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## Development of hyaluronan-based dissolving microneedle arrays for dermal vaccination

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### Citation

Leone, M. (2020, December 10). *Development of hyaluronan-based dissolving microneedle arrays for dermal vaccination*. Retrieved from <https://hdl.handle.net/1887/138252>

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**Title:** Development of hyaluronan-based dissolving microneedle arrays for dermal vaccination

**Issue date:** 2020-12-10

# Chapter 7

Summary, prospects and conclusion

## SUMMARY

Vaccination is one of the most effective method for reducing infection-related deaths and morbidity around the world. The principle of vaccination was first published by Edward Jenner in 1796 [1] and, since the advent of the microbiology era in the second half of the 19th century, numerous types of vaccines have been developed and licensed for both human and veterinary use.

Many factors have an impact on vaccination coverage [2-4]. These include costs, concerns about vaccine safety and efficacy by the general public [5], religious believes, the spread of mis-information by “anti-vax” groups [6]. Some other factors are related with the way vaccines are administered. Injected vaccines cause discomfort by a substantial part of the target group. Furthermore, needlestick injuries and re-use of needles and syringes are health risks for medical personnel as well as the vaccinated. To overcome these problems, vaccination in a minimally invasive and pain-free manner may improve vaccination coverage.

Microneedle-based dermal immunization is a promising alternative to the classical conventional administration of vaccine by means of hypodermic needles such as intramuscular and subcutaneous injections. The skin is an excellent immune competent organ containing many antigen presenting cells, such as Langerhans cells and dendritic cells, to induce an effective immune response. This is a consequence of the fact that the skin, differently than muscle and subcutaneous tissue, is directly exposed to the surrounding environment and protects the body against pathogens, not only by forming an effective physical barrier but also by extensive immune surveillance just beneath the barrier.

Of the several types of microneedles [7], the focus of this thesis is on the development of dissolving microneedles, using hyaluronan as matrix polymer.

This thesis starts with a detailed overview of dissolving microneedle research and development (*Chapter 2*), describing methods to produce dissolving microneedles and their challenges, as well as the microneedle characterization methods and antigen stability aspects. Furthermore, this chapter contains a detailed overview of the immunogenicity of several antigens encapsulated in dissolving microneedles. Immune responses generated after immunization by dissolving microneedles have been compared with conventional injection. Frequently, the responses after dermal immunization are at least comparable to conventional injection. Additionally, several factors influencing the immunogenicity have been discussed: besides the use of conventional adjuvants, the microneedle design such as needle spacing and needle geometry may also influence the immunogenicity. Finally, the current status of the clinical development of dissolving microneedles is discussed. Dissolving microneedle devices are in early clinical development [8, 9] and may reach the market within a decade.

The research described in *Chapter 3* focuses on the development of a digitally-controlled microneedle applicator to insert microneedles into the skin via impact insertion (velocity) or via pressing force insertion. Six microneedle arrays with different geometries, needle density and/or consisting of different materials were applied onto *ex vivo* human skin varying velocities and pressing forces to assess differences in penetration efficiency of the skin and antigen delivery in the skin. Application of microneedle arrays by impact application, with a specific angle of application, could generate a more efficient piercing of the skin than application via pressing force. The delivery of the antigen in the skin could be increased by increasing the velocity or pressure, demonstrating the importance of a controlled application of the microneedle array on the skin by means of an applicator.

In *Chapter 4* a novel mold design is described for the manufacturing of dissolving microneedles by micromolding, avoiding waste of antigen in the backplate. During the original manufacturing one backplate with 9 arrays was produced. This single backplate resulted in non-homogeneous antigen distribution between arrays. The new method eliminates this problem as one mold contains 9 separate templates for the arrays. Using this mold, dissolving microneedles with increasing antigen loading were fabricated to assess the physicochemical effects of maximal antigen content. Dissolving microneedles could be fabricated with an ovalbumin:hyaluronan ratio of 1:1 (w/w), with excellent sharpness and efficient skin piercing properties, even after storage at high temperature and high humidity. The protein did not aggregate during the fabrication of dissolving microneedles. However, when using the same skin dissolution time, increased antigen loading led to a decrease in dissolution volume of microneedles in *ex vivo* human skin. Finally an immunization study in mice by using dissolving microneedles induced antibody responses comparable to those obtained by conventional immunization and there was a faster antibody response compared to dermal immunization by means of a single hollow microneedle.

