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| ESRF | **Experiment title: Study of the capacity of CeO nanoparticles to reduce the oxidative effects of Alzheimer's amyloid peptide aggregates in cell culture by microFTIR and microXRF.** | **Experiment number**: LS-2777 |
| **Beamline**: ID16B | **Date of experiment**: from: 7 May 2018 to: 12 May 2018 | **Date of report**: 20/09/2022 |
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**Report:** Alzheimer Disease (AD) is characterized by the presence of Neurofibrillary Tangles (NFT) and Senile Plaques (SP). The pathological hallmarks are the formation of extracellular deposits of amyloid aggregates in the central nervous systems affected by AD. The main components of SP are the fibrillar aggregates formed by Aβ(1-40) and Aβ(1-42). In vitro studies show that these fibrillar structures are formed at physiological pH following a nucleationdependent polymerization process where at a certain concentration of monomeric peptides they interact forming oligomers that finally aggregate forming fibrils. According to the current prevalent idea, it is believed that the more toxic agents are the non-fibrillar aggregated species, thus, the oligomeric species. Among these toxic species, our group has described the formation of other non-fibrillar structures, the amorphous aggregates also called Granular Non-fibrillar Aggregates (GNAs) which can be formed at acidic pH or near negatively charged biological membranes. In addition, another characteristic of AD is the presence of increased lipid peroxidation observed on the amyloid aggregated area. It has been reported in human AD tissue samples and PC12 cultured neuronal cells incubated with amyloid aggregates in our group (Benseny et al. 2014, Pereira Mater Thesis, Benseny et al., 2018). Hence, the study of peptide aggregation and oxidative stress levels may be used as markers for the evaluation of potential antiamyloidogenic and antioxidant compounds on AD models.

**Objective**: Evaluate the potential antioxidant effect of CeO2 nanoparticles (CNPs) and its consequent ability to prevent/ameliorate the deleterious effects of the amyloid peptide aggregates on a cellular neuronal cell line model (SHSY-5Y), since these nanoparticles have been previously described as promising agents in the therapy of several diseases involving free radicals or oxidative stress (Ferraro et al., 2017).

**Materials and methods**: The neuronal cell line SHSY-5Y (from neuroblastoma) grown at 70% confluence was incubated for 48 hours with the different treatments indicated below:

- SHSY-5Y cells incubated with buffer at pH5.5 and pH 7.4.
- SHSY-5Y cells treated with 25 μM Aβ(1-40) pre-incubated O/N at pH 5.5 (formation of GNAs).
- SHSY-5Y cells treated with 25 μM Aβ(1-40) prepared at pH 7.4 (formation of oligomers).
- SHSY-5Y cells treated with 25 μM Aβ(1-40) pre-incubated O/N at 37ºC, pH 5.5 (formation of GNAs) in the presence of 2 μg/mL of CNPs.
- SHSY-5Y cells treated with 25 μM Aβ(1-40) prepared at 7.4 (formation of oligomers) in the presence of 2 μg/mL of CNPs.

- SHSY-5Y cells treated with 2 μg/mL CNPs at pH 5.5 and pH 7.4.

The final CNPs concentrations added to the cell medium were chosen after an MTT toxicity test that revealed that 2 μg/mL was the highest concentration not toxic for this cell line, whereas for HeLa cells was reported to be 200 μg/mL (Ferraro et al., 2017).

**Results:** To detect the areas with Aβ(1-40) aggregates microFTIR at ALBA synchrotron was used. The analysis of the infrared data with PCA performed on the region of the Amide I to determine the peptide aggregation levels shows that the results obtained corresponding to the aggregation of the peptide compared to the control groups are similar to the ones previously reported by our group for the PC12 neuronal cell line (Benseny et al., 2018), validating the model of study for this cell line. When analyzing a possible effect of the CNPs using these experimental conditions on the aggregation state we found that no significant differences can be observed between cells treated with aggregates and cells treated with aggregates together with the CNPs. This conclusion was obtained for both amyloid aggregates: amorphous or oligomers. When the spectroscopic ratios of aggregation ($A_{1627}/A_{1665}$) and lipid oxidation ($A_{1740}/A_{2925+2960}$) shown that when cells are treated with amyloid oligomers (Aβ peptide prepared at pH 7.4) there is no lipid oxidation, whereas when cells are treated with amorphous aggregates (incubated at pH 5.5) the oxidation ratio is significantly higher than the control one, both in the presence or absence of nanoparticles.

