Nucleic Acid Nanocapsules for Enhanced Cytosolic Delivery of Therapeutic DNA-Surfactant Conjugates

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Purpose
Therapeutic nucleic acid delivery inside the cytosol after the successful bypassing of the lysosome is hard to achieve. Our Nucleic Acid Nanocapsules (NANs) are DNA-functionalized micelles that have been chemically designed to achieve enzyme triggered release of therapeutic nucleic acids with improved delivery into the cytosol of cells. As a biocompatible nanocarrier, NANs have demonstrated attractive properties such as prolonged resistance to degradation of nucleic acid cargo, receptor-mediated cellular uptake followed by endosomal escape, and the tunable delivery of bioactive DNA-surfactant conjugates. Additionally, upon further functionalization RNA modified NANs can be generated for a variety of therapeutic purposes.

Methods
We synthesized NANs starting from nontoxic small surfactant molecule based micelle formation followed by crosslinking it with target-enzyme responsive cross-linker. Finally we functionalized them with therapeutic DNA/ RNA and disease cell targeted RNA-aptamer to make it more relevant for real therapeutic purposes. Upon successful synthesis we studied their cellular uptake mechanism by commercially available inhibitors and tracked their pathways to bypass lysosome in details. Finally we estimated their cytosolic delivery efficiency followed by real therapeutic efficacy in terms of gene-knockdown. For these detailed studies, we used confocal microscopy-based optical imaging technique, flow-cytometry-based quantitative assays and PCR-based RNA evaluation methods in detailed. We also examined few parts of the therapeutic efficacy in in-vivo mice model.

Results
Here we confirmed caveolae as the key pathway involved in the cellular uptake of NANs using pharmacological inhibitors and we got further evidence for the importance of the surfactant in cellular entry. We also have evidence that the triggered degradation of NANs produces DNA-surfactant conjugates (DSCs) that is capable of passively entering the cytosol from the plasma membrane whereas NANs require energy dependent uptake mechanisms. Also we proved that even in presence of serum, our NANs are capable to bypass lysosome when the targeted enzyme acts as a trigger. Gene-knockdown is more effective when we construct NANs with C10 surfactant than that of other long chain surfactant. In vivo gene-knockdown results are also supporting our delivery efficacy by NANs.

Conclusion
Here we conclude that surfactant chain length in NANs construction is very important for better cytosolic delivery. Also, crosslinking plays a role in endosomal escape due to enzyme cleavable properties. Due to its lipophilic character and receptor mediated uptake, our NANs is a unique cytosol targeted nano-capsule to deliver therapeutic DNA/ RNA for better therapeutic purposes. In vivo efficacy of NANs is also noticeable.

Keywords: Nucleic Acid Nanocapsules, Cytosolic Delivery, endosomal escape, Therapy