FORMULATION AND CLINICAL TRANSLATION OF MICRONEEDLES FOR VACCINATION IN DEVELOPING COUNTRIES

A Thesis
Presented to
The Academic Faculty

by

Jaya Arya

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy in Bioengineering

Georgia Institute of Technology
August 2016

FORMULATION AND CLINICAL TRANSLATION OF MICRONEEDLES FOR VACCINATION IN DEVELOPING COUNTRIES

Approved by:

Dr. Mark R. Prausnitz, Advisor
School of Chemical and Biomolecular Engineering
Georgia Institute of Technology

Dr. Julie A. Champion
School of Chemical and Biomolecular Engineering
Georgia Institute of Technology

Dr. Jonathan S. Colton
School of Mechanical Engineering
Georgia Institute of Technology

Dr. Krishnendu Roy
School of Biomedical Engineering
Georgia Institute of Technology

Dr. William C. Weldon
Polio and Picornavirus Laboratory Branch
Centers for Disease Control and Prevention

Date Approved: April 5th, 2016

To
My grandfather Bhagwandev Arya
And
My parents Lajwanti and Mahesh Arya

For giving me the gift of opportunity

ACKNOWLEDGEMENTS

As I think back to the day I moved to Georgia Tech for graduate school and reflect upon the past five years, I feel extremely grateful for this rewarding journey to get my PhD. I do also remember how trying and difficult some of those days were and am very thankful to all the people who provided help and support along the way. Dr. Mark Prausnitz has been an excellent advisor and mentor which has allowed me to have a well-rounded and fulfilling graduate school career. He encouraged me to try new ideas, was supportive when I changed projects two years into my PhD and was always available for feedback despite his extremely busy schedule. I got to work along the entire timeline of medical device development – formulation, pre-clinical and clinical testing during my time in the lab and I am aware that these opportunities are rare. He was always encouraging of presenting my research at conferences which allowed me to meet and learn from researchers from across the globe. He has provided excellent feedback in communication and I truly believe that these lessons will be broadly applicable to my career in the future. He was also supportive of my pursuits outside of the lab – I was able to take seventeen classes across various disciplines and participate in and lead student organizations and events. For all of this and more, I am grateful.

I would also like to thank my committee members Dr. Julie Champion, Dr. Jonathan Colton, Dr. Krishnendu Roy and Dr. William Weldon for their time and feedback. My thesis would be incomplete without the support of my collaborators at Georgia Tech, Centers for Disease Control and Prevention and Merial. I am grateful for the opportunity to work with the CDC and am thankful to Dr. Steve Oberste, Dr. Paul

Rota and the members of the polio and measles group for their enthusiasm about my project, expertise and time. Kristopher DeWitt, Dr. Maya-Scott Garrard and Dr. Yu-Wei Chiang at Merial have provided constant input through their expertise with rabies vaccine, assays and veterinary applications.

I am grateful to Donna Bondy for subtracting one year from my PhD by always being on top of paperwork, lab orders, IACUC and IRB protocols. Thank you for the encouragement, laughs and for being my motivation to meet my fitness and outdoors goals. Christopher Ruffin, Laura Paige and Janice Whatley-Nwanze – thank you for keeping me up to date with all of my academic requirements and ensuring that I submit my thesis and graduate in time.

My time in graduate school would not have been nearly as fun if not for the wonderful colleagues at the Drug Delivery Lab – thank you all the past and present members for your help in the lab - feedback at group meetings, troubleshooting and training in the lab, volunteering for my studies and brainstorming discussions. Thank you for creating a fun, inclusive and supportive culture – I am leaving this group with beautiful memories and I hope to remain friends with you all in the future. I can confidently say that every single one of you has helped me at some point during the course of my PhD and for that I am extremely grateful.

The world is not flat for opportunities. I am extremely grateful for the opportunities that I have received and want to celebrate the family and friends who made these opportunities possible for me. My parents, Lajwanti and Mahesh Arya and my brother Yash Arya – Thank you for your unconditional love and support, teaching me the value of rational thinking and education, creating an environment where it was okay for

me to fail and learn from my mistakes and for your incredible sense of humor. I also want to thank my boyfriend Dr. Pratik Darvekar for being my biggest champion, believing in me and pushing me to follow my dreams. When I landed in Atlanta five years ago, I had only two checked bags with me and did not know any person in Atlanta. I'm grateful that it is no longer true thanks to my roommates and friends who became my Atlanta family over these years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
WHO	World Health Organization
MN	Microneedle
EPI	Expanded Program on Immunization
OPV	Oral Polio Vaccine
IPV	Inactivated Polio Vaccine
HIV	Human immunodeficiency virus
UNICEF	United Nations Children's Emergency Fund
MNP	Microneedle patch
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
CDC	Centers for Disease Control and Prevention
ORISE	Oak Ridge Institute for Science and Education
PDMS	Polydimethylsiloxane
IACUC	Institutional Animal Care and Use Committees
RFFIT	Rapid Fluorescent Focus Inhibition Test
ANOVA	Analysis of Variance
IM	Intramuscular
VAS	Visual Analog Scale

SUMMARY

Most vaccines are currently administered by healthcare personnel using a needle and syringe. This delivery method poses significant hurdles in vaccine delivery, especially in developing countries. We propose dissolving microneedle patches to be a suitable alternative to needle and syringe vaccination in developing countries. Dissolving microneedle patches contain micron sized needles made out of water-soluble biodegradable polymers that dissolve in the skin to deliver the vaccine. They offer the simplicity of patch application and the possibility to mitigate the logistical and safety challenges associated with conventional hypodermic needles.

The overall goal of this thesis was to develop dissolving microneedle patches to further clinical translation of this technology in the context of vaccinations in developing countries. We studied two specific scenarios, development of microneedle patches for rabies vaccination of dogs and assessment of dissolving microneedle patches in human subjects. Human rabies is eliminated in most developed countries by employing control measures of vaccinations in animals. However, dogs account for nearly all human rabies infections in developing countries and vaccinations are difficult to employ in animals due to the need of a needle and syringe and the cost of administration. While microneedle patches are in pre-clinical development for different vaccines, limited information is available about their use in human subjects, which will be important for clinical translation.

The central hypothesis was that rabies vaccine can be stabilized in a dissolving microneedle patch and be at least as immunogenic as conventional needle and syringe while enabling simple administration and that dissolving microneedle patches could be

easily administered without the need of an applicator, be well tolerated in the skin and preferred over needle and syringe administration. This was assessed by engineering patches for veterinary rabies vaccination and evaluating immune response in dogs and determining tolerability, usability and acceptability of placebo microneedle patches in human subjects.

The first study reports on rabies vaccination in dogs using a dissolving microneedle patch to enable simple and reliable intradermal rabies vaccination of dogs. The results show that the vaccine was stable upon formulation and storage for at least 3 weeks at 4 °C in a microneedle patch. Microneedle patches were well tolerated in the skin, with mild erythema, minimal wheal formation and complete resolution of skin reactions within 7 days, and generated no systemic adverse events. Microneedle patches were at least as immunogenic as intramuscular injection at the same dose, as demonstrated by similar serum neutralizing antibody titers. A ten-fold lower vaccine dose administered by microneedle patch generated a weaker immune response compared to full-dose intramuscular vaccination.

The second study reports on tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects without the use of an applicator. The results show that microneedle patches were very well tolerated in the skin with minimal erythema that resolved fully within seven days and caused no pain or swelling. Microneedle patches were administered reliably by hand without the need of an applicator and delivery efficiencies were similar between investigator-administration and self-administration. Microneedle patch administration was not painful and the large majority of subjects were at least somewhat confident that they self-administered the

patch correctly. Microneedle patch administration was overwhelmingly preferred over conventional needle and syringe injection for delivery of medications.

Altogether, the positive results from these studies should further clinical translation of microneedles in the context of vaccination in developing countries.

CHAPTER 1

INTRODUCTION

1.1 Motivation

Vaccination is one of the most effective ways to reduce morbidity and mortality associated with vaccine-preventable diseases [1]. Most vaccines are currently administered by healthcare personnel using a needle and syringe. This delivery method poses significant hurdles in vaccine delivery, especially in developing countries. It requires trained personnel to administer each dose, creates medical sharps waste that must be safely disposed of to prevent reuse and necessitates the need of cold chain for vaccine stability [2, 3]. Dissolving microneedle patches contain micron-sized needles made out of water-soluble biodegradable polymers that dissolve in the skin to deliver the vaccine [4-6]. They offer the simplicity of patch application and the possibility to mitigate the logistical and safety challenges associated with conventional hypodermic needles.

Human rabies is eliminated in most developed countries by employing control measures of vaccinations in animals [7]. Dogs account for transmission of nearly all human rabies infections in developing countries [8] and vaccinations are difficult to employ in animals due to the need of a needle and syringe and the cost of administration [9]. We propose that dissolving microneedle patches can be a substitute to needle and syringe injection this vaccination scenario.

Dissolving microneedle patches have previously been studied for delivery of other drugs and vaccines in pre-clinical models, but limited information is available about

clinical administration of microneedle patches [4, 5, 10-16]. As microneedle patches continue to be developed for delivery of vaccines, it will also be important to characterize their use in human subjects. We therefore propose to evaluate dissolving microneedle patches in human subjects to understand the reactions in the skin after administration and dissolution of microneedles as well as the efficiency of microneedle delivery.

1.2 Specific aims

The overall goal of this thesis is to develop dissolving microneedle patches to further clinical translation in the context of vaccination in developing countries. The overall hypothesis is that rabies vaccine can be stabilized in a dissolving microneedle patch and be at-least as immunogenic as needle and syringe injection while enabling simple administration. To study this hypothesis, we investigated the following specific aims:

Aim 1: Engineer dissolving microneedle patches for rabies vaccination and evaluate immunogenicity and dose sparing in dogs.

We hypothesize that rabies DNA vaccine can be suitably stabilized in a dissolving microneedle patch, and that the patches can be reliably and easily inserted into dog ears, are safe and well tolerated and are at least as immunogenic as intramuscular injection in Beagle dogs.

Aim 2: Evaluate tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects.

We hypothesize that a controlled-bioburden process can be developed to fabricate dissolving microneedle patches suitable for use in humans and that microneedle patches

can be inserted into skin by forces generated with the thumb without the need for an applicator. After insertion and dissolution, we hypothesize that the microneedles are well-tolerated in the skin and preferred over conventional hypodermic needles for delivery of vaccines.

1.3 Outline of remaining chapters

Chapter 2 contains background information on the polio and rabies vaccine delivery challenges and clinical translation of microneedle patches. Chapter 3 contains a review on microneedle patches for vaccination in developing countries. Chapters 4 - 6 contain work done on the specific aims. Chapter 7 contains the key conclusions and Chapter 8 provides recommendations and future work.

1.4 References

1. WHO. *Global Immunization Data July 2014*. [July 30 2015]; Available from: http://www.who.int/immunization/monitoring_surveillance/global_immunization_data.pdf.

2. LaFond, A., N. Kanagat, R. Steinglass, R. Fields, J. Sequeira, and S. Mookherji, *Drivers of routine immunization coverage improvement in Africa: findings from district-level case studies.* Health Policy Plan, 2015. **30**(3): p. 298-308.

3. Favin, M., R. Steinglass, R. Fields, K. Banerjee, and M. Sawhney, *Why children are not vaccinated: a review of the grey literature.* Int Health, 2012. **4**(4): p. 229-38.

4. Kim, Y.C., J.H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Adv Drug Deliv Rev, 2012. **64**(14): p. 1547-68.

5. Sullivan, S.P., D.G. Koutsonanos, M. Del Pilar Martin, J.W. Lee, V. Zarnitsyn, S.-O. Choi, N. Murthy, R.W. Compans, I. Skountzou, and M.R. Prausnitz,

Dissolving polymer microneedle patches for influenza vaccination. Nat Med, 2010. **16**(8): p. 915-20.

6. van der Maaden, K., W. Jiskoot, and J. Bouwstra, *Microneedle technologies for (trans)dermal drug and vaccine delivery.* J Control Release, 2012. **161**(2): p. 645-55.

7. CDC. *Rabies in the U.S.* 2011 [11 Feb]; Available from: http://www.cdc.gov/rabies/location/usa.

8. Publication, W.H.O., *Rabies vaccines: WHO position paper--recommendations.* Vaccine, 2010. **28**(44): p. 7140-2.

9. World Health, O., *WHO Expert Consultation on Rabies. Second report.* World Health Organization technical report series, 2013(982): p. 1-139, back cover.

10. Edens, C., N.C. Dybdahl-Sissoko, W.C. Weldon, M.S. Oberste, and M.R. Prausnitz, *Inactivated polio vaccination using a microneedle patch is immunogenic in the rhesus macaque.* Vaccine, 2015. **33**(37): p. 4683-90.

11. Mistilis, M.J., A.S. Bommarius, and M.R. Prausnitz, *Development of a Thermostable Microneedle Patch for Influenza Vaccination.* Journal of Pharmaceutical Sciences, 2015. **104**(2): p. 740-749.

12. Carey, J.B., A. Vrdoljak, C. O'Mahony, A.V. Hill, S.J. Draper, and A.C. Moore, *Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route.* Sci Rep, 2014. **4**: p. 6154.

13. Torrisi, B.M., V. Zarnitsyn, M.R. Prausnitz, A. Anstey, C. Gateley, J.C. Birchall, and S.A. Coulman, *Pocketed microneedles for rapid delivery of a liquid-state botulinum toxin A formulation into human skin.* Journal of Controlled Release, 2013. **165**(2): p. 146-152.

14. Raphael, A.P., M.L. Crichton, R.J. Falconer, S. Meliga, X. Chen, G.J. Fernando, H. Huang, and M.A. Kendall, *Formulations for microprojection/microneedle vaccine delivery: Structure, strength and release profiles.* J Control Release, 2016.

15. Kommareddy, S., B.C. Baudner, A. Bonificio, S. Gallorini, G. Palladino, A.S. Determan, D.M. Dohmeier, K.D. Kroells, J.R. Sternjohn, M. Singh, P.R. Dormitzer, K.J. Hansen, and D.T. O'Hagan, *Influenza subunit vaccine coated microneedle patches elicit comparable immune responses to intramuscular injection in guinea pigs.* Vaccine, 2013. **31**(34): p. 3435-3441.

16. Arya, J. and M.R. Prausnitz, *Microneedle patches for vaccination in developing countries.* Journal of Controlled Release.

CHAPTER 2

BACKGROUND

2.1 Rabies vaccination

2.1.1 Rabies virus and disease

Rabies virus is a RNA virus shaped like a bullet, 200 nm long and 75 nm wide. The virus contains multiple copies of the five structural proteins – virion transcriptase (L), glycoprotein (G), nucleoprotein (N), phosphoprotein (P) and matrix protein (M) [1]. The rabies virus G protein is the major antigen responsible for production of virus neutralizing antibodies and for conferring immunity against lethal infection with rabies [2].

Rabies is an acute, often fatal encephalitis caused by viruses in the Rhabdoviridae family [3]. The disease is zoonotic and human infection usually results from a bite or scratch from an infected animal or direct contact of skin wounds with virus-containing saliva. After a bite, the virus in saliva attaches to the nerve endings and travels to the brain. Once the virus reaches the central nervous system and symptoms begin to show, the disease is almost always fatal. Rarely infections due to inhalation of the virus, inoculations with improperly inactivated vaccine or through transplantation of infected corneas, tissues and organs have been reported [3]. Although all warm-blooded animals can be reservoirs of rabies, dogs account for 99% of human deaths due to rabies and pose a potential threat to more than 3.3 billion people [4]. Globally, an estimated 26,000 to 61,000 deaths are caused by rabies each year, more than 95% of which occur in Africa

and Asia due to dog bites [5]. Rabies occurs mainly in remote rural communities where children between the age of 5–14 years are the most frequent victims [6]. Human rabies has been almost eliminated in industrialized countries by widespread and often mandatory vaccination of dogs and other animals, as well as the availability of vaccines for humans [5].

2.1.2 Vaccination in humans

Since their development many decades ago, concentrated, purified cell culture and embryonated egg-based vaccines have proved to be safe and effective in preventing rabies [5]. These vaccines are given for both pre-exposure and post-exposure prophylaxis. Humans are not typically vaccinated for rabies prevention but pre-exposure prophylaxis is recommended for anyone who will be at continual, frequent or increased risk of exposure to the rabies virus. Laboratory workers, veterinarians, animal handlers and travelers at high risk are recommended. An older vaccine derived from animal nerve tissues is no longer recommended for use in people because they have a higher number of adverse events and are less immunogenic than cell culture vaccines [5]. Upon exposure, it is recommended that the wound be thoroughly cleaned with soap and water, and the rabies vaccine be administered [7]. Rabies immunoglobulin is also administered once and multiple booster shots of the vaccine are given [8]. The vaccines are usually given intramuscularly, but in some countries are approved to be given at a low-dose via the intradermal route [9]. Intradermal vaccination using one-fifth to one-tenth the dose of rabies vaccine has been shown to be effective in humans, thereby enabling significant cost savings [4, 10-12].

2.1.3 Vaccination in animals

Veterinary vaccines have been developed for use against rabies in domestic mammals and wildlife. Injectable live virus vaccines are no longer used in domestic animals due to their inherent ability to cause rabies [4]. An injectable live recombinant vectored vaccine is available for use in cats in the US. The most widely used vaccines worldwide are injectable inactivated vaccines because they are safe and inexpensive. There is only one oral rabies vaccine approved for use in dogs but its use has been limited when vaccinating dogs because the bait in which the vaccine is contained does not always lead to complete delivery of vaccine and in developing countries where the contact with street dogs is high the use of oral vaccines is usually limited to areas with minimal human activity so as to ensure safe distribution [5]. Limited vaccination of dogs and availability of post exposure prophylaxis for people is the cause of most of the deaths due to rabies in developing countries. The WHO recommends mass vaccination of at least 70% of the dog population to control canine rabies in endemic areas [5].

Different rabies DNA vaccines are currently being studied for vaccination of dogs and other animals and their efficacy has been shown in companion animals [13-15]. DNA vaccines could be much less costly to manufacture compared to inactivated virus vaccines like the conventional rabies vaccine for human vaccination [16, 17]. This is because DNA vaccines can be produced in large quantities by bacterial fermentation processes and may not require expensive facilities with high biosafety levels for production [18]. DNA vaccines could also show stability at high temperatures reducing the need for cold chain [17].

Currently, there are a few DNA-based products approved for animal use. West Nile virus vaccine for horses, infectious hematopoietic necrosis virus vaccine for salmon fish, canine melanoma vaccine for dogs and a growth hormone releasing hormone gene therapy product for swine and food animals [16]. These licensures are important validations of the DNA vaccine platform and illustrate its commercial potential. It shows that DNA vaccines can be manufactured to scale and at low cost and that large animals can be successfully protected by specific DNA approaches. In addition, the generic nature of production and purification of plasmid vaccines can enable tech transfer to developing countries and low cost of vaccines [17].

2.2 Microneedles

Microneedles are micron-sized needles up to 1 mm in length which are able to minimally penetrate past the biological barrier membranes for targeted drug delivery [19]. Microneedles are being studied for drug delivery to the skin by penetrating the stratum corneum [20], drug delivery to the back of the eye by penetrating the sclera [21] and drug delivery to cells by penetrating the cell membrane [22]. In the case of drug delivery to the skin, microneedles are able to penetrate the skin's protective physical layer called the stratum corneum, which is about 20 μm thick [23]. It has been shown that by penetrating this upper most layer of skin, drug delivery to the skin can be increased by orders of magnitude [20].

Microneedles are generally of the following four types – solid microneedles, coated microneedle patch, dissolving microneedle patch and hollow microneedles. Figure 2.1 shows the delivery mechanism of each of the microneedle types [24]. This thesis

project covers microneedle patches for drug or vaccine delivery. Chapter 3 discusses background regarding microneedle patches and their advantages specific to vaccine delivery in developing countries.

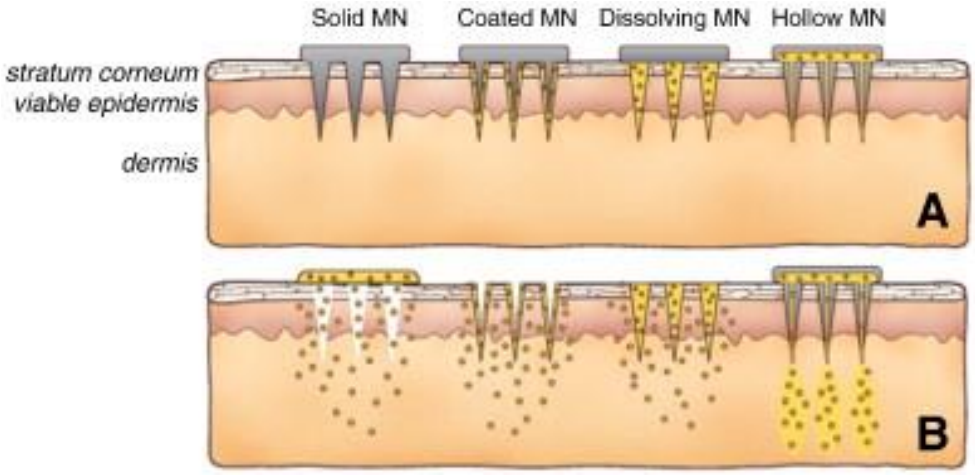


Fig 2.1 Methods of drug delivery to the skin using microneedles (MN). Microneedles are first applied to the skin (A) and then used for drug delivery (B). Solid microneedles are used as a pretreatment, after which drug can diffuse through residual holes in skin from a topical formulation (solid MN). After insertion of drug-coated microneedles into the skin, the drug coating dissolves off the microneedles in the aqueous environment of the skin (coated MN). Drug-loaded microneedles are made of water-soluble or biodegradable materials encapsulating drug that is released in the skin upon microneedle dissolution (dissolving MN). Hollow microneedles are used to inject liquid formulations into the skin (hollow MN) [24].

2.2.1 Dissolving microneedle patches

Dissolving microneedle patches are made up of water soluble biocompatible or biodegradable polymers and sugars and the drug or vaccine is contained within the needle such that when the microneedle patch is applied to the skin, the microneedles dissolve in the skin and deliver the payload contained within them. After insertion and dissolution into the skin, dissolving microneedle patches do not leave behind any sharps waste. They offer the key advantage of minimizing the possibility of needle stick injury to healthcare

provider and do not require safe disposal of biohazardous sharps waste after drug or vaccine delivery [25].

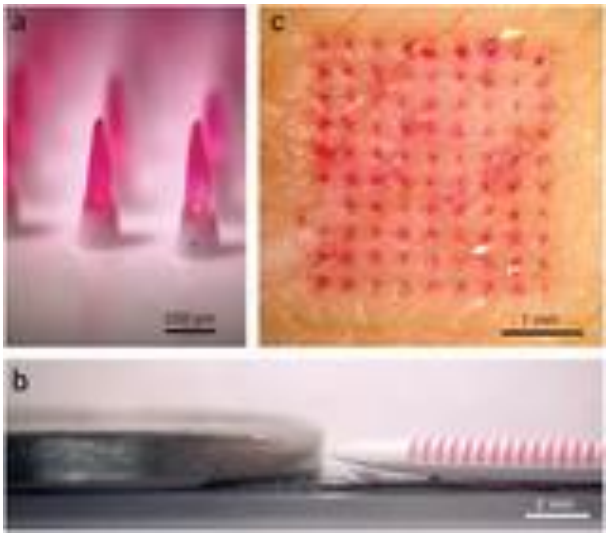


Fig 2.2 Dissolving polymer microneedle patches. (A) Side view of dissolving polymer microneedles. (B) Relative height of an array of microneedles next to a US nickel coin. (C) *En face* view of porcine cadaver skin after insertion and removal of microneedles, showing delivery of the encapsulated compound (sulforhodamine) [26].

Different materials have been used in the formulation of dissolving microneedle patches resulting in dissolution times ranging from few minutes to hours, varying microneedle strengths and varying stability of the encapsulated antigen. Dissolving microneedle patches have been studied for delivery of different antigens – for example, Adenovirus [27-30], Amyloid β peptide [31], Diphtheria [32-36], HIV [37], Influenza [35, 38-64], Malaria [29, 32, 65], Measles [66], Poliovirus [67], Tetanus [32].

2.3 References

1. Graham, S.C., R. Assenberg, O. Delmas, A. Verma, A. Gholami, C. Talbi, R.J. Owens, D.I. Stuart, J.M. Grimes, and H. Bourhy, *Rhabdovirus matrix protein structures reveal a novel mode of self-association.* PLoS Pathog, 2008. **4**(12): p. e1000251.

2. Albertini, A.A., A.K. Wernimont, T. Muziol, R.B. Ravelli, C.R. Clapier, G. Schoehn, W. Weissenhorn, and R.W. Ruigrok, *Crystal structure of the rabies virus nucleoprotein-RNA complex.* Science, 2006. **313**(5785): p. 360-3.

3. Plotkin, S., W. Orenstein, and P. Offit, *Rabies vaccines*, in *Vaccines*. Elsevier p. 687-714.

4. Publication, W.H.O., *Rabies vaccines: WHO position paper--recommendations.* Vaccine, 2010. **28**(44): p. 7140-2.

5. World Health, O., *WHO Expert Consultation on Rabies. Second report.* World Health Organization technical report series, 2013(982): p. 1-139, back cover.

6. WHO. *Rabies Factsheet Updated September 2015*. [January 28 2016]; Available from: http://www.who.int/mediacentre/factsheets/fs099/en/.

7. Manning, S.E., C.E. Rupprecht, D. Fishbein, C.A. Hanlon, B. Lumlertdacha, M. Guerra, M.I. Meltzer, P. Dhankhar, S.A. Vaidya, S.R. Jenkins, B. Sun, and H.F. Hull, *Human rabies prevention--United States, 2008: recommendations of the Advisory Committee on Immunization Practices.* MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control, 2008. **57**(RR-3): p. 1.

8. Nigg, A.J. and P.L. Walker, *Overview, Prevention, and Treatment of Rabies.* Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2009. **29**(10): p. 1182-1195.

9. Ambrozaitis, A., A. Laiskonis, L. Balciuniene, A. Banzhoff, and C. Malerczyk, *Rabies post-exposure prophylaxis vaccination with purified chick embryo cell vaccine (PCECV) and purified Vero cell rabies vaccine (PVRV) in a four-site intradermal schedule (4-0-2-0-1-1): an immunogenic, cost-effective and practical regimen.* Vaccine, 2006. **24**(19): p. 4116-21.

10. Briggs, D.J., A. Banzhoff, U. Nicolay, S. Sirikwin, B. Dumavibhat, S. Tongswas, and C. Wasi, *Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine.* Bulletin of the World Health Organization, 2000. **78**(5): p. 693-698.

11. Khawplod, P., H. Wilde, S. Sirikwin, M. Benjawongkulchai, S. Limusanno, W. Jaijaroensab, N. Chiraguna, C. Supich, Y. Wangroongsarb, and V. Sitprija, *Revision of the Thai Red Cross intradermal rabies post-exposure regimen by eliminating the 90-day booster injection.* Vaccine, 2006. **24**(16): p. 3084-6.

12. Quiambao, B.P., E.M. Dimaano, C. Ambas, R. Davis, A. Banzhoff, and C. Malerczyk, *Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals.* Vaccine, 2005. **23**(14): p. 1709-14.

13. Lodmell, D.L., L.C. Ewalt, M.J. Parnell, C.E. Rupprecht, and C.A. Hanlon, *One-time intradermal DNA vaccination in ear pinnae one year prior to infection protects dogs against rabies virus.* Vaccine, 2006. **24**(4): p. 412-6.

14. Lodmell, D.L., M.J. Parnell, J.T. Weyhrich, and L.C. Ewalt, *Canine rabies DNA vaccination: a single-dose intradermal injection into ear pinnae elicits elevated and persistent levels of neutralizing antibody.* Vaccine, 2003. **21**(25-26): p. 3998-4002.

15. Bahloul, C., D. Taieb, M.F. Diouani, S.B. Ahmed, Y. Chtourou, I. B'Chir B, H. Kharmachi, and K. Dellagi, *Field trials of a very potent rabies DNA vaccine which induced long lasting virus neutralizing antibodies and protection in dogs in experimental conditions.* Vaccine, 2006. **24**(8): p. 1063-72.

16. Kutzler, M.A. and D.B. Weiner, *DNA vaccines: ready for prime time?* Nat Rev Genet, 2008. **9**(10): p. 776-88.

17. Ullas, P.T., *Rabies DNA Vaccines: Current Status and Future.* World Journal of Vaccines, 2012. **02**(01): p. 36-45.

18. Redding, L. and D.B. Werner, *DNA vaccines in veterinary use.* Expert review of vaccines, 2009. **8**(9): p. 1251-1276.

19. van der Maaden, K., W. Jiskoot, and J. Bouwstra, *Microneedle technologies for (trans)dermal drug and vaccine delivery.* J Control Release, 2012. **161**(2): p. 645-55.

20. Henry, S., D.V. McAllister, M.G. Allen, and M.R. Prausnitz, *Microfabricated microneedles: A novel approach to transdermal drug delivery.* Journal of Pharmaceutical Sciences, 1998. **87**(8): p. 922-925.

21. Patel, S.R., A.S. Lin, H.F. Edelhauser, and M.R. Prausnitz, *Suprachoroidal drug delivery to the back of the eye using hollow microneedles.* Pharm Res, 2011. **28**(1): p. 166-176.

22. Park, S., S.O. Choi, S.J. Paik, S. Choi, M. Allen, and M. Prausnitz, *Intracellular delivery of molecules using microfabricated nanoneedle arrays.* Biomed Microdevices, 2016. **18**(1): p. 10.

