Nanoparticles and microfluidics for future tuberculosis vaccines

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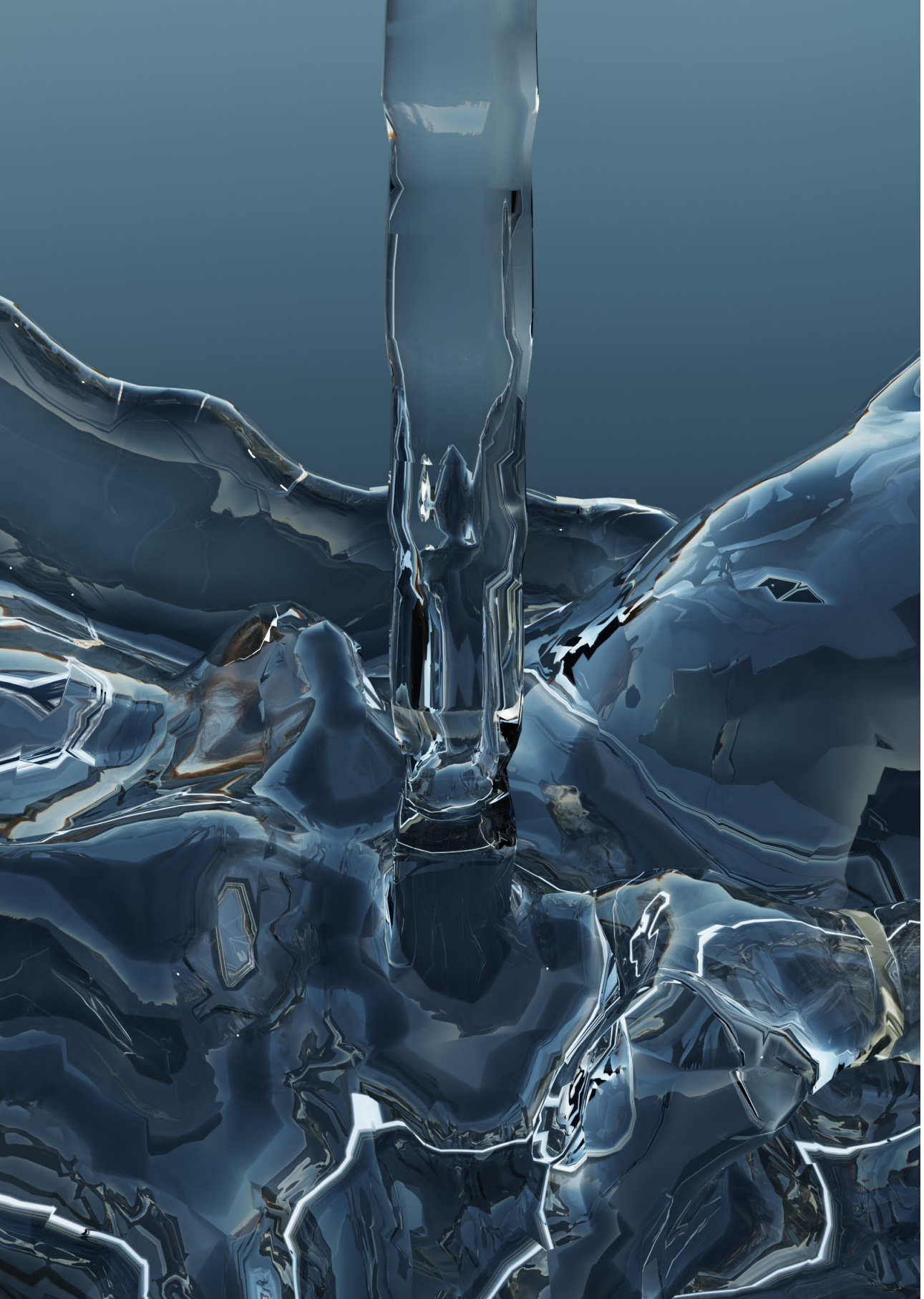

CHAPTER 6

SUMMARY OF THE DISSERTATION
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SUMMARY OF THE DISSERTATION

This dissertation focuses on nanoparticulate vaccine formulations and delivery routes to enhance antigen-specific induction of proinflammatory immune responses in the pursuit of creating new tuberculosis (TB) vaccination strategies.

