Evaluation of Polyelectrolyte Multilayer Thin-Film Coated Microneedle Arrays for Transcutaneous Vaccine Delivery

by

Peter W. Fung

S.B. Chemical-Biological Engineering
S.B. Biology
Massachusetts Institute of Technology, 2009

Submitted to the Department of Materials Science and Engineering
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF ENGINEERING IN MATERIALS SCIENCE AND ENGINEERING

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2011

Signature of Author:_____
Department of Materials Science and Engineering
February 9, 2011

Certified by:_____
Darrell J. Irvine
Associate Professor of Materials Science and Engineering and Biological Engineering
Thesis Supervisor

Accepted by:_____
Christopher Schuh
Chair, Departmental Committee on Graduate Students

(Page Intentionally Left Blank)

Evaluation of Polyelectrolyte Multilayer Thin-Film Coated Microneedle Arrays for Transcutaneous Vaccine Delivery

by

Peter W. Fung

Submitted to the Department of Materials Science and Engineering
on February 9, 2011 in partial fulfillment of the requirements for the degree of
Master of Engineering in Materials Science and Engineering

Abstract

The skin is an ideal organ for the safe and convenient delivery of vaccines, small molecules, and other biologics. Members of the Irvine and Hammond groups have developed a polyelectrolyte multilayer thin film-coated microneedle platform that can achieve simultaneous DNA and nanoparticle delivery. This delivery platform has the advantage of direct delivery of DNA or polymer nanoparticles to immune-active cells at the interface between the dermis and epidermis, enhancing uptake of the delivered cargo by resident immune cells. Ideal for the delivery of DNA vaccines, this platform aims to bridge the gap in the lack of efficient delivery platforms hampering the effectiveness of DNA vaccines. The ability to co-deliver polymer nanoparticles can serve as a conduit for delivering immune stimulating adjuvants or other drugs for therapeutic applications.

An overview of current vaccine and delivery system research is presented. Market factors for the commercialization of the polyelectrolyte multilayer thin film-coated microneedle delivery platform are considered along with the risk factors in bringing this invention to market. An assessment of the intellectual property surrounding the platform is performed and a preliminary market entry strategy is developed for minimizing the risks commercialization.

Thesis Supervisor: Darrell J. Irvine
Title: Associate Professor of Materials Science and Engineering and Biological Engineering

(Page Intentionally Left Blank)

Acknowledgements

I would like to thank Professor Darrell Irvine and Peter Demuth for the opportunity to work with the project that made this thesis possible. I would also like to thank Professor Eugene Fitzgerald for the advice that helped me finish this thesis. I am also very grateful to the faculty and the staff that have made this program rewarding and enjoyable this past year.

This thesis is dedicated to my family, whose support and encouragement has allowed me to succeed thus far.

(Page Intentionally Left Blank)

Table of Contents

Table of Figures

Table of Tables

1 Introduction

Vaccination is undoubtedly one of the most significant advances in medical science to prevent infection-related morbidity and mortality. First documented in China as early as the 10th century and implemented on a wide scale in 1796 by Edward Jenner for smallpox, vaccination is currently credited for preventing more than three million deaths per year worldwide [1] and attributed to a significant portion of the thirty year increase in human lifespan during the 20th century [2]. Major triumphs such as the eradication of smallpox more than three decades ago and the inevitable eradication of polio [3-4] underscore the current efforts to replicate the experience from these successful campaigns to other diseases and infectious agents.

While the danger of a polio epidemic is waning, the threat of a human influenza pandemic is on the rise, with many experts predicting the imminent and inevitable spread of pandemic influenza [5]. Major governments around the world have sought to prepare for an influenza pandemic by ordering millions of doses of vaccine to stockpile. However, with a worldwide production capacity of approximately 300 million doses per year, [6] the demand for vaccine would easily outstrip available and anticipated supplies for a rapidly mutating and contagious strain [7]. In addition, current influenza vaccine production is haphazard, relying on a hit or miss approach of screening inoculated eggs for recombinant strains that can provide protection against circulating strains and can grow well *in ova*, lengthening the already long egg-based production process. A logical course of action to minimize this lag time is to stockpile vaccine; however, pandemic strains cannot be predicted with certainty and any advantage from a stockpile would be negated if forecasts were inaccurate.

Varying strategies have been put forth by the World Health Organization (WHO) in an attempt to alleviate vaccine supply problems including exchange of clinical trial data from different strains of influenza, development of antigen sparing strategies, increased funding and tax incentives for industry, and lowering regulatory licensing fees [8]. However, none of these suggestions addresses the core problem: adequate supply of vaccine in times of high demand (**Figure 1)**. While commercial processes of existing vaccine strategies such as attenuated, whole killed and subunit vaccines are well characterized, major issues such as the suitability of an unknown or poorly characterized pathogen for attenuation in the short term after an outbreak and the need for relatively higher dosing for clinical effect in whole

killed and subunit vaccines put considerable strain on the vaccine supply chain in the event of a pandemic outbreak. DNA vaccines have been proposed as an alternative to alleviate this production bottleneck. Mechanistically able to induce robust humoral and cellular responses in small animals, DNA vaccines also promise to alleviate issues related to mass production given existing robust cell-based and cell-free manufacturing capabilities. The success of the DNA vaccine platform has been demonstrated with products on the market approved for veterinary use in horses, dogs, pigs, and fish for varying indications such as West Nile virus and melanoma (**Table 1)**.

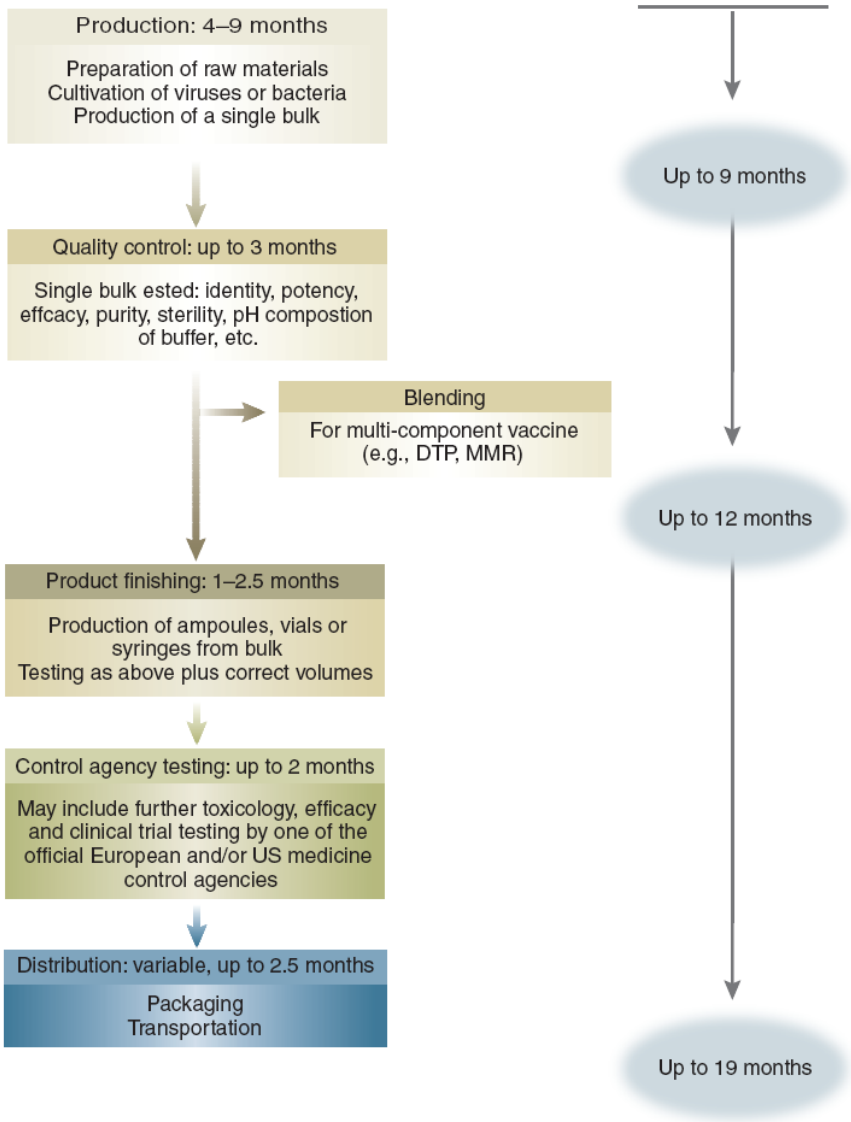

Figure 1: Manufacturing and production timeline for producing influenza vaccines in eggs [1]

Vaccine target	Product name	Company involved	Date licensed and country	Target organisms	Benefits
West Nile virus	West Nile Innovator	Centers for Disease Control and Prevention and Fort Dodge Laboratories	2005 USA	Horses	Protects against West Nile virus infection
Infectious haematopoietic necrosis virus	Apex-IHN	Novartis	2005 Canada	Salmon	Improves animal welfare, increase food quality and quantity
Growth hormone releasing hormone	LifeTide-SW5	VGX Animal Health	2007 Australia	Swine and food animals*	Increases the number of piglets weaned in breeding sows; significantly decreases perinatal mortality and morbidity
Melanoma	Canine Melanoma Vaccine	Merial, Memorial Sloan–Kettering Cancer Center and The Animal Medical Center of New York	2007 USA, conditional license	Dogs	Treats aggressive forms of cancer of the mouth, nail bed, foot pad or other areas as an alternative to radiation and surgery

*Refers to agricultural animals as opposed to pet animals — a higher level of stringency is required for approval for animals that are part of the food chain.

Table 1: DNA vaccines approved for use [9]

However, there have been difficulties during stage II and stage III clinical trials in replicating the robust responses seen in small animals that have been attributed to poor expression of the delivered plasmid [9]. Nevertheless, the proven efficacy of the veterinary products on the market and positive results in ongoing human trials for melanoma and prostate cancer therapeutic DNA vaccines [9-11] demonstrate that focusing efforts to boost expression and response levels in humans is one of the keys to the success of the platform. Among the areas ripe for investigation include optimization of transcriptional elements for stronger transcription activity, gene optimization for more stable mRNA and more efficient translation, addition of formulation adjuvants in the delivery vehicle and on the plasmid to stimulate a more potent response, and optimizing the delivery method to increase cellular delivery of the plasmid (**Figure 2)**.

