Development and Testing of Novel Vaccine Technologies

Holly Wright

Project Aims

1. Design and create a novel self-amplifying DNA vaccine that can be delivered transdermally via ultrasound-mediated cavitation.
2. Create and test solid protein cavitation nuclei (pCaNs).
3. Combine these two technologies in vivo experiments.

What is the issue?

Needle & Stick vaccine delivery has several key problems, namely **phobia/pain** (leading to vaccine hesitancy), **reuse of non-sterile equipment, and needlestick injuries** (leading to hepatitis or HIV). New delivery techniques are needed to provide painless, safe, patient-friendly technologies.
The current **vaccine production process** (for traditional live-attenuated vaccines) **is too slow**, highlighted by the SAR—CoV-2 pandemic. New methods are required to tackle rapidly emerging and shifting diseases.

How do we solve it?

Nucleic acid vaccines (DNA and RNA) can be designed within days, produced within weeks, and can be easily adjusted at the gene sequence level to account for mutations as they occur. **DNA vaccines** provide some potential advantages, over RNA, in that they have **stability**, and therefore don't have to be kept at ultra-cold temperatures. However, poor results in human trials have meant that no DNA vaccines have been approved for use yet - **further optimisation is required**.

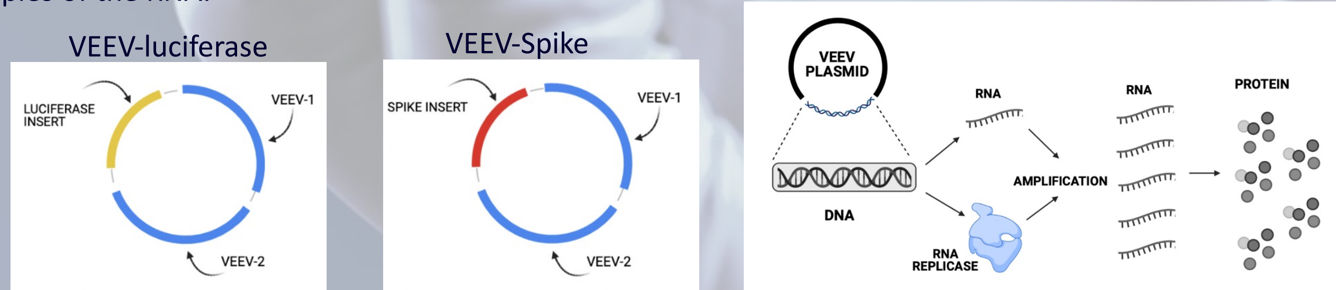
Ultrasound can induce a mechanical process called cavitation, defined as the formation of vapour filled cavities (bubbles), which, when they collapse violently, can lead to the opening of the pores in the skin, and this allows for drugs to pass through. Cavitation agents help lower cavitation pressures to **safe levels** and aid in **prolonged and enhanced drug delivery**. They have already been successful in improving the delivery of chemotherapeutics in tumours.

This 4YP builds on published data **(S. Bhatnagar, 2016)** which demonstrates that ultrasound-mediated cavitation, in combination with polymeric, inert, solid cavitation nuclei can be used to drive **the delivery of DNA vaccines across the skin** for needle-free delivery approaches.

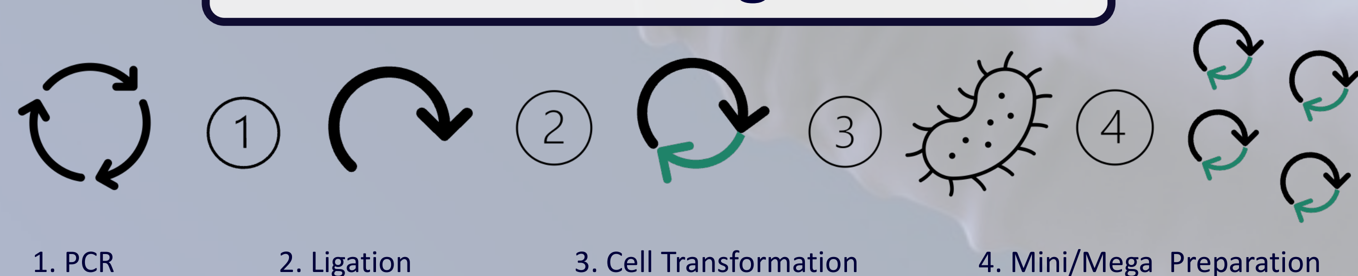
Plasmid Design

DNA vaccines use pieces of DNA, called plasmids, to deliver genetic information to the cells in the body. Plasmids are circular strands of DNA which can be manipulated, through a process called cloning to insert a gene of choice. Once the plasmids enter a cell, their code can begin to be transcribed into mRNA and then translated into the corresponding protein, expressing the gene coded for in the plasmid. Here two separate plasmids were designed - their insert genes were luciferase (a luminescent biomarker) and subunit-1 of spike protein (antigen of SARS-CoV-2) respectively.