To formulate a new TB vaccine, a TB antigen or antigen-encoding part is needed. The recombinant protein Ag85B-ESAT6-Rv2034 (AER), which consists of three *Mycobacterium tuberculosis* (Mtb)-expressed proteins fused together, is a promising TB antigen that provides T-cell epitopes in the human immune system [1]. However, subunit vaccines, which are vaccines based on purified antigens, such as the AER protein, are often poorly immunogenic [2–4]. Therefore, it is necessary to include molecular or particulate adjuvants in the vaccine formulation or, ideally, a combination of both, as adjuvants can help improve the potency and redirect the immune system toward an effective response [3, 4]. The emphasis in this thesis was on three particulate adjuvant types: i) cationic liposomes, ii) poly(D,L-lactic- *co*-glycolic acid) (PLGA) particles, and iii) PLGA- lipid hybrids. The research described in this dissertation focused on preparing and characterising these nanoparticles and comparing the immune responses induced by these three particulate adjuvant types.

i) Cationic liposomes are excellent subunit vaccine delivery systems that can induce cluster of differentiation (CD)4^+ (skewed towards a T helper type 1 (Th1) response) and $CD8^+$ T-cell responses [5–7]. These responses are deemed important in protection against TB [8]. A subgroup of cationic liposomes is the pH-sensitive cationic liposomes. They are stable at physiological pH; however, when they are internalised by antigen-presenting cells and exposed to the decreasing pH in the endosomes, where the liposomal bilayer becomes unstable, fuses with the endosomal membrane and the content leaks into the cytosol [9, 10]. This can promote $CD8^+$ T-cell responses [11]. The research described in this dissertation focused on optimising the lipid composition of liposomes to determine which lipid compositions could activate dendritic cells (DCs) and $CD4^+$ T cells.
ii) The second particulate adjuvant type described in this dissertation is PLGA nanoparticles. Particles made of this material have an excellent safety profile, being both biodegradable and biocompatible [12], and their properties (hydrophilicity/hydrophobicity, drug loading, drug release rate, etc.) are tuneable, which allows for the customisation of their properties to fit specific applications [13]. PLGA nanoparticles, without any added molecular adjuvants, do generally not elicit much of an immune response [5]. However, with a molecular adjuvant included, such nanoparticles can induce Th1-biased responses in mice [5].
iii) The third nanoparticle type investigated in the studies described in this dissertation is based on lipid-PLGA hybrids, which combine liposomes and PLGA nanoparticles by being nanoparticles with a PLGA core covered by lipids or vice versa [14]. Lipid-PLGA hybrids have successfully been used in drug and vaccine delivery preclinical research, where they induced equal IFNγ^+CD4^+CD44^{high}(Th1)-cell responses to liposomes with the same lipid composition in vivo [14].

While PLGA nanoparticles and lipid-PLGA hybrids are promising as drug delivery systems and nanoparticulate adjuvants, the typical bulk production methods for producing PLGA nanoparticles and lipid-PLGA hybrids are time-consuming and complex to control. It is crucial to improve and develop novel nano-preparation methods to increase their applicability, which can be done using microfluidics. Microfluidics is a technique that enables the manipulation of fluid streams through microscale fluidic channels [15]. It has emerged as a method to prepare PLGA nanoparticles with controlled diameters, which results in excellent batch-to-batch reproducibility and a narrow particle size distribution [15]. The studies described in this dissertation focus on the production of PLGA nanoparticles and PLGA hybrids using microfluidics.

Finally, a potent vaccination strategy could be to target the skin. The dermis is highly populated with different subsets of DCs, in contrast to subcutaneous and muscle tissue, which are the conventional administration routes [16]. Therefore, intradermal delivery of a TB vaccine could be of interest. Indeed, the only available TB vaccine, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG), is mainly administered intradermally. Among the intradermal administration techniques, dissolvable microneedle arrays (dMNAs) are of special interest, as they: i) can secure the stability of loaded drugs by keeping them in their dry form, ii) be self-administered because of the easy application of the microneedle patch with microneedle lengths that would target the dermis, and iii) create zero needle waste as the microneedle dissolves, preventing needle-associated spread of blood-borne pathogens [17]. The research described in this dissertation investigated the first steps required for incorporating PLGA nanoparticles into dMNAs.

