Engineering of antigen-saving dissolving microneedles for intradermal vaccine delivery

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CHAPTER 6

SUMMARY, PERSPECTIVES, AND CONCLUSION

SUMMARY

Skin is the largest organ in the human body and contains a high number of antigen presenting cells (APCs). Therefore, vaccine delivery via the intradermal route is expected to improve immune responses by increasing the uptake of antigens by APCs. Currently, there are only a few intradermally delivered vaccines in the market including Bacillus Calmette-Guérin (BCG) and rabies, and these vaccines are delivered using a conventional needle via the Mantoux technique[1]. However, this technique requires trained medical staff for the administration. Besides, the use of conventional needles has many drawbacks, including the risk of cross-contamination, injection pain, and needle phobia. In order to solve these issues, a novel vaccine delivery device is required.

In recent decades, dissolving microneedle arrays (dMNAs) have been introduced for intradermal vaccine delivery. With many advantages, it can compensate for the drawbacks of conventional needles. First, self-administration of complex drug formulations is possible, circumventing patients to go to the healthcare facility. Second, it can avoid sharp waste by dissolving in the skin. Third, a cold chain during storage and delivery is preventable because antigens are dried in a thermostable solid state. Finally, microneedle-based delivery reduces pain sensation as they are too short to reach the deeper laying pain receptors.

The antigen containing dMNA should penetrate the skin and dissolve rapidly in the skin to deliver the antigen effectively. Therefore, sufficient mechanical strength and a timely dissolution ability are necessary properties of dMNA. Another important property of dMNA is thermal stability. dMNA should maintain the functionality of antigen during storage to secure vaccine immunogenicity. Excellent thermal stability allows vaccines to avoid the cold chain and consequently can increase vaccine coverage rates in low-income countries. Therefore, this thesis focuses on designing adaptable dMNA formulations for individual antigens, which ensures sufficient mechanical strength and fast enough dissolution ability of the microneedles, while protecting the vaccine against environmental influences.

Once a dMNA dissolves within the skin, the released vaccine should provoke adequate immune responses. Initially, APCs in the skin take up the antigen that is entrapped within the microneedle matrix material. These APCs sequentially get activated and migrate to the draining lymph nodes, upon which they start presenting the antigen to T cells. Upon recognition, antigen-specific T cells get activated and start proliferating. Generally, vaccination via the skin is favourable for inducting Th1 responses and cytotoxic T cell (CTL) responses ($CD8^+$), thereby making vaccination via the skin very attractive for novel cancer vaccination strategies.

Skin APCs consist of a myriad of different cell types, including Langerhans cells in the epidermis and dermal dendritic cells in the dermis. These cells are involved in both innate and adaptive immunity, as they can be stimulated with different adjuvants (different cells express different toll-like receptors) and thereby can induce different types of immune responses. Therefore, intradermal vaccination can provoke both types of immune responses[2,3] and promote both CD4 and CD8 T helper (Th) cells.

T helper cells are essential for the induction of antibody responses (via B cells), which get activated by APCs. Because the skin contains abundant numbers of APCs, it is an excellent site for prophylactic vaccination. This thesis found that intradermal influenza vaccine delivery using dMNAs evokes comparable immune responses compared to subcutaneous administration that was able to protect against a lethal virus dose. This shows that antibody levels induced by intradermal vaccination via dMNAs are sufficiently high and of high quality to protect against disease.

The most common method of dMNA production is by centrifugation. However, this method increases the economic burden, especially on industrial scales since it results in significant antigen waste, the most expensive part of a vaccine. Therefore, this thesis also focuses on engineering a novel antigen-saving production method for dMNAs and its utilisation by incorporating an influenza vaccine.

In **Chapter 2**, a novel composition of dMNAs was selected that ensures the thermostability of an influenza vaccine. To this end, whole inactivated influenza virus (WIV) was incorporated into different trehalose:pullulan ratios, and dMNAs were produced using the centrifugation method. The most suitable dMNA formulation was researched among the ratios, and a 1:1 trehalose:pullulan ratio was selected as it showed effective skin piercing as well as the fastest intradermal dissolution. The thermostability of WIV in dMNAs was analysed after four weeks of storage at three different conditions. The influenza vaccine functionality was comparably retained to the positive control (non-stored aqueous WIV formulation) when formulated in dMNA, while it was nearly dysfunctional in the stored liquid formulation. Using near-infrared fluorescence imaging, 10-12.5% of WIV delivery was observed from the dMNA into the skin, resulting in delivery of 1 µg (target dose). The formulation of WIV dMNA provided a sharp microneedle tip with fast dissolution and also proved its excellent thermostability by inducing protective immune responses.

