# Improving Vaccine Equity with Novel Technology Platforms

by

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The goal of global vaccine equity, the idea that vaccines should be allocated across all countries based on needs rather than economic status, comes across as a goal that everyone can agree on. The emergence of a global pandemic gave clarity to how constrained our vaccination methods currently are, shining a blinding light of realism on how out of reach our objective of global vaccine equity may be. If we are to continue with our current methods of vaccine development and distribution, standard intramuscular injection, this recurring global vaccine inequity crisis will likely prevail.

To address the inefficiencies of today's approach toward global vaccination, we propose a new approach. Our thesis is that the solution to this global vaccine inequity crisis is new technology. Here, we propose to improve vaccine equity using novel technology platforms, specifically through the emerging biotechnology and key attributes of the microneedle array patch.

As a collective, the outline for this thesis is as follows: First, it is necessary to establish that there is a problem of vaccination inequity around the world. More so, though our current methods for global vaccine administration are not entirely ineffective, they fall short of fulfilling the need for global vaccine equity. After this has been established, we will analyze why it is necessary to take the risk in pivoting to a novel vaccination strategy, via dissolvable microneedle array patch delivery, as opposed to continuing our current strategy: why it is necessary to change what we are doing now. Then, we will support the argument for change by describing the technology and benefits it enables, the rationale for the vaccination approach, and preliminary studies that support

feasibility. Together, we present the ethical, public health, technical, and scientific basis for a new focus on emerging technology to address global vaccine equity, and how this strategic shift can work.

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## Preface

I would like to thank everyone who has helped and supported me in making my undergraduate journey at the University of Pittsburgh one I will always remember. This research was made possible by my thesis advisor, Dr. Zhaoyang You, who granted me the opportunity to pursue what I love. Dr. Zhaoyang You's guidance, expertise, and encouragement have made this pursuit more than worth it.

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Finally, I would like to thank all administration associated with the Frederick Honors College. Because of them, I, with many of my undergraduate colleagues, have a valuable and unique opportunity to share what we love and are passionate about. It is my hope, that as a consequence of doing so, we all may inspire future students at the Frederick Honors College to do the same.

## 1.0 Technology Is the Next Step Forward in Pursuit of Vaccine Equity

When it comes to the moral responsibility of vaccine equity, fear of the unknown repercussions associated with COVID-19 in conjunction with the limitations of our technology has complicated the ethics of the developed world. At the surface level, the idea that vaccines should be allocated across all countries based on needs rather than economic status comes across as a goal that everyone can agree on. However, the emergence of a global pandemic gives clarity to how constrained our vaccination distribution methods currently are, shining a blinding light of realism on how out of reach our objective of global vaccine equity may actually be.

This is not to say the responsibility of vaccine equity has entirely gone by the way side, but speaks more to the harsh reality of how feasible the objective of vaccine equity is given the factors of overwhelming urgency, the resources that we have, and our current strategies for using them. Today's standard approach to hypodermic needle intramuscular vaccine development and distribution has created a gap in vaccine equity on an international scale.

As of April 5th, 2022, over 60% of the population in high income countries have received at least one dose of the COVID-19 vaccine, while only 2% of people of low-income countries have received at least one dose (Vanderslott et al., 2022). More so, reports have shown only 0.3% of total vaccine doses administered globally have been administered in low-income countries (Vanderslott et al., 2022).

In agreement with these statistics, the United Nations further substantiates the existence of vaccine inequity. Specifically, the UN reports that over 90% of high-income countries have

reached a high level of vaccine coverage, while only 10% of low-income countries have reached the same level (United Nations, 2023).

Additionally, studies done here in the United States confirm global vaccine inequity. NYU School of Global Health reports that COVID-19 vaccine administration in African countries did not reach 60% coverage until 2023, given our current rate and strategy rate of vaccine administration. Meanwhile, 86% of all vaccines today are still being distributed between high income countries, like the U.S. and the United Kingdom.

The World Health Organization (WHO) has taken notice of this. As they confirm, this limited vaccine supply and unequal distribution drive global disparities. According to the WHO, as of September 2021, only 2% of the population in Africa had been fully vaccinated against COVID-19, compared to 60% in Europe and 49% in the Americas (World Health Organization, 2023). Beyond merely the SARS-COV2 vaccine, the WHO reports that the HPV vaccine against cervical cancer has been introduced in only 41 percent of low-income countries (despite carrying a significant portion of the disease burden) compared to 83 percent of high-income countries (World Health Organization, 2023).

This data suggests that our current methods and strategies for vaccine distribution, provided by our standard model of intramuscular injection, leads to global vaccine allocation that is far from equitable for every country. If we are to continue with our traditional intramuscular approach to developing and distributing vaccines, this vaccine inequity crisis will continue to be felt across the world.

Needless to say, coronavirus has demonstrated a critical need for the development of effective and rapidly deployable vaccines against a broad range of pathogens. Importantly, experience gained through the pandemic has defined the strengths and limitations of current

vaccine technologies. Ethical and public health questions, including important considerations of vaccine equity, have been brought to the forefront now more than ever. In the exploration of these persisting limitations, I will review the idea of vaccine equity and the issues in vaccine development and distribution that contribute to vaccine inequity.

### 1.1 Developing New Technologies to Improve Vaccine Equity

To address these limitations, I ask the reader to consider the possibility of using emerging technology to address the age-old vaccine inequity crisis felt around the world. As an alternative to major and costly infrastructure revisions in the current care delivery model, I propose that efforts to develop next generation vaccines should include efforts to assure that they are shelf-stable, easily distributed, self-administered, and cost-effective. In this way, new vaccine technologies could more effectively narrow the gap in vaccine access.

Thus, in the larger context, I propose that an example of this is the development of needle-free vaccine technologies, and specific strategies to effectively deliver vaccines to the skin using a simple patch: a microneedle array patch.

Microneedle patches offer the potential for more efficient and less expensive vaccine distribution, especially when considered on an international scale. Microneedle patches embody temperature-independent technology in contrast to standard vaccines that require highly demanding and expensive refrigeration when packaged and shipped across significant distances. By removing the challenge of keeping vaccines at freezing temperatures from an already challenging supply chain, the efficiency of distribution and accessibility of vaccines could increase

dramatically. I suggest that this emerging technology exemplifies an opportunity to change the global vaccine delivery paradigm. Large-scale distribution of microneedle patches can offer a less complicated, less expensive, and more transparent alternative than our current approach to vaccine distribution at the domestic and international levels.

With this, we are nearing the emergence of a less expensive and more easily distributive method for vaccine administration. Novel technology urges us to reevaluate our current methods for vaccine administration taking into consideration the efficacy of vaccination protection and the potential to overcome the economic challenges of large-scale distribution. These medical, public health, and ethical factors ultimately funnel into one moral question regarding the current vs. novel approaches for global vaccine distribution in relation to their contributions to vaccine equity: Is it best to continue with and expand our current strategy for vaccine administration, or is it worth pivoting to new technology and a new strategy for vaccine administration all together with the hope of closing the gap in vaccine equity.

It is necessary to flesh out both potential solutions to this moral question. First off, when analyzing the current approach to vaccine administration, the question arises as to whether or not we should continue with the proven methods and confirmed efficacy behind the standard, intramuscular needle vaccine delivery model when presented with a potential alternative. Although the current method of vaccine delivery has been shown to be successful in developed countries, it carries the burden of being a relatively expensive and less accessible method for developing countries. Because of distribution limitations, developing countries continue to have much lower access to vaccinations: vaccine inequity remains.

Alternatively, when analyzing a novel approach to vaccine administration, such as intradermal needle-free vaccination via microneedle delivery, the question arises as to whether we

ought to explore unproven methods associated with the unconfirmed protection efficacy that is associated with novel intradermal delivery. Although this method of vaccine delivery has yet to be confirmed to be as effective as intramuscular delivery in protection from disease, on an international distribution scale, its technology directly addresses vaccine logistics, economics, and distribution concerns. It is a considerably less expensive, more efficient, and more accessible method of vaccination for developing countries. Although these logistical efficiencies are untested for large-scale distribution, vaccine distribution limitations for developing countries are directly addressed: improvement toward vaccine equity could prevail.

After further evaluation of these two options, we are faced with the question of whether we should continue with the approach for vaccine administration that we have been using for decades, and that is supported by proven success in many developed countries. The alternative option, on the other hand, is one that has largely been untested on the global stage, and thus may come across as more of a risk, especially in response to the crisis of a pandemic felt across the world. In our current world of limited resources, is risking the development of a new vaccination strategy worth the potential achievement of closing the gap in vaccine equity? Or should we continue with what we know to be successful, ensuring millions will be effectively vaccinated, but not necessarily with as much care for where and to whom the accessibility of vaccines is gifted?

### 1.2 Taking the Risk – The Capability Approach

I will argue in favor of taking the risk; it is worth pivoting to a novel vaccine administration strategy in pursuit of closing the gap in vaccine inequity. To support my position, I will demonstrate how this action falls under the ethical foundation and moral perspective of the

capability approach. Specifically, I will provide a sufficient exploration of the creation and distinctive qualities associated with the capability approach, and how exactly it has come to be such a supported and aspirational approach to public health ethics today. Then, I will evaluate the capability approach's consideration of the six detriments of well-being along, with its underlying mission of pursuing human well-being with priority for the people and nations that are inherently disadvantaged. Taken together, the capability approach as a whole, with these two unique contributions to public health ethics most of all, will be used to support my position on why pivoting to a novel vaccine administration strategy is a better alternative than holding on to the vaccine administration strategy that is in use today.

After it has been established why the current gap in vaccine equity must be addressed, and thus why it is necessary to risk evolving today's approach to health and vaccine administration strategies that we use on a global stage, it is then necessary to transition to an in-depth analysis of what we are going to do about it. Specifically, we will shift our focus to what microneedle arrays exactly are, how microneedle arrays work, what makes them different compared to vaccination methods used today, the particular advantages they bring to the table in the promotion of vaccine equity, and most importantly why we all ought to be confident that microneedle arrays hold the potential to promote global vaccine equity to a level far greater than our current administration strategies can achieve.

Finally, it is necessary to explore how exactly the biocargo within microneedles operates within our skin's immune system once effectively delivered. Discovering safe and effective adjuvants to deliver with antigens via microneedle arrays is an essential piece of the puzzle for the development of effective MNA vaccines. In later chapters, we evaluate the effects of MNA delivered adjuvants on the immune response in the skin microenvironment. Specifically, we

develop a series of adjuvant incorporating MNAs and evaluate their delivery and effect on cytokine expression in the skin as a marker of a proinflammatory skin immune microenvironment favorable for the induction of vaccine-induce pathogen-specific antibodies and T cells. And so, the final third of this paper will be dedicated to explain specifically how immune responses work in relation to the adjuvants we have created specifically to be delivered via dissolvable microneedle array delivery. To support this explanation, data we have collected over the years will be presented to demonstrate adequate cytokine expression in response to the specific adjuvants we have engineered to comprise the biocargo of microneedle arrays.

Taken together, our data suggest that adjuvants can be integrated into MNAs and can induce skin immune responses associated with the induction of potent antigen-specific Immunity, further supporting the potential of MNAs as a more effective, efficient, and patient-friendly immunization approach compared to our current vaccine strategies.

As a collective, the outline for this thesis is as follows: First, it is necessary to establish that there is the problem of a vaccination inequity crisis around the world. More so, though our current methods for global vaccine administration are not entirely ineffective, they fall short of fulfilling the need for global vaccine equity. After this has been established, we will then analyze why it is necessary to take the risk in pivoting to a novel vaccination strategy, via dissolvable microneedle array delivery, as opposed to continuing our current strategy: why it is necessary to change what we are doing in the first place. Then, it will be clarified what specifically this novel strategy and technology are: what we are going to do about this problem. Finally, we will discuss how skin immunization, the biocargo we offer, and ultimately how the vaccinations we develop will actually work: why our solution can work.