In *Chapter 5* the optimal hyaluronan molecular weight for the fabrication of dissolving microneedles with regard to microneedle integrity and immune modulating properties was identified. These studies were initiated as it was reported that low MW HA showed immune modulating properties. Hyaluronan ranged from a molecular weight of 4.8 kDa up to 1.8 MDa and all demonstrated to be inert material. No effects on the antibody response generated in mice or on the CD4 T-cell responses after immunization or after *in vitro* stimulation with the model antigen ovalbumin were detected. However, not all the hyaluronan molecular weights of the selected range were suitable for microneedle fabrication: too high molecular weight resulted in a too viscous formulation to be used for micromolding while too low molecular weight generated fragile microneedle arrays showing a lack of structure. Furthermore, longer application time of dissolving microneedles in the skin was necessary for a complete dissolution of high hyaluronan molecular weight dissolving microneedles than for low hyaluronan molecular weight ones, leading to the selection of the 20 kDa HA to fabricate dissolving microneedles.

The aim of *Chapter 6* was to determine whether repeated-fractional intradermal administration of the antigen diphtheria toxoid could enhance the response compared to a single administration in the presence or absence of adjuvants with both hollow and dissolving microneedles. After a selective immunization screening, poly(I:C) and gibbsite, a nanoparticulate alum based adjuvant, were selected as adjuvants and encapsulated with diphtheria toxoid in dissolving microneedles in full or fractional diphtheria toxoid(-adjuvant) dose. Regardless the composition, it was possible to fabricate sharp dissolving microneedles capable to penetrate the skin and dissolve within 20 minutes depositing the intended diphtheria toxoid(-adjuvant) dose. Vaccination by dissolving microneedles without adjuvant, led to a superior response as compared to a hollow microneedle. Repeated dosing with dissolving microneedles did not further increase the immune responses. However, repeated-fractional dosing with a hollow microneedle led to a higher immune response than single-full dose and reached the same level of response as using dissolving microneedles. Furthermore, adjuvanted diphtheria toxoid co-encapsulated in dissolving microneedle did not increase further the immune response. The response after applying dissolving microneedles without adjuvant was comparable to conventional subcutaneous injections of diphtheria toxoid- $\text{AlPO}_4$  in a 15 times higher antigen dose as well as diphtheria toxoid-poly(I:C) in a similar antigen dose. In conclusion, single-full dermal dose diphtheria toxoid administration by means of dissolving microneedle led to a superior response without the use of adjuvants. Based on results in this study, it may be possible that immunization by dissolving microneedle will result in efficient immune responses while only using a single administration with a lower antigen dose as compared to subcutaneous administration.

## PROSPECTS

### **Dissolving microneedles: challenges and next steps in development**

Although dissolving microneedles as vaccine delivery device have huge potential, pharma companies are still facing hurdles that must be overcome. Before vaccine-loaded dissolving microneedles will appear as licensed products, the accuracy of vaccine delivery need to be increased and the ease of application of microneedle arrays on the skin need to be improved. To achieve this, the research should focus on i) the formulation of a vaccine product, not only taking into account the shelf life of the final product, but also antigen loss during manufacturing should be acceptable, ii) increase in antigen loading and its stability in dMNs, iii) an efficient insertion, and dissolution, of the microneedle in the skin, iv) product sterility for which new production procedures have to be developed, v) adjuvants that may need to be added for less immunogenic antigens, vi) the scale-up of the manufacturing process.

A first challenge encountered during the fabrication of dissolving microneedles, especially in micromolding, is the **loss of antigen** in the backplate of the microneedle array, that increases the cost of vaccination. In the literature hardly any data are available on formulation volume

and antigen amount used for the fabrication of dMN array, the antigen amount present in the dMN tips and on the dose delivered in the skin. This would highlight the important drawback related to a low fabrication efficiency in terms of antigen incorporation. In this thesis we attempted to optimize the PDMS mold design to overcome this problem. However, future research should keep focus on developing methods to cast the vaccine formulation only in the microneedle tips of the mold in order to reduce vaccine loss in the backplate [10, 11]. This would make vaccination using dissolving microneedles more affordable, especially for developing countries.