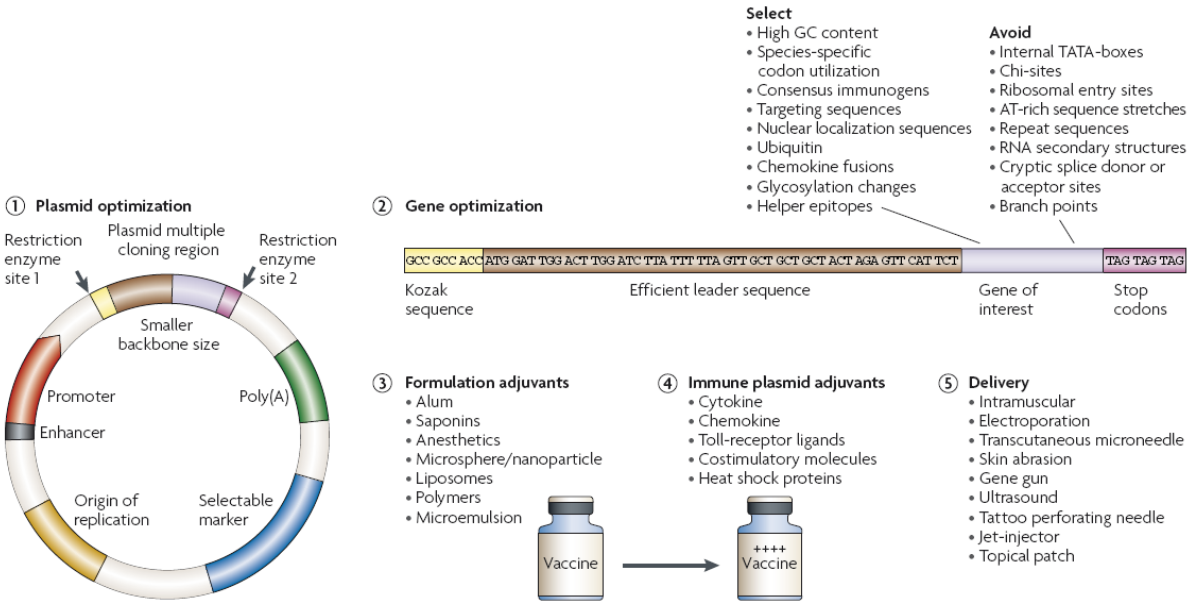

Figure 2: Areas for improvement for the development of a DNA vaccine [9]

Focusing on the delivery of vaccines, intramuscular (IM) injection has been the most common route for administration. To induce a more potent immune response for DNA vaccines, studies have demonstrated the importance of transfecting antigen presenting cells (APCs) directly to engage both the endogenous and exogenous antigen presentation pathways for robust humoral and cellular responses [12-13]. However, most DNA vaccine trials have been administered via intramuscular injection, a method that is known to transfect mostly myocytes [14] as opposed to APCs. The lack of available alternative delivery methods has spurred the development of innovative means for targeted delivery of DNA to APCs. One such alternative is the microneedle array, a device that is able to increase the permeability of the skin by piercing the stratum corneum with pain-free micrometer length projections. Once disrupted, the skin is drastically more amendable for delivery of small molecules and other agents. Current methods for microneedle delivery of bioactive molecules involve coating thin films of dried formulations on the surfaces of the microneedle array [15]. While delivery is achieved, this coating method is notably limited in the range of molecules that can stay active after a drying process, especially for fragile bioactive molecules, and provides few means for controlling release kinetics [16].

To address these issues, the Irvine and Hammond groups have devised a biodegradable polymer microneedle device with surfaces that allow layer-by-layer (LbL) deposition and self-assembly of polyelectrolyte films for concurrent delivery of nanoparticles (NP) and

plasmid DNA [17]. Using the mild aqueous conditions for building polyelectrolyte multilayered (PEM) films, they have demonstrated a general platform for *in vivo* transfection and nanoparticle co-delivery that preserves the activity of bioactive molecules. This combination addresses the challenge of targeted delivery to APCs by exploiting their relative abundance in the skin, where they are two times more abundant than in the circulation [18], to boost vaccine efficacy. The platform can potentially reduce the strain on vaccine manufacturing via dose sparing [19] of current vaccine formulations and by enabling the use of DNA vaccines, which can exploit the superior transport properties of a PEM film-coated microneedle device to overcome the poor immunogenic responses elicited with traditional IM delivery methods. In addition, solid-state stabilization can alleviate logistical concerns related to stockpiling and distribution by allowing for stable room temperature storage of solid as opposed to liquid formulations [20-24]. When compared to hypodermic needles, the use of microneedle arrays can also lower costs to a healthcare system by reducing the incidence of sharps related injuries and allowing for self-administration of a vaccine patch where there is a lack of trained medical personnel [23]. These features of the layer-by-layer (LbL) assembled PEM film microneedle arrays make the technology attractive for commercialization, which will be the focus of this paper.

2 Background

2.1 Current Vaccine Options

A successful vaccine is marked by adherence to several design criteria including safety, cost, efficacy, and level of invasiveness to administer [25]. Since Jenner's observations more than two centuries ago of immunity that a related infection can confer, the concept of a live-attenuated vaccine has been applied to numerous other pathogens including polio, chickenpox, and yellow fever. (**Table 2)** Efforts to improve the safety profile of the first vaccines resulted in Pasteur's findings over a century ago of the effects of administering a killed pathogen to elicit an immune response and led to development of vaccines based on whole-killed pathogens to treat illnesses including whooping cough, typhoid fever, and influenza. Further efforts to improve safety and efficacy have employed reductionist principles for eliciting immunity and have resulted in the development and use of purified or recombinant subunit vaccines for indications such as HPV and meningococcus. Ongoing efforts over the last two centuries have indeed resulted in ever-safer vaccines; however, not all of these improvements have resulted in equal progress for all design criteria.

Vaccine type	Selected disease targets	Vaccine preparation
Live attenuated	Smallpox	Crude preparation of cowpox infected calf skin
	Tuberculosis	*Mycobacterium bovis* BCG grown in media
	Yellow fever	Purified, attenuated virus grown in eggs
	Polio	Purified, attenuated virus grown in tissue culture cells
	Chickenpox	Purified, attenuated virus grown in tissue culture cells
	Rotavirus	Purified, attenuated virus grown in tissue culture cells
	Influenza	Purified, attenuated virus grown in eggs
Killed	Typhoid fever	Inactivated *Salmonella typhi* grown in media
	Plague	Inactivated *Yersinia pestis* grown in media
	Whooping cough	Inactivated whole-cell *Bordetella pertussis* grown in media
	Influenza	Inactivated virus grown in eggs
	Polio	Inactivated virus grown in tissue culture cells (see **Table 2**)
	Hepatitis A	Inactivated virus grown in tissue culture cells
Purified subunit	Diphtheria	Inactivated toxin from *Corynebacterium diphtheriae* grown in media
	Tetanus	Inactivated toxin from *Clostridium tetani* grown in media
	Pneumococcus	Polysaccharides from 23 *Streptococcus pneumoniae* strains grown in media
	Meningococcus	Polysaccharides from four *Neisseria meningitidis* strains grown in media
	Haemophilus *influenzae B*	Polysaccharides from *H. influenzae* chemically conjugated to carrier protein (see **Table 2**)
	Pertussis	Acellular extract of *B. pertussis* grown in media (see **Table 2**)
	Anthrax	Culture supernatant of *Bacillus anthracis* grown in media
Recombinant subunit	Hepatitis B	Purified, recombinant HBsAg VLP produced in tissue culture cells (see **Table 2**)
	Borrelia burgdorferi	Purified, recombinant OspA protein produced in tissue culture cells (no longer available)

Table 2: Sample of disease targets and method used to generate vaccine [1]

2.1.1 Live-Attenuated Vaccines

The concept behind a live-attenuated vaccine lies in separating illness-causing virulence from factors stimulating protective immunity. If virulence can be decoupled from a pathogen, a live-attenuated vaccine typically elicits strong immunogenic responses from its qualities as an invasive organism capable of efficient delivery to the cytosol, generating antigens for a memory response, and displaying pathogen-associated molecular patterns (PAMP) to provoke an innate response [1]. These qualities provoke the infected host into treating the attenuated organism as a serious threat and mounting robust humoral and cellular immune responses to clear the pathogen.

The attenuation process to generate a live-attenuated vaccine typically starts by subjecting the pathogen to non-ideal growth conditions and selecting for variants of the strain that are viable. In the case of influenza, attenuation involves growth at lower than ideal temperatures to select for temperature sensitive mutants. Since the attenuation process relies on the generation of viable but weakened mutants that are less able to cause disease, the process may not ever achieve a suitable strain that successfully decouples virulence from the invasive properties of the organism. In addition, attenuated strains risk reversion to a virulent form by acquiring mutations in the wild or encountering wild-type strains after distribution, which raises concerns for both consumers of the vaccine and healthcare providers who administer the vaccine [26].

2.1.2 Whole-Killed Vaccines

Another general strategy to generate vaccines is via killing or inactivating a pathogen. This procedure permanently disables the ability of the pathogen to invade, replicate, and cause disease. When a killed pathogen is administered as a vaccine, its antigens are still available to provoke an immune response via class II antigen presentation that APCs use to sample the extracellular space. Disabling of the invasive properties removes the reversion risk when exposed to the environment, which makes whole-killed vaccines preferable when compared to an attenuated vaccine formulation for vulnerable populations.

While whole-killed vaccines are perceived to be safer than live-attenuated vaccination strategies, issues with efficacy are more pronounced. The lack of *in vivo* activity for an inactivated pathogen preferentially activates a skewed immune response resulting in

primarily humoral immunity and the generation of antibodies. This biased reaction results in poor cell-mediated immunity and the poor activation of cytotoxic T-cells (CTLs), which is important for sustained and effective long-term immunity to the pathogen in question. The inability to replicate *in vivo* also renders a whole-killed vaccine ineffective at provoking a strong immune response without an adjuvant to achieve adequate activation of APCs [1].

2.1.3 Subunit Vaccines

Purified and recombinant subunit vaccines take the concept of a whole-killed or inactivated vaccine one step further by isolating or exogenously expressing in another organism the immune stimulating epitopes from a pathogen and administering the purified antigens as a vaccine. The strategy has the advantage of removing harmful debris that may stimulate unwanted side effects from a whole-killed preparation and has no risk of causing disease. Similar to a whole-killed vaccine, subunit vaccines may also suffer from poor immunogenicity from the lack of *in vivo* activity and PAMP to generate a balanced immune response and may require an adjuvant or chemically conjugated carrier protein to be added to the final vaccine formulation [1].

2.1.4 Manufacturing for Mass Distribution

Manufacturing processes of existing vaccine technologies, while mature in the ability to produce a highly pure compound, lacks the ability to scale to higher production capacities at a low enough cost and a short enough timeframe for prompt worldwide distribution. In the case of the influenza vaccine, which uses an egg-based production process to produce a whole-killed formulation, large-scale production of the vaccine is extremely time-consuming and labor-intensive but the economics of the influenza vaccine preclude investment in alternative means of production. Under the most optimistic scenarios and a production timeframe of one year, approximately 17% of the world population can be covered; a timeframe of almost five years is required for 100% worldwide coverage (**Table 3)**. The relatively short timeframe for the spread of a novel pathogen such as H5N1 avian influenza would have had a devastating effect on the human population by the time enough vaccine were produced to take advantage of herd immunity effects, making any effort to stem the spread of a particularly virulent and infectious strain a task with a bleak outlook.