As will be discussed in detail in subsequent chapters, vaccines offered by microneedle arrays have considerable advantages over current delivery methods, particularly in the context of enabling global vaccine equity. For example, microneedles offer key attributes such as temperature stability, storage, and stockpiling facilitation, and serve as an overall inexpensive substitute in comparison to standard intramuscular injection models. The innovations and innate advantages microneedles offer to vaccine delivery serve as a step forward toward promoting global vaccine equity.

### 1.3 Applying the Capability Approach to Vaccine Equity

Beginning with a general understanding of the capability approach, it is necessary to give sufficient background on what the approach entails and why it is important to health policy administration today. The capability approach largely stems from the concept that the majority of the world's conventional, economic indicators are primarily used for broad assessments and are valued as averages within a population (Nussbaum, 2013). A well-known example of one of these indicators is Gross Domestic Product. Although GDP gives insight into the overall devolvement of a nation and its population as a collective average, it gives little to no recognition to the economic status of people on an independent, individual level. Thus, an indicator like GDP makes it difficult to pose challenges towards genuine insight into how the world's billions of individuals are experiencing aspects of their well-being on a personal level.

For example, take a situation where a conventual economic indicator, like GDP, shows a promising increase but other attributes, such as health care or basic education, are not being developed appropriately. Regardless of painfully slow, or perhaps even reversive, growth to

integral attributes to one's wellbeing, they are ultimately glanced over when collecting an average that takes into consideration an overwhelming number of other factors eluding to a result of an overall increase in GDP. The question then arises how telling is a factor like this really in reference to whether or not a country is making real and significant "progress"?

As an alternative, the capability approach does not merely focus on the success of a country's economy and whether or not one merely has "the right" or the freedom to succeed within that economy, but takes it to the next level; *it analyzes the realistic capability for each individual to do so.* The capability approach is specifically dedicated not just to the formal freedom to be able to do or be something; it is dedicated to the substantial opportunity to achieve it. The sense of freedom and measure used in the capability approach is the idea that one has all the required means necessary to achieve whatever level of wellbeing one wishes too, and to do so to their highest potential.

Within the ethical foundation of the capability approach emerge two focus points that are particularly applicable in favor of the argument for pivoting to an alternative vaccine administration plan. The first being the idea that proper measures of equality are not in agreement with the methodology of using strict equality as a proper distributive ideal of justice. Strict equality, in this sense, means, everyone in both the developed and developing world ought to receive the same standard of health distribution. The capability approach argues that, instead, priority for the worse off should be emphasized or at the least ensured that the worse off will 'have enough' to thrive under the universal standard of well-being.

More so, the capability approach is not focused on merely ensuring the aspects of income and wealth for the worse off as most sufficiency theories are. Rather, the capability approach is geared towards fulfilling six distinct social detriments that makeup one's well-being. These six

detriments are health, personal security, reasoning, respect, attachment, and self-determination (Nussbaum, 2013). Thus, it follows that the ethical foundations of the capability approach ought to support a global vaccination strategy that best suits the fulfillment of these six factors, and should specifically do so with the aim to prioritize countries in worse-off situations than others.

With this, it is integral to analyze how a pivot to the alternative method for vaccine administration via microneedle array delivery aligns with the promotion of each of the six detriments of well-being described by the capability approach. Beginning with health, it goes without saying that the promotion of vaccination availability is a benefit to an individual that may have not had access to this health advantage to begin with. Similarly, having the option of becoming defended from a virus, which may not have been possible in the first place, is a massive improvement towards the attribute of personal security. Even more, the dimensions of health and personal security are addressed in a way where its promotion is specifically targeted towards less developed communities as vaccination via a microneedle array is a more economically favorable, and thus more easily distributive, modality than our current, traditional methods for distribution. Thus, increased accessibility to vaccination is not just a benefit to the dimension of health and personal security as a whole, but is specifically aimed towards the improvement of those who are most disadvantaged.

A transition to this novel method for vaccine administration addresses the remaining four dimensions of well-being, too. Regarding the dimension of reasoning, this aspect of well-being goes hand in hand with what we ought to do and how we ought to live on an everyday basis. By increasing accessibility to vaccination, and therefore expanding on potential options for how to live, the novel vaccine administration approach generates options facilitating the capability to reason to one's highest potential.

Increased availability of vaccine access, particularly in underdeveloped countries, will improve the aspects of respect and attachment. In regard to the dimension of respect, a life lacking in respect for others is seriously deficient in something crucial to well-being (along with a life lacking in self-respect). More so, when an individual or country is perceived as having a lesser value because of identification with a particular race, gender, or economic class, it bleeds into the dignity of the well-being of both the victim and the accuser. It follows that we must address our vaccine administration system with a distribution strategy that will respect neighboring nations, regardless of associated races or economic status, as equals when it addresses the concern of vaccine accessibility.

The dimension of attachment, which is integral for promoting feelings of love, engagement, and compassion for one's own community and between other communities, ties into this as well. It is of ethical obligation and duty to both our own as well as our neighbors well-being to strengthen our international attachment between countries and cultures. With this, it follows that we ought to address vaccine administration that supports this aim of building upon the elements of international respect and attachment. With the suggested approach of novel vaccine administration via microneedle patches, we will not only be able to increase the accessibility to vaccination, but hence our respect and attachment to one another along with it.

A novel vaccination approach also addresses the last dimension of well-being: the aspect of self-determination. As described by the capability approach, self-determination serves as a foundation for other conclusions about what a just social structure requires. With this, it is to be believed that carrying out an action that supports the previous five dimensions, as well as having priority for those who are worse off, would coincide with the notion that this is something that is just and we ought to do. Following this belief, promotion, and action of self-determination would

follow suit with the promotion of the novel approach of vaccine administration via microneedle patches.

Beyond the improvement of the six dimensions of well-being, the capability approach speaks to how the ethical foundation and the foundational moral justification underlying public health is social justice. The aspect of this moral justification that is especially applicable to the novel vaccination approach is the idea that public health systems ought to address the systematic disadvantages that undermine the capacity for well-being, and draw attention to the moral urgency of the health needs of oppressed and subordinated groups. More specifically towards our current vaccine distribution system: countries with overwhelming low vaccination access and thus highly unvaccinated populations.

Additionally, the capability approach's view of public health specifically supports a change in vaccine administration in how it addresses the matter of healthcare distribution. It particularly criticizes standard views of public health in that they are primarily driven toward health outcomes and not matters of health distribution. From this, the capability approach emphasizes that public health systems ought to have a primary focus on necessary distribution to those most disadvantaged. With this, it follows that the moral justifications and ethical foundation of the capability approach support a strategy for vaccine administration that embodies the impulses for improving human well-being by improving health and all of its associated dimensions and does so specifically by focusing on the needs of those who are most disadvantaged.

This ethical outlook strongly supports a system for vaccine distribution that poses the best possibility of closing the gap in vaccine inequity. When considering the current methods for vaccine administration via standard intramuscular injection, there are economic obstacles to its distribution potential that ultimately can only close the gap in vaccine inequity so much between

developed and developing countries. Although this vaccine administration strategy has proven successful, it has limited potential for improving its distribution methods to disadvantaged countries and populations.

The capability approach would argue in favor of an alternative vaccination strategy that has the potential to address the most disadvantaged, puts the concern of international vaccine equity at the forefront, and holds the likelihood of still being an effective vaccine in it. Presented with the options of maintaining our current methods for vaccine administration or pivoting to new technology that directly addresses the concern of vaccine equity and all six dimensions of human wellbeing, as opposed to putting health above the rest, the capability approach would favor the latter. It follows that a vaccine administration strategy consisting of intradermal vaccination via microneedle patches is the superior option in improving the six dimensions of human well-being while embodying a focus on most disadvantaged countries.

As COVID-19 has demonstrated, the demand for addressing the unbalance in vaccine equity is as high as ever. Although our current vaccination delivery methods have proven to be successful, they have continued to show little promise in prioritizing the demand for vaccination access for countries in need. As the capability approach suggests, we ought to alter our strategy for vaccine distribution that incorporates social justice into our public health systems. All six dimensions of well-being should be factored into this decision as opposed to prioritizing health outcomes above the rest.

## 1.4 New Technology is the Answer

Current global economic limitations prevent the simultaneous pursuit of both improvements in existing distribution chains, and new approaches enabled by new technologies. Thus, if we must choose, new technology is the answer. Although never tested on a global stage, a novel approach to vaccine administration via delivery by microneedle patches holds the potential to be a more effective vaccination method in multiple dimensions. More than just its measures of effectiveness, this novel approach enables new distribution strategies that present solutions that promote vaccine equity: a social justice concern that seems to have been brushed aside for decades. Given the concern that vaccine inequity imposes, it follows that we ought to make a change in our vaccine technology and distribution system under the ethical foundation of the capability approach.

Given these considerations, in subsequent chapters, we address the feasibility of MNA vaccines, including the immunological rationale for targeting the skin immune system, the novel engineering advances that enable this new technology platform, and lastly, our own studies that support the feasibility of harnessing the skin immune system for effective vaccination by utilizing MNA delivered adjuvants.

## 2.0 The Skin Immune System and Skin-Targeting Vaccine Technologies

Now that we have identified the issue of global vaccine inequity and the ultimate limitations of our current vaccine development and distribution strategies in promoting a fair and right opportunity for vaccination for all people across the world, it is clear that vaccine inequity persists in our world today. We have established an immoral concern that has no obvious solution if we continue to do things the way we do them now.

After clearly identifying the context and harmful implications associated with this problem, we have then evaluated whether or not it is worth the risk of risking attempting a new vaccination strategy to pursue a more virtuous outcome in global vaccine availability. Specifically, we have concluded that it is worth the risk of pivoting to novel technologies to enable new vaccination strategies to promote global vaccine equity. Although yet to be tested on the world stage, the risk of diving into the depths of unknown outcomes is worth the effort in the attempt to bring us one meaningful step closer to resolving this vaccine equity crisis.

Continuing with our current and traditional intramuscular vaccine administration strategy, which we know to be successful, and can result in the effective vaccination of millions of individuals, is certainly the safer option. But this is without attention and concern for where and to whom the accessibility of the vaccines is gifted. The risk of change is necessary, and we should cultivate the courage to make it effective.

Thus, the answer to why this problem must be addressed, as well as the answer to why we are doing what we are doing, is clear. New vaccination technologies and strategies must be cultivated to address a vaccine inequity crisis felt across the world. Ideally, the current and future strategies will not be mutually exclusive. If sufficient funding for both could be secured, current

strategies would guarantee a minimum of the status quo while novel technologies emerge and eventually are implemented to replace them.

Now that we have addressed the why, we ought to turn to what we are specifically going to do about this problem. Skin targeted vaccines may be the answer. Specifically, skin-targeted vaccination holds the potential to bring our world one step closer to a common health equity standard for every human being.

We will now evaluate the feasibility of skin-targeted vaccines and the enabling technology that is emerging. First, we will consider the advantages of skin targeted vaccine delivery over traditional intramuscular needle injection. Specifically, why one should look to target human skin as opposed to muscle, and why the structural and functional characteristics of skin pose a promising target for vaccination. Next, we will explore current examples of emerging technologies that are enabling for skin targeted vaccine delivery. Specifically, we will consider dissolvable microneedle array patches (MAPs). We will seek a practical understanding of how this technology platform works, the advantages it brings to the table in comparison to intramuscular delivery techniques, and how they specifically could be used to promote the next generation of vaccine delivery.

## 2.1 Skin as a Preferred Target for Vaccine Delivery

There are two important advantages of targeting skin for vaccine delivery: 1) the skin is readily accessible, and 2) the skin is highly immunogenic. The structural and functional characteristics of human skin pose a promising opportunity for vaccination. A sophisticated organ, the skin is capable of integrating multifunctional structural and immune cells, a vascular network,

and an extensive lymphatic system. The skin has evolved for the purpose of protection. This includes physical protection provided by a tough outer layer referred to as the stratum corneum, and an underlying epidermis made primarily of keratinocytes, which as their name implies, produce protective keratin. Beneath the epidermis lies the dermis, which includes numerous fibroblasts that contribute to a fibrous scaffold. The dermis has both an extensive and organized vascular system that provides a constant supply of oxygen and nutrients to the skin cells, and a well-developed lymphatic system that serves as a series of highways connecting skin immune cells and their products to the skin draining lymph nodes. These lymph nodes are a critical component of the immune system. They facilitate immune cell communication which is critical to mount an effective local and systemic immune response.

