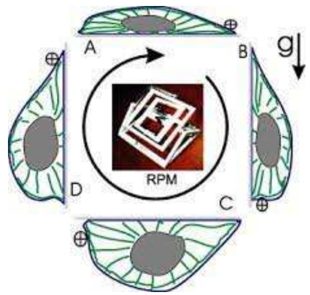


Organic: polydopamine



- Identification of the most suitable 3D rotation conditions for microgravity simulation and promotion of nanoparticle internalization.

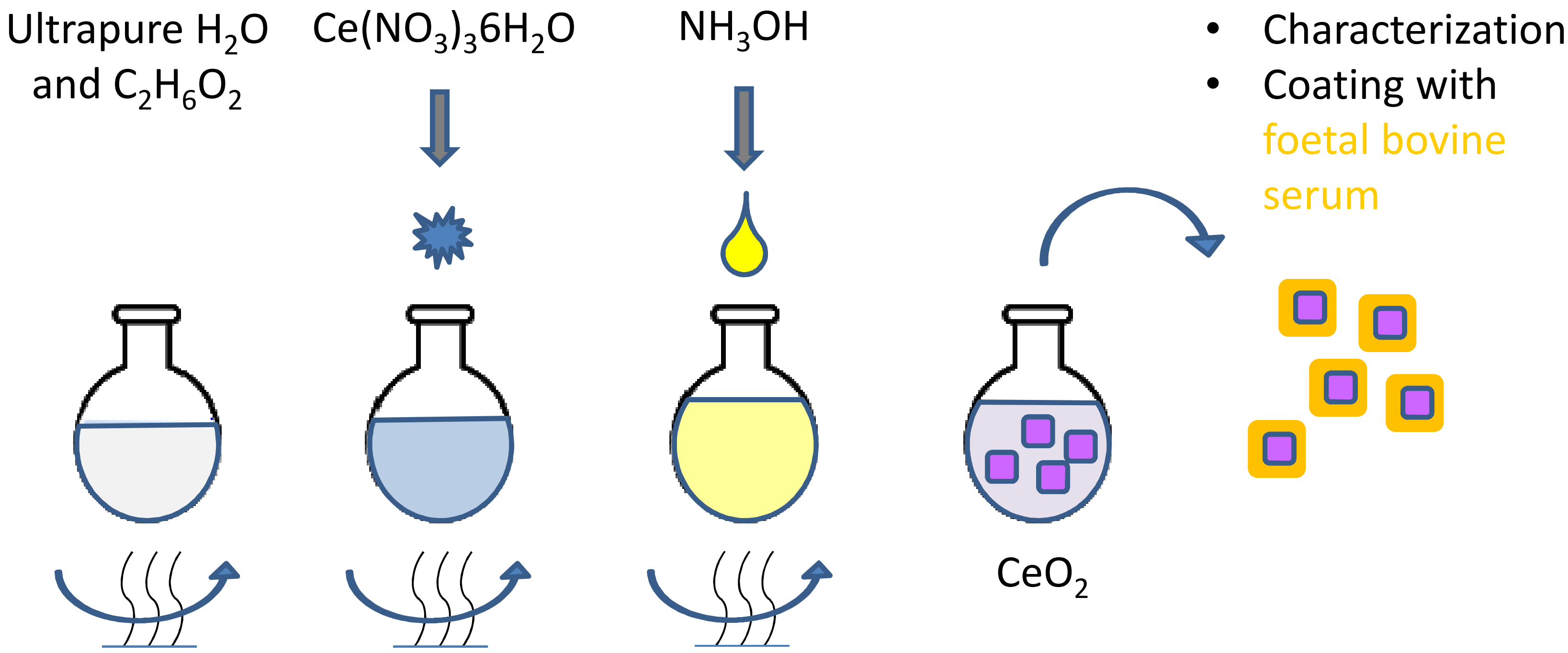
Focus on CeO_2 nanoparticles-NC and human myoblasts



Van Loon J.J.W.A. "Some history and use of the random positioning machine, RPM, in gravity related research" Advances in Space Research 39(7) 1161-1165, 2007 doi: 10.1016/j.asr.2007.02.016

Cerium oxide nanoparticle synthesis

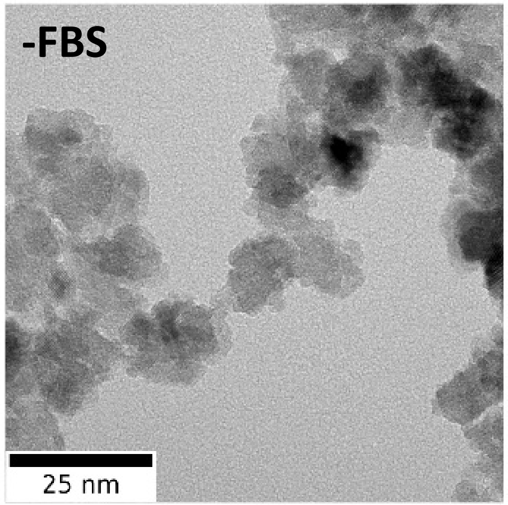
NC were synthetized by ethylene glycol-assisted direct precipitation. Briefly, $Ce(NO_3)_3 \times 6H_2O$ salt (5.16 gr) was dissolved in an 8% (v/v) ethylene glycol solution in water (100 ml). The solution was heated at 70°C, and then a 28%-30% NH_3OH solution in water was added dropwise under mild stirring until pH became 9.2. After 1 h of incubation, nanoparticles were collected by several cycles of centrifugation (at 8,000 g for 20 min) and resuspension in water.

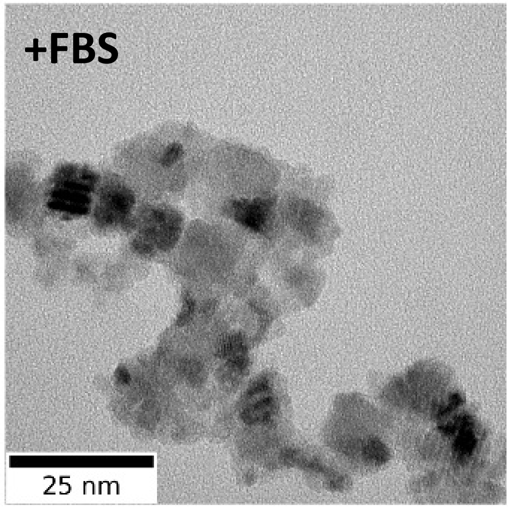


Cerium oxide nanoparticle characterization: TEM and Raman

a

8 nm size

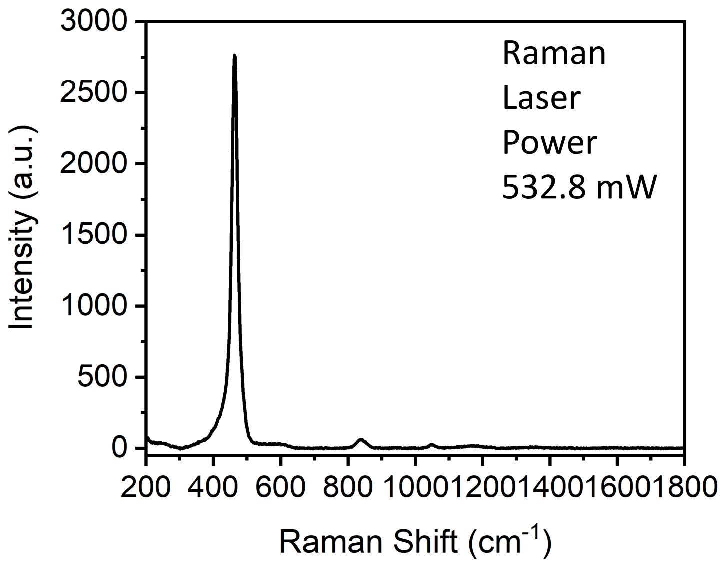




b

Thin conformal layer of FBS coating NC

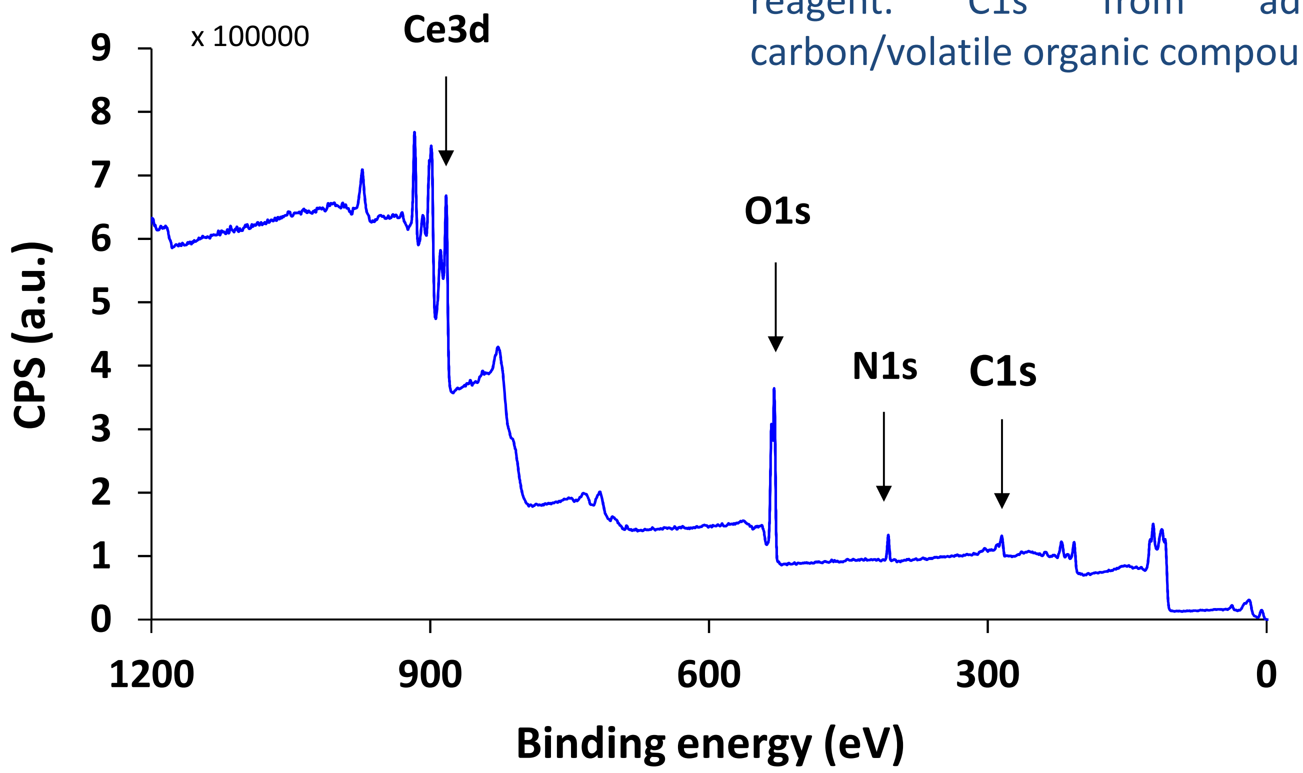
c



Single characteristic NC peak in Raman spectrum

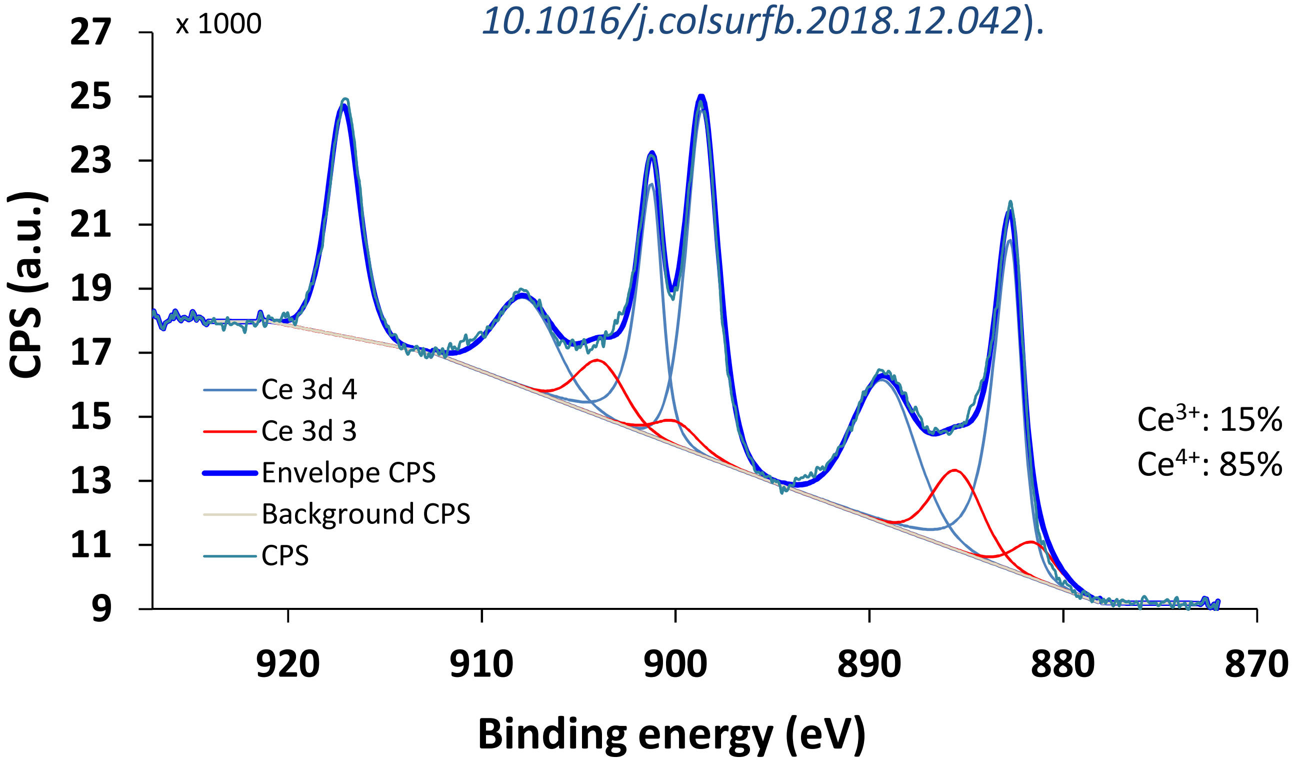
Cerium oxide nanoparticle characterization: XPS, wide spectrum

Expected elemental composition due to Ce3d and O1s. N1s peak from unreacted reagent. C1s from adventitious carbon/volatile organic compounds.

