β-AMYLOID-INDUCED OXIDATIVE STRESS BOOSTS CeO_2 NANOPARTICLES UPTAKE BY CHANGING BRAIN ENDOTHELIUM MICROVILLI PATTERN

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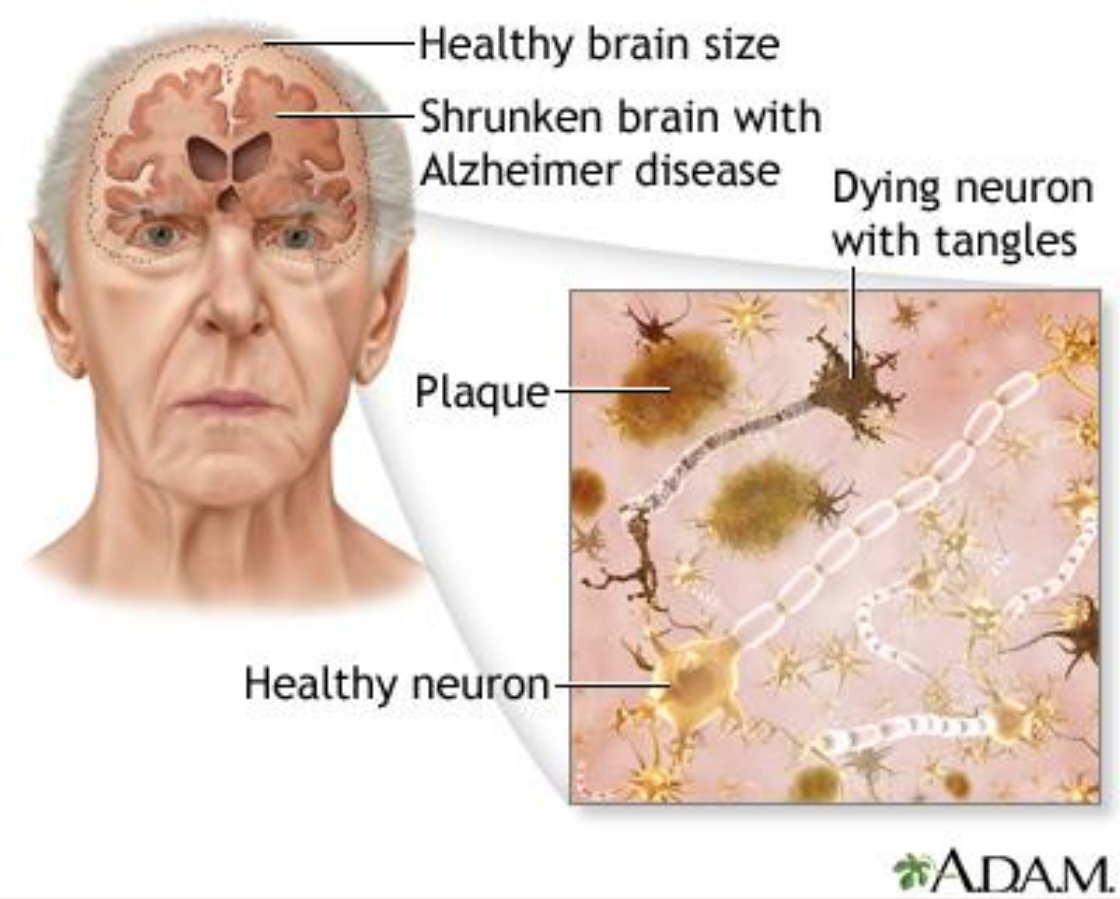
Alzheimer’s Disease (AD)

