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INVESTIGATION OF INTRACELLULAR DELIVERY OF THERAPEUTIC NUCLEIC ACIDS WITH THE HELP OF LIPID CONJUGATES AND DNA NANOSTRUCTURES

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Annotation. A big branch of therapeutic nucleic acid (TNA) research is correlated to developing stable and effective delivery systems for these gene therapy drug products to the targeted cells. While currently used delivery systems may exert significant toxicity for the surrounding tissues and face the significant obstacle of endosomal escape, bioconjugates are emerging as non-toxic alternatives with great potential in this field. We aim to investigate cholesterol integrated into DNA nanostructures as a delivery system for TNAs.

Introduction. DNA nanotechnology has shown significant advancements in gene therapeutics during the past decades. TNAs with unique properties, capable of gene modulation or suppression through RNA interference, have been developed. However, such TNAs still have to face significant obstacles in order to become useful in clinical practice. One of the most significant obstacles is the targeted cell delivery of TNAs. To address this problem, several technologies have been extensively studied and developed; nanotechnology carriers such as lipid and polymeric nanoparticles, chemical modifications, viral vectors, and so on. Nonetheless, most of these technologies still cannot achieve efficient delivery and exert high toxicity for the surrounding cells and tissues. Bioconjugates are in the spotlight as potential facilitators of cell delivery, as biomolecules have repeatedly demonstrated diverse functionalities, including effective drug delivery. Biomolecules as conjugates to TNAs can show high stability and minimal cytotoxicity, as they are highly biocompatible. The goal of this study was to use DNA nanostructure conjugates with cholesterol to achieve highly efficient delivery of TNA in human cells.

Main part. We chose to use cholesterol as the bioconjugate for the development of our stable and non-toxic Cholesterol-DNA nanostructure delivery system. Cholesterol acquires all the aforementioned advantages of bioconjugates. However, it has a critical disadvantage; the formation of hydrophobic intra-cholesterol interactions in aqueous environments. To surpass this, we designed a unique Cholesterol-DNA nanostructure in the shape of a dumbbell that will be able to protect the lipid from the aqueous environment by hiding it with the help of DNA loops while at the same time remaining available enough to develop interaction with the cell membrane. It is proven that cholesterol has the unique property of spontaneous penetration of lipid bilayers, a trait that distinguishes it from other conjugates, such as peptides. While conjugates such as peptides rely on a receptor-mediated mechanism to enter the cytoplasm, cholesterol can independently anchor on the cell membrane giving a chance for our nanostructure to diffuse through it. With this strategy, we aim to avoid the step of endosomal escape, which is a dead-end for most developing drug delivery systems. To achieve this, we carefully investigated the lipid-nucleic acid interactions in our nanoconstruct in order to select the optimal lipid-nucleic acid ratio that could lead to the desired interaction of our nanoconstruct with the cell membrane while avoiding micelle formation. For this, we designed three different variants of conjugated DNA nanoconstructs with varying numbers of cholesterol tags that would carry antisense oligonucleotides of different lengths.

Conclusions. This gene therapy agent delivery system was expected to prevent micelle formation and achieve immediate release of our TNA inside the cytoplasm. Three different variants of conjugated constructs were assembled to investigate the optimal cholesterol to nucleic acid ratio. In polyacrylamide gel electrophoresis, we observed that our nanoconstructs' design indeed prevented

micelle formation. However, these constructs did not display any positive results regarding effective delivery in cell culture experiments.

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