Connectosomes for Direct delivery of siRNA into the Cytoplasm

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Introduction: The plasma membrane protects a cell from the extracellular environment. As such it presents an obstacle that therapeutics needs to traverse in order to achieve efficacy. For example, small interfering RNAs (siRNAs) need to be delivered to the cytoplasm, where they can interact with the RNA interference machinery and initiate gene silencing. However, these macromolecules have poor membrane permeability, largely limiting their therapeutic potential. To address this challenge, current strategies involve encapsulating siRNAs into nanoparticles. However, upon cellular uptake, these nanoparticles are trapped in endosomes, which lack access to the cytoplasm. Towards developing an alternative strategy that provides direct access to the cytoplasm, we have been inspired by the unique capabilities of gap junctions to establish passageways between the cytoplasm of neighboring cells. Specifically, six connexins hexamerize to form a connexon hemichannel. Two hemichannels from neighboring cells dock to each other to form a complete gap junction channel, facilitating the exchange of molecular cargoes such as ions and siRNA. Therefore, incorporating the gap junction network into therapeutic delivery materials has the potential to enhance the delivery efficiency of siRNAs by directly depositing siRNAs into the cytoplasm.

Materials and Methods: We began by genetically engineering retinal pigmented epithelial (RPE) cells to overexpress mRFP-tagged connexin 43 (cx43-mRFP). The expression of cx43-mRFP was examined using a confocal fluorescence microscopy and flow cytometry. To test the functionality of the gap junctions (A), a scratch loading assay was performed. Specifically, a scratch was made across a confluent layer of cells while Lucifer yellow dye was released. Damaged cells along the scratch can take up the dye, and over time the dye will spread to neighboring cells through functional gap junctions, forming a fluorescence gradient (B). Next, we extracted the plasma membrane of these cells to form Connectosomes, cell-derived vesicles with high concentration of connexon hemichannels (C and D). Finally, siRNAs were loaded into Connectosomes through electroporation.

Results and Discussion: The engineered RPE cells showed strong mRFP fluorescence on the plasma membrane, indicating successful expression of chimeric connexin. Using flow cytometry, we estimated that the average mRFP fluorescence of these cells was four times higher in comparison to the wild-type RPE cells. Further, spreading of Lucifer yellow dye in the scratch loading experiment suggested that chimeric connexins were able to assemble into functional gap junctions. Towards harvesting functional gap junctions, we extracted Connectosomes from the plasma membrane of the engineered RPE cells. Confocal fluorescence images showed clear mRFP fluorescence on the membrane of Connectosomes, suggesting the display of connexon hemichannels on the membrane surface. To load siRNAs into Connectosomes, we electroporated Connectosomes in the presence of 6-FAM labeled siRNA. The 6-FAM fluorescence inside Connectosomes following electroporation indicated efficient siRNA loading.

Conclusion: The results above demonstrate the formation of Connectosomes as delivery vehicles for siRNAs. Specifically, our results showed the expression and the incorporation of functional gap junctions into plasma membrane vesicles for the delivery of siRNAs. Moving forward, the effectiveness of this novel delivery strategy will be evaluated by measuring gene knockdown efficiency. More broadly, gap junction-mediated delivery can be extended to other classes of macromolecular therapeutics such as peptides.

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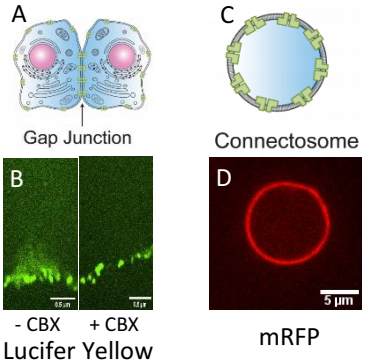

Figure 1: A) Schematic of cells overexpressing of connexin B) Fluorescent gradient produced from scratch loading assay with and without gap junction inhibitor CBX C) Connectosome with high expression of connexin D) Fluorescent image of Connectosome