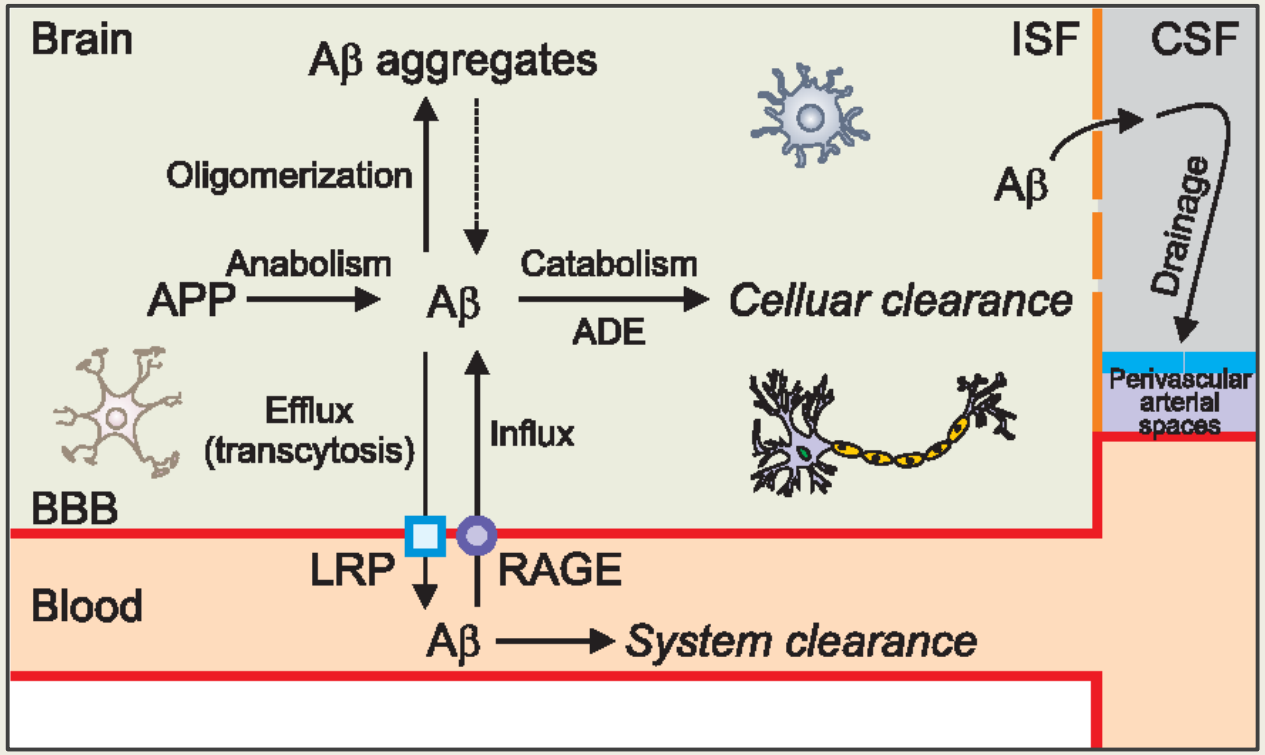


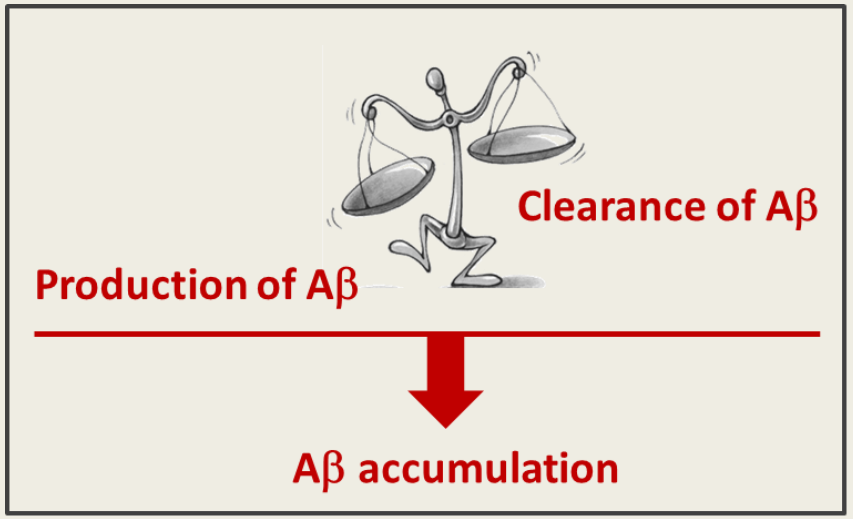
Alzheimer's disease is a chronic illness with long preclinical and prodromal phases (20 years) and an average clinical duration of 8–10 years. The disease has an estimated prevalence of 10–30% in the population >65 years of age with an incidence of 1–3%. Most patients with Alzheimer's disease (>95%) have the sporadic form, which is characterized by a late onset (80–90 years of age), and is the consequence of the failure to clear the amyloid-β (Aβ) peptide from the interstices of the brain.

[Colin LM 2015 Nature Reviews Dis]

Beta-amyloid peptide (Aβ)

Aβ is still best known as a molecule to cause Alzheimer's disease (AD) through accumulation and deposition within the frontal cortex and hippocampus in the brain. Since accumulation of Aβ depends on the rate of its synthesis and clearance, the metabolic pathway of Aβ in the brain and the whole body should be carefully explored for AD research.

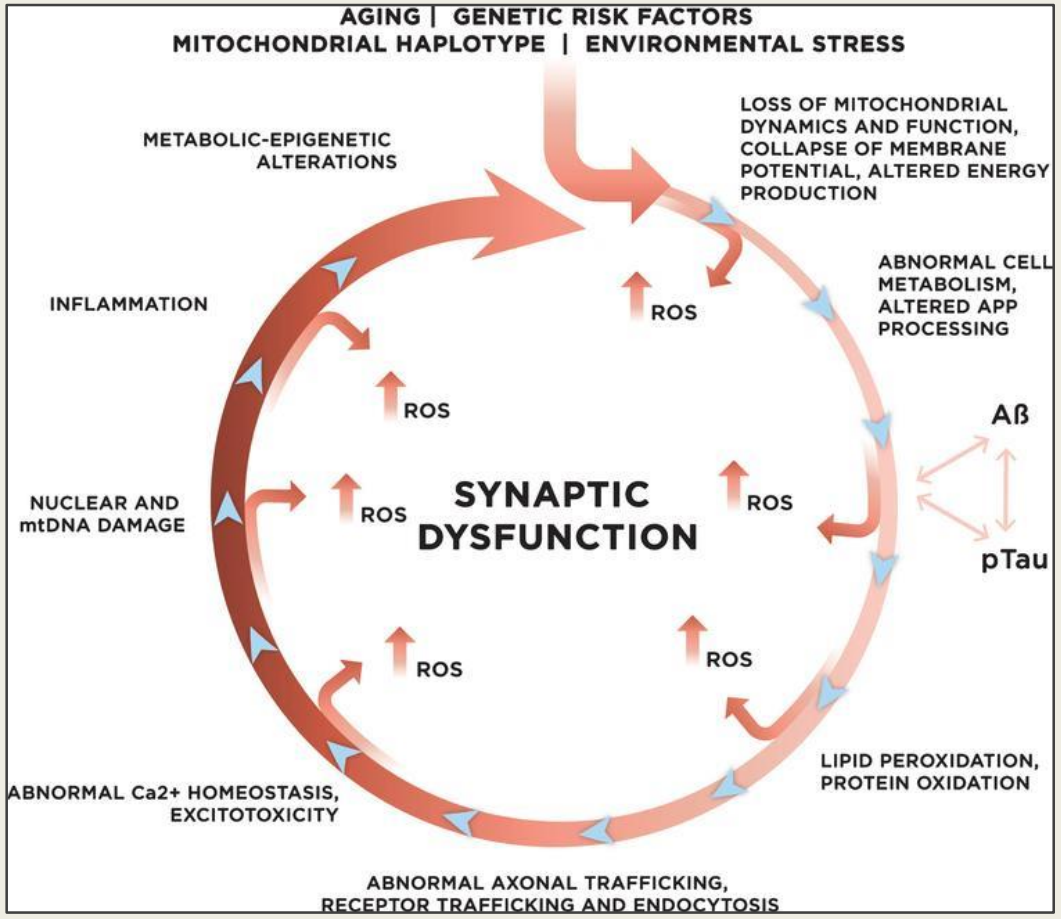




[Yoon S 2013 Biomol Ther]

Oxidative stress in AD

Oxidative stress is a serious imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and antioxidant defences, and has been shown in a wide range of studies to contribute significantly to the pathogenesis and progression of AD.