DNA vaccines still require optimisation of their performance before they can be used in humans. The optimisation technique explored here is to create a DNA plasmid that have genetic code that allows for the **self-amplification of its RNA**, with the goal to increase the amount of antigen protein translated per DNA plasmid delivered. This is achieved through using part of the Venezuelan equine encephalitis virus **(VEEV) genome** which codes for a **viral replicase** complex. The replicase is used to produce more copies of the RNA.

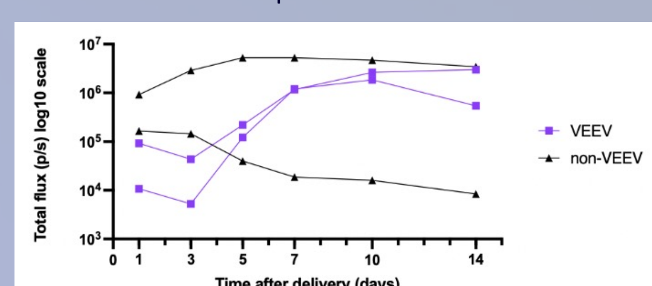


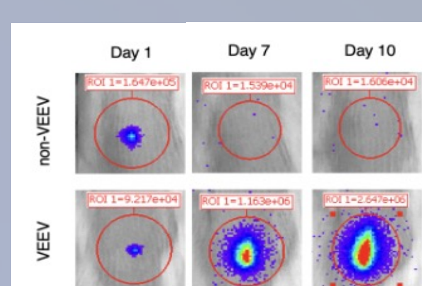
Cloning



Plasmid Characterisation

Before attempting to deliver the self-amplifying VEEV-luciferase DNA vaccine via ultrasound, the ability of the plasmids to express luciferase was first tested using intradermal (ID) injection *in vivo*.
The mice injected with the VEEV-luciferase DNA show expression profiles that increase over time, which is characteristic of self-amplifying DNA, compared to the non-VEEV luciferase expression profiles, which have a short-lived increase and then plateau or fall back below its initial level. (the shape of the profile is more important than the initial total flux value).
This is also clearly illustrated by the In Vivo Imaging System images on the right which show the VEEV injected mice increasing in expression over time. These figures, together with Sanger sequencing data which was obtained from samples sent to an external lab to be read, provide good evidence of a correctly cloned VEEV-luciferase plasmid.



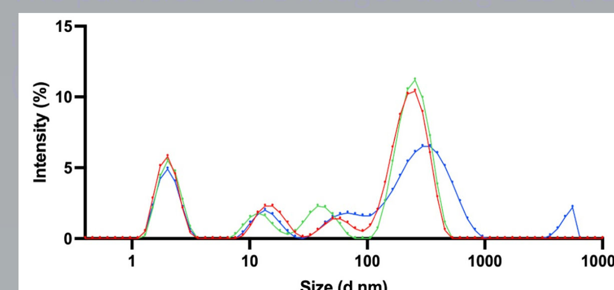


pCaN Synthesis

Here **novel protein cavitation nucle**i (pCaNs) were made and tested. The polymeric nanocups used in the experiments done by **(S. Bhatnagar, 2016)** are inert and nondegradable. Thus, motivating the production of protein cup cavitation nuclei, which are both naturally degradable and may even be designed to invoke a specific immune response by creating them out of 'non-self' protein.

The pCaNs were made by **sonicating** (applying ultrasound via a probe) **protein** (BSA) and a **volatile solution** (hexane) to form protein bubbles entrapping the volatile. The hexane was **evaporated away**, using rotary evaporator and **freeze-drying** cycles, to leave single cavity nano-sized protein cups.

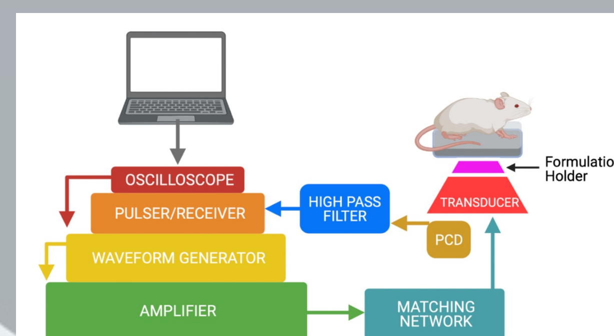
pCaN Characterisation



The particles were imaged using **Dynamic Light scattering (DLS).** Data (left) indicates the presence of the pCaNs at the expected ~200nm (diameter).

The **ultrasound set-up** used for the experiments is shown in the bottom left. The generated electric signal is amplified and then converted to an ultrasonic acoustic wave via the **piezoelectric transducer.**

At a high enough voltage and hence pressure, this instigates **cavitation** in the sample held in the formulation holder. The natural frequency response of the **passive cavitation detector** (PCD) is used to filter out most of the drive signal yet remain very sensitive to the acoustic emissions of cavitation events. Here the PCD is located coaxially inside the transducer and converts the measured acoustic signals back into electrical signals, which are transmitted to the oscilloscope, where they can be analysed.