This dissertation delved into various aspects of developing a new TB subunit vaccine. **Chapter 1** comprehensively introduces TB immunology and the imperative for innovative vaccine solutions, culminating in this dissertation's aim and outline.

Chapter 2 describes how AER was formulated into cationic liposomal formulations with different lipid compositions and how the immune responses were assessed in vitro. The AER-containing liposomal formulations were formulated using the thin-film dehydration-rehydration method, followed by tip-sonication. The liposomes consisted of a positively charged lipid, cholesterol and a helper lipid (zwitterion) in different molar ratios. The physiochemically stable formulations were subsequently studied in a series of in vitro assays: i) a human monocyte-derived DC (MDDC) assay, where the viability and activation of DCs were assessed post-incubation with the formulations, ii) an uptake assay, whereby the uptake of liposomes was measured in MDDCs and M1 (classically activated macrophages that exhibit a proinflammatory phenotype) and M2 (alternatively activated

macrophages that exhibit an anti-inflammatory phenotype) macrophages, and iii) a T-cell assay in which the best-performing formulations were tested by incubating the activated DCs with specific $CD4^+$ T cells, to determine if the latter upregulated the activation marker CD154 and their interferon (IFN)-γ production.

The formulations containing cholesterol and the cationic lipids 1,2-dioleoyl-3-trimethylammonium-propane chloride (DOTAP), dimethyldioctadecylammonium bromide (DDA), 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine chloride (EPC), or N^4-cholesteryl-spermine hydrochloride (GL-67) induced the highest upregulation of the MDDC-activation markers CD40, CD80, and CCR7. However, the formulation containing GL-67 induced high cell death and was therefore excluded from the T-cell assay. Among the remaining formulations, the AER/DOTAP:cholesterol:1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and AER/EPC:cholesterol:DOPC formulations significantly increased the level of IFN-γ^+CD154^+ T cells compared to their AER-empty counterparts. However, in a separate study (unpublished), the DOTAP liposomes were less immunogenic. Therefore, only the AER/EPC:cholesterol:DOPC formulation's lipid ratio was optimised further, where a molar lipid ratio of 2:1:2 was the most promising formulation, as it induced the highest level of DC activation markers and cytokine/chemokine production. In conclusion, the chapter describes a screening method for particulate vaccine formulations, where the most promising liposomal formulation in regards to inducing Th1 responses was the EPC:cholesterol:DOPC formulation.

Chapter 3 presents the set-up of a novel low-cost modular microfluidic system for producing PLGA nanoparticles and describes how the flow rates, solvents, and PLGA concentrations impact the PLGA nanoparticle formation in this system. The usability of this system for producing particles for controlled drug delivery was explored by incorporating positively and negatively charged proteins into PLGA nanoparticles. The simplest form of the modular microfluidic system involves a co-flow configuration, where an inner flow of PLGA-containing organic solvent meets an outer flow of an aqueous fluid. Mixing these two solvents triggers PLGA precipitation, leading to nanoparticle formation.

The results presented in this chapter show that the formation of nanoparticles is affected by the PLGA concentration, where an increasing PLGA concentration leads to larger particle diameters. Furthermore, it was observed that increasing the total flow rate results in the formation of smaller nanoparticles. Utilising ultrapure water as an aqueous phase resulted in negatively charged nanoparticles and uncontrolled precipitation at the outlet with high PLGA concentrations. Meanwhile, adding poly(vinyl alcohol) to the aqueous phase created neutral particles and eliminated precipitation issues. Negatively charged particles were controllably obtained utilising ethanol-water mixtures. Incorporation of the proteins ovalbumin or lysozyme (negatively and positively charged, respectively) with a three-syringe system resulted in encapsulation efficiencies above 40%. In conclusion, a cheap and easily adjustable modular microfluidic system was developed to prepare PLGA nanoparticles with precise control over the particle diameter and the possibility of including proteins, making it an excellent tool for drug and vaccine delivery applications.

In **Chapter 4**, studies on three different nanoparticulate adjuvants were reported: cationic pH-sensitive liposomes, prepared with sonication, and two modular-microfluidic-system prepared nanoparticles: PLGA nanoparticles and lipid-PLGA hybrids.

