Development and permeability evaluation through BBB-on-a-chip model of Gold nanorods with therapeutic potential for Alzheimer's disease

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by a progressive loss of cognitive capacity and memory. AD is mainly associated to two neuropathological processes: hyperphosphorylation of tau protein (P-tau) and the accumulation of toxic aggregates of amyloid β peptide (Aβ) in the brain. In pathological conditions, Aβ peptide is synthetized from a transmembrane protein (APP) which is cleavage by the beta and gamma secretase enzymes [1]. Then, the Aβ peptide is aggregated in oligomeric species, fibers and amyloid plaques which produce oxidative stress and neurotoxicity [2].

In the last years, multiple efforts have been performed in order to develop new molecules for AD's treatment based on the disaggregation of Aβ cumulates [1]. However, most of them do not reach the action site due the strict permeability in the brain by the blood brain barrier (BBB). By the way, nanotechnology is an attractive field that offers several alternatives for the treatment and diagnosis of AD. Specifically, a previous work reported gold nanorods (GNRs) functionalized with polyethylene glycol (PEG), a peptide that acts as a β sheet breaker (D1 peptide) and other one to allow the shuttling through the BBB (Angiopep-2) by LRP1 receptor present in the brain endothelium. The results revealed that the nanosystem (GNRs-PEG-Ang2/D1) performed Aβ growth inhibition *in vitro*. Remarkably, this effect was enhanced by irradiation due GNRs present photothermal properties. In addition, the nanosystem decreased the toxicity of Aβ aggregates in a *Caenorhabditis elegans in vivo* model [2].

Therefore, promising therapy/diagnostic agents are being developed and need to be evaluated quickly and easily for the early provision of new alternatives for AD. BBB-on-a-chip is an interesting alternative due their versatile, controlled, repeatable and lower cost design to mimic both *in vivo* physiological and pathological conditions for the study of drug permeability, disease progression, efficacy of treatment and others [3].

In the present work, we proposed the development of GNRs-PEG-Ang2/D1 and evaluate its permeability in a BBB-on-a-chip model which allows a 3D arrangement closer to the biological structure and tuning different flow conditions.

For this, we synthetized GNRs by a seed-mediated growth method and then were conjugated with PEG, Angiopep-2 and D1. These nanosystems were characterized by dynamic light scattering (DLS), zeta potential, electron microscopy (TEM) and UV-Vis-NIR spectroscopy. On the other hand, for the construction of the BBB-on-a-chip we will use brain endothelial cells and pericytes; therefore, the cytotoxicity of the nanosystems was determined by Annexin V/DAPI assay. Then, we expect to assess the cell uptake by flow cytometry. Finally, we will evaluate the permeability of the GNRs at different flow conditions in BBB-on-a-chip by atomic absorption and Nano tracking analysis (NTA). Also, the integrity of the BBB after GNRs administration will be assessed by immunofluorescence and trans-endothelial electric resistance (TEER).

From UV-vis-NIR spectroscopy of GNRs and its conjugates showed two absorption peaks about 520 and 710nm, directly regarding to this type of nanoparticles. Also, DLS and zeta potential revealed that the GNRs surface functionalization caused an increase in the hydrodynamic diameter and electrostatic change from positive to negative charge (+40 to -5 mV). Finally, electron microscopy displayed the shape and size of GNRs of length 40nm and 10nm width. On the other hand, these nanosystems did not show cytotoxic effects over endothelial cells and pericytes between 0.05 to 0.4nM for 24 hours.

We conclude that obtained GNRs-PEG-Ang2/D1 in a reproducible manner and they were not toxic for the endothelial and pericytes cells for BBB-on-a-chip. In future, we will evaluate the permeability of the GNRs at different flow conditions in BBB-on-a-chip and the integrity of the barrier after GNRs exposure.

References

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Figures

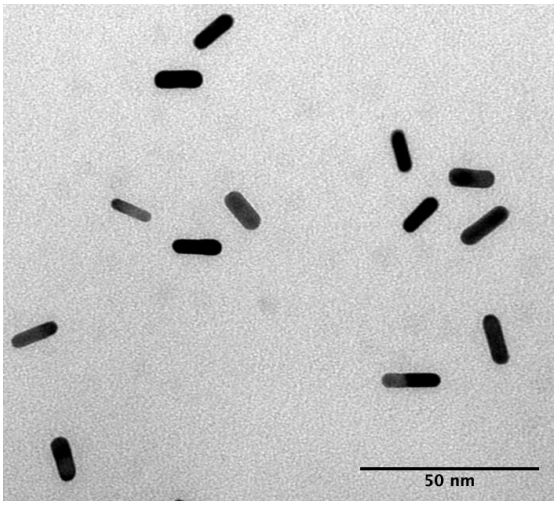

Figure 1. Electron micrograph of GNRs-PEG-Ang2/D1.