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# Assessing Lipid Nanoparticle Protein Corona Formation and Cytocompatibility

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SUMMARY

Lipid nanoparticles (LNPs) represent an emerging new modality for mRNA delivery. Following administration and interaction with blood constituents, LNPs form a corona complex consisting of proteins adsorbed on the surface altering their stability, biological identity, and fate. Cytocompatibility of the LNPs is an important factor when considering their safety efficacy in delivering the encapsulated drug dose, lipid choice and the specific target cells. The aim of this study was to investigate the changes in LNP physical parameters in physiologically-relevant media. Key attributes such as particle size, polydispersity index and zeta-potential were measured using Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). Cytocompatibility was assessed via CellTiter-Glo® assay. Following 24-hour incubation of LNPs with Bovine Serum Albumin (BSA), the LNP z-average increased from 92.4 (± 49.0) nm to 131.4 (± 64.9) nm indicating interaction between LNPs and BSA. A decrease in percentage cell viability was demonstrated with increased lipid concentration for MCF-7 and A549 cell lines. This work shows changes in LNP physicochemical properties in the presence of protein and biologically relevant conditions consistent with protein surface adsorption. The cytocompatibility of LNPs can be associated with the type of lipids used in the synthesis of LNPs.



## INTRODUCTION

Lipid nanoparticles (LNPs) are emerging new modalities for the delivery of therapeutic mRNA. Upon intravenous administration of the LNPs, they interact with constituents in the biological fluid forming nanoparticle-protein corona complexes (Capriotti *et al*, 2014). This complex has a new biological identity with different physicochemical changes in size, shape and surface charge altering their chemical identity and biological fate. These physicochemical changes can be analysed through initial low resolution analytical methodologies followed by orthogonal high resolution analysis of the corona complex.

The primary requirement of a drug delivery system is non-toxicity to cells. The composition of the LNP can be altered to improve biocompatibility and decrease immunogenicity and toxicity. The *in vitro* cytocompatibility profile of these carriers play a major role in determining their safety and use for clinical application.

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The aim of this study was evaluating changes in LNP physical parameters in physiologically-relevant media and its cytocompatibility profile following interaction with cells.

## MATERIALS AND METHODS

Poly(A) LNPs were manufactured using 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP): 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC): Cholesterol: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000) at a molar ratio of 50:10:38.5:1.5 mol%. Key attributes were measured for following incubation with Bovine Serum Albumin (BSA) at 37 °C.

Cell cytocompatibility measurements were carried out using Michigan Cancer Foundation-7 (MCF-7) breast cancer and alveolar-549 (A549) cell lines. Cell viability was determined after 24 hours incubation at 37 °C with the CellTiter-Glo® assay.

## RESULTS AND DISCUSSION

The intensity based distribution in Figure 1 shows an increase in size evident of the free LNP compared to the corona complex. This size increase is due to the formation of non-covalent and hydrophobic interactions between the BSA constituents and LNPs.

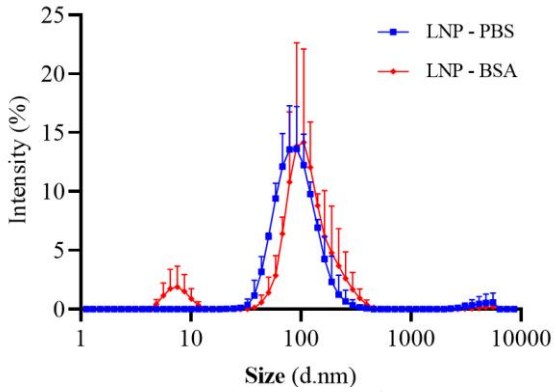


***Fig.1.*** *Size distribution by intensity for Poly(A) DOTAP LNP incubated for 24 hours with Bovine Serum Albumin (BSA). Results are expressed as mean ± standard deviation (n=3).*

The results in Figure 2 indicate a reduction in percentage cell viability with increased LNP concentration for both cell lines.

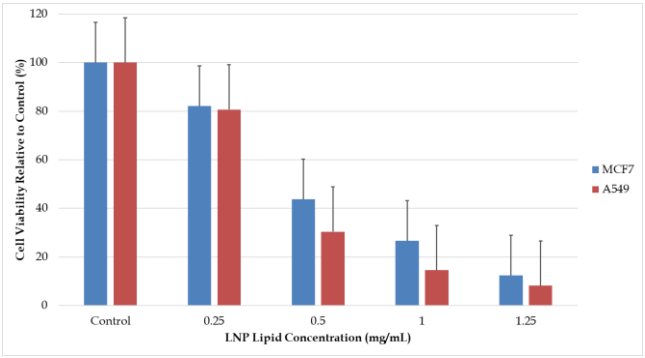


***Fig.2.*** *Effect of different Poly(A) DOTAP LNP concentration (0 – 1.25 mg/mL) on viability of MCF-7 and A549 cells following 24-hour incubation. Results are expressed as mean ± standard deviation (n=3).*

The target dose of LNP will expose target cells and lipids may impact the cytocompatibility of cells *in vitro*. DOTAP can be cytotoxic to cells as it interact with protein kinase C enzymes which are involved in controlling the function of other cellular proteins (Ly *et al,* 2006).

## CONCLUSIONS

Our preliminary work demonstrates changes in LNP properties in biologically relevant conditions. Incorporating the early assessment of LNP interactions with biomolecules can support a more fundamental understanding of the biological fate of LNPs. Cytocompatibility of LNP prototypes will be tested on the cell lines used in this study in addition to other cell lines such as human monocytic cell line THP-1.

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## REFERENCES

Capriotti, A.L., et al., 2014. Analytical Methods for Characterizing the Nanoparticle-Protein Corona. Chromatographia, 77, 755-769.

Lv, H., Zhang, S., Wang, B., Cui, S. & Yan, J, 2006. Toxicity of cationic lipids and cationic polymers in gene delivery. J. Control. Release , 114, 100–109.