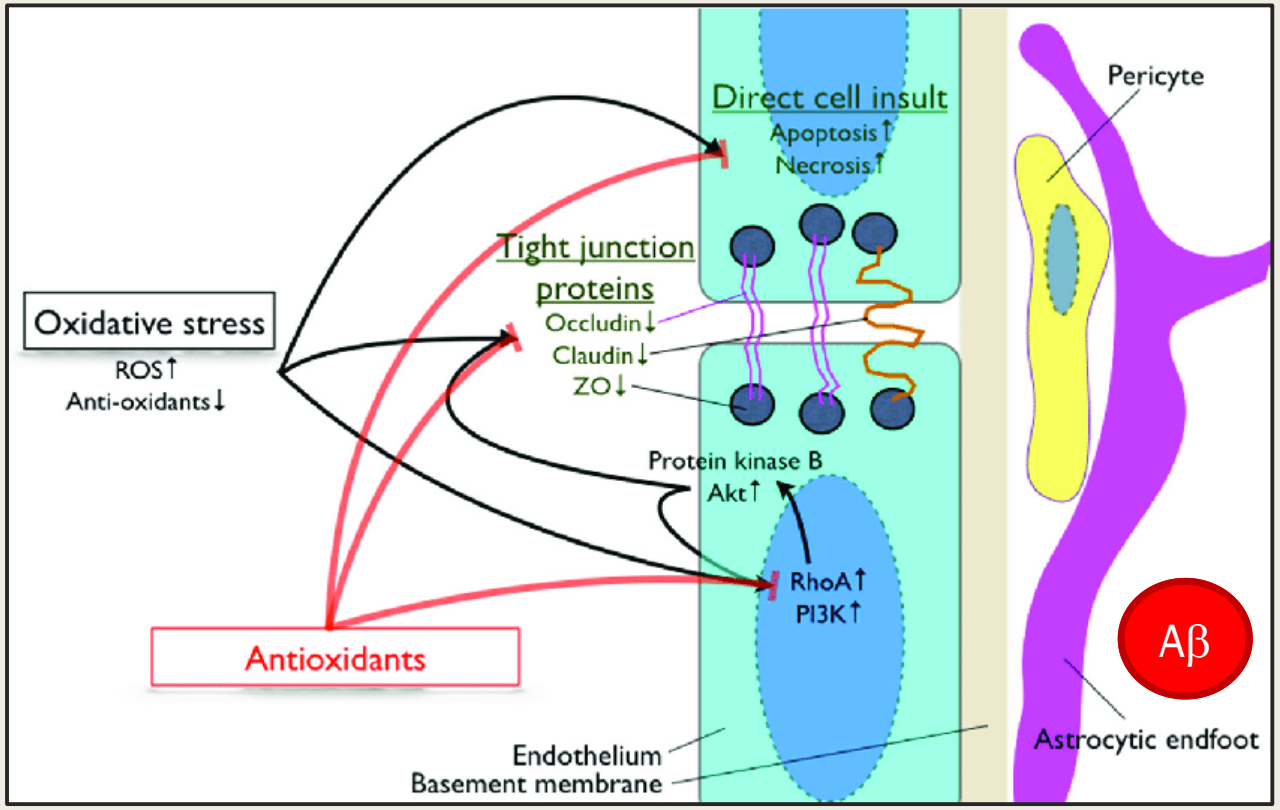


With age, increased ROS production could initiate a vicious cycle where multiple systems and mechanisms affected by ROS exacerbate ROS production, accelerating cellular damage, and leading to synaptic dysfunction.
Abnormal cellular metabolism in turn could affect the production and accumulation of amyloid-β (Aβ) and hyperphosphorylated Tau protein.

Oxidative stress is an important factor in the pathogenesis of AD and contributed to Aβ generation.

[Tonnies E 2017 J Alzh Dis]

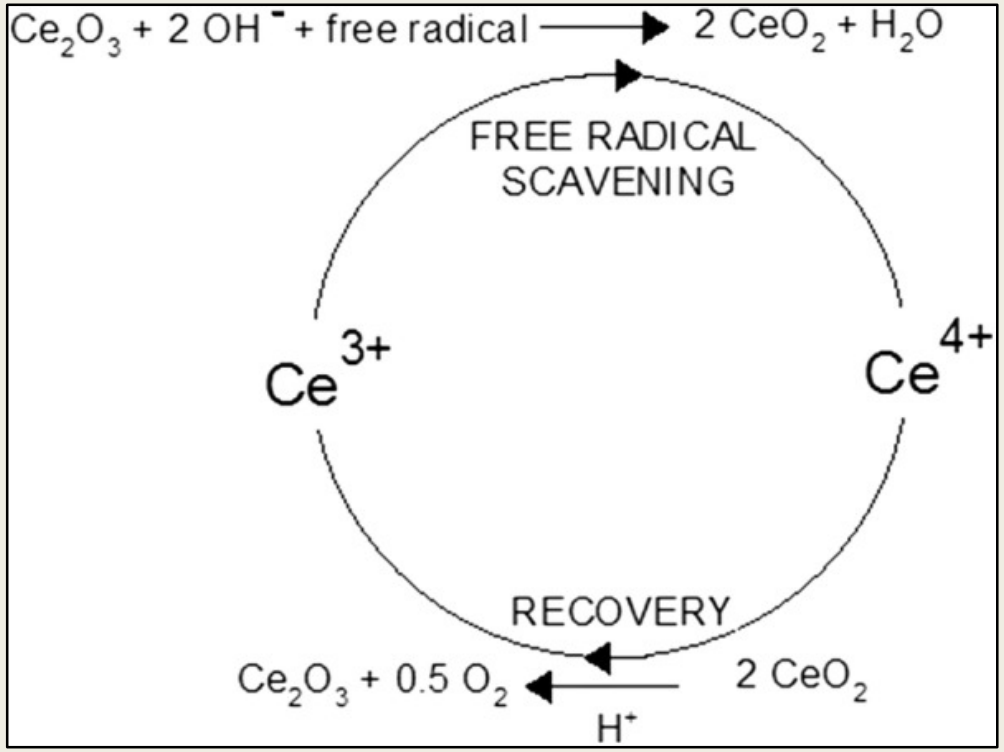
Oxidative stress ⇄ Aβ → BBB damage



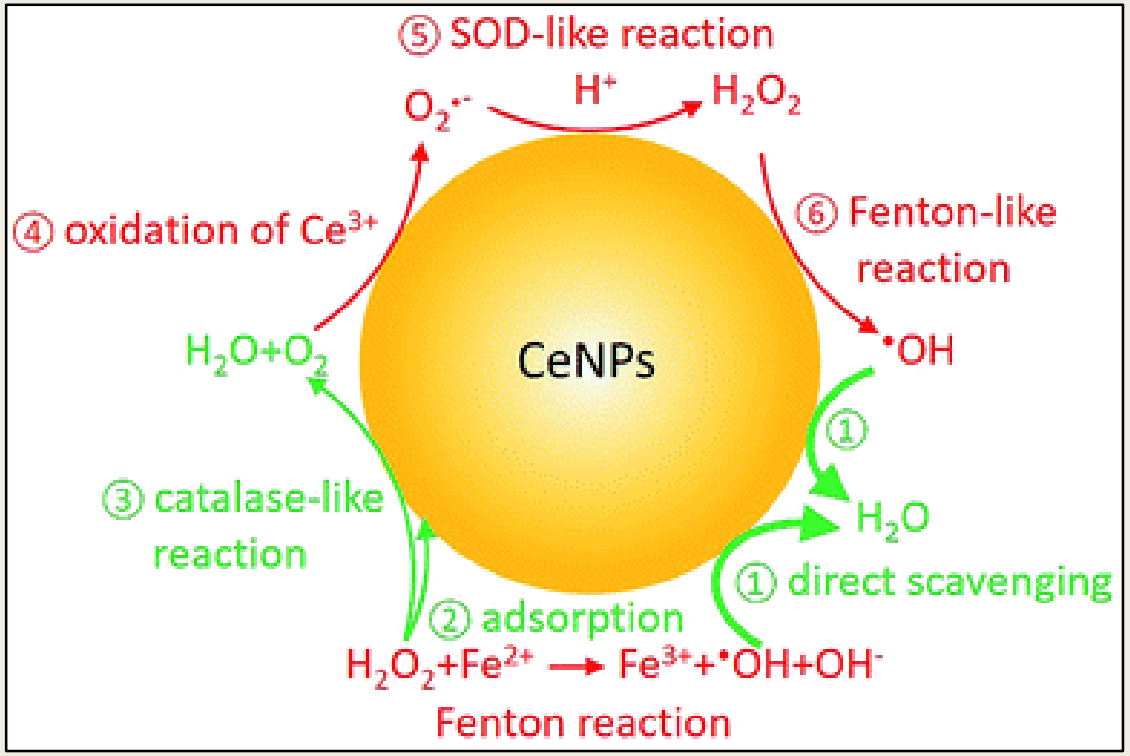
Antioxidants have been proposed to counteract oxidative stress in this disease

Cerium oxide nanoparticles (CeO_2 NPs = nanoceria)

The ability of CeNPs in switching the oxidation state of Ce3+ and Ce4+ makes it a good antioxidant candidate



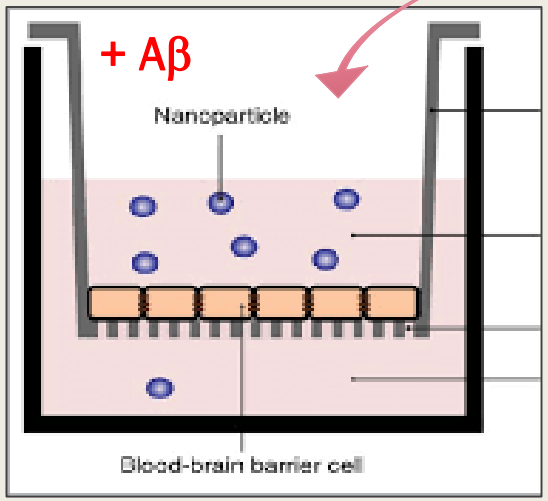
Cerium exists in two different oxidation states and their interchangeability makes them regenerative.