The aim of **Chapter 3** was to design highly-immunogenic intradermal T-cell vaccines. In order to achieve this, two approaches were investigated: 1) designing antigen-containing poly-(DL)-lactide-co-glycolide nanoparticles (PLGA NPs) vaccine formulations to improve

uptake by APCs and 2) developing microneedle compositions that secure the physicochemical properties of the NPs. PLGA NPs were prepared using a microfluidics system and incorporated into dMNA formulation. dMNA was produced by using centrifugation, and 5% (w/v) PVA was selected for the formulation among three different formulations since PLGA NPs retained the size and size distribution after incorporation into 5% (w/v) PVA dMNA. However, it displayed too-slow dissolution. Therefore, the formulation was further optimised by decreasing the PVA concentration and PLGA proportion. Ex vivo human skin penetration and dissolution tests revealed that the optimised formulation had excellent penetration ability and a sufficient dissolution rate. According to the results of dMNA dissolution and OVA quantification in dMNA, 13% of the loading amount of OVA could be delivered to the skin. Although the nanoparticles themselves induced higher T-cell responses, intradermal delivery via dMNA didn't yield T-cell responses probably due to poor dissolution in mice skin. However, PLGA NPs were successfully integrated into dMNA which ensured the physicochemical properties of PLGA NPs.

As shown in the previous two chapters, the centrifugation method yields significant antigen loss. In order to provide an economic production of dMNA, in **Chapter 4**, an antigen-saving production method was engineered: the automatic dispensing system. The system mainly consisted of a dispenser and linear stages. Because the dispenser allowed limited viscosity, the viscosity of the formulation was an important factor together with the penetration and dissolution abilities of dMNA to select the suitable dMNA formulation. Among the various polymer formulations, PVP/PEG was selected as the most optimal candidate. The dispenser produced droplets of the drug formulation, and the linear stages aligned the dispenser with a microneedle cavity in the PDMS mould. During dispensing and aligning, low pressure was applied on the PDMS mould via a costume-made vacuum chamber in order to suck the entrapped air from the cavity. Using this automatic dispensing system, ovalbumin-incorporated dMNA was successfully produced with 98.1% loading efficiency. Compared to the centrifugation method, it resulted in a day shorter production time and achieved a significant volume reduction of antigen formulation by 98.5%.

Chapter 5 addressed the utilisation of the automatic dispensing system by developing WIV dMNA. This was done by adapting the novel dMNA composition (**Chapter 2**) for the dispensing system (**Chapter 4**). First, the formulation was optimised for the dispenser, since the formulation developed as described in **Chapter 2** exceeded the viscosity range for the dispenser. During these studies, it was discovered that the trehalose/pullulan combined with the influenza vaccine had a close interplay on the viscosity of formulation to dispense, but also on the formation of sharp and strong dMNA. Based on this, the concentration of formulation was optimised, and 1% (w/v) trehalose/pullulan was used for WIV dMNA. WIV

dMNA displayed 100% penetration efficiency and the majority of the microneedle volume dissolved within 10 minutes. Furthermore, it successfully reduced antigen waste by 95% compared to the centrifugation method. WIV dMNA also proved its thermostability after long-term storage at ambient and higher temperatures. Finally, in an immunisation study, it was shown that anti-influenza immune responses were effectively induced from WIV dMNA administrated mice, offering a potential alternative to intramuscular immunisation.

PERSPECTIVES

Boost dissolution ability of PLGA NPs incorporated dMNA

Poor dissolution of dMNA results in low immune responses since the released amount of the drug highly depends on the dissolved volume of dMNA[6]. This was illustrated in Chapter 3. PLGA NPs incorporated dMNA failed to show sufficient dissolution in mice skin, therefore, the induced T-cell responses were not significantly different from those of the negative control. In order to elicit robust immune responses, therefore, it is important to improve the dissolution of dMNAs.