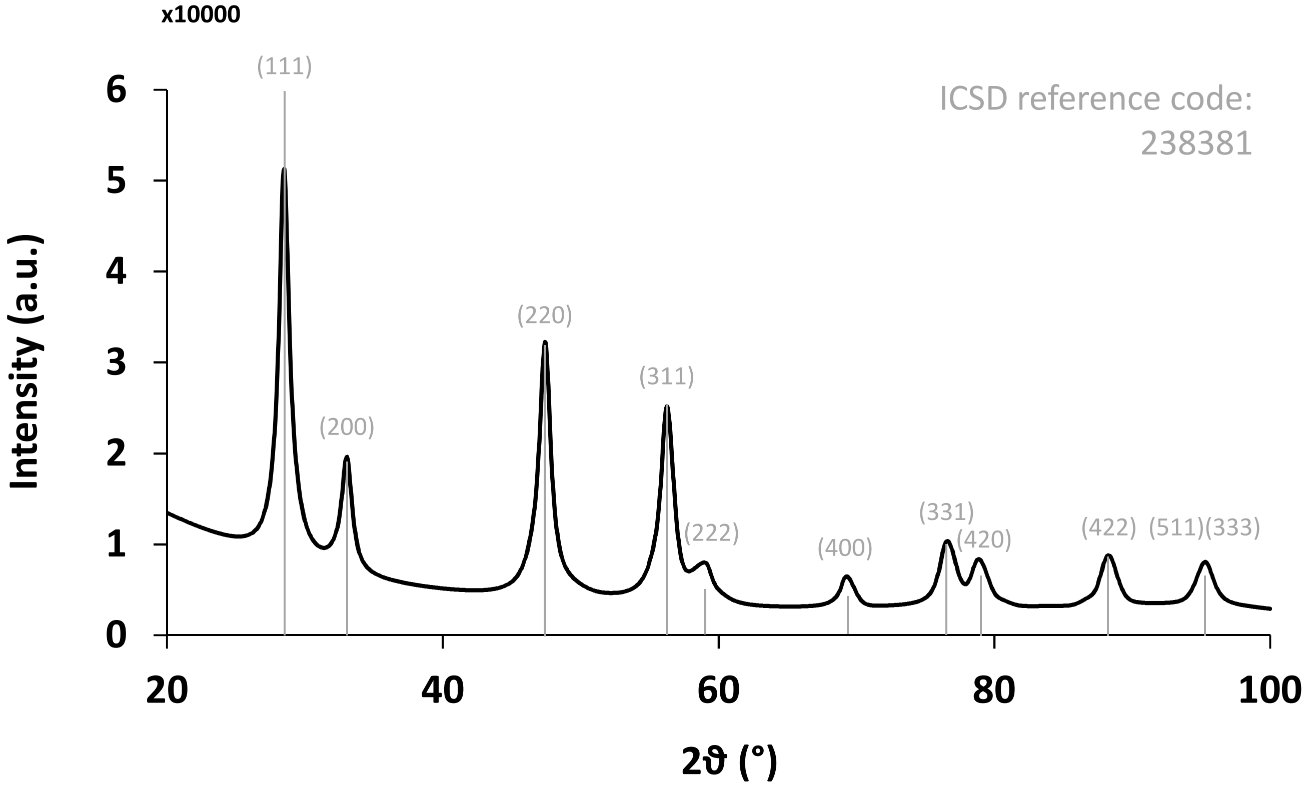


Cerium oxide nanoparticle characterization: XPS, narrow spectrum

Optimal ratio for antioxidant activity (*Celardo I. et al. 2011 doi: 10.1021/nn200126a*) and Ce^{4+} % ascribable to catalase-like activity (*Singh R. et al. 2019 doi: 10.1016/j.colsurfb.2018.12.042*).



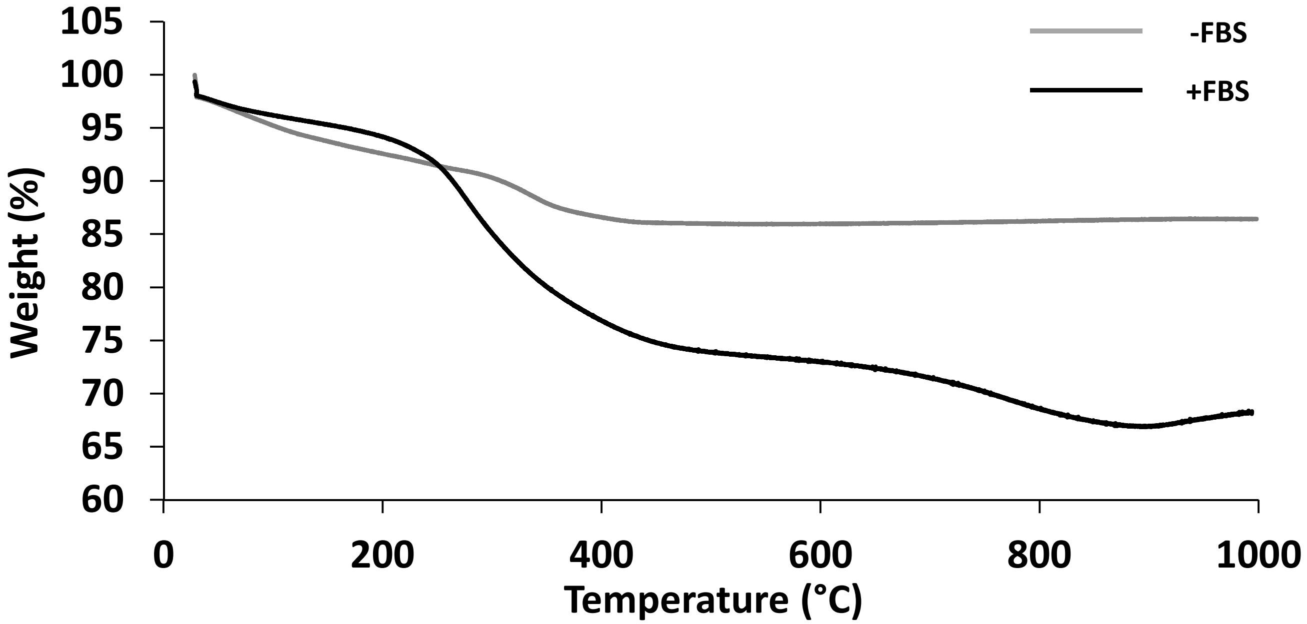
Cerium oxide nanoparticle characterization: XRD



- Single cubic phase, fluorite structure Fm-3m (225) typical of NC
- Large strain

XRD	Cristallite size [nm]	Lattice strain [%]
IIT NC	8.6	0.2610

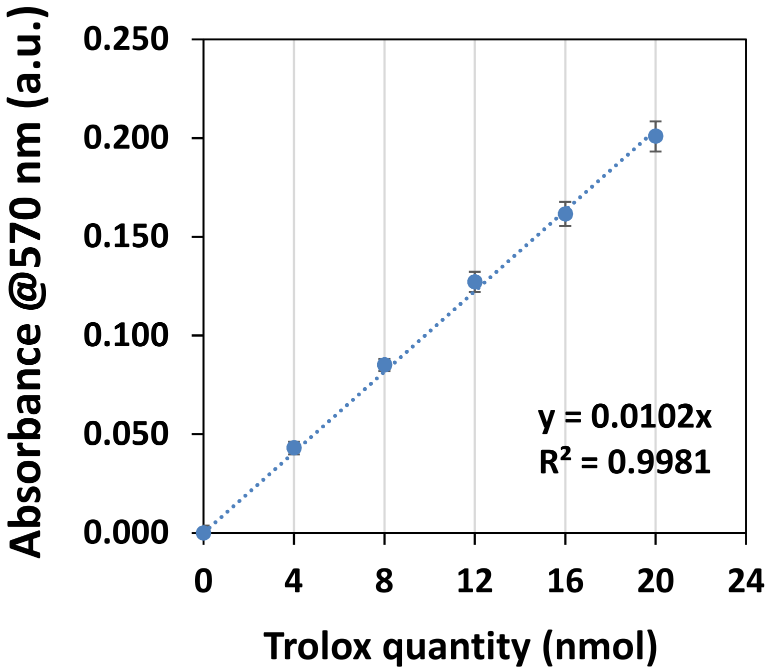
Cerium oxide nanoparticle characterization: TGA



TGA	Weight loss	%	T (°C)
-FBS	I	4.1	335
	II	1.1	412
+FBS	I	23.5	280
	II	7.5	796

~5-fold weight gain upon FBS coating.

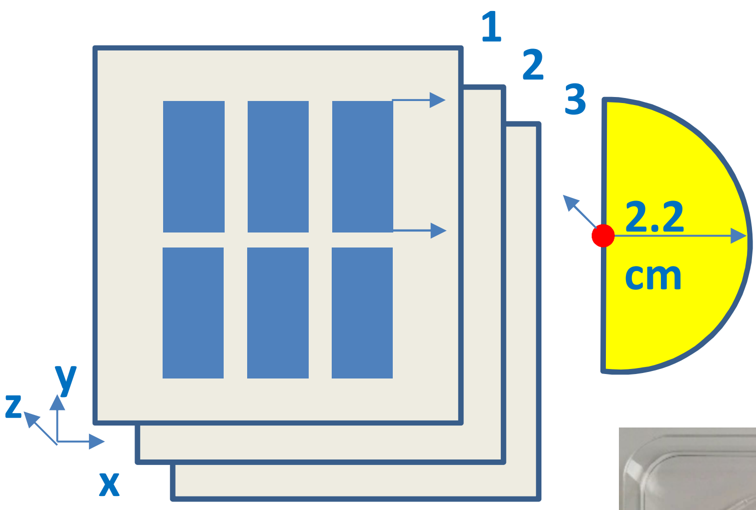
Nanoparticle characterization: photometry



Higher antioxidant activity compared to soluble and biodegradable antioxidants.

Nanoparticle / compound	Weight (mg)	Trolox equivalent (nmol)
NC @70°C	**1**	**235.0**
Commercial NC	10	1.3
Ascorbic acid	2	9.4
Curcumin	2	0.2
Resveratrol	2	10.8

Equipment and restraints

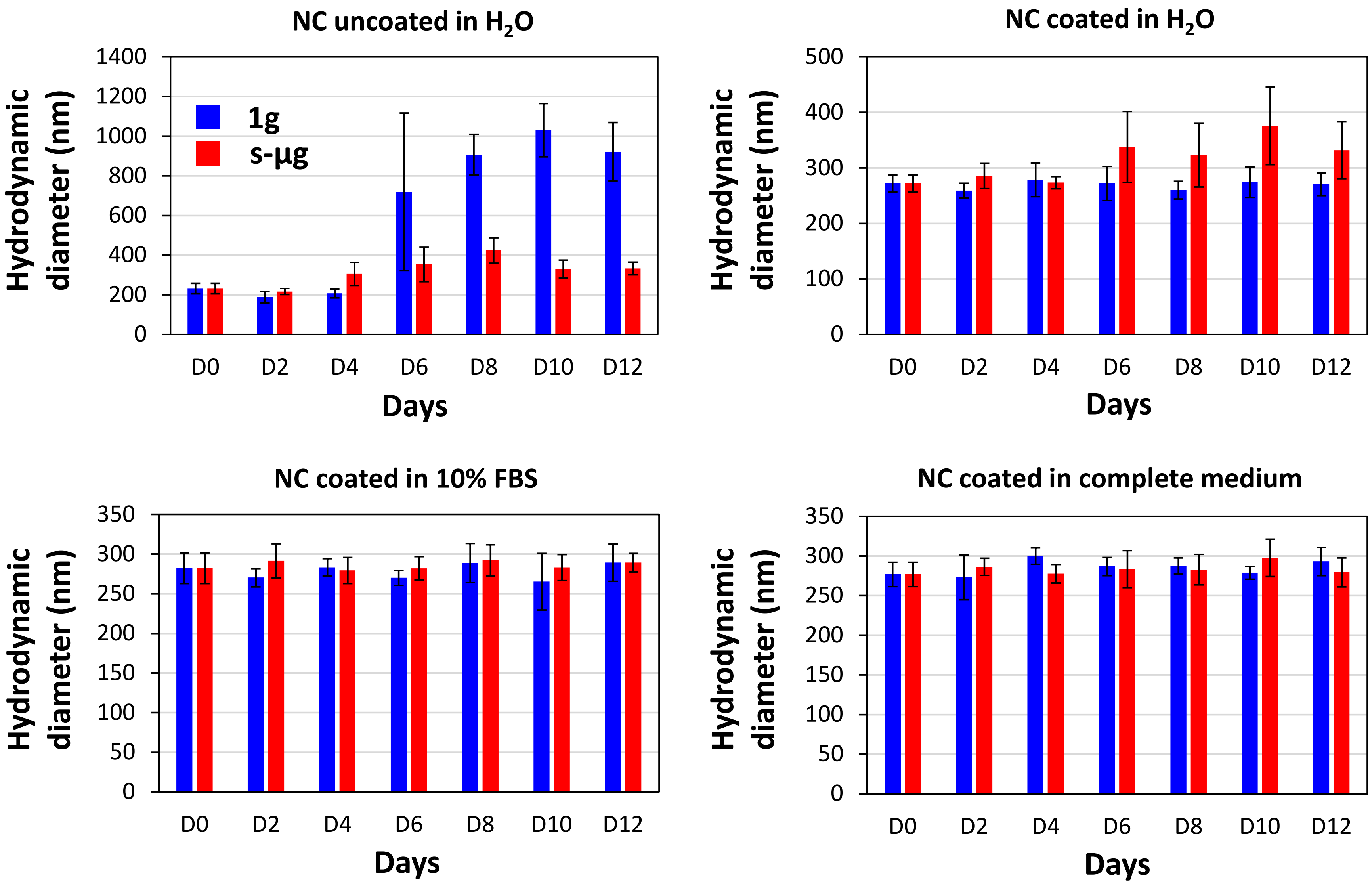


Acceleration loads < 0.001 in a sphere of 2.2 cm radius from center of rotation (•) for rotations within 30 deg/s.