Production time (including lead time)	Worldwide capacity (monovalent doses of 15 μg)	Worldwide coverage (%)
1 year	~1,000,000,000	~17
2 years	~2,500,000,000	~40
4 years and 9 months	~6,500,000,000	100

Table 3: Worldwide manufacturing capacity and time needed to achieve population coverage [1]

While the production process for attenuated, whole-killed, or subunit vaccines is relatively straightforward since cultures can be expressed to high concentrations with existing technologies [1], major issues remain that require assessment of alternatives to stem the spread of unanticipated outbreaks. In the case of attenuated vaccines, not all pathogens are amenable to attenuation. For pathogens that can be attenuated, the process can be very labor and time intensive, which would make it a poor candidate for controlling a fast-spreading novel pathogen in the wild. Both whole-killed and subunit processes generate relatively safe vaccines but are poorly immunogenic without an adjuvant and primarily skews a normally balanced response between humoral and cellular immune reactions. In addition, the poor immunogenicity frequently requires large doses of a vaccine in conjunction with an adjuvant to elicit protective levels of immunity, which can strain a production and distribution chain for a rapidly spreading novel pathogen. Downstream manufacturing, especially in the case for subunit vaccines, can be an enormous bottleneck exacerbated by higher than expected dosing requirements. In combination, increased dosage and processing exerts upward pressure on pricing, narrowing the market for a vaccine product to populations that can bear the cost burden. Innovation that can blend the effectiveness of a live-attenuated strain with the safety of subunit vaccines at an accessible price point with low production lead times would be well equipped to flourish in the present and foreseeable market.

2.2 DNA Vaccines

Introduced in the early 1990's, the concept of a DNA vaccine was presented as a technically simple means of inducing robust humoral and cellular immune responses against pathogens and tumor antigens [27-30]. With promises to eliminate reversion concerns associated with the use of live-attenuated pathogens [31] and contamination risks during the

manufacture of whole-killed vaccine stocks, [32] high hopes were placed on the generalized platform for combating the most vexing chronic and infectious disease problems of the day including cancer, influenza, malaria, hepatitis B, and HIV. However, the concept proved too early for its time and early clinical trials in humans for these targets failed to demonstrate effective levels of immunity but did generate enough data to demonstrate the safe nature of the platform [33-38]. Advances over the last two decades to improve the immunogenicity of the platform have begun to address the initial shortcomings of the original design.

While the exact means by which DNA vaccines are capable of eliciting protective levels of humoral and cellular immunity *in vivo* is still under investigation [9], idealized mechanistic schematics exist outlining its mechanism of action (**Figure 3**). After delivery and uptake, the delivered plasmid DNA is thought to enter the nucleus of a transfected cell and direct the production of the encoded antigen of interest. Depending on the cell type transfected, the antigens produced by the delivered plasmid engage both the type I and type II presentation pathways to activate APCs via interaction with MHC I and II receptors to direct proliferation of CD8+ and CD4+ in lymph nodes and select for antigen-specific CTLs and B-cells. The activated CTLs and plasma cells direct a robust immune response to mobilize the host immune system to fight off the pathogen or tumor cells of interest.

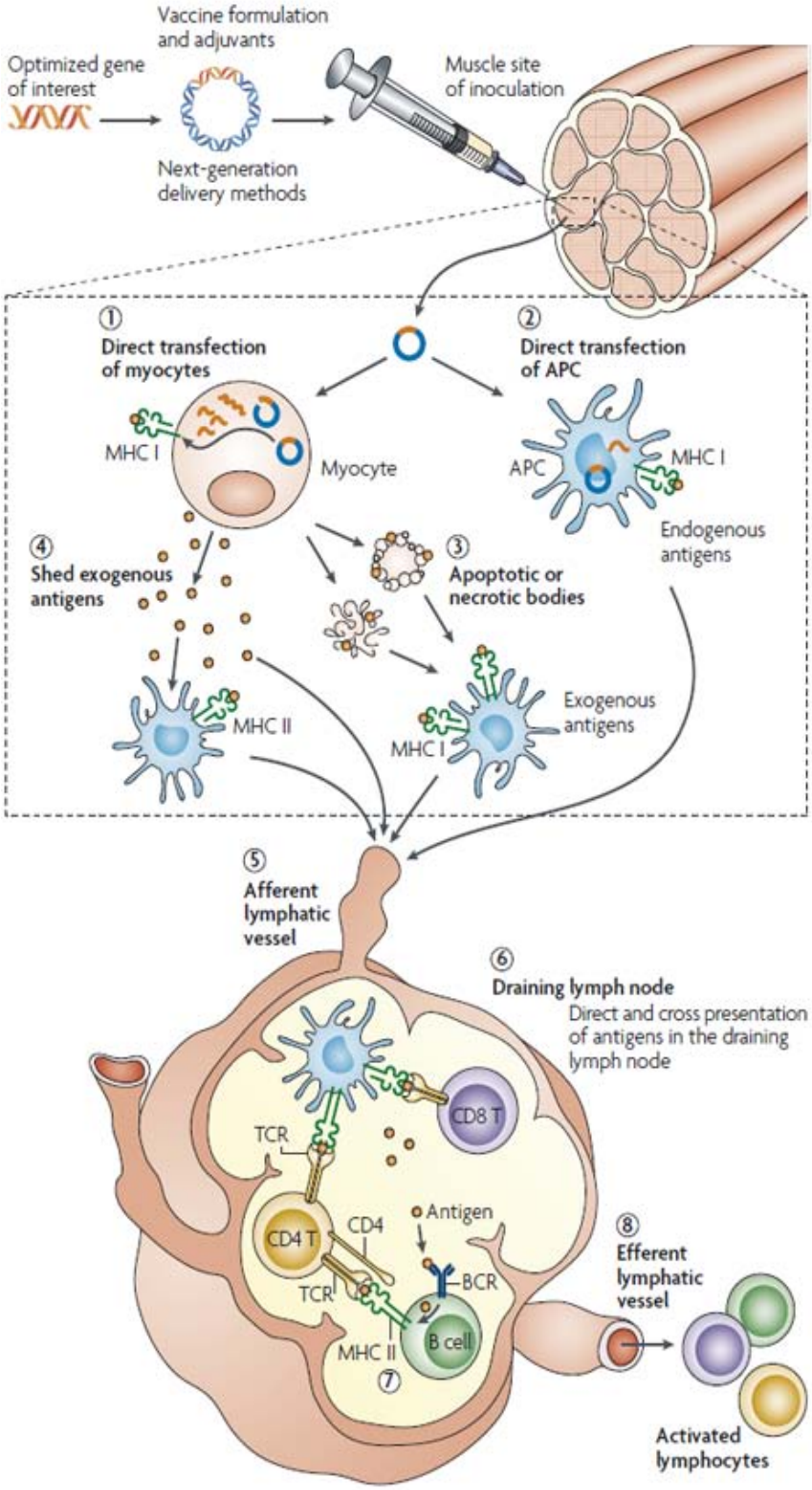

Figure 3: Mechanism of action for DNA vaccines [9]

Initially deemed a failure after results of early clinical trials yielded poor effectiveness data, DNA vaccines have made a comeback in the last five years with products licensed for use in small and large animals (**Table 1**) including fish, dogs, pigs, and horses. Research activity to translate the successes in large animals to humans remains intense as the allure of a truly universal, effective, safe, and easily mass producible vaccine (**Table 5**) draws researchers to work on the multifaceted challenges related to boosting expression in a human subject. Areas that have been targeted include plasmid optimization, gene optimization, formulation and immune plasmid optimization, and delivery vehicle optimization (**Figure 2**). Among the areas that are currently targets for research, optimization of the delivery method poses the greatest technical challenge with the largest amount of diversity and variability in the means that the challenge can be resolved. Initial trial failures can be partly attributed to the lack of alternative delivery methods to administer DNA beyond a traditional IM injection, which has been shown to transfect mostly myocytes, a cell type that is located beyond the APC-rich regions of the skin. Studies have shown the importance of transfecting APCs to generate a potent immune response [12-13] and these findings have directed the development of next-generation delivery methods that target the skin directly.

Commendable qualities	Attributes
Design	Synthetic and PCR methods allow simple engineering design modifications
	Optimization of plasmids through codon and RNA structure changes
	Brings the power of genomics to vaccine construction
Time to manufacture	Rapid production and formulation
	Reproducibile, large-scale production and isolation
Safety	Unable to revert into virulent forms, unlike live vaccines
	In contrast to some killed vaccines, efficiency does not require use of toxic treatments
	No significant adverse events in any clincal trial — many thousands vaccinated so far
Stability	More temperature-stable than conventional vaccines
	Long shelf life
Mobility	Ease of storage and transport
	Likely not to require a cold chain
Immunogenicity	Induction of antigen-specific T and B cell responses similar to those elicited by live attenuated platforms

Table 5: Qualities of a DNA vaccine [9]

2.3 Vaccine Delivery

The predominant mode of vaccine delivery has been and continues to be by needle and syringe. The World Health Organization estimates that approximately 12 billion needle injections are administered worldwide where 5% of that total accounts for immunization-related injections [39]. Despite refined protocols for administration, this mature but antiquated delivery technique is not without its risks, with the most prominent concern resting in safety from needle pricks for the healthcare provider and the reuse and spread of disease from improperly used or reused needles for patients and the community. Recent geopolitical and pandemic events have placed renewed focus on immunization and the resource-, and labor-intensive nature for the proper handling of the needle and syringe, especially in developing nations where the population is already predisposed to increased morbidity and mortality from infectious agents (**Figure 4**) and it is estimated that 50% of all injections are given in an unsafe manner [40].