23. Huzil, J.T., S. Sivaloganathan, M. Kohandel, and M. Foldvari, *Drug delivery through the skin: molecular simulations of barrier lipids to design more effective noninvasive dermal and transdermal delivery systems for small molecules, biologics, and cosmetics.* Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2011. **3**(5): p. 449-62.

24. Kim, Y.C., J.H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Adv Drug Deliv Rev, 2012. **64**(14): p. 1547-68.

25. Arya, J. and M.R. Prausnitz, *Microneedle patches for vaccination in developing countries.* Journal of Controlled Release.

26. Sullivan, S.P., D.G. Koutsonanos, M. Del Pilar Martin, J.W. Lee, V. Zarnitsyn, S.-O. Choi, N. Murthy, R.W. Compans, I. Skountzou, and M.R. Prausnitz, *Dissolving polymer microneedle patches for influenza vaccination.* Nat Med, 2010. **16**(8): p. 915-20.

27. Vrdoljak, A., M.G. McGrath, J.B. Carey, S.J. Draper, A.V.S. Hill, C. O'Mahony, A.M. Crean, and A.C. Moore, *Coated microneedle arrays for transcutaneous delivery of live virus vaccines.* Journal of Controlled Release, 2012. **159**(1): p. 34-42.

28. Bachy, V., C. Hervouet, P.D. Becker, L. Chorro, L.M. Carlin, S. Herath, T. Papagatsias, J.B. Barbaroux, S.J. Oh, A. Benlahrech, T. Athanasopoulos, G.

Dickson, S. Patterson, S.Y. Kwon, F. Geissmann, and L.S. Klavinskis, *Langerin negative dendritic cells promote potent CD8(+) T-cell priming by skin delivery of live adenovirus vaccine microneedle arrays.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(8): p. 3041-3046.

29. Carey, J.B., A. Vrdoljak, C. O'Mahony, A.V.S. Hill, S.J. Draper, and A.C. Moore, *Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route.* Sci. Rep., 2014. **4**.

30. Erdos, G., C. Donahue, J.Y. Zhang, B. Ozdoganlar, A. Gambotto, and L. Falo, *Dissolvable microneedle arrays deliver live adenovirus to the skin for genetic immunization.* Journal of Immunology, 2012. **188**: p. 1.

31. Matsuo, K., H. Okamoto, Y. Kawai, Y.-S. Quan, F. Kamiyama, S. Hirobe, N. Okada, and S. Nakagawa, *Vaccine efficacy of transcutaneous immunization with amyloid β using a dissolving microneedle array in a mouse model of Alzheimer's disease.* Journal of Neuroimmunology, 2014. **266**(1–2): p. 1-11.

32. Matsuo, K., S. Hirobe, Y. Yokota, Y. Ayabe, M. Seto, Y.-S. Quan, F. Kamiyama, T. Tougan, T. Horii, Y. Mukai, N. Okada, and S. Nakagawa, *Transcutaneous immunization using a dissolving microneedle array protects against tetanus, diphtheria, malaria, and influenza.* Journal of Controlled Release, 2012. **160**(3): p. 495-501.

33. Ding, Z., S. Bal, S. Romeijn, G.A. Kersten, W. Jiskoot, and J. Bouwstra, *Transcutaneous Immunization Studies in Mice Using Diphtheria Toxoid-Loaded Vesicle Formulations and a Microneedle Array.* Pharmaceutical Research, 2011. **28**(1): p. 145-158.

34. Bal, S., Z. Ding, G.A. Kersten, W. Jiskoot, and J. Bouwstra, *Microneedle-Based Transcutaneous Immunisation in Mice with N-Trimethyl Chitosan Adjuvanted Diphtheria Toxoid Formulations.* Pharmaceutical Research, 2010. **27**(9): p. 1837-1847.

35. Ding, Z., F.J. Verbaan, M. Bivas-Benita, L. Bungener, A. Huckriede, D.J. van den Berg, G. Kersten, and J.A. Bouwstra, *Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice.* Journal of Controlled Release, 2009. **136**(1): p. 71-78.

36. Ding, Z., E. Van Riet, S. Romeijn, G.F.A. Kersten, W. Jiskoot, and J.A. Bouwstra, *Immune Modulation by Adjuvants Combined with Diphtheria Toxoid Administered Topically in BALB/c Mice After Microneedle Array Pretreatment.* Pharmaceutical Research, 2009. **26**(7): p. 1635-1643.

37. Pattani, A., P.F. McKay, M.J. Garland, R.M. Curran, K. Migalska, C.M. Cassidy, R.K. Malcolm, R.J. Shattock, H.O. McCarthy, and R.F. Donnelly, *Microneedle mediated intradermal delivery of adjuvanted recombinant HIV-1 CN54gp140 effectively primes mucosal boost inoculations.* Journal of Controlled Release, 2012. **162**(3): p. 529-537.

38. Kim, Y.-C., F.-S. Quan, R.W. Compans, S.-M. Kang, and M.R. Prausnitz, *Formulation and coating of microneedles with inactivated influenza virus to improve vaccine stability and immunogenicity.* Journal of Controlled Release, 2010. **142**(2): p. 187-195.

39. Alarcon, J.B., A.W. Hartley, N.G. Harvey, and J.A. Mikszta, *Preclinical evaluation of microneedle technology for intradermal delivery of influenza vaccines.* Clinical and Vaccine Immunology, 2007. **14**(4): p. 375-381.

40. Arnou, R., G. Icardi, M. De Decker, A. Ambrozaitis, M.P. Kazek, F. Weber, and P. Van Damme, *Intradermal influenza vaccine for older adults: A randomized controlled multicenter phase III study.* Vaccine, 2009. **27**(52): p. 7304-7312.

41. Beran, J., A. Ambrozaitis, A. Laiskonis, N. Mickuviene, P. Bacart, Y. Calozet, E. Demanet, S. Heijmans, P. Van Belle, F. Weber, and C. Salamand, *Intradermal influenza vaccination of healthy adults using a new microinjection system: a 3-year randomised controlled safety and immunogenicity trial.* Bmc Medicine, 2009. **7**: p. 15.

42. Choi, H.J., B.J. Bondy, D.G. Yoo, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Stability of whole inactivated influenza virus vaccine during coating onto metal microneedles.* Journal of Controlled Release, 2013. **166**(2): p. 159-171.

43. Choi, H.J., D.G. Yoo, B.J. Bondy, F.S. Quan, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Stability of influenza vaccine coated onto microneedles.* Biomaterials, 2012. **33**(14): p. 3756-3769.

44. Fernando, G.J.P., X.F. Chen, C.A. Primiero, S.R. Yukiko, E.J. Fairmaid, H.J. Corbett, I.H. Frazer, L.E. Brown, and M.A.F. Kendall, *Nanopatch targeted*

delivery of both antigen and adjuvant to skin synergistically drives enhanced antibody responses. Journal of Controlled Release, 2012. **159**(2): p. 215-221.

45. Fernando, G.J.P., X.F. Chen, T.W. Prow, M.L. Crichton, E.J. Fairmaid, M.S. Roberts, I.H. Frazer, L.E. Brown, and M.A.F. Kendall, *Potent Immunity to Low Doses of Influenza Vaccine by Probabilistic Guided Micro-Targeted Skin Delivery in a Mouse Model.* Plos One, 2010. **5**(4): p. 11.

46. Holland, D., R. Booy, F. De Looze, P. Eizenberg, J. McDonald, J. Karrasch, M. McKeirnan, H. Salem, G. Mills, J. Reid, F. Weber, and M. Saville, *Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: A randomized controlled trial.* Journal of Infectious Diseases, 2008. **198**(5): p. 650-658.

47. Kim, Y.C., F.S. Quan, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Stability Kinetics of Influenza Vaccine Coated onto Microneedles During Drying and Storage.* Pharmaceutical Research, 2011. **28**(1): p. 135-144.

48. Kim, Y.C., F.S. Quan, D.G. Yoo, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Enhanced Memory Responses to Seasonal H1N1 Influenza Vaccination of the Skin with the Use of Vaccine-Coated Microneedles.* Journal of Infectious Diseases, 2010. **201**(2): p. 190-198.

49. Kim, Y.C., D.G. Yoo, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Cross-protection by co-immunization with influenza hemagglutinin DNA and inactivated virus vaccine using coated microneedles.* Journal of Controlled Release, 2013. **172**(2): p. 579-588.

50. Kommareddy, S., B.C. Baudner, A. Bonificio, S. Gallorini, G. Palladino, A.S. Determan, D.M. Dohmeier, K.D. Kroells, J.R. Sternjohn, M. Singh, P.R. Dormitzer, K.J. Hansen, and D.T. O'Hagan, *Influenza subunit vaccine coated microneedle patches elicit comparable immune responses to intramuscular injection in guinea pigs.* Vaccine, 2013. **31**(34): p. 3435-3441.

51. Kommareddy, S., B.C. Baudner, S. Oh, S.Y. Kwon, M. Singh, and D.T. O'Hagan, *Dissolvable microneedle patches for the delivery of cell-culture-derived influenza vaccine antigens.* Journal of Pharmaceutical Sciences, 2012. **101**(3): p. 1021-1027.

52. Koutsonanos, D.G., E.V. Vassilieva, A. Stavropoulou, V.G. Zarnitsyn, E.S. Esser, M.T. Taherbhai, M.R. Prausnitz, R.W. Compans, and I. Skountzou, *Delivery of*

subunit influenza vaccine to skin with microneedles improves immunogenicity and long-lived protection. Scientific Reports, 2012. **2**: p. 10.

53. Levin, Y., E. Kochba, and R. Kenney, *Clinical evaluation of a novel microneedle device for intradermal delivery of an influenza vaccine: Are all delivery methods the same?* Vaccine, 2014. **32**(34): p. 4249-4252.

54. Martin, M.D., W.C. Weldon, V.G. Zarnitsyn, D.G. Koutsonanos, H. Akbari, I. Skountzou, J. Jacob, M.R. Prausnitz, and R.W. Compans, *Local Response to Microneedle-Based Influenza Immunization in the Skin.* Mbio, 2012. **3**(2): p. 8.

55. Nougarede, N., H. Bisceglia, A. Rozieres, C. Goujon, F. Boudet, P. Laurent, B. Vanbervliet, K. Rodet, A. Hennino, and J.F. Nicolas, *Nine mu g intradermal influenza vaccine and 15 mu g intramuscular influenza vaccine induce similar cellular and humoral immune responses in adults.* Human Vaccines & Immunotherapeutics, 2014. **10**(9): p. 2713-2720.

56. Pearton, M., S.M. Kang, J.M. Song, Y.C. Kim, F.S. Quan, A. Anstey, M. Ivory, M.R. Prausnitz, R.W. Compans, and J.C. Birchall, *Influenza virus-like particles coated onto microneedles can elicit stimulatory effects on Langerhans cells in human skin.* Vaccine, 2010. **28**(37): p. 6104-6113.

57. Pearton, M., D. Pirri, S.M. Kang, R.W. Compans, and J.C. Birchall, *Host Responses in Human Skin After Conventional Intradermal Injection or Microneedle Administration of Virus-Like-Particle Influenza Vaccine.* Advanced Healthcare Materials, 2013. **2**(10): p. 1401-1410.

58. Puig-Barbera, J., A. Natividad-Sancho, J. Calabuig-Perez, J.A. Lluch-Rodrigo, E. Pastor-Villalba, S. Martinez-Ubeda, and J. Diez-Domingo, *Intradermal and virosomal influenza vaccines for preventing influenza hospitalization in the elderly during the 2011-2012 influenza season: A comparative effectiveness study using the Valencia health care information system.* Vaccine, 2014. **32**(42): p. 5447-5454.

59. Quan, F.S., Y.C. Kim, R.W. Compans, M.R. Prausnitz, and S.M. Kang, *Dose sparing enabled by skin immunization with influenza virus-like particle vaccine using microneedles.* Journal of Controlled Release, 2010. **147**(3): p. 326-332.

60. Quan, F.S., Y.C. Kim, D.G. Yoo, R.W. Compans, M.R. Prausnitz, and S.M. Kang, *Stabilization of Influenza Vaccine Enhances Protection by Microneedle Delivery in the Mouse Skin.* Plos One, 2009. **4**(9): p. 10.

61. Song, J.M., Y.C. Kim, P.G. Barlow, M.J. Hossain, K.M. Park, R.O. Donis, M.R. Prausnitz, R.W. Compans, and S.M. Kang, *Improved protection against avian influenza H5N1 virus by a single vaccination with virus-like particles in skin using microneedles.* Antiviral Research, 2010. **88**(2): p. 244-247.

62. Weldon, W.C., V.G. Zarnitsyn, E.S. Esser, M.T. Taherbhai, D.G. Koutsonanos, E.V. Vassilieva, I. Skountzou, M.R. Prausnitz, and R.W. Compans, *Effect of Adjuvants on Responses to Skin Immunization by Microneedles Coated with Influenza Subunit Vaccine.* Plos One, 2012. **7**(7): p. 8.

63. Yan, L., Y. Yang, W.J. Zhang, and X.F. Chen, *Advanced Materials and Nanotechnology for Drug Delivery.* Advanced Materials, 2014. **26**(31): p. 5533-5540.

64. Zhu, Q.Y., V.G. Zarnitsyn, L. Ye, Z.Y. Wen, Y.L. Gao, L. Pan, I. Skountzou, H.S. Gill, M.R. Prausnitz, C.L. Yang, and R.W. Compans, *Immunization by vaccine-coated microneedle arrays protects against lethal influenza virus challenge.* Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(19): p. 7968-7973.

65. Carey, J.B., F.E. Pearson, A. Vrdoljak, M.G. McGrath, A.M. Crean, P.T. Walsh, T. Doody, C. O'Mahony, A.V.S. Hill, and A.C. Moore, *Microneedle Array Design Determines the Induction of Protective Memory CD8⁺ T Cell Responses Induced by a Recombinant Live Malaria Vaccine in Mice.* PLoS ONE, 2011. **6**(7): p. e22442.

66. Edens, C., M.L. Collins, J.L. Goodson, P.A. Rota, and M.R. Prausnitz, *A microneedle patch containing measles vaccine is immunogenic in non-human primates.* Vaccine, 2015. **33**(37): p. 4712-8.

67. Edens, C., N.C. Dybdahl-Sissoko, W.C. Weldon, M.S. Oberste, and M.R. Prausnitz, *Inactivated polio vaccination using a microneedle patch is immunogenic in the rhesus macaque.* Vaccine, 2015. **33**(37): p. 4683-90.

CHAPTER 3

MICRONEEDLE PATCHES FOR VACCINATION IN DEVELOPING COUNTRIES

Reprinted (adapted) with permission from Elsevier (Jaya Arya, Mark R. Prausnitz, Microneedle patches for vaccination in developing countries, Journal of Controlled Release).

3.1 Abstract

Millions of people die of infectious diseases each year, mostly in developing countries, which could largely be prevented by the use of vaccines. While immunization rates have risen since the introduction of the Expanded Program on Immunization (EPI), there remain major challenges to more effective vaccination in developing countries. As a possible solution, microneedle patches containing an array of micron-sized needles on an adhesive backing have been developed to be used for vaccine delivery to the skin. These microneedle patches can be easily and painlessly applied by pressing against the skin and, in some designs, do not leave behind sharps waste. The patches are single-dose, do not require reconstitution, are easy to administer, have reduced size to simplify storage, transportation and waste disposal, and offer the possibility of improved vaccine immunogenicity, dose sparing and thermostability. This review summarizes vaccination challenges in developing countries and discusses advantages that microneedle patches offer for vaccination to address these challenges. We conclude that microneedle patches offer a powerful new technology that can enable more effective vaccination in developing countries.

3.2 Barriers to vaccination in developing countries

According to 2014 WHO estimates, 1.5 million children die each year from vaccine-preventable diseases for which there are vaccines recommended by the WHO and 29% of deaths among children 1-59 months old are vaccine preventable [1]. For example, measles vaccine is 97% effective after two doses [2], yet, as of 2010, more than 100,000 children under the age of five died each year from measles, most of whom were unvaccinated children [3].

Vaccines are currently administered in developing countries primarily in two scenarios: routine vaccination and mass vaccination campaigns. Routine vaccination is used to achieve high immunization coverage on an on-going basis, but can fall short by itself due to infrastructural challenges in developing countries. Instead, or in addition, mass vaccination campaigns are employed to target large populations in specific regions more effectively [4, 5]. Mass vaccination campaigns can be performed at fixed-post clinics, which is typically required for injectable vaccines, or can be carried out door-to-door, usually by minimally trained personnel administering non-injectable vaccines [6].

While immunization rates have risen since the introduction of the Expanded Program on Immunization (EPI), there remain significant barriers to more effective vaccination in developing countries (Table 3.1). We summarize these barriers in the rest of this section.

Table 3.1. Barriers to more effective vaccination in developing countries.

Barriers to more effective vaccination in developing countries [7, 8]
Need for increased vaccine effectiveness
Need for trained healthcare providers
Need for effective supply chain
Risk of sharps
Vaccine wastage due to multi-dose vials
Need for vaccine reconstitution
Cost of vaccine/vaccination

3.2.1 Need for increased vaccine effectiveness

While many vaccines are extremely effective and offer life-long protection, other vaccines provide only moderate protection rates, especially in developing countries where nutrition levels may be low and individuals may have a compromised immune system due to presence of other infections [9, 10]. Most vaccines need booster doses in order to mount an appropriate immune response; this requires vaccinating the same people multiple times, which can be difficult to execute in places with poor healthcare infrastructure and recordkeeping.

For example, the efficacy of oral polio vaccine (OPV) is known to be sub-optimal in densely populated tropical countries [9] and the immunogenicity of rotavirus vaccine has been shown to be much worse in resource-poor countries in Africa and Asia [11-13]. Measles vaccine can be less efficacious in the presence of vitamin A deficiency in

developing countries and vitamin A supplementation along with measles vaccination is often recommended [10].

3.2.2 Need for trained healthcare providers

Most vaccines are administered by hypodermic needle and syringe injection. A trained healthcare provider is needed to safely administer these injections as well as to safely dispose of the resulting sharps waste. The lack of trained healthcare providers in developing countries can be a significant barrier to attaining high vaccination rates, especially in the case of vaccination campaigns [14].

Smallpox eradication was achieved in part due to the ability to achieve high vaccination coverage using minimally trained personnel administering the vaccine using the scarification technique with a bifurcated needle [15]. Similarly, OPV is being administered orally by minimally trained personnel as part of polio eradication efforts [14], and the anticipated switch to inactivated polio vaccine (IPV) that is given by injection is of great concern to public health officials due to its increased cost and complexity [16] .

3.2.3 Need for effective supply chain

Vaccines must be maintained at the correct temperature (i.e., usually refrigerated) during storage and distribution as well as during use after reconstitution. Heat and freezing temperatures are both detrimental to most vaccines. The resulting need for a cold chain during storage and distribution can be difficult to maintain due to limited infrastructure in developing countries, leading to vaccine wastage [17, 18]. Size and

volume of vaccine vials and syringes are thus also important considerations to utilize the supply chain most effectively [19, 20].

The cost of the cold chain is estimated to be $200 to $300 million per year [18] and can even experience failures in industrialized countries with established cold chain systems [17], indicating that developing countries with less-established cold chain systems can be especially susceptible to losses in the cold chain.. As an example of the variation in cold-chain space occupied by a given vaccine presentation, estimates suggest that one dose of a given vaccine in a 10-dose vial occupies 3 cm^3 of cold-chain volume, where as one dose of vaccine in a single-dose vial presentation occupies 12.9 cm^3 of cold-chain volume [21].

3.2.4 Risk of sharps

Hypodermic needles need to be handled carefully to prevent needle-stick injuries to healthcare providers and others. Hypodermic needles also create biohazardous sharps waste after use that needs to be disposed of safely to ensure that the needles are not reused intentionally or accidentally. During vaccination campaigns it may be more difficult to safely collect and dispose of needles in developing countries [22, 23]

Both healthcare workers and patients are at risk due to unsafe injection practices. A study estimated that up to 33,800 HIV infections, 1.7 million hepatitis B infections and 315,000 hepatitis C infections are caused every year due to unsafe injection practices [24].

3.2.5 Vaccine wastage due to multi-dose vials

Many vaccines are available in multi-dose (e.g., ten-dose) vials for injection. On a per-dose basis, multi-dose vials are less expensive than single dose vials, take up less space during transportation and in the cold-chain and create less waste. However, the actual cost savings can be difficult to evaluate based on the amount of vaccine that gets wasted because opened vials need to be used quickly to prevent microbial growth and, if not used in time, must be discarded. Vaccine wastage can be very high in developing countries for some vaccines [25-27].

In general vaccine wastage rates increase as the number of vaccine doses per vial increases and an estimate suggests wastage rates for 10 dose vials could be as high as 25% for liquid vaccines and 40% for lyophilized vaccines [21]. The WHO Vaccine Presentation and Packaging Advisory Group's guidelines recommend vaccines to be presented in formats to minimize the number of steps and potential for error during administration when possible [20].

3.2.6 Need for vaccine reconstitution

Some vaccines are lyophilized and need to be reconstituted with a diluent at the time of use for injection, which adds additional challenges in developing countries [28]. Reconstitution adds another step that requires additional reconstitution needles, syringes and vials that also need to be stored and transported in part in the cold chain, further complicating the supply chain. Time and expertise is needed to reconstitute the vaccine since there is room for error if an incorrect diluent is used or mixing is not carried out

using sterile devices. Reconstitution errors lead to vaccine wastage, ineffective vaccination or, in some cases, injury to patients.

As an example, measles vaccine contamination by *Staphylococcus Aureus* from non-sterile diluent has been documented in many countries and accidental injection of other drugs stored in the diluent's container have resulted in infant deaths [28]. In a recent case in Syria, the use of an incorrect diluent for the reconstitution of measles vaccine caused the death of 15 children [24].

3.2.7 Cost of vaccine/vaccination

The cost of vaccination is the cost of vaccine plus the logistical costs associated with making the vaccine available for use. Healthcare provider, waste disposal, vaccine transportation, cold-chain and vaccine wastage all contribute to the cost of vaccination [29, 30]. While vaccine manufacturers often sell vaccine at significantly reduced cost for use in developing countries, the logistical costs to vaccinate can remain a significant barrier.

As evidence of the significance of vaccination costs other than the cost of the vaccine itself, a study of the average cost to administer vaccines in Senegal found that logistics comprise approximately 50% of the total average cost of each dose delivered [29]. As another example, the 2015 UNICEF price for measles/rubella vaccine is US$0.578 per dose [31], but the cost to administer a dose of measles and rubella vaccine is estimated at approximately US$1.50 per dose [32].

3.3 Microneedle patches address challenges to vaccination in developing countries

3.3.1 Overview of microneedles for vaccination

Microneedle patches (MNPs) have been proposed to improve vaccination in developing countries and are the subject of increasing research in academia and industry (Figure 3.1). Microneedles are less than one millimeter long and deliver vaccines to the skin's epidermis and dermis, as compared to conventional injection into deeper tissues in the muscle or subcutaneous space by hypodermic needle and syringe. In a MNP, an array of microneedles is attached to a backing such that it can be applied to the skin by hand like a bandage [33, 34] .

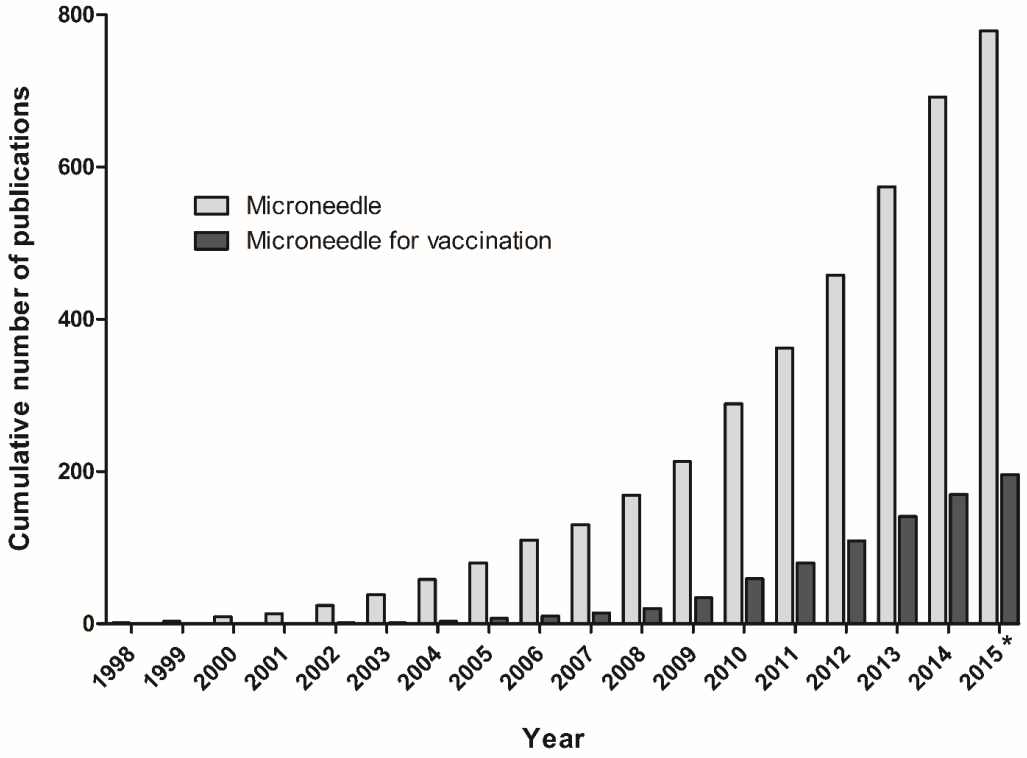


Figure 3.1 Cumulative number of publications on microneedles and on microneedles for vaccination. The total number of microneedle publications was determined by searching the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) on 2nd August 2015 using the search terms "microneedle", "microfabricated needle", or "nanopatch". The subset of microneedle publications with focus on vaccination was determined by adding "vaccin*" or "immuniz*" terms to the previous search. Conference proceedings were excluded. *Publications from 2015 only represent those posted on PubMed by 2nd August 2015.

MNPs are typically designed either as solid metal, silicon or polymer microneedles coated with vaccine that releases the vaccine upon dissolution of the coating in the skin or as solid, dissolving microneedles made of water-soluble materials that encapsulate vaccine and releases the vaccine when the microneedles dissolve in the skin. While this review focuses on MNP, microneedles have also been employed for vaccination as solid microneedles used for skin pretreatment followed by application of a topical vaccine formulation for delivery through residual holes in the skin and as hollow microneedles for liquid vaccine formulation delivery into the skin.

In contrast to hypodermic needles that deliver vaccine in a liquid form, MNPs contain the vaccine in a dried solid form which dissolves within the skin upon administration. Each MNP contains a single dose of the vaccine and can be easily applied by pressing down against the skin with the thumb or with the use of an applicator. Upon application of a MNP to the skin, the microneedles penetrate the skin and the patch is left on the skin for a few minutes to allow for dissolution to deliver the payload contained in it. In the case of coated MNP, the coating dissolves but not the microneedles themselves. In the case of dissolving MNPs, the microneedles dissolve within the skin, thus leaving behind only the backing and no biohazardous sharps waste.

MNPs inherently target vaccine delivery to the skin, which is the largest immunological organ in the body and is densely populated by antigen-presenting cells, which play a crucial role in induction of immune responses. As a result, skin vaccination has been shown to be beneficial for many vaccines [35]. However, conventional intradermal injection using a hypodermic needle by the Mantoux technique can be difficult to perform reproducibly [36]. MNPs offer a simple and reliable way to target the

skin and have been studied for delivery of many vaccines [33, 34, 37, 38]. Table 3.2 summarizes the vaccines that have been studied using microneedles; although not otherwise part of this review, hollow microneedles have been included in the table for completeness.

Table 3.2 Vaccines studied with microneedles.

Microneedle type		
Coated	Dissolving	Hollow
Adenovirus [39-42]	Adenovirus [39-42]	Anthrax [43-46]
BCG [47, 48]	Amyloid β peptide [49]	Botulism [45, 50]
Chikungunya virus [51]	Diphtheria [52-56]	Influenza [55, 57-83]
Hepatitis B [84-87]	HIV [88]	Japanese encephalitis [89]
Hepatitis C [90]	Influenza [55, 57-83]	Poliovirus [91]
Herpes simplex virus [92, 93]	Malaria [41, 52, 94]	Rabies virus [95]
HPV [96]	Measles [97]	Staphylococcus aureus [43, 45]
Influenza [55, 57-83]	Poliovirus [98]	Yersinia pestis [45, 99]
Measles [100]	Tetanus [52]	
Modified Vaccinia Ankara [39, 94]		
Rotavirus [101]		
Small Pox [102]		
West Nile virus [51]		

3.3.2 Potential impact of microneedle patches for vaccination in developing countries

In addition to effectively targeting the skin, MNPs offer many other advantages for vaccination, including addressing logistical challenges to vaccine delivery, which are extremely important for vaccination in developing countries. Table 3.3 summarizes the main advantages that MNPs offer to vaccination in developing countries.

Table 3.3 Advantages of microneedle patches for vaccination in developing countries.

Increased vaccine effectiveness
Reduced need for trained healthcare providers
Simplified supply chain
Reduced risk of sharps
Reduced vaccine wastage
No need for vaccine reconstitution
Reduced cost of vaccine/vaccination

3.3.3 Increased vaccine effectiveness

3.3.3.1 Skin vaccination enables dose sparing

Delivering vaccines in the epidermis or dermis puts the antigen in close contact with the skin's rich population of antigen-presenting cells and can result in lower doses of antigens being used. For example, dose-sparing using the intradermal route has been demonstrated in clinical studies for IPV, seasonal influenza and rabies vaccines [36, 103]. Since MNPs also target the skin for delivery, they could offer improved protection in terms of vaccine dose sparing or a wider range of immune response. In support of that

hypothesis, vaccination using MNPs has demonstrated dose-sparing in pre-clinical studies with influenza [63, 78], rotavirus [101] and herpes simplex virus [92], among other vaccines.