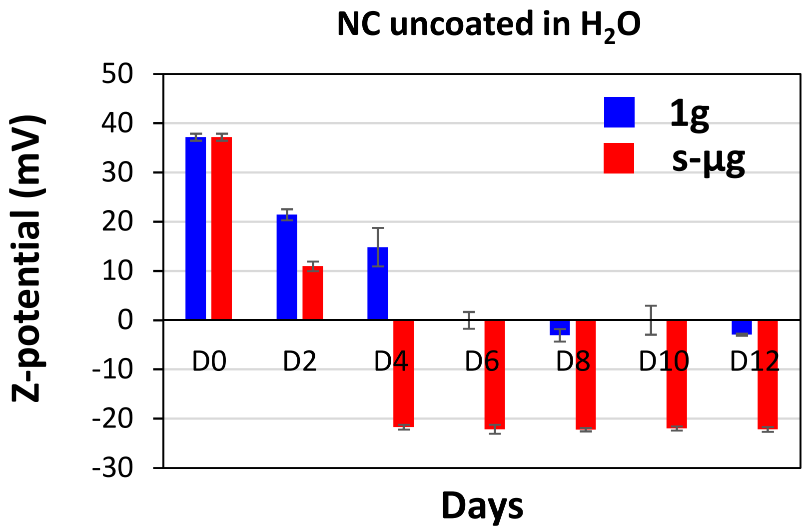
Acceleration loads < 0.002 for rotations within 60 deg/s.

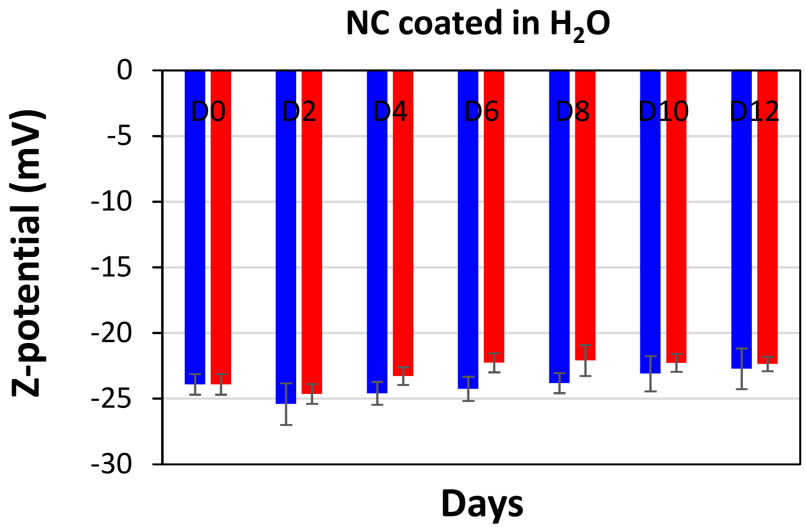
Fast prototyping with laser cutter and PDMS silicone casting for fabrication of disposable, transparent, sticky vessels for cell culture on plastic substrates (Thermanox or polystyrene) in single or multiple compartments that can be sealed with transparent film for prevention of liquid spills and gas exchanges during rotation.

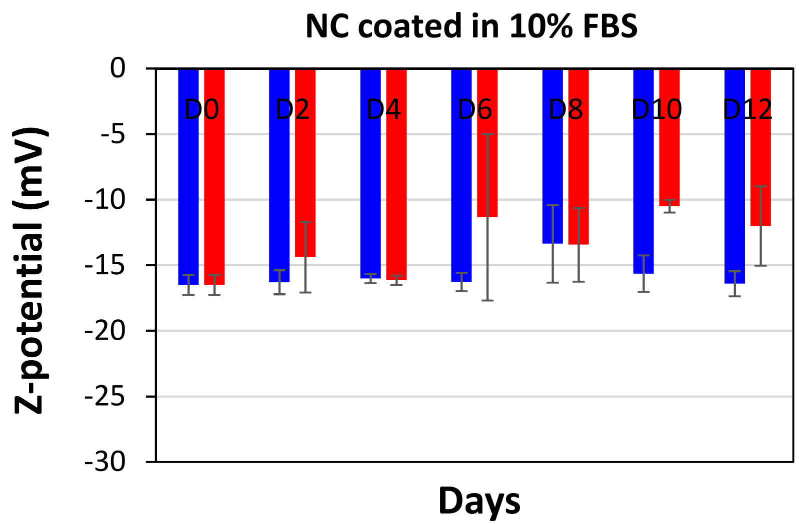
Cerium oxide nanoparticle characterization: DLS 1/2



Cerium oxide nanoparticle characterization: DLS 2/2

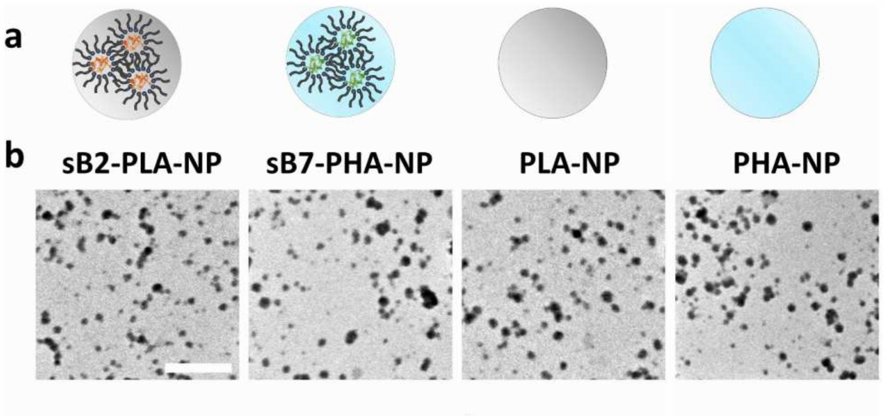


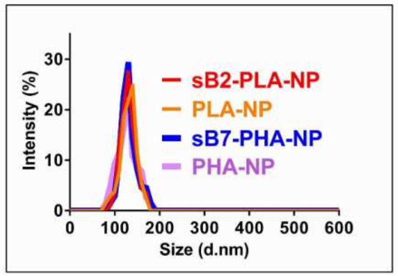




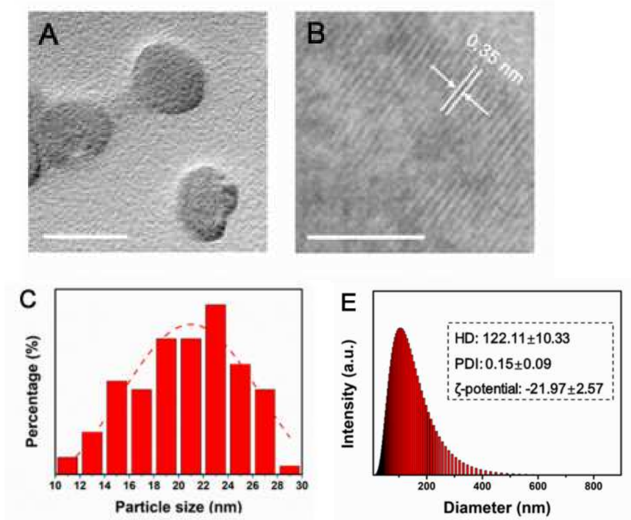
FBS coating improves NC dispersion colloidal stability in terms of hydrodynamic diameter and Z-potential.

Nanoparticle stability under simulated microgravity

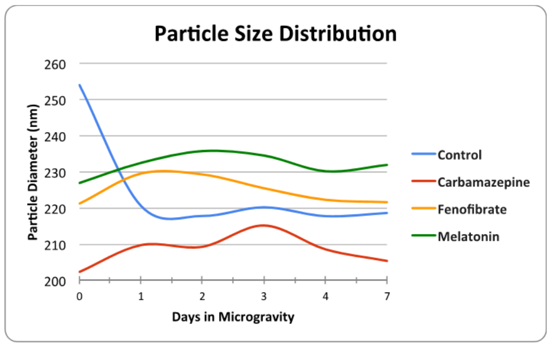


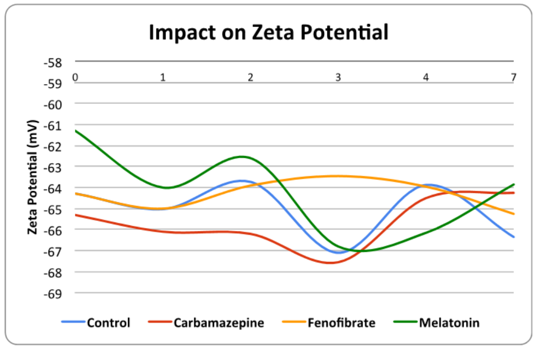


Zhao. X.-H. et al. "Osteogenic differentiation system based on biopolymer nanoparticles for stem cells in simulated microgravity". Biomedical Materials 16, 044102, 2021. doi: 10.1088/1748-605X/abe9d1



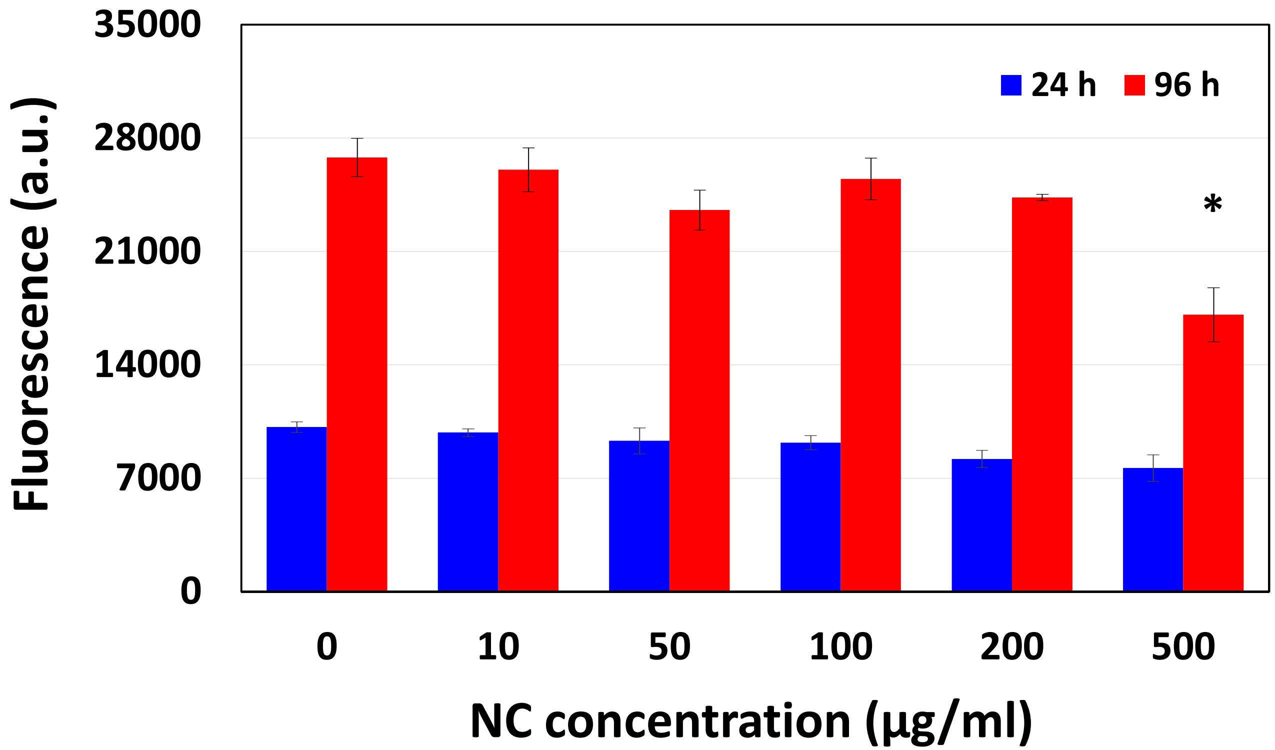
Wang M. et al. "Effects of Titanium Dioxide Nanoparticles on Cell Growth and Migration of A549 Cells under Simulated Microgravity". Nanomaterials 12, 1879, 2022. doi: 10.3390/nano12111879





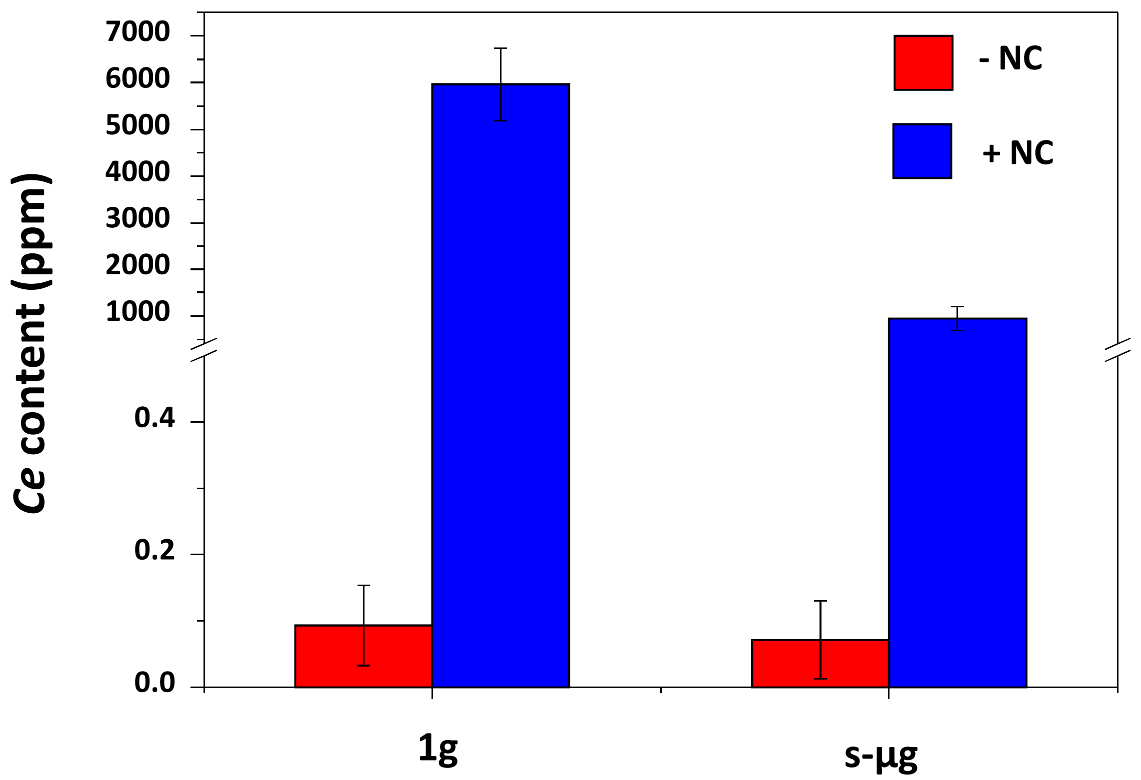
Dantuma D. et al. "Impact of Simulated Microgravity on Nanoemulsion Stability – A Preliminary Research." American Journal of Medical and Biological Research, 3(4) 102-106, 2015. doi: 10.12691/ajmbr-3-4-4

Cytotoxicity tests with HSkM: ds-DNA quantitation



Human Skeletal Myoblasts retain their viability at increasing concentrations of NC.