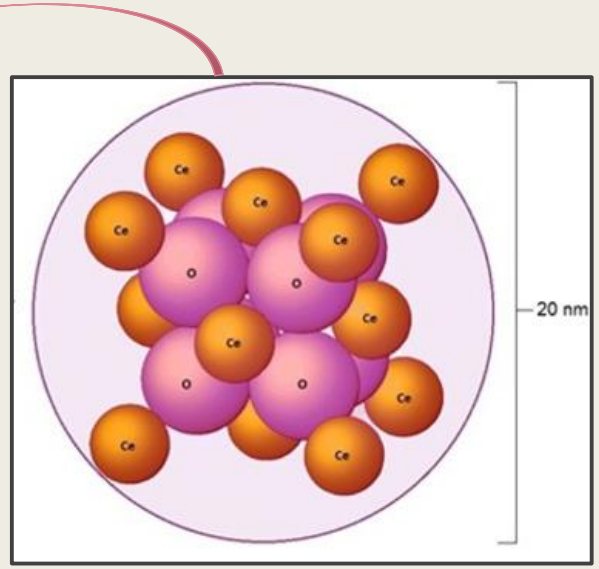
Most of interest is related to the self-regenerating mechanism of CNP anti-oxidant activity, which results from the continuous shift between Ce3+ and Ce4+.

AIM OF THE STUDY

To investigate the potentiality of CNP in controlling the Aβ-induced oxidative stress on human cerebral microvascular endothelial cells



In vitro AD-like BBB model (hCMEC/D3 cells)



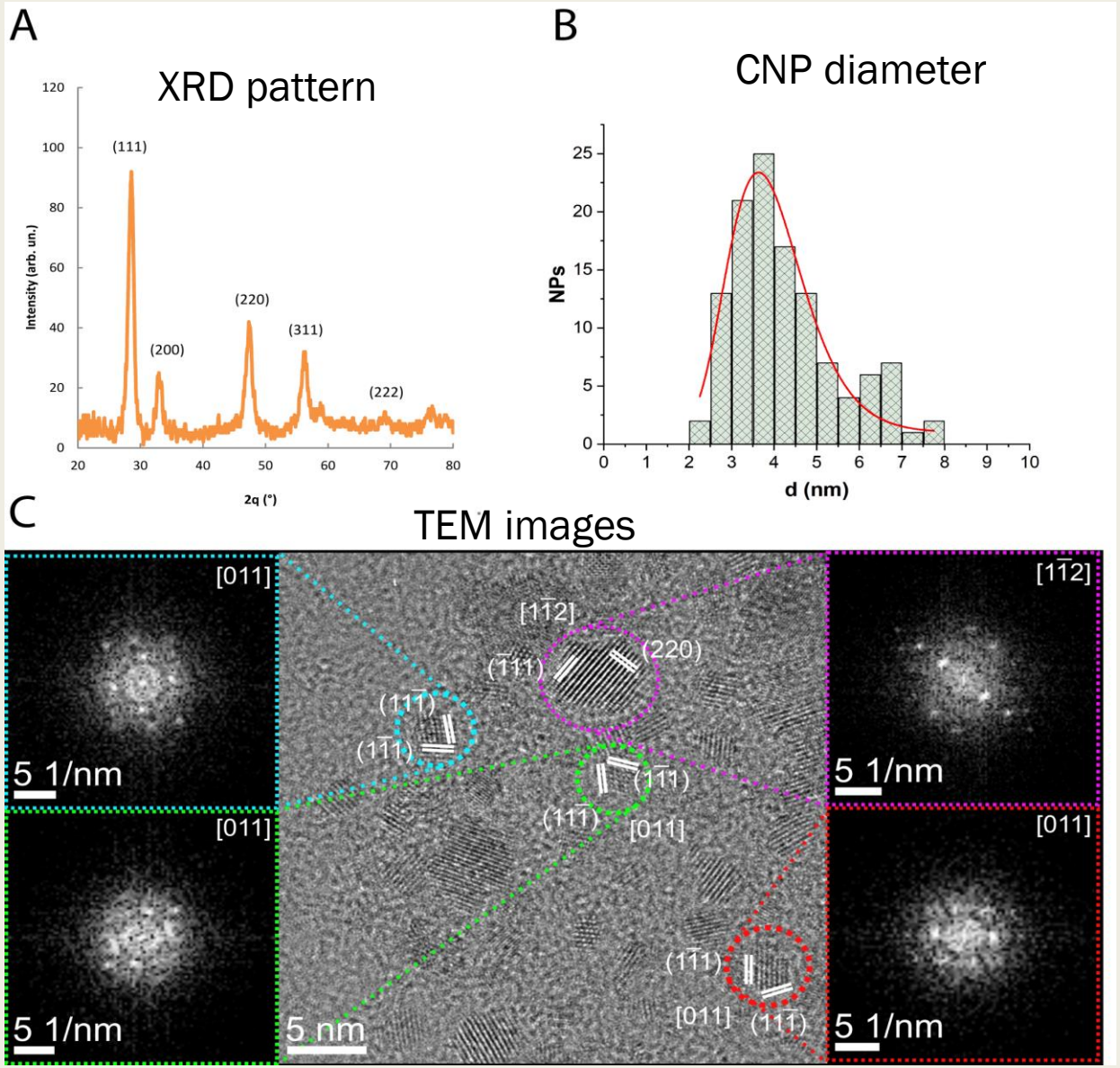
Polyacrylic acid (PAA)-coated CeO_2-NP

- CNP biocompatibility
- CNP free radical scavenging activity
- CNP cellular uptake