3.3.3.2 Skin vaccination offers improved protection

MNP vaccination has been shown to provide superior immunological responses by other measures as well. Vaccination at the same dose has been shown to produce stronger antibody and/or cellular responses when performed using MNPs compared to hypodermic injection [83, 104, 105], including improved immune responses in very young animals [104]. As a measure of protection, animals vaccinated against influenza using MNPs have been shown to clear virus from the lungs after challenge with live influenza virus better than those vaccinated intramuscularly [67, 105, 106]. Immune response and protection after vaccination have also been shown to last longer after MNP vaccination compared to intramuscular injection [107].

While the mechanisms responsible for the increased immunogenicity of vaccination using MNPs is still under study, evidence suggests that it may be due to vaccine delivery targeted to the unique collection of antigen-presenting cells found in the skin (e.g., Langerhans cells) [75, 76, 94, 108], transport of antigen and antigen-presenting cells from the skin to draining lymph nodes [73], adjuvanted immune response due to cell death caused by the trauma of microneedle insertion into skin [64, 109] , and other factors.

3.3.4 Reduced need for trained healthcare providers

The simple and minimally invasive approach of MNP delivery could allow administration by personnel with minimal training and also offer the possibility of self-

administration – with or without the presence of a healthcare provider. This could enable vaccines that currently must be injected by trained healthcare personnel at fixed-post clinics to instead be administered by minimally trained personnel in house-to-house campaigns.

In focus group studies of the public as well as healthcare professionals, MNPs were generally viewed favorably as compared to hypodermic needle injections, suggesting good acceptance of MNPs [110, 111]. In human studies with placebo MNPs, naïve subjects with no prior experience with microneedles were able to successfully administer MNPs when provided with only a brief set of instructions [112, 113]. MNPs for drug delivery have been taken home and used repeatedly by patients without supervision with excellent outcomes [114]. Additional analysis showed that the use of self-administered MNPs could improve vaccination coverage [113] and their use was shown to be cost effective in the majority of scenarios considered in an analysis of influenza vaccination in the United States [111].

3.3.5 Simplified supply chain

3.3.5.1 Simplified storage, distribution and disposal

MNPs are much smaller in size than a vaccine vial and needle-syringe system, which could allow MNPs to be stored in a smaller volume and enable simpler storage and distribution [115]. For example, microneedle arrays are typically on the order of 1 cm^2 in area and, once assembled onto a patch, could have a representative volume on the order of 1 cm^3 [33, 37]. Although packaging, possibly in multi-dose presentations, would increase the product size, it is clear that MNPs have the potential to dramatically reduce the size of vaccines during storage, distribution and disposal.

3.3.5.2 Reduction or elimination of cold chain

MNPs contain vaccines in a dried form, and suitable excipients can be used in the formulation to make vaccines thermostable. If sufficiently stabilized, MNP could be stored at ambient temperature, eliminating the cold chain completely. If only partial thermostability is achieved, MNPs could be refrigerated during storage at major distribution hubs, but then removed from the cold chain during transportation, storage at village clinics or mass vaccination campaigns.

Influenza vaccine MNPs have been studied extensively for stability at elevated temperatures. A recent study identified formulations stable for at least 6 months at 25 °C and for at least a few weeks at 40 °C [116]. Thermostability has also been studied for MNPs with adenovirus-based vaccines [40] and measles vaccine, which was shown to be stable for at least 4 months at 25 °C and lost less than 10-fold potency after 4 months at 40 °C [97].

3.3.6 Reduced risk of sharps

MNPs contain microneedles that are a few hundred microns tall and are assembled on a patch backing that is applied to the skin either with thumb pressure or the use of a high-velocity applicator. Casual contact with a MNP is unlikely to result in accidental penetration of microneedles into the skin of an unintended subject, because the MNP needs to be placed flat against the surface of the skin and a significant force needs to be applied for a successful insertion [113]. MNPs could in this way reduce the risks associated with accidental needle stick injury to healthcare providers.

After use, MNPs may offer additional safety advantages. Dissolving MNPs contain microneedles made of water-soluble, biocompatible materials that dissolve in the

skin after administration. Thus, they do not leave behind biohazardous sharps waste; only an adhesive backing that can be discarded as non-sharps waste (e.g., similar to a used bandage). This eliminates the risk of injury and disease transmission from used needles. Coated MNPs do not completely eliminate sharps waste. However, used MNPs cannot be reloaded with vaccine absent special coating equipment, making reuse unlikely. Accidental exposure to used MNPs is also expected to be safer than for hypodermic needles because, as mentioned above, it is difficult to get microneedles to penetrate the skin without an intentional, forceful application.

3.3.7 Reduced vaccine wastage

Each MNP contains a single dose of vaccine and is intended as a single-use product. In comparison to multi-dose vials, single-dose MNPs avoid the problem of vaccine wastage because vaccine in a multi-dose vial must be discarded before all of the doses are used. The single-dose format also avoids patients being turned away without vaccination, as sometimes occurs when an insufficient number of patients need a vaccine on a given day and the vaccinator does not want to open a new vial, knowing that much of the vaccine will be wasted [26].

3.3.8 No need for vaccine reconstitution

Vaccines are often lyophilized to increase vaccine stability, but this requires vaccine reconstitution before use. MNPs contain vaccine that is administered in a dried form without reconstitution that rapidly dissolves in the skin upon administration. In this way, MNPs can have the increased stability of a dry formulation without the time of clinical personnel and risk of errors associated with reconstitution.

3.3.9 Reduced cost of vaccine/vaccination

3.3.9.1 Low-cost manufacturing

In developing countries, a critical concern is the cost of vaccination. Part of that cost is the cost the vaccine itself. The cost-of-goods for a vaccine manufactured in a MNP may be similar to that of conventional vaccine vials or pre-filled syringes, depending in part on the type of MNP technology used. The cost of MNP manufacturing can be low in part because the materials are generally low-cost medical-grade polymers, metals and other excipients that are used in very small amounts, e.g., a representative microneedle array (not including the backing, adhesive and packaging) weighs less than 1 g, and the backing, adhesive and packaging are typically made of conventional pharmaceutical materials used in transdermal patches and other products.

Manufacturing of coated MNPs typically involves a metal, polymer or silicon microneedle structure than can be mass-produced at low cost (e.g., < US$ 0.10), upon which a vaccine is coated by dipping or spraying, allowed to dry and packaged. Manufacturing of dissolving MNPs typically involves a polymer microneedle mold that can be mass produced at low cost (e.g., < US$ 0.10), onto which a vaccine is cast, allowed to dry and packaged. Dipping, spraying, coating and drying are all commonly performed in the pharmaceutical industry, which suggests that MNP manufacturing methods can be compatible with conventional pharmaceutical manufacturing environments and equipment. Much of the cost of MNP manufacturing is the need to perform it under aseptic conditions, which is similar to the cost structure of manufacturing vaccines in vials and syringes.

Terminal sterilization after manufacturing of microneedle patches may be possible, but the sterilization method will need to maintain stability of the vaccine as well as be compatible with the materials that microneedle patches are made of. Although terminal sterilization of vaccine patches has not been studied yet, electron beam and gamma irradiation of a microneedle patch containing a peptide therapeutic was found to unacceptably alter the product [117].

While companies have not released detailed information about their manufacturing methods and costs, 3M offers a solid microneedle device (sMTS) that has undergone FDA-approval and is available for purchase as a stand-alone device with no vaccine or other active. Their proprietary GMP manufacturing and aseptic coating technology has a capacity of up to 10,000 patches per day [118].

3.3.9.2 Reduced cost of vaccination

In addition to the cost of the vaccine, the complete cost of vaccination should be considered, by accounting for the logistical costs of getting a vaccine delivered to a patient. Thus, even if the cost of a MNP vaccine is greater than a conventional one, those increased costs may be more than offset by reduced logistical costs, including direct costs of vaccine delivery and indirect costs of reduced vaccine safety, efficacy and coverage.

As discussed throughout this section, the costs of vaccination could be reduced through the use of MNPs to increase vaccine effectiveness, reduce the need for trained healthcare providers, simplify the supply chain, reduce the risk of sharps, reduce vaccine wastage and eliminate the need for vaccine reconstitution.

3.4 **Directions for future research and development**

MNPs have great potential to improve vaccination in developing countries, but more work needs to be done to realize this potential. Overall, translation of preclinical studies into clinical trials of MNP vaccination is strongly needed, as is commercial manufacturing that can mass produce MNPs at suitable cost. Additional considerations follow.

- Increased vaccine effectiveness has been shown for a number of vaccines in animal models, but has not yet been established in human subjects, and the mechanisms associated with improved immunogenicity need further elucidation.
- Initial studies suggest that MNPs can be reliably used by minimally trained personnel, including patients themselves, but more widespread assessment and possible improved MNP designs are needed to assure reliable vaccine delivery.
- Reduced product size and increased vaccine thermostability are expected to simplify the supply chain, but the true extent of thermostability and the actual impact on healthcare systems have not yet been determined.
- Reduced risk of sharps is expected, especially for dissolving MNPs. While MNPs reduce this risk associated with hypodermic needles, MNPs may introduce new, unanticipated risks that may only become apparent once they are placed in the hands of diverse users in diverse scenarios and cultures.
- Reduced vaccine waste and elimination of vaccine reconstitution appear to be inherent capabilities of MNP vaccines, but, again, unintended consequences of these changes may present new challenges.

- The cost of MNP manufacturing remains a significant uncertainty and an opportunity for advances that bring down costs. Modeling can predict the possible cost savings associated with MNP vaccination balancing cost of goods and costs of vaccine delivery, but commercial and clinical implementation will be needed to determine the true cost, which will vary based on vaccine and use scenario. Identification of terminal sterilization methods that avoid the need for costly aseptic manufacturing could significantly reduce the costs of MNP products.

3.5 Conclusions

Many lives could be saved by improved vaccination in developing countries. MNPs offer advantages that could improve vaccination through increased vaccine effectiveness, reduced need for trained healthcare providers, simplified supply chain, reduced risk of sharps, reduced vaccine wastage, no need for vaccine reconstitution and reduced cost of vaccine/vaccination. With continued development, especially translation into clinical trials and advanced manufacturing, MNPs have great potential to address the limitations of current vaccination methods and thereby improve vaccination in developing countries.

3.6 Acknowledgements

The authors would like to thank Darin Zehrung for a critical reading of the manuscript and providing insight and references concerning the barriers to vaccination in developing countries, and Kimberly Haight and Daniel Pardo for their help in gathering information for this review. This work was supported in part by a grant to Mark Prausnitz from the National Institutes of Health and an ORISE fellowship to Jaya Arya funded by

the Centers for Disease Control and Prevention. Mark Prausnitz is an inventor on patents and has a significant financial interest in a company that is developing microneedle-based products (Micron Biomedical). This potential conflict of interest has been disclosed and is being managed by Georgia Tech and Emory University.

3.7 References

1. WHO. *Global Immunization Data July 2014*. [July 30 2015]; Available from: http://www.who.int/immunization/monitoring_surveillance/global_immunization_data.pdf.

2. CDC. *Measles Vaccination*. [July 30 2015]; Available from: http://www.cdc.gov/measles/vaccination.html.

3. *Institute for Health Metrics and Evaluation (IHME). GBD Database. Seattle, WA: IHME, University of Washington, 2014. Available from [http://www.healthdata.org/search-gbd-data?s=measles]. (Accessed [July 30 2015].).*

4. LaMontagne, D.S., S. Barge, N.T. Le, E. Mugisha, M.E. Penny, S. Gandhi, A. Janmohamed, E. Kumakech, N.R. Mosqueira, N.Q. Nguyen, P. Paul, Y. Tang, T.H. Minh, B.P. Uttekar, and A.O. Jumaan, *Human papillomavirus vaccine delivery strategies that achieved high coverage in low- and middle-income countries.* Bulletin of the World Health Organization, 2011. **89**(11): p. 821-830.

5. Shen, A.K., R. Fields, and M. McQuestion, *The future of routine immunization in the developing world: challenges and opportunities.* Global Health: Science and Practice, 2014. **2**(4): p. 381-394.

6. Linkins, R.W., E. Mansour, O. Wassif, M.H. Hassan, and P.A. Patriarca, *Evaluation of house-to-house versus fixed-site oral poliovirus vaccine delivery strategies in a mass immunization campaign in Egypt.* Bulletin of the World Health Organization, 1995. **73**(5): p. 589-595.

7. LaFond, A., N. Kanagat, R. Steinglass, R. Fields, J. Sequeira, and S. Mookherji, *Drivers of routine immunization coverage improvement in Africa: findings from district-level case studies.* Health Policy Plan, 2015. **30**(3): p. 298-308.

8. Favin, M., R. Steinglass, R. Fields, K. Banerjee, and M. Sawhney, *Why children are not vaccinated: a review of the grey literature.* Int Health, 2012. **4**(4): p. 229-38.

9. Grassly, N.C., C. Fraser, J. Wenger, J.M. Deshpande, R.W. Sutter, D.L. Heymann, and R.B. Aylward, *New Strategies for the Elimination of Polio from India.* Science, 2006. **314**(5802): p. 1150-1153.

10. Sudfeld, C.R., A.M. Navar, and N.A. Halsey, *Effectiveness of measles vaccination and vitamin A treatment.* International Journal of Epidemiology, 2010. **39**(suppl 1): p. i48-i55.

11. Madhi, S.A., N.A. Cunliffe, D. Steele, D. Witte, M. Kirsten, C. Louw, B. Ngwira, J.C. Victor, P.H. Gillard, B.B. Cheuvart, H.H. Han, and K.M. Neuzil, *Effect of Human Rotavirus Vaccine on Severe Diarrhea in African Infants.* New England Journal of Medicine, 2010. **362**(4): p. 289-298.

12. Vesikari, T., D.O. Matson, P. Dennehy, P. Van Damme, M. Santosham, Z. Rodriguez, M.J. Dallas, J.F. Heyse, M.G. Goveia, S.B. Black, H.R. Shinefield, C.D.C. Christie, S. Ylitalo, R.F. Itzler, M.L. Coia, M.T. Onorato, B.A. Adeyi, G.S. Marshall, L. Gothefors, D. Campens, A. Karvonen, J.P. Watt, K.L. O'Brien, M.J. DiNubile, H.F. Clark, J.W. Boslego, P.A. Offit, and P.M. Heaton, *Safety and Efficacy of a Pentavalent Human–Bovine (WC3) Reassortant Rotavirus Vaccine.* New England Journal of Medicine, 2006. **354**(1): p. 23-33.

13. Bhandari, N., T. Rongsen-Chandola, A. Bavdekar, J. John, K. Antony, S. Taneja, N. Goyal, A. Kawade, G. Kang, S.S. Rathore, S. Juvekar, J. Muliyil, A. Arya, H. Shaikh, V. Abraham, S. Vrati, M. Proschan, R. Kohberger, G. Thiry, R. Glass, H.B. Greenberg, G. Curlin, K. Mohan, G.V.J.A. Harshavardhan, S. Prasad, T.S. Rao, J. Boslego, and M.K. Bhan, *Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial.* The Lancet, 2014. **383**(9935): p. 2136-2143.

14. Jafari, H., J.M. Deshpande, R.W. Sutter, S. Bahl, H. Verma, M. Ahmad, A. Kunwar, R. Vishwakarma, A. Agarwal, S. Jain, C. Estivariz, R. Sethi, N.A. Molodecky, N.C. Grassly, M.A. Pallansch, A. Chatterjee, and R.B. Aylward,

Polio eradication. Efficacy of inactivated poliovirus vaccine in India. Science (New York, N.Y.), 2014. **345**(6199): p. 922-925.

15. Fenner, F., *Smallpox and its eradication*, in *History of international public health ; no. 6*. 1988, Geneva : World Health Organization, c1988.

16. Initiative, W.G.P.E., *Polio Eradicatio & Endgame Strategic Plan 2013-2018.* Geneva, Switzerland: World Health Organization, 2013.

17. *The Cost of a Broken Vaccine Cold Chain Part Two, Financial Cost*. 2014 Wednesday, September 17 [February 25]; Available from: http://www.csafeglobal.com/the-cost-of-a-broken-vaccine-cold-chain-part-two-financial-cost-1.

18. Das, P., *Revolutionary vaccine technology breaks the cold chain.* The Lancet Infectious Diseases, 2004. **4**(12): p. 719.

19. WHO, *Guidelines on the international packaging and shipping of vaccines.* 2005.

20. VPPAG, *Generic Preferred Product Profile for Vaccines*.

21. Alliance, T.W.B.G. *Immunization Financing Toolkit: A Resource for Policy-Makers and Program Managers*. 2010 [August 2 2015].

22. Miller, M.A. and E. Pisani, *The cost of unsafe injections.* Bull World Health Organ, 1999. **77**(10): p. 808-11.

23. Kermode, M., *Unsafe injections in low-income country health settings: need for injection safety promotion to prevent the spread of blood-borne viruses.* Health Promotion International, 2004. **19**(1): p. 95-103.

24. WHO. *Injection safety policy and global campaign*. [July 30 2015]; Available from: www.who.int/injection_safety/global-campaign/en/.

25. Drain, P.K., C.M. Nelson, and J.S. Lloyd, *Single-dose versus multi-dose vaccine vials for immunization programmes in developing countries.* Bulletin of the World Health Organization, 2003. **81**(10): p. 726-731.

26. Haidari, L.A., B. Wahl, S.T. Brown, L. Privor-Dumm, C. Wallman-Stokes, K. Gorham, D.L. Connor, A.R. Wateska, B. Schreiber, H. Dicko, P. Jaillard, M. Avella, and B.Y. Lee, *One size does not fit all: The impact of primary vaccine container size on vaccine distribution and delivery.* Vaccine, 2015. **33**(28): p. 3242-7.

27. WHO, *WHO policy statement: multi-dose vial policy (MDVP): handling of multi-dose vaccine vials after opening.* 2014.

28. Clements, C.J., G. Larsen, and L. Jodar, *Technologies that make administration of vaccines safer.* Vaccine, 2004. **22**(15-16): p. 2054-8.

29. PATH. *Some vaccine costs are hidden below the surface*. [March 15 2015]; Available from: http://www.path.org/publications/files/TS_opt_banner_hippo.pdf.

30. Lydon, P., G. Gandhi, J. Vandelaer, and J.-M. Okwo-Bele, *Health system cost of delivering routine vaccination in low-and lower-middle income countries: what is needed over the next decade?* Bulletin of the World Health Organization, 2014. **92**(5): p. 382-384.

31. UNICEF. *MR vaccine supplier prices*. [3 August 2015]; Available from: http://www.unicef.org/supply/files/MR.pdf.

32. *Measles and Rubella move fast*. [3 August 2015]; Available from: http://www.cdc.gov/globalhealth/measles/pdf/measles-factsheet2015.pdf.

33. Kim, Y.C., J.H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Adv Drug Deliv Rev, 2012. **64**(14): p. 1547-68.

34. Pettis, R.J. and A.J. Harvey, *Microneedle delivery: clinical studies and emerging medical applications.* Therapeutic Delivery, 2012. **3**(3): p. 357-71.

35. Nestle, F.O., P. Di Meglio, J.Z. Qin, and B.J. Nickoloff, *Skin immune sentinels in health and disease.* Nat Rev Immunol, 2009. **9**(10): p. 679-91.

36. Zehrung, D., C. Jarrahian, and A. Wales, *Intradermal delivery for vaccine dose sparing: overview of current issues.* Vaccine, 2013. **31**(34): p. 3392-5.

37. van der Maaden, K., W. Jiskoot, and J. Bouwstra, *Microneedle technologies for (trans)dermal drug and vaccine delivery.* J Control Release, 2012. **161**(2): p. 645-55.

38. Vrdoljak, A., *Review of recent literature on microneedle vaccine delivery technologies.* Vaccine: Development and Therapy, 2013.

39. Vrdoljak, A., M.G. McGrath, J.B. Carey, S.J. Draper, A.V.S. Hill, C. O'Mahony, A.M. Crean, and A.C. Moore, *Coated microneedle arrays for transcutaneous delivery of live virus vaccines.* Journal of Controlled Release, 2012. **159**(1): p. 34-42.

40. Bachy, V., C. Hervouet, P.D. Becker, L. Chorro, L.M. Carlin, S. Herath, T. Papagatsias, J.B. Barbaroux, S.J. Oh, A. Benlahrech, T. Athanasopoulos, G. Dickson, S. Patterson, S.Y. Kwon, F. Geissmann, and L.S. Klavinskis, *Langerin negative dendritic cells promote potent CD8(+) T-cell priming by skin delivery of live adenovirus vaccine microneedle arrays.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(8): p. 3041-3046.

41. Carey, J.B., A. Vrdoljak, C. O'Mahony, A.V.S. Hill, S.J. Draper, and A.C. Moore, *Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route.* Sci. Rep., 2014. **4**.

42. Erdos, G., C. Donahue, J.Y. Zhang, B. Ozdoganlar, A. Gambotto, and L. Falo, *Dissolvable microneedle arrays deliver live adenovirus to the skin for genetic immunization.* Journal of Immunology, 2012. **188**: p. 1.

43. Ryan, E., M.J. Garland, T.R.R. Singh, E. Bambury, J. O'Dea, K. Migalska, S.P. Gorman, H.O. McCarthy, B.F. Gilmore, and R.F. Donnelly, *Microneedle-mediated transdermal bacteriophage delivery.* European Journal of Pharmaceutical Sciences, 2012. **47**(2): p. 297-304.

44. Mikszta, J.A., J.P. Dekker, N.G. Harvey, C.H. Dean, J.M. Brittingham, J. Huang, V.J. Sullivan, B. Dyas, C.J. Roy, and R.G. Ulrich, *Microneedle-based intradermal delivery of the anthrax recombinant protective antigen vaccine.* Infection and Immunity, 2006. **74**(12): p. 6806-6810.

45. Morefield, G.L., Tammariello, R. F., Purcell, B. K., Worsham, P. L., Chapman, J., Smith, L. A., ... & Ulrich, R. G. , *An alternative approach to combination*

vaccines: intradermal administration of isolated components for control of anthrax, botulism, plague and staphylococcal toxic shock. Journal of immune based therapies and vaccines. Journal of Immune Based Therapies and Vaccines 2008. **6**(5).

46. Mikszta, J.A., V.J. Sullivan, C. Dean, A.M. Waterston, J.B. Alarcon, J.P. Dekker, J.M. Brittingham, J. Huang, C.R. Hwang, M. Ferriter, G. Jiang, K. Mar, K.U. Saikh, B.G. Stiles, C.J. Roy, R.G. Ulrich, and N.G. Harvey, *Protective immunization against inhalational anthrax: A comparison of minimally invasive delivery platforms.* Journal of Infectious Diseases, 2005. **191**(2): p. 278-288.

47. Hiraishi, Y., S. Nandakumar, S.-O. Choi, J.W. Lee, Y.-C. Kim, J.E. Posey, S.B. Sable, and M.R. Prausnitz, *Bacillus Calmette-Guérin vaccination using a microneedle patch.* Vaccine, 2011. **29**(14): p. 2626-2636.

48. Wong, Y.L., S. Sampson, W.A. Germishuizen, S. Goonesekera, G. Caponetti, J. Sadoff, B.R. Bloom, and D. Edwards, *Drying a tuberculosis vaccine without freezing.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(8): p. 2591-2595.

49. Matsuo, K., H. Okamoto, Y. Kawai, Y.-S. Quan, F. Kamiyama, S. Hirobe, N. Okada, and S. Nakagawa, *Vaccine efficacy of transcutaneous immunization with amyloid β using a dissolving microneedle array in a mouse model of Alzheimer's disease.* Journal of Neuroimmunology, 2014. **266**(1–2): p. 1-11.

50. Torrisi, B.M., V. Zarnitsyn, M.R. Prausnitz, A. Anstey, C. Gateley, J.C. Birchall, and S.A. Coulman, *Pocketed microneedles for rapid delivery of a liquid-state botulinum toxin A formulation into human skin.* Journal of Controlled Release, 2013. **165**(2): p. 146-152.

51. Prow, T.W., X. Chen, N.A. Prow, G.J.P. Fernando, C.S.E. Tan, A.P. Raphael, D. Chang, M.P. Ruutu, D.W.K. Jenkins, A. Pyke, M.L. Crichton, K. Raphaelli, L.Y.H. Goh, I.H. Frazer, M.S. Roberts, J. Gardner, A.A. Khromykh, A. Suhrbier, R.A. Hall, and M.A.F. Kendall, *Nanopatch-Targeted Skin Vaccination against West Nile Virus and Chikungunya Virus in Mice.* Small, 2010. **6**(16): p. 1776-1784.

52. Matsuo, K., S. Hirobe, Y. Yokota, Y. Ayabe, M. Seto, Y.-S. Quan, F. Kamiyama, T. Tougan, T. Horii, Y. Mukai, N. Okada, and S. Nakagawa, *Transcutaneous immunization using a dissolving microneedle array protects against tetanus,*

diphtheria, malaria, and influenza. Journal of Controlled Release, 2012. **160**(3): p. 495-501.

53. Ding, Z., S. Bal, S. Romeijn, G.A. Kersten, W. Jiskoot, and J. Bouwstra, *Transcutaneous Immunization Studies in Mice Using Diphtheria Toxoid-Loaded Vesicle Formulations and a Microneedle Array.* Pharmaceutical Research, 2011. **28**(1): p. 145-158.

54. Bal, S., Z. Ding, G.A. Kersten, W. Jiskoot, and J. Bouwstra, *Microneedle-Based Transcutaneous Immunisation in Mice with N-Trimethyl Chitosan Adjuvanted Diphtheria Toxoid Formulations.* Pharmaceutical Research, 2010. **27**(9): p. 1837-1847.

55. Ding, Z., F.J. Verbaan, M. Bivas-Benita, L. Bungener, A. Huckriede, D.J. van den Berg, G. Kersten, and J.A. Bouwstra, *Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice.* Journal of Controlled Release, 2009. **136**(1): p. 71-78.

56. Ding, Z., E. Van Riet, S. Romeijn, G.F.A. Kersten, W. Jiskoot, and J.A. Bouwstra, *Immune Modulation by Adjuvants Combined with Diphtheria Toxoid Administered Topically in BALB/c Mice After Microneedle Array Pretreatment.* Pharmaceutical Research, 2009. **26**(7): p. 1635-1643.

57. Kim, Y.-C., F.-S. Quan, R.W. Compans, S.-M. Kang, and M.R. Prausnitz, *Formulation and coating of microneedles with inactivated influenza virus to improve vaccine stability and immunogenicity.* Journal of Controlled Release, 2010. **142**(2): p. 187-195.

58. Alarcon, J.B., A.W. Hartley, N.G. Harvey, and J.A. Mikszta, *Preclinical evaluation of microneedle technology for intradermal delivery of influenza vaccines.* Clinical and Vaccine Immunology, 2007. **14**(4): p. 375-381.

59. Arnou, R., G. Icardi, M. De Decker, A. Ambrozaitis, M.P. Kazek, F. Weber, and P. Van Damme, *Intradermal influenza vaccine for older adults: A randomized controlled multicenter phase III study.* Vaccine, 2009. **27**(52): p. 7304-7312.

60. Beran, J., A. Ambrozaitis, A. Laiskonis, N. Mickuviene, P. Bacart, Y. Calozet, E. Demanet, S. Heijmans, P. Van Belle, F. Weber, and C. Salamand, *Intradermal influenza vaccination of healthy adults using a new microinjection system: a 3-year randomised controlled safety and immunogenicity trial.* Bmc Medicine, 2009. **7**: p. 15.

61. Choi, H.J., B.J. Bondy, D.G. Yoo, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Stability of whole inactivated influenza virus vaccine during coating onto metal microneedles.* Journal of Controlled Release, 2013. **166**(2): p. 159-171.

62. Choi, H.J., D.G. Yoo, B.J. Bondy, F.S. Quan, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Stability of influenza vaccine coated onto microneedles.* Biomaterials, 2012. **33**(14): p. 3756-3769.

63. Fernando, G.J.P., X.F. Chen, C.A. Primiero, S.R. Yukiko, E.J. Fairmaid, H.J. Corbett, I.H. Frazer, L.E. Brown, and M.A.F. Kendall, *Nanopatch targeted delivery of both antigen and adjuvant to skin synergistically drives enhanced antibody responses.* Journal of Controlled Release, 2012. **159**(2): p. 215-221.

64. Fernando, G.J.P., X.F. Chen, T.W. Prow, M.L. Crichton, E.J. Fairmaid, M.S. Roberts, I.H. Frazer, L.E. Brown, and M.A.F. Kendall, *Potent Immunity to Low Doses of Influenza Vaccine by Probabilistic Guided Micro-Targeted Skin Delivery in a Mouse Model.* Plos One, 2010. **5**(4): p. 11.

65. Holland, D., R. Booy, F. De Looze, P. Eizenberg, J. McDonald, J. Karrasch, M. McKeirnan, H. Salem, G. Mills, J. Reid, F. Weber, and M. Saville, *Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: A randomized controlled trial.* Journal of Infectious Diseases, 2008. **198**(5): p. 650-658.

66. Kim, Y.C., F.S. Quan, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Stability Kinetics of Influenza Vaccine Coated onto Microneedles During Drying and Storage.* Pharmaceutical Research, 2011. **28**(1): p. 135-144.

67. Kim, Y.C., F.S. Quan, D.G. Yoo, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Enhanced Memory Responses to Seasonal H1N1 Influenza Vaccination of the Skin with the Use of Vaccine-Coated Microneedles.* Journal of Infectious Diseases, 2010. **201**(2): p. 190-198.