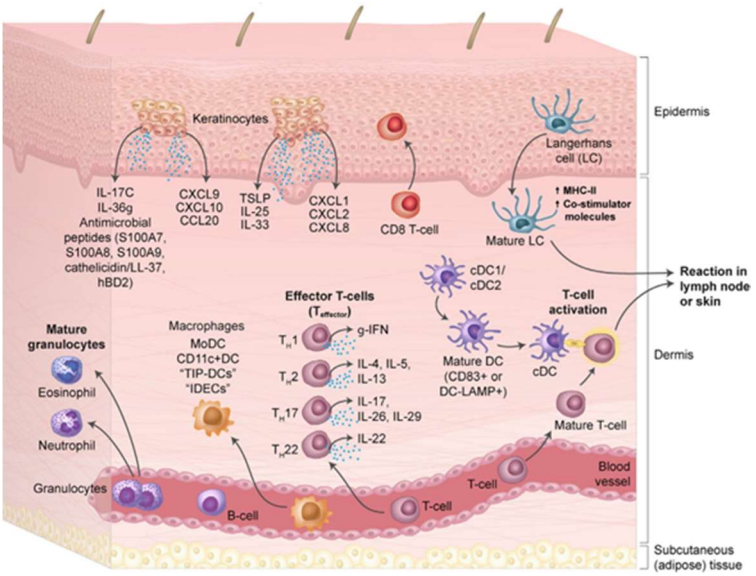



**Figure 1. Functional Anatomy of the Skin.** The skin is a sophisticated protective organ that integrates multifunctional structural and immune cells, a vascular network, and an extensive lymphatic system.

*(Image from Guttman-Yassky E, et all, 2019)*

There are multiple interacting immune cell types in the skin (**Fig.1**). These include:

**Dendritic Cells.** Dendritic cells (DCs) are present in both the dermis and epidermis (where they are referred to as Langerhans cells). Dendritic cells are known as professional antigen presenting cells (APCs) because of their capacity to internalize antigens from the environment, process them internally, and then present antigen fragments to T-cells to induce their activation against the specific antigen presented. Importantly, DCs present antigen to T-cells in the context of multiple costimulatory molecules that influence T-cell function and hence the overall immune response. DCs themselves are remarkably plastic in their function and are influenced by their environment. Thus, they serve as sentinel cells. They take up antigens in the skin, and deliver those antigens to T-cells in the draining lymph nodes in the context of costimulatory signals programmed in their skin environment. In response to "danger" signals, they program protective proinflammatory T-cell function. In the context of a normal baseline or suppressive signals they can prevent or reduce immune responses. In the context of vaccines, the introduction of antigens into the skin with "danger" signals via a vaccine result in a stimulated or proinflammatory dendritic cell that presents antigen to activate T-cell responses in the node. This leads to the production of antigen-specific antibodies and T-cells that can access the skin at the site of the invasion, and importantly can also travel throughout the body to localize in other mucosal tissues like those found in the lung and the gut. By integrating both environmental cues and antigen-specificity DCs induce antigen-specific adaptive (or "trained") immunity in response to environmental insults. Hence, they are critical orchestrators of vaccine responses.

**Macrophages and Granulocytes**. Macrophages are dermal resident cells that can engulf and degrade foreign pathogens and proteins and respond to "danger" signals by producing proinflammatory cytokines. They are part of the innate immune system that recognizes and responds to patterns of molecules from a broad range of pathogens. Innate immunity is rapid and not antigen specific and serves to initiate the development of adaptive immune responses that are antigen specific, typically by activating dendritic cells. Granulocytes, including neutrophils, basophils, and eosinophils can be recruited from the bloodstream to the skin to sites of infection or inflammation and contribute to innate immunity.

**Skin Resident T-Cells.** Multiple types of T-cells are present in the skin at relatively low frequencies. These include memory T-cells generated from past exposures that recognize their specific pathogens. On re-exposure to a pathogen, memory T-cells can quickly differentiate into active T-cells that can quickly target the pathogen. Thus, they can provide rapid antigen-specific protection to more commonly encountered pathogens.

**Innate Lymphoid Cells.** Innate lymphoid cells include subsets of cells that are part of the innate immune system. They are considered to be like T-cells, but they lack antigen specific T-cell receptors. They release cytokines in response to pathogen stimuli, contributing to the innate proinflammatory environment. They also play a role in skin homeostasis and tissue repair.

**Mast Cells.** Mast cells are located in the dermis and when activated release granules that contain histamine and a wide variety of cytokines and neuropeptides associated with "allergic" reactions. Typically triggered by the binding of antigens to antigen-specific IgE on their cell surface, they

generally induce a proinflammatory environment associated with the activation of dendritic cells and dendritic cell migration to the draining lymph nodes.

In addition to these cell types which are generally considered to be immune cells, the skin has several cell types that, while not classified as immune cells, can make important contributions to innate skin immunity. These include **keratinocytes** which play a critical structural role but also express pattern recognition receptors that recognize diverse danger signals. In response to these signals, keratinocytes produce cytokines, chemokines, and other immunologically active molecules that influence the function of other immune cells. While they can have either proinflammatory or anti-inflammatory effects, they generally induce a proinflammatory environment in response to "danger" signals. In addition, skin **fibroblasts** which are prevalent in the dermis and have primarily connective tissue functions have also been shown to secrete various cytokines and immune mediators in response to stress. Under certain circumstances, they can also secrete anti-inflammatory signals that are important for skin homeostasis. **Melanocytes**, whose primary function is to produce melanin and thus protect the skin from UV exposure can also affect immune responses. Melanin itself can scavenge free radicals to reduce oxidative stress in the skin and thus prevent DNA damage and skin inflammation. Under certain "danger" conditions, melanocytes can also express pattern recognition receptors and produce proinflammatory cytokines and chemokines.

Taken together, it is evident that the skin, unlike muscle, has an intricate immune system with multiple communicating immune cell types, and that it is a uniquely active immunological organ capable of communicating with draining lymph nodes to regulate both local and systemic immune responses. The immune system of the skin is the result of evolutionary pressures imposed

on the body's protective outer covering toward the primary goal of protection. In addition to physical protection, the skin has evolved an intricate immune system that can adapt to ever-changing environmental conditions and a broad range of noxious agents and pathogens. A network of immune cell crosstalk serves to rapidly adapt to the nature of the insult through the release of cytokines, chemokines, and other immune mediators to create a microenvironment in which dendritic cells, the sentinel cells of the immune system, are "trained" to internalize antigens, migrate to the draining lymph nodes, and present these antigens to T-cells in the context of signals "learned" in the skin environment. These signals then educate the B-cells and T-cells in the draining lymph node to recognize the specific antigen and perform the immune functions that are appropriate to respond to the changes in the skin environment. These features are characteristic of the skin and mucosal tissues perhaps as a result of the evolutionary pressures from constant exposure to the outside world. Other potential vaccine targets, like muscle, lack these innately "responsive" immune networks. Thus, the skin is very well suited to induce appropriate immunity in response to vaccine delivery. By including "danger" signals in vaccines, skin targeted vaccines could "engineer' the skin to induce effective anti-pathogen immune responses. Collectively, it is these important characteristics that make the skin an attractive target for vaccine delivery. In the next chapter, we will discuss the use of adjuvants as danger signals to engineer the skin to induce effective systemic immunity.

## 2.2 Overcoming the Skin Barrier to Vaccine Delivery

While the skin is the largest organ in the body, and directly accesses the external environment, the protective features of the skin can present a significant barrier to drug delivery.

For any intradermal vaccination attempt, our outermost layer of skin, the stratum corneum, is the protective barrier that must be overcome. With being as successful as it is at its job, that of fulfilling the role of an organic human shield, there comes the tradeoff of it being a challenging target to penetrate for vaccination. The stratum corneum is essentially composed of dead keratin filled keratinocytes in a lipid matrix. This structure protects us from physical injury and regulates water loss and temperature to maintain homeostasis. The lipid matrix and cross-linked keratinocytes restrict the movement of large, complex, or charged, molecules across the skin. Despite the development of various penetration enhancers, the large protein antigens or antigen-encoding nucleic acids that make up most modern vaccines do not penetrate this protective barrier. This prevents the development of topical creams or lotion-like vaccines. The use of small needles for intradermal delivery, such as those used for tuberculin skin tests, has also been attempted but is problematic. Intradermal injections are not straightforward, and typically require trained medical professionals. User variability is substantial, and additional inconstancies can arise due to variations in skin properties between different individuals, and in the presence of dermatologic abnormalities. Interestingly, it should be noted that the first successful vaccine, Edward Jenner's smallpox vaccine, was delivered by literally scratching the antigen (in this case the virus itself) into the skin with a crude device (**Fig. 2**). While effective, this approach lacked in reproducibility. Currently, there are many efforts underway to develop technology to enable vaccine delivery to the immune environment within the skin. Next, we describe an example of this technology in detail to demonstrate the potential of technological advances to safely penetrate the skin barrier and improve vaccine effectiveness and vaccine equity.

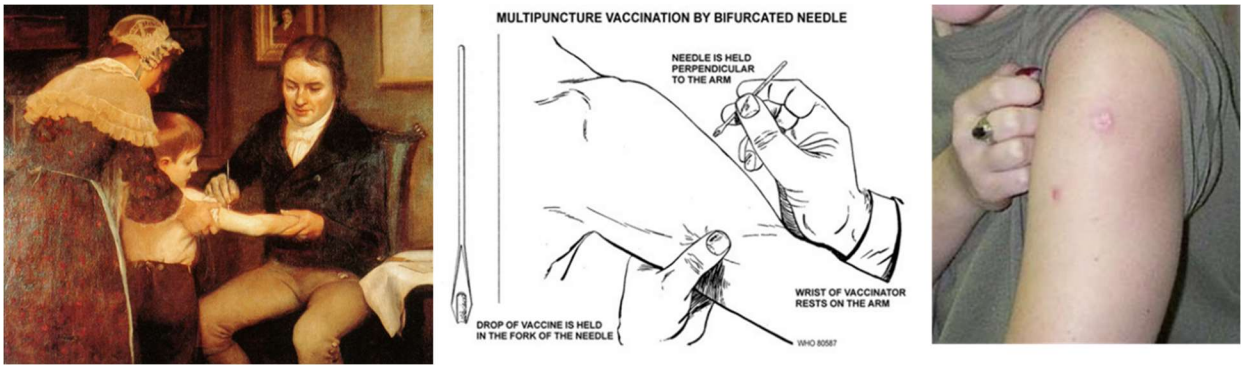



**Figure 2. Breaching the Skin Barrier for Vaccination – Edward Jenner and Scarification.** In Edward Jenner's time, smallpox killed approximately 10% of the population, with the number as high as 20% in towns and cities. On 14 May 1796, Jenner tested his hypothesis by inoculating James Phipps, an eight-year-old boy who was the son of Jenner's gardener.

## 2.3 Microneedle Array Patch Vaccine Delivery

Ideally, what is needed is a skin vaccine delivery approach that is highly reproducible, painless, and readily applied without a requirement for training or medical expertise. These were the desired performance characteristics that guided the development of dissolvable microneedle array technology. Put simply, dissolvable microneedle arrays (MNAs) were designed as a clinically feasible, patient-friendly platform technology for safe, precise, and consistent intradermal and transdermal biocargo delivery (Ingrole et al., 2021).

Microneedle array (MNA) technology is a rapidly developing field which has inspired considerable interest. The term microneedle array is broad and includes 4 predominate categories of microneedle array types (**Fig. 3**).