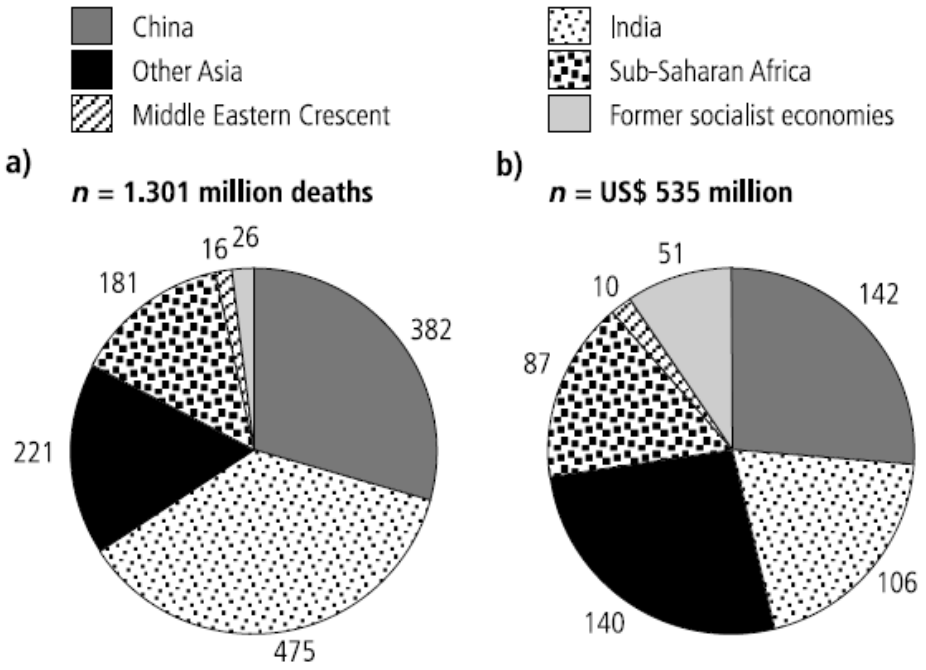

Figure 4: Mortalities per year from unsafe injections [39]

In addition to the safety and resource matters that pervade the use of needles and syringes, there are also issues of compliance with recommended vaccination schedules, fears of pain from a needle prick, and general convenience matters related to the proper

administration of a needle and syringe delivered vaccine [41] that drives patients away from being proactive about receiving their vaccinations and plays an important role in changing morbidity and mortality for populations in developing countries. Cost also plays a very important role in motivating the design of alternative delivery methods for vaccinations. While the materials cost for an injection amounts to $0.06, the social costs of increased risks of infection and the need for trained personnel to administer an injection increases resource outlays more than 450-fold to $26.77 per injection [42]. In addition, needle syringe delivery obligates the use of liquid formulations that require a cold chain to sustain vaccine integrity at a worldwide annual cost of between $200 and $300 million that can be redirected to vaccinating 100 million more children annually [43]. These factors combined have catalyzed innovation in the field to change the standard of needle and syringe delivery with promising results. Among the alternatives to an IM injection with a needle and syringe are delivery candidates that target mucosal and cutaneous surfaces.

Mucosal delivery of vaccine was first practiced centuries ago in China with the nasal delivery of smallpox scabs and oral delivery of fleas from cows infected with cowpox as a means to prevent smallpox [44]. However, the field was not brought to prominence until the 1960's with the introduction of Sabin's oral polio vaccine, which has since resulted in several vaccines developed exclusively for use on mucosal surfaces including the influenza nasal spray. Other mucosal surfaces of interest include pulmonary and vaginal or rectal surfaces; however, the inherent qualities of mucosal surfaces as the first line of defense against insults from the environment has made development of effective vaccines difficult without the use of strong adjuvants. In addition, mucosal surfaces frequently display high levels of degradative enzymatic activity, making the delivery of intact antigens difficult (**Table 6**).

Method	Advantages	Limitations
Cutaneous immunization		
Liquid-jet injection	Long history of use, ability to work with existing formulations, and success with many forms of vaccine	Issues associated with cross-contamination when using MUNJIs, high cost of device, and occasional pain and bleeding
Epidermal powder immunization	Use of powders facilitates storage, strong data for DNA vaccines, and natural targeting of Langerhans cells	High cost of device, occasional bleeding, limited clinical data for non-DNA vaccines, and limited clinical history
Topical application	Ease of access, natural targeting of Langerhans cells, generation of mucosal and systemic immunity, and high patient compliance	Strong adjuvants or permeabilizing agents required, some permeabilization methods require expensive devices, and most delivery methods have limited clinical history
Mucosal immunization		
Oral	Ease of administration, high patient compliance, no complex devices necessary in most cases, primary site of infection of many pathogens, and long history of use for live attenuated pathogens	Gastrointestinal deactivation of vaccines, high doses required, variability of response, and mixed clinical data
Nasal	Easier access to mucosal membrane than for oral delivery, low cost, and one of the main sites of infection for airborne pathogens	Short contact time, enzymatic activity of nasal tissue, adjuvants required, safety concerns with earlier nasal vaccine, and limited applicability in patients with upper respiratory-tract infections
Pulmonary	Large surface area, one of the main sites of infection for airborne pathogens, and history of use for measles vaccine	Strong adjuvants required, high cost of some devices, and interference from upper respiratory-tract infections
Vaginal or rectal	High relevance for HIV and causative agents of other sexually transmitted diseases	Poor patient compliance for general applications, and strong adjuvants required

MUNJI, multi-use-nozzle jet injector.

Table 6: Needle-free delivery methods [44]

Potent systemic and mucosal responses elicited through cutaneous immunization have made skin immunization a desirable means by which to prevent infection at the point of entry [45-46]. Skin immunization offers an effective means to elicit robust CD8+ CTL mucosal responses simultaneously in the gut, lung, saliva, and the female reproductive tract [47]. Cutaneous immunization is thus vastly superior to traditional immunization routes including intramuscular or intranasal techniques that typically elicit compartmentalized mucosal responses specific to a subset of the surfaces stimulated via cutaneous immunization [45]. Cutaneous delivery vehicles designed to take advantage of the robust immune response have had a long history of development since the introduction of the liquid-jet injector (**Table 6**). However, its usefulness as an alternative to the needle and syringe is limited because of the same risks of infection and contamination in common with needles as well as the high cost associated with the device [44,48]. An alternative developed to better target APCs for intracellular delivery was embodied in epidermal powder devices that propel dried

formulations under high pressure. While powder injectors enhanced targeting for more efficacious delivery (**Table 6**), the devices also suffered from the same drawbacks as liquid-jet injectors and needles at significantly higher costs [44,48].

Recent efforts for cutaneous delivery have focused on topical delivery devices that attempt to permeate the outermost layers of the skin to make the skin more amenable for delivery of small molecules and biologics. Initial efforts have focused on passive delivery patches but these devices were limited to small molecules that were readily diffusible past the stratum corneum layers of the skin [44,49]. Iterations of the platform have made it possible to deliver higher molecular weight compounds and biologics but at the cost of adding strong adjuvants and permeabilizing agents such as cholera toxin [44,49], which comes with risks such as inflammation and damage from retrograde transport to neuronal cell bodies, causing unintended side effects to the central nervous system [50]. Most recently, efforts have been focused on electroporation as a candidate for reversible physical disruption of the stratum corneum and success has been documented with the delivery of a peptide into the skin of a mouse that generated a strong CTL response [51]. Positive results for the electroporation platform has been documented in small and large animals for the transcutaneous administration of therapeutic genes [9] although complexities with device design have inhibited study in humans [49].

An alternative effort at cutaneous delivery has focused on microneedle patches dip-coated with small molecule or biologic compounds [49]. These devices have been shown to be painless or near painless by careful design of short micrometer projections that do not penetrate the dermis where pain receptors are found [52] (**Figure 5**). However, dip-coating comes with many disadvantages including limited control of delivery kinetics and the relatively few molecules that stay active after a drying process [16]. Iterations of the concept have resulted in the production of a dissolvable microneedle array molded from biologically compatible monomers and cross-linked to encapsulate a vaccine payload [53]. However, photopolymerization with UV light to form the final array precludes the use of more sensitive biomolecules such as DNA due to photochemical damage [54] to the payload, restricting the range of compounds that a dissolving microneedle patch can deliver. Recent efforts in thin film engineering have sought to deal with the challenge of coatings on

micrometer surfaces while preserving bioactivity of a therapeutic agent [55] and results point to a generalizable platform for potent and cost effective vaccine delivery vehicle [17].

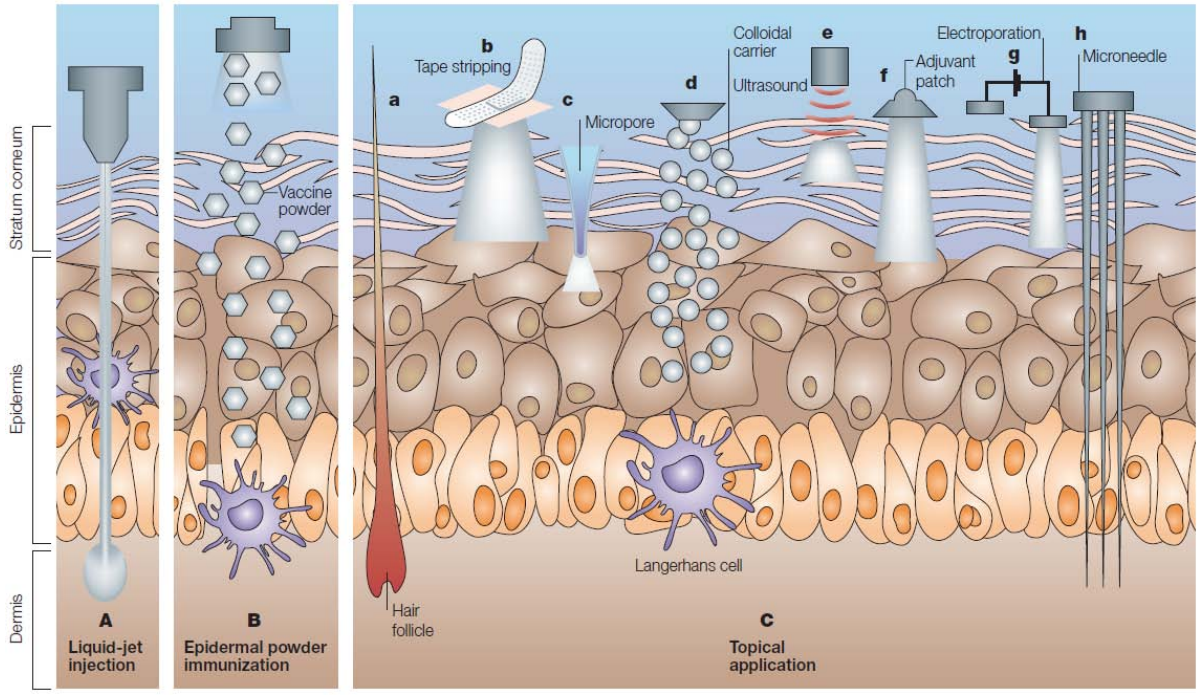

Figure 5: Delivery methods in development to replace needle and syringe delivery of vaccines [44]

3 Invention Overview

3.1 Motivation

Transdermal delivery has been extensively studied as an alternative to mucosal delivery and hypodermic needle injection of drugs and vaccines. The ease of access and stratification of bioactive layers [17] makes the skin a very desirable target for immunization and has been the focus of innovative solutions that aim to permeabilize this robust barrier for the delivery of bioactive compounds. One solution that has garnered much attention involves the use of microneedle patches composed of micrometer arrays of projections coated with thin films of dried drug or biologic formulations [15,56-58] that efficiently permeabilizes the stratum corneum to deliver the coated compounds directly to APCs. However, the use of a dry-coat dipping process has inherent disadvantages that prevent uniform deposition and controllable release kinetics [16]. Another proposed solution for transcutaneous delivery involves the use of dissolvable microneedle arrays that release its encapsulated contents upon application of the microneedle patch [53]. However, UV photopolymerization to crosslink the polymer matrix during production has been shown to damage sensitive biomolecules such as DNA [54], which places a limitation on the breadth of biomolecules that this platform can deliver.