68. Kim, Y.C., D.G. Yoo, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Cross-protection by co-immunization with influenza hemagglutinin DNA and inactivated virus vaccine using coated microneedles.* Journal of Controlled Release, 2013. **172**(2): p. 579-588.

69. Kommareddy, S., B.C. Baudner, A. Bonificio, S. Gallorini, G. Palladino, A.S. Determan, D.M. Dohmeier, K.D. Kroells, J.R. Sternjohn, M. Singh, P.R.

Dormitzer, K.J. Hansen, and D.T. O'Hagan, *Influenza subunit vaccine coated microneedle patches elicit comparable immune responses to intramuscular injection in guinea pigs.* Vaccine, 2013. **31**(34): p. 3435-3441.

70. Kommareddy, S., B.C. Baudner, S. Oh, S.Y. Kwon, M. Singh, and D.T. O'Hagan, *Dissolvable microneedle patches for the delivery of cell-culture-derived influenza vaccine antigens.* Journal of Pharmaceutical Sciences, 2012. **101**(3): p. 1021-1027.

71. Koutsonanos, D.G., E.V. Vassilieva, A. Stavropoulou, V.G. Zarnitsyn, E.S. Esser, M.T. Taherbhai, M.R. Prausnitz, R.W. Compans, and I. Skountzou, *Delivery of subunit influenza vaccine to skin with microneedles improves immunogenicity and long-lived protection.* Scientific Reports, 2012. **2**: p. 10.

72. Levin, Y., E. Kochba, and R. Kenney, *Clinical evaluation of a novel microneedle device for intradermal delivery of an influenza vaccine: Are all delivery methods the same?* Vaccine, 2014. **32**(34): p. 4249-4252.

73. Martin, M.D., W.C. Weldon, V.G. Zarnitsyn, D.G. Koutsonanos, H. Akbari, I. Skountzou, J. Jacob, M.R. Prausnitz, and R.W. Compans, *Local Response to Microneedle-Based Influenza Immunization in the Skin.* Mbio, 2012. **3**(2): p. 8.

74. Nougarede, N., H. Bisceglia, A. Rozieres, C. Goujon, F. Boudet, P. Laurent, B. Vanbervliet, K. Rodet, A. Hennino, and J.F. Nicolas, *Nine mu g intradermal influenza vaccine and 15 mu g intramuscular influenza vaccine induce similar cellular and humoral immune responses in adults.* Human Vaccines & Immunotherapeutics, 2014. **10**(9): p. 2713-2720.

75. Pearton, M., S.M. Kang, J.M. Song, Y.C. Kim, F.S. Quan, A. Anstey, M. Ivory, M.R. Prausnitz, R.W. Compans, and J.C. Birchall, *Influenza virus-like particles coated onto microneedles can elicit stimulatory effects on Langerhans cells in human skin.* Vaccine, 2010. **28**(37): p. 6104-6113.

76. Pearton, M., D. Pirri, S.M. Kang, R.W. Compans, and J.C. Birchall, *Host Responses in Human Skin After Conventional Intradermal Injection or Microneedle Administration of Virus-Like-Particle Influenza Vaccine.* Advanced Healthcare Materials, 2013. **2**(10): p. 1401-1410.

77. Puig-Barbera, J., A. Natividad-Sancho, J. Calabuig-Perez, J.A. Lluch-Rodrigo, E. Pastor-Villalba, S. Martinez-Ubeda, and J. Diez-Domingo, *Intradermal and virosomal influenza vaccines for preventing influenza hospitalization in the*

elderly during the 2011-2012 influenza season: A comparative effectiveness study using the Valencia health care information system. Vaccine, 2014. **32**(42): p. 5447-5454.

78. Quan, F.S., Y.C. Kim, R.W. Compans, M.R. Prausnitz, and S.M. Kang, *Dose sparing enabled by skin immunization with influenza virus-like particle vaccine using microneedles.* Journal of Controlled Release, 2010. **147**(3): p. 326-332.

79. Quan, F.S., Y.C. Kim, D.G. Yoo, R.W. Compans, M.R. Prausnitz, and S.M. Kang, *Stabilization of Influenza Vaccine Enhances Protection by Microneedle Delivery in the Mouse Skin.* Plos One, 2009. **4**(9): p. 10.

80. Song, J.M., Y.C. Kim, P.G. Barlow, M.J. Hossain, K.M. Park, R.O. Donis, M.R. Prausnitz, R.W. Compans, and S.M. Kang, *Improved protection against avian influenza H5N1 virus by a single vaccination with virus-like particles in skin using microneedles.* Antiviral Research, 2010. **88**(2): p. 244-247.

81. Weldon, W.C., V.G. Zarnitsyn, E.S. Esser, M.T. Taherbhai, D.G. Koutsonanos, E.V. Vassilieva, I. Skountzou, M.R. Prausnitz, and R.W. Compans, *Effect of Adjuvants on Responses to Skin Immunization by Microneedles Coated with Influenza Subunit Vaccine.* Plos One, 2012. **7**(7): p. 8.

82. Yan, L., Y. Yang, W.J. Zhang, and X.F. Chen, *Advanced Materials and Nanotechnology for Drug Delivery.* Advanced Materials, 2014. **26**(31): p. 5533-5540.

83. Zhu, Q.Y., V.G. Zarnitsyn, L. Ye, Z.Y. Wen, Y.L. Gao, L. Pan, I. Skountzou, H.S. Gill, M.R. Prausnitz, C.L. Yang, and R.W. Compans, *Immunization by vaccine-coated microneedle arrays protects against lethal influenza virus challenge.* Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(19): p. 7968-7973.

84. Andrianov, A.K., D.P. DeCollibus, H.A. Gillis, H.H. Kha, A. Marin, M.R. Prausnitz, L.A. Babiuk, H. Townsend, and G. Mutwiri, *Poly[di(carboxylatophenoxy)phosphazene] Is a Potent Adjuvant for Intradermal Immunization.* Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(45): p. 18936-18941.

85. Guo, L., Y. Qiu, J. Chen, S. Zhang, B. Xu, and Y. Gao, *Effective transcutaneous immunization against hepatitis B virus by a combined approach of hydrogel patch*

formulation and microneedle arrays. Biomedical Microdevices, 2013. **15**(6): p. 1077-1085.

86. Yin, D., W. Liang, S. Xing, Z. Gao, W. Zhang, Z. Guo, and S. Gao, *Hepatitis B DNA Vaccine-Polycation Nano-Complexes Enhancing Immune Response by Percutaneous Administration with Microneedle.* Biological and Pharmaceutical Bulletin, 2013. **36**(8): p. 1283-1291.

87. Mikszta, J.A., J.B. Alarcon, J.M. Brittingham, D.E. Sutter, R.J. Pettis, and N.G. Harvey, *Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery.* Nature Medicine, 2002. **8**(4): p. 415-419.

88. Pattani, A., P.F. McKay, M.J. Garland, R.M. Curran, K. Migalska, C.M. Cassidy, R.K. Malcolm, R.J. Shattock, H.O. McCarthy, and R.F. Donnelly, *Microneedle mediated intradermal delivery of adjuvanted recombinant HIV-1 CN54gp140 effectively primes mucosal boost inoculations.* Journal of Controlled Release, 2012. **162**(3): p. 529-537.

89. Dean, C.H., J.B. Alarcon, A.M. Waterston, K. Draper, R. Early, F. Guirakhoo, T.P. Monath, and J.A. Mikszta, *Cutaneous Delivery of a Live, Attenuated Chimeric Flavivirus Vaccine Against Japanese Encephalitis (ChimeriVax (TM)-JE) in Non-Human Primates.* Human Vaccines, 2005. **1**(3): p. 106-111.

90. Gill, H.S., J. Soderholm, M.R. Prausnitz, and M. Sallberg, *Cutaneous vaccination using microneedles coated with hepatitis C DNA vaccine.* Gene Ther, 2010. **17**(6): p. 811-814.

91. van der Maaden, K., S.J. Trietsch, H. Kraan, E.M. Varypataki, S. Romeijn, R. Zwier, H.J. van der Linden, G. Kersten, T. Hankemeier, W. Jiskoot, and J. Bouwstra, *Novel Hollow Microneedle Technology for Depth-Controlled Microinjection-Mediated Dermal Vaccination: A Study with Polio Vaccine in Rats.* Pharmaceutical Research, 2014. **31**(7): p. 1846-1854.

92. Chen, X., A.S. Kask, M.L. Crichton, C. McNeilly, S. Yukiko, L. Dong, J.O. Marshak, C. Jarrahian, G.J.P. Fernando, D. Chen, D.M. Koelle, and M.A.F. Kendall, *Improved DNA vaccination by skin-targeted delivery using dry-coated densely-packed microprojection arrays.* Journal of Controlled Release, 2010. **148**(3): p. 327-333.

93. Kask, A.S., X.F. Chen, J.O. Marshak, L.C. Dong, M. Saracino, D. Chen, C. Jarrahian, M.A. Kendall, and D.M. Koelle, *DNA vaccine delivery by densely-packed and short microprojection arrays to skin protects against vaginal HSV-2 challenge.* Vaccine, 2010. **28**(47): p. 7483-7491.

94. Carey, J.B., F.E. Pearson, A. Vrdoljak, M.G. McGrath, A.M. Crean, P.T. Walsh, T. Doody, C. O'Mahony, A.V.S. Hill, and A.C. Moore, *Microneedle Array Design Determines the Induction of Protective Memory CD8⁺ T Cell Responses Induced by a Recombinant Live Malaria Vaccine in Mice.* PLoS ONE, 2011. **6**(7): p. e22442.

95. Laurent, P.E., H. Bourhy, M. Fantino, P. Alchas, and J.A. Mikszta, *Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults.* Vaccine, 2010. **28**(36): p. 5850-5856.

96. Corbett, H.J., G.J.P. Fernando, X. Chen, I.H. Frazer, and M.A.F. Kendall, *Skin Vaccination against Cervical Cancer Associated Human Papillomavirus with a Novel Micro-Projection Array in a Mouse Model.* PLoS ONE, 2010. **5**(10): p. e13460.

97. Edens, C., M.L. Collins, J.L. Goodson, P.A. Rota, and M.R. Prausnitz, *A microneedle patch containing measles vaccine is immunogenic in non-human primates.* Vaccine, 2015. **33**(37): p. 4712-8.

98. Edens, C., N.C. Dybdahl-Sissoko, W.C. Weldon, M.S. Oberste, and M.R. Prausnitz, *Inactivated polio vaccination using a microneedle patch is immunogenic in the rhesus macaque.* Vaccine, 2015. **33**(37): p. 4683-90.

99. Huang, J., A.J. D'Souza, J.B. Alarcon, J.A. Mikszta, B.M. Ford, M.S. Ferriter, M. Evans, T. Stewart, K. Amemiya, R.G. Ulrich, and V.J. Sullivan, *Protective Immunity in Mice Achieved with Dry Powder Formulation and Alternative Delivery of Plague F1-V Vaccine.* Clinical and Vaccine Immunology, 2009. **16**(5): p. 719-725.

100. Edens, C., M.L. Collins, J. Ayers, P.A. Rota, and M.R. Prausnitz, *Measles vaccination using a microneedle patch.* Vaccine, 2013. **31**(34): p. 3403-3409.

101. Moon, S., Y. Wang, C. Edens, J.R. Gentsch, M.R. Prausnitz, and B. Jiang, *Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch.* Vaccine, 2013. **31**(34): p. 3396-3402.

102. Hooper, J.W., J.W. Golden, A.M. Ferro, and A.D. King, *Smallpox DNA vaccine delivered by novel skin electroporation device protects mice against intranasal poxvirus challenge.* Vaccine, 2007. **25**(10): p. 1814-1823.

103. Laurent, P.E., H. Bourhy, M. Fantino, P. Alchas, and J.A. Mikszta, *Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults.* Vaccine, 2010. **28**(36): p. 5850-6.

104. Koutsonanos, D.G., E.S. Esser, S.R. McMaster, P. Kalluri, J.W. Lee, M.R. Prausnitz, I. Skountzou, T.L. Denning, J.E. Kohlmeier, and R.W. Compans, *Enhanced immune responses by skin vaccination with influenza subunit vaccine in young hosts.* Vaccine, 2015. **33**(37): p. 4675-82.

105. Sullivan, S.P., D.G. Koutsonanos, M. Del Pilar Martin, J.W. Lee, V. Zarnitsyn, S.-O. Choi, N. Murthy, R.W. Compans, I. Skountzou, and M.R. Prausnitz, *Dissolving polymer microneedle patches for influenza vaccination.* Nat Med, 2010. **16**(8): p. 915-20.

106. Quan, F.S., Y.C. Kim, A. Vunnava, D.G. Yoo, J.M. Song, M.R. Prausnitz, R.W. Compans, and S.M. Kang, *Intradermal vaccination with influenza virus-like particles by using microneedles induces protection superior to that with intramuscular immunization.* J Virol, 2010. **84**(15): p. 7760-7769.

107. Koutsonanos, D.G., E.V. Vassilieva, A. Stavropoulou, V.G. Zarnitsyn, E.S. Esser, M.T. Taherbhai, M.R. Prausnitz, R.W. Compans, and I. Skountzou, *Delivery of subunit influenza vaccine to skin with microneedles improves immunogenicity and long-lived protection.* Sci Rep, 2012. **2**: p. 357.

108. Pulit-Penaloza, J.A., E.S. Esser, E.V. Vassilieva, J.W. Lee, M.T. Taherbhai, B.P. Pollack, M.R. Prausnitz, R.W. Compans, and I. Skountzou, *A protective role of murine langerin(+) cells in immune responses to cutaneous vaccination with microneedle patches.* Sci Rep, 2014. **4**: p. 6094.

109. Ruutu, M.P., X. Chen, O. Joshi, M.A. Kendall, and I.H. Frazer, *Increasing mechanical stimulus induces migration of Langerhans cells and impairs the immune response to intracutaneously delivered antigen.* Exp Dermatol, 2011. **20**(6): p. 534-6.

110. Birchall, J.C., R. Clemo, A. Anstey, and D.N. John, *Microneedles in clinical practice--an exploratory study into the opinions of healthcare professionals and the public.* Pharm Res, 2011. **28**(1): p. 95-106.

111. Lee, B.Y., S.M. Bartsch, M. Mvundura, C. Jarrahian, K.M. Zapf, K. Marinan, A.R. Wateska, B. Snyder, S. Swaminathan, E. Jacoby, J.J. Norman, M.R. Prausnitz, and D. Zehrung, *An economic model assessing the value of microneedle patch delivery of the seasonal influenza vaccine.* Vaccine, 2015. **33**(37): p. 4727-36.

112. Donnelly, R.F., K. Moffatt, A.Z. Alkilani, E.M. Vicente-Perez, J. Barry, M.T. McCrudden, and A.D. Woolfson, *Hydrogel-Forming Microneedle Arrays Can Be Effectively Inserted in Skin by Self-Application: A Pilot Study Centred on Pharmacist Intervention and a Patient Information Leaflet.* Pharm Res, 2014.

113. Norman, J.J., J.M. Arya, M.A. McClain, P.M. Frew, M.I. Meltzer, and M.R. Prausnitz, *Microneedle patches: usability and acceptability for self-vaccination against influenza.* Vaccine, 2014. **32**(16): p. 1856-62.

114. Cosman, F., N.E. Lane, M.A. Bolognese, J.R. Zanchetta, P.A. Garcia-Hernandez, K. Sees, J.A. Matriano, K. Gaumer, and P.E. Daddona, *Effect of transdermal teriparatide administration on bone mineral density in postmenopausal women.* J Clin Endocrinol Metab, 2010. **95**(1): p. 151-8.

115. Toon, J. *Self-Administration of Flu Vaccine with a Patch May be Feasible*. [March 2 2015]; Available from: http://www.news.gatech.edu/2014/02/26/self-administration-flu-vaccine-patch-may-be-feasible-study-suggests.

116. Mistilis, M.J., A.S. Bommarius, and M.R. Prausnitz, *Development of a Thermostable Microneedle Patch for Influenza Vaccination.* Journal of Pharmaceutical Sciences, 2015. **104**(2): p. 740-749.

117. Ameri, M., X. Wang, and Y.F. Maa, *Effect of irradiation on parathyroid hormone PTH(1-34) coated on a novel transdermal microprojection delivery system to produce a sterile product--adhesive compatibility.* J Pharm Sci, 2010. **99**(4): p. 2123-34.

118. Zhang, Y., K. Brown, K. Siebenaler, A. Determan, D. Dohmeier, and K. Hansen, *Development of lidocaine-coated microneedle product for rapid, safe, and prolonged local analgesic action.* Pharm Res, 2012. **29**(1): p. 170-7.

CHAPTER 4

RABIES VACCINATION IN DOGS USING A DISSOLVING MICRONEEDLE PATCH

4.1 Abstract

Because humans get rabies primarily through dog bites, mass vaccination of domestic dogs and other animals has virtually eliminated human rabies in industrialized countries. However, thousands of people in developing countries die of rabies each year due to lack of mass vaccination because of financial, logistical and other challenges. Here, we propose the use of dissolving microneedle patches for simple, cost-effective rabies vaccination and assess the safety and immunogenicity of microneedle patch vaccination using a rabies DNA vaccine in dogs. The vaccine was stable upon formulation and storage for at least 3 weeks at 4 °C in a microneedle patch. For vaccination, the patches were applied to the inner ear by hand without an applicator, Microneedle patches were well tolerated in the skin, with mild erythema, minimal wheal formation and complete resolution of skin reactions within 7 days, and generated no systemic adverse events. Microneedle patches were at least as immunogenic as intramuscular injection at the same dose, as demonstrated by similar serum neutralizing antibody titers. A ten-fold lower vaccine dose administered by microneedle patch generated a weaker immune response compared to full-dose intramuscular vaccination. We conclude that dissolving microneedle patches may serve as an innovative approach to mass vaccination of dogs.

4.2 Introduction

Rabies is an acute, often fatal encephalitis caused by viruses in the Rhabdoviridae family [1]. The disease is zoonotic and human infection usually results from a bite or scratch from an infected animal or direct contact of skin wounds with virus containing saliva. Although all warm-blooded animals can be reservoirs of rabies, dogs account for 99% of human deaths due to rabies and pose a potential threat to more than 3.3 billion people [2].

Human rabies has been almost eliminated in industrialized countries by widespread and often mandatory vaccination of dogs and other animals, as well as the availability of vaccines for humans [3]. Humans are not typically vaccinated against rabies for prevention, but post-exposure prophylactic vaccines and immunoglobulins are available to people who become exposed to the virus [4]. These measures have caused the number of deaths due to rabies in the United States to drop to just one to two per year [5]. However, globally, an estimated 26,000 to 61,000 deaths are caused by rabies each year, more than 95% of which occur in Africa and Asia due to dog bites [3]. Rabies occurs mainly in remote rural communities where children between the age of 5–14 years are the most frequent victims [6], and limited access to healthcare facilities with the high cost and complex schedule of post-exposure vaccines for humans often makes it difficult to provide medical care to people that become exposed to the virus [7].

In developing countries, more extensive vaccination of dogs and humans is often limited by the high cost of vaccination and a lack of trained personnel to administer the vaccines. Intradermal vaccination using one-fifth to one-tenth the dose of rabies vaccine has been shown to be effective in humans, thereby enabling significant cost savings [2, 8-

10]. This dose sparing is believed to be due to targeting of the vaccine to resident dendritic cells in the skin, such as Langerhans and dermal dendritic cells, which are able to mount a more robust immune response [11-13]. However, intradermal injection requires specifically trained healthcare personnel and successful injection into the skin is unreliable [14, 15]. Thus, low-cost intradermal post-exposure prophylaxis of humans is sometimes available, but intradermal pre-exposure vaccination in dogs is generally not. A simple and reliable method of intradermal rabies vaccination could therefore enable more widespread vaccination at lower cost.

Another method of cost savings is through DNA vaccination. Human DNA vaccines could be much less costly to manufacture compared to inactivated virus vaccines. This is because human DNA vaccines can be produced in large quantities by bacterial fermentation processes and may not require expensive facilities with high biosafety levels for production [16].

In this study, we propose that delivery of a rabies DNA vaccine using a microneedle patch could enable more widespread rabies vaccination of dogs and humans by enabling minimally trained personnel to carry out vaccination. Microneedles are less than one millimeter long and deliver vaccines to the skin's epidermis and dermis using a patch that is simply and painlessly applied to the skin by personnel with minimal training [17-22]. In a dissolving microneedle patch, an array of microneedles is attached to a backing such that it can be applied to the skin by hand like a bandage. After insertion into the skin, the microneedles dissolve in the skin within minutes, thereby delivering the vaccine contained in them and not generating sharps waste [23-33].

Vaccination using a microneedle patch could simplify rabies vaccination of dogs, especially stray dogs in developing countries, since the microneedle patches could be easily applied by hand on a dog's ears by personnel with minimal training. While oral rabies vaccine exist, it has limited use when vaccinating dogs because the bait in which the vaccine is contained does not always lead to complete delivery of vaccine and the use of oral vaccines is usually limited to areas with minimal human activity so as to ensure safe distribution [3]. Post-exposure rabies prophylaxis could also reach more patients by enabling vaccination by minimally trained personnel without the need to go to qualified healthcare facilities. Microneedle patch vaccination could also be attractive in industrialized countries, where dogs and their owners may prefer a painless, less-invasive method of vaccination.

Microneedle patches have previously been studied for delivery of a number of vaccines for eventual human applications [34-41], but have not previously been studied for rabies vaccination or for veterinary vaccination applications. The goal of this project is to develop an easy-to-administer rabies vaccine suitable for use in dogs that enables cost savings, is safe and is at least as immunogenic as conventional intramuscular vaccination. We therefore developed and characterized dissolving microneedle patches for rabies vaccination and then assessed safety and immunogenicity in a small clinical study in beagle dogs.

4.3 Materials and methods

4.3.1 Fabrication of microneedle patch

Polydimethylsiloxane (PDMS) molds containing a 10 x 10 array of conical microneedles (base diameter 300 µm and height 650 µm) were used for microneedle patch fabrication by a two-step micromolding process. (i) Vaccine fill: The vaccine (i.e., proprietary DNA plasmid provided by Merial Inc. isolated from E. coli culture using the EndoFree Plasmid Giga Kit (Qiagen, Germantown, ND)) was mixed 1:1 with 15% w/v sucrose (Sigma-Aldrich, St. Louis, MO) and applied to the microneedle mold. Vacuum was then applied for 45 min. Excess vaccine was removed and the mold was allowed to dry for 90 min. (ii) Polymer matrix fill: The polymer matrix solution was composed of polyvinyl alcohol (EMD Millipore, Billerica, MA) and sucrose (Sigma-Aldrich) in sterile water. The solution was heated to 80 °C for 6 h before use to facilitate dissolution of the polyvinyl alcohol. The matrix solution was applied onto the mold and exposed to vacuum for 4 h. The mold was left in a chemical hood overnight to dry.

To remove the dried microneedle patches, a 2.3 cm-diameter disc of polymethylmethacrylate (McMaster-Carr, Atlanta, GA) was covered on one side with double-sided tape (MacTac, Stow, OH) and applied to the back of the mold. The resulting patch was gently peeled away from the mold and stored in a dark, sealed pouch with desiccant at 4 °C until use.

As a quality control measure, a representative sample of patches from each batch was tested for DNA loading, supercoiling and sterility, as described below. Microneedle patches were imaged by brightfield microscopy (SZX12, Olympus, Center Valley,

Pennsylvania). Microneedle patches were applied to the animals for vaccination three weeks after fabrication, as described below, after all testing had been completed.

4.3.2 Quantification of DNA loaded into microneedle patch

DNA concentrations were measured using the nucleic acid setting on Nanodrop 2000 (Thermo Fisher, Waltham, MA). The patch was dissolved in deionized autoclaved water to determine the dose contained in the patch. A placebo patch containing no vaccine was used as a negative control to subtract any interference from the microneedle matrix materials.

4.3.3 Quantification of DNA supercoiling

DNA supercoiling was measured using agarose gel electrophoresis. A 0.8% agarose gel was run with Tris-acetate buffer and the gel was stained with ethidium bromide. The gel was imaged using a Kodak 200 gel logic camera system (Kodak, Rochester, NY) and the relative intensities of the bands were used to calculate the percentage of supercoiled DNA.

4.3.4 *In-vitro* expression assay for DNA stability

An *in-vitro* expression assay was used to confirm the ability of the vaccine to express the rabies G protein *in-vitro* (i.e., the vaccine antigen). CHO-K1 cells (ATCC CCL-61, American Type Culture Collection, Manassas, VA) were transfected with rabies DNA obtained from reconstituted patches using Lipofectamine (Life Technologies, Carlsbad, CA) and stained with mouse anti-rabies glycoprotein monoclonal antibody clone 24-3F-10 (EMD Millipore, Billerica, MA) and FITC-conjugated rabbit anti-mouse IgG (Sigma, St. Louis, MO). For a sample to be declared satisfactory, cells needed to show easily visible and similar level of green fluorescence as compared to the control.

4.3.5 Insertion of microneedle patches into dog ears *ex-vivo*

Excised dog ears were obtained from animals euthanized as part of a separate study and the skin was carefully shaved with a razor to remove fur. Microneedle patches containing sulforhodamine dye (to simulate vaccine) were applied to the skin on the inner ear pinna by pressing down with the thumb, left on the skin for 15 min and then removed. The skin site and microneedle patches were imaged before and after insertion.

4.3.6 Safety and immunization study

The study was approved by the Institutional Animal Care and Use Committees (IACUC) at Merial and Georgia Tech. Male and female beagle dogs aged 5 to 11 months were used in the clinical study. The dogs were seronegative for rabies and were excluded from the study if they had eczema or inflammation at the injection sites at the time of the study. The dogs were vaccinated by intramuscular (IM) injection (50 µg DNA), microneedle patch (50 µg DNA) and microneedle patch (5 µg DNA) (n=5 per group). A placebo (i.e., no vaccine) microneedle patch group was also included in the study (n=2). Four weeks after the first dose, all dogs were given a booster using the same route and dose as the initial vaccination (Figure 4.1).

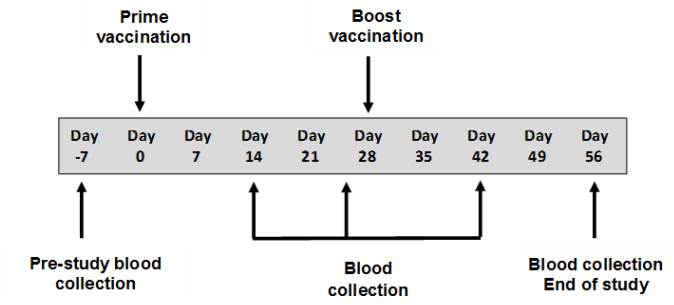


Figure 4.1. Outline of vaccination timeline. After a 7 day acclimation period, prime vaccination was carried out, followed by a boost vaccination 28 days later. The skin was shaved three days prior to each vaccination. Blood was collected 14, 25, 42 and 56 days after the prime vaccination.

Microneedle patches were applied on the inner ear pinna. Fur was removed from the inner ear surface by shaving with a disposable razor and shaving cream three days prior to each vaccination. During vaccination, the ear was gently held on top of one hand and the patch was applied using the other hand by pressing down on the backing with the thumb for 1 min. The patches were secured onto the ear with 3M™ VetRap™ Bandaging Tape and left on the skin for up to15 min. The patches were then removed from the skin and stored for imaging. Intramuscular injections were administered to the rear leg in the caudal thigh muscle using a conventional 22 gauge needle and syringe. All dogs were awake during the vaccinations without sedation or pain relieving drugs.

Tolerance to injection was noted during each vaccination. Dogs were considered intolerant of injection if they vocalized, withdrew or tried to bite upon injection. Dogs were observed for local injection site reactions on the day of the vaccination, daily for the first three days following vaccination and intermittently for any dogs with reactions persisting for more than 3 days. Local injection sites were assessed by blinded personnel for erythema, wheal formation, swelling, pain upon palpation and ulceration. Rectal temperatures were recorded in conjunction with injection site observations. Blood was collected prior to beginning the study and every two weeks until the end of the study at eight weeks.

4.3.7 Measurement of neutralizing antibodies

Serum was separated from blood and stored at -20 °C. Serum samples were submitted to Atlanta Health Associates (Cumming, GA) for analysis of anti-rabies neutralizing antibody titers using the rapid fluorescent focus inhibition test (RFFIT).

Results were expressed in international units per milliliter of serum (IU/ml) and titers greater than 0.2 IU/ml were considered seropositive.

4.3.8 Statistics

Statistical analysis was carried out using Prism software version 5 (Graphpad, La Jolla, CA). P values < 0.05 were considered significant. Average values of degree of supercoiling were analyzed using one-way ANOVA with Dunnett's multiple comparison post-test.

4.4 Results

4.4.1 Vaccine stabilization in microneedle patches

Microneedle patches were formulated with biocompatible, water-soluble excipients so that microneedles could dissolve in the skin, thereby releasing encapsulated vaccine. These dissolving microneedle patches were fabricated as a 10 x 10 microneedle array in a ~1 cm^2 area affixed to a clear plastic backing (Figure 4.2A). Compared to conventional needle-and-syringe vaccination, the microneedle patches were designed to be small (Figure 4.2A, B), generate no sharps waste and be simple to apply by minimally trained personnel.