The immunogenicity of the particulate adjuvants formulated with the antigen AER with and without the molecular adjuvants monophosphoryl lipid A (MPLA) and cytosine-phosphate-guanine oligodeoxynucleotides (CpG ODNs) 1826 was assessed in vitro in MDDCs. The uptake of the particulate adjuvants in MDDCs without the molecular adjuvants was evaluated. Lipid-PLGA hybrids and pH-sensitive liposomes were taken up efficiently by MDDCs, but PLGA nanoparticles were not. MDDCs were stimulated with the particulate adjuvants, with and without the molecular adjuvants, and examined in terms of activation markers and cytokine production. Among the particulate formulations without molecular adjuvants, the cationic pH-sensitive liposomes were less efficient than the lipid-PLGA hybrids at upregulating DC surface markers and cytokine production, while PLGA nanoparticles were the least efficient. PLGA particles and pH-sensitive liposomes without molecular adjuvants hardly induced the excretion of cytokines/chemokines. The lipid-PLGA hybrids, PLGA nanoparticles, and the pH-sensitive liposomes with molecular adjuvants were all efficient at upregulating DC surface markers and cytokine production.

The protective efficacy of the liposomes, PLGA nanoparticles, and the lipid-PLGA hybrids formulated with the molecular adjuvants were tested in vivo in C57Bl/6 mice that were challenged sequentially with Mtb to determine possible efficacy as a TB vaccine. The candidate vaccines we developed were compared head-to-head with the current BCG vaccine and AER mixed with adjuvants MPLA and CpG ODN 1826. All vaccines (BCG, liposomes, PLGA nanoparticles, and the lipid-PLGA hybrids), except the AER-molecular-adjuvant mix, induced protection in Mtb-challenged C57/Bl6 mice, as indicated by a significant reduction in bacterial burden in the lungs and spleens of the animals compared to Mtb-challenged unvaccinated mice. Mice vaccinated with PLGA nanoparticles had a lower median number of Mtb bacteria in the spleens and lungs compared to BCG and the other two nanoparticle-based vaccines; however, this difference was not statistically significant between these relatively small groups. In conclusion, the nanoparticle-based formulation vaccines lowered the Mtb bacterial burden in the mice. The PLGA particles tended to have the best protective efficacy, even though the lipid-PLGA hybrids induced slightly better results in vitro.

In **Chapter 5**, studies are described in which the effect of intradermal administration of PLGA nanoparticles with CpG ODN 1826 and ovalbumin in dMNAs versus intradermal administration with hypodermic needles in vivo was determined. Intradermal injection of nanoparticles has been an effective administration route for vaccines.

In this study, we first had to design stable dMNAs with PLGA nanoparticles because the different polymers used for dMNA preparation affected the nanoparticle integrity. The dMNAs prepared with poly(vinyl alcohol) showed almost no aggregation of PLGA nanoparticles. The PLGA:poly(vinyl alcohol) weight ratio of 1:9 resulted in 100% penetration efficiency and the fastest dissolution in ex-vivo human skin (below 30 min). Subsequently, aqueous formulations and dMNAs with ovalbumin and CpG ODN 1826 with and without PLGA nanoparticles were tested in mice. The aqueous formulations with ovalbumin and CpG ODN 1826 with and without PLGA nanoparticles induced significant $CD4^+$ T-cell responses in mice compared to the other formulations. The formulation with ovalbumin and CpG ODN 1826 with PLGA nanoparticles induced significant $CD8^+$ T-cell responses compared to the other formulations. Unfortunately, the dMNAs did not dissolve entirely in the mouse skin, which could be why they did not induce $CD4^+$ and $CD8^+$ T-cell responses.

In conclusion, the aqueous formulations performed better than the dMNAs, probably due to the poor dissolution of the dMNAs in murine skin in the in vivo experiment. However, the dissolution was good in the ex-vivo human skin, demonstrating the differences between models. The dMNA formulation should, therefore, be adapted for murine testing. Even though we did not induce immune responses utilising dMNAs, dMNAs with incorporated PLGA nanoparticles were successfully prepared as the particles retained their physicochemical properties after dissolution. The aqueous formulation with PLGA nanoparticles prepared with the modular microfluidic system was especially potent at inducing $CD4^+$ and $CD8^+$ T-cell responses.