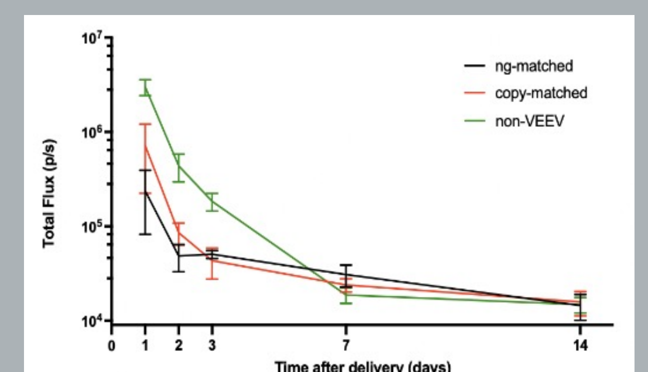


The pCaNs were tested for cavitation on an increasing transducer **pressure ramp**. The plots show the measured energy at each frequency against a time increasing pressure ramp: the red and yellow are indicative of higher energy and cavitation. The pCaNs substantially outperformed control samples of water and free BSA protein.

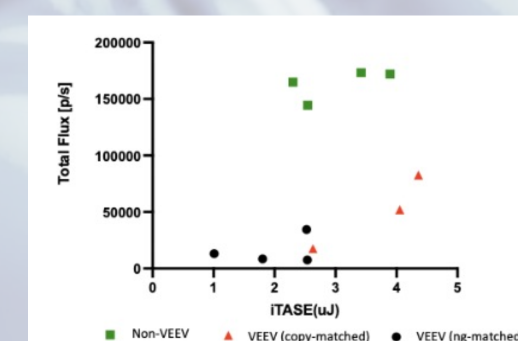
Transdermal Delivery

The experiments in this section tested if the **VEEV-luciferase plasmids**, in combination with **pCaNs**, could **improve the delivery** profile and **immune response** when **delivered transdermally using ultrasound**.
All 11 mice in the study showed luciferase expression above baseline levels, thus indicating successful delivery of the plasmid via ultrasound. However, all three groups of mice (two VEEV groups and one control) followed a similar trend in their luciferase time expression profiles: a rapid initial fall of the total flux measured, and a subsequent levelling out to a total flux measurement similar to baseline levels.

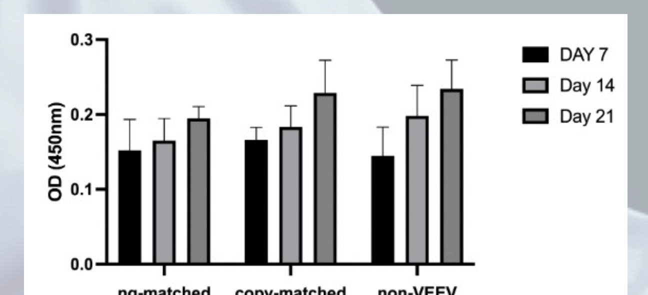
The **notable conclusion** from this experiment is that the expression profile of VEEV-luciferase plasmid, delivered transdermally, did not mimic that of the ID injection. Namely, **no prolonged or delayed protein expression** was observed. Reasons for this require further study but may include a minimum required DNA threshold to observe self-amplifying properties, higher skin cell turnover and cell shuttling.



Acoustic emissions that can be recorded from cavitation provide **an immediate and noninvasive feedback mechanism**, which is of paramount advantage in a clinical setting, thus separating this technique from other transdermal delivery methods. The measured luminescence, total flux (p/s), was plotted against the calculated total inertial cavitation energy, iTASE (μJ) value. To explore the **luminescence/cavitation relationship**. A somewhat **threshold relationship** between the total flux (p/s) and inertial cavitation energy was observed.



It was next necessary to test whether **antibodies** to luciferase protein could be detected and whether such levels differed between the mice. This was achieved using an enzyme-linked immunosorbent assay **(ELISA)**. The measured deviations from the expected baseline level (~1.5) are small. Hence, the immune responses **are inconclusive** As luciferase is not the strongest immunogenic protein.



Next Steps...

1. Further optimisation of the **pCaNs production procedure** is needed. Variables that are still being optimised include the purity and volume ratio of the volatile (hexane) used, the duration and intensity of sonication, and the rotary evaporation and freeze-drying cycles.
2. More experiments must be done to understand the reasons for the **uncharacteristic** VEEV-luciferase time expression when delivered transdermally and to establish a more **robust relationship** between luminescence and cavitation.
3. Additionally, repeating the experiments for a **longer time period** using the **VEEV-spike plasmid** (which is likely to produce a stronger immune response than luciferase) would be useful to properly assess the impact of the self-amplifying DNA on the immune system through ELISAs.

Supervisor: Robert Carlisle