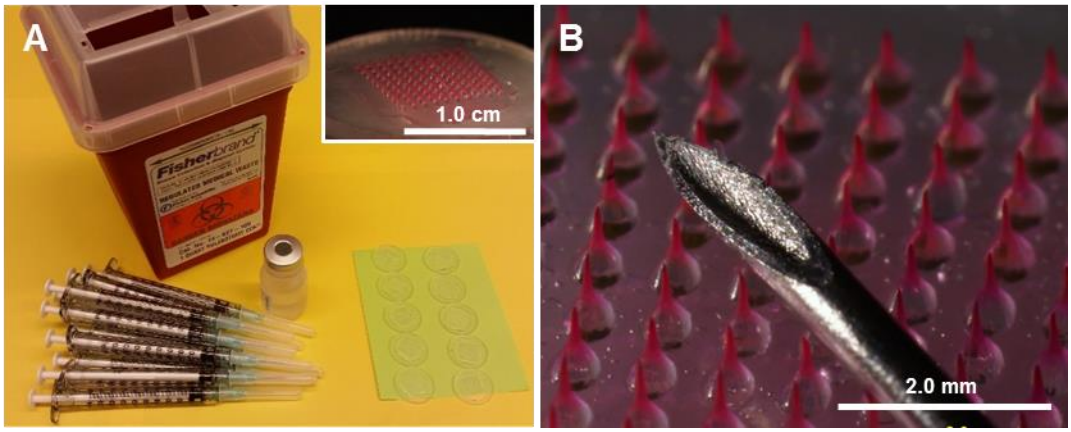


Figure 4.2 (A) Picture of a 10-dose vaccine vial, 10 conventional needles and syringes and a sharps waste container next to 10 microneedle patches. Inset: A 100-microneedle array made of water-soluble polymers and sugars containing sulforhodamine dye. **(B)** Magnified image of a microneedle patch containing sulforhodamine dye placed adjacent to a 22-gauge needle for scale.

Because vaccine is dried during fabrication of microneedle patches, a suitable formulation was developed to maintain vaccine activity during the fabrication process. Sucrose has previously been used in formulations to stabilize various vaccines during drying [42, 43]. Microneedle patches were therefore fabricated with sucrose as the stabilizing excipient during the vaccine fill step and stored in a sealed foil pouch with desiccant. Before use in the clinical study, the patches had to be stored for three weeks to allow sufficient time to complete sterility testing, during which time the vaccine in the patch needed to remain stable. We therefore stored the microneedle patches for three weeks at 4 °C and assessed DNA vaccine stability by two methods: maintenance of DNA supercoiling and *in-vitro* transfection of cells. The patches containing 50 µg DNA vaccine were able to meet the stability requirements for the study as seen by no significant loss in supercoiling (Figure 4.3B) as compared to the liquid control and successful *in-vitro* transfection of cells demonstrated by expression of rabies G protein ($p > 0.05$, Figure 4.3A). Microneedle patches containing the 5 µg DNA vaccine dose

showed some loss in supercoiling ($p < 0.05$, Figure 4.3B), but exhibited successful *in-vitro* transfection (Figure 4.3A). Both microneedle patch groups also passed the sterility test (data not shown) and were therefore considered suitable for clinical testing.

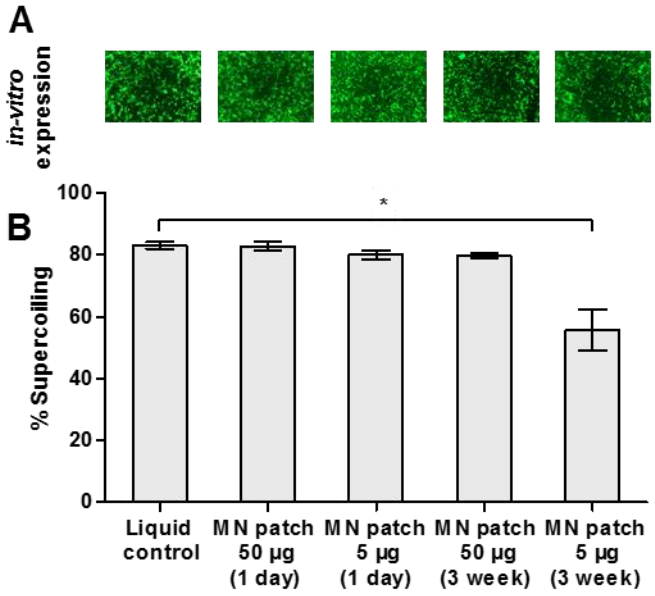


Figure 4.3 Effect of microneedle fabrication and storage on DNA vaccine stability. Microneedle patches were packaged in a foil pouch with desiccant and stored in a stability chamber at 4 °C for three weeks. **(A)** Representative images from *in-vitro* expression assay (green-stained cells indicate expression of rabies G protein encoded in the DNA vaccine). **(B)** Degree of supercoiling of DNA. Data points represent the average ± standard deviation (SD) from n=3 independently tested samples. Asterisk (*) indicates a significant difference ($p < 0.05$) from liquid control.

4.4.2 Microneedle patch insertion into skin

In addition to vaccine stability, microneedle patches also needed to be mechanically strong in order to pierce the stratum corneum and insert into the skin. Microneedle patches containing a pink dye (i.e., sulforhodamine, to simulate vaccine) were applied to the skin of dog ears *ex-vivo* by pressing on the patch backing with the thumb (i.e., no applicator was used). We wanted to avoid the use of an applicator device because it adds

bulk and cost to the microneedle patch, and the goal was to design a device that is small and easy to administer. The microneedles dissolved in the skin within 15 min of application, as seen by the 10 x 10 grid of pink dye deposited in the skin (Figure 4.4A, B), as well as the disappearance of microneedle tips containing the dye shown by microscopy (Figure 4.4C, D). It is important to note that most of the dye (or vaccine) is concentrated in the tips of the microneedles, such that complete insertion and dissolution of the microneedle is not needed to deliver the dye/vaccine into the skin.

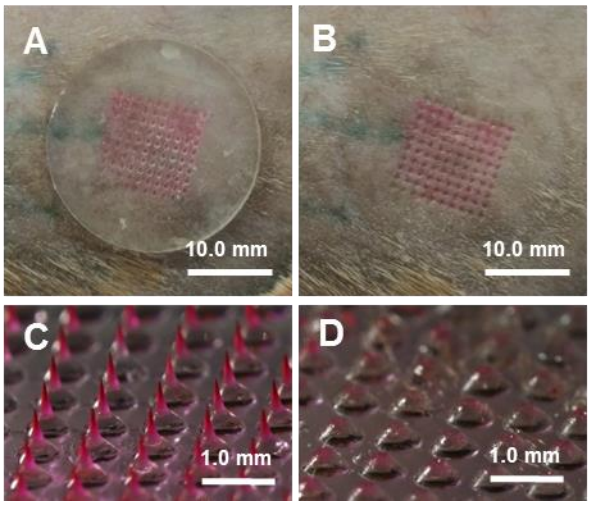


Figure 4.4 Representative images of insertion and dissolution of microneedles after patch application to dog ears *ex vivo*. Microneedle patches were applied to shaved skin by pressing down with the thumb, left on the skin for 15 min and then removed. **(A)** Microneedle patch containing sulforhodamine dye applied to skin. **(B)** Same section of skin imaged after microneedle patch application and removal, which shows a grid where microneedles punctured the skin and delivered the dye. Microneedle patches **(C)** before and **(D)** after insertion into skin. The dye was concentrated in the tip of the microneedles whereas the base of the microneedles contained very little dye.

4.4.3 Safety of rabies vaccination of dogs using a microneedle patch

Beagle dogs were vaccinated with a prime dose and a booster dose 28 days later using microneedle patches at a 50 µg or 5 µg dose, IM injection at a 50 µg dose, and a placebo microneedle patch containing no vaccine. During vaccination, the dogs were noted for

intolerance to vaccine administration as indicated by vocalization, withdrawal or attempted biting. During prime vaccination, 60% of the dogs in the IM injection group were intolerant of injection, whereas none of the dogs in any of the microneedle patch groups showed signs of intolerance. During boost vaccination none of the dogs in the IM injection group, the 50 µg patch group or the placebo patch groups showed signs of intolerance, whereas one dog (20%) in the 5 µg microneedle patch group showed signs of intolerance (Table 4.1).

Table 4.1 Tolerance of dogs to vaccination by IM injection and microneedle patch.

	Prime (Day 0)	Boost (Day 28)
Intramuscular injection 50 µg		
Microneedle patch 50 µg		
Microneedle patch 5 µg		
Placebo microneedle patch		


Dogs were tolerant


Dogs were intolerant

[1]Dogs were considered intolerant of injection if they vocalized, withdrew or tried to bite upon injection.

The vaccination sites were monitored for local skin reactions. After removal of microneedle patch from the skin, a faint grid of the needles puncturing the skin was visible with slight erythema as well as minor redness along the edges of the patch. A small drop of blood (< 1 µL) was also visible in most insertions (Figure 4.5).

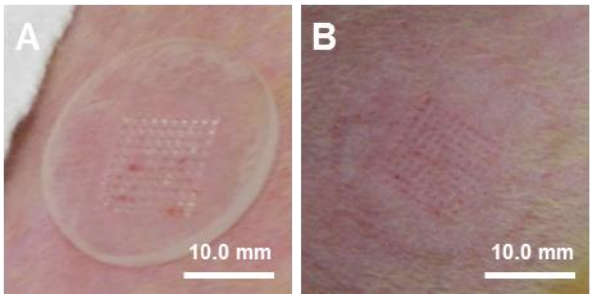


Figure 4.5 Dog ear during and after microneedle patch vaccination *in vivo*. Microneedle patches were applied onto dog ears with hair removed, left on the skin for 15 min and then removed. **(A)** Microneedle patch applied to skin. **(B)** Same section of skin immediately imaged after microneedle patch application and removal showing a faint grid where microneedles inserted and slight skin erythema.

All (100%) of the dogs in the microneedle vaccination groups showed mild transient erythema at the vaccination site, whereas only 50% of the dogs in the microneedle placebo group showed erythema. None of the dogs in the IM injection group showed erythema at the injection site (Table 4.2). Most skin erythema resolved within 4 days and all erythema resolved within 7 days (Table 4.3 and 4.4).

Table 4.2 Number and type of local injection site reactions.

	Erythema	Wheal Formation	Swelling	Pain upon palpation	Ulceration
IM 50 µg	○	○	○	○	○
MN 50 µg	●	◔	○	○	○
MN 5 µg	●	◑	○	○	○
MN Placebo	◑	◑	○	○	○

□ Skin reactions absent
■ Skin reactions present

[1]The dogs were observed for local injection site reactions on the day of the vaccination, daily for the first three days following each vaccination and intermittently for any dogs with reactions persisting for more than three days. This table reports the cumulative percentage of dogs with injection site reactions after both vaccinations.

Table 4.3 Duration of erythema after prime vaccination.

	% Of dogs with Erythema	% Resolving in		
		3 Days	4 Days	7 Days
IM 50 µg	○	○	○	○
MN 50 µg	●	◔	◑	○
MN 5 µg	◕	◔	◔	◔
MN Placebo	◑	◑	○	○

Skin reactions absent

Skin reactions present

Table 4.4 Duration of erythema after boost vaccination.

	% Of dogs with Erythema	% Resolving in			
		2 Days	3 Days	4 Days	7 Days
IM 50 µg	○	○	○	○	○
MN 50 µg	●	◔	◔	◔	◔
MN 5 µg	●	○	◔	○	◕
MN Placebo	○	○	○	○	○

Skin reactions absent

Skin reactions present

Vaccination sites were also monitored for wheal formation: 20% of dogs in the 50 µg microneedle group, 40% of dogs in the 5 µg microneedle group and 50% of dogs in the placebo microneedle group showed wheal formation (Table 4.2). None of the dogs in the IM injection group showed wheal formation. All wheal formation resolved within 2 days (Table 4.5 and 4.6). There was no swelling, pain upon palpation, ulceration or any other abnormality noted at the vaccination site. The veterinary staff noted no other study related health problems in the dogs, and there were no systemic adverse events reported.

Table 4.5 Duration of wheal formation reaction after prime vaccination.

	% Of dogs with Wheal	% Resolving in 2 Days
IM 50 µg	○	○
MN 50 µg	◔	◔
MN 5 µg	◔	◔
MN Placebo	◑	◑

▭ Skin reactions absent
▬ Skin reactions present

Table 4.6 Duration of wheal formation reaction after boost vaccination

	% Of dogs with Wheal	% Resolving in 2 Days
IM 50 µg	○	○
MN 50 µg	○	○
MN 5 µg	◔	◔
MN Placebo	○	○

▭ Skin reactions absent
▬ Skin reactions present

4.4.4 Immunogenicity of rabies vaccination of dogs using a microneedle patch

Immune response following rabies vaccination was also evaluated. Beagle dogs were selected as the study subjects because they are representative of a major population that receives rabies vaccination (i.e., dogs) and can serve as a model for humans and other animals. Fourteen days after prime vaccination, none of the groups showed meaningful increases in rabies-specific, neutralizing antibody titers (Figure 4.6). On day 25, 60% of dogs in the IM vaccination group, 40% of dogs in the 50 µg microneedle patch group and

20% of dogs in the 5 µg microneedle patch group were seropositive (Figure 4.7), but most had very low antibody titers. On day 42, fourteen days after the boost vaccination, antibody responses were much higher, with mean titers similar between the 50 µg microneedle group and the IM vaccination group at the same dose, whereas on day 56 the mean titer in the 50 µg microneedle group was higher than after IM vaccination at the same dose. However, due to the small number of animals per group and the variability in titers within each group, statistically significant differences were not seen. Overall, 100% of animals were seropositive in both the 50 µg microneedle and IM vaccination groups on day 42 and 56 with antibody titers well above threshold, which indicates that microneedle patch vaccination produced an equivalent immune response to conventional IM vaccination.

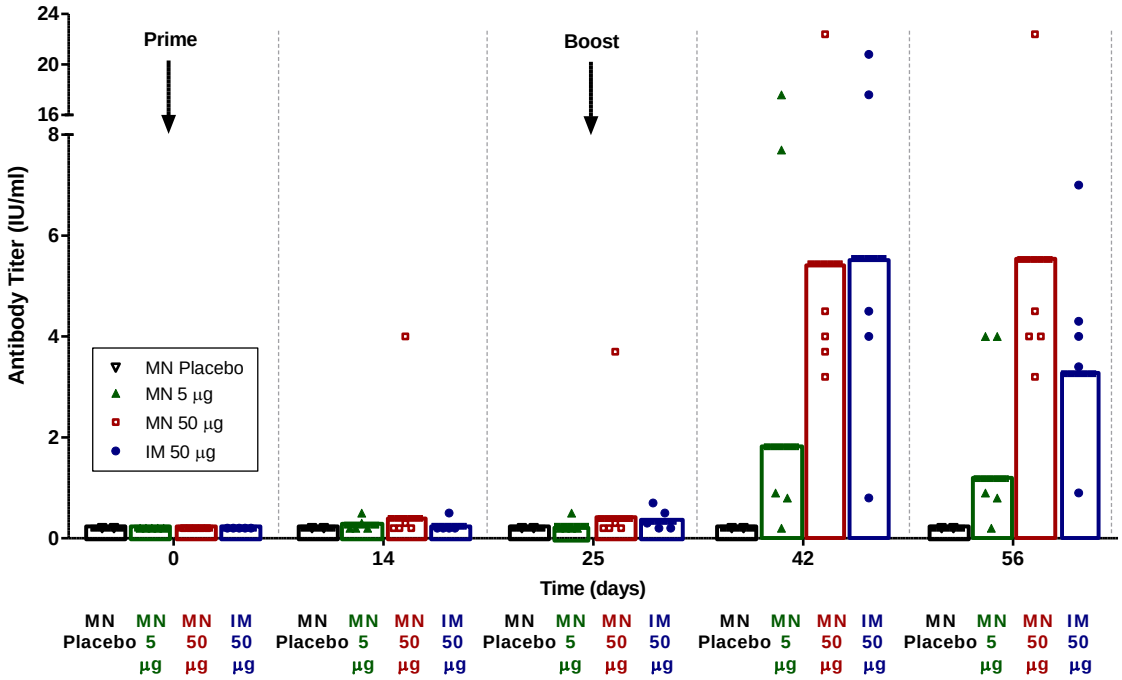


Figure 4.6 Neutralizing antibody titers after vaccination using microneedle patch as compared to conventional intramuscular injection. Dogs were vaccinated with either a microneedle patch (MN) containing no vaccine (placebo), a microneedle patch containing 5 µg or 50 µg vaccine or a conventional intramuscular injection (IM) containing 50 µg vaccine. Blood was collected from the dogs at each of the given time points and tested independently. Neutralizing antibodies were measured using the RFFIT assay and expressed in International Units per milliliter (IU/ml). Data points show the individual antibody titers and column bars represent the geometric mean titer.

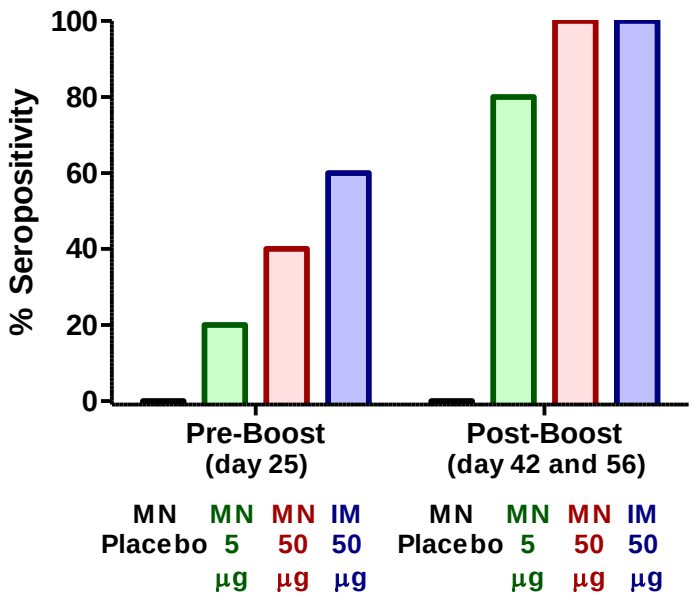


Figure 4.7 Percentage of dogs seropositive within the different microneedle (MN) and intramuscular (IM) groups before and after the boost. Pre-boost data are shown for day 25. Post-boost seropositivity remained the same at day 42 and 56. Seropositivity was defined as a titer > 0.2 IU/ml.

While the 5 µg microneedle patch group exhibited an increased immune response, there was no evidence of dose sparing, since the 5 µg microneedle patch group had lower mean titers and seropositivity rates compared to the IM vaccination group. The placebo microneedle group had no rabies-specific neutralizing antibodies, and none of the animals were seropositive, as expected.

4.5 Discussion

The goal of this study was to develop a small, sharps-free and easy-to-administer device for rabies vaccination of dogs such that it contains the required dose of the vaccine, is safe, is at least as immunogenic as conventional IM vaccination and enables cost savings.

Dissolving microneedle patches could enable simpler administration of vaccines. In this study, microneedle patches were applied to dog ears with gentle manual force (i.e., without the need for an applicator device) and left in place for a few minutes. The microneedles dissolved in the skin and did not leave behind sharps waste. The dogs tolerated the microneedle patch very well, and better than the first intramuscular injection. Future work will address reducing the time of patch application to as little as a few seconds and elimination of the need to shave the skin. Altogether, microneedle patch vaccination could enable simpler administration of rabies vaccine to dogs, including stray dogs in developing countries, and could be applied by minimally trained workers, which would reduce the need for personnel trained to give hypodermic injections. In industrialized countries, microneedle patches could be an attractive sharps-free alternative to intramuscular injection in the veterinary setting.

Rabies vaccination using microneedle patches was well-tolerated in the skin, and there were no systemic adverse events reported. Only mild, transient erythema was observed, which resolved within a few days, was not sensitive to touch and did not result in other sequelae.

Prior studies have shown excellent thermostability of vaccines in microneedle patches [27, 44, 45] and DNA has been shown to be stable during storage in a dried state [34, 46-49]. While this study only assessed stability for three weeks at 4 °C, future studies should evaluate stability at higher temperatures for extended periods to determine if microneedle patches can avoid the need for storage and transportation in the cold chain, which would be of significant value in developing countries that often lack access to reliable refrigeration [50, 51].

Microneedle patch vaccination produced a strong rabies-specific immune response. Antibody titers after vaccination by microneedle patch were similar to intramuscular vaccination at the same dose in this study and were similar to responses to intramuscular vaccination in previous studies using rabies DNA vaccine [52]. The neutralizing antibody titers were well above 0.5 IU/ml, which is considered protective in humans [53]. However, the dogs were not challenged with rabies virus to evaluate survival in this study.

Intradermal delivery has been shown to enable dose sparing for a number of vaccines, including rabies [11, 32, 54, 55]. However, we did not see evidence for ten-fold dose sparing in this study. It is possible that the vaccine patch developed in this study could enable dose sparing at reductions less than ten-fold.

One of the goals of this study was to assess possible cost savings due to rabies vaccination using a microneedle patch. Cost savings relative to conventional intramuscular vaccination could result from the use of minimally trained (i.e., lower cost) personnel to perform vaccination and the use of a DNA vaccine, which is expected to be relatively inexpensive to manufacture compared to the cost of traditional human vaccines [56]. Further cost savings could result from possible thermostability, which has been demonstrated for other microneedle patch vaccines and DNA vaccines [44, 45, 57, 58], and the possibility of dose sparing, which was not seen in this study at the doses used but has been demonstrated for microneedle patch vaccines and, more specifically, intradermal delivery of rabies vaccines [8-10, 59]. Also, manufacturing of microneedle patches is expected to be inexpensive, i.e., less than the cost of a needle, syringe and vaccine-filled vial [19].

This study used beagle dogs, which are commonly used in veterinary research as a model dog breed [60]. This study therefore serves as a first-in-dogs clinical trial for veterinary vaccine applications. Dogs have been used before as an animal model for insulin delivery using microneedles [61].

4.6 Conclusions

This study has shown for the first time that dissolving microneedle patches can safely and effectively administer rabies DNA vaccine to dogs using a delivery technology that is easy to administer and may enable cost savings. The vaccine was stable upon formulation and storage for at least 3 weeks at 4 °C in a microneedle patch. The patches were administered manually to dog ears by pressing with the thumb, without the need of an applicator, and the microneedles dissolved in the skin within 15 min, thereby leaving no sharps waste.

Microneedle patches were well tolerated in the skin with mild erythema, minimal wheal formation and complete resolution of skin reactions within 7 days, and generated no systemic adverse events. Microneedle patches were at least as immunogenic as intramuscular injection at the same dose, as demonstrated by similar serum neutralizing antibody titers. A ten-fold lower vaccine dose administered by microneedle patch generated a weaker immune response compared to full-dose intramuscular vaccination.

In contrast to traditional needle-and-syringe vaccination, these microneedle patches were designed to enable administration by minimally trained personnel, increase safety by generating no sharps waste and utilize a DNA vaccine that is relatively inexpensive to manufacture compared to traditional human vaccines, all of which are

expected to enable increased vaccination coverage at reduced cost. For these reasons, dissolving microneedle patches may serve as an innovative and effective approach to mass vaccinate dogs and humans.

4.7 Acknowledgements

The authors would like to thank Yu-Wei Chen of Merial for help with the DNA vaccine assays, Donna Bondy of Georgia Tech for administrative support and the clinical technicians and animal care personnel who assisted with the study. This work was supported by a grant from Merial. Mark Prausnitz is an inventor of patents that have been or may be licensed to companies developing microneedle-based products, is a paid advisor to companies developing microneedle-based products and is a founder/shareholder of companies developing microneedle-based products, including Micron Biomedical. This potential conflict of interest has been disclosed and is being managed by Georgia Tech and Emory University.

4.8 References

1. Plotkin, S., W. Orenstein, and P. Offit, *Rabies vaccines,* in *Vaccines*. Elsevier p. 687-714.

2. Publication, W.H.O., *Rabies vaccines: WHO position paper--recommendations.* Vaccine, 2010. **28**(44): p. 7140-2.

3. World Health, O., *WHO Expert Consultation on Rabies. Second report.* World Health Organization technical report series, 2013(982): p. 1-139, back cover.

4. Manning, S.E., C.E. Rupprecht, D. Fishbein, C.A. Hanlon, B. Lumlertdacha, M. Guerra, M.I. Meltzer, P. Dhankhar, S.A. Vaidya, S.R. Jenkins, B. Sun, and H.F.

Hull, *Human rabies prevention--United States, 2008: recommendations of the Advisory Committee on Immunization Practices.* MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control, 2008. **57**(RR-3): p. 1.

5. CDC. *Rabies in the U.S.* 2011 [11 Feb]; Available from: http://www.cdc.gov/rabies/location/usa.

6. WHO. *Rabies Factsheet Updated September 2015*. [January 28 2016]; Available from: http://www.who.int/mediacentre/factsheets/fs099/en/.

7. Nigg, A.J. and P.L. Walker, *Overview, Prevention, and Treatment of Rabies.* Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2009. **29**(10): p. 1182-1195.

8. Briggs, D.J., A. Banzhoff, U. Nicolay, S. Sirikwin, B. Dumavibhat, S. Tongswas, and C. Wasi, *Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine.* Bulletin of the World Health Organization, 2000. **78**(5): p. 693-698.

9. Khawplod, P., H. Wilde, S. Sirikwin, M. Benjawongkulchai, S. Limusanno, W. Jaijaroensab, N. Chiraguna, C. Supich, Y. Wangroongsarb, and V. Sitprija, *Revision of the Thai Red Cross intradermal rabies post-exposure regimen by eliminating the 90-day booster injection.* Vaccine, 2006. **24**(16): p. 3084-6.

10. Quiambao, B.P., E.M. Dimaano, C. Ambas, R. Davis, A. Banzhoff, and C. Malerczyk, *Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals.* Vaccine, 2005. **23**(14): p. 1709-14.

11. Zehrung, D., C. Jarrahian, and A. Wales, *Intradermal delivery for vaccine dose sparing: overview of current issues.* Vaccine, 2013. **31**(34): p. 3392-5.

12. Nestle, F.O., P. Di Meglio, J.Z. Qin, and B.J. Nickoloff, *Skin immune sentinels in health and disease.* Nat Rev Immunol, 2009. **9**(10): p. 679-91.

13. Lambert, P.H. and P.E. Laurent, *Intradermal vaccine delivery: will new delivery systems transform vaccine administration?* Vaccine, 2008. **26**(26): p. 3197-208.

14. Flynn, P.M., J.L. Shenep, L. Mao, R. Crawford, B.F. Williams, and B.G. Williams, *INfluence of needle gauge in mantoux skin testing.* Chest, 1994. **106**(5): p. 1463-1465.

15. Tarnow, K. and N. King, *Intradermal injections: Traditional bevel up versus bevel down.* Applied Nursing Research, 2004. **17**(4): p. 275-282.

16. Redding, L. and D.B. Werner, *DNA vaccines in veterinary use.* Expert review of vaccines, 2009. **8**(9): p. 1251-1276.

17. Kim, Y.C., J.H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Adv Drug Deliv Rev, 2012. **64**(14): p. 1547-68.

18. van der Maaden, K., W. Jiskoot, and J. Bouwstra, *Microneedle technologies for (trans)dermal drug and vaccine delivery.* J Control Release, 2012. **161**(2): p. 645-55.

19. Arya, J. and M.R. Prausnitz, *Microneedle patches for vaccination in developing countries.* Journal of Controlled Release.

20. Pettis, R.J. and A.J. Harvey, *Microneedle delivery: clinical studies and emerging medical applications.* Therapeutic Delivery, 2012. **3**(3): p. 357-71.

21. Coulman, S.A., J.C. Birchall, A. Alex, M. Pearton, B. Hofer, C. O'Mahony, W. Drexler, and B. Povazay, *In vivo, in situ imaging of microneedle insertion into the skin of human volunteers using optical coherence tomography.* Pharm Res, 2011. **28**(1): p. 66-81.

22. Liu, S., M.N. Jin, Y.S. Quan, F. Kamiyama, H. Katsumi, T. Sakane, and A. Yamamoto, *The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin.* J Control Release, 2012. **161**(3): p. 933-41.

23. Teunissen, M.B. and D. Zehrung, *Cutaneous vaccination - Protective immunization is just a skin-deep step away.* Vaccine, 2015. **33**(37): p. 4659-62.

24. Wendorf, J.R., E.B. Ghartey-Tagoe, S.C. Williams, E. Enioutina, P. Singh, and G.W. Cleary, *Transdermal delivery of macromolecules using solid-state biodegradable microstructures.* Pharm Res, 2011. **28**(1): p. 22-30.

25. Raphael, A.P., M.L. Crichton, R.J. Falconer, S. Meliga, X. Chen, G.J. Fernando, H. Huang, and M.A. Kendall, *Formulations for microprojection/microneedle vaccine delivery: Structure, strength and release profiles.* J Control Release, 2016.

26. Matsuo, K., S. Hirobe, Y. Yokota, Y. Ayabe, M. Seto, Y.-S. Quan, F. Kamiyama, T. Tougan, T. Horii, Y. Mukai, N. Okada, and S. Nakagawa, *Transcutaneous immunization using a dissolving microneedle array protects against tetanus, diphtheria, malaria, and influenza.* Journal of Controlled Release, 2012. **160**(3): p. 495-501.

27. Bachy, V., C. Hervouet, P.D. Becker, L. Chorro, L.M. Carlin, S. Herath, T. Papagatsias, J.B. Barbaroux, S.J. Oh, A. Benlahrech, T. Athanasopoulos, G. Dickson, S. Patterson, S.Y. Kwon, F. Geissmann, and L.S. Klavinskis, *Langerin negative dendritic cells promote potent CD8(+) T-cell priming by skin delivery of live adenovirus vaccine microneedle arrays.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(8): p. 3041-3046.