In Chapter 4 of the present thesis, increase in **antigen loading** in dMNs has been investigated and it was observed that, up to a weight ratio antigen:hyaluronan of 1:1, it was still possible to fabricate sharp dMNs resulting in **antigen stability** after fabrication. However, these studies have been conducted using OVA, a very stable model antigen. Thus, several hurdles related to the antigen loading in dMNs should be taken into account, such as antigen stability when working with less stable antigens, solubility issues when high antigen concentration is needed to deliver sufficient antigen dermally for a proper response, etc. It is known that the physical stability of the antigen is relevant to avoid uncontrolled immune response, thus these aspects should trigger new studies to explore themes hardly reported in literature.

Another critical issue is an efficient and complete microneedle insertion into the skin. When the microneedles have a geometry and length causing an incomplete insertion in the skin, this results in **incomplete dissolution**. This could lead to inconsistent dosing and waste of antigen. Furthermore vaccinees should wear patches long enough to ensure complete microneedle dissolution avoiding failure in the vaccine delivery related to the dissolution [12]. Patches may be removed too early, introducing an unwanted variable in the delivery of the vaccine. To overcome these problems of too early removal of the patch or incomplete microneedle insertion into the skin, some ideas have been proposed, such as i) arrow-head dissolving microneedles [13] composed of polymer arrowheads that, upon insertion in the skin, separate from the metal shaft on which they are mounted and remain embedded in the skin for subsequent dissolution and drug release and ii) patchless dissolving microneedle with a system capable of inserting drug-loaded dissolving microneedles in the skin as individual microneedles [14]. However, these ideas have their drawbacks including i) generation of long microneedles (>600  $\mu\text{m}$  [13]) that, although potentially enhancing the immune response more than short microneedles [15], are more likely to induce pain [16], ii) use of a device for the dissolving microneedle insertion in the skin that may need trained personnel and can increase costs for their application. It would be then necessary to work on the choice of the matrix material and the formulation composition in order to reach very short application time (1-2 minutes) needed for the dissolution of the dMNs. In this regard, an application time of hours [12] would result in too long applications for a real-life vaccination.

It is important that microneedle products are sterile. In light of this, the **sterile manufacture** of microneedles is essential and should be performed accordingly to specific sterility requirements to guarantee product safety [17]. Sterilization methods should be incorporated in the manufacturing processes avoiding modification of the microneedle loaded vaccine and also an increase in manufacturing costs. For example, terminal sterilization using gamma irradiation, moist heat or microwave heating can be less expensive than aseptic manufacturing, however it can damage the microneedles or the product cargo. Several materials used for dissolving microneedle production have been shown to possess antimicrobial properties, showing no microbial growth upon storage and are, therefore, highly unlikely to cause skin or systemic infection [18]. In such cases, a fabrication of microneedles with the assurance of a low bioburden may be sufficient to avoid sterile manufacture and obtain regulatory approval.

For some less immunogenic antigens the addition of **adjuvants** may still be essential to obtain a high enough immune response. However, because of safety concerns, not all adjuvants are feasible for dermal application. An example is the classical aluminum preparations inducing palpable persistent intradermal injection-site nodules in mice [19]. On the one hand the avoidance of adjuvant would make the vaccine product less complex and thus less likely to undergo interactions affecting stability, on the other hand including adjuvants may lead to antigen dose sparing.

An important step to progress is the **scale-up** of the manufacturing process. Currently, an abundance of small-scale production methods are present in literature (see Chapter 2) by which a number of steps need to be undertaken (e.g. centrifuge and vacuum steps for the micromolding) that pose a challenge in terms of transfer to a larger scale. Furthermore, guidance related to good manufacturing practice, pharmacopoeial standards and appropriate quality control tests specific to microneedle devices are required [20]. Specific regulatory guidelines concerning packaging, disposal, assurance of correct use are needed. However, due to the innovative nature of the technology, the lack of regulation remains a barrier to the availability of a dissolving microneedle product. Although the use of dissolving microneedles for vaccination is promising, there are not yet dissolving microneedle products on the market. This is expected to change in the next decade as a high number of studies in the field continues to accumulate and some products are in clinical development [8, 9]. Currently some companies are setting up a production of dissolving microneedles for skin care cosmetics [21] and for delivery of biologics across the skin [22]. The latter with a successfully completed Phase 2a clinical evaluation.