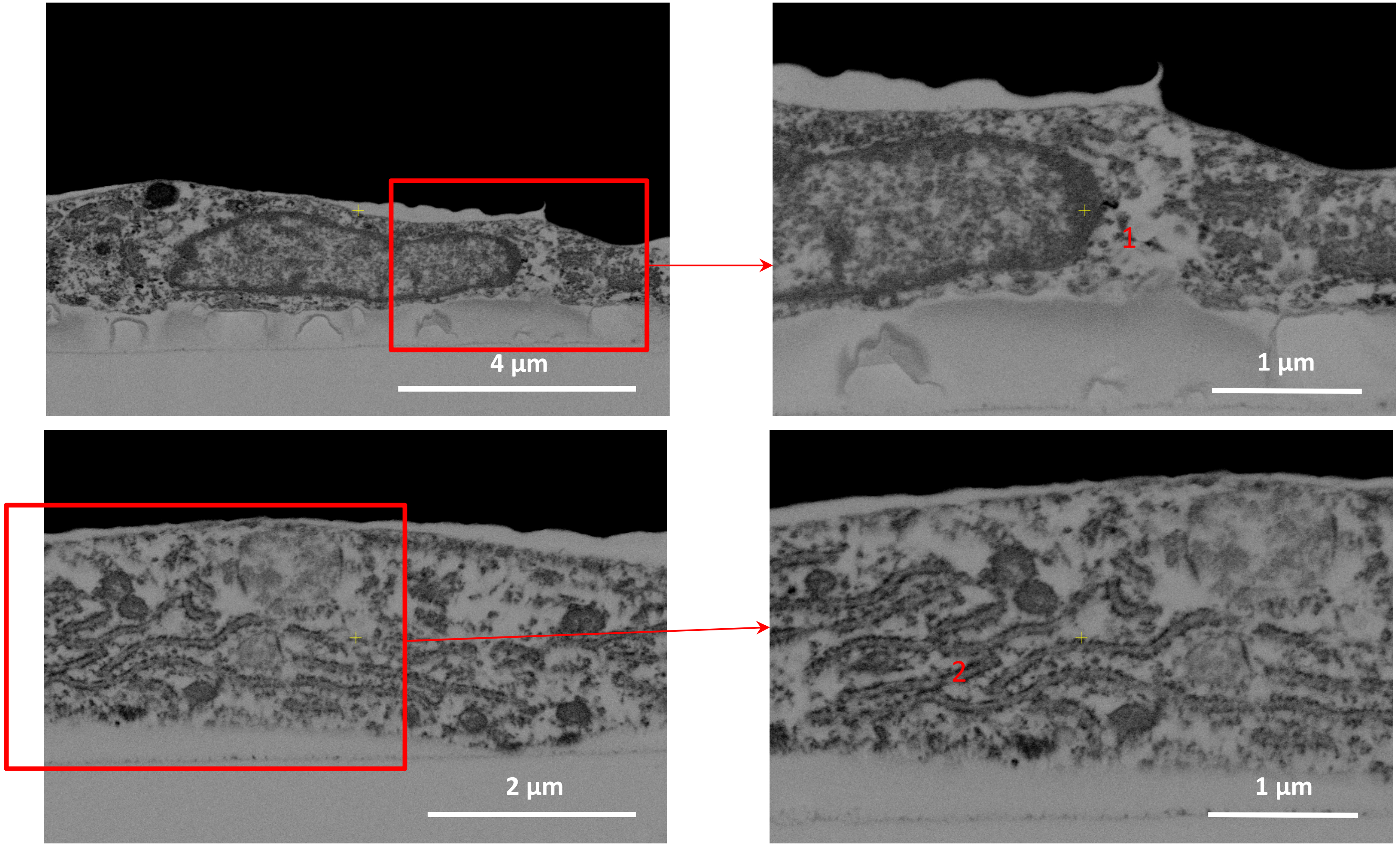
Nanoparticle internalization: ICP



Evidence of nanoparticle internalization by HSkM at 48 h after NC administration and exposure to s-µg obtained by random speed rotation at 8-20 deg/s.

Nanoparticle internalization: Electron Microscopy 1/4

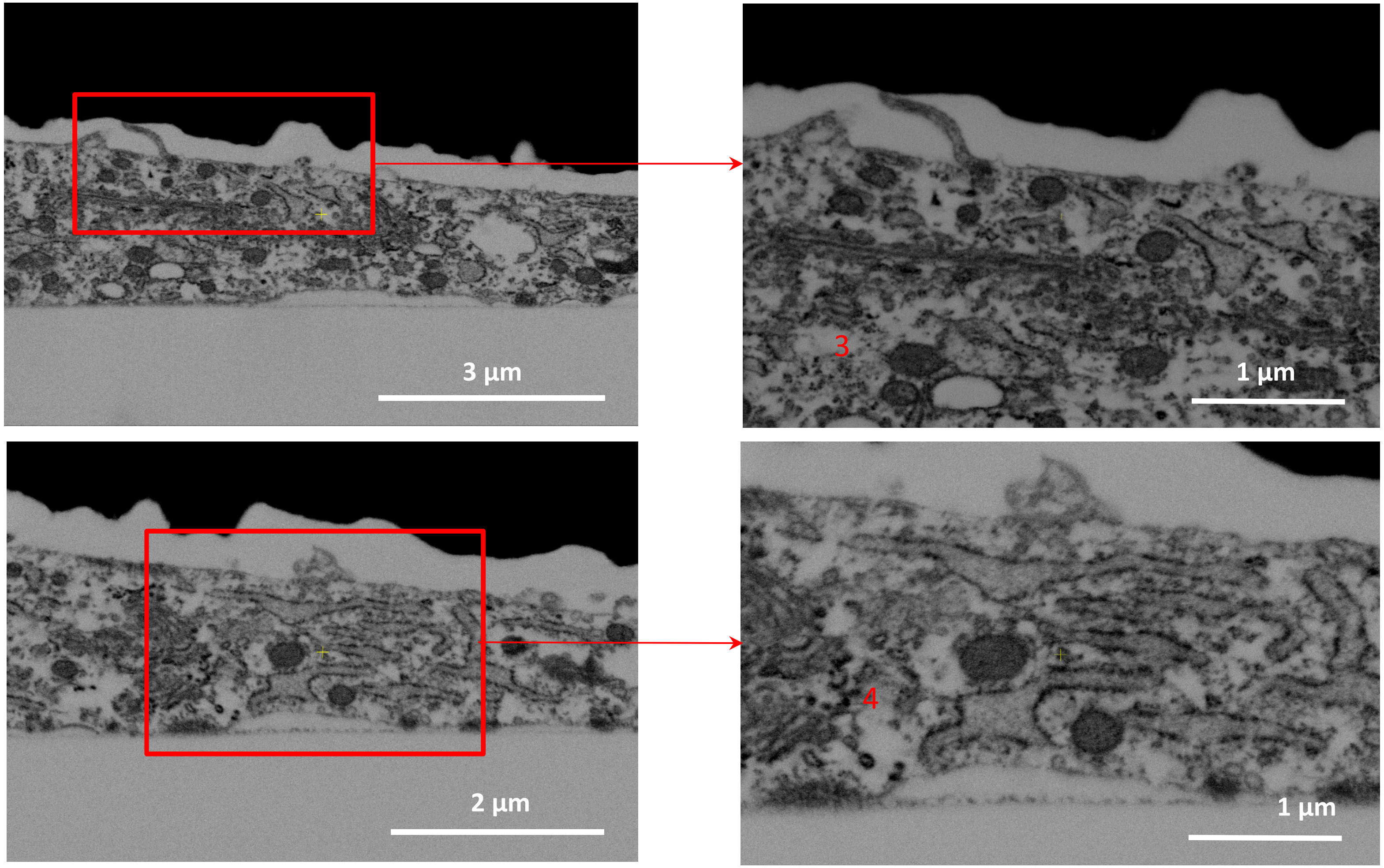
+NC, 1g for 6 h



1: perinuclear localization of intense dark spots ascribable to nanoceria; 2: rough endoplasmic reticulum covered by ribosomes (less-intense dark spots).

Nanoparticle internalization: Electron Microscopy 2/4

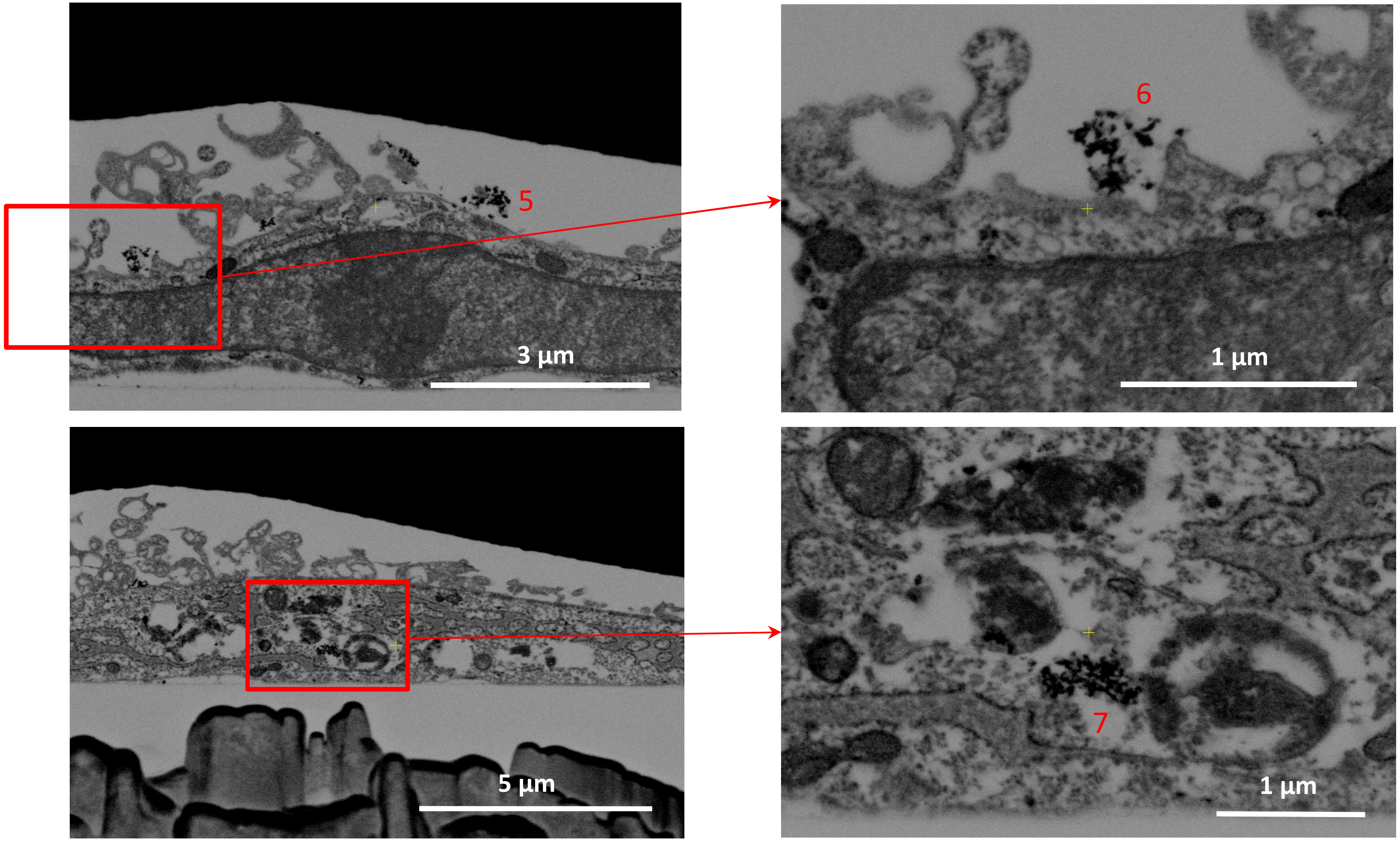
+NC, s-µg for 6 h



3, 4: cytoplasmic localization of intense-dark spots ascribable to nanoceria.

