Automated biofunctionalization of lipid nanoparticles for CAR T cell therapy

Quinn Matthews, Masoud Norouzi, Fahim Masum, Ryan Fobel, Pouriya Bayat, Soheil Talebi, Dominik Witzigmann, Keith Pardee

UNIVERSITY OF TORONTO
LESLIE DAN FACULTY OF PHARMACY

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Nanomedicine

- Nanomedicine has revolutionized drug delivery.
- Therapeutics can be packaged into lipid nanoparticles (LNPs) functionalized with target-specific antibodies for precise delivery.
- However, nanomedicine production is challenging, requiring infrastructure and skilled workers, limiting access in remote and low-resource areas and at the point of care.

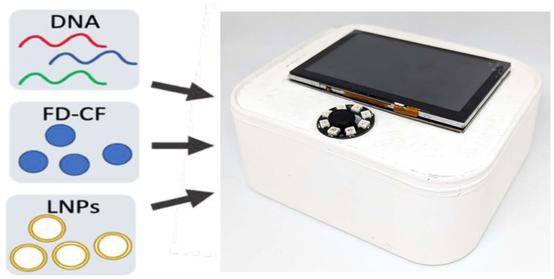
CAR T cell therapy

- In chimeric antigen receptor (CAR) T cell therapy, a patients T cells are isolated, removed, and engineered *ex vivo* to express a unique receptor targeting the patient's cancer, and then reinjected into the patient.
- This process can be expensive and requires time spent in a hospital and is only available in resourced areas.

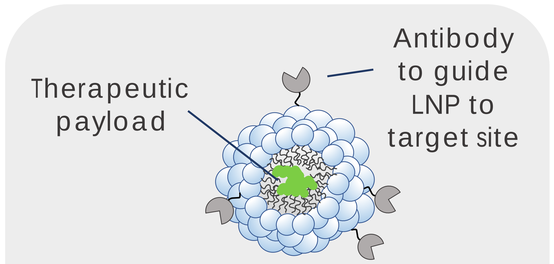
Goal and Motivation

- We have recently demonstrated the capacity for portable, cell-free production of biologics from freeze-dried cell-free reaction pellets (FD-CF) that can be stored and distributed at room temperature.
- Using this technology, we aim to engineer a portable device to produce antibody functionalized therapeutic LNPs at the point of care.
- This will enable nanomedicine to be brought to a broader population and to the laboratory benchtop.
- As a proof of concept, we will use our device to produce LNPs loaded with CAR encoding DNA and targeted to T cells, allowing modification of T cells to CAR T cells *in vivo* rather than *ex vivo*

Research Design

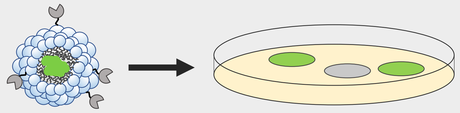


LNPs, reaction pellets, and DNA encoding the antibody are added into the device

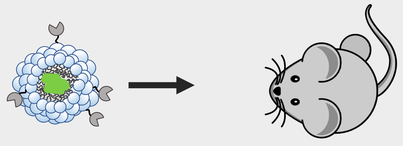


Functionalized Lipid Nanoparticle (LNP)

LNPs functionalized with anti – T cell antibodies and loaded with CAR DNA are produced



LNPs are tested *in vitro* with human T cells to determine if CAR T cells can be produced



LNPs are tested *in vivo* with mice to determine if CAR T cells are made and if tumour regression can be achieved

Results

- A panel of anti CD3 and CD8 antibodies were expressed within the device (fig. 1A) and conjugation was achieved using the enzyme Sortase (fig. 1B)
- Two CAR-GFP cassettes (CAR8 and CAR28) were expressed and validated in HEK293T cells (fig. 2A) and confirmed via anti-CAR western blot (fig. 2B)

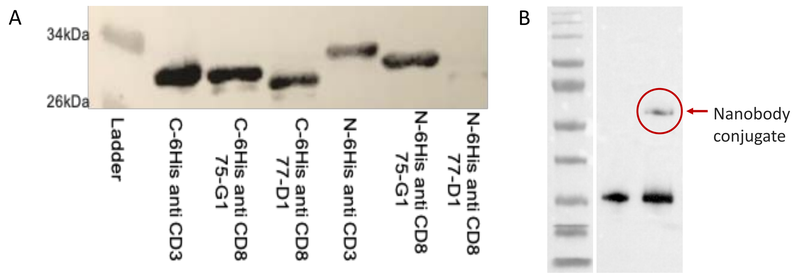


Figure 1. A) Antibody expression and B) conjugation

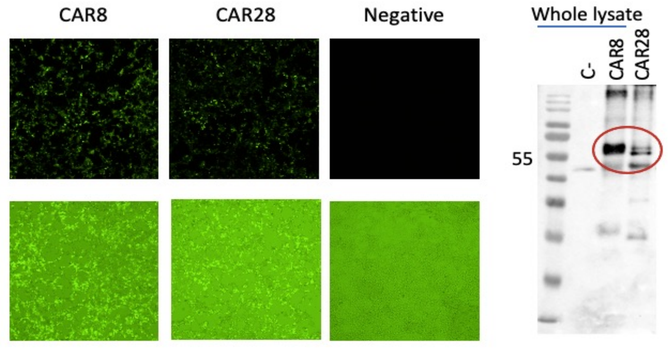


Figure 2. A) Expression of CAR-GFP and B) confirmation via anti CAR western blot

Future work and Conclusions

- We are currently working to integrate these tools into the automated device and are setting up mouse experiments to test our technology.
- If successful, this work will improve accessibility to custom nanomedicines to patients in remote and low-resource areas and will expand access for researchers.