Nanostructured materials | Jožef Stefan Institute Ljubljana, Slovenia

SEMINAR III

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Bio-inspired nanocarriers for therapeutic nucleic acid delivery

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Despite many advances in gene therapy, the delivery of small-interfering RNAs (siRNAs) remains challenging. Liposomal and polymeric nanostructures have been used as siRNA carriers, leading to the approval of patisiran in 2017. However, the fast clearance and short plasma residency of artificial carriers necessitate finding a suitable, biocompatible alternative. Extracellular vesicles (EVs) are liposomal-like objects secreted by cells, but their laborious isolation and low yield hamper their clinical translation. Conversely, erythrocytes are the most abundant cells in the human body, and their membrane possesses unique features. We present a siRNA nano-carrier based on erythrocyte ghosts.

Human erythrocytes were depleted of hemoglobin via osmotic shock. Subsequently, they were extruded in the presence of 1-20 µg/ml of siRNA, and the resulting objects were termed EMVs (Erythrocytes Membrane Vesicles). An ultracentrifugation-based method was applied to select only the densest EMVs, i.e., those containing siRNA. Their stability at 4°C, cytotoxicity against multiple cell lines, ability to protect the cargo against RNAse A, particle size, and Z-potential were characterized. qRT-PCR was used to evaluate knockdown ability against tdTomato fluorescent protein in B16F10 cells in vitro, and in B16F10 tumor-induced mice as a single injection. EMVs' plasma residency time and biodistribution were assessed via TaqMan PCR and fluorescence microscopy of Cy-5-siRNA on the heart, brain, liver, kidneys, lungs, spleen, and melanoma-induced solid tumors. EMVs showed exceptional plasma residence time, excellent biocompatibility, and, at a dose of 2.5 mg/kg of siRNA, caused an 80% target gene knockdown after 48 hours.

It is important to note that the most widespread class of nanocarriers for nucleic acids are cationic lipids and polymers, with more than ten FDA/EMA-approved formulations. However, the high positive charge of such nanoparticles often leads to the activation of the mitochondrial apoptotic cascade, increasing cytotoxicity and hampering therapeutic effects. Genipin is a naturally occurring alkaloid from Gardenia jasminoides, capable of crosslinking un-protonated amines during prolonged reaction times (>36 h). Using genipin as a crosslinker, we developed a set of genipin-spermine-based nano-sized polymers with moderate positive charge, self-fluorescence, nucleic acid complexing ability, and mild cytotoxicity. We were able to tune the size of the nanoparticles and control their zeta potential by adding different molar ratios of glycine in the reaction batch. We termed our polymers GXS5, where X is the molar ratio of glycine-to-genipin, and 5 is the molar ratio of spermine-to-genipin in the initial reaction batch. They effectively transfected B16F10 murine melanoma cells with siRNA, with the best performer being G10S5.

Coating artificial nanoparticles with erythrocyte membranes is a widespread approach to create a "stealth" and biocompatible nanocarrier. Therefore, we are currently in the process of coating G10S5 nanoparticles with dipalmitoylphosphatidylcholine (DPPC)-doped erythrocyte ghosts.

Kindly invited.