GENERAL DISCUSSION

Physicochemical properties of nanoparticles

To elicit robust $CD8^+$ T-cell responses and Th1-skewed $CD4^+$ T-cell profiles, we utilised particle diameters of approximately 150 nm for both the in vivo and in vitro experiments, as previous studies have demonstrated that particles within the size range of 10 to 200 nm tend to induce Th1-skewed $CD4^+$ and $CD8^+$ T-cell responses [18]. In contrast, larger particles (200-500 nm) often lead to Th2 responses [18]. However, the optimal size for liposomes may be different. It has been observed that small liposomes (below a size of 200 nm) generate Th2-skewed immune responses, whereas liposomes above 200 nm induce Th1-skewed responses [18]. This might be because small antigen-containing liposomes are degraded fast in the lysosomes, which could lead to ineffective antigen presentation, whereas the larger liposomes are degraded slower in the phagosomes [18]. This might explain why we see a tendency of lower protective efficacy against TB for the liposomes described in **Chapter 4** when compared with the TB vaccine based on PLGA particles and lipid-PLGA hybrids.

Particle rigidity also plays a crucial role in immune responses. Studies have shown that rigid particles are more readily taken up by macrophages, endothelial cells, and DCs than less rigid particles [18, 19]. They are also more likely to induce Th1 responses [18, 20]. When cholesterol is incorporated into the bilayer of liposomes with a liquid-disordered organisation, a liquid-ordered phase, which is more rigid, is formed [19, 21]. Our results in **Chapter 2** align with these findings, as liposomes containing cholesterol were generally more efficiently taken up by cells and could induce Th1-skewed $CD4^+$ T-cell responses (the $CD8^+$ T-cell responses were not tested). We have previously characterised the rigidity of PLGA particles, finding them significantly more rigid than liposomes (Young's modulus value of around 14.4 MPa, which is the same for cartilage [19]). In comparison, liposomes have, dependent on the lipid composition, Young's modulus values of around 500 kPa to 4 MPa [19]. The rigidity of lipid-PLGA hybrids is expected to be between these two values, as they often consist of a PLGA core surrounded by lipid layers [14]. Despite this, MDDCs took up lipid-PLGA hybrids more efficiently in our experiments. This suggests that the positive charge of the lipid-PLGA hybrids, in contrast to the negative charge of PLGA particles, mainly plays a role in cellular uptake, which also is seen for liposomes in literature, where positively charged particles are taken up more than neutral and negatively-charged liposomes [7].

Conventional methods versus microfluidics for PLGA nanoparticle production

PLGA particles prepared with conventional methods do not induce high antigen-specific $CD4^+$ and $CD8^+$ T-cell responses (the PLGA particles induced ~1% and 0.2-1% antigen-specific $CD4^+$ and $CD8^+$ T cells, respectively, out of the total $CD4^+$ or $CD8^+$ T cell population in the spleen [5]). However, the PLGA particles produced with the modular microfluidic system show high antigen-specific $CD4^+$ and $CD8^+$ T-cell responses in a similar experiment (5% and 30% antigen-specific $CD4^+$ and $CD8^+$ T cells in the spleen), as shown in **Chapter 3**. This is despite the physicochemical characteristics of the particles being more or less the same when prepared with the conventional method (average particle diameter: 157-160 nm, polydispersity index (PDI): 0.052-0.060, and zeta potential: -18 to -22 mV [5]) and the modular microfluidic system (average particle diameter: 96 nm, PDI 0.09, and zeta potential: -0.8 mV in **Chapter 3**). The differences in the responses could be due to the different ovalbumin (antigen) doses (0.31 µg and 4.4 µg for PLGA particles prepared with the conventional method and microfluidics, respectively). However, it could also be due to the particle preparation method. From personal experience, I have observed that even though PLGA particle formulations prepared with the conventional double-emulsion

method often seem acceptable when the particle diameter is measured with dynamic light scattering, the particles are usually visible to the naked eye. The PLGA particles are often spun down and washed, which removes the surfactant and lowers the zeta potential, and the steric hindrance between the particles leads to more aggregation. It is possible to avoid these aggregates in the measurement by sampling from the surface. It could also be due to differences in the surface morphology, density, or porosity, or other non-tested factors. In general, it appears that microfluidic methods are better at preparing PLGA particles with uniform particle diameters throughout the sample that can induce higher $CD4^+$ or $CD8^+$ T-cell responses than the conventional methods.