Features of CeO_2 NPs

Department of chemistry
Prof. Sommi
Prof. Anselmi-Tamburini

UNIVERSITÀ DI PAVIA

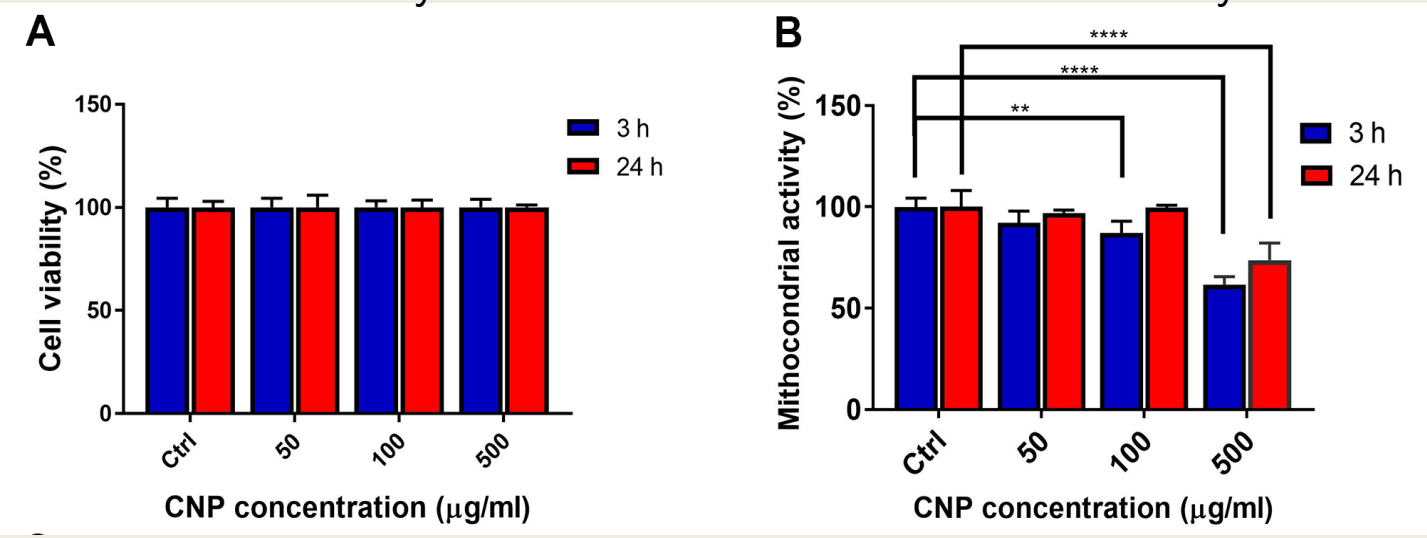


- CNP were obtained by direct precipitation from aqueous solution in presence of an excess of PAA
- Size by XRD = 4.5 nm
- Hydrodinamic diameter by DLS = 15 nm