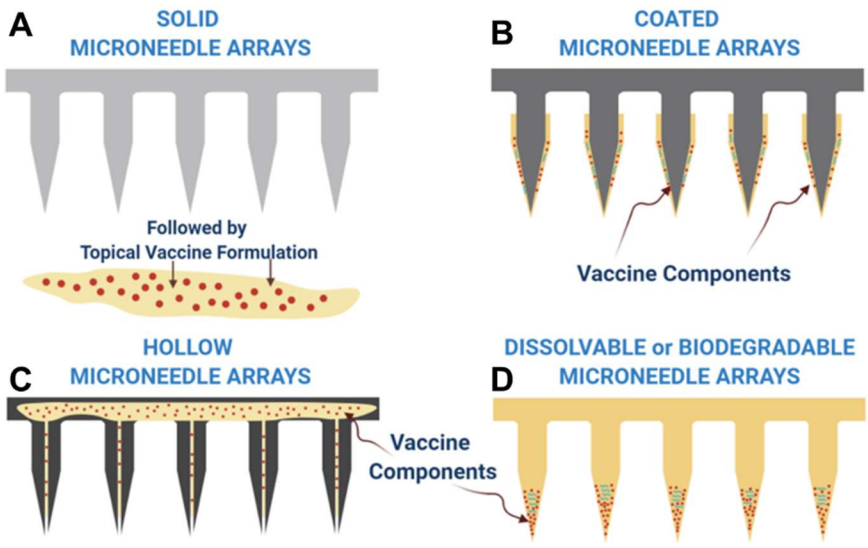



**Figure 3. Microneedle Arrays.** A. Solid, B. Coated, C. Hollow, and D. Dissolvable Microneedle Arrays.

Solid MNAs are typically made from metal and are used to poke holes in the stratum corneum and epidermis. This is followed by the application of a topical formulation that then diffuses through the openings created by the metal needles. This approach is limited by the extent and variability of diffusion of the cargo into the skin. Coated MNAs are also typically made of metal but have the cargo sprayed onto the solid metal needles. These coated MNAs are then pushed into the skin where the coated material comes in contact with the amount of material that can be coated onto the needles. Hollow MNAs typically consist of metal needles with a hollow center, much like miniaturized versions of the common hypodermic needle. The cargo is typically in a chamber above the needles and after the needles are inserted into the skin pressure is applied to the cargo reservoir pushing the cargo down the needle channels and into the skin. This approach is limited by the capacity of the cargo chamber and the ability to push the cargo into the skin against the hydrostatic pressure in the skin and skin substance that can clog the needles. Finally, the MNAs we will focus on are known as dissolvable MNAs. These MNAs are made from

biocompatible dissolvable materials (generally sugars) that, following skin insertion, rapidly dissolve on exposure to the skin's interstitial fluid. For dissolvable MNAs, the cargo is integrated into the dissolvable matrix, so that when the matrix dissolves, the cargo is released into the skin. This approach is readily adaptable for the delivery of a wide array of cargo types including small molecules, peptides, large proteins, DNA, and RNA. Skin penetration is dependent on material hardness and tip sharpness. Delivery is limited by the volume of the needles in the MNA patch.

As engineered in our labs, dissolvable MNA patches (MAPs) are typically fabricated from water-soluble biomaterials to incorporate vaccine components such as antigens and adjuvants (Balmert et al., 2021) (**Fig 4**).

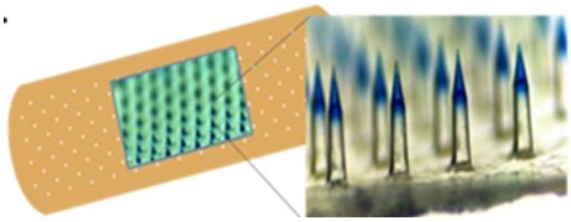


**Figure 4. Microneedle Array Patch.** Diagrammatic representation of a MAP with microneedle projections integrating a cargo (blue dye) in the needle tips**.**

Included in the patch are the "needles" which are sharp micro-scale projections integrated with a backing layer or substrate that creates a ready-to-use, potentially self-applicable vaccination device. The needles and backing are made through a spin-casting process in which the contents are dried into a prefabricated mold. Importantly no heat is used in the process, assuring that the cargo is not denatured or otherwise adversely affected by thermal stress. The material and geometric properties of MNAs are designed to deliver the integrated antigen and adjuvants loaded

into the biocargo while simultaneously providing a patient-friendly, painless, delivery approach (Korkmaz et al., 2021).

As far as application methods are concerned, dissolvable MNAs can be applied to the skin without medical expertise. Needle penetration can be accomplished by providing sufficient manual pressure to the back of the patch with a finger, and the needles dissolve delivering the biocargo within minutes. In this process, microneedles breach the stratum corneum in a minimally invasive way, rapidly dissolving into the skin microenvironment while actively delivering the contents within the defined skin layers (Amani et al., 2021; Paredes et al., 2021). Notably, the length of the needles is such that they do not reach the skin's blood vessels or nerves, thus enabling pain-free and bloodless vaccine delivery. In summary, dissolvable microneedle array patches (MAPs) are physical, single-unit, skin targeted biocargo delivery platforms that can persevere the embedded biocargos and mechanically penetrate the superficial cutaneous layers in a way that is minimally invasive to actively deposit their contents into the viable skin microenvironment (Balmert et al., 2022).

The needles in MAPs can be made with a broad range of geometries optimized for different applications. To provide an initial idea and visualization of this potential, **Figure 5** shows a three-dimensional representative dissolvable MNA platform that consists of an array of tip loaded microscale needles integrated with a backing substrate or layer (**Fig. 5a**).

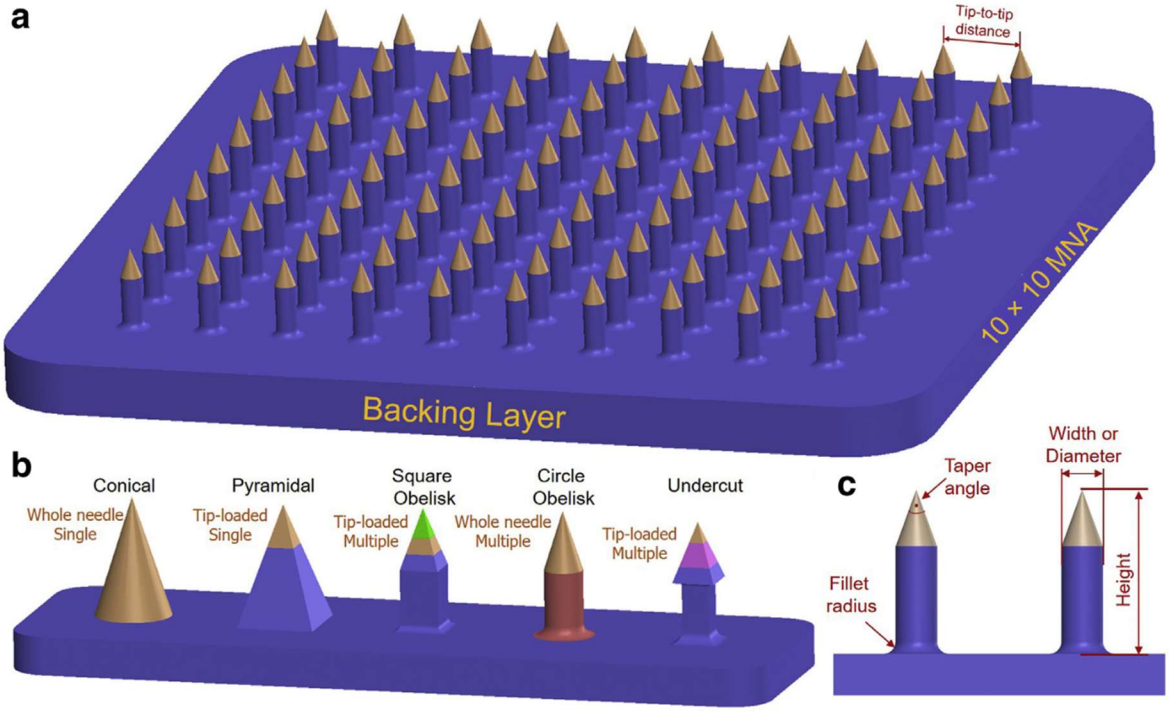



**Figure 5. Design Parameters** are engineered for the development of dissolvable MNA based biocargo delivery systems. (Image from Balmart, et all, 2022)

The needles themselves can be of various geometries and can integrate cargos throughout the needles, or distinct cargoes can be layered within the body of the needle (**Fig. 5b**) (Balmert et al., 2022). Specific design parameters (**Fig. 5c**) are chosen to achieve optimal skin penetration of the maximum number of microneedles with efficient dissolution of microneedles in the cutaneous environment. Importantly, needle design plays a role in the functional preservation of the integrated biocargo during drug delivery, especially for an extended period without refrigeration (Faraji Rad et al., 2017; Makvandi et al. 2021). Importantly, several types of cargo have been shown to be stable once integrated into microneedles without the need for refrigeration, thus obviating the cold chain for distribution.

To further address these factors, specific design parameters capable of controlling biocargo delivery performance of dissolvable MNAs have been evaluated. Especially impactful microneedle dimensions include aspects such as tip radius, width/diameter, height, and taper/apex angle. MNA patch size and the density of the needles across the entirety of the patch can influence delivery performance (Balmert et al., 2022). As an example, sharper tips decrease the skin penetration forces while the fillets towards the base of the needles reduce the overall stress concentration. This prevents the needles from breaking when the applied insertion pressure is not absolutely vertical. These features will improve the mechanical performance of the needles themselves and improve penetration and delivery efficiency (Balmert et al., 2020; Faraji Rad et al. 2017). Aligned with this design concept, needles that are too closely spaced or that are relatively short or blunt in design often fail to overcome the viscoelasticity of the skin (Makvandi et al., 2021). This has been referred to as the "bed of nails" effect.

Microneedle and array design parameters play a crucial role in controlling the reliability, reproducibility, and efficiency of skin insertion that results in a successful biocargo delivery. It follows that the biometric and geometric parameters in MNAs should be engineered in a way that ensures effective and reproducible skin delivery of specific cargo.

An important aspect of the bioengineering and manufacturing strategy behind MAPs are the designs of the molds themselves. Specifically, **Figure 6** shows representative and optical stereochemistry images of master MNA molds produced using 3D adaptive fabrication and 3D printing microscale (**Figs. 6a, 6b**) (Balmert et al., 2022).

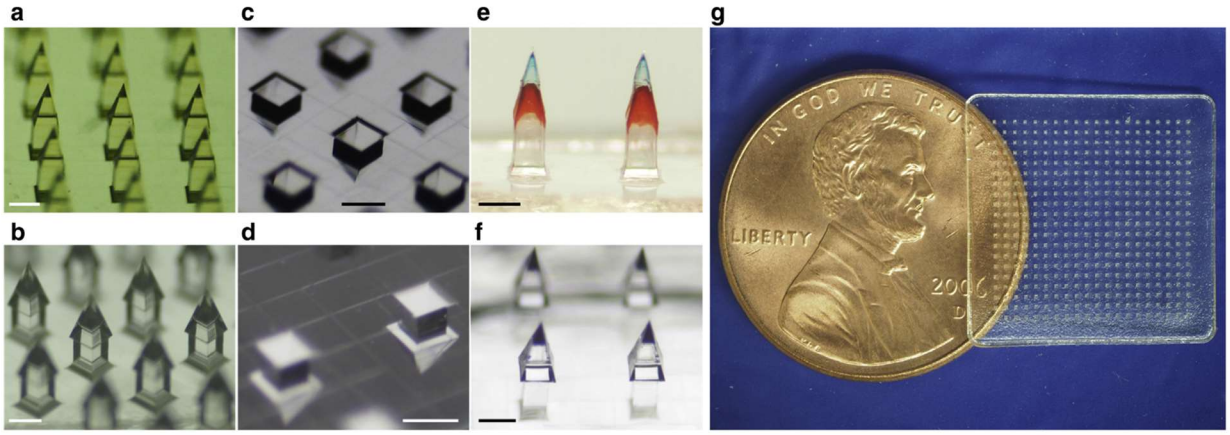



**Figure 6. MNA Manufacturing.** Representative products from the distinct processing steps of traditional three-stage MNA manufacturing strategy (Image from Balmert, et al, 2022)

These molds are then used to produce production elastomer production molds typically fabricated through soft lithography and micromolding (**Figs. 6c, 6d**). These molds can then be used to produce single or multiple cargo-loaded dissolvable MNAs (**Figs 6e, 6f)**. The needles shown here were created using different solvent-based spin casting strategies through centrifugation. To provide a birds-eye view visualization of these a finalized microneedle patch ready to use for clinical trials, a 20 by 20 (400 microneedle) MAP is shown in (**Fig. 6g**). This patch was manufactured from carboxymethylcelluose; a water-soluble biomaterial that is generally regarded as safe (GRAS) by the United States Food and Drug Administration.