Once manufacturing processes are optimized and regulatory hurdles are solved, vaccination by means of dissolving microneedles may become a keystone in improving vaccination coverage around the world.



### **The big potential of dissolving microneedles**

Although dissolving microneedles may have still a long development route before being ready as product for marketing authorization, they have a great potential over the other microneedle types: dissolving microneedles consist of dry formulations which enhances the vaccine stability compared to liquid formulation used in traditional vaccination routes [23, 24]. Because of this increased stability, these microneedles could circumvent the need for a cold-chain supply and be ideal for vaccine campaigns in low income countries. This would significantly reduce vaccination costs for transportation and storage and therefore increase the vaccination coverage and the efficiency of vaccination programs.

Additionally, vaccination by means of dissolving microneedles showed, in most of the cases, a comparable or even higher response than by conventional injection (see *Chapter 2*). The antigen dose sparing, as illustrated by the results described in *Chapter 6*, provides a further strong potential of dissolving microneedles for dermal vaccination.

### **The importance of microneedle application**

In this thesis, two microneedle types have been used for administration of vaccines: a hollow microneedle and dissolving microneedles. Both microneedle types require application devices.

Controlled and precise injection volume of the vaccine in the skin by means of a hollow microneedle may require a complex device with an applicator for controlled depth piercing of the skin and a pump for a controlled dermal microinjection.

Dissolving microneedles can be applied instantaneous and would take short time for dissolution in the skin. However, the use of an applicator may not be feasible in areas with a lack of infrastructure. Applicator failure or applicator loss may disrupt immunization programs locally. Additionally, a device for the application may increase the vaccination costs.

In this regard, an important improvement would be skin application of microneedles without the use of a device. This may avoid the need for trained personnel and should make self-administration possible especially in remote locations in the world or in case of pandemic disease outbreaks. Studies show that using an applicator improves the efficiency and reproducibility of microneedle insertion [25] and that short microneedles (300  $\mu\text{m}$ ), but also less sharp microneedles, may have a lower penetration efficiency in the skin than longer ones (>550  $\mu\text{m}$ ) [26-28] and thus the use of an applicator can be crucial for their efficient piercing in the skin. Manual application avoiding the use of an applicator is possible and successful [8, 9] if longer microneedles are used. In this case, long needles can still be considered much less invasive than hypodermic needles, keeping their advantage in a

reduced generation of pain sensation. Table 1 summarizes the pros and cons of applicator use in microneedle application.

**Table 1.** Advantages and disadvantages of manual application and application by the use of an applicator.

Manual application		Application by the use of an applicator	
Advantages	Disadvantages	Advantages	Disadvantages
Low production costs	Limitation in microneedle length: application of long microneedles only	No limitation in microneedle length (application of short microneedles)	Higher production costs of the system
No need for trained personnel	Use of long microneedles may generate pain sensation	High efficiency and reproducibility in microneedle piercing	Need for trained personnel
			More dependent on technology on site

### The role of hyaluronan in vaccination by means of dissolving microneedles

As demonstrated in *Chapter 5*, the hyaluronan used as matrix material does not have an effect on the immune response after vaccination by dissolving microneedles despite the reported immune modulating properties of low molecular weight hyaluronan [29]. However, in literature hyaluronan conjugated with ovalbumin can activate naive dendritic cells *in vitro* more efficiently than ovalbumin only [30] and conjugation can increase the size of compounds to promote lymphatic delivery [31]. Furthermore, intramuscular injection of hyaluronan-ovalbumin conjugates induced a higher immune response than ovalbumin alone [30]. To this end, it would be interesting to encapsulate hyaluronan-antigen conjugates in dissolving microneedles and assess the immunogenicity.

## CONCLUSION

The research described in this thesis showed that dissolving microneedles used for dermal delivery of vaccines can evoke an antigen specific immune response comparable with the conventional subcutaneous route. However, further research is needed to optimize this technology and overcome several manufacturing and application hurdles in order to translate research to commercial products beneficial for the patients.

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