A functional study of tubing material for the spray drying of mRNA-lipoplexes

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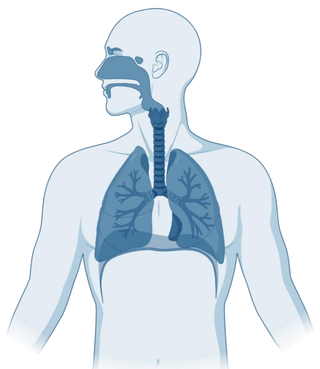
INTRODUCTION

- The overall aim of this project is the development of an inhalable dry powder platform for mRNA vaccines.
- As many viral infections enter the body through the respiratory tract, an application of mRNA vaccines directly into the lungs might lead to more effective protection.
- Liquid vaccines face the risk of chemical degradation and physical instability[1], a solid formulation might secure stability without challenging storage conditions.
- DOTAP:DOPE is a safe transfection agent for human administration[2] and was used as lipoplex builder. The lipoplex composition and transfection efficacy was determined in a previous study[3].
- In the first spray drying experiments decreased transfection capabilities of the processed mRNA-lipoplexes compared to control were observed.



➡ We focused on each individual step of the process: pumping, spraying, drying

RESULTS

1. Spraying and pumping of naked mRNA and Lipoplexes using a silicone tube

The transfection efficiency of naked mRNA to which DOTAP:DOPE was added after pumping was still above 75% of the transfection efficiency of untreated lipoplexes (Figure 1). This indicates that mRNA integrity as such was not affected by the pumping process.
In contrast, the sprayed naked mRNA solution showed very low transfection efficiency. The reason for this observation could be that the naked mRNA did not survive the stress of the spraying process or the impact on the surface of the sampling vessel.
However, the preformed lipoplexes could not be transfected to a sufficient extent after either pumping or spraying. The pumping process disrupted the functional integrity of the lipoplexes.

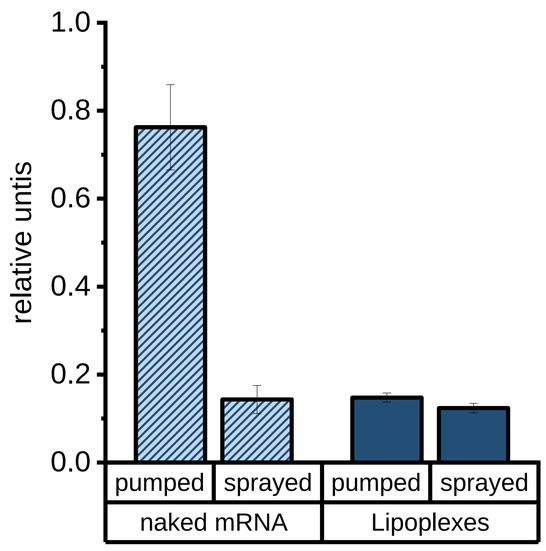


Figure 1 - Transfection efficiency after pumping and spraying (silicone tube): Luminescence in RLU/mg protein of sample normalised to untreated lipoplexes. A value of 1 relative unit represents an equal transfection efficiency for the pumped or sprayed samples and the untreated lipoplexes. Error bars show SD, n = 3.

➡ possible reason: adsorption of lipoplexes to the silicone tube

2. Pumping of naked mRNA and Lipoplexes using two alternative tubes

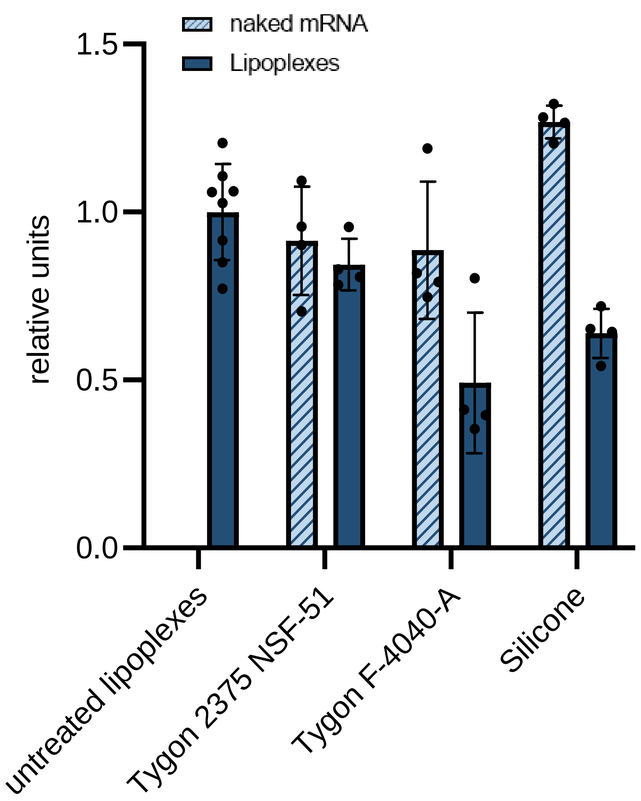


Figure 2 - Transfection efficiency of naked mRNA and lipoplexes after flowing through the tubes: Luminescence in RLU/mg protein of sample normalised to untreated lipoplexes. A value of 1 relative unit represents an equal transfection efficiency for the pumped or sprayed samples and the untreated lipoplexes. Error bars show SD, n = 4.