Recent work in the field of PEM thin film engineering has demonstrated the versatility of self-assembled films to encapsulate small molecules and biologics, enabling control over release kinetics and solid-state stabilization of coated compounds on the nanometer scale [16-17,21-22,59-60]. However, cutaneous patches require a means of disrupting the stratum corneum of the skin for the delivery of larger and more charged compounds such as DNA. The synergy of PEM thin films deposited on microneedle arrays would combine efficient and pain-free disruption of the stratum corneum with the superior release kinetics and solid-state stabilization intrinsic to PEM films. As a combination platform, this PEM film-coated microneedle device would be ideal for the delivery of vaccines, small molecules, and other biologics.

3.2 Device Fabrication

Fabrication of the PEM film-coated microneedle arrays requires serial processing of the raw constituents to form the final device. While most bench-scale processes require

retooling and adaptation of methods for mass manufacture, the utilization of processes prevalent in microelectronics and techniques refined to fabricate microfluidic devices for the fabrication of this coated microneedle device would most likely only require swapping of fabrication machinery to accommodate higher throughput in an industrial process.

3.2.1 Poly(lactide-co-glycolide) Microneedle Array

Poly(lactide-co-glycolide) (PLGA), a biocompatible and biodegradable copolymer compound, was used as the raw starting material to fabricate the microneedle arrays. A polydimethylsiloxane (PDMS) mold was fabricated and laser ablated to the required depth and geometry to serve as a reusable negative mold. PLGA pellets were then placed on the PDMS surface and the PLGA-loaded mold was placed in a vacuum oven for 40 minutes at 145^{o} C. After melting into the mold, the microneedle-mold device was placed in a -20^{o} C freezer for 1 hour to allow for solidification before separation (**Figure 6**). Microneedle devices fabricated in this way yielded PLGA arrays with either pyramidal or conical geometry and needle lengths ranging from 800 to 1200 µm. For the purposes of this study, a needle length of 900 µm was selected (**Figure 7**).

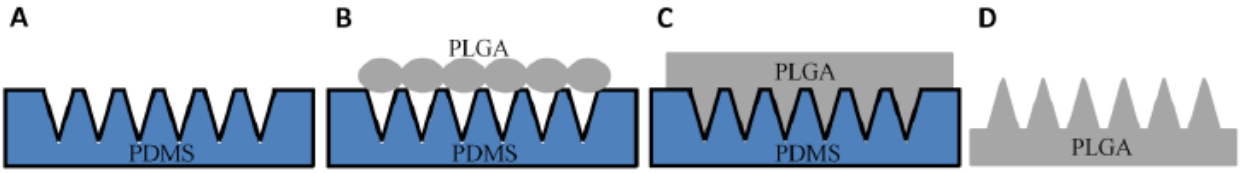

Figure 6: Schematic for the production of PLGA microneedle arrays from a PDMS mold [17]

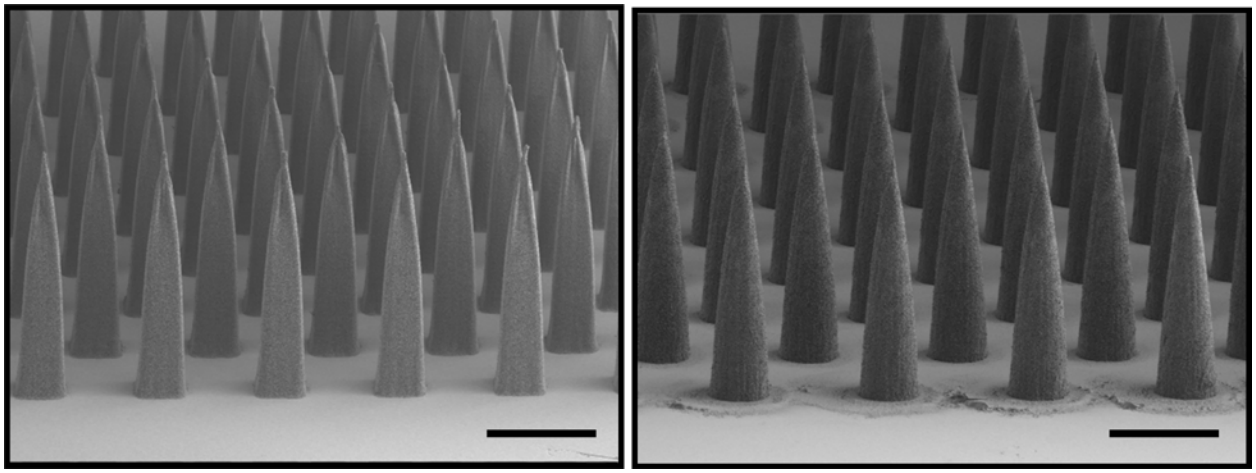

Figure 7: Microneedle arrays with pyramidal (left) and conical (right) cross sections [17]

3.2.2 Polyelectrolyte Multilayered Thin Film Coatings

After separation of the PLGA microneedle device from the PDMS mold, the microneedle surfaces were treated in plasma cleaner with O_2 plasma for 2 minutes. The PLGA microneedle array was then dipped in an alternating fashion into a solution of protamine sulfate (PS) and poly(4-styrene sulfonate) (SPS) for 10 and 5 minutes respectively with intervening 1 minute rinses with deionized water to provide a uniform surface charge density for PEM functional layer deposition. Following 20 coats of PS/SPS, plasmid DNA was deposited on the PS/SPS coated microneedle surfaces by dipping in the same fashion as described above but alternating with polymer-1, a hydrolysable polyelectrolyte in the poly(B-amino ester) family, in 5 minute dipping intervals separated by 30 second rinsing steps with deionized water to achieve an alternating stacked structure of cationic and anionic charges that self assemble onto the microneedle surface (**Figure 8**). Lipid-coated PLGA nanoparticles were also deposited onto the PS/SPS prepared surfaces in an alternating fashion with polymer-1 but via an airbrush spray method for 3 seconds punctuated by 6 second deionized water rinse sprays. Spray coating followed by dip-coat deposition of the microneedle surfaces was also attempted and combination plasmid DNA/lipid-coated PLGA nanoparticle delivery arrays were studied as a platform for concurrent DNA and nanoparticle delivery.

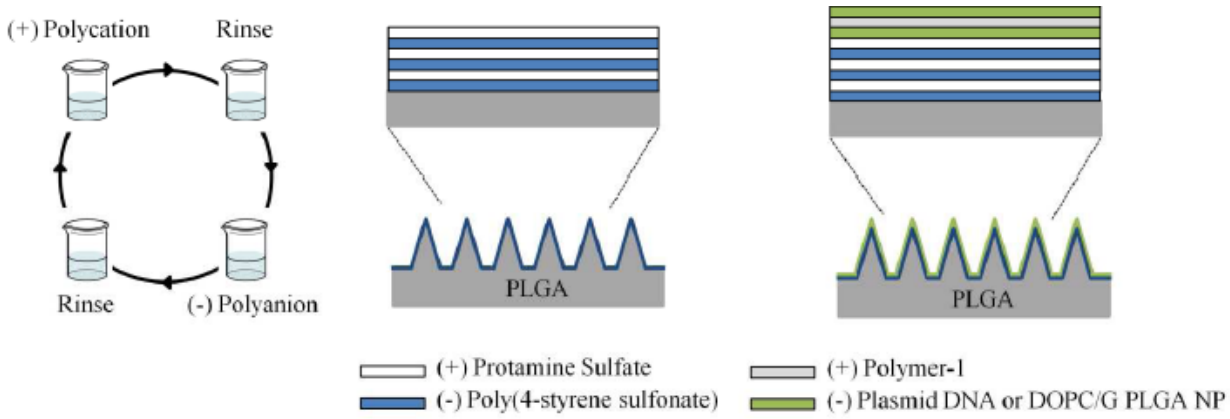

Figure 8: Alternating polycation and polyanion coating strategy for layer-by-layer assembly of thin films [17]

3.3 PEM Microneedle Performance

3.3.1 Delivery Performance

Delivery performance of the DNA and/or nanoparticle loaded microneedle arrays was visualized using a MHC II-GFP transgenic mouse model that expresses an *in situ* marker for discerning viable epidermis. Microneedle devices were placed on the dorsal ear skin of the mouse model and delivery performance was visualized with fluorescent confocal microscopy after applications for 1 min, 5 min, and 24 hours. Delivery kinetics for labeled DNA-loaded microneedle arrays differed from that of nanoparticle-loaded arrays (**Figure 9**). Insertion of the DNA-loaded microneedle array for short time periods (5 min) revealed poor delivery characteristics as shown in **Figure 9A** whereas a 24 hour insertion period revealed highly localized and efficient delivery into the viable epidermis (**Figure 9B**), suggesting that the polymer-1 layers require time for hydrolytic degradation to release the DNA cargo. Nanoparticle-loaded microneedle arrays were found to deliver nanoparticles even after a 5-minute insertion period as shown in **Figure 9C**, suggesting that the act of inserting the microneedle is associated with nanoparticle delivery. When the microneedle array was coated with nanoparticles followed by DNA, controlled delivery of nanoparticles and DNA was observed after 24 hours as shown in **Figure 9D**, suggesting that the profile of the thin film should be a design criterion dependent on the delivery application.

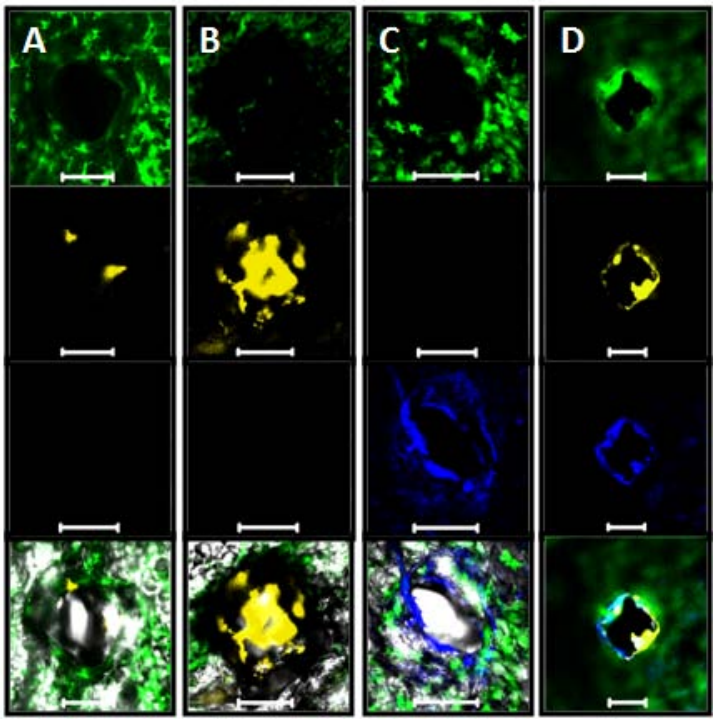

Figure 9: Confocal microscopy for visualizing delivery of labeled DNA and labeled nanoparticles by microneedle application to the dorsal ear skin of transgenic MHC II-GFP fusion mouse. A) DNA loaded array applied for 5 minutes B) DNA array applied for 24 hours C) Lipid-coated nanoparticle array applied for 5 minutes D) DNA/lipid-coated NP co-delivery after 24 hour application [17]

3.3.2 Transfection Performance

DNA transfection performance was evaluated with a DNA-coated microneedle device carrying a plasmid expressing the firefly luciferase gene. Transfection was evaluated for different coating densities (1, 5, 24 bilayers) and at two times (5 minutes and 24 hours) (**Figure 10**). When comparing **Figures 10A** and **10D**, there is a notable time dependence for transfection of a 24-bilayer coated microneedle array that correlates well with the co-localization trend observed with the delivery of labeled DNA in **Figures 9A** and **9B**. In addition, there was a discernable difference in expression level between mice treated with an array loaded with more DNA as measured in the differences in luminescence between mice in **Figures 10B**, **10C**, and **10D**. Mice treated with an array loaded with 1-bilayer (**Figure 10B**) showed no expression whereas mice treated with arrays loaded with 24-bilayers (**Figure 10D**) showed strong luciferase expression. These findings indicate that dosage can be tuned with modification of simple parameters to suit the needs of the desired delivery application.