Nanoparticle internalization: Electron Microscopy 3/4

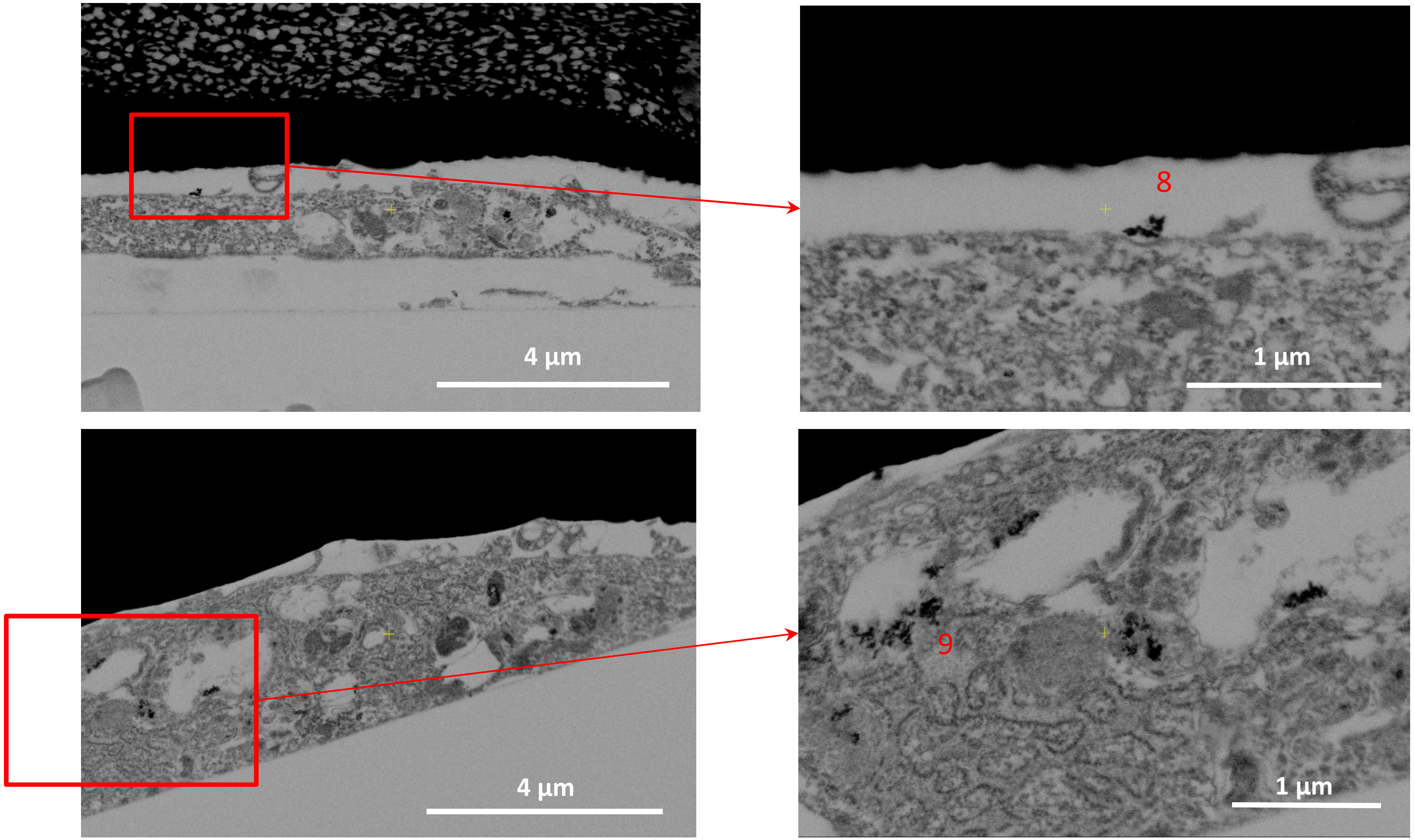
+NC, 1g for 24 h



5: cell membrane ruffles suggesting persistent nanoparticle internalization by macropinocytosis;
6: nanoparticle aggregates on the cell membrane; 7: cytoplasmic nanoparticle aggregates.

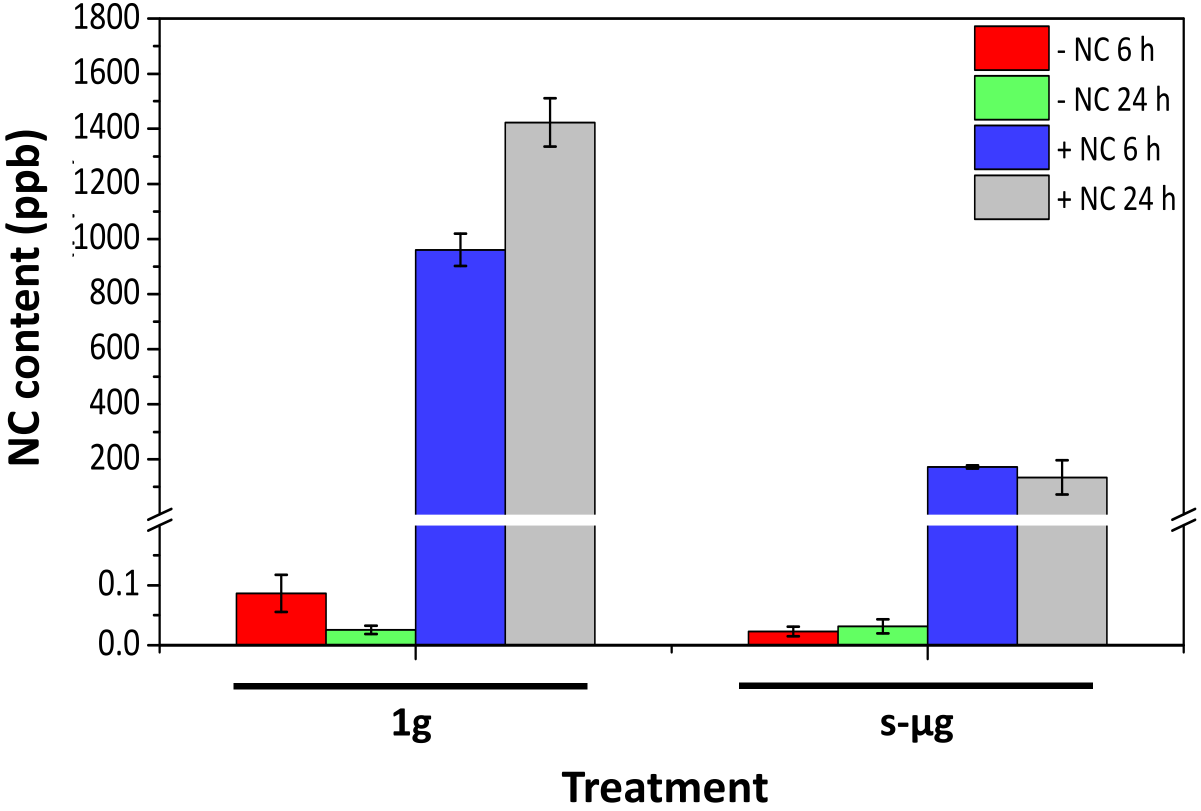
Nanoparticle internalization: Electron Microscopy 4/4

+NC, s-µg for 24 h



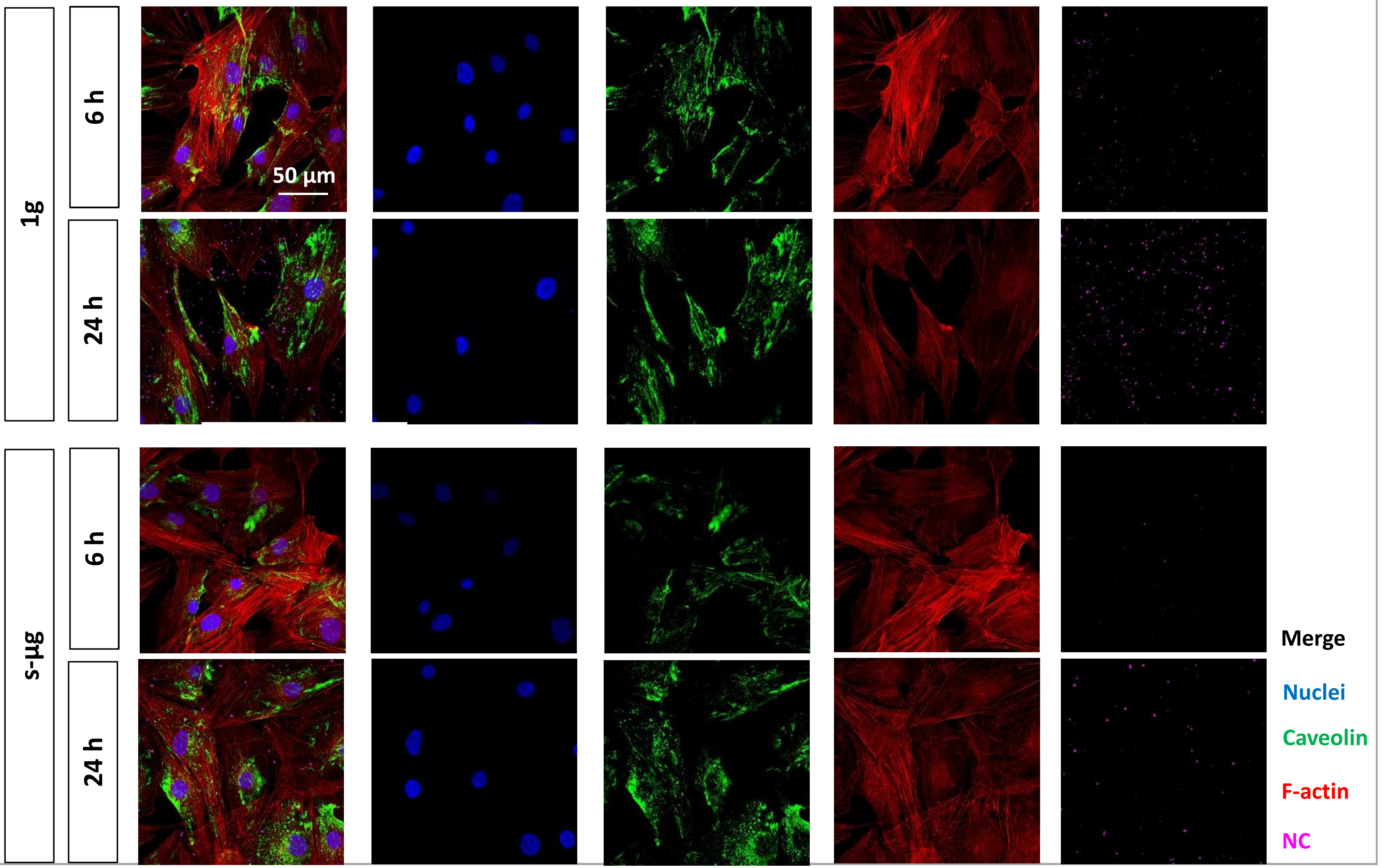
8: nanoparticle aggregates on the cell membrane; 9: nanoparticle aggregates in proximity of vacuoles.

Nanoparticle internalization: ICP

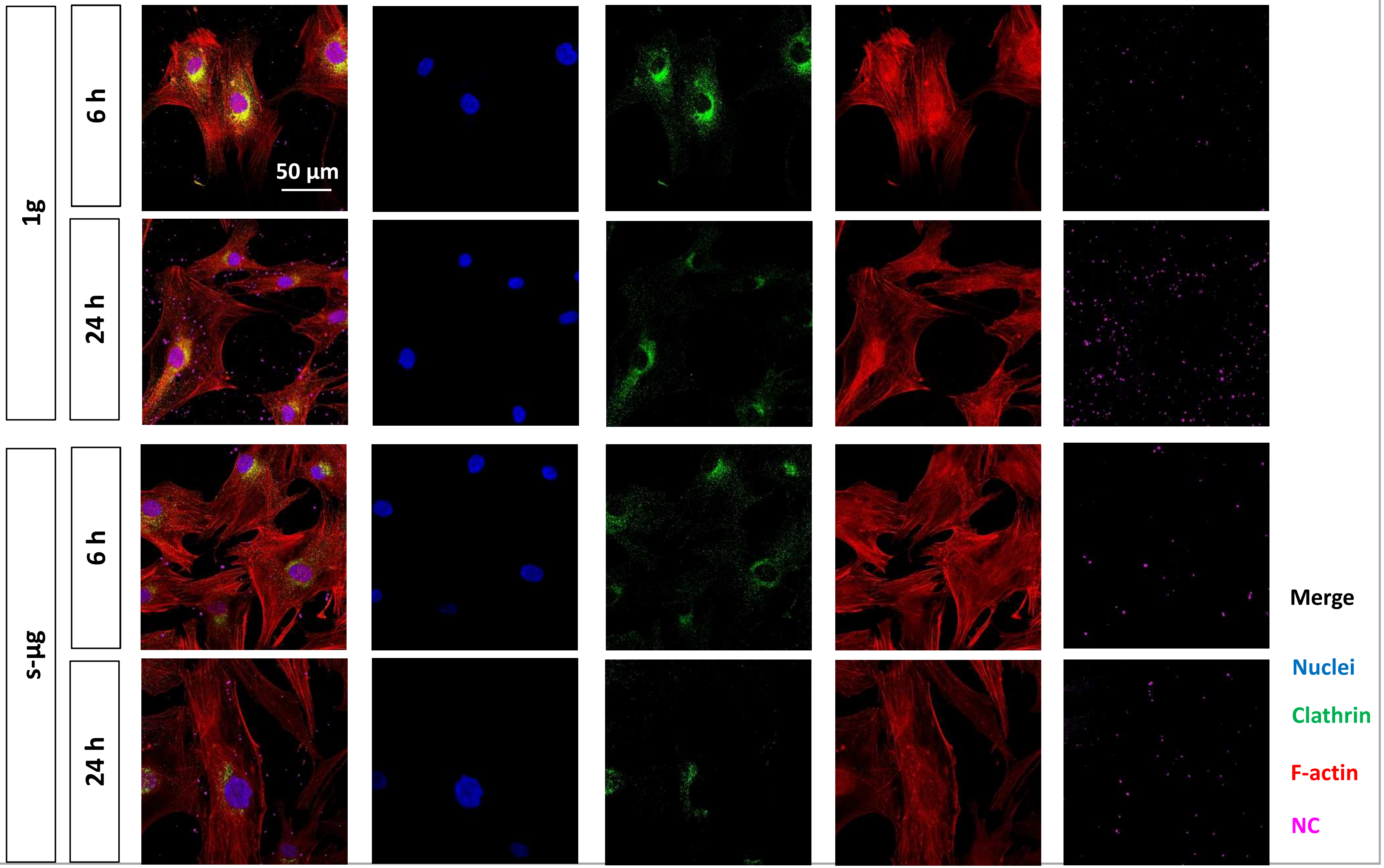


Evidence of nanoparticle internalization by HSkM within 24 h from NC administration and exposure to s-µg obtained by random speed rotation at 25-60 deg/s.

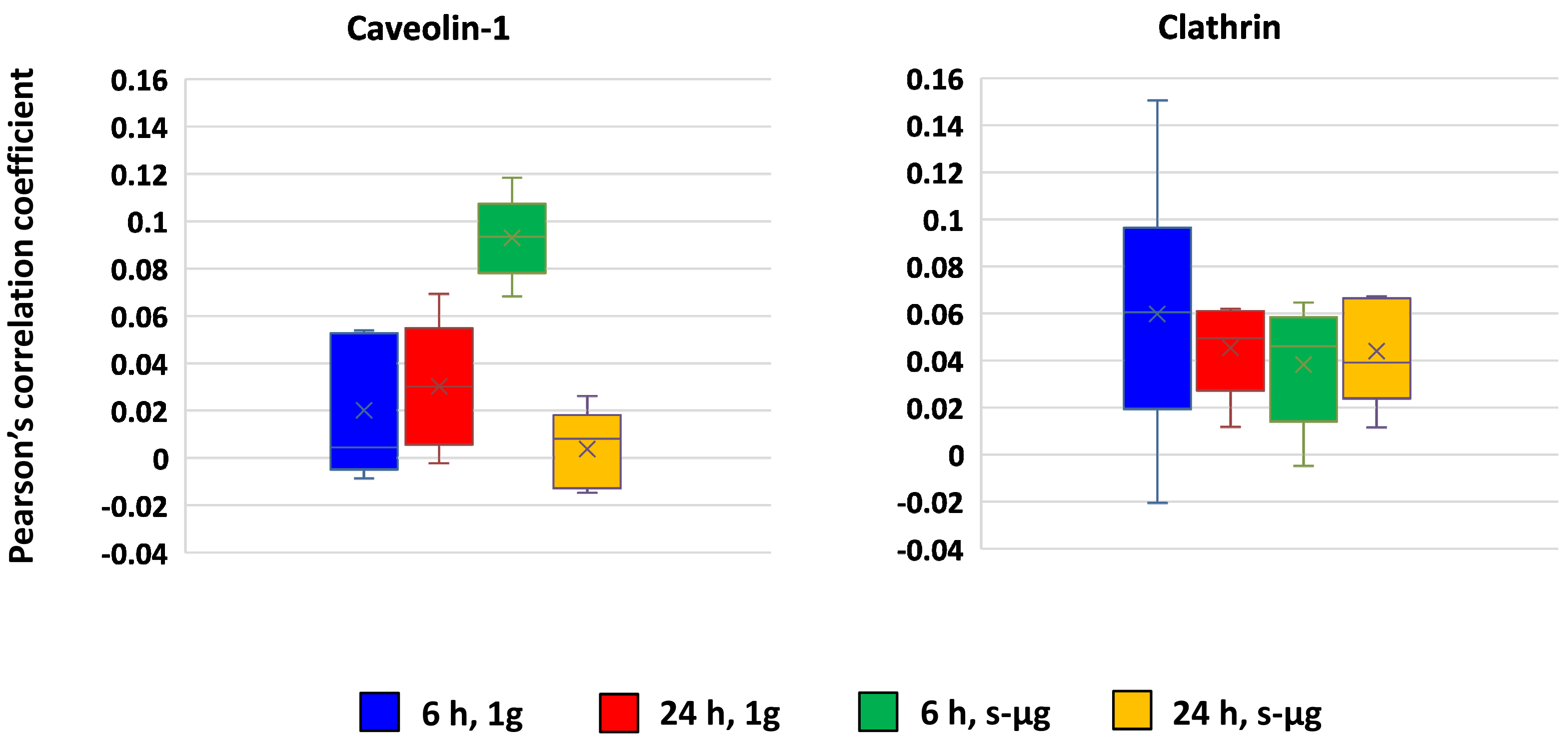
Nanoparticle internalization: confocal microscopy, caveolin-1



Nanoparticle internalization: confocal microscopy, clathrin



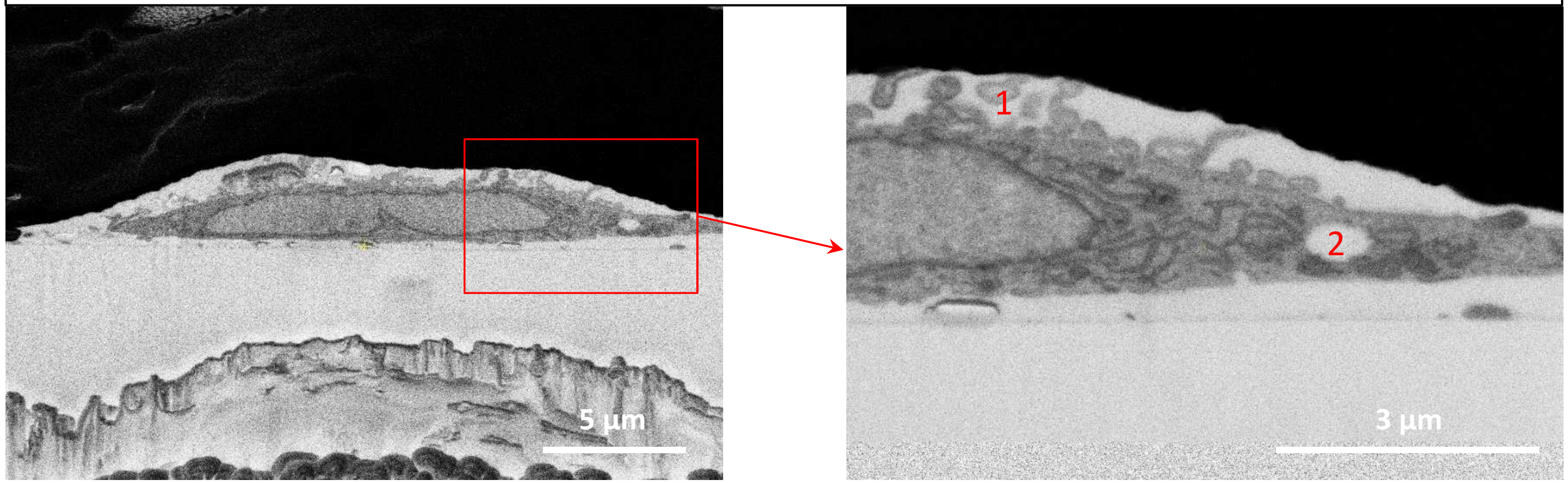
Nanoparticle internalization: image analysis



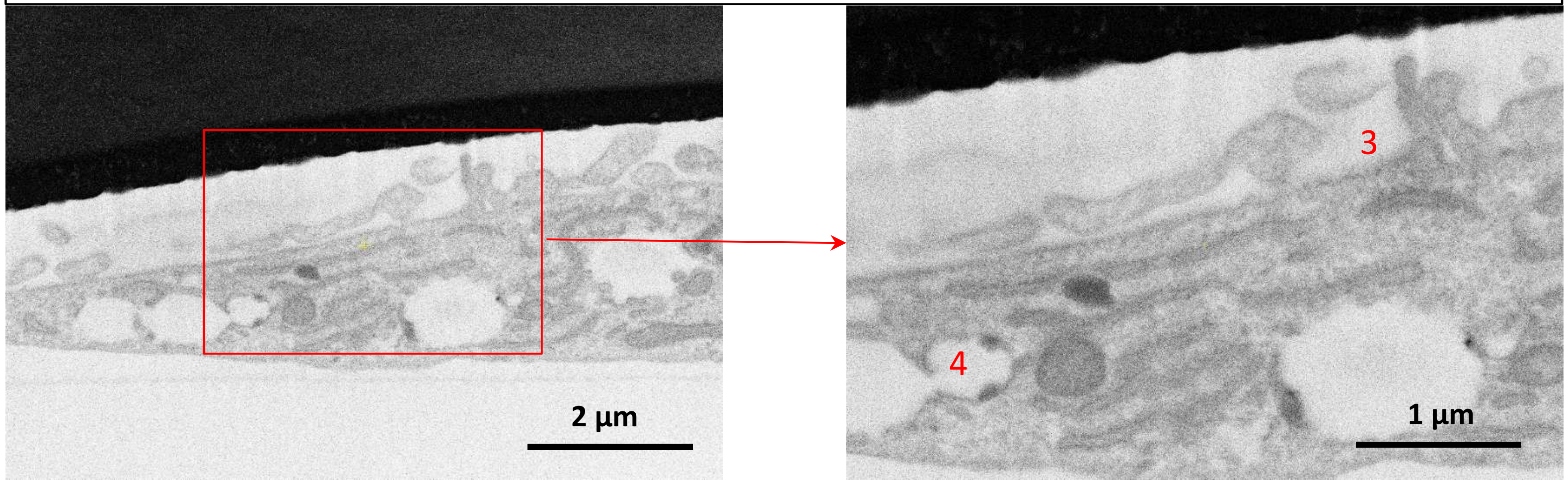
Light signal from NC aggregates poorly co-localizes with signal from fluorophore-conjugated antibodies: NC aggregates are not internalized by caveolin-1 and clathrin-mediated mechanisms.

Nanoparticle internalization: electron microscopy 1/2

+NC, 1g for 6 h



+NC, s-µg for 6 h

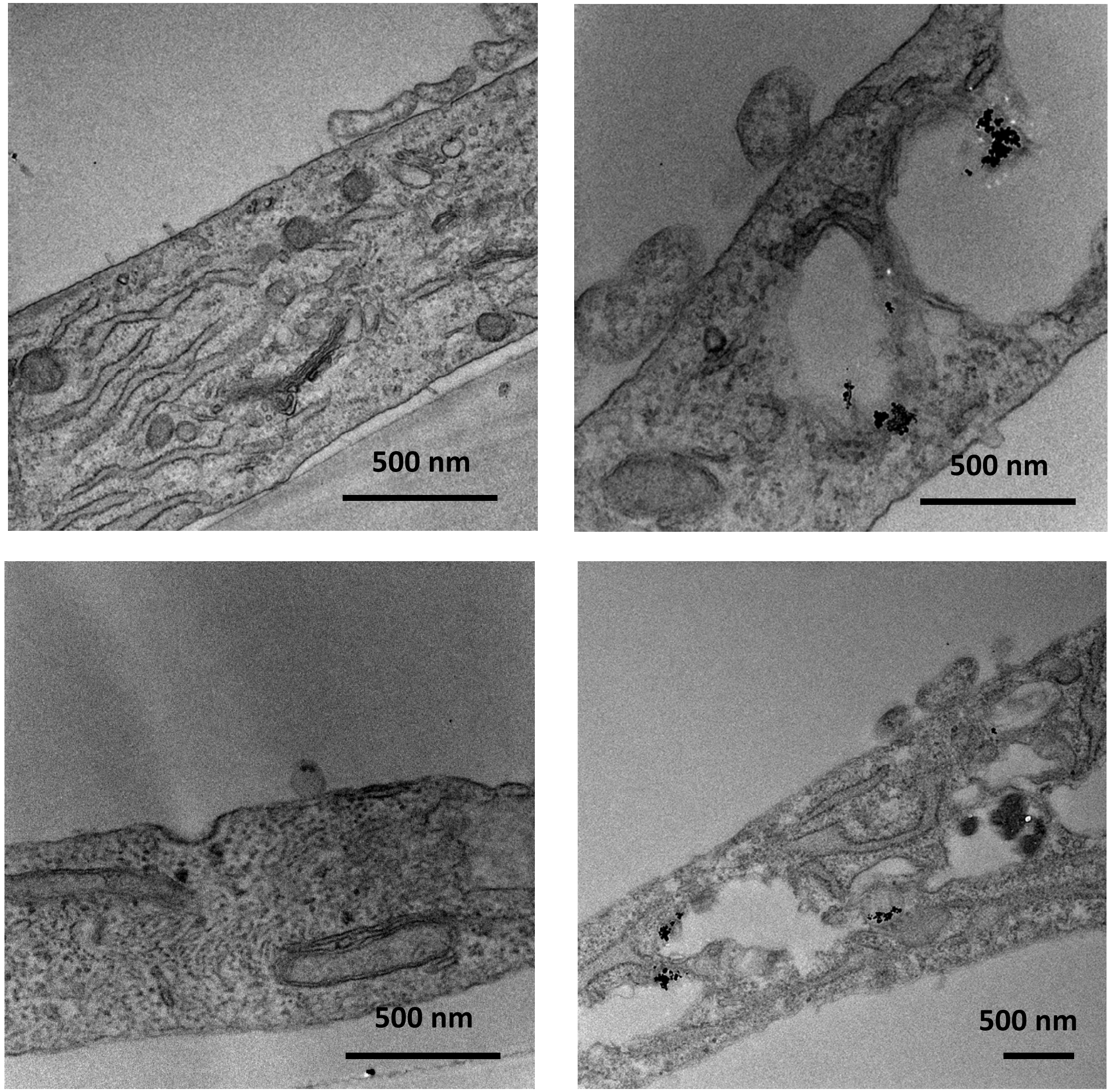


Confirmation of previous data on NC macropinocytosis.

Nanoparticle internalization: electron microscopy 2/2

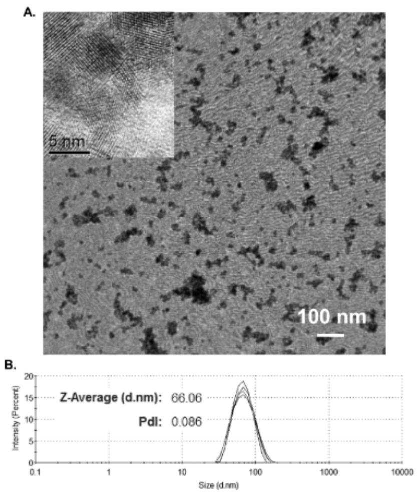
+NC, 1g for 24 h

+NC, s-µg for 24 h

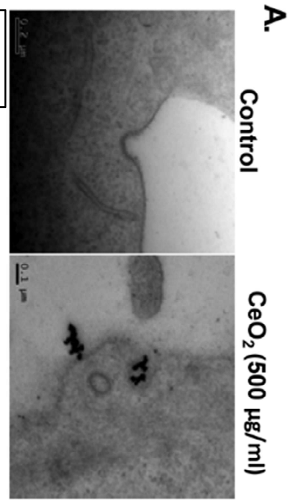


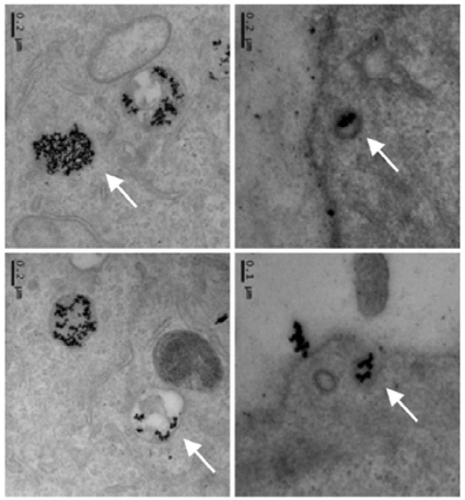
Ultrastructure well retained.

Nanoparticle internalization in the literature 1/2



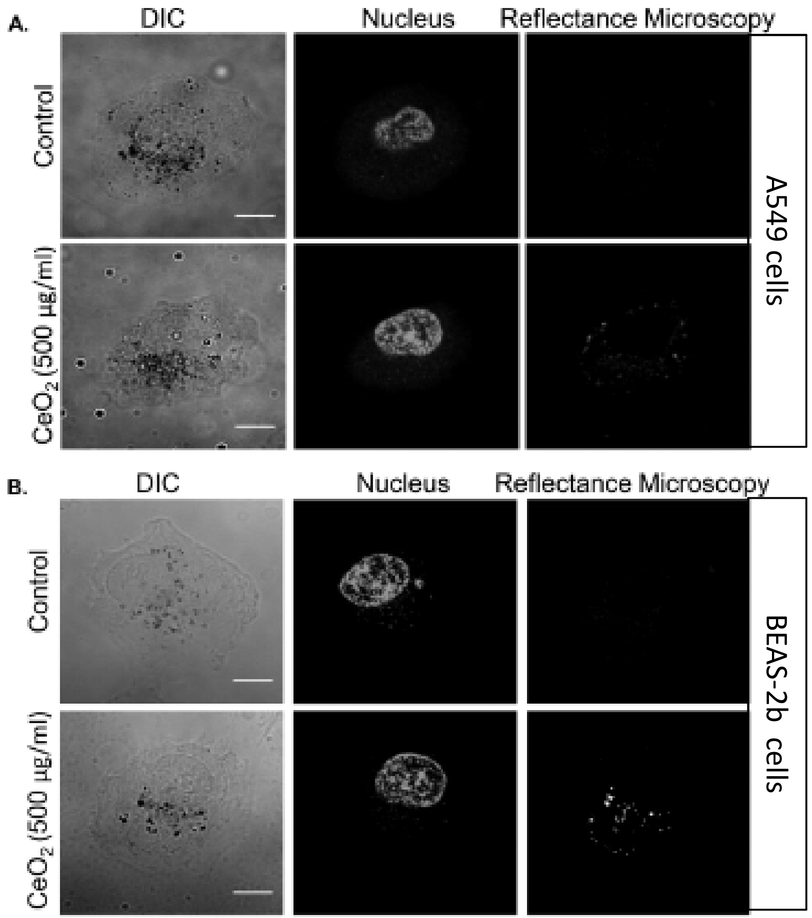
A549 cells





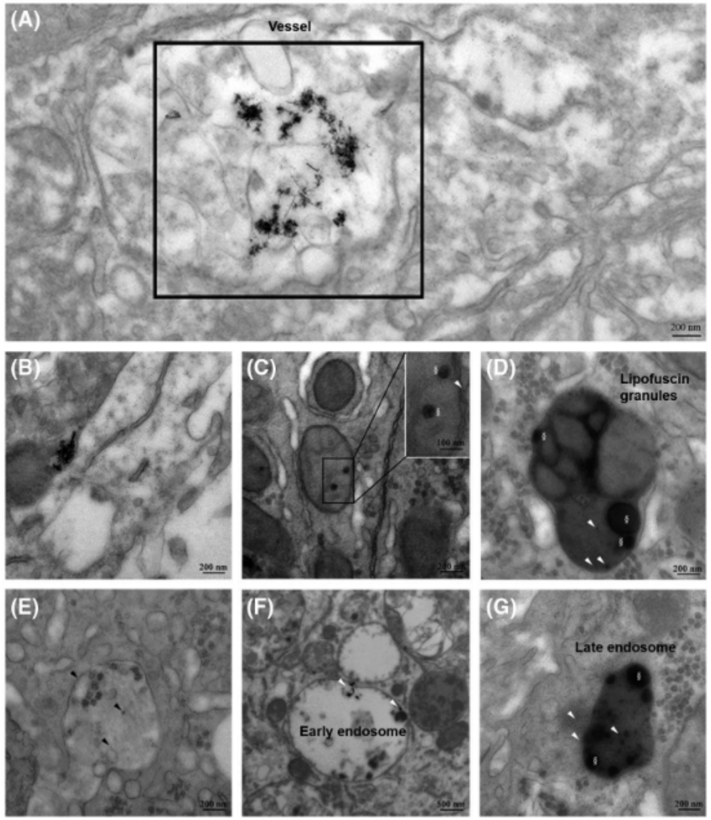
Endosomal compartments **Vesicles**

Interaction of protein corona (from serum) wrapping 8 nm-size NC with transferrin receptor promotes clathrin-mediated nanoparticle internalization.

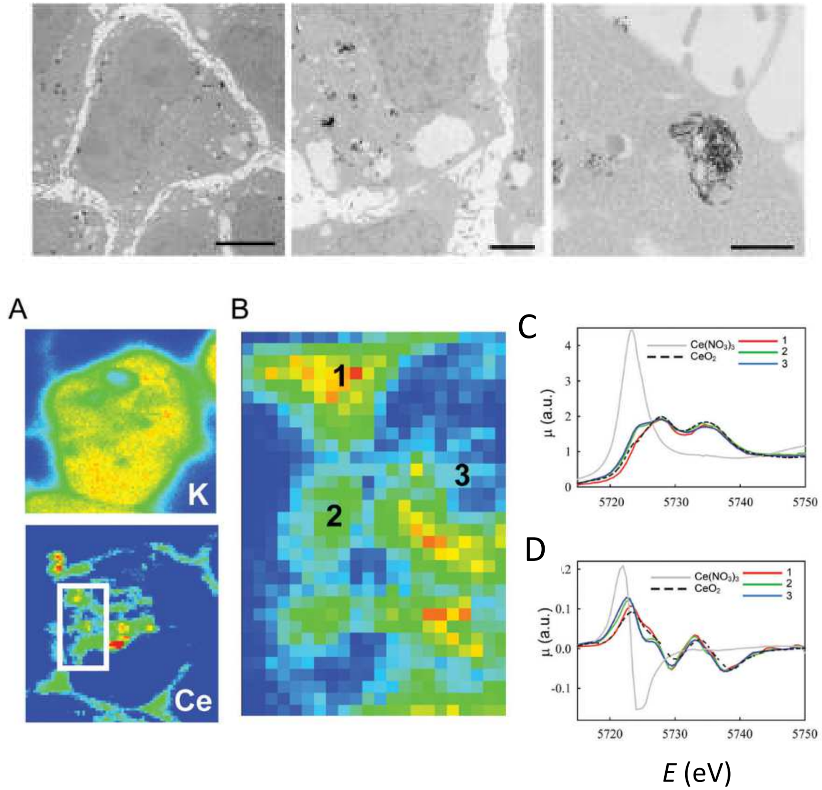


Mazzolini J. et al. "Protein Corona Modulates Uptake and Toxicity of Nanoceria via Clathrin-Mediated Endocytosis" Biology Bulletin 231, 40-60. doi: 10.1086/689590

Nanoparticle internalization in the literature 2/2



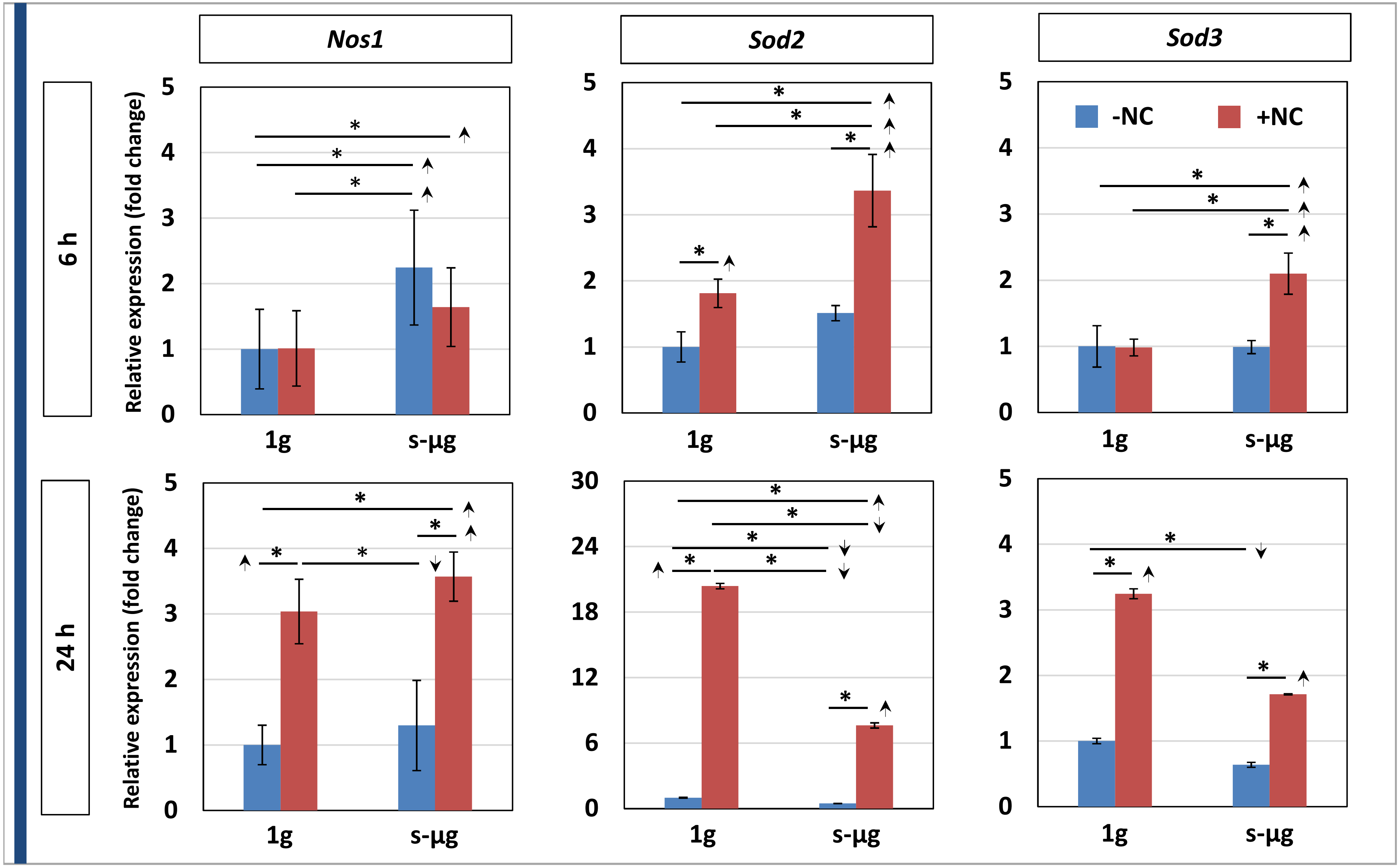
Del Turco S. et al. "Cerium oxide nanoparticles administration during machine perfusion of discarded human livers: A pilot study" Liver Transplantation 28, 1173–1185, 2022 doi: 10.1002/lt.26421



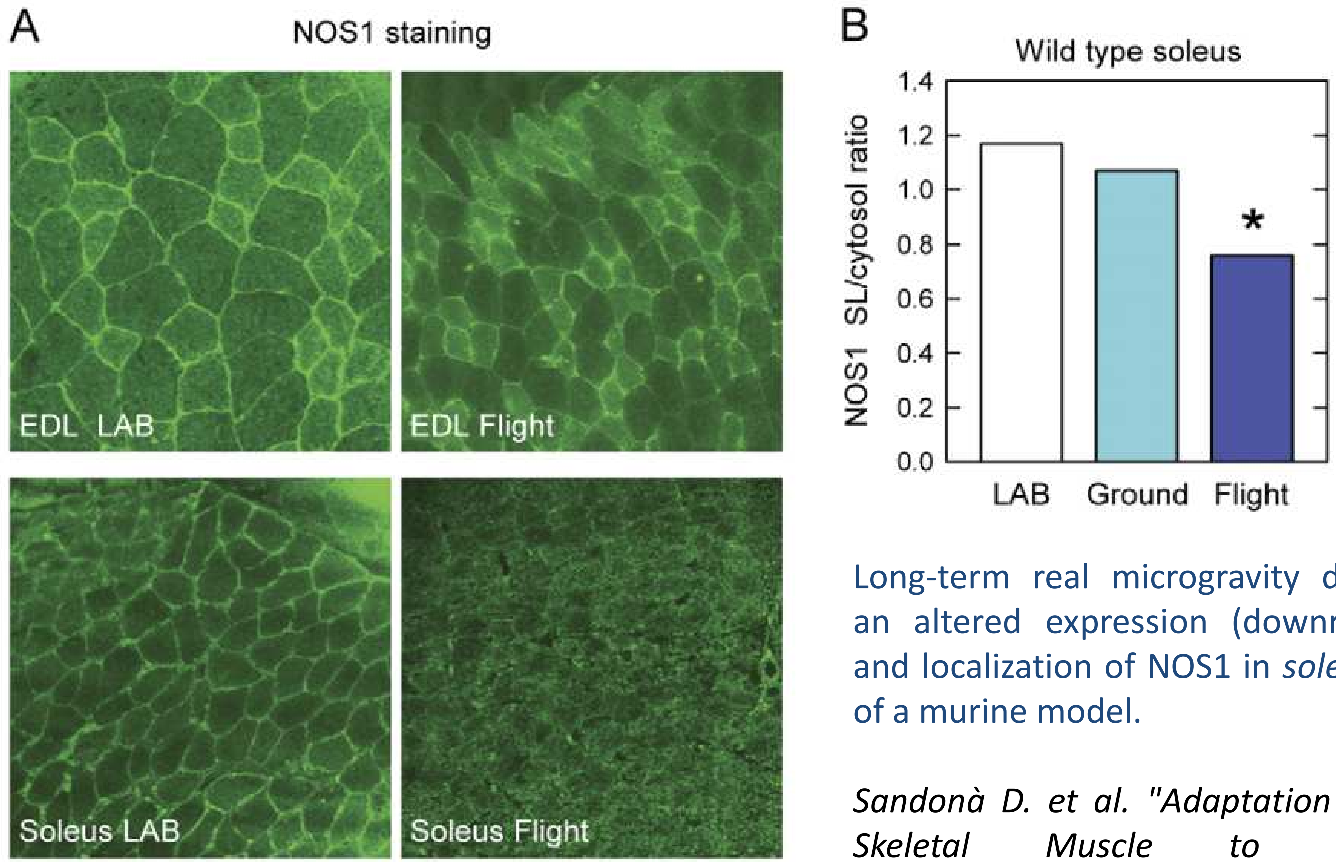
Ferraro D. et al. "Dependence of the Ce(III)/Ce(IV) ratio on intracellular localization in ceria nanoparticles internalized by human cells" Nanoscale 9, 1527, 2017 doi: 10.1039/c6nr07701c

Nanoparticle effects under s-µg: qRT-PCR

*p< 0.001, regulation threshold: 2



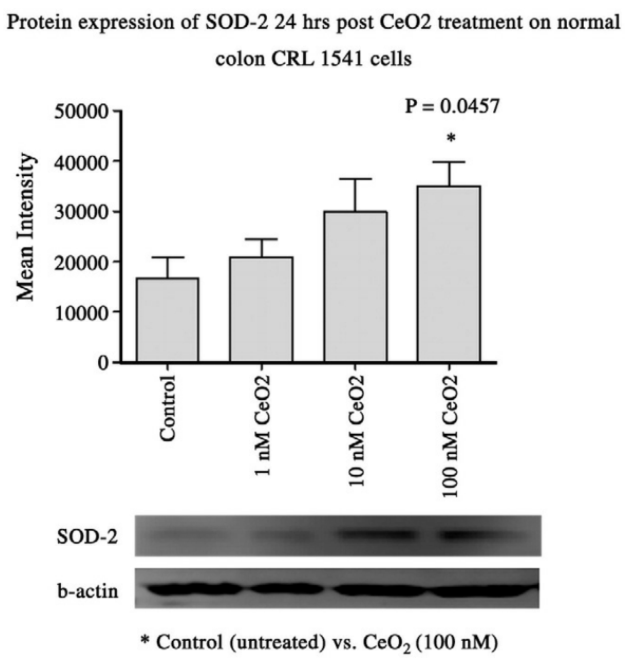
Nitric oxide synthase 1 regulation



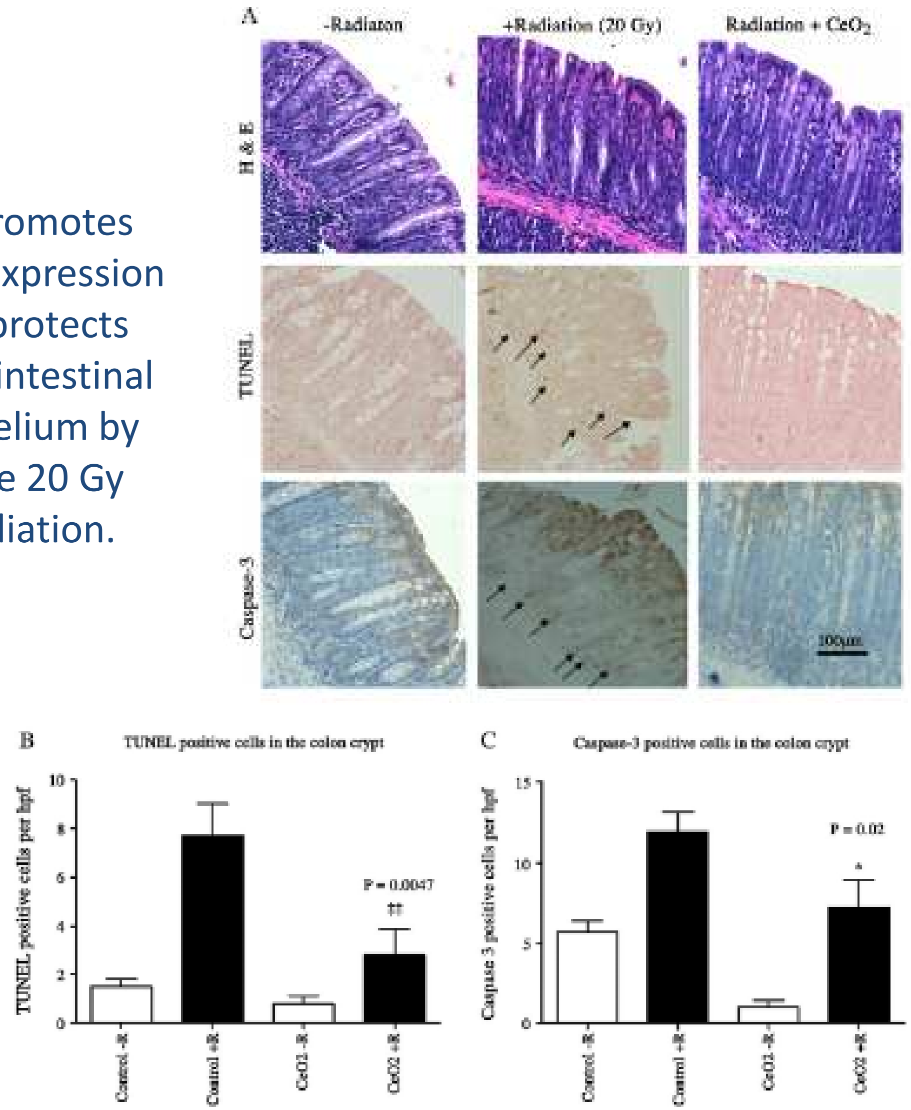
Long-term real microgravity determines an altered expression (downregulation) and localization of NOS1 in *soleus* muscle of a murine model.

Sandonà D. et al. "Adaptation of Mouse Skeletal Muscle to Long-Term Microgravity in the MDS Mission" PLoS ONE 7(3) e33232, 2012 doi: 10.1371/journal.pone.0033232

Superoxide dismutase 2 regulation

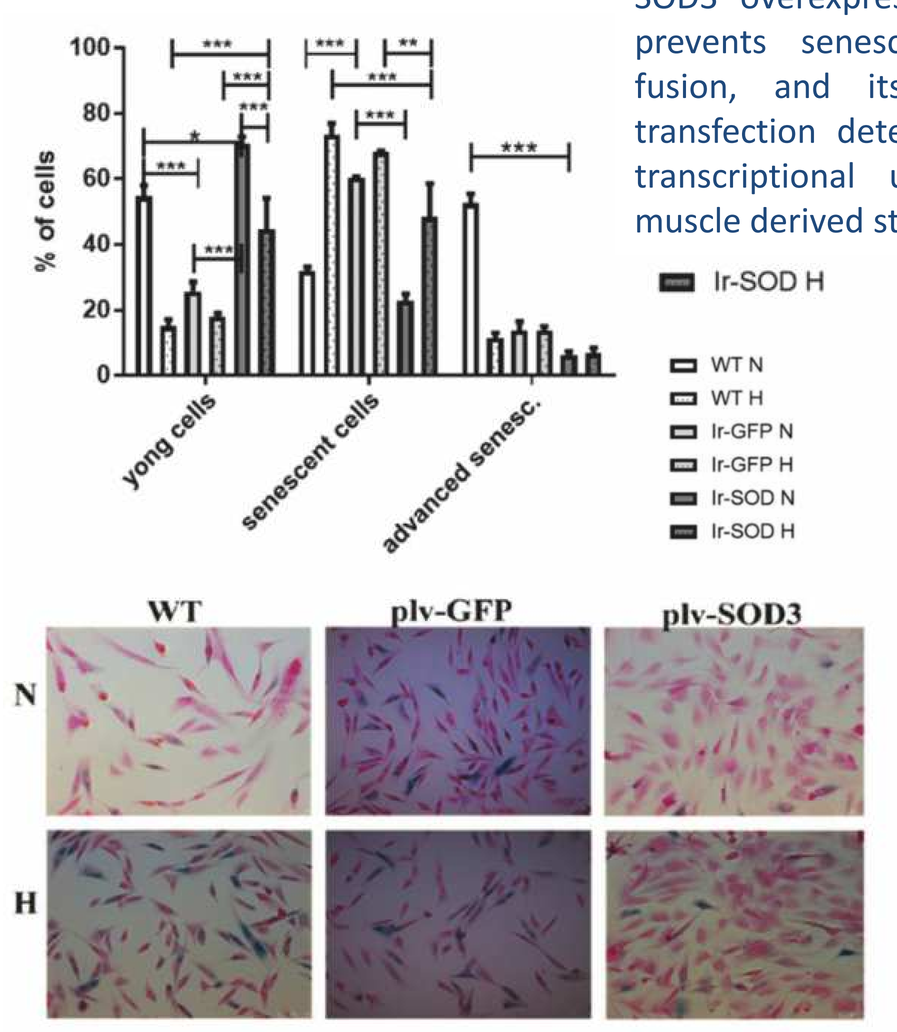


NC promotes SOD2 expression and protects gastrointestinal epithelium by single 20 Gy irradiation.

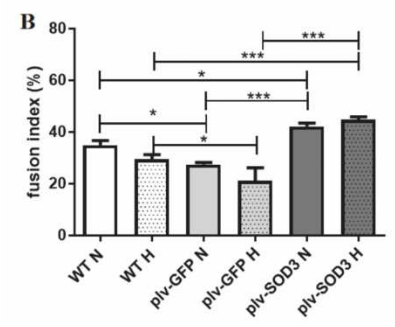


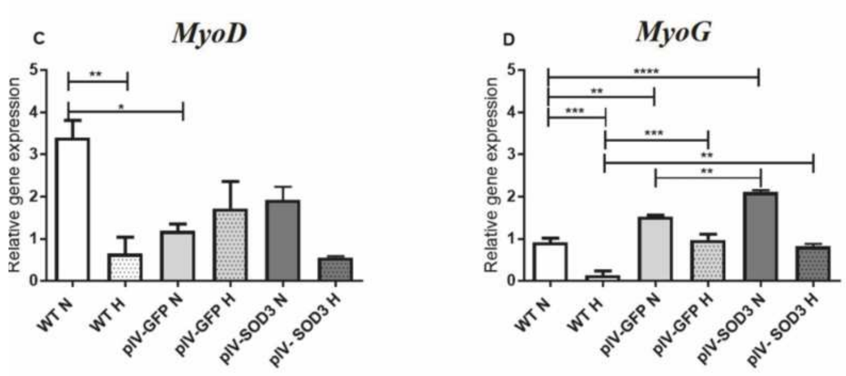
Colon J. et al. "Cerium oxide nanoparticles protect gastrointestinal epithelium from radiation-induced damage by reduction of reactive oxygen species and upregulation of superoxide dismutase 2" Nanomedicine: Nanotechnology, Biology, and Medicine 6, 698-705, 2010 doi: 10.1016/j.nano.2010.01.010.

Superoxide dismutase 3 regulation



SOD3 overexpression upon transduction prevents senescence while promoting fusion, and its overexpression upon transfection determines myogenic factor transcriptional upregulation in human muscle derived stem/progenitor cells.





Nowaczyk M. et al. "Transient and Stable Overexpression of Extracellular Superoxide Dismutase is Positively Associated with the Myogenic Function of Human Skeletal Muscle-Derived Stem/Progenitor Cells" Antioxidants 9, 817, 2020 doi: 10.3390/antiox9090817.

Conclusion and future perspectives

Cerium oxide nanoparticles seem to prevalently undergo internalization by macropinocytosis in the applied experimental conditions.

Internalization occurs with a time-dependent mode under normal gravity whereas it remains constant within the observation period under simulated microgravity.

Antioxidant nanoparticles regulate transcription of key enzymes involved in cellular antioxidant response.

Future perspectives to be focused on intracellular vesicles by selective staining and/or isolation and following analyses by confocal microscopy, and on validation at translational level of regulation of markers involved in antioxidant response.

Communication activities 1/2

Abstract #284 "Interaction of antioxidant nanoparticles with myoblasts in simulated microgravity: possible strategies for muscle maintenance under mechanical unloading"

<u>ORAL presentation </u>to the 31st Conference of the European Society for Biomaterials (ESB2021) Porto (Portugal) September 5-9, 2021

Abstract #88 "Administration of antioxidant nanomaterials in simulated microgravity: the ESA-IIT InterGravity project"

<u>ORAL presentation </u>to the 10th ISS R&D Conference, to be held online August 3-5, 2021

Communication activities 2/2

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Master Degree in Biomedical Engineering

Master Thesis

Cerium Oxide Nanoparticles as antioxidant agents: Study of interaction with muscle cells under simulated microgravity

Politecnico di Torino

1859

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Thanks for your attention