The studies performed in X-ray fluorescence microscopy of the same samples in ID16-B in ESRF showed that the CNPs co-localized with the aggregates (Figure 1).

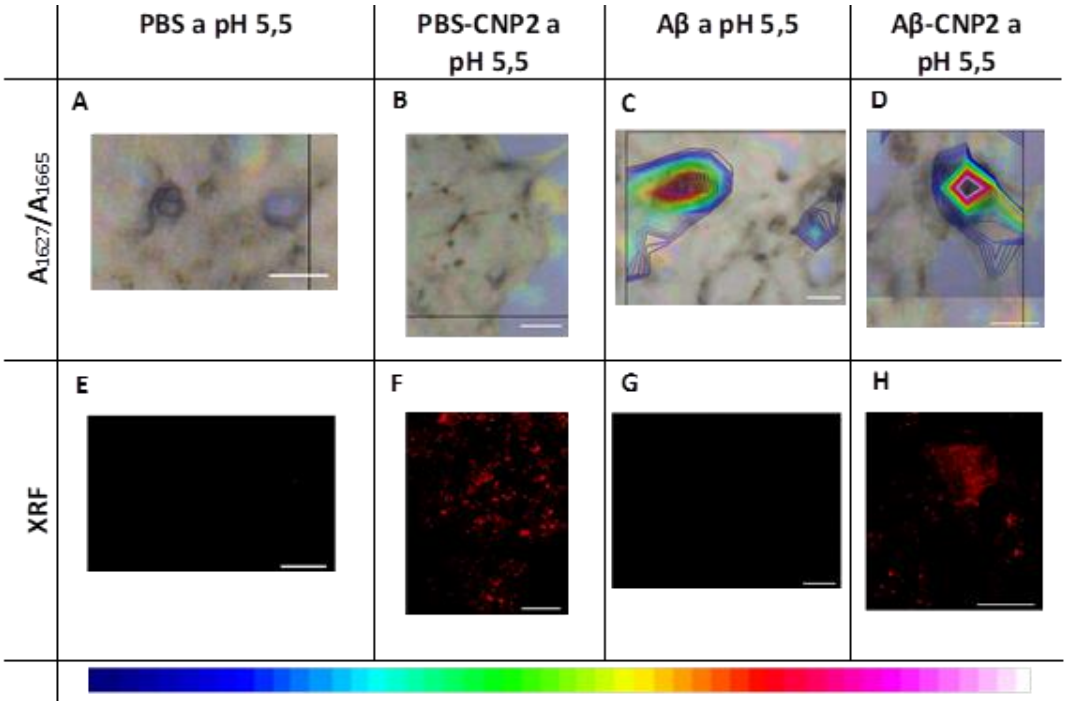


Figure 1. Infrared and XRF maps of the cells incubated with (A, E) PBS at pH 5.5; (B, F) PBS at pH5.5 with CNP;(D, H) Aβ40 at pH 5.5 and (D, H) peptide Aβ40 at pH 5.5 with CNP. The first row shows the amyloid aggregation ratio detected using the ratio ($A_{1627}/A_{1665}$) from IR data and the second row show the Ce fluorescence from XRF data.

So, although at the conditions studied CNP are not able to reduce amyloid oxidation it is clear that this molecules accumulate in amyloid aggregates. A different functionalization of the nanoparticles or way of administration could be used to change their action and even be directed to a particular cell compartment. For instance, the conjugation of cerium nanoparticles with Levan has been proved successful reducing ROS levels (Kim et al., 2016). Thus, further studies could be done in this direction. On the other hand, we can also conclude that the use of SH-SY5Y cells treated with pre-incubated amorphous aggregates or oligomers is a good AD model to test antiamoloidogenic and antioxidant agents, and in the future an improved CNP system could be tested using the same AD cellular model.

The results of this experiments were included in Sthefany Ortiz master thesis at UAB (2018).

**Bibliography**

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