Biocompatibility of CeO_2 NPs

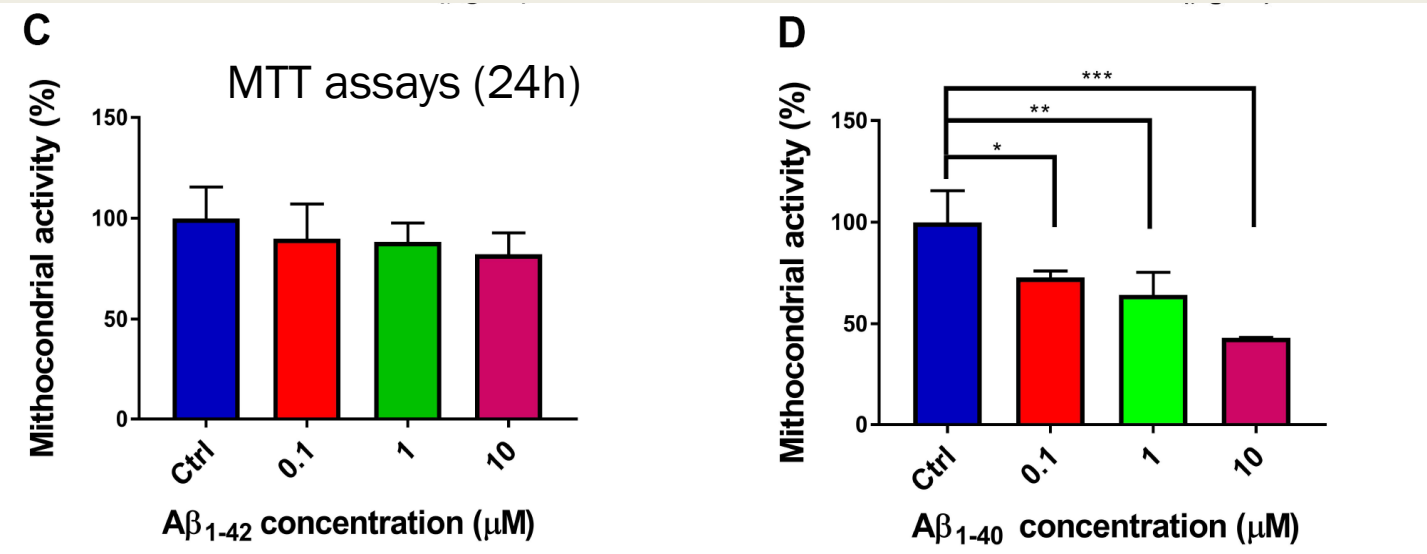
LDH assay

MTT assay



hCMEC/D3 cell viability after incubation with CNP

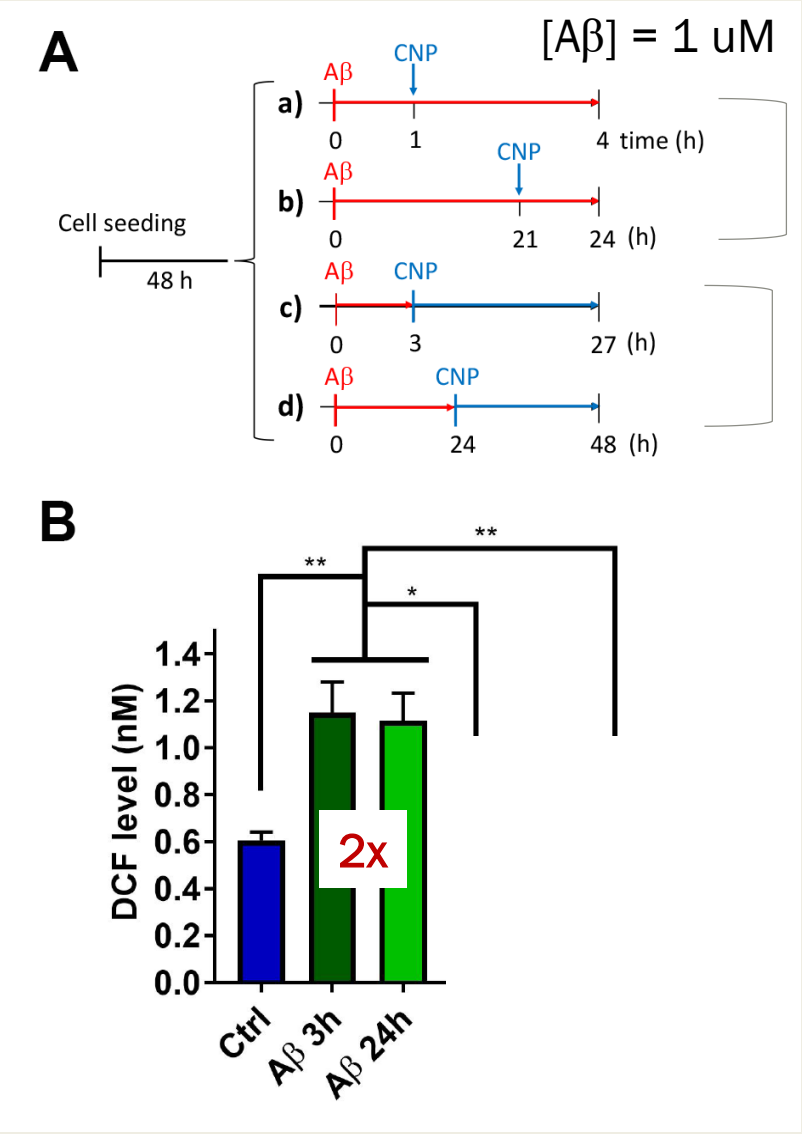
→ **50 ug/ml**



hCMEC/D3 cell viability after incubation with Aβ 42 or 40

→ **1 uM**

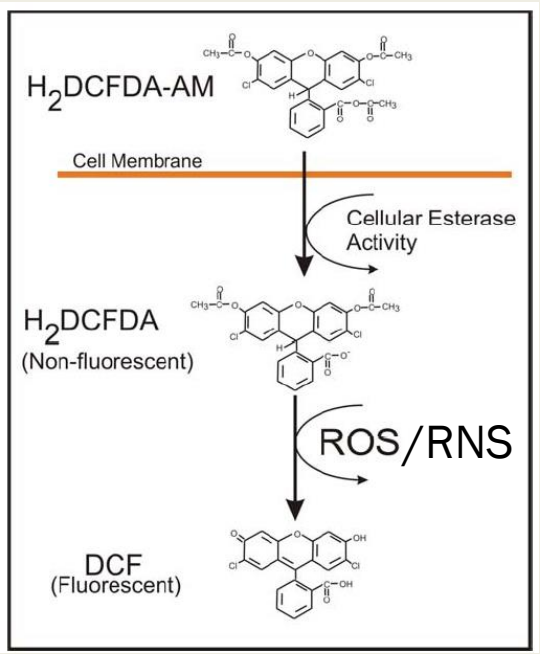
Scavenging activity of CeO_2 NPs



Co-treatment CNP-Aβ

Pre-treatment with Aβ

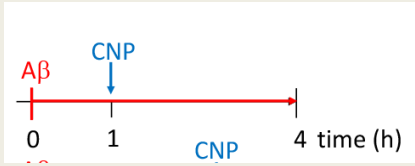
DCF assay

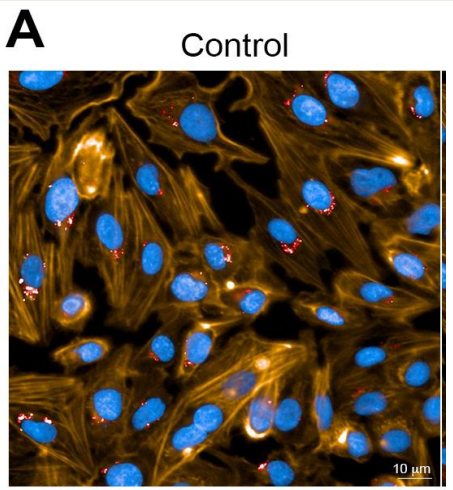


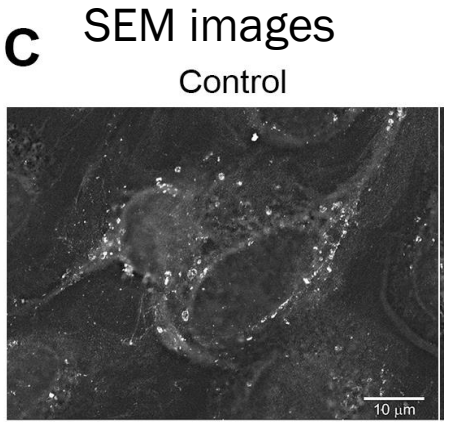
- 1 μM Aβ induced ROS/RNS production
- treatment with CNP leads to a significant reduction of ROS/RNS production by hCMEC/D3 cells after exposure to Aβ

Uptake of CeO_2 NPs

Fluorescent (DiI)-labelled CNP







- after 3 h of incubation, CNP were internalized by hCMEC/D3 cells and gathered mainly in the perinuclear region
- an increased internalization of CNP was detected in cells exposed to 1 µM Aβ1-42, compared to untreated cells.

ces some modifications in brain
enhance the internalization of CNP

hCMEC/D3 microvilli-like protrusions

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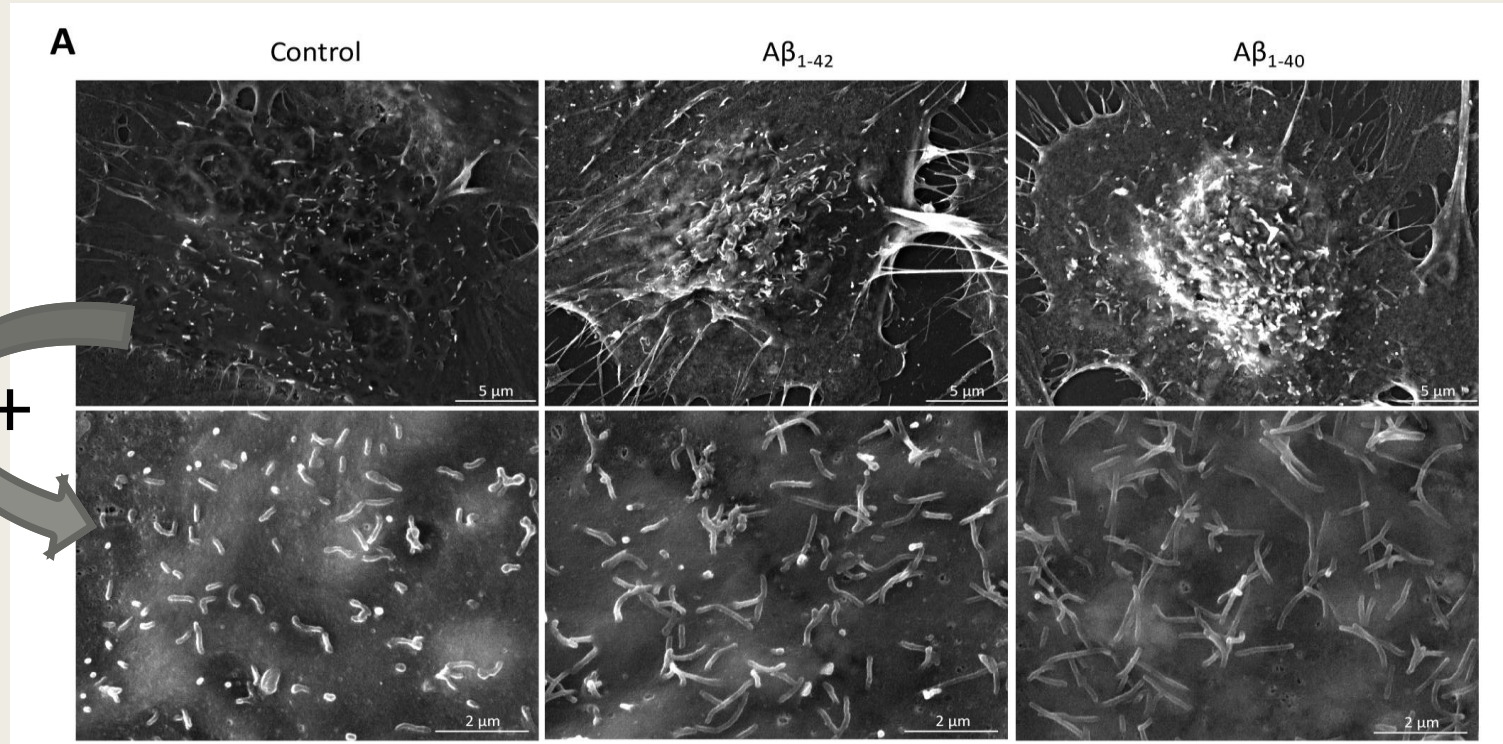
King Abdullah University of Science and Technology