With this, it is clear that the bioengineering strategies for the design of dissolvable MAPs play an integral role in enabling skin delivery of vaccines to induce an effective immune response. It is also important to take into account that the design strategies will impact the manufacturing approach, which in turn impacts clinical applicability. Thus, the optimal engineering parameters may not be practical in the context of large-scale clinical production. MAP design must take into account the need for clinical scale standard operating procedures essential to validate the

fabrication process for the quality control of biocargo loaded dissolvable MNAs for clinical applications (Leone et al., 2017; Zhang et al. 2021).

Because dissolvable MNAs can breach the outermost skin layers, they can effectively deliver a wide variety of bioactive compounds including proteins, hydrophobic and hydrophilic small molecule agents, and even recombinant viral vectors. Specific to this project, we will be focusing on biocargos that include adjuvants that serve as "danger" or "stress" to create a proinflammatory skin microenvironment necessary for the induction of an effective systemic immune response. For this reason, it is necessary to validate cargo delivery. MNA cargo delivery can be validated by histology and fluorescence image analysis. **Figure 7** demonstrates skin targeted cargo delivery using dissolvable MNAs.

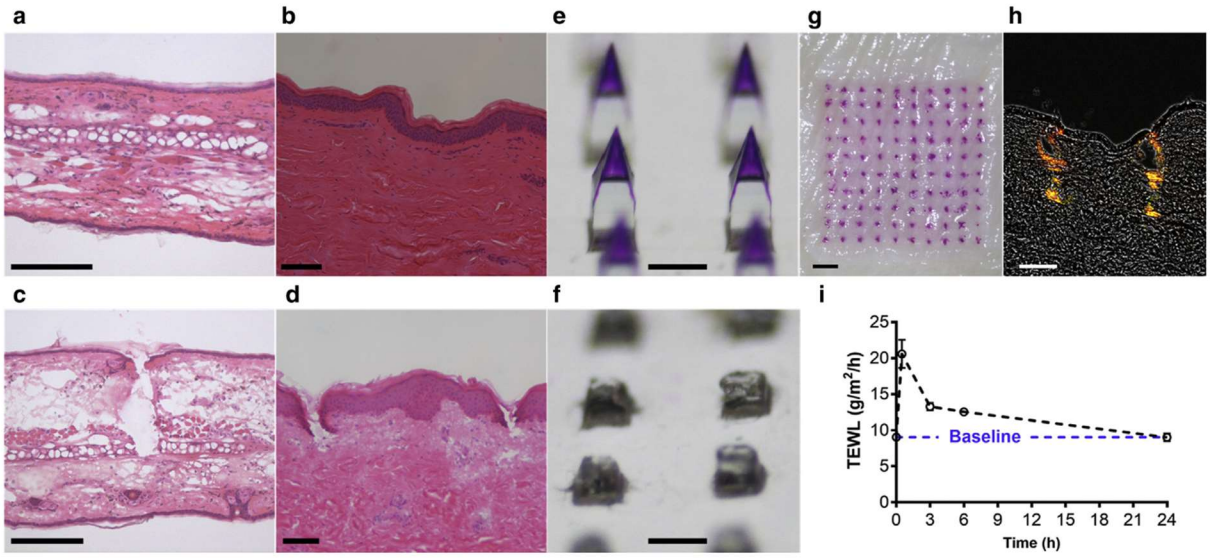



**Figure 7. Skin Targeted Cargo Delivery Using Dissolvable MNAs.** (Image from Balmert, et all, 2022)

This figure shows mouse skin before (**Fig. 7a, b, e**) and after (**Fig. 7c, d, f**) microneedle application and removal. MNA needle tracks can clearly be seen after MNA administration in **Figs 7c, d.** These tracks penetrate through the epidermis and well into the dermis of mouse skin. **Fig.**

**7e** shows the needles before MNA insertion, with a fluorescent dye loaded in the needle tips. **Fig. 7f** is a representative image of the needles in an MNA after 5 min. administration and removal in which the bodies of the needles are absent, having dissolved in the tissue. To evaluate needle insertion in human skin, freshly excised human skin samples (deidentified discarded normal skin from cosmetic procedures) were obtained from the Institutional Tissue Bank, and the tip-load MNAs were applied to the skin using pressure from the fingertip. **Fig 7g** shows a top surface visualization of human skin demonstrating the pattern of the delivered cargo in the skin after MNA removal, which corresponds to the needle pattern of the MNA. **Fig. 7h** is a histological cross section of the same skin, showing the depth of the deposited cargo in the needle tracks. Note that the cargo is delivered into the upper dermis of the skin, an area that is rich in dendritic cells and innate immune cells. **Fig. 7i** is a graphical depiction of the change in transepidermal water loss during and after MNA application. This demonstrates a rapid increase in TEWL immediately after MNA removal due to the holes in the epidermis at the sites of needle penetration. Over the next several hours, the TEWL returns to normal, indicating closure of the needle holes. MNAs shown in this figure typically dissolve in the aqueous skin microenvironment within minutes. Additionally, cutaneous delivery of dry cargo by these dissolvable MNAs has been shown to improve skin residence compared to intradermal needle injection of liquid biocargo (Zhao et al., 2016).

The dissolvable MNAs we are using are currently in clinical trials as a potential treatment for skin cancer. Shown in **Figure 8** is the clinical application of an MNA delivering a chemotherapeutic agent to a patient's skin (Balmert, 2022). The MNA is applied with fingertip pressure (**Fig. 8b**). **Figs 8c, and d** show the area of MNA application after removal (**Fig. 8c**) and

a dermatoscopic magnified image of the same area (**Fig. 8d**) demonstrating cargo delivery (red dots) consistent with the needle pattern of the MNA.

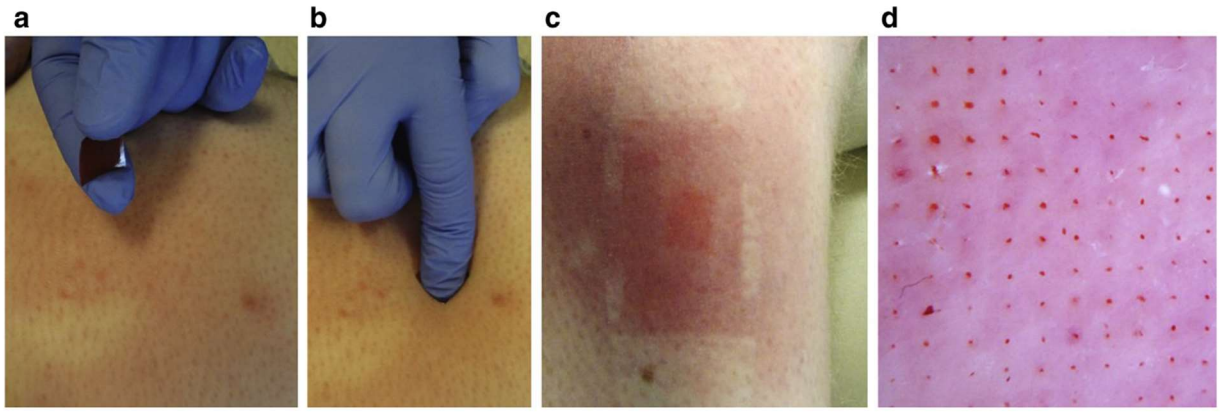



**Figure 8. Dissolvable MNA Based Delivery: Clinical Application.** (Image from Balmart, et all, 2022)

### 2.4 Skin Targeted MNA Vaccines Could Promote Global Vaccine Equity

Several features of skin-targeted MNA vaccine delivery support the idea that the development of this novel technology could promote global vaccine equity. We have discussed in detail, the sophisticated immune system of the skin. This system has resulted from evolutionary pressures as part of the body's protective outer covering toward the primary goal of protection. In addition to physical protection, the skin has evolved an intricate immune system that is capable of adapting to ever-changing environmental conditions and a broad range of noxious agents and potential pathogens. A network of immune cell crosstalk serves to rapidly adapt to the nature of the insult through the release of cytokines, chemokines, and other immune mediators to create a microenvironment in which dendritic cells are "trained" to internalize antigens, migrate to the draining lymph nodes, and present these antigens to T-cells in the context of signals "learned" in

the skin environment. These signals then educate the T-cells to recognize the specific antigen and perform the immune functions that are appropriate to the changes in the skin environment. These features are characteristic of the skin and mucosal tissues perhaps as a result of the evolutionary pressures from constant exposure to the outside world. Other potential vaccine targets, like muscle, lack these innately "responsive" immune networks. Thus, the skin is very well suited to induce appropriate immunity in response to vaccine delivery.

Several features of skin-targeted MNA vaccine delivery support the idea that the development of novel technologies could promote global vaccine equity. MNA delivery technology itself provides important advantages for broad global vaccination campaigns (summarized in **Fig. 9**).

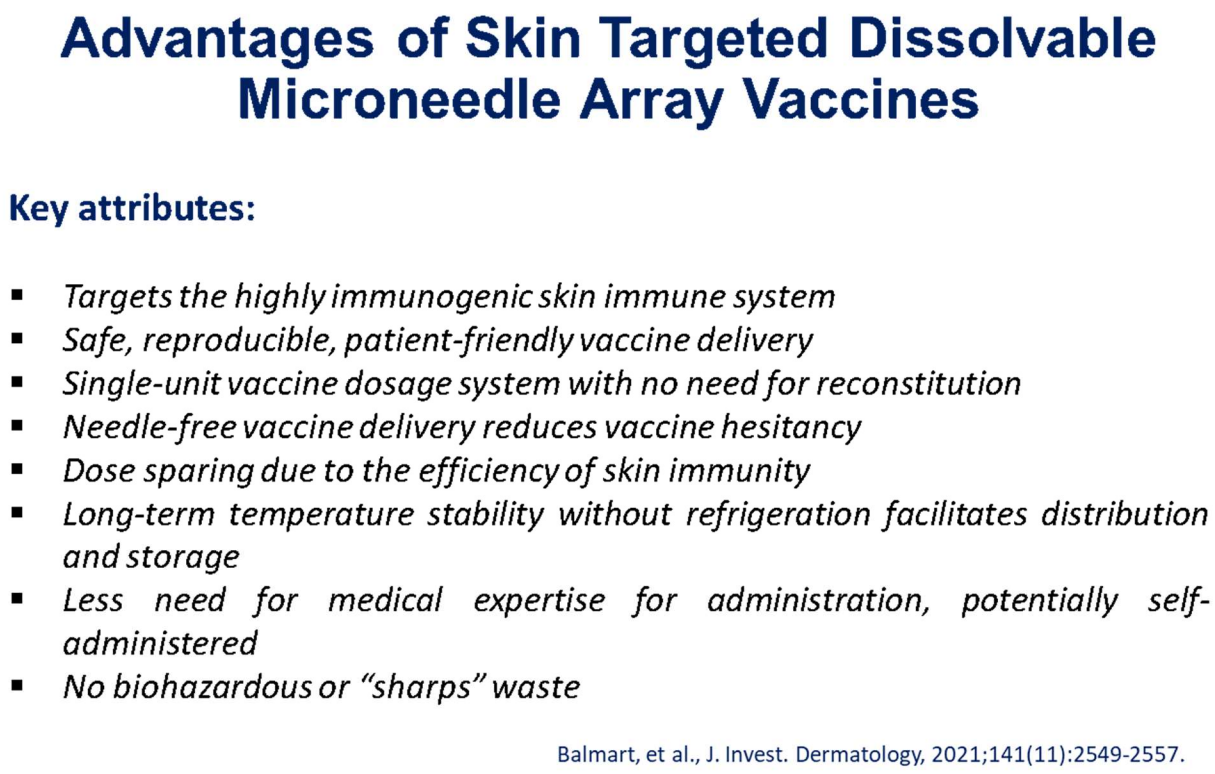



**Figure 9. Advantages of Skin-Targeted Dissolvable Microneedle Array Vaccines.**

The technology is reproducible, providing the same drug dose reliably across a wide range of conditions. It provides a single-unit drug and vaccine dosage system with no need for reconstitution. It does not require sharp needles, and is painless and bloodless, and is thereby patient friendly and thus supports patient compliance. Further, there is no biohazardous or sharps waste. The integration of vaccine components into the MNA stabilizes them. This results in long term stability without refrigeration, thereby eliminating the "cold chain", which currently is a major barrier to vaccine storage and global distribution. The MNAs are band-aid like and easy to apply, requiring no specific medical expertise, and may even enable self-application. Finally, the MNAs themselves are inexpensive to manufacture and readily scalable. This, combined with the advantage of dose-sparing due to the efficiency of skin immunity and the packaging and temperature stability provide substantial economic benefits that could significantly reduce the costs of global vaccine campaigns. Taken together, these features provide the potential to overcome many of the current obstacles to global vaccination campaigns, thus enabling progress toward vaccine equity across the world.