28. Pattani, A., P.F. McKay, M.J. Garland, R.M. Curran, K. Migalska, C.M. Cassidy, R.K. Malcolm, R.J. Shattock, H.O. McCarthy, and R.F. Donnelly, *Microneedle mediated intradermal delivery of adjuvanted recombinant HIV-1 CN54gp140 effectively primes mucosal boost inoculations.* Journal of Controlled Release, 2012. **162**(3): p. 529-537.

29. Sullivan, S.P., D.G. Koutsonanos, M. Del Pilar Martin, J.W. Lee, V. Zarnitsyn, S.-O. Choi, N. Murthy, R.W. Compans, I. Skountzou, and M.R. Prausnitz, *Dissolving polymer microneedle patches for influenza vaccination.* Nat Med, 2010. **16**(8): p. 915-20.

30. Edens, C., N.C. Dybdahl-Sissoko, W.C. Weldon, M.S. Oberste, and M.R. Prausnitz, *Inactivated polio vaccination using a microneedle patch is immunogenic in the rhesus macaque.* Vaccine, 2015. **33**(37): p. 4683-90.

31. Edens, C., M.L. Collins, J. Ayers, P.A. Rota, and M.R. Prausnitz, *Measles vaccination using a microneedle patch.* Vaccine, 2013. **31**(34): p. 3403-3409.

32. Moon, S., Y. Wang, C. Edens, J.R. Gentsch, M.R. Prausnitz, and B. Jiang, *Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch.* Vaccine, 2013. **31**(34): p. 3396-3402.

33. Zhu, Q.Y., V.G. Zarnitsyn, L. Ye, Z.Y. Wen, Y.L. Gao, L. Pan, I. Skountzou, H.S. Gill, M.R. Prausnitz, C.L. Yang, and R.W. Compans, *Immunization by vaccine-coated microneedle arrays protects against lethal influenza virus challenge.* Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(19): p. 7968-7973.

34. Gill, H.S., J. Soderholm, M.R. Prausnitz, and M. Sallberg, *Cutaneous vaccination using microneedles coated with hepatitis C DNA vaccine.* Gene Ther, 2010. **17**(6): p. 811-4.

35. Norman, J.J., J.M. Arya, M.A. McClain, P.M. Frew, M.I. Meltzer, and M.R. Prausnitz, *Microneedle patches: usability and acceptability for self-vaccination against influenza.* Vaccine, 2014. **32**(16): p. 1856-62.

36. Al-Zahrani, S., M. Zaric, C. McCrudden, C. Scott, A. Kissenpfennig, and R.F. Donnelly, *Microneedle-mediated vaccine delivery: Harnessing cutaneous immunobiology to improve efficacy.* Expert opinion on drug delivery, 2012. **9**(5): p. 541-550.

37. Pearson, F.E., C.L. McNeilly, M.L. Crichton, C.A. Primiero, S.R. Yukiko, G.J.P. Fernando, X. Chen, S.C. Gilbert, A.V.S. Hill, and M.A.F. Kendall, *Dry-Coated Live Viral Vector Vaccines Delivered by Nanopatch Microprojections Retain Long-Term Thermostability and Induce Transgene-Specific T Cell Responses in Mice.* PLoS ONE, 2013. **8**(7): p. e67888.

38. Carey, J.B., A. Vrdoljak, C. O'Mahony, A.V. Hill, S.J. Draper, and A.C. Moore, *Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route.* Sci Rep, 2014. **4**: p. 6154.

39. Pulit-Penaloza, J.A., E.S. Esser, E.V. Vassilieva, J.W. Lee, M.T. Taherbhai, B.P. Pollack, M.R. Prausnitz, R.W. Compans, and I. Skountzou, *A protective role of murine langerin(+) cells in immune responses to cutaneous vaccination with microneedle patches.* Sci Rep, 2014. **4**: p. 6094.

40. Birchall, J.C., R. Clemo, A. Anstey, and D.N. John, *Microneedles in clinical practice--an exploratory study into the opinions of healthcare professionals and the public.* Pharm Res, 2011. **28**(1): p. 95-106.

41. Hirobe, S., H. Azukizawa, K. Matsuo, Y. Zhai, Y.S. Quan, F. Kamiyama, H. Suzuki, I. Katayama, N. Okada, and S. Nakagawa, *Development and Clinical*

Study of a Self-Dissolving Microneedle Patch for Transcutaneous Immunization Device. Pharm Res, 2013.

42. Mistilis, M.J., A.S. Bommarius, and M.R. Prausnitz, *Development of a Thermostable Microneedle Patch for Influenza Vaccination.* Journal of Pharmaceutical Sciences, 2015. **104**(2): p. 740-749.

43. Kumru, O.S., S.B. Joshi, D.E. Smith, C.R. Middaugh, T. Prusik, and D.B. Volkin, *Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies.* Biologicals, 2014. **42**(5): p. 237-259.

44. Edens, C., M.L. Collins, J. Ayers, P.A. Rota, and M.R. Prausnitz, *Measles vaccination using a microneedle patch.* Vaccine, 2013. **31**(34): p. 3403-9.

45. Mistilis, M.J., A.S. Bommarius, and M.R. Prausnitz, *Development of a Thermostable Microneedle Patch for Influenza Vaccination.* J Pharm Sci, 2014.

46. Kim, Y.C., J.M. Song, A.S. Lipatov, S.O. Choi, J.W. Lee, R.O. Donis, R.W. Compans, S.M. Kang, and M.R. Prausnitz, *Increased immunogenicity of avian influenza DNA vaccine delivered to the skin using a microneedle patch.* Eur J Pharm Biopharm, 2012. **81**(2): p. 239-47.

47. Song, J.M., Y.C. Kim, E. O, R.W. Compans, M.R. Prausnitz, and S.M. Kang, *DNA vaccination in the skin using microneedles improves protection against influenza.* Mol Ther, 2012. **20**(7): p. 1472-80.

48. Guo, L., Y. Qiu, J. Chen, S. Zhang, B. Xu, and Y. Gao, *Effective transcutaneous immunization against hepatitis B virus by a combined approach of hydrogel patch formulation and microneedle arrays.* Biomedical Microdevices, 2013. **15**(6): p. 1077-1085.

49. Quaak, S.G., J.B. Haanen, J.H. Beijnen, and B. Nuijen, *Naked plasmid DNA formulation: effect of different disaccharides on stability after lyophilisation.* AAPS PharmSciTech, 2010. **11**(1): p. 344-50.

50. Das, P., *Revolutionary vaccine technology breaks the cold chain.* The Lancet Infectious Diseases, 2004. **4**(12): p. 719.

51. *The Cost of a Broken Vaccine Cold Chain Part Two, Financial Cost*. 2014 Wednesday, September 17 [February 25]; Available from: http://www.csafeglobal.com/the-cost-of-a-broken-vaccine-cold-chain-part-two-financial-cost-1.

52. Patial, S., V.K. Chaturvedi, A. Rai, M. Saini, R. Chandra, Y. Saini, and P.K. Gupta, *Virus neutralizing antibody response in mice and dogs with a bicistronic DNA vaccine encoding rabies virus glycoprotein and canine parvovirus VP2.* Vaccine, 2007. **25**(20): p. 4020-8.

53. Organization, W.H., *WHO guide for rabies pre and post-exposure prophylaxis in human (updated 2013).* Geneva: WHO. Available online at: http://www. who. int/rabies/WHO_Guide_Rabies_ Pre_Post_Exposure_Prophylaxis_Humans_2013. pdf (accessed February 2014), 2014.

54. Quan, F.S., Y.C. Kim, R.W. Compans, M.R. Prausnitz, and S.M. Kang, *Dose sparing enabled by skin immunization with influenza virus-like particle vaccine using microneedles.* J Control Release, 2010. **147**(3): p. 326-332.

55. Chen, X., A.S. Kask, M.L. Crichton, C. McNeilly, S. Yukiko, L. Dong, J.O. Marshak, C. Jarrahian, G.J.P. Fernando, D. Chen, D.M. Koelle, and M.A.F. Kendall, *Improved DNA vaccination by skin-targeted delivery using dry-coated densely-packed microprojection arrays.* Journal of Controlled Release, 2010. **148**(3): p. 327-333.

56. Kutzler, M.A. and D.B. Weiner, *DNA vaccines: ready for prime time?* Nat Rev Genet, 2008. **9**(10): p. 776-88.

57. WHO. *Temperature sensitivity of vaccines*. 2006; Available from: http://whqlibdoc.who.int/hq/2006/WHO_IVB_06.10_eng.pdf.

58. PATH. *Summary of stability data for investigational formulations of vaccines*. [January 28 2016]; Available from: http://www.path.org/publications/files/TS_vaccine_stability_table_invest.pdf.

59. Laurent, P.E., H. Bourhy, M. Fantino, P. Alchas, and J.A. Mikszta, *Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults.* Vaccine, 2010. **28**(36): p. 5850-6.

60. Meunier, L.D., *Selection, acclimation, training, and preparation of dogs for the research setting.* Ilar j, 2006. **47**(4): p. 326-47.

61. Fukushima, K., T. Yamazaki, R. Hasegawa, Y. Ito, N. Sugioka, and K. Takada, *Pharmacokinetic and pharmacodynamic evaluation of insulin dissolving microneedles in dogs.* Diabetes Technol Ther, 2010. **12**(6): p. 465-74.

CHAPTER 5

TOLERABILITY, USABILITY AND ACCEPTABILITY OF DISSOLVING MICRONEEDLE PATCH ADMINISTRATION IN HUMAN SUBJECTS WITHOUT AN APPLICATOR

5.1 Abstract

To support translation of microneedle patches from pre-clinical development into clinical trials, this study examined the effect of microneedle patch application to local skin reactions, reliability of use and acceptability to patients. Placebo patches containing dissolving microneedles were administered to fifteen human participants. Microneedle patches were very well tolerated in the skin with no pain or swelling and only minimal erythema localized to the site of patch administration that resolved fully within seven days. Microneedle patches could be administered reliably by hand without the need of an applicator and delivery efficiencies were similar for investigator-administration and self-administration. Microneedle patch administration was not considered painful and the large majority of subjects were at least somewhat confident that they self-administered patches correctly. Microneedle patches were overwhelmingly preferred over conventional needle and syringe injection. Altogether, these results demonstrate that dissolving microneedle patches were well tolerated, easily usable and strongly accepted by human subjects, which will facilitate further clinical translation of this technology.

5.2 Introduction

Microneedle patches contain hundreds of microneedles less than one millimeter long to deliver drugs and vaccines into the skin. In a dissolving microneedle patch, an array of microneedles is attached to a backing such that it can be applied to the skin. The microneedles dissolve in the skin within minutes, thereby delivering the vaccine contained in them and not generating sharps waste. Microneedle patches offer advantages over conventional drug delivery by needle and syringe.

Microneedle patches have previously been studied for delivery of a number of drugs and vaccines in pre-clinical studies [1-9] but limited information is available about the use of dissolving microneedle patches in human subjects. Microneedle patches are typically designed either as coated microneedle patches made of solid metal, silicon or polymer microneedles coated with vaccine that releases the vaccine upon dissolution of the coating in the skin or as dissolving microneedle patches containing solid, dissolving microneedles made of water-soluble materials that encapsulate vaccine and release the vaccine when the microneedles dissolve in the skin [9, 10].

Coated microneedle patches are being evaluated in clinical trials for delivery of parathyroid hormone to treat osteoporosis [11], glucagon to treat hypoglycemia [12] and zolmitriptan to treat migraine [13]. However, given the difference in delivery mechanisms of coated and dissolving microneedles, all the results from coated microneedle patches cannot be directly applied towards studying dissolving microneedle patches. Dissolving microneedle patches are being evaluated in clinical trials for delivery of parathyroid hormone [14] as well as influenza vaccination [15, 16].

Both of these microneedle patch types have been studied using a high velocity insertion device, which while effective in delivery of microneedle patches, adds additional bulk and cost to the microneedle device. In this study, we are examining the usability of dissolving microneedle patches without the use of an applicator for the first time in human subjects. To our knowledge, no other study has evaluated the puncture and delivery efficiencies of dissolving microneedle patches in humans or the acceptability preferences regarding vaccination using dissolving microneedle patches. As dissolving microneedle patches continue being developed for clinical translation in the next few years, it is important to fully characterize the insertion and dissolution of microneedles in humans. Reliably administering the microneedle patches in a way that ensures complete insertion and delivery of the vaccine into the skin will be key factors to enable clinical use, including the possible use of microneedle patches applied by hand without the use of an applicator.

The goal of this study is to evaluate skin tolerability, usability and acceptability of dissolving microneedle patches to further clinical translation of microneedle patches for delivery of drugs and vaccines. In order to prepare for a phase 1 clinical trial of influenza vaccination using microneedle patches of a similar design [17], we conducted a human study with placebo microneedle patches to study these parameters in greater detail. We therefore developed placebo dissolving microneedle patches and conducted a human study assessing reactions in the reactions in the skin after microneedle patch application, microneedle patch delivery efficiency in investigator-administration and self-administration and conducted a survey about participant's preferences about microneedle patch administration.

5.3 Materials and methods

5.3.1 Fabrication of dissolving microneedle patch

Microneedle arrays were fabricated using a micromolding process similar to that described before [1] to produce microneedle patches containing 100 microneedles in a ~1 cm^2 area that were adhered to a flexible paper backing that incorporated a force-feedback indicator that made a clicking sound when a force greater than 13 lbf is applied. Microneedle patches were stored in a sealed foil pouch with silica gel desiccant until used at the time of study.

5.3.2 Study approval and study subjects

This study was approved by the Georgia Institute of Technology Institutional Review Board and informed consent was obtained from all participants. To be eligible, participants had to be healthy non-pregnant adults with normal skin, no known problems with pain perception and no known allergies to the materials used in the study. Participants could not have previously seen or worked with microneedle patches to be eligible for the study. Fifteen subjects (seven females and eight males), ages 18 - 57 were recruited from the Georgia Institute of Technology and other sites in Atlanta, GA.

5.3.3 Experimental design

Participants received three microneedle patches – one self-administered and two investigator-administered. Participants were provided a brief overview of the study and watched a short presentation on self-administration of microneedle patches. An outline of microneedle patch administration process is outlined in Figure 5.1.

Participants first self-administered a microneedle patch to their forearm without assistance from the investigator. The investigator stained this skin site (see below). The

investigator then applied two microneedle patches to the participant, one on each forearm. Only one of these skin sites was stained by the investigator. The site not stained was used to make measurements of skin tolerability (see below). The investigator-administration stained site and the self-administration stained site were used for usability measurements (see below).

Participants then answered a questionnaire about microneedle patch administration for acceptability measurements. Participants returned to the study site on days 1, 2, 3, 4 and 7 after microneedle patch administration for skin tolerability measurements.

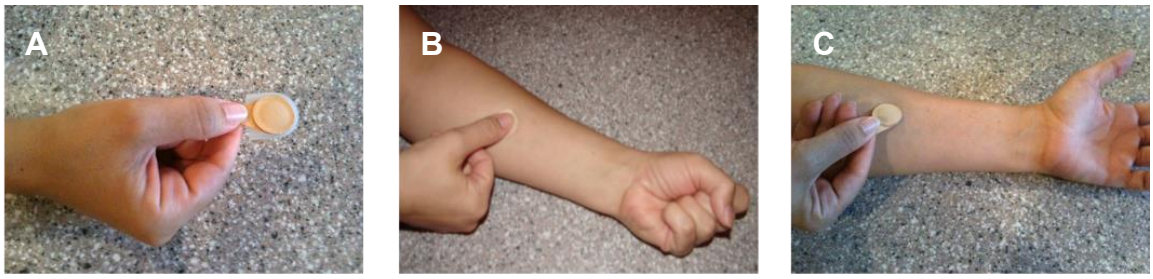


Figure 5.1 Procedure to apply a microneedle patch to the skin. **(A)** Subject or investigator picks up the patch with the dominant hand and removes the protective cap. **(B)** Subject forms a fist in the non-dominant hand and then subject or investigator places the patch on the forearm. Subject or investigator pushes on the patch with the thumb and continues to apply force until hearing a 'click' sound, indicating that enough force has been applied. **(C)** Subject leaves the patch on the skin for 20 minutes after which patch application is complete. Subject or investigator peels away the patch from the skin and the investigator saves the patch for additional analysis.

5.3.4 Skin tolerability measurements

Skin tolerability was measured using the skin scoring scale listed in the appendix table A.1. The scale was created for microneedle patches using guidelines available for vaccine clinical trials and clinical testing of transdermal patches [18, 19]. The skin site was scored for pain, tenderness, erythema (size and intensity) and induration or swelling

on a grading scale of 0 to 4. Pain and tenderness were scored based on the participant's response whereas erythema and swelling were measured by the investigator.

Participants were asked if they felt any pain at the skin site after microneedle patch administration was complete. This pain is separate from the pain during microneedle patch application, which is noted in usability measurements. Tenderness was defined as any pain felt at the skin site when the investigator gently touched the skin site. Erythema size was measured using a ruler scale and intensity by visual observation of the skin site. Since there were no erythema scale for microneedle patches already in place, the investigator was trained on erythema measurements using guidelines and training available for Psoriasis Area Severity Index (PASI) scores [20]. Swelling was measured by the investigator by gently moving the thumb over the skin site to notice any raised surfaces in the skin. Investigator noted a score for all of the criteria and photographically imaged the skin at each time point.

5.3.5 Skin staining and microscopy to measure usability

Usability was measured in terms of microneedle puncture efficiency by skin staining (percentage of microneedles that penetrated the skin surface) and delivery efficiency by microscopy (percentage volume of microneedles that dissolved after administration). Skin was stained using gentian violet 1% solution (Humco, Texarkana, TX). Immediately after microneedle patch administration, gentian violet was pooled on the skin site for 1 min, dabbed with gauze and cleaned with alcohol after 10 min. The stained skin site was imaged and microneedle puncture efficiency was measured by counting the number of stained skin sites, which appeared as blue dots. It has been previously shown that the number of stained skin sites visible after microneedle patch

insertion is correlated with skin puncture by measuring trans-epidermal water loss [21]. Microneedle patches were imaged using brightfield microscopy (SZX12 Olympus, Center Valley, PApl990) before and after administration, and the microneedle dimensions were measured to calculate the volume dissolved after microneedle patch administration. Since placebo microneedle patches (i.e., containing no drug or other active substance) were used in this study, it was not possible to assay the microneedle patches for delivery efficiency of a drug or active, and therefore this method of usability from staining and microscopy was used.

5.3.6 Survey about microneedle patch administration to measure acceptability

Participants answered a short questionnaire to solicit information about the acceptability of microneedle patches for delivery of drugs or vaccines. We surveyed the subjects about pain during microneedle patch application, confidence during self-administration and subject preferences regarding microneedle patches, conventional intramuscular injection and conventional oral delivery using pills.

Pain during microneedle patch administration was reported by participants using a visual analog scale from 0 (no pain) to 10 (worst pain ever). Participants were also asked to score their confidence during self-administration of microneedle patches on a score of 1 to 5 using the following scale:

1: I'm confident that I applied the patch incorrectly

2: I'm somewhat confident that I applied the patch incorrectly

3: I do not know if I applied the patch correctly or incorrectly

4: I'm somewhat confident that I applied the patch correctly

5: I'm confident that I applied the patch correctly

Subjects were then asked their preferences regarding obtaining medications by microneedle patches versus hypodermic needles and microneedle patches versus conventional oral delivery by pill.

5.3.7 Statistical methods

Statistical analysis was carried out using Prism software version 5 (Graphpad, La Jolla, CA). P values < 0.05 were considered significant. Average values of delivery efficiency by microscopy were analyzed using a paired t test.

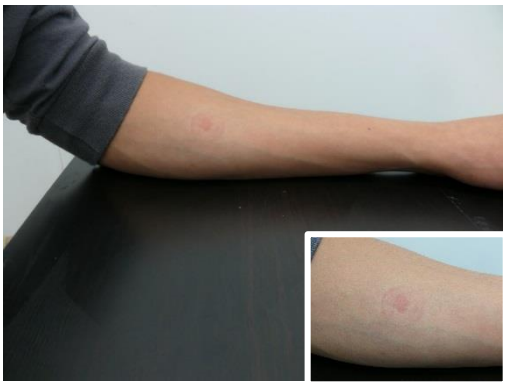
5.4 Results

5.4.1 Skin tolerability

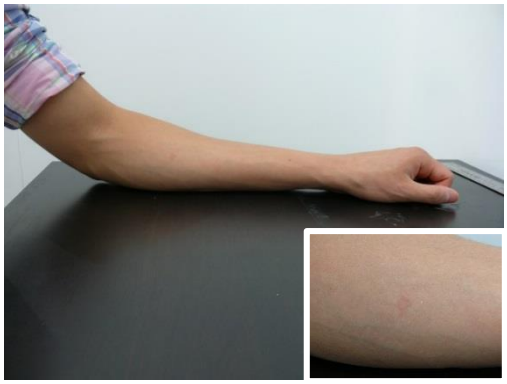
We studied skin tolerability of microneedle patch administration to understand the local reactions in the skin that occur. These reactions are associated with the patch administration process and the patch excipients left in the skin after microneedle dissolution. This study did not assess the possible additional effects of delivery of a drug or other active to the skin on tolerability. The investigator administered one patch onto the subject's skin and the skin site was monitored, imaged and scored once per day for 7 days.

Figure 5.2 shows a series of images from a single representative subject after microneedle patch application. Immediately after microneedle patch application at Day 0, a rectangular area equal to the patch size exhibited mild erythema with faint redness around the rectangle in the area where the adhesive backing was applied to the skin. On Day 1, redness from the adhesive backing was fully resolved and the rectangular area decreased in redness and size. For the subject in Figure 5.2, all erythema resolved by Day

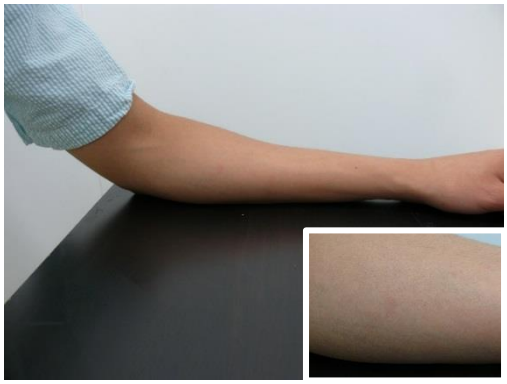
4. There were no other skin conditions or adverse effects noted, and overall the microneedle patch was well tolerated.



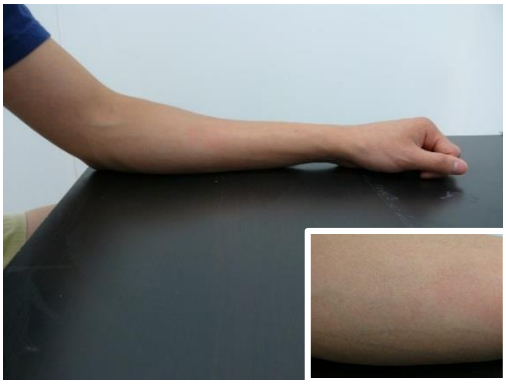
Day 0



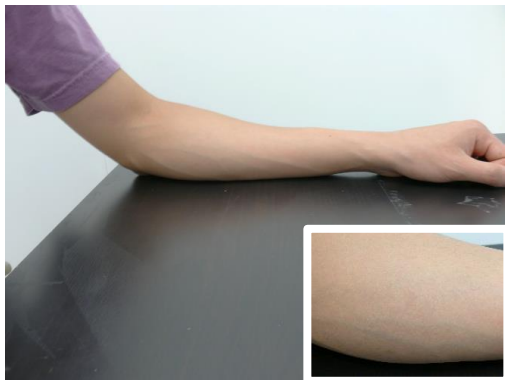
Day 1



Day 2



Day 3



Day 4

Figure 5.2 Representative images of the site of microneedle patch application on the skin over time. Inset shows magnified images of the skin site. Day 0 is immediately after patch application and removal. These images are all from the same subject.

We quantified the skin reactions noted after microneedle patch application over time in Figure 5.3. Subjects were asked if they felt pain or tenderness at the skin site. Pain in this case was assessed after microneedle patch application was complete, which differs from possible pain experienced during microneedle patch insertion, which is addressed in the context of the usability analysis. None of the subjects reported pain at the skin site after microneedle patch application on Day 0 through Day 7. Only one subject (out of 15) reported tenderness at the skin site on Day 0 which was fully resolved by Day 1 and later. The tenderness was reported of Grade 1 (i.e., mild discomfort to touch on a scale of 0 to 4). None of the other subjects reported tenderness at the skin site through Day 7.

The investigator also scored the skin site for erythema and induration/swelling. Erythema was scored based on size and intensity. Based on size, erythema, when present, was always of Grade 0.5, which corresponds to a size equal to or less than the patch. As noted in Figure 5.3A, erythema was present in 100% of subjects on Day 0 and 80% of subjects on Day 1. Erythema continued to subside over time with 33% of subjects having erythema on Day 2, 20% of subjects on Day 3 and 13% of subjects on Day 4. By Day 7 all erythema was fully resolved.

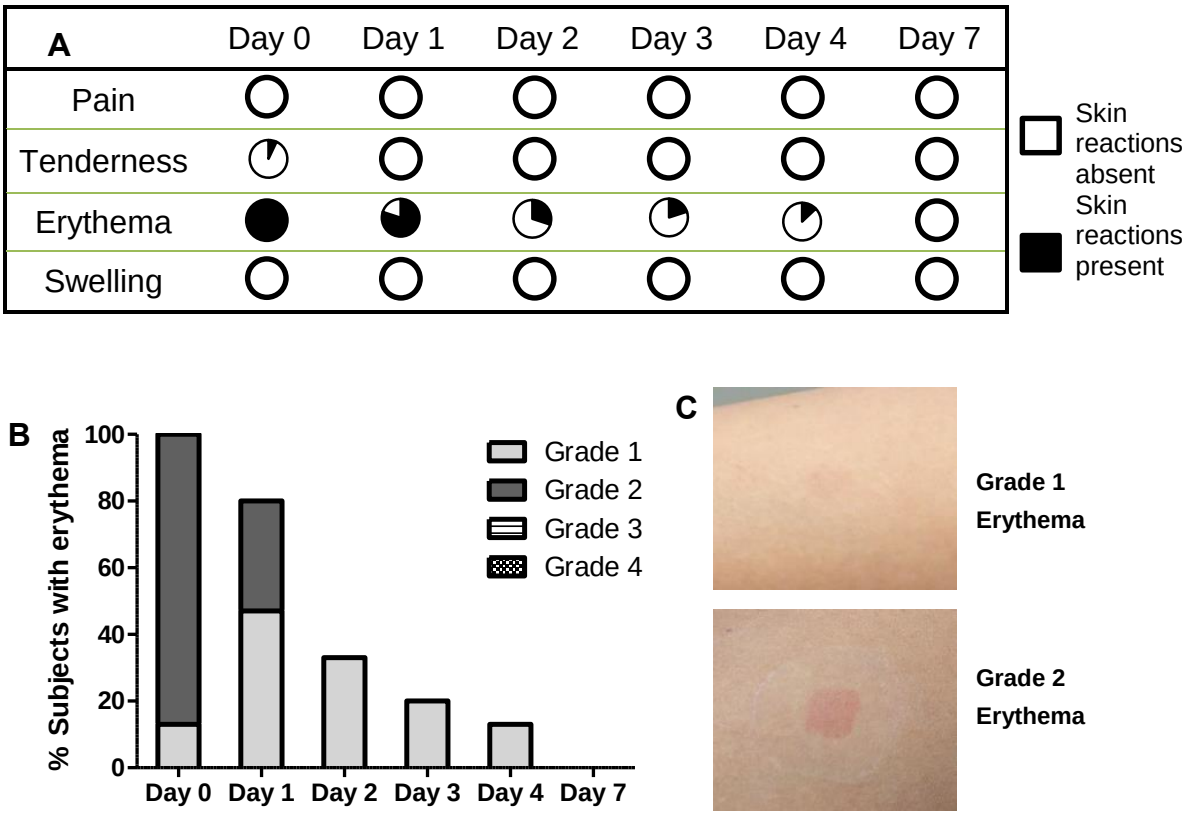


Figure 5.3 Skin tolerability after microneedle patch application. Skin sites were monitored and scored over a period of one week. **(A)** Summary of the prevalence of skin reactions at different time points. **(B)** Intensity of erythema in the skin after microneedle patch application. Column bars show the percentage of subjects with different grades of erythema. None of the subjects had Grade 3 or 4 erythema scores. **(C)** Representative images of skin showing Grade 1 and Grade 2 erythema.

Figure 5.3B charts the score of erythema intensity on a scale of 0 – 4 over time. Move to methods Representative examples of Grade 1 and 2 erythema seen in the study are shown in Figure 5.3C. On Day 0, 13% (2 out of 15) subjects had very slight erythema (Grade 1) while 87% (13 out of 15) subjects had erythema (Grade 2). On Day 1 47% (7 out of 15) subjects showed Grade 1 erythema and 33% (5 out of 15) subjects showed Grade 2 erythema. From Day 2 onwards, erythema, when present, was only Grade 1. None of the subjects showed Grade 3 or Grade 4 erythema at any point in the study. In addition, none of the subjects showed induration or swelling at the skin site at any time

during the study. Overall the patches were tolerated very well in the skin with mild transient erythema that resolved fully by Day 7, almost no tenderness and no pain or swelling.

5.4.2 Usability

We next determined if microneedle patches could be inserted and dissolved in the skin in a reliable manner. We also determined if subjects could self-administer microneedle patches after only minimal training.

Figure 5.4A charts the puncture efficiency as measured by the percentage of microneedles in a given patch that inserted into the skin after investigator-administration and self-administration. Figure 5.4B shows an example of the subject's skin stained with gentian violet after microneedle patch application. The blue dots show the number of sites that were punctured in the skin during microneedle patch application. The mean puncture efficiency by skin staining was 99% after investigator-administration and 98% after self-administration.