Which response is needed against tuberculosis

The immune response that correlates with protection against TB is not entirely established. It is, therefore, hard to determine if a new TB vaccine is protective in preclinical studies without performing an Mtb challenge study. Historically, it was believed that Th1 responses were essential for an effective TB vaccine, and a conventional strategy was to aim to induce Th1/Th17 responses while minimising Th2/Treg responses [22]. However, recent understanding suggests that this theory should be complemented by the interplay between Th1, Th2, and B-cell responses [22].

All the AER-containing nanoparticle vaccines in **Chapter 4** induced polyfunctional IL-2, IFN-γ, and TNF-α producing $CD4^+$ and $CD8^+$ T cells and monofunctional IFNγ-producing $CD8^+$ T cells with a central memory phenotype ($CD62L^+$). Even though the lipid-PLGA hybrids and pH-sensitive liposomes seemed to have the best cellular responses in vitro in MDDCs and in AER restimulated splenocytes from immunised non-Mtb-challenged mice, the PLGA nanoparticles with molecular adjuvants tended to have the most protective effect against TB, which demonstrates that the immune correlate is still not established.

PROSPECTS

Future directions of TB vaccine formulations

The work in this dissertation offers valuable insights into developing future TB vaccines. To translate these findings clinically, the field should focus on three significant aspects:

- Dosing and release kinetics
- Dissolvable microneedles to improve global vaccine distribution
- Production of the vaccine: assembly line with Quality Control (QC)

Dosing and release kinetics

The antigen and molecular adjuvant dose administered in the animal experiments described in this dissertation were based on previous work within the BioTherapeutics research group (dose of ovalbumin per immunisation: 5 μg [5], ovalbumin-to-CpG-ODN ratio 1:1 [23], antigen-to-liposome weight ratio 1:50 [23], dose of AER: antigen dose in antigen adjuvant mix: 25 μg) and have not been further optimised. To further improve the vaccine, the antigen dose, molecular adjuvant concentration, and antigen-to-particle weight ratio in the vaccine formulation can be further optimised. Given that the antigen dose can significantly influence both B-cell and T-cell responses [24], determining the optimal dose and antigen-to-particle weight ratio in mice and, subsequently, in humans is essential for future translation of the vaccine.

As dosing schedules have been shown to influence immune responses in both mice [25] and humans [26], the number of doses and interval between the prime and possible booster(s), as well as the release profile of the antigen, are crucial. Prolonged dosing schedules have been observed to increase neutralising antibody titres [26], suggesting several potential strategies to improve vaccination efficacy. These include exploring different intervals between prime and booster doses. This could be implemented by testing different intervals between prime and boosters or by incorporating the antigen (and molecular adjuvant) into delivery systems with diverse release profiles (e.g., mimicking the natural course of an infection). Consequently, characterising the release profiles of the different produced particles; liposomes, PLGA nanoparticles, and polymer-lipid hybrids, is of relevance. This would determine whether the vaccines release antigen in discrete bursts or continuously, and establish if a combination of these delivery systems could achieve a prolonged dosing schedule.

Dissolvable microneedles

Maintaining an expensive cold chain is often necessary to distribute liquid vaccines. This can be circumvented by incorporating vaccines into dissolvable microneedles. Furthermore, dissolvable microneedles offer additional benefits, including reduced pain compared to conventional intradermal injections and elimination of biohazardous needle waste [17].

The dissolution properties of the dissolvable microneedles with nanoparticles incorporated, described in **Chapter 5,** require improvement. While demonstrating acceptable dissolution kinetics in ex vivo human skin, the in vivo animal experiments yielded suboptimal results. Therefore, to better inform subsequent in vivo studies, initial ex vivo experiments should be conducted using mouse skin, which exhibits significant differences from human skin (e.g., reduced thickness, increased flexibility). The influence of compromised human skin (various skin conditions) on microneedle dissolution [27] could also be considered. Furthermore, for the development of a dissolvable microneedle-based vaccine, the stability of the microneedle arrays should be evaluated under a range of environmental conditions, including variations in humidity and temperature.