Various factors affect the dissolution of dMNA. The composition of dMNA is the most frequently optimised one to enhance the dissolution. In general, the concentration or molecular weight of the polymer suspension can be decreased to speed up the dissolution[7,8]. For multiple polymers-composed dMNA, the dissolution rate can be increased by either raising the proportion of rapidly dissolvable polymers or decreasing the proportion of the slowly dissolvable polymers[9,10]. In order to boost the dissolution of PLGA NPs incorporated dMNA, similarly, the concentration of polymer and PLGA proportion in the formulation were lowered. Although the dissolution was improved, the dissolved volume was not sufficient to potentiate T-cell responses. However, the concentration and PLGA proportion couldn't be further reduced since a lower concentration couldn't build a structure of dMNA and a lower PLGA proportion required more dMNA to deliver the target dose into the skin. Therefore, the dissolution of PLGA NPs incorporated dMNA could be enhanced by combining rapidly dissolvable polymers and PVA, providing faster dissolving than only PVA while maintaining the structure of dMNA.

The dissolution rate can also be increased by choosing a proper production method of dMNA. To produce PLGA NPs incorporated dMNA, centrifugation was employed. However, this method separated the formulation by weight and localised PLGA NPs in the tip layer. Although this PLGA layer in the tip layer enhanced the mechanical strength of dMNA[11,12], it was more hydrophobic than the mixture of PLGA and PVA, which yielded a slower dissolution[13]. Therefore, for better dissolution, a homogeneous dispersion of the formulation is needed.

There are various methods that can disperse the formulation evenly in dMNA including spraying and 3D-printing of the formulation[14,15]. This can also be achieved by using the automatic dispensing system which was engineered in the studies described in Chapter 4. In order to visualise the distribution, fluorescent dye labelled ovalbumin was encapsulated in PLGA NPs, and added into PVA. With this formulation, dMNAs were produced by using the automatic dispensing system and it displayed the uniform distribution of formulation (Figure 1). Additionally, the automatic dispensing system required a lower concentration of formulation than the centrifugation approach to achieve a low viscosity, and a lower concentration of the formulation enhanced the dissolution of dMNAs. As demonstrated in Chapters 2 and 5, WIV dMNA produced by the centrifugation method used a higher concentration and dissolved slower than WIV dMNA produced by the automatic dispensing system. Therefore, via the automatic dispensing system, PLGA NPs incorporated dMNA is expected to have better dissolution due to its uniformly distributed and lower concentration of the formulation.

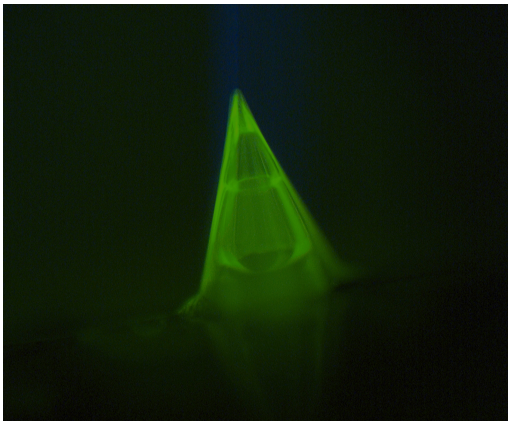

Figure 1. Alexa Fluor 647 dye labelled Ovalbumin encapsulated PLGA NPs incorporated dissolving microneedle produced by the automatic dispensing system. The fluorescent image indicates that the formulation containing dye is spread homogenously in the microneedle tip.

Lastly, an alternative dMNA shape can be adopted in order to increase the dissolution rate. The shape of dMNA influences the dissolution profile since different dMNA shapes provide varied insertion depths. For instance, although all three dMNA shapes (conical, funnel, and candlelight) had the same heights, the candlelit shape of dMNA showed the lowest residue height and biggest dissolved volume (Figure 2)[16]. It was because a continuously increasing diameter like conical or funnel-shaped dMNA limited further skin insertion after reaching the insertion limit, and this resulted in a relatively less dissolved volume of dMNA. However, choosing a candlelight-shaped dMNA, which has more inserted volume in the skin, might improve the dissolution of PLGA NPs incorporated dMNA.