Figure 5.5 charts the delivery efficiency as measured by the percentage volume of microneedles in a given patch that dissolve after patch administration. The mean delivery efficiency by microscopy was 74% after investigator-administration and 67% after self-administration, and the two groups were not statistically different from each other. This suggests that overall usability was similar between investigator-administration and self-administration with minimal training and that microneedle patches can be administered reliably.

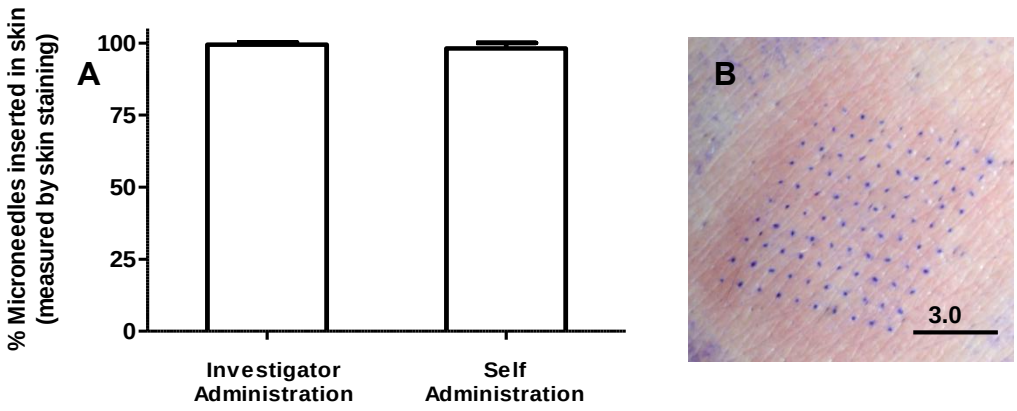


Figure 5.4 Puncture efficiency of microneedle patch application as determined by the percentage of microneedles that inserted into the skin. Skin sites were stained with gentian violet dye and the number of dots were counted to measure the number of microneedles that punctured into the skin. **(A)** Puncture efficiency of microneedle patches after investigator-administration and self-administration. Column bars represent the average percentage of microneedles that inserted into the skin with standard deviation error bars shown. **(B)** Representative magnified image of a stained skin site showing a 10 x 10 array where the microneedles punctured into the skin.

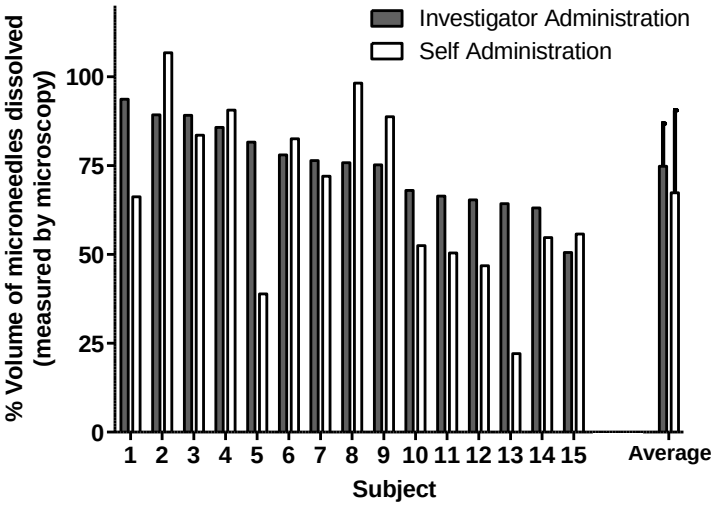


Figure 5.5 Delivery efficiency as determined by the percentage of the volume of microneedles that dissolved during microneedle patch application to the skin. Microneedle patches were imaged by brightfield microscopy before and after insertion into the skin and image analysis was used to determine the volume of microneedles dissolved. Volume of microneedles dissolved is interpreted as a measure of the dose delivered, i.e., if a drug or vaccine had been incorporated into the microneedles. Each bar represents the result from an individual subject. The 'average' bars represent the averages of the 15 individual bars, with standard deviation error bars shown.

5.4.3 Acceptability

We studied acceptability of microneedle patches by surveying the subjects about pain during microneedle patch application, confidence during self-administration and subject preferences regarding microneedle patches compared to conventional intramuscular injection and conventional oral delivery by pills.

Figure 5.6A shows the pain score reported by subjects during microneedle patch administration. Fourteen out of the 15 subjects reported a pain score of 0 or 'no pain' during microneedle patch administration. One subject reported a pain score of 1 (on a scale of 0 to 10). This indicates that microneedle patch administration was not considered painful.

Figure 5.6B shows the confidence score reported by subjects during self-administration of microneedle patch. Among the subjects, 53% (8 out of 15) reported that they were confident that they applied the microneedle patch correctly (score of 5), and 33% (5 out of 15) subjects reported that they were somewhat confident that they applied the microneedle patch correctly (score of 4). Only 14% (2 out of 15) subjects reported that they did not know if they applied the microneedle patch correctly or incorrectly (score of 3). None of the subjects reported that they thought that they applied the patch incorrectly (score of 1 or 2). Therefore, 85% of the subjects were at least somewhat confident that they applied the microneedle patch correctly (confidence score of 4 or 5). This shows that the large majority of subjects felt confident self-administering a microneedle patch.

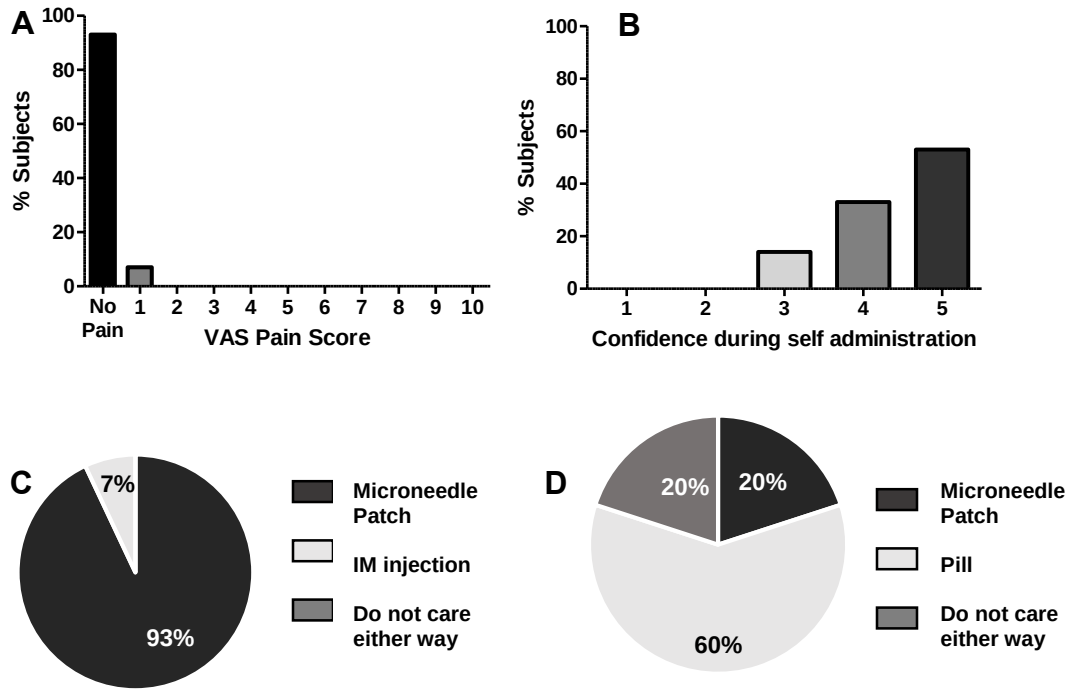


Figure 5.6 Acceptability survey results from subjects about microneedle patch administration. **(A)** Assessment of pain during microneedle patch administration. Pain was scored by subjects on a visual analog scale (VAS) of 0 (No pain) to 10 (Worst pain). **(B)** Confidence of subjects during self-administration of microneedle patches. Confidence during self-administration was scored by subjects on a scale of 1 (least confident) to 5 (most confident). **(C)** Preference of subjects for application of microneedle patch as compared to intramuscular injection for delivery of medications. **(D)** Preference of subjects for microneedle patch administration as compared to oral administration by conventional pill for delivery of medications.

Figures 5.6C and 5.6D compare the preferences reported by subjects about microneedle patches versus conventional delivery methods. Figure 5.6C shows that 93% (14 out of 15) of subjects would prefer to obtain their medication by a microneedle patch as compared to a conventional intramuscular injection. Only 7% (1 out of 15) of subjects reported a preference for intramuscular injection over microneedle patch. That subject stated that the longer wear time of the microneedle patch as compared to intramuscular injection was the primary reason for preferring intramuscular injection. This suggests that

microneedle patches are overwhelmingly preferred over intramuscular injections for administration of medications.

Figure 5.6D shows that 20% (3 out of 15) subjects would prefer a microneedle patch over conventional oral delivery by pill for obtaining medication, while another 20% reported that they do not care either way. The remaining 60% (9 out of 15) subjects reported that they would prefer obtaining their medication by a pill over a microneedle patch. This indicates that although oral delivery was preferred, a significant fraction of subjects found the microneedle patch to be similar or better than oral administration.

5.5 Discussion

The goal of the study was to evaluate skin tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects to further clinical translation of dissolving microneedle patches.

In this study, microneedle patches were very well tolerated in the skin with mild, transient erythema that resolved within 7 days. It is, however, important to note that the microneedle patches used in this study were placebos and did not contain any drug or vaccine. The presence of drug or vaccine within the microneedle patches could change the number or intensity of skin reactions. Tolerability in the skin should also be dependent upon the materials used for microneedle patch fabrication and the properties of these materials upon dissolution in the skin. Therefore, dissolving microneedle patches made of different materials may have different skin tolerability. For example, Hirobe S. *et al* reported significantly greater erythema associated with application of dissolving microneedle patches containing influenza vaccine to human subjects [16].

This study also showed that 98% – 99% of microneedles in a given patch punctured the skin's surface based on data from skin staining, which leaves little room for improvement. Based on microscopy analysis about 70% of the volume of microneedles was dissolved after microneedle patch administration. The amount of dissolution should depend on microneedle patch geometry, the materials that comprise the microneedle patch, the force of microneedle patch application and other factors. Further optimization of these factors could lead to greater delivery efficiency.

For most subjects, investigator-administration and self-administration delivery efficiencies were similar to each other with no statistically significant difference. However, there were certain subjects (e.g., subjects 5 and 13 where self-administration delivery was much lower than investigator-administration. That being said, there were also subjects for whom self-administration yielded more efficient delivery than investigator-administration. Future studies should expand upon these proof-of-concept results to assess self-administration protocols in larger populations and using microneedle designs that are further optimized for simple, reliable administration with less variability.

Microneedle patches were well accepted in this study with minimal pain of insertion and were overwhelmingly preferred over intramuscular injection. This is consistent with previous studies reporting less pain from microneedle administration compared to injection [21-23] and reporting overall preference of microneedle patches over drug or vaccine delivery by injection [22, 24].

The large majority of participants were at least somewhat confident that they self-administered the microneedle patch correctly, and none reported that they thought they had applied the patch incorrectly. Almost all participants preferred microneedle patch

administration to intramuscular injection and some preferred the patch to oral administration. This indicates that offering microneedle patch administration for medications that otherwise require injection could be a strategy to increase patient compliance with these therapies.

5.6 Conclusions

This study evaluated skin tolerability, usability and acceptability of dissolving microneedle patch administration in humans. The microneedle patches were very well tolerated in the skin with minimal erythema that resolved fully within seven days and caused no pain or swelling. Microneedle patches were administered reliably by hand without the need of an applicator and delivery efficiencies were similar between investigator-administration and self-administration. Microneedle patch administration was not painful and the large majority of subjects were at least somewhat confident that they self-administered the patch correctly. Microneedle patch administration was overwhelmingly preferred over conventional needle and syringe injection for delivery of medications.

Altogether the results of this study show the feasibility of using dissolving microneedle patches for investigator-administration as well as self-administration for future applications in drug and vaccine delivery in a well-tolerated and reliable manner that offers an attractive alternative to conventional hypodermic needles.

5.7 Acknowledgements

The authors would like to thank Donna Bondy for administrative support; Danny Pardo for help with microscopy assay development, microscopy measurements and

participant recruitment; and Matt Mistilis and other colleagues at Georgia Tech for help with preliminary clinical assessment of microneedle patches. This work was supported by a grant from National Institutes of Health. Mark Prausnitz is an inventor of patents that have been or may be licensed to companies developing microneedle-based products, is a paid advisor to companies developing microneedle-based products and is a founder/shareholder of companies developing microneedle-based products, including Micron Biomedical. This potential conflict of interest has been disclosed and is being managed by Georgia Tech and Emory University.

5.8 References

1. Edens, C., N.C. Dybdahl-Sissoko, W.C. Weldon, M.S. Oberste, and M.R. Prausnitz, *Inactivated polio vaccination using a microneedle patch is immunogenic in the rhesus macaque.* Vaccine, 2015. **33**(37): p. 4683-90.

2. Sullivan, S.P., D.G. Koutsonanos, M. Del Pilar Martin, J.W. Lee, V. Zarnitsyn, S.-O. Choi, N. Murthy, R.W. Compans, I. Skountzou, and M.R. Prausnitz, *Dissolving polymer microneedle patches for influenza vaccination.* Nat Med, 2010. **16**(8): p. 915-20.

3. Mistilis, M.J., A.S. Bommarius, and M.R. Prausnitz, *Development of a Thermostable Microneedle Patch for Influenza Vaccination.* Journal of Pharmaceutical Sciences, 2015. **104**(2): p. 740-749.

4. Carey, J.B., A. Vrdoljak, C. O'Mahony, A.V. Hill, S.J. Draper, and A.C. Moore, *Microneedle-mediated immunization of an adenovirus-based malaria vaccine enhances antigen-specific antibody immunity and reduces anti-vector responses compared to the intradermal route.* Sci Rep, 2014. **4**: p. 6154.

5. Torrisi, B.M., V. Zarnitsyn, M.R. Prausnitz, A. Anstey, C. Gateley, J.C. Birchall, and S.A. Coulman, *Pocketed microneedles for rapid delivery of a liquid-state botulinum toxin A formulation into human skin.* Journal of Controlled Release, 2013. **165**(2): p. 146-152.

6. Raphael, A.P., M.L. Crichton, R.J. Falconer, S. Meliga, X. Chen, G.J. Fernando, H. Huang, and M.A. Kendall, *Formulations for microprojection/microneedle vaccine delivery: Structure, strength and release profiles.* J Control Release, 2016.

7. Kommareddy, S., B.C. Baudner, A. Bonificio, S. Gallorini, G. Palladino, A.S. Determan, D.M. Dohmeier, K.D. Kroells, J.R. Sternjohn, M. Singh, P.R. Dormitzer, K.J. Hansen, and D.T. O'Hagan, *Influenza subunit vaccine coated microneedle patches elicit comparable immune responses to intramuscular injection in guinea pigs.* Vaccine, 2013. **31**(34): p. 3435-3441.

8. Kim, Y.C., J.H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Adv Drug Deliv Rev, 2012. **64**(14): p. 1547-68.

9. Arya, J. and M.R. Prausnitz, *Microneedle patches for vaccination in developing countries.* Journal of Controlled Release.

10. Kim, Y.-C., J.-H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Advanced Drug Delivery Reviews, 2012. **64**(14): p. 1547-1568.

11. Daddona, P.E., J.A. Matriano, J. Mandema, and Y.F. Maa, *Parathyroid hormone (1-34)-coated microneedle patch system: clinical pharmacokinetics and pharmacodynamics for treatment of osteoporosis.* Pharm Res, 2011. **28**(1): p. 159-65.

12. *Zosano Pharma Announces Positive Phase 2 Results for Its ZP-Glucagon Patch Program for Treatment of Severe Hypoglycemia*. 2015 [Feb 8, 2016].

13. *Zosano Pharma Announces Positive Phase 1 Results for Its ZP-Triptan Patch Program for Treatment of Migraine*. 2015 [Feb 8, 2016].

14. *Corium Announces Positive Topline Results From Phase 2a Study of Transdermal MicroCor(R) PTH in Post-Menopausal Women*. 2015 [Feb 8, 2015].

15. Hirobe, S., H. Azukizawa, K. Matsuo, Y. Zhai, Y.S. Quan, F. Kamiyama, H. Suzuki, I. Katayama, N. Okada, and S. Nakagawa, *Development and Clinical Study of a Self-Dissolving Microneedle Patch for Transcutaneous Immunization Device.* Pharm Res, 2013.

16. Hirobe, S., H. Azukizawa, T. Hanafusa, K. Matsuo, Y.S. Quan, F. Kamiyama, I. Katayama, N. Okada, and S. Nakagawa, *Clinical study and stability assessment of a novel transcutaneous influenza vaccination using a dissolving microneedle patch.* Biomaterials, 2015. **57**: p. 50-8.

17. *Clinical Trial: Inactivated Influenza Vaccine Delivered by Microneedle Patch or by Hypodermic Needle*, in *US Fed News Service, Including US State News*2015: Washington, D.C.

18. FDA. *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*. 2007 [Feb 7, 2016]; Available from: http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm074775.htm.

19. FDA. *Guidance for Industrv: Skin Irritation and Sensitization Testing of Generic Transdermal Drug Products* 1999 [Feb 7, 2016]; Available from: http://www.fda.gov/ohrms/dockets/98fr/990236Gd.pdf.

20. PASI. *PASI Score Training: How to Calculate the PASI.* [Feb 7, 2016]; Available from: http://www.pasitraining.com/pasi_score/.

21. Haq, M.I., E. Smith, D.N. John, M. Kalavala, C. Edwards, A. Anstey, A. Morrissey, and J.C. Birchall, *Clinical administration of microneedles: skin puncture, pain and sensation.* Biomed Microdevices, 2009. **11**(1): p. 35-47.

22. Norman, J.J., J.M. Arya, M.A. McClain, P.M. Frew, M.I. Meltzer, and M.R. Prausnitz, *Microneedle patches: usability and acceptability for self-vaccination against influenza.* Vaccine, 2014. **32**(16): p. 1856-62.

23. Gill, H.S., D.D. Denson, B.A. Burris, and M.R. Prausnitz, *Effect of microneedle design on pain in human volunteers.* Clin J Pain, 2008. **24**(7): p. 585-594.

24. Birchall, J.C., R. Clemo, A. Anstey, and D.N. John, *Microneedles in clinical practice--an exploratory study into the opinions of healthcare professionals and the public.* Pharm Res, 2011. **28**(1): p. 95-106.

CHAPTER 6

CONCLUSIONS

The goal of this study was to develop dissolving microneedle patches to further their clinical translation in the context of vaccination in developing countries. This thesis evaluated rabies vaccination in dogs with a microneedle patch and the tolerability, usability and acceptability of placebo dissolving microneedle patches in human subjects. The key conclusions from these studies are listed below:

6.1 Rabies vaccination in dogs using a microneedle patch

We have shown for the first time that dissolving microneedle patches can safely and effectively administer rabies DNA vaccine to dogs using a delivery technology that is easy to administer and may enable cost savings.

- The vaccine was stable upon formulation and storage for at least 3 weeks at 4 °C in a microneedle patch.
- The patches were administered manually to dog ears by pressing with the thumb, without the need of an applicator, and the microneedles dissolved in the skin within 15 min, thereby leaving no sharps waste.
- Microneedle patches were well tolerated in the skin with mild erythema, minimal wheal formation and complete resolution of skin reactions within 7 days, and generated no systemic adverse events.

- Microneedle patches were at least as immunogenic as intramuscular injection at the same dose, as demonstrated by similar serum neutralizing antibody titers.
- A ten-fold lower vaccine dose administered by microneedle patch generated a weaker immune response compared to full-dose intramuscular vaccination.

In contrast to traditional needle-and-syringe vaccination, these microneedle patches were designed to enable administration by minimally trained personnel, increase safety by generating no sharps waste and utilize a DNA vaccine that is relatively inexpensive to manufacture compared to traditional human vaccines, all of which are expected to enable increased vaccination coverage at reduced cost. For these reasons, dissolving microneedle patches may serve as an innovative and effective approach to mass vaccinate dogs and humans. This first study on veterinary applications for microneedle patches should motivate further research in this field.

6.2 Tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects

6.2.1 Tolerability

- Dissolving microneedle patches were very well tolerated in the skin with minimal erythema that resolved fully within seven days.
- Based on size, erythema when present was always of Grade 0.5, i.e., size equal to or less than the microneedle patch.
- Based on intensity, on Day 0, 13% (2 out of 15) subjects had Grade 1 erythema while 87% (13 out of 15) subjects had Grade 2 erythema. On Day 1, 47% (7 out of 15)

subjects showed Grade 1 erythema and 33% (5 out of 15) subjects showed Grade 2 erythema. From Day 2 onwards, erythema, when present in subjects, was only Grade 1 erythema. None of the subjects showed Grade 3 or Grade 4 erythema at any point in the study.

- There was no induration or swelling noted at the skin site on any of the days through Day 7.
- Only 6.7% (1 out of 15) of subjects reported tenderness at the skin site on Day 0 after microneedle patch administration that resolved by Day 1. The tenderness was reported of Grade 1 or mild discomfort to touch on a scale of 0 to 4. None of the other subjects reported any tenderness at the skin site through Day 7.
- There was no pain reported at the skin site on any of the days through Day 7.

6.2.2 Usability

- Microneedle patches were mechanically strong enough to insert into skin as assessed by gentian violet staining. Mean puncture efficiencies were 99% for investigator-administration and 98% for self-administration.
- The mean delivery efficiency assessed by microscopic examination of microneedle dissolution in the skin was 74% for investigator-administration and 67% for self-administration, and the two groups were not statistically different from each other. Thus overall usability was similar between investigator-administration and self-administration.

6.2.3 Acceptability

- Microneedle patch administration was not considered painful. Fourteen out of the 15 subjects reported a pain score of 0 or ‘no pain’ during microneedle patch administration. 1 subject reported a pain score of 1 (on a scale of 0 to 10).
- 85% of the subjects were at least somewhat confident that they applied the microneedle patch correctly (confidence score of 4 or 5).
- 93% (14 out of 15) subjects would prefer to obtain their medication by a microneedle patch as compared to a conventional intramuscular injection.
- 20% (3 out of 15) subjects would prefer a microneedle patch over conventional oral delivery by pill for obtaining medication, while another 20% reported that they do not care either way. 60% (9 out of 15) subjects reported that they would prefer obtaining their medication by a pill over a microneedle patch.

We have shown that dissolving microneedle patches can be administered reliably by a simple administration process without the need of an applicator, the microneedles are safe after insertion and dissolution in the skin and overwhelmingly preferred over intramuscular injection. The results of this study show the feasibility of using dissolving microneedle patches for investigator-administration as well as self-administration of drug or vaccines in a safe and reliable manner and offer an attractive alternative to conventional hypodermic needles. This study should motivate further studies on clinical translation of microneedles.

The results of this thesis have shown positive data for rabies vaccination of dogs as well as positive data for clinical use of microneedle patches. Altogether, these results

should further clinical translation of dissolving microneedle patches for vaccination in developing countries.

CHAPTER 7

FUTURE DIRECTIONS

7.1 Improving rabies vaccination in dogs using a microneedle patch

- Stability: The study showed that the DNA vaccine was stable upon formulation and storage for at least 3 weeks at 4 °C in a microneedle patch. However, we did not test long term stability or stability at elevated temperatures. Future work can address stability of DNA vaccine within the microneedle patch at 40°C over a period of months. Eliminating the cold chain during storage will be a big step towards vaccination in developing countries. It will also be important to characterize mechanical strength and insertion ability of microneedle patches after long term storage at elevated temperatures. Microneedle patches were stored in a foil pouch with desiccant until their time of use and this study did not evaluate the effect of humidity on the mechanical strength of microneedles. Since microneedles were made of highly water soluble excipients, it is possible that they may absorb moisture from the air and become soft before insertion into skin. Future work can address the rate at which this occurs and how soon after opening the foil pouch will the microneedle patches be suitable for use.
- Administration of microneedle patches: The patches were administered manually to dog ears by pressing with the thumb without an applicator and the microneedles dissolved in the skin within 15 minutes. While the 15 minutes wear time was used for this first proof of concept study, it would not be viable during vaccination of dogs in

developing countries. Future work can address development of fast dissolving microneedle patches that can dissolve fully within 1 to 2 minutes.

- Immunogenicity: Microneedle patches were as immunogenic as intramuscular injection at the same dose, but a 10-fold lower microneedle patch dose resulted in a lower immune response than full dose intramuscular injection. Future work can address if microneedle patches can enable dose sparing at doses lower than 10-fold. Future work should also address longevity of immune response and a challenge in dogs with rabies virus to test survival after vaccination.

`

7.2 Tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects

- Tolerability: Dissolving microneedle patches were well tolerated in the skin with minimal erythema that resolved within 7 days. However, no drug or vaccine was used in this study. Future studies should address skin tolerability with the presence of an active, as this may be different from placebo microneedle patches.
- Usability: Puncture efficiencies were over 95% for investigator-administration and self-administration and delivery efficiencies were around 70%. Future work can address improving the delivery efficiencies further by optimizing the microneedle geometry, excipients used in microneedle fabrication as well as optimizing the force feedback indicator and developing a more robust administration protocol.
- Acceptability: Dissolving microneedle patch administration was not painful and was overwhelmingly preferred over intramuscular injection. Future work can address studying acceptability in a larger diverse group to get further insights into people's

preferences for microneedle patches and self-administration. Future work should also address quick dissolving microneedles to further improve acceptability.

7.3 **Recommendations for microneedle patches**

- Quick dissolving: Future work should address development of dissolving microneedle patches that can dissolve and deliver drugs or vaccines within 1 minute. It should also be noted to test the mechanical strength of these microneedles when exposed to short periods of humidity outside of storage packaging.
- Limiting vaccine wastage: Future manufacturing processes for microneedle patches should focus on minimizing the wastage of vaccine used for production of microneedle patches.
- Identification of terminal sterilization methods: For clinical translation and large scale manufacturing, identification of terminal sterilization methods for microneedle patches could greatly reduce the costs associated with low-bioburden or aseptic manufacturing.
- Evaluating self-administration in larger groups: Future work should evaluate self-administration in larger groups to get a better sense for people's preferences towards self-administration. Policy implications of self-administration also need to be studied.

Overall, dissolving microneedle patches have a great potential to improve vaccination in developing countries and continued efforts in these areas can help realize their potential.

APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTER 5

Table A.1 Skin tolerability scoring scale to evaluate local reactions in the skin.

Local reaction to patch	Grade 0	Grade 0.5*	Grade 1	Grade 2	Grade 3	Grade 4	Score
Pain**	Pain is absent	N/A	Mild pain that does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization	
Tenderness**	No discomfort to touch	N/A	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization	
Erythema (Size)	0 cm	0.1 – 1 cm [erythema less than or equal to patch size ≤ 1 cm]	1.1 – 5 cm [erythema spreading beyond patch site > 1cm]	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis	
Erythema (Intensity)	No erythema	N/A	Very slight erythema (barely perceptible)	Well-defined erythema	Moderate to severe erythema	Severe erythema (beet redness)	
Induration/ Swelling	0 cm	0.1 – 1 cm [swelling less than or equal to patch size ≤ 1 cm]	1.1 – 5 cm and does not interfere with activity [swelling spreading beyond patch size > 1cm]	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis	

* Used only with erythema (size) and induration/swelling.

** Pain and tenderness scored based on the response from the subject.

APPENDIX B

INACTIVATED POLIO VACCINE FORMULATION IN MICRONEEDLE PATCHES

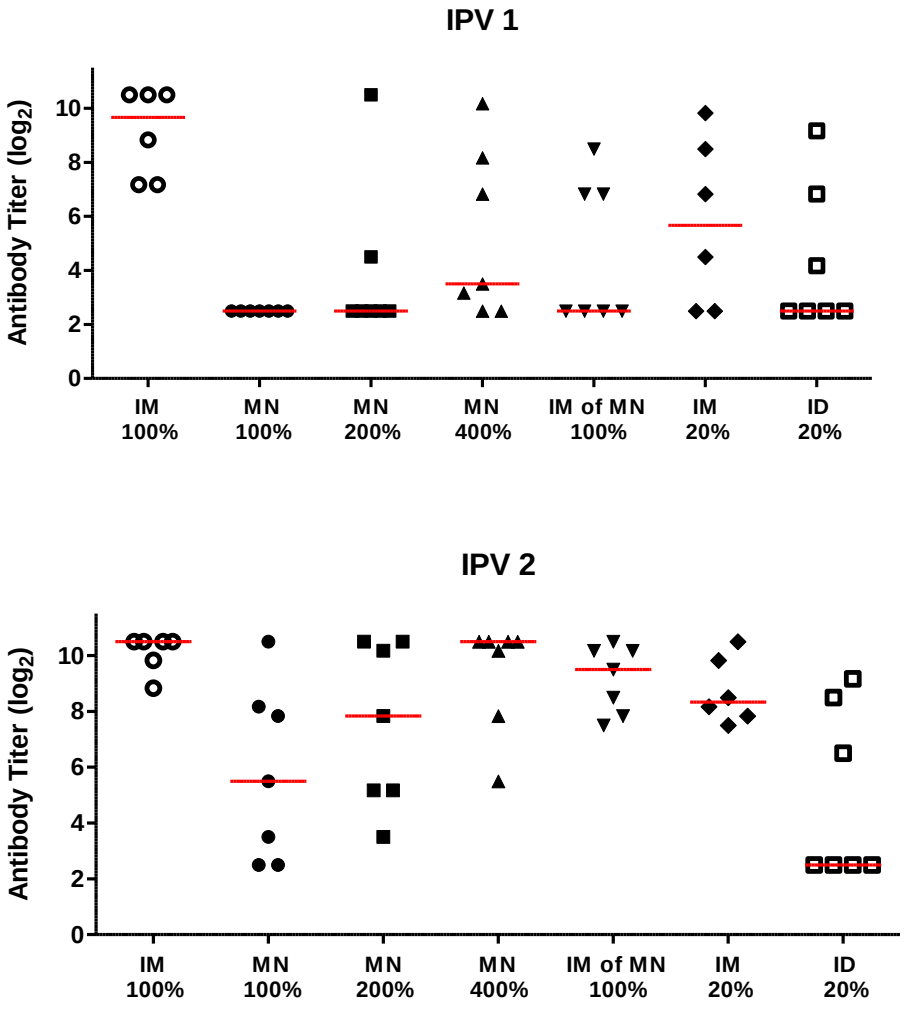
The overall goal was to study formulations to stabilize Inactivated Polio Vaccine (IPV) in a dry state in a microneedle patch and test immunogenicity in rats. We have presented some preliminary data that can guide further development of an inactivated polio vaccine microneedle patch. These studies were performed as a component of a much larger project involving multiple researchers. These studies provide a look into some of the early work done on this project that helped shape it subsequent direction and successes.