Our long-term goal is to develop a novel technology for effective skin-targeted immunization. Given this background, we believe that the MNA platform may enable society to reach this goal. We propose that the ideal MNA vaccine platform will include a protein antigen and an adjuvant in the same MNA. Given the extensive discovery efforts focused on adjuvants, surprisingly little is known about their effects on innate immunity in the skin. Thus, our immediate goal is to identify an adjuvant that, when delivered to the skin by MNA delivery, will simulate "danger" signals and induce a proinflammatory skin environment that will result in the stimulation of effective systemic immunity.

In the following chapter, we describe our initial studies in which we deliver representative well-defined small molecule adjuvants to the skin, and evaluate innate immune responses by quantifying changes in gene expression of proinflammatory cytokines and chemokines in the skin.

## 3.0 Engineering the Skin Immune System to Promote Vaccine Equity

We have presented rationale for the development of skin targeted vaccines as an approach to address the challenge of global vaccine equity. We have described the skin immune system and the rationale for targeting the skin for more effective vaccination. Further, we have presented examples of an emerging vaccination technology that can target the skin's immune system and provide unique advantages in vaccine efficacy, safety, economics, production, storage, distribution, administration, and patient acceptance.

Having established these key concepts, we now turn to one of the remaining challenges that must be addressed if we are to advance these technologies from concept to clinical reality. Optimally effective skin immunization, and in turn the success of each of the evolving skin-targeted vaccine technologies, requires the introduction of both a target pathogen antigen and a "danger" signal into the skin. The target antigen is essential for the antigen-specific adaptive immune response, including such effector mechanisms as antibody responses and helper and cytotoxic T-cell responses. These effector mechanisms are adaptive, and hence antigen-specific which restricts their activities to the target pathogen to avoid harm to normal self-tissues. In turn, "danger" signals are critical to activate the innate immune response, which though not specific to any pathogen, is required to create a proinflammatory environment necessary to train dendritic cells and other antigen presenting cells in the skin to stimulate the appropriate adaptive effector functions of T and B cells.

The capacity to identify and manufacture pathogen antigens is reasonably well established. Several large pharmaceutical companies are producing protein-based vaccines that target a wide variety of pathogens. Further, the COVID pandemic accelerated efforts to develop nucleic acid

(DNA and RNA) based vaccines, including the mRNA vaccines by Pfizer and Moderna that became the first approved vaccines against the SARS-CoV-2 virus. The COVID pandemic crisis accelerated these later efforts and demonstrated that the mRNA antigen format could be developed and produced to scale within months, rather than the years necessary to produce traditional protein antigen vaccines. On the other hand, despite extensive scientific development efforts, very few adjuvants have been approved for clinical use. Further, given the extensive discovery efforts focused on adjuvants, surprisingly little is known about their specific effects on innate immunity in the skin. Thus, our immediate goal is to support the feasibility of activating skin immunity by delivering adjuvants in MNAs. Thus, our experimental goal is to identify an adjuvant that, when delivered to the skin by MNAs, will simulate "danger" signals and induce a proinflammatory skin environment that will enable the stimulation of effective adaptive systemic immunity.

### 3.1 The Selection of Adjuvants for Skin Targeted Vaccines

Several cell types in the skin have evolved to express receptors that recognize signals associated with invading pathogens or other insults. Toll-like receptors are a type of pattern recognition receptor (PRR) that can be expressed by many types of skin cells (reviewed in Li and Wu, 2021). They play an important role in skin immunity by recognizing pathogen-associated molecular patterns (PAMPs) associated with bacteria, viruses, and other pathogens (**Fig. 10**).

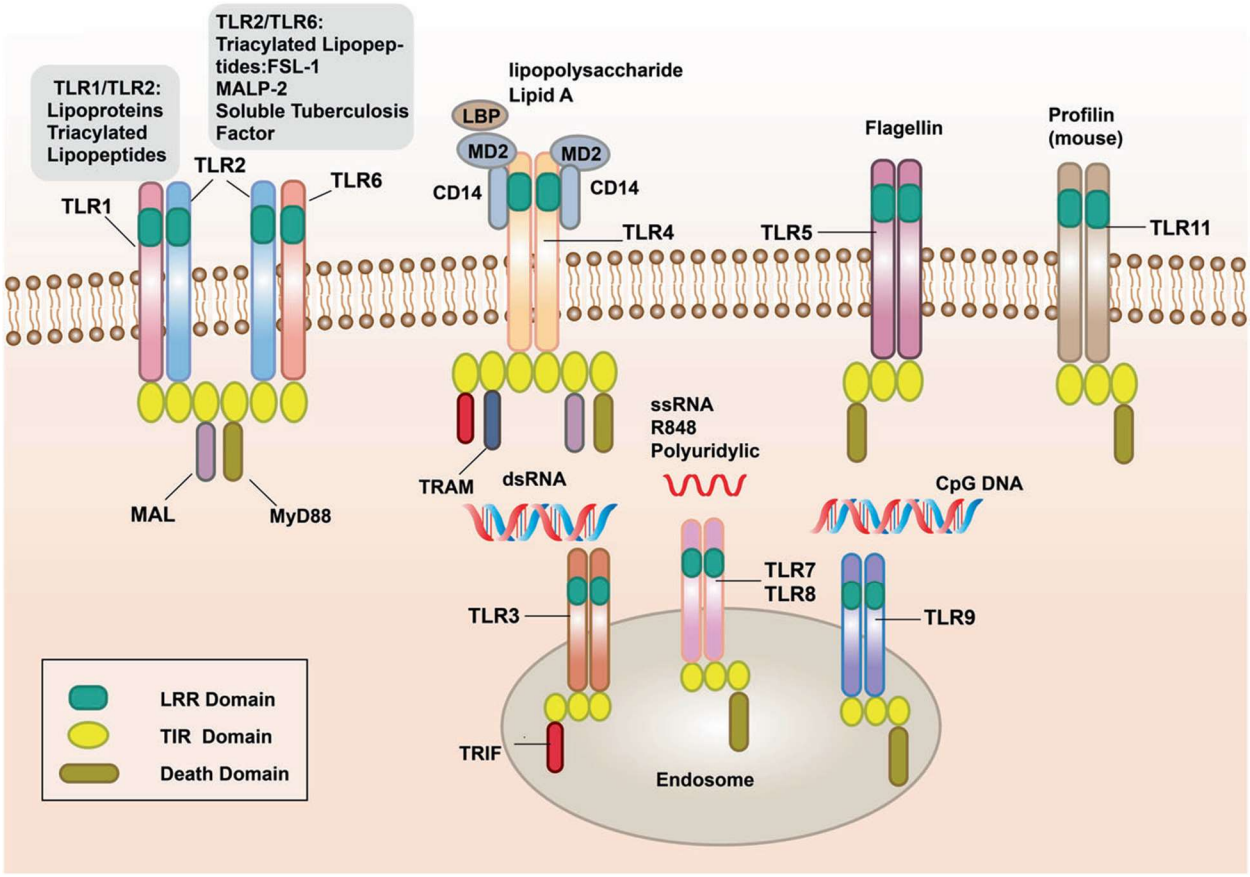



**Figure 10. Toll-like Receptors Recognize Molecular Patterns from Pathogen Molecules to Stimulate Innate Immune Responses.** Shown are the common TLR ligands and their signal transduction pathways. TLRs can recognize one or more PAMPs through LRR domain. (Image from Li and Wu, 2021)

When stimulated, TLR pathways activate cells to produce innate immune mediators that induce a localized proinflammatory environment essential for the induction of effective systemic immune responses. When TLRs bind their specific ligand(s), a signal cascade is triggered within the cell that leads to the activation of various transcription factors and the expression and secretion of proinflammatory mediators, including various cytokines and chemokines (reviewed in Li and Wu, 2021). Specific TLRs and their ligands are shown in **Fig. 10**. The following TLRs and their ligands are directly relevant to this effort:

**TLR3:** TLR3 recognizes double-stranded RNA, which is produced by viruses during replication. **Poly(I:C)** is an adjuvant that is a synthetic double-stranded RNA molecule. It imitates viral double-stranded RNA and is recognized by TLR3. It is a potent stimulator of innate immunity through the stimulation of proinflammatory cytokine secretion.

**TLR4:** TLR4 recognizes lipopolysaccharide (LPS), which is found on the outer membrane of Gram-negative bacteria. The adjuvant **MPLA** (monophosphoryl lipid A), which is a derivative of the lipopolysaccharide (LPS) molecule, also binds to TLR4 and has potent innate immune stimulating activity. The adjuvant activity of MPLA has been extensively studied in the context of vaccine development, and it is currently used in licensed vaccines including the human papillomavirus (HPV) vaccine and the hepatitis B vaccine.

Though not a member of the TLR family, the Stimulator of Interferon Genes **(STING)** pathway is an important stimulator of innate immune responses and is responsible for detecting viral and bacterial DNA inside infected cells (Decout, et al 2021). As the name implies, when activated the STING pathway induces the production of interferons and other inflammatory cytokines. **ADU-S100** is a synthetic small molecule adjuvant that activates the STING pathway and is currently in clinical trials.

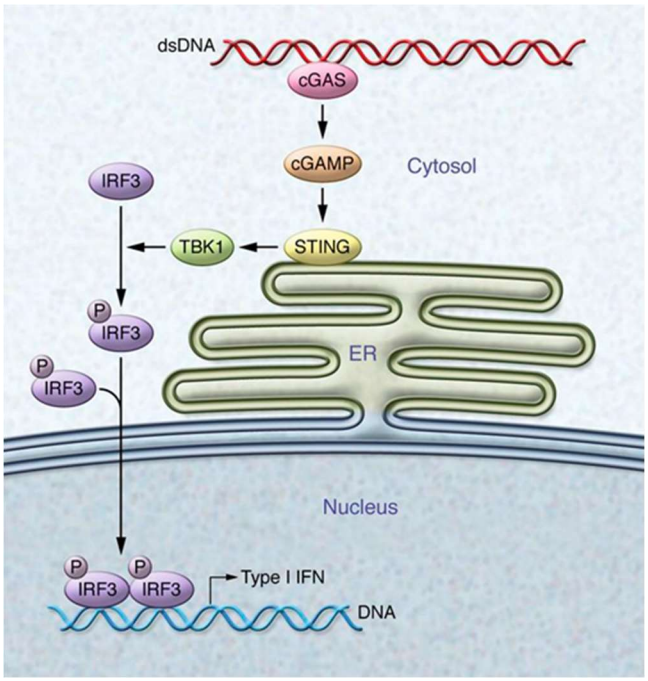



**Figure 11. The cGAS -STING innate immune pathway.** (Image from Corrales et al, 2016)

Each of these adjuvants exerts its activity through distinct receptors on various skin cells. Importantly, Poly(I:C), MPL, and ADU-S100 are all small molecules that are compatible with MNA delivery. They can be fabricated into the matrix of the needles of the MNA with the protein antigen, enabling both the adjuvant and the antigen to be delivered to the same microenvironment within the skin. This is an important feature because cells in the same anatomic site will be exposed to both the antigen and the proinflammatory signals necessary for the induction of the systemic immune response.

