photothermal therapy (PTT) in comparison to CD44 low expressing cells (Thesc) and fibroblast cell line (NIH-3T3). The study characterized pegylated gold nanospheres (PEG-GNPs) and gold nanostars (PEG-GNSs) conjugated with an anti-CD44 antibody using UV-visible spectroscopy (UV-Vis), dynamic light scattering (DLS) and Bicinchoninic Acid (BCA). The biocompatibility of both nanovectors were assessed using 2D and 3D cell models through MTT assay. Receptor recognition and internalization were confirmed using confocal microscopy (CLSM) and inductively coupled plasma-mass spectrometry (ICP). Finally, the lines treated with the conjugates, were exposed to an appropriate laser wavelength for PTT and cell viability was evaluated. The results from DLS, Zeta potential, UV-Vis analysis, dot blot, and BCA studies confirmed the antibody conjugation to both types of PEG-GNPs and PEG-GNSs. The viability assays demonstrated the biocompatibility of the conjugates with all three cell lines in 2D cell model. CLSM and ICP data revealed that the nanoconjugate recognized the receptor on Z12 cells better, resulting in higher internalization compared to the other cell lines. The nanosystem's PTT was observed *in vitro* only on Z12 cells, as compared to Thesc and NIH-3T3. Similar results were also obtained with the 3D model, in where both nanovectors were able to recognize the cells embedded into hydrogel and their PTT was observed only in Z12 cells compared to the other two cell lines. Further investigations are required to assess toxicity and the effect on endometriotic cells that overexpress CD44.

P-35-034
Effect of curcumin on platelet activation and ROS production induced by physiological and pathological agonists

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Platelets are key players in the processes of haemostasis and thrombosis. Recent evidence showed that platelets are also involved in Alzheimer's disease (AD) [1]. Fibrillar amyloid peptides, which accumulate in senile plaques in AD patients, are also present in cerebrovascular vessels and has been shown to induce platelet activation, aggregation, and ROS generation increasing the risk of vascular complications. Different natural compounds, such as curcumin, have been extensively used to treat human diseases for their anti-inflammatory, anti-oxidant, and anti-amyloidogenic effects [2]. The aim of this study is to investigate the possible effects of curcumin on platelet activation and oxidative stress induced by physiological agonists and by pathological amyloid peptides. Amyloid peptides Aβ40 and Aβ42, and corresponding scrambled peptides, were diluted in phosphate buffered saline at 37°C for 24 hours to promote amyloid fibril formation. Platelet aggregation was analysed by light transmission aggregometry, and protein phosphorylation was evaluated by immunoblotting with specific phospho-antibodies. Platelet intracellular ROS release was measured on washed platelets using H2DCFDA dye by flow cytometry. 25 μM curcumin reduced platelet aggregation induced by fibrillar amyloid peptides and standard agonists such as thrombin, GPVI agonist convulxin and TxA2 analogue U46619. Curcumin also diminished MAP kinases and phosphoinositide 3-kinase signalling pathways induced by fibrillar Aβ40 and Aβ42. Convulxin, thrombin and fibrillar amyloid peptides promoted significant intracellular ROS production in platelets, that is strongly reduced by curcumin. These results demonstrate the ability of curcumin to modulate platelet activation and ROS production induced by amyloid peptides and physiological agonists, paving the way to the use of curcumin in the treatment of cardiovascular disease and AD. *The authors marked with an asterisk equally contributed to the work. References: [1] Canobbio, I. et al. (2013) FEBS Lett 587(16):2606-11. doi: 10.1016/j.febslet.2013.06.041. [2] Bisceglia F. et al. (2019) ACS Chem Neurosci. 10(3):1420-1433. doi: 10.1021/acschemneuro.8b00463

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Optimisation and characterisation of different strategies for siRNA loading into exosome

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The use of therapeutic RNAs has opened up a new avenue in drug discovery; however, their application is limited by the lack of appropriate systems ensuring efficient delivery and tissue specificity. Exosomes are extracellular vesicles produced by the majority of eukaryotic cells and appear to naturally host endogenous RNAs [1]. Given their biocompatibility, nanosized scale, low immunogenicity, and surface engineering feasibility, exosomes represent interesting biological carriers to deliver therapeutic RNAs. However, RNA loading into exosomes remains challenging, with poor loading efficiency and lack of technology standardization [2]. Here, electroporation, sonication, and transfection were evaluated as methods to improve loading efficiency of a siRNA against GFP transcript into HEK293T cells-derived exosomes, compared to passive loading. 10 μg of exosomes (~7.5×10^{10}) were mixed with 100 pmol of siRNA. Each loading method was carried out, followed by a clearing step to remove unloaded siRNA. All methods showed improvement of siRNA loading into exosomes compared to passive loading, with differences that are currently being investigated by gel electrophoresis and digital-PCR. None of the techniques induced physical alterations of exosomes, evaluated by tuneable resistive pulse sensing (TRPS) and TEM analysis, or in the amount of exosome markers, measured by western blot. Notably, ~50% of GFP silencing was observed after treatment of stably GFP-expressing HEK293T cells with selected loaded exosomes, confirming their ability to efficiently embed and deliver siRNA. Further studies on the effects of loading on the endogenous RNA content of exosomes by transcriptomic analysis are in progress. Overall, this study paves the way to the development and validation of a loading technology for using exosomes as RNA delivery systems. Previously published in: [1] O'Brien K et al. (2020) Nat Rev Mol Cell Biol. 21:585-606. [2] Zeng H et al. (2023) Cells, 12:1416.

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ATM variants activation by endogenous ATM

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Ataxia telangiectasia (AT) is a rare neurodegenerative disease caused by biallelic mutations in the Ataxia telangiectasia mutated (*ATM*) gene, that codes for the protein of the same name ATM.