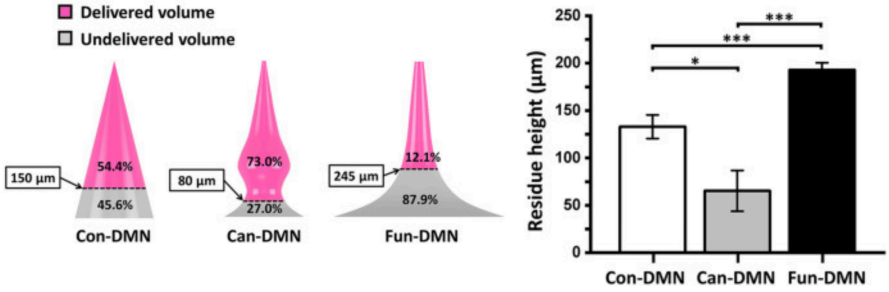

Figure 2. Residue height of dMNAs after dissolution according to their shapes[16]

Improve the antigen-saving production method of dMNA

In this thesis, antigen-saving dMNA was developed by using the automatic dispensing system (Chapter 4). The system mainly consisted of a nano-dispenser and linear stages, and their operation was automised by programming with Python. However, it still required a manual calibration step prior to repeating the dispensing and aligning process. It is because the PDMS mould was deformed when the low pressure was applied to it, and the level of deformation varied depending on the thickness of the PDMS mould. Therefore, each mould required the calibration step to align the dispenser with all microneedle cavities. In order to develop a completely automised system, this calibration step should also be automated by incorporating optical shape recognition of microneedle cavities. To achieve this, the movement of linear stages can be designed to halt once the camera positioned beneath the mould detects the shape of the microneedle cavity[17]. Via this shape recognition, the accuracy of dispensing can be raised, thus, more antigens can be saved.

The main drawback of the dispensing system is its restriction on formulation viscosity since the dispenser only accepts liquid formulations with a viscosity of below 50 m.Pas. Therefore, a highly concentrated formulation is not dispensable since it frequently has a high viscosity, despite the fact that the mechanical strength of dMNA which is obtained from the highly concentrated formulation is desired for skin penetration.

Similar issues might arise when dMNA is produced using a spraying approach[18]. The spraying technique uses an automised spray which scatters drug compositions into the mould by controlling an air pressure and liquid input rate. Although this technique also reduces antigen waste, it is constrained by the viscosity of formulations because it has an impact on droplet size. In contrast, the droplet-born air-blowing approach requires a viscous formulation[19]. This technique elongates the dispensed drop formulation using upper and bottom plates. In order to control the length of dMNA, this method limits to formulations with high viscosity. In order to regulate the viscosity and facilitate efficient use of these techniques including the

automatic dispensing system, the temperature can be regulated as temperature and viscosity are inversely proportional[20]. By raising the process temperature during dispensing, the number of dispensable formulations can be increased. Regarding the thermal stability of antigens, several antigens proved their functionality during dMNA production at high temperatures by inducing immune responses[21–23].

Utilise the automatic dispensing system

As discussed in *Boost dissolution ability of PLGA NPs incorporated dMNA* section, dissolution profiles of dMNA can be regulated by its composition. Therefore, dMNA consisting of multiple formulations can allow controlled release and significantly enhance patient compliance by reducing the number of doses. For instance, most of vaccines require repeated doses over time including hepatitis B and human papillomavirus vaccines. By loading multiple formulations with different release profiles into a single dMNA, the entire dose can be administrated at once. To accomplish this, the base layer of the microneedle containing the prime dose should rapidly dissolve and separate the microneedle tip from the backplate. Thus, the detached microneedle tip stays in the skin and releases the booster dose on schedule. This multi-layered dMNA can be achieved via the automatic dispensing system by adding several nozzles for each formulation and dispensing them in order. It also can improve the penetration ability by placing the mechanically stronger formulation in the tip layer[24]. Multi-layered dMNA can be produced with various methods including centrifugation, spraying, or a mixture of centrifugation and dispensing[24–26].

CONCLUSION

In this thesis, various formulations of dMNA were designed and optimised for each antigen. The design of formulation is based on the stability of antigens, penetration and dissolution abilities of dMNA, and the dMNA production method as well. Successful production of antigen-incorporated dMNAs with the desired penetration efficiency and dissolution rate was demonstrated for several antigens. To develop antigen-saving dMNA, the automatic dispensing system was engineered and it successfully reduced the antigen waste. Although further research is required to develop a completely automated system, it is a promising system to scale up and prepare multi-layered dMNA.

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