### 3.2 Evaluating Adjuvant Responses in the Skin

The qRT-PCR technique enables a quantitative measurement of gene expression through the quantitation of specific mRNAs in a tissue sample. To accomplish this, at various time points after adjuvant delivery, the target skin is harvested and mRNA is isolated. mRNA is then converted into complementary DNA (cDNA) by reverse transcription (RT) using reverse transcriptase. The cDNA is then amplified using the polymerase chain reaction (PCR) with primers specific to the gene under investigation. The amount of PCR product is quantified using an appropriate probe (e.g. TaqMan or SYBR green probes). The data is then analyzed to determine the cycle threshold (Ct) value, which is a measure of the number of PCR cycles required for the probe to exceed a certain threshold. The Ct value is thus inversely proportional to the amount of mRNA in the sample, enabling comparison of the gene expression levels between samples.

As shown previously in **Fig.1**, the skin is capable of producing a broad range of proinflammatory cytokines, chemokines, and other mediators. Although it is not practical to measure all of the potential mediators that can be expressed in the skin, we assembled a pattern of mediators to evaluate based on functions important for vaccine efficacy. The selected molecules and the rationale for their selection are summarized below from information available in Janeway, 2022:

**CCL2.** The chemokine (C-C motif) ligand 2 (CCL2) is also referred to as monocyte chemoattractant protein 1 (MCP1) or small inducible cytokine A2. CCL2 is a small cytokine that belongs to the CC chemokine family. CCL2 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by tissue injury or infection.

**CCL3.** Chemokine (C-C motif) ligand 3 (CCL3), also known as macrophage inflammatory protein 1-alpha (MIP-1-alpha), is a CC chemokine that is involved in the acute inflammatory state and recruits monocytes, dendritic cells, and polymorphonuclear leukocytes.

**CXCL9.** Chemokine (C-X-C motif) ligand 9 (CXCL9) is a small cytokine belonging to the CXC chemokine family that is also known as monokine and is induced by gamma interferon. The CXCL9 is one of the chemokines that induce chemotaxis, and the migration and differentiation and T-cells toward Th1.

**CXCL10.** C-X-C motif chemokine ligand 10 (CXCL10) is also known as Interferon gamma-induced protein 10 (IP-10). CXCL10 is secreted by several cell types in response to IFNγ including monocytes, endothelial cells, and fibroblasts. CXCL10 has been implicated in chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells, and the promotion of T cell adhesion to endothelial cells.

**CXCL11.** C-X-C motif chemokine 11 (CXCL11) is also called Interferon-inducible T-cell alpha chemoattractant (I-TAC) and Interferon-gamma-inducible protein 9 (IP-9). This chemokine elicits its effects on its target cells by interacting with the cell surface chemokine receptor CXCR3, with a higher affinity than do the other ligands for this receptor, CXCL9, and CXCL10. CXCL11 is chemotactic for activated T cells.

**IFNβ.** IFNβ is a representative type-I interferon. Type-I interferons are cytokines that play essential roles in inflammation, immunoregulation, tumor cell recognition, and T-cell

responses. IFNβ is secreted by many cell types including lymphocytes (NK cells, B-cells, and T-cells), macrophages, fibroblasts, endothelial cells, osteoblasts, and others. It stimulates both macrophages and NK cells to elicit anti-viral responses. Plasmacytoid dendritic cells have been identified as being the most potent producers of type I IFNs.

**IL-6.** IL-6 is an interleukin secreted by macrophages in response to pathogen-associated molecular patterns (PAMPs). IL-6 is an important mediator of fever and of the acute phase response. It is responsible for stimulating acute phase protein synthesis and stimulates neutrophils. It supports the growth of B cells and is antagonistic to regulatory T cells. IL-6 can also function as an anti-inflammatory cytokine due to its inhibitory effects on TNF-alpha and IL-1.

**IL-1α.** Interleukin-1 alpha is also known as hematopoietin 1. It is produced by macrophages, neutrophils, epithelial cells, and endothelial cells. It plays one of the central roles in the regulation of immune responses. It binds to the interleukin-1 receptor and induces the production of other cytokines and chemokines, and promotes leukocyte recruitment and activation.

**OAS1a.** 2'-5'-oligoadenylate synthetase 1 is an enzyme induced by interferons and is involved in the proinflammatory innate immune response to viral infection. OAS1a is thought to play a role in the antiviral response by enhancing the activity of RNase L and promoting the degradation of viral RNA. In addition to its antiviral activity, OAS1a has also been implicated in the regulation of cell proliferation, differentiation, and apoptosis.

**IL-1β.** Interleukin-1 beta, is also known as leukocytic pyrogen and is produced by macrophages. It is an important mediator of the inflammatory response and is involved in a variety of cellular activities. IL-1β, in combination with IL-23, induces expression of IL-17, IL-21 and IL-22.

Taken together, the evaluation of the expression of this panel of innate immune system genes will provide a general assessment of the nature and magnitude of the innate immune response induced by the adjuvants that are our focus.

### 3.3 Experimental Design and Methods

To evaluate the innate immune response induced by adjuvants delivered by MNAs, MNAs integrating a specific adjuvant were applied to the skin of mice for 5 minutes and then removed and discarded. Either 6hr (early events) or 24h (late events) later, the skin was harvested from the MNA application site and mRNA was extracted for analysis by qRT-PCR. Experimental methods are described in detail below:

**Mice.** C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used at 8-12 weeks of age. All mice were maintained under specific pathogen-free conditions at the University of Pittsburgh, and experiments were conducted with the approval of the Institutional Animal Care and Use Committee and in accordance with NIH guidelines.

**Microneedle Array Fabrication.** Dissolvable MAPs integrating adjuvants were fabricated using our published manufacturing strategy (Balmert et al., 2020). High-quality master MAPs, which consist of obelisk-shaped micro-scale projections with the projection length and width of 750μm and 225 μm, respectively, in a 10x10 array, were manufactured using two-photon polymerization 3D printing (Nanoscribe Photonic Professional, GT; Nanoscribe GmbH & Co. KG, Germany). To facilitate skin penetration without mechanical failure, these obelisk-shaped microprotrusions included filleted bases and were spaced in the array with a tip-to-tip distance of 675 μm. To show the quality of 3D-printed microprotrusions, master MAPs were imaged by optical stereomicroscopy. Next, micromolding steps, enabled by master MAPs were employed to create MAP production molds from an elastomer, polydimethylsiloxane (PDMS: SYLGARD 184 from Dow Corning, Midland, MI; 10:1 base material to curing agent ratio), resulting in flexible molds with obelisk-shaped wells. Adjuvant-loaded MAPs were then produced from carboxymethylcellulose sodium salt (CMC, 90kDaMW; #C5678, Sigma-Aldrich) by spin-casting of adjuvants and CMC into the wells of MAP production molds in a sequential fashion with centrifugation at room temperature. The PDMS molds were then used to spin-cast MNAs. MNAs were tip-loaded with the indicated amounts of either Poly(I:C), MPL, or ADU-S100. Unloaded Blank MNAs were fabricated for controls. For all formulations, adjuvant was added to a 2% (w/v) solution of low-viscosity sodium carboxymethyl cellulose (CMC, Mw 90 kDa; Aldrich) in sterile-filtered cell-culture grade water (Sigma). For tip-loading, 15 μL of the solution of adjuvant was dispensed onto each MNA production mold, followed by centrifugation in covered rotors for 1 min at 3500 rpm. Excess solution was removed, leaving ~ 2.3 μL per MNA in the obelisk-shaped cavities, and the molds were centrifuged uncovered for 30 min at 3500 rpm, with 20 L/min filtered airflow, leaving dry bioactive agent(s) in the tips of the microneedles. After tip-loading, molds

were loaded with 80 mg of 25 wt% hydrogel consisting of 3:2 CMC:trehalose (Sigma) to fill the remainder of the microneedle cavities and form the MNA backing. Molds were centrifuged in covered rotors for 15 min at 4500 rpm, followed by covered incubation for 15 min. Rotor bucket covers were then removed, and molds were centrifuged for 4 hours and 3500 rpm, with 20 L/min filtered airflow, leaving dry MNAs. All spin-casting steps were carried out at room temperature (~22 °C) in a Sorvall Legend XTR centrifuge (TX-750 rotor, rectangular buckets; Thermo Scientific).

**Microneedle Array Characterization.** MNA geometry was evaluated by optical microscopy using a dissecting microscope (ZEISS Stemi 2000-C with an Olympus OM-D E-M5II camera). MNAs were viewed at an angle of 45° with respect to the objective lens. We verified that endotoxin content in MNAs was below acceptable levels by chromogenic LAL assay (ToxinSensor; GenScript, Piscataway, NJ).

**Evaluation of cytokine expression in the skin.** After 6 or 24 hours, skin tissue was homogenized at 4 °C in TRI-reagent (Molecular Research Center, Cincinnati, OH) using a Bullet Blender Storm 24 with stainless steel beads in Navy RINO tubes (Next Advance, Averill Park, NY). Total RNA was extracted according to the TRI-reagent manufacturer's protocol and quantified using a DeNovix DS-11 spectrophotometer (Wilmington, DE). cDNA was synthesized from mRNA using One-Step RT-PCR Kit with gDNA wipeout as per vendor's instruction (Qiagen). TaqMan® Assay-based real-time PCR was performed with the Applied Biosystems StepOnePlusTM Instrument following standard protocols using primers specific for IL1α, IL1β, IFNβ, IL-6, TNFα, and OAS1a as well as the chemokines CCL2, CCL3, CXCL9, CXCL10, and CXCL11 purchased

from commercial vendors. Relative fold changes in expression were calculated and normalized based on the 2-ΔΔCt method, with naïve ear skin as the untreated control.

### 3.4 Results

To determine the effect of MNA delivery of adjuvants, mice were treated with MNAs delivering Poly(I:C) (100 ugs), ADU-S100 (5ug), or MPLA (2.5ug), or treated with MNAs alone without adjuvant (to determine inflammation induced by the MNA application process alone), or were left untreated as a negative control. The amounts of adjuvant delivered for each adjuvant were previously optimized based on functional antibody responses against a model antigen (not shown). To evaluate the effects of MNA delivery of these adjuvants on the expression of proinflammatory mediators in the skin microenvironment, we specifically evaluated changes in the expression of the proinflammatory cytokines IL1α, IL1β, IFNβ, IL-6, TNFα, and OAS1a as well as the chemokines CCL2, CCL3, CXCL9, CXCL10, and CXCL11, all of which are associated with proinflammatory skin microenvironments. We described the general functions of these mediators in innate immunity previously. Relevant to the skin, **IL1α** can be produced by various cell types in the skin including keratinocytes, fibroblasts, and immune cells, and is a proinflammatory cytokine that plays a key role in the regulation of immune and inflammatory responses (Jensen, 2007). In the context of infection, inflammasome-dependent production of the critical innate immune mediator **IL1β** by keratinocytes has been observed to induce the production of proinflammatory cytokines by multiple cell types and to drive human beta-defensin 2 (HBD2) production (Cai et al, 2019). **IFNβ** was of considerable interest given known associations with inflammatory skin diseases, and the clinically observed skin inflammation at the sites of IFNβ

injection in patients, an observation that suggests the potential of locally released IFNβ as a cutaneous adjuvant (Maurelli M. et. al., 2018). Recent data also suggests that **TNFα**, a potent cytotoxic cytokine, contributes to immunogenicity when present at the site of vaccination (Kamensek et. al., 2018). **CCL3** at the site of vaccination has been shown to be critical for DC migration (Mitchell et. al., 2015). **IL-6** can also be produced by a variety of skin cells and is proinflammatory and has been shown to be involved in a range of autoinflammatory skin diseases (Baran,et al, 2019). The **OAS1** family of proteins can be produced by keratinocytes and other skin cells in response to interferons and has been implicated in the regulation of skin immunity and anti-viral responses (Liu, Y., et al. 2019). **CCL2** was selected as a chemokine found in inflamed or damaged skin and is a critical factor in the recruitment of monocytes and DCs (Gschwandtner et. al., 2019). **CCL3** at the site of vaccination has been shown to be critical for DC migration (Mitchell et al, 2015). The **C-X-C motif chemokines** including **CXCL9**, **CXCL10**, and **CXCL11** are proinflammatory chemokines produced by a variety of skin cells. They have multiple proinflammatory functions that include serving as chemoattractants for activated T-cells and antigen presenting cells (Clark, et. al, 2010; Luster et.al. 2005).

Importantly, we found that MNA delivery of these adjuvants resulted in a general increase in the expression of the genes encoding several of these proinflammatory mediators shortly (6hrs) after delivery (**Fig 12**). In particular, the MNA-delivered STING agonist ADU-S100 induced significant increases in the expression of IL1a, IL6, OAS1a, and TNFa, and all 3 of the C-X-C motif chemokines (CXCL9, CXCL10, and CXCL11) tested. Though not statistically significant, MNA delivery of ADU-S100 also resulted in increases in all of the other mediators tested. MNA delivery of Poly(I:C) also had substantial proinflammatory effects on the skin at the 6hr timepoint. Significant increases in IL1a, OAS1a, TNFa, CCL3, and all 3 C-X-C motif cytokines were induced

by Poly(I:C), and as seen with ADU-S100, the expression of all other mediators tested trended upward. Interestingly, MNA-delivered MPLA appeared to be the least inflammatory of the adjuvants tested. No statistically significant increases in production were found for the proinflammatory mediators tested. This is noteworthy because MPLA is the only adjuvant of the group that is FDA-approved as a vaccine adjuvant. While Poly(I:C) and ADU-S100 are now in clinical studies for cancer immunotherapy, they have not yet been approved for clinical applications.

Given these results, we also determined the proinflammatory effects of these adjuvants on the skin at a later time point (24hr). Unlike the early time point, by 24hr post-delivery one could expect to see effects of immune cells that infiltrated into the skin from proinflammatory effects observed at the early time points.

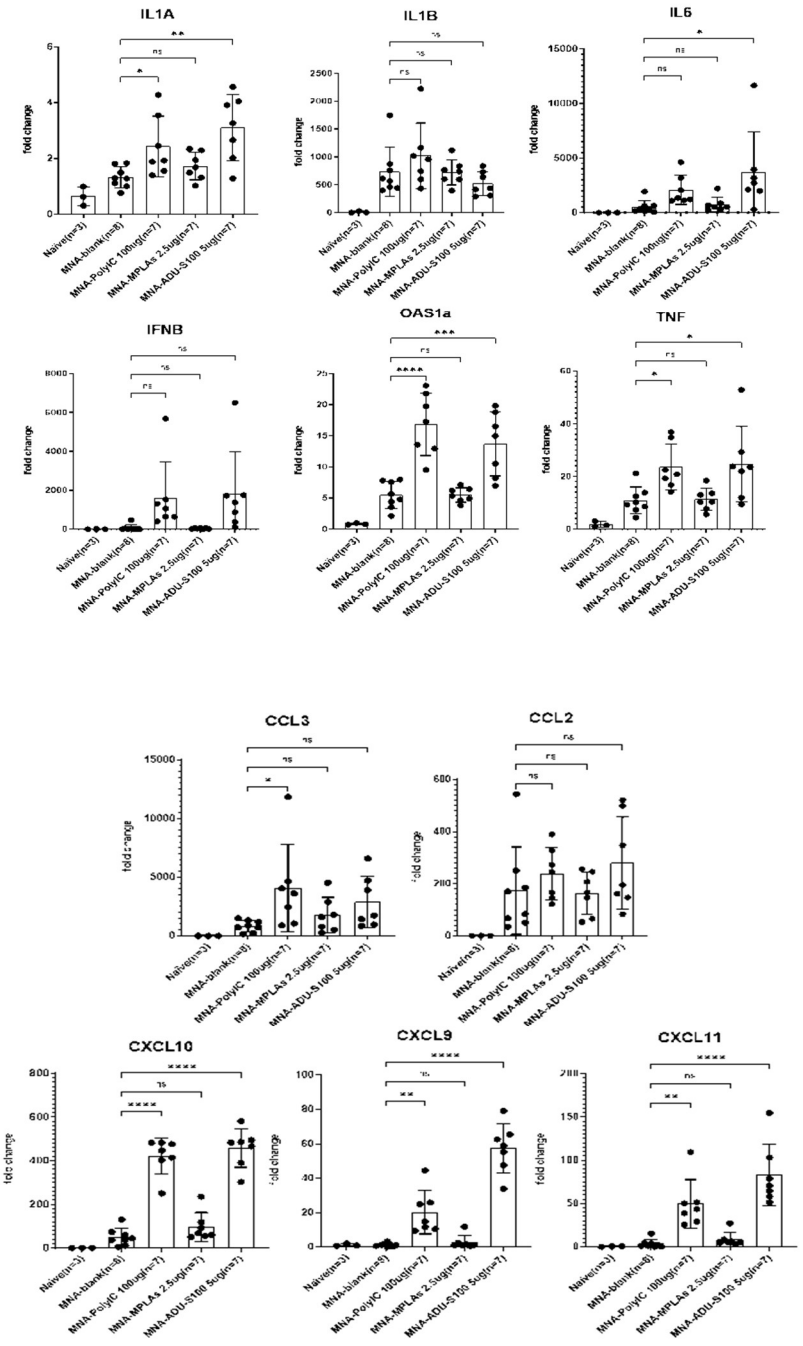
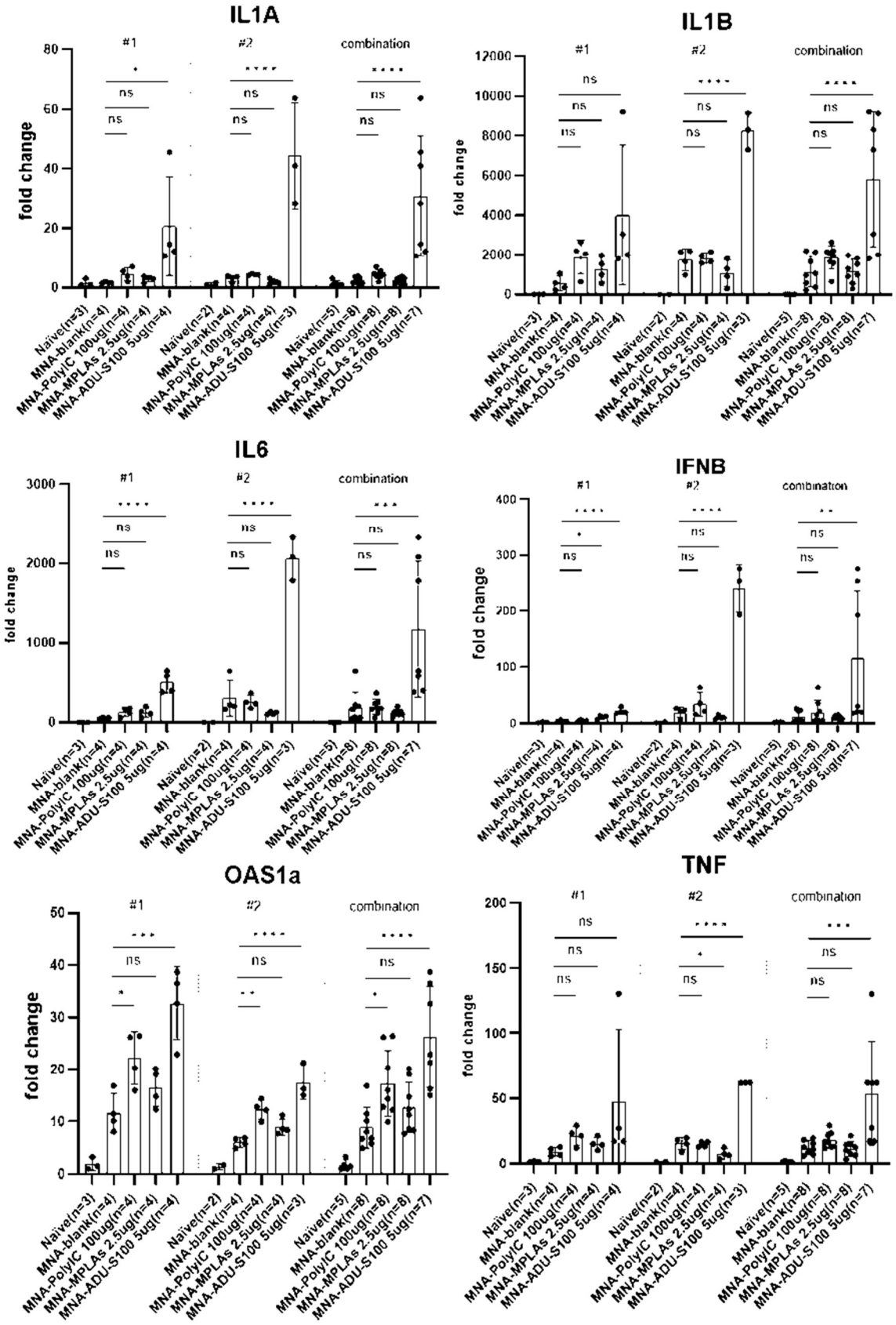



**Figure 12. (6hr) Effects of MNA Delivery of Adjuvants on Innate Inflammatory Responses in Skin.** Murine skin was treated with MNAs delivering the indicated adjuvants and cutaneous expression of the indicated inflammatory mediators was evaluated after 6h by qRT-PCR. Fold changes in expression ($2^{-\Delta\Delta Ct}$) are relative to untreated skin (mean ± SD of the indicated numbers of samples per group). Data were analyzed by ordinary one way ANOVA using PRISM. Significant differences are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

We repeated the same experimental approach, except for this study skin was collected for evaluation 24hrs after MNA-adjuvant administration. We repeated this experiment on two separate occasions and in **Fig. 13**, data from both of the individual experiments and the combined data are presented. Notably, delivery of ADU-S100 by MNAs resulted in statistically significant increases in gene expression of all mediators tested. MNA-Poly(I:C) resulted in significant increases in the expression of IL1B, OAS1a, CCL2, and CXCL9 and expression trended upward for several others. We did not observe any significant increases in the expression of any of the mediators tested 24hr after MNA-MPLA delivery. Importantly, none of these studies induced toxic responses (e.g. erythema, ulceration, scaling) at the delivery site. Taken together, these results suggest that MNA delivery of STING agonist (ADU-S100) or a TLR3 agonist (Poly(I:C)) induces a proinflammatory microenvironment in the targeted skin. These results provide support for the idea that these adjuvants may be useful for co-delivery with antigen in the context of skin-targeted microneedle array vaccines.


IL1A
IL1B
IL6
IFNB
OAS1a
TNF
fold change
#1
#2
combination
ns
Naïve(n=3)
MNA-blank(n=4)
MNA-PolyIC 100ug(n=4)
MNA-MPLAs 2.5ug(n=4)
MNA-ADU-S100 5ug(n=4)
Naïve(n=2)
MNA-blank(n=4)
MNA-PolyIC 100ug(n=4)
MNA-MPLAs 2.5ug(n=4)
MNA-ADU-S100 5ug(n=3)
Naïve(n=5)
MNA-blank(n=8)
MNA-PolyIC 100ug(n=8)
MNA-MPLAs 2.5ug(n=8)
MNA-ADU-S100 5ug(n=7)