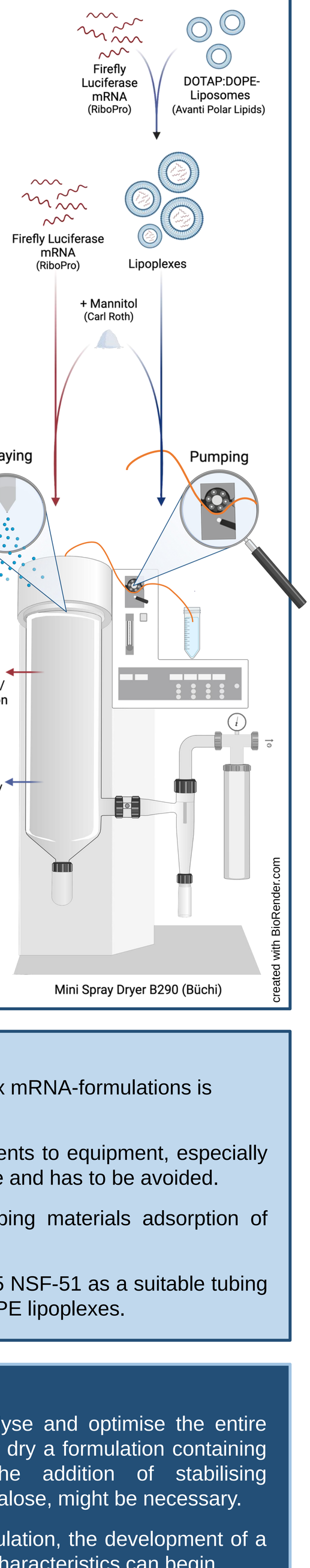
Three tubes were tested with naked mRNA and lipoplexes (Figure 2):
- Tygon® F-4040-A (TPE)
- Tygon® 2375 NSF-51 (TPO)
- silicone tube

The transfection efficiency of naked mRNA was not reduced by any tubing. In contrast, the transfection efficiency of lipoplexes was decreased to 39% when given through the Tygon® F-4040-A tube and to 64% when the silicone tube was used.
The Tygon® 2375 NSF-51 tube showed the highest and the Tygon® F-4040-A tube the lowest transfection efficiency for the DOTAP:DOPE-lipoplexes.

➡ The Tygon® 2375 NSF-51 tube showed the lowest adsorption of lipids.

METHODS AND MATERIALS

1. Preparation of naked mRNA solution with a concentration of 0.002 µg/µl.
2. Preparation of mRNA-lipoplex dispersion:
   a. Formation of DOTAP:DOPE (DD) liposomes via lipid film method and homogenisation with a sonicator
   b. Addition of Firefly Luciferase mRNA in a weight ratio of 5:1 (DD:mRNA)
3. Addition of mannitol as matrix material to a concentration of 20 µg/µl.
4. Pumping and spraying of naked mRNA and lipoplexes, respectively, using 3 tubing materials. Samples were collected with a falcon tube positioned underneath the two-fluid nozzle.
5. Addition of DOTAP:DOPE (DD) liposomes to processed naked mRNA solution in a weight ratio of 5:1 (DD:mRNA).
6. Transfection of mRNA-lipoplexes into CALU-3 cells, measuring of luciferase expression using a luminescence assay. For normalisation to protein content per well, a Bradford-Assay was performed.



CONCLUSION

➡ Liquid handling and processing complex mRNA-formulations is challenging.

➡ Adsorption of lipid formulation components to equipment, especially tubing material, can be a major obstacle and has to be avoided.

➡ In contrast, using the investigated tubing materials adsorption of mRNA itself was not a problem.

➡ In this study, we identified Tygon® 2375 NSF-51 as a suitable tubing material for the handling of DOTAP:DOPE lipoplexes.

FUTURE WORK

❖ As a next step, we are going to analyse and optimise the entire spray drying process. In order to spray dry a formulation containing fully functional mRNA-lipoplexes, the addition of stabilising excipients such as leucine, PEG or trehalose, might be necessary.

❖ Once we have produced a stable formulation, the development of a formulation with suitable aerodynamic characteristics can begin.

REFERENCES

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