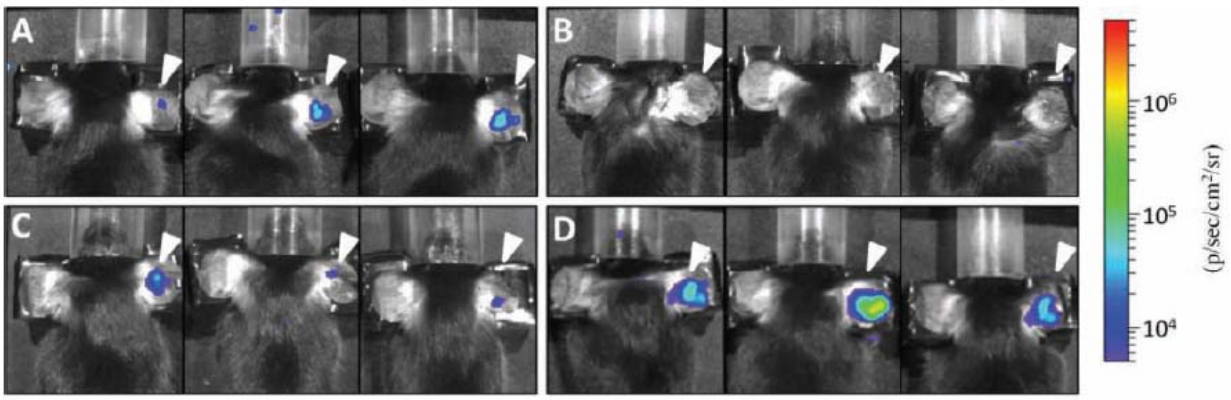

Figure 10: Bioluminescence assay to visualize transfection efficiency of pLUC loaded microneedle arrays. A) 24-bilayer applied for 5 minutes. B) 1-bilayer applied for 24 hours. C) 5-bilayers applied for 24 hours. D) 24-bilayers applied for 24 hours [17]

3.4 Suitability and Market Potential as a Vaccine Delivery Platform

The Hammond and Irvine groups have demonstrated a proof of concept device that synergizes the permeabilizing efficiency of a microneedle array with the versatility of PEM thin films coatings. Tunable layer-by-layer deposition of DNA and/or nanoparticles allows for robust control of dosing and release kinetics, a feature absent or lacking in dip coated and dissolvable microneedle arrays. In addition, the conditions for deposition of thin films are mild and do not require the use of drying or radiation to produce the final product, expanding the range of molecules that can be encapsulated in a PEM thin film-coated array to include fragile biomolecules.

Initial characterization of the device on viable murine dorsal ear epidermis with fluorescently labeled cargo showed co-localization of delivered cargo with epidermal Langerhans cells, which is indicative of successful delivery at the proper depth to induce an immunogenic response. Subsequent characterization with an *in vivo* transfection assay demonstrated stable DNA transfection by a microneedle-supported PEM film, a key finding that allows for the translation of this device to vaccination-related uses for the delivery of a DNA vaccine.

4 Market Dynamics and Commercialization Strategy

4.1 Global Vaccine Market

Traditionally a high cost, low profit margin segment, the pharmaceutical industry has found renewed interest in the vaccine market and have begun to invest in the segment since the blockbuster introduction of Prevnar, a 7-valent conjugate vaccine for pneumococcal infections, by Wyeth in 2000 [61]. With the rise of critical public health issues in the face of H1N1, H5N1, and the specter of bioterrorism, pharmaceutical giants have begun a conversion to hybrid biotechnology/pharmaceutical companies to take advantage of the growth opportunities through mergers and acquisitions of key market players including Chiron by Novartis in 2006 and Wyeth by Pfizer in 2009. Attrition from this high cost vaccine industry, especially after the 1980 introduction of good manufacturing practices (GMP), has thinned the number of market players significantly from over a dozen in 1967 to five major players that control almost 90% of the market [62]: Novartis, GlaxoSmithKline, Merck, Sanofi-Pasteur, and Pfizer [61].

While the global vaccine market accounted for only 2.8% of pharmaceutical industry revenue of $21.28 billion in 2008, revenue is expected to grow with a compound annual growth rate (CAGR) of 12.1% in the global market to over $47 billion in 2015 [62] (**Figure 11**). Emerging markets such as India, China, and Brazil are becoming key drivers for revenue growth as the government finds renewed focus in public vaccination campaigns [62].

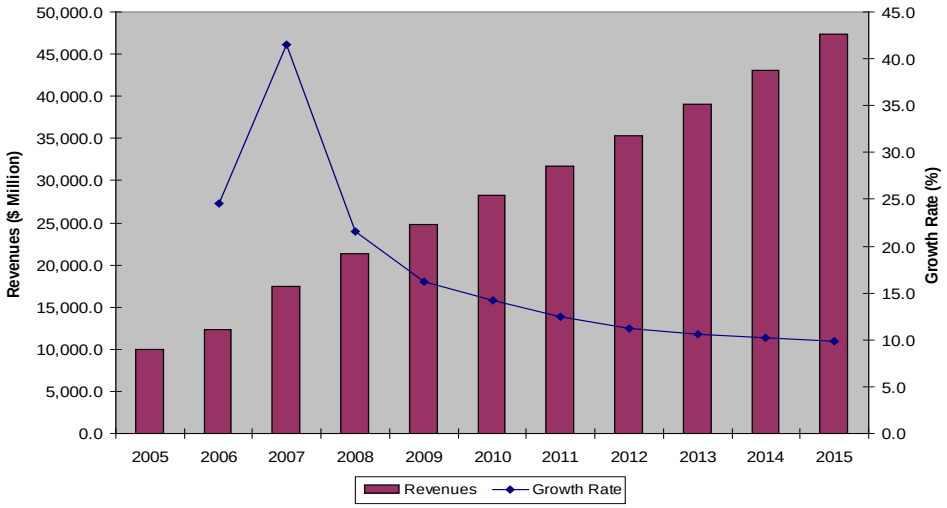

Figure 11: Global vaccine market revenue projections from 2008-2015 [62]

4.2 Industry Challenges for Incumbent and Potential Market Players

Growth potential in the vaccine market remains high and is expected to grow at a sustained rate but the path to higher revenues remains rocky. Major barriers to entry and/or growth include crushing upfront capital expenditures, a highly fragmented global market, and the absence of healthcare infrastructure in developing nations for effective penetration of the market. These barriers are not insurmountable for emerging players but do require large amounts of capital and is a strong deterrent for smaller players that aim to challenge the 89.8% market share amongst the market leaders [62].

For aspiring market entrants planning to tap into this growth sector, large investment outlays are required at the outset for manufacturing capabilities since the introduction of GMP and the requirement to document and validate each step in the supply chain. Because vaccines are typically produced in or are parts of living organisms, FDA regulatory guidelines pose an enormous barrier for entrants that attempt to penetrate to market with a new product since there is an approximately 15-year lag time before market approval [62].

A highly fragmented market also poses a challenge for both established and aspirant vaccine manufacturers. A patchwork of differing regulatory guidelines, price controls, and monopsony purchasing power of governments in developing countries and Europe pose challenges for maximizing revenue, especially for a startup (**Table 7**).

While developing nations possess enormous growth potential for vaccines, inadequate or non-existing healthcare infrastructure poses a challenge for getting any product to market no matter the size of the company. By far, the greatest issue remains the maintenance of the cold chain, the low temperature storage needed for most biologics until the point of use. Manufacturing and delivery innovation that can alleviate this barrier can be extremely attractive to existing market players and can be used as leverage for a small company with the proper intellectual property holdings looking to penetrate the market due to the cost savings and disruption of the current supply chain model this innovation may have [17,43,62-63].

Vaccines Market: Impact of Top Industry Challenges (World), 2009-2015			
Challenge	**1-2 Years**	**3-4 Years**	**5-7 Years**
Large investments required to produce vaccines limits number of market participants	High	High	High
Highly fragmented markets present unique barriers for manufacturers	High	High	High
Sustaining growth in developed markets proves challenging	High	High	High
Absence of healthcare infrastructures restricts access to vaccines in developing regions	High	Medium	Medium
Intellectual property issues curb innovation and increase R&D costs	Medium	Medium	Medium
Lingering concerns about risks of childhood immunization perpetuates negative image	Low	Low	Low
Success of immunization programs creates complacency	Low	Low	Low

Table 7: Challenges in vaccine market [62]

4.3 Initial Analysis of Intellectual Property

For any innovation to succeed on the market, clear intellectual property (IP) rights need to be established in order to attract sources of funding as this is the only way to ensure that a market exists for a return on investment. The patentability of the PEM thin film-coated degradable microneedle device was assessed through a patent and literature search.

4.3.1 Patents Related to Microneedle Arrays

Extensive patent coverage has already been issued for the use and manufacture of polymer microneedle arrays. Patent 6,334,856 held by the Georgia Tech Research Corporation describes a microneedle device that is extensively similar to the device employed by the Irvine and Hammond system [64]. Among the most substantial claims that prevent the use and manufacture of a polymer microneedle device includes claims 1 and 23, which are especially vague in its claims to any microneedle device constructed from a polymer. In addition, extensive coverage of the molding process including the use of polymer to make the mold and the use of laser ablation to generate the holes used to make the microstructures is covered in claims 2, 5, and 22.

In addition to claims made in patent 6,334,856, a follow-up patent application number 20090131905 [65] was filed on 5/21/2009 that attempts to expand the coverage of the uses and manufacture of microneedle devices. In this patent application, Allen, et al. clarify their claims to polymer microneedle devices to include biodegradable polymers composed of poly(hydroxy acid)s, polyanhydrides, poly(ortho)esters, polyurethanes, poly (butyric acid)s,

poly(valeric acid)s, and poly(lactide-co-caprolactone)s. While the Irvine and Hammond microneedle platform uses a polymer that is not specifically covered in this claim, a literature search revealed public disclosures [66] by Allen, et al. documenting use of PLGA microneedles. Depending on the outcome of the new patent application and its claims to the use of other classes of biodegradable polymers, licensing of this patent may be required.