B.1 IPV stabilization in microneedle patches with Human Serum Albumin

Based on an excipient screen by drying vaccine on PDMS surface, maltodextrin 13 and Human Serum Albumin (HSA) were identified as suitable excipients for IPV stabilization upon drying (data not shown). Dissolving microneedle patches were fabricated using these excipients and tested in Wistar rats for immunogenicity. Neutralizing antibody titers specific to each serotype were measured in the serum to determine immunogenicity.

Figure B.1 shows the antibody titers at week 4 after vaccination. For IPV type 1, dissolving microneedle patches at 100%, 200% and 400% of the human dose were not immunogenic as compared to the intramuscular injection at 100%. Microneedle patches were reconstituted in phosphate-buffered saline and injected intramuscularly at 100%

human dose (labeled as "IM of MN 100%" in Figure B.1), but were not immunogenic. This suggests that the vaccine activity as measured by ELISA in the reconstituted microneedle patch solution was not correlative with immunogenicity in the rat model. Intradermal injection at 20% of the human dose was inferior in immune response to intramuscular injection at 20%. Similar trends were noted for IPV types 2 and 3. In a follow-up study, microneedle patches were fabricated without HSA in the formulation and the reconstitutions of microneedle patches were found to be immunogenic (data not shown). Our hypothesis was that HSA interferes with immune response in the rats after drying (and reconstitution) with IPV.



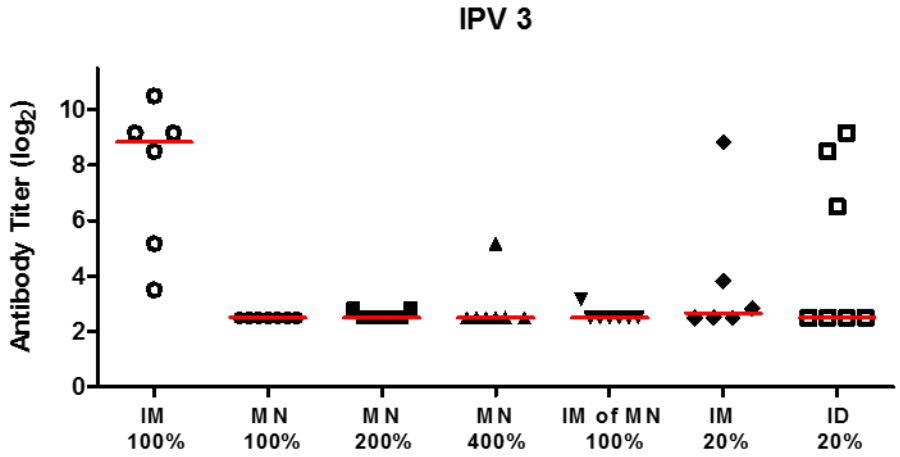


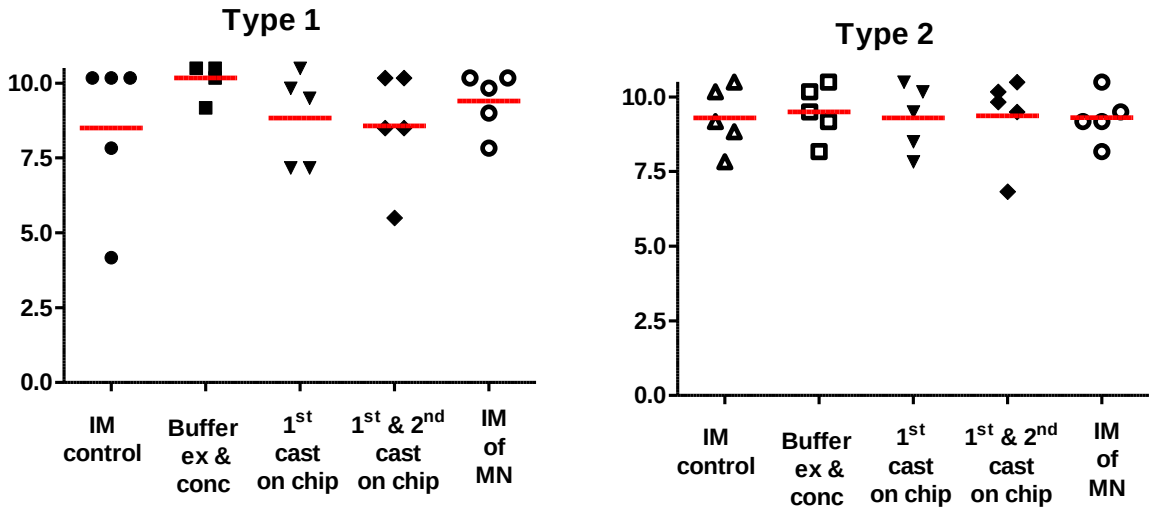
Figure B.1 Antibody titers after vaccination of rats using IPV microneedle patch containing HSA. Individual data point represent data from an individual rat. The red lines represent the median values.

B.2 IPV stabilization in microneedle patches using sorbitol, magnesium chloride and monosodium glutamate

Based on a previously published study on stabilization of IPV after lyophilization [1], we wanted to evaluate the suitability of sorbitol, magnesium chloride and monosodium glutamate for IPV stabilization in microneedle patches. Microneedle patch fabrication differs from the lyophilization drying process used to originally identify this formulation. In our study, the vaccine was concentrated and buffer-exchanged into McIlvaine buffer (phosphate-citrate buffer) using a spin-filter before microneedle fabrication. Microneedle fabrication was modeled using a first cast – casting onto a PDMS chip (to simulate a PDMS microneedle mold) with vaccine and stabilizing excipients (i.e., sorbitol, magnesium chloride and monosodium glutamate) followed by air drying. A second cast was applied using a polymer solution (20% PVA, 20% sucrose) followed by air drying. Finally, we made actual microneedle patches using these two

casting steps but with microneedle molds rather than chips. Our goal was to evaluate the stability of the vaccine after each of these steps by measuring activity by ELISA and injecting the solutions into rats for testing immunogenicity. No microneedle patches were applied to the rats

Figure B.2 shows the antibody titers after vaccination in rats at 4 weeks. For all three serotypes, similar titers are noted across all the groups, suggesting that no vaccine activity was lost after each additional fabrication step.



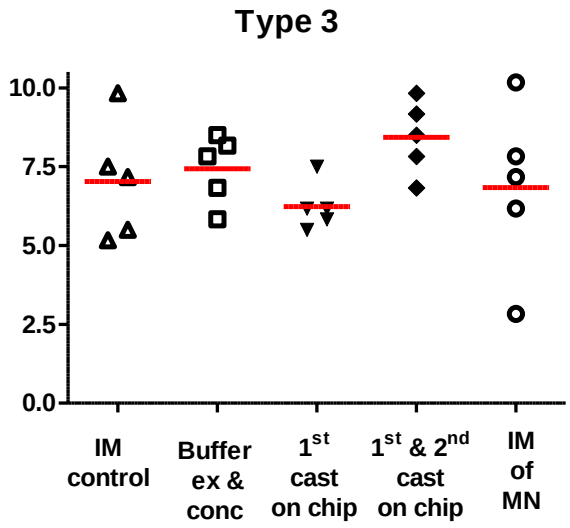


Figure B.2 Antibody titers after vaccination of rats with intramuscular injections of formulations at different stages of microneedle patch fabrication. Individual data point represent data from an individual rat. The red lines represent the median values.

As a next step, we fabricated microneedle patches using this formulation and administered them to Wistar rats to test immunogenicity of microneedle patches containing IPV. Figure B.3 shows the antibody titers for IPV Type 1 and Type 2 were equivalent between the positive control of intramuscular injection of liquid vaccine and intramuscular injection of a reconstituted microneedle patch, which was consistent with the previous study. For Type 3, antibody titers for the reconstituted microneedle patch were slightly lower than the positive control, suggesting that some loss may have occurred during microneedle patch fabrication. For all the serotypes, the microneedle patch administered to the rat generated lower antibodies than the intramuscular injection control. We hypothesized this was due to inefficient delivery from microneedle patches.

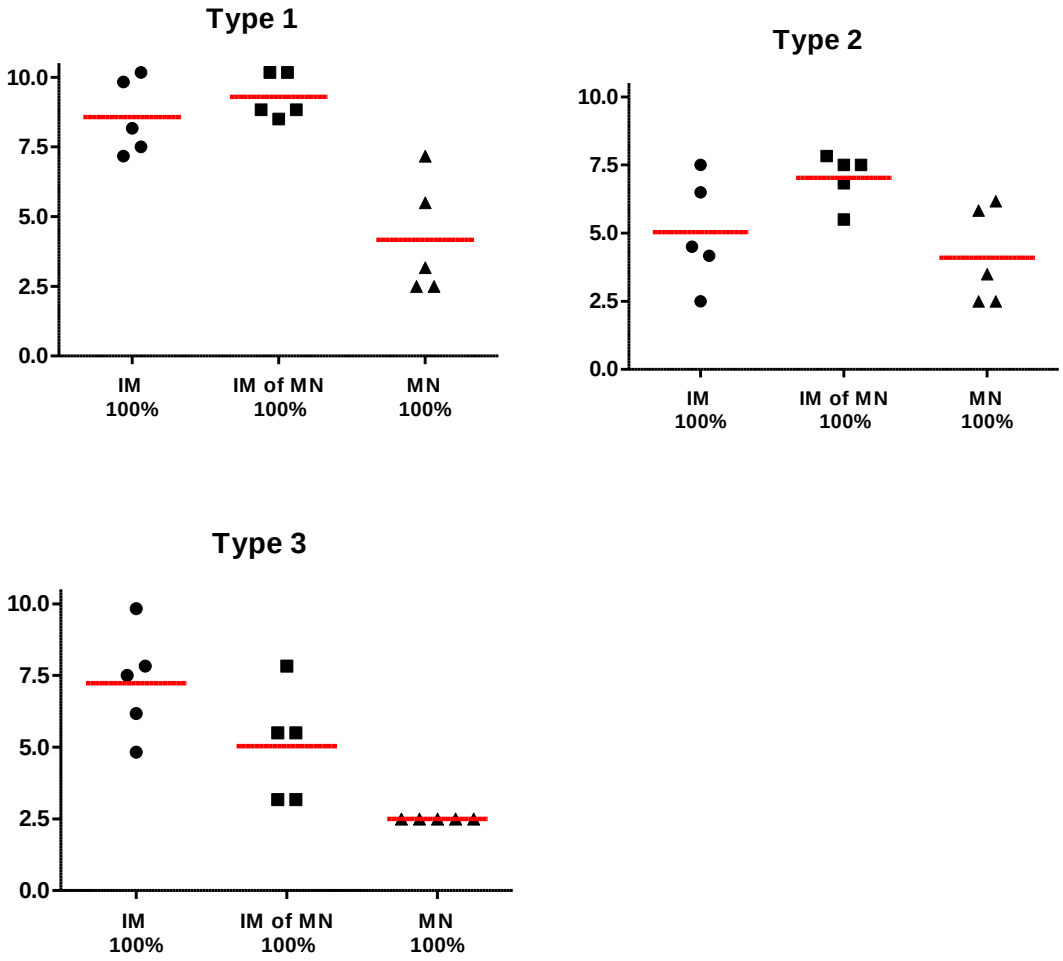


Figure B.3 Antibody titers after vaccination of rats using IPV microneedle patch. Individual data point represent data from an individual rat. The red lines represent the median values.

Upon further inspection of microneedle patches fabricated for the study, we noted that the sharp tips of the microneedles were broken during fabrication, which would make penetrating the skin difficult and greatly reduce vaccine delivery to the skin (Figure B.4). We believe this is the reason why the microneedle patches provided poor immunogenicity in this study. Additional studies are needed to further assess the stability of this formulation.

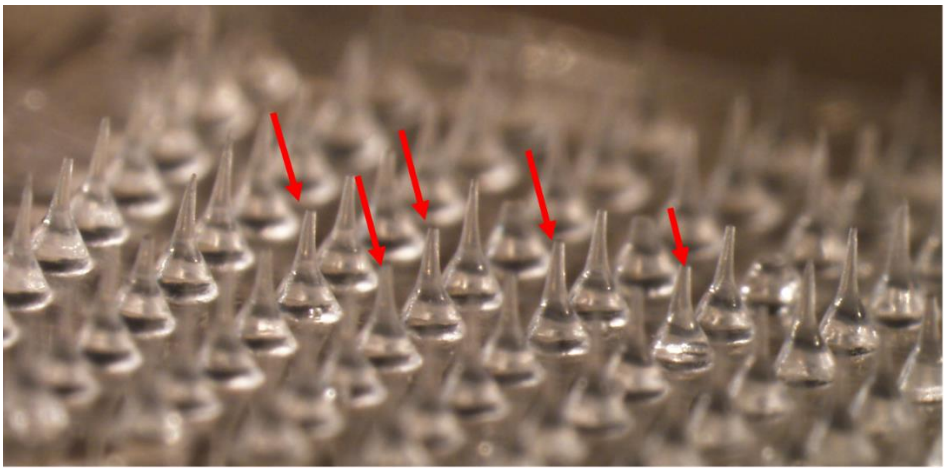


Figure B.4 IPV microneedle patch showing tips that are not sharp due breakage during microneedle patch fabrication.

1. Kraan, H., P. van Herpen, G. Kersten, and J.P. Amorij, *Development of Thermostable Lyophilized Inactivated Polio Vaccine.* Pharm Res, 2014.

APPENDIX C

DESIGN, DEVELOPMENT AND FABRICATION OF MICRONEEDLE PATCHES

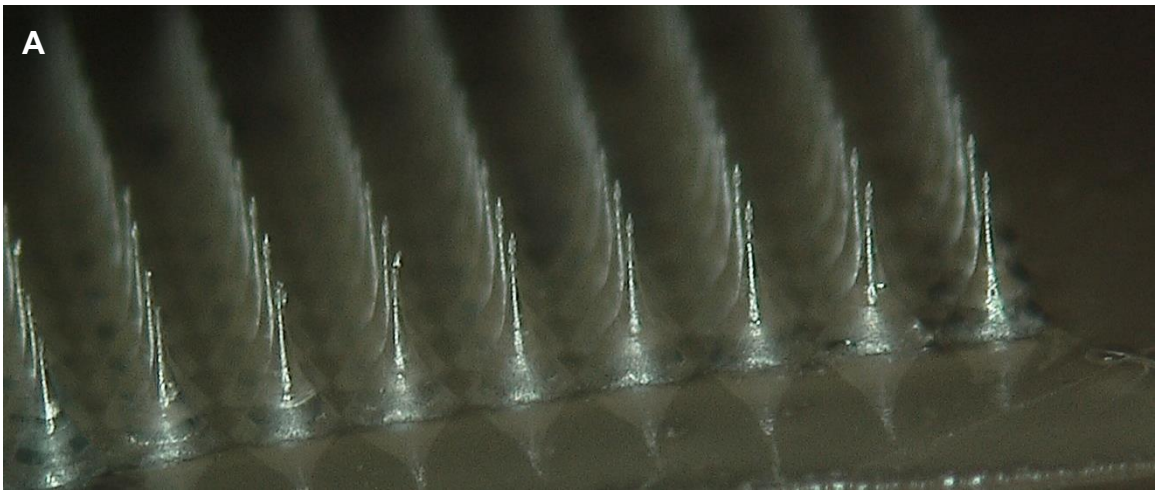
To support translation of microneedle patches from pre-clinical development into clinical trials, this thesis has examined the effect of microneedle patch application to local skin reactions, reliability of use and acceptability to patients. This appendix outlines the studies that helped guide the development of the placebo dissolving microneedle patch used in the study. Microneedle patches contain a force feedback indicator (FFI) device incorporated within them, such that when pressing down on the microneedles with the thumb, the FFI produces a click sound to indicate that enough force has been applied. The patch is then left on the skin for 20 minutes for dissolution of microneedles and then peeled away. The following studies provide background information and preliminary results on how the FFI and the microneedle patch insertion protocol were developed.

C.1 Optimization of insertion force using the FFI

Previous results with insertion of stainless steel metal microneedle patches in human subjects reported that an insertion force of 37 N was sufficient for microneedles to puncture the skin, as noted by gentian violet skin staining [1]. We used this force as a starting range for insertion of dissolving microneedle patches. Dissolving microneedles have less mechanical strength than metal microneedles and the tips of dissolving microneedles are less sharp than metal microneedles. For these reasons, it was

hypothesized that the force of insertion of dissolving microneedles could be higher than the reported force for metal microneedles.

We tested FFIs with two forces 8 lbf (35 N) and 13 lbf (57 N). The investigator applied the microneedle patch with these two FFIs to the same subject, while keeping all other aspects of the insertion protocol similar. The microneedles were imaged after insertion into the skin. Figure C.1 shows representative images of the microneedle patch from this study. Based on these studies it was noted that more uniform and complete dissolution was observed in the higher force 13 lbf FFI. It was not significantly more difficult to apply this force with the thumb as compared to the lower force FFI and it was determined that the 13 lbf FFI would be an appropriate choice for the insertion of microneedle patch.



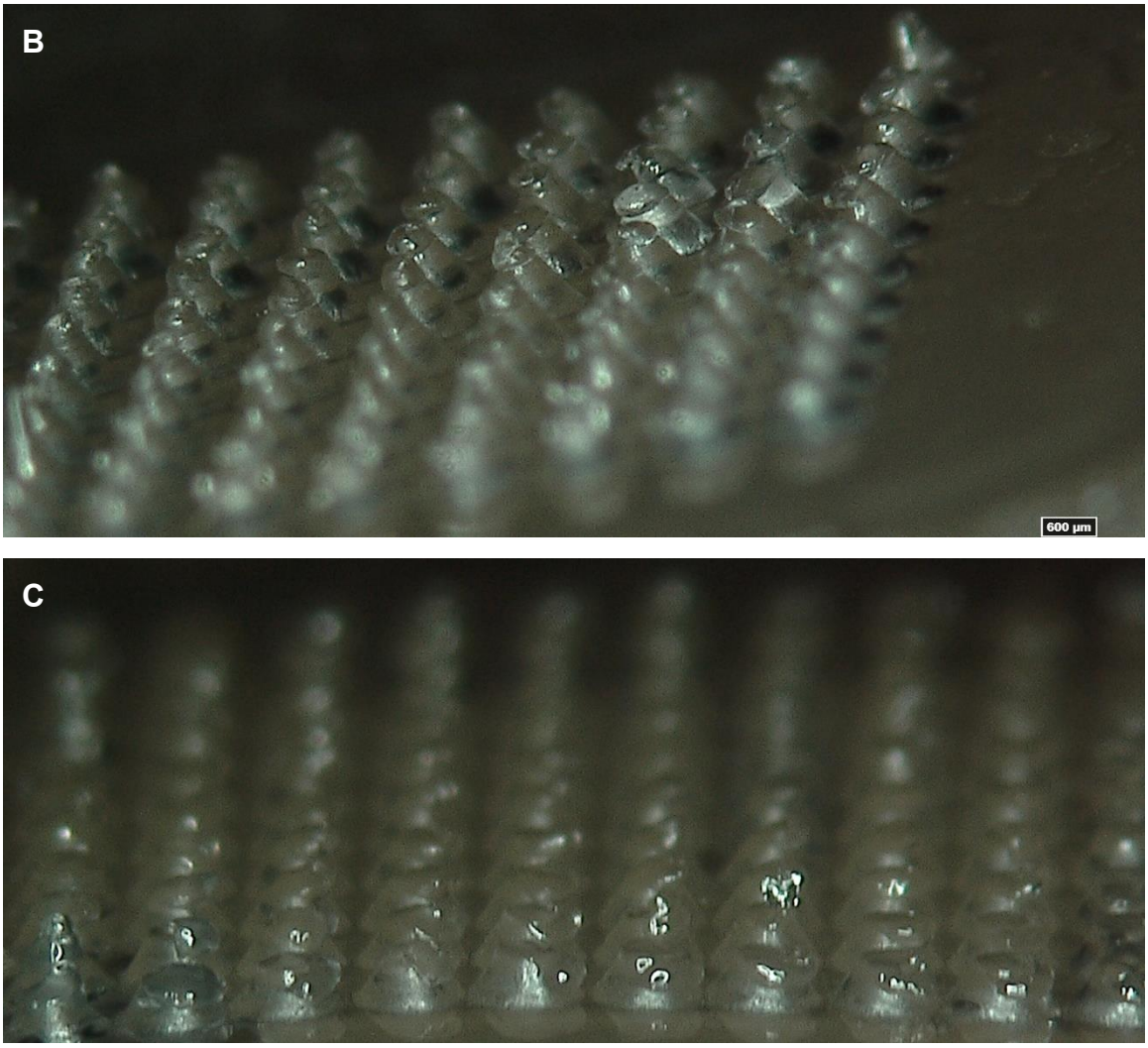
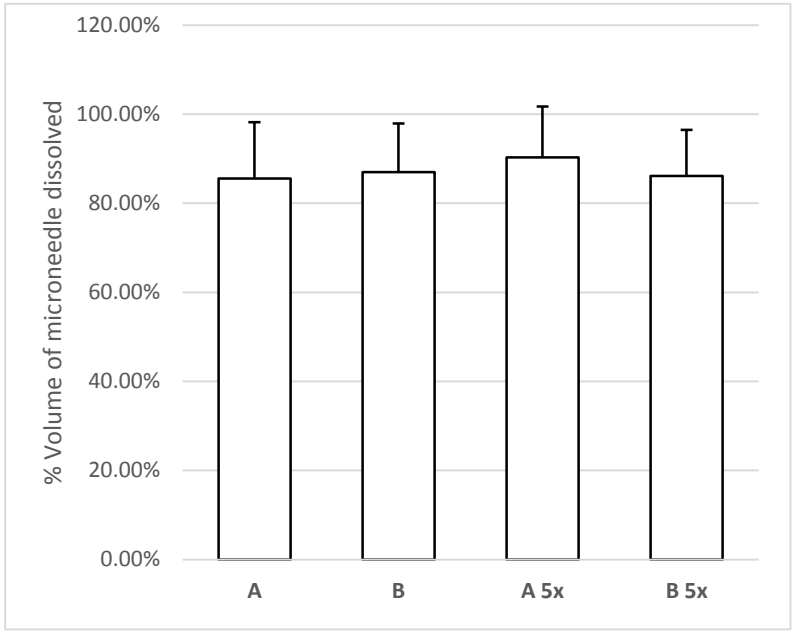


Figure C.1 Representative images of microneedles (A) Before insertion (B) After insertion with 8 lbf FFI and (C) After insertion with 13 lbf FFI.

C.2 Optimization of number of clicks using the FFI

We wanted to study the effect of number of clicks using the FFI and the delivery efficiency of microneedles. The FFI produces a click sound when sufficient force has been applied. We studied the effect of applying the force once (one click sound) or applying the force five consecutive times (five click sounds) on microneedle delivery efficiency in pig skin *in-vitro*.

Figure C.2 shows the volume of microneedle dissolved between one click and five clicks. Two investigators, A and B, applied three microneedle patches each by one click and five clicks (A 5x and B 5x) to pig skin *in-vitro.* As seen in the figure, no significant difference is seen between groups A and A 5x, B and B 5x, and there is minimal variation between the two investigators. Based on these results, only a one click insertion was determined to be necessary for the microneedle patch insertion process.



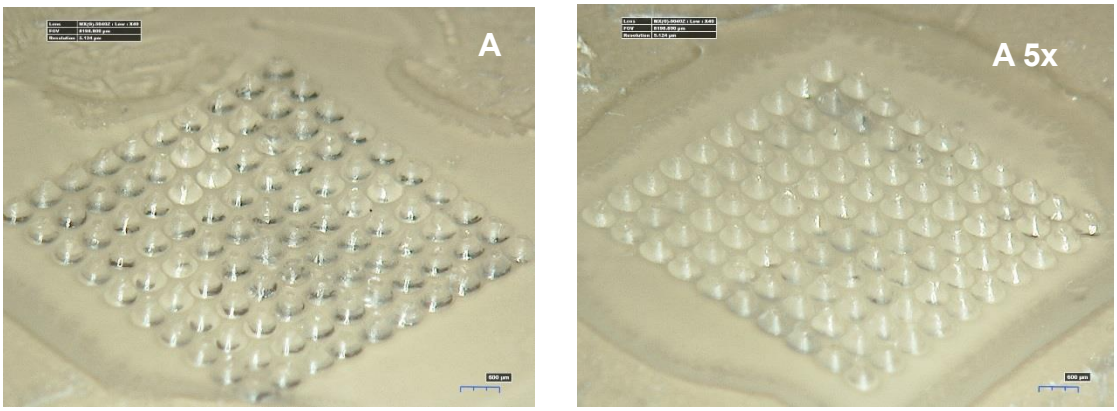


Figure C.2 Volume of microneedle dissolved and representative images of microneedles between one click and five clicks.

C.3 Optimization of microneedle patch wear time

While preliminary information on microneedle patch dissolution pig skin *in-vitro*, limited information was available on microneedle patch dissolution time in human subjects. We wanted to test the effect of patch wear time on microneedle dissolution in human subjects. An earlier formulation of dissolving microneedle patches consisted of gelatin and sucrose and was tested in human subjects with a patch wear time ranging from 30 seconds to 15 minutes.

Figure C.3 shows the representative images of microneedle patches from the time course study in human subjects. The pink border marks the size of the microneedle at different time points. It can be seen that at 2 minutes more than half of the microneedle length is dissolved in the skin, at 5 to 10 minutes most of the microneedle length is dissolved in the skin. It is important to note that the microneedles sit on top a pedestal base that does not contain any vaccine and only provides a mechanical function. Therefore, a patch wear time of 10 minutes was considered suitable for microneedles with the gelatin and sucrose formulation.

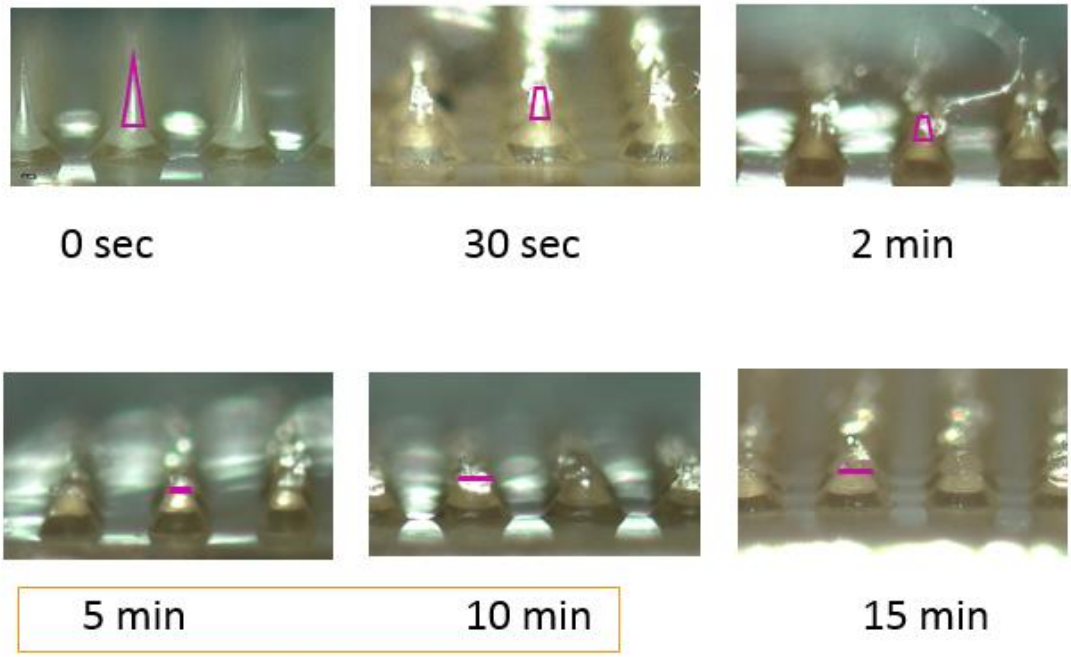


Figure C.3. Representative images of microneedles before insertion into human subjects and at different time points after insertion and dissolution into the skin.

As a next step, a similar study was carried out with the polyvinyl alcohol and sucrose formulation studied in Chapter 5 and a patch wear time of 20 minutes was found to be optimal (data not shown) and chosen as the patch wear time for the human study.

1. Norman, J.J., J.M. Arya, M.A. McClain, P.M. Frew, M.I. Meltzer, and M.R. Prausnitz, *Microneedle patches: usability and acceptability for self-vaccination against influenza.* Vaccine, 2014. **32**(16): p. 1856-62.