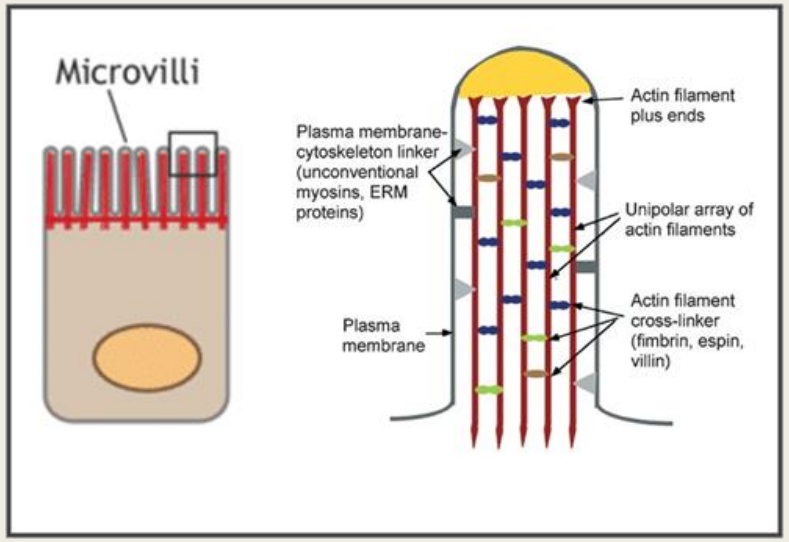
Dott. Alberto Casu
Dott. Andrea Falqui

SEM images

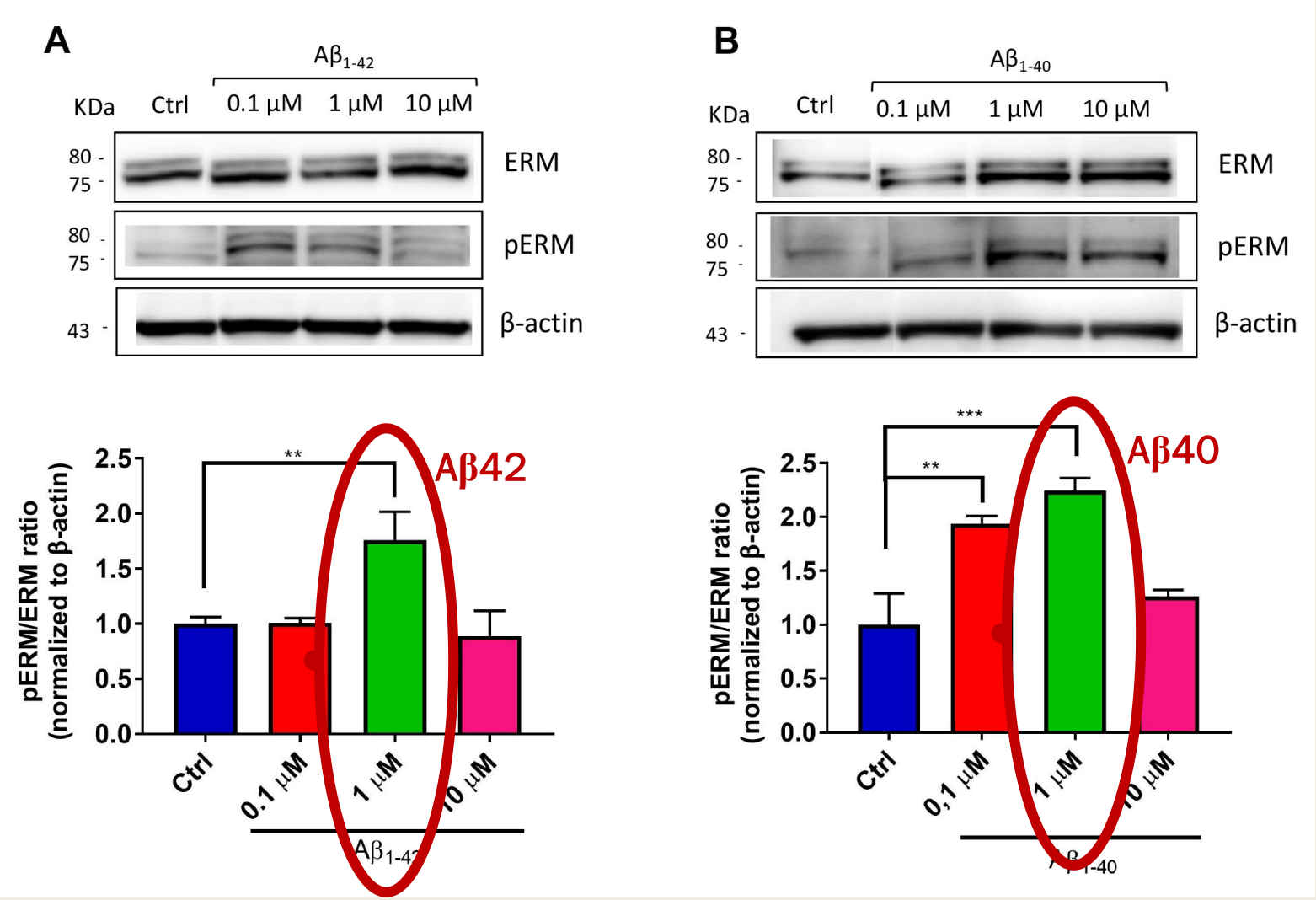


- No changes in microvilli density on hCMEC/D3 surface (0.5 microvilli/µm^2)
- The patter of microvilli changes after incubation with Aβ.