4.3.2 Patents Related to Microneedle Coatings

In the realm of coated microneedle structures, patent application 20080213461 [67] has been filed by Gill et al. claiming coverage for coatings that are composed of at least one drug and a viscosity enhancer. The restriction of the use of a drug and a viscosity enhancer should post no threat to the patentability of the Irvine and Hammond system as neither a drug nor a viscosity enhancer was used. A point of caution should be noted if further development requires a means of securing a microneedle array to the skin with adhesive; claim 65 specifically claims the use of adhesive material between two or more needles in the array. This claim poses a minor annoyance if it remains in final approval since a different means of fixating the device to the skin, if needed for complete delivery, may need to be developed.

4.3.3 Patents Related to Thin Films

Methods for constructing decomposable thin films of polyelectrolytes and their uses has been patented in patent 7,112,361 [68] by a group that includes Professors Hammond and Langer at MIT. This patent provides broad coverage of the alternating polycation and polyanion structure similar to the scheme used in the Irvine and Hammond system. The patent also provides coverage for deposition of this film on polymer substrates. Hammond, et al. has also further clarified claims to self-assembled thin film structures in patent application 20080311177 [55] to lay claim to films that self assemble specifically with proteins and drugs. The Irvine and Hammond microneedle system employs a PEM thin film structure that layers DNA and lipid-coated nanoparticles, which is not explicitly claimed by patent 7,112,361 or patent application 20080311177. The wording of both patents covering decomposable thin films, however, should not be of any issue as Hammond is a collaborator for this PEM film coated microneedle system and will be an integral part of any commercialization process.

4.3.4 Patents Related to Manufacturing Processes

Issues related to the patentability of certain manufacturing processes associated with the Irvine and Hammond microneedle array tie back to the IP surrounding polymer microneedle arrays described in patent 6,334,856 issued to Allen, et al. of Georgia Tech. The patent provides broad coverage for a mold-produced microneedle array composed of a polymer. Patent application 20090131905 further excludes the use of degradable polymers that are not already in the public domain. Licensing of this technology may likely need to happen if innovation to circumvent the existing patent and patent application does not occur.

4.3.5 Patents Related to Vaccines and Targets

At its core, the PEM film-coated microneedle array is a delivery vehicle and offers no therapeutic benefit on its own. In order to confer therapeutic qualities to the microneedle device, an IP portfolio for particular antigen targets and/or vaccine candidates must be obtained. Since the development of an IP portfolio for antigen targets and DNA vaccines is not a core competency, it would be very time- and cost-prohibitive. A strategic partnership to license an IP portfolio of disease targets would be the most effective solution.

4.4 Market Strategy for Commercialization

With the assumption that the initial analysis of intellectual property revealed most of the major roadblocks to successful commercialization of the Irvine and Hammond microneedle system, the IP holdings for this delivery system would be most amenable to commercialization via a strategic partnership with a pharmaceutical company and with the stakeholders at Georgia Tech while grouping all the IP holdings into an intellectual property licensing company. This partnership would be mutually beneficial for all parties involved. Pharmaceutical companies have large portfolios of disease and antigen targets that can be adapted for use in a DNA plasmid delivered in a vaccine formulation. Georgia Tech's holdings for the underlying microneedle device would be inadequate if used in its current form to controllably deliver a plasmid for transcutaneous delivery. This gap can be bridged with the patent holdings between Irvine and Hammond for a LbL PEM film coating that can be self-assembled onto the surface of a degradable polymer microneedle.

Once suitable partners that possess disease target portfolios, negotiations should take place for the disease target IP holders to acquire a non-exclusive license to use the Irvine and Hammond system for the delivery of the vaccine candidate. In this way, the IP surrounding the Irvine and Hammond platform will not be limited to the disease targets with earlier partners and the company holding the IP for the Irvine and Hammond delivery system would be able to maximize revenue. Once the rights to use the IP from the disease target holders are acquired, the company should work to maximize the efficient delivery of the construct and contract with a contract manufacturing organization to produce this specific formulation. The pharmaceutical partners who hold the rights to the disease target antigens would then use the formulations produced by the contract manufacturing organization to conduct clinical trials, which can also be outsourced if the pharmaceutical partners wish to do so.

This business arrangement would be optimal for the Irvine and Hammond holding company as all partners would be involved with their core competencies. The Irvine and Hammond holding company would be charged with optimizing the layered film formulations and then passing this formulation to a contract manufacturer, which would have the expertise to produce the devices at a large scale and at low cost. The IP for the target vaccine that did not have a feasible delivery vehicle that the pharmaceutical partners held would now have an effective delivery vehicle for use in clinical trials, a core competency that requires resources that a large pharmaceutical company would already possess. In this way, the Irvine and Hammond IP holding company and the pharmaceutical partners would be able to minimize risk throughout the entire process. The lack of a need to develop manufacturing facilities would drastically reduce costs related to capital expenditure, the primary barrier for competitors to the main players in the vaccine market.

4.4.1 Value-Added in the Vaccine Supply Chain

In order to be a successfully commercialized technology, there has to be an incentive for the existing market players to employ the novel technology. One of the main benefits of the Irvine and Hammond microneedle delivery platform is the solid-state stabilization of the loaded vaccine, which has enormous implications for reducing cost throughout the supply chain. The pharmaceutical partners that participate in the joint venture would be able to reduce logistical costs associated with ensuring the vaccine does not spoil at ambient

temperatures. The contract manufacturing partners would also be able to reduce overhead from the lack of cold chain maintenance throughout the manufacturing process. The savings associated with the lack of a cold chain alone can either be converted into higher profits or, if the partnership terms allow, enable the sale of the resultant vaccine at significantly lower costs and generate good will and publicity for all partners involved in the venture.

4.4.2 Risks of the Commercialization Process

While strategic planning can reduce most of the risks associated with the commercialization process, unexpected events should be expected to occur. Variables that need to be considered during the commercialization process include the variability that contract manufacturing can introduce to the supply chain, regulatory hurdles for approval by the FDA, and the long-term investments required to last through a FDA approval process.

While contract manufacturing can reduce the risks of failure in terms of upfront capital costs to all parties, manufacturing by a third party can lead to unintended and unexpected variability in the final product. Bench-scale operations typically do not scale well to industrial processes and tweaking of the production process for manufacturing may be required to achieve feasibility and cost goals. In order to minimize the level of unintended roadblocks during the contract manufacturing stage, it would be useful to work closely with the contract manufacturing partners to co-develop a process that does not deviate from the function of the final device.

FDA approval for a novel delivery device can be a long and arduous process. Successful preclinical data in animals may not translate to successful outcomes in human studies as was experienced with first generation DNA vaccines [9]. Even in cases where clinical trials uphold the data obtained from animal models, unexpected occurrences can take place including adverse events and demands for more trial data. Should these occurrences take place, the enormous capital outlays that the pharmaceutical partners have budgeted might be exceeded and threaten the future of the project. Since these events cannot be predicted with any certainty, adequate budgeting and working closely with pharmaceutical partners is the only recourse for mitigation.

Collaborating with a large pharmaceutical company provides the benefits of reduced risks, especially for a startup company, but the results of failure are more profound for the

startup partner due to limited resources. There is always risk of a disruptive technology that can make the delivery platform obsolete before it has a chance to recoup costs from selling on the market. It is therefore wise to diversify and recruit a group of pharmaceutical partners and target various disease targets to hedge the risk of inevitable failure given the low rates of FDA approval [69].

5 Conclusion

An evaluation of the polyelectrolyte multilayer thin film-coated microneedle vaccine delivery system has been performed throughout this paper. Combining the advantages of a microneedle array to permeabilize the stratum corneum in the skin with the superior control characteristics of a self-assembling thin film loaded with bioactive cargo, the Irvine and Hammond microneedle platform is poised to supplant existing microneedle coating solutions that rely on surface tension and drying during manufacture [23]. The solid-state stabilization of cargo allows for streamlined handling of vaccine formulations by potentially eliminating the need for refrigeration and allowing storage at ambient temperatures [16]. The ability to eliminate the cold chain can confer any vaccine therapeutic with a competitive advantage in cost and in logistical concerns for developing nations where an adequate cold chain does not exist.

The intellectual property landscape for this new technology was also discussed in this paper. While most of the delivery platform is clear of any potentially infringing elements, the need to license intellectual property from Georgia Tech may pose some issues if the infringing elements cannot be circumvented. Even if the potential overlap cannot be feasibly circumvented, a strategic partnership can and should be formed to add partners to the joint venture, which can mitigate risk if FDA approval should fail.

The case to form a purely intellectual property company was presented in this paper as a means to maximize revenue and hedge away most of the potential risks of bringing a new biologic and delivery device to market. While contracting out all manufacturing does have risks, the consequences of failure far exceed the costs associated with developing expertise outside the scope of the company's core competencies. The proposal to develop a partnership should be viable as value is added across the supply chain where all partners stand to gain from the efficiencies generated from this delivery platform.

Risks during the commercialization process were also discussed that may hamper progress during a joint venture. While the risk factors may be hard to hedge away, especially in the case of the FDA approval process, this should not deter the company from attempting the joint venture as the technology underpinning the delivery platform is sound and has the potential to disrupt the status quo for vaccine delivery in a positive manner.