Several strategies can be explored to enhance the dissolution rate of the microneedles. These include:

- Evaluating alternative matrix materials: Previous investigations involving poly(vinyl alcohol), polyvinylpyrrolidone, and trehalose revealed that the latter two induced PLGA nanoparticle aggregation upon resuspension of the nanoparticles from the microneedles. Further studies should, therefore, examine a broader range of polymers, sugars, or combinations to identify formulations that minimise dissolution time while preventing aggregation of the nanoparticles.
- Investigating alternative fabrication methodologies: The centrifugation method, which was employed in **Chapter 5**, led to PLGA particle accumulation at the microneedle tips due to the higher density of the nanoparticles relative to the polymer matrix. This might be the reason for the high dissolution rate. Alternative methods that enable homogeneous particle distribution within the microneedles, such as dispensing with robotics and nanodispensing [28], or incorporating a rapidly dissolving layer just above the microneedle tip, could address this issue.
- Investigating alternative microneedle array designs, such as increasing the amount of microneedles in the array. This could lead to a reduction in the concentration of PLGA particles per microneedle while maintaining the overall dose.
- Examining alternative microneedle geometries to optimise the dissolution rate (e.g., increase the microneedle surface area).

Assembly Line

For vaccine production, the goal is to establish a continuous production assembly line with integrated QC to ensure product consistency over time. This approach aims to reduce production costs and increase speed by minimising manual labour. All processes must adhere to Good Manufacturing Practice (GMP) to ensure the vaccine is consistently produced according to quality standards and meets regulatory requirements. The modular microfluidic system is well-suited, as the vaccine formulation could be produced continuously. However, the purification method used in this dissertation (using dialysis chambers) requires improvement, as it creates batches. Alternative purification methods that are already available on the market, i.e., continuous-flow dialysis, could be explored to continuously remove organic solvents, free antigens, and non-encapsulated molecular adjuvants.

Quality Control

During the assembly line production of vaccines, the product should be analysed and assessed at critical points to ensure consistent quality. This begins with the components introduced into the microfluidic system.

There are no standardised interlaboratory methods for assessing the antigen or antigen-encoding component before its incorporation into a vaccine. In this dissertation, the antigen AER was not monomeric when dissolved (analysed with size-exclusion chromatography and asymmetric flow field-flow fractionation), and gel electrophoresis indicated inconsistent protein synthesis. This aggregation made it difficult to formulate the antigen into liposomes using the extrusion method, necessitating the sonication method instead. Even the model antigen ovalbumin, obtained from major manufacturers, was sometimes dimeric and sometimes only partially passed through a 1000 kDa dialysis membrane. Furthermore, lipophilic antigens tend to form particles that cannot penetrate a dialysis chamber, making it challenging to determine the amount of antigen incorporated into the nanoparticles.

To address these issues, thorough product analysis and interlaboratory standards for antigen characterisation, including standard characterisation methods for the nanoparticles, should be established, as they are crucial for advancing the field and improving GMP compliance.

CONCLUSION

The findings in the studies described in this dissertation highlight the interplay between nanoparticle physicochemical properties and their ability to induce robust T-cell responses. The preparation method of nanoparticles can influence their immunogenicity. Compared to conventional methods, we observed that PLGA nanoparticles produced using the modular microfluidic system induced significantly higher antigen-specific $CD4^+$ and $CD8^+$ T-cell responses. This might be attributed to the improved uniformity and reduced aggregation achieved through microfluidic fabrication. The choice of nanoparticle platform, such as liposomes, PLGA particles, or lipid-PLGA hybrids, can also impact the type of immune response elicited, as the different particle types have different physicochemical properties in regard to rigidity and charge. While each platform has its advantages and disadvantages, our results suggest that PLGA nanoparticles with incorporated antigen and molecular adjuvants produced with the modular microfluidic system may be a promising candidate for inducing robust T-cell responses with protective potential against TB.

In conclusion, the studies described in this dissertation underscore the importance of carefully considering the physicochemical properties of nanoparticles to obtain the desired immunological response. As discussed in the prospects, several other things should be considered before the research can be carried out further, such as optimising the vaccine dosage, streamlining the production method, and improving the dissolvable microneedle dissolution rate.

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