ERM = ezrin-radixin-moesin protein complex

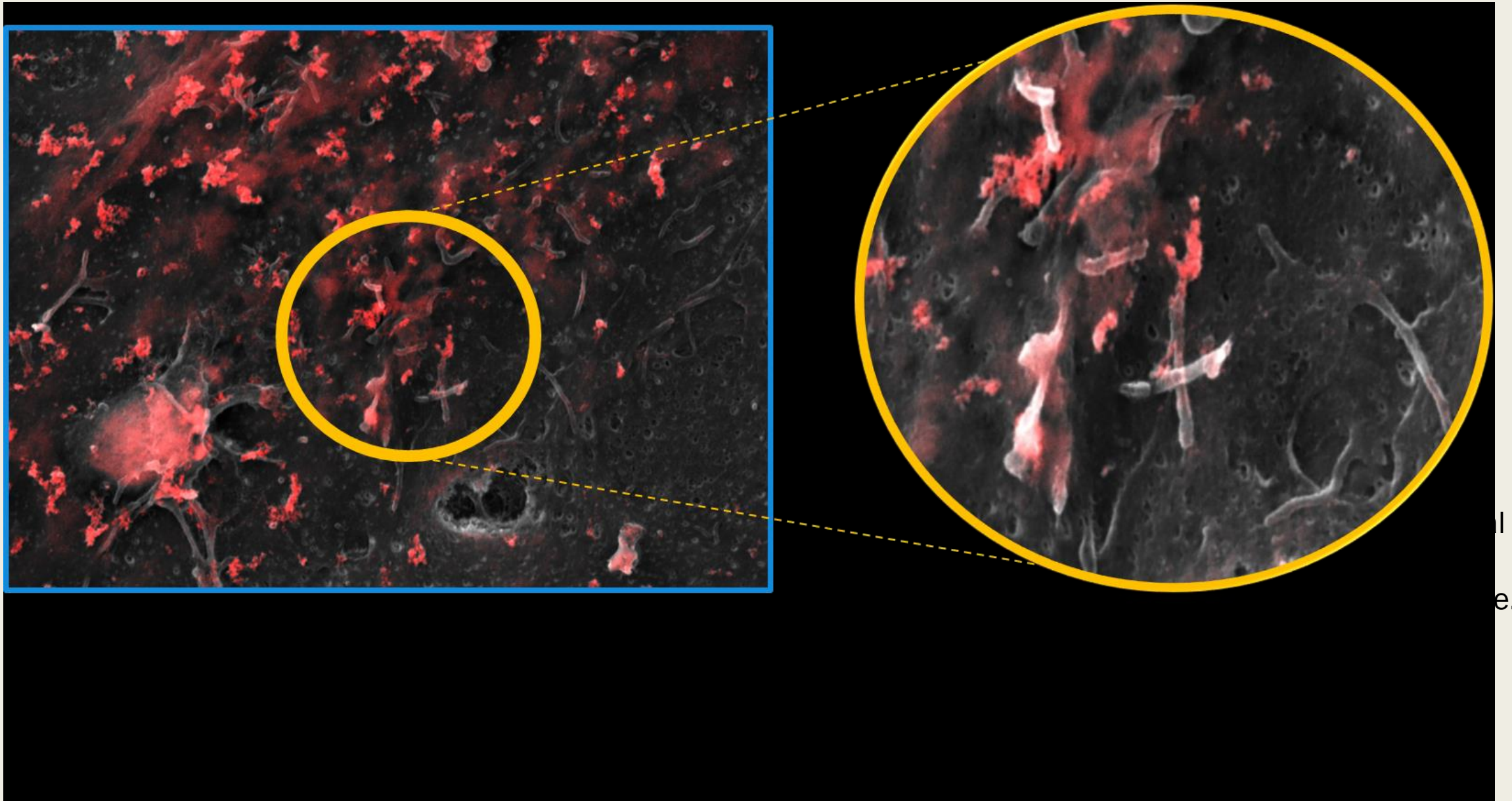


Detection of pERM/ERM as markers of microvilli

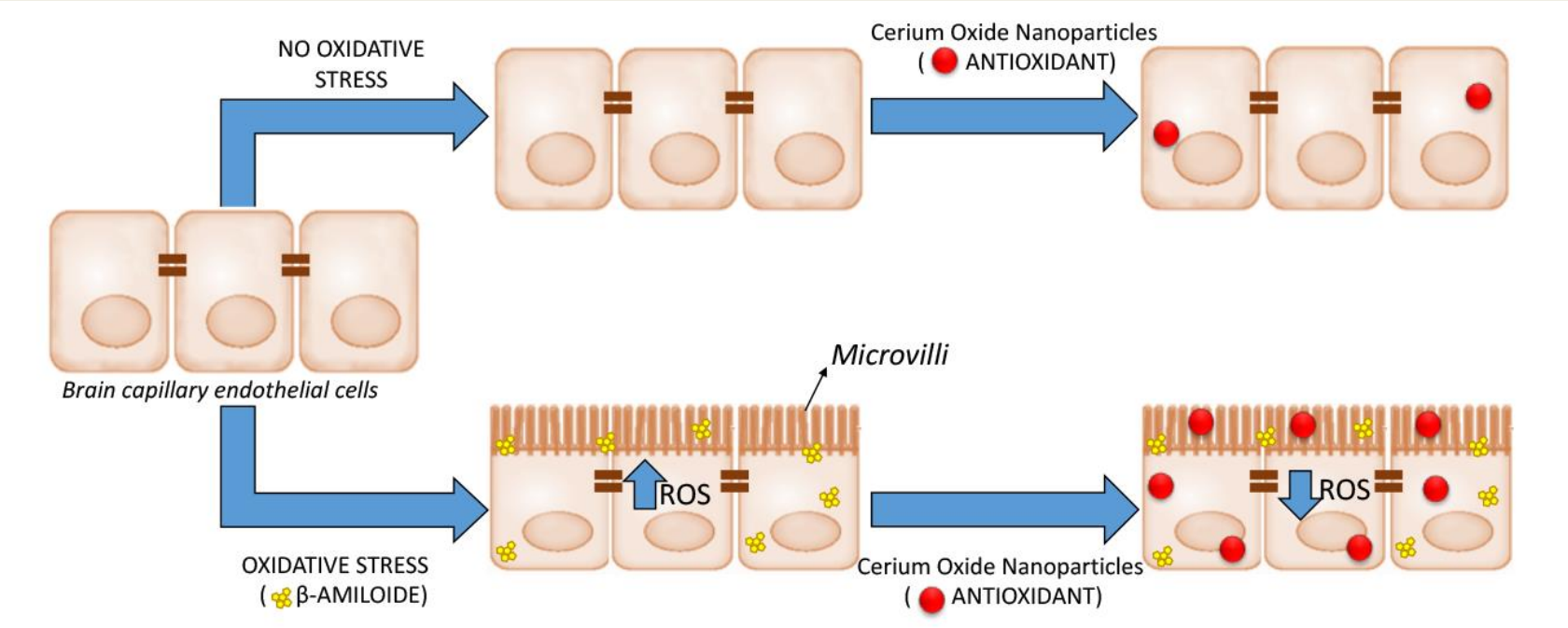


The exposure of hCMEC/D3 cells to Aβ induces a significantly increase of pERM/ERM ratio of 1.4-fold for Aβ1-42 and of 1.6-fold for Aβ1-40, compared to untreated cells.

Microvilli-Aβ-CNP interactions



Take home message



Conclusions

This opens the possibility to exploit endothelial microvilli formation under oxidative stress

to boost the uptake of anti-oxidant particles at the vascular level

as potential therapy for ROS-mediated (cerebro)vascular dysfunctions

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Thanks for your attention