References

1. Ulmer, J.B., Valley, U. & Rappuoli, R. Vaccine manufacturing: challenges and solutions. *Nat Biotech* **24**, 1377-1383 (2006).
2. Cooper, M.R., Stewart, D.C., Kahl, F.R., Brown, W.M. & Cordell, A.R. Medicine at the medical center then and now: One hundred years of progress. *South.Med.J.* **95**, 1113-1121 (2002).
3. Global Polio Eradication Initiative. *Bull. World Health Organ.* **84**, 595-595 (2006).
4. Wassilak, S. & Orenstein, W. Challenges faced by the global polio eradication initiative. *Expert Review of Vaccines* **9**, 447-449 (2010).
5. Webby, R.J. & Webster, R.G. Are We Ready for Pandemic Influenza? *Science* **302**, 1519-1522 (2003).
6. Check, E. Avian flu special Is this our best shot? *Nature* **435**, 404-406 (2005).
7. Hehme, N., Engelmann, H., Kuenzel, W., Neumeier, E. & Saenger, R. *Virus Res.* **103**, 163-171 (2004).
8. Epidemic Alert and Response: Vaccines for Pandemic Influenza. Summary Report. (World Health Organization, 2004).
9. Kutzler, M.A. & Weiner, D.B. DNA vaccines: ready for prime time? *Nat. Rev. Genet.* **9**, 776-788 (2008).
10. Rice, J., Ottensmeier, C.H. & Stevenson, F.K. DNA vaccines: precision tools for activating effective immunity against cancer. *Nat. Rev. Cancer* **8**, 108-120 (2008).
11. Buchan, S. Electroporation as a 'prime/boost' strategy for naked DNA vaccination against a tumor antigen. *J. Immunol.* **174**, 6292-6298 (2005).
12. Porgador, A., *et al.* Predominant Role for Directly Transfected Dendritic Cells in Antigen Presentation to CD8+ T Cells after Gene Gun Immunization. *The Journal of Experimental Medicine* **188**, 1075-1082 (1998).
13. Condon, C., Watkins, S.C., Celluzzi, C.M., Thompson, K. & Falo, L.D. DNA-based immunization by in vivo transfection of dendritic cells. *Nat Med* **2**, 1122-1128 (1996).
14. Danko, I., *et al.* High expression of naked plasmid DNA in muscles of young rodents. *Hum. Mol. Genet.* **6**, 1435-1443 (1997).
15. Gill, H.S. & Prausnitz, M.R. Coated microneedles for transdermal delivery. *Journal of Controlled Release* **117**, 227-237 (2007).
16. Su, X., Kim, B.-S., Kim, S.R., Hammond, P.T. & Irvine, D.J. Layer-by-Layer-Assembled Multilayer Films for Transcutaneous Drug and Vaccine Delivery. *ACS Nano* **3**, 3719-3729 (2009).
17. DeMuth, P.C., Su, X., Samuel, R.E., Hammond, P.T. & Irvine, D.J. Nano-Layered Microneedles for Transcutaneous Delivery of Polymer Nanoparticles and Plasmid DNA. *Advanced Materials* **22**, 4851-+ (2010).
18. Clark, R.A., *et al.* The Vast Majority of CLA+ T Cells Are Resident in Normal Skin. *J Immunol* **176**, 4431-4439 (2006).
19. Widera, G., *et al.* Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system. *Vaccine* **24**, 1653-1664 (2006).
20. Jewell, C.M., Zhang, J., Fredin, N.J. & Lynn, D.M. Multilayered polyelectrolyte films promote the direct and localized delivery of DNA to cells. *J. Controlled Release* **106**, 214-223 (2005).

21. Kim, B.-S., Park, S.W. & Hammond, P.T. Hydrogen-Bonding Layer-by-Layer-Assembled Biodegradable Polymeric Micelles as Drug Delivery Vehicles from Surfaces. *ACS Nano* **2**, 386-392 (2008).
22. Macdonald, M., Rodriguez, N.M., Smith, R. & Hammond, P.T. Release of a model protein from biodegradable self assembled films for surface delivery applications. *J. Controlled Release* **131**, 228-234 (2008).
23. Prausnitz, M.R., Mikszta, J.A., Cormier, M. & Andrianov, A.K. Microneedle-based vaccines. *Curr Top Microbiol Immunol* **333**, 369-393 (2009).
24. Fuller, D.H., Loudon, P. & Schmaljohn, C. Preclinical and clinical progress of particle-mediated DNA vaccines for infectious diseases. *Methods* **40**, 86-97 (2006).
25. Babiuk, S., Baca-Estrada, M., Babiuk, L.A., Ewen, C. & Foldvari, M. Cutaneous vaccination: the skin as an immunologically active tissue and the challenge of antigen delivery. *Journal of Controlled Release* **66**, 199-214 (2000).
26. AnneMorris, H., Joseph, A.B. & Max, P.O. Live Attenuated Bacterial Vaccines: New Approaches for Safety and Efficacy. *The Lancet* **325**, 1472-1474 (1985).
27. Tang, D.-c., DeVit, M. & Johnston, S.A. Genetic immunization is a simple method for eliciting an immune response. *Nature* **356**, 152-154 (1992).
28. Ulmer, J., *et al.* Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* **259**, 1745-1749 (1993).
29. Wang, B., *et al.* Gene inoculation generates immune responses against human immunodeficiency virus type 1. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 4156-4160 (1993).
30. Fynan, E.F., *et al.* DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 11478-11482 (1993).
31. Ruprecht, R.M. Live attenuated AIDS viruses as vaccines: promise or peril? *Immunological Reviews* **170**, 135-149 (1999).
32. Offit, P.A. The Cutter Incident, 50 Years Later. *New England Journal of Medicine* **352**, 1411-1412 (2005).
33. Ulmer, J.B., Wahren, B. & Liu, M.A. Gene-based vaccines: recent technical and clinical advances. *Trends in Molecular Medicine* **12**, 216-222 (2006).
34. Le, T.P., *et al.* Safety, tolerability and humoral immune responses after intramuscular administration of a malaria DNA vaccine to healthy adult volunteers. *Vaccine* **18**, 1893-1901 (2000).
35. Mincheff, M., *et al.* Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: A phase I/II clinical trial. *European Urology* **38**, 208-217 (2000).
36. MacGregor, R.R., *et al.* First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: Safety and host response. *Journal of Infectious Diseases* **178**, 92-100 (1998).
37. Tacket, C.O., *et al.* Phase 1 safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine* **17**, 2826-2829 (1999).
38. Liu, M.A. & Ulmer, J.B. Human Clinical Trials of Plasmid DNA Vaccines. in *Advances in Genetics*, Vol. Volume 55 (eds. Jeffrey C. Hall, J.C.D.T.F. & Veronica van, H.) 25-40 (Academic Press, 2005).

39. 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* **19**, 95-103 (2004).
40. Simonsen, L., Kane, A., Lloyd, J., Zaffran, M. & Kane, M. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. *Bull World Health Organ* **77**, 789-800 (1999).
41. Miller, M.A. & Pisani, E. The cost of unsafe injections. *Bull World Health Organ* **77**, 808-811 (1999).
42. Ekwueme, D.U., Weniger, B.G. & Chen, R.T. Model-based estimates of risks of disease transmission and economic costs of seven injection devices in sub-Saharan Africa. *Bull. World Health Organ.* **80**, 859-870 (2002).
43. Das, P. Revolutionary vaccine technology breaks the cold chain. *Lancet Infect Dis* **4**, 719 (2004).
44. Mitragotri, S. Immunization without needles. *Nat. Rev. Immunol.* **5**, 905-916 (2005).
45. Belyakov, I.M., Hammond, S.A., Ahlers, J.D., Glenn, G.M. & Berzofsky, J.A. Transcutaneous immunization induces mucosal CTLs and protective immunity by migration of primed skin dendritic cells. *Journal of Clinical Investigation* **113**, 998-1007 (2004).
46. Gockel, C.M., Bao, S.S. & Beagley, K.W. Transcutaneous immunization induces mucosal and systemic immunity: a potent method for targeting immunity to the female reproductive tract. *Mol. Immunol.* **37**, 537-544 (2000).
47. Berry, L.J., *et al.* Transcutaneous immunization with combined cholera toxin and CpG adjuvant protects against Chlamydia muridarum genital tract infection. *Infection and Immunity* **72**, 1019-1028 (2004).
48. Giudice, E.L. & Campbell, J.D. Needle-free vaccine delivery. *Adv. Drug Deliv. Rev.* **58**, 68-89 (2006).
49. Prausnitz, M.R. & Langer, R. Transdermal drug delivery. *Nat. Biotechnol.* **26**, 1261-1268 (2008).
50. van Ginkel, F.W., Jackson, R.J., Yuki, Y. & McGhee, J.R. Cutting Edge: The Mucosal Adjuvant Cholera Toxin Redirects Vaccine Proteins into Olfactory Tissues. *J Immunol* **165**, 4778-4782 (2000).
51. Zhao, Y.L., *et al.* Induction of cytotoxic T-lymphocytes by electroporation-enhanced needle-free skin immunization. *Vaccine* **24**, 1282-1290 (2006).
52. Haq, M.I., *et al.* Clinical administration of microneedles: skin puncture, pain and sensation. *Biomed Microdevices* **11**, 35-47 (2009).
53. Sullivan, S.P., *et al.* Dissolving polymer microneedle patches for influenza vaccination. *Nat Med* **16**, 915-920 (2010).
54. Cariello, N.F., Keohavong, P., Sanderson, B.J.S. & Thilly, W.G. DNA damage produced by ethidium bromide staining and exposure to ultraviolet light. *Nucleic Acids Research* **16**, 4157-4157 (1988).
55. Hammond, P.T. & Macdonald, M. Self Assembled Films for Protein and Drug Delivery Applications. in *USPTO* (Massachusetts Institute of Technology (Cambridge, MA, US), United States, 2008).
56. Prausnitz, M.R. Microneedles for transdermal drug delivery. *Adv. Drug Delivery Rev.* **56**, 581-587 (2004).

57. Jiang, J., *et al.* Coated microneedles for drug delivery to the eye. *Invest Ophthalmol Vis Sci* **48**, 4038-4043 (2007).
58. Gill, H.S. & Prausnitz, M.R. Coating formulations for microneedles. *Pharm. Res.* **24**, 1369-1380 (2007).
59. Lynn, D.M. Peeling back the layers: Controlled erosion and triggered disassembly of multilayered polyelectrolyte thin films. *Advanced Materials* **19**, 4118-4130 (2007).
60. Jessel, N., *et al.* Multiple and time-scheduled in situ DNA delivery mediated by Î²-cyclodextrin embedded in a polyelectrolyte multilayer. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 8618-8621 (2006).
61. Sheridan, C. The business of making vaccines. *Nat Biotech* **23**, 1359-1366 (2005).
62. Global Vaccines Market. (Frost & Sullivan, 2009).
63. Weir, E. & Hatch, K. Preventing cold chain failure: vaccine storage and handling. *CMAJ* **171**, 1050 (2004).
64. Allen, M.G., Prausnitz, M.R., Mcallister, D.V. & Cros, F., Paul Marcel. Microneedle devices and methods of manufacture and use thereof. (ed. USPTO) (Georgia Tech Research Corporation (Atlanta, GA), United States, 2002).
65. Allen, M.G., Prausnitz, M.R., Mcallister, D.V. & Cros, F., Paul Marcel. Microneedle devices and methods of manufacture and use thereof. (ed. USPTO) (Georgia Tech Research Corporation (Atlanta, Georgia), United States, 2009).
66. Park, J.H., Allen, M.G. & Prausnitz, M.R. Polymer microneedles for controlled-release drug delivery. *Pharmaceutical Research* **23**, 1008-1019 (2006).
67. Gill, H.S. & Prausnitz, M.R. Coated Microstructures and Methods of Manufacture Thereof. (ed. USPTO) (Georgia Tech Research Corporation (Atlanta, GA, US), United States, 2008).
68. Lynn, D.M., Vazquez, E., Langer, R. & Hammond, P.T. Methods of making decomposable thin films of polyelectrolytes and uses thereof. in *USPTO* (Massachusetts Institute of Technology (Cambridge, MA, US), United States, 2006).
69. Czerepak, E.A. & Ryser, S. Drug approvals and failures: implications for alliances. *Nature Reviews Drug Discovery* **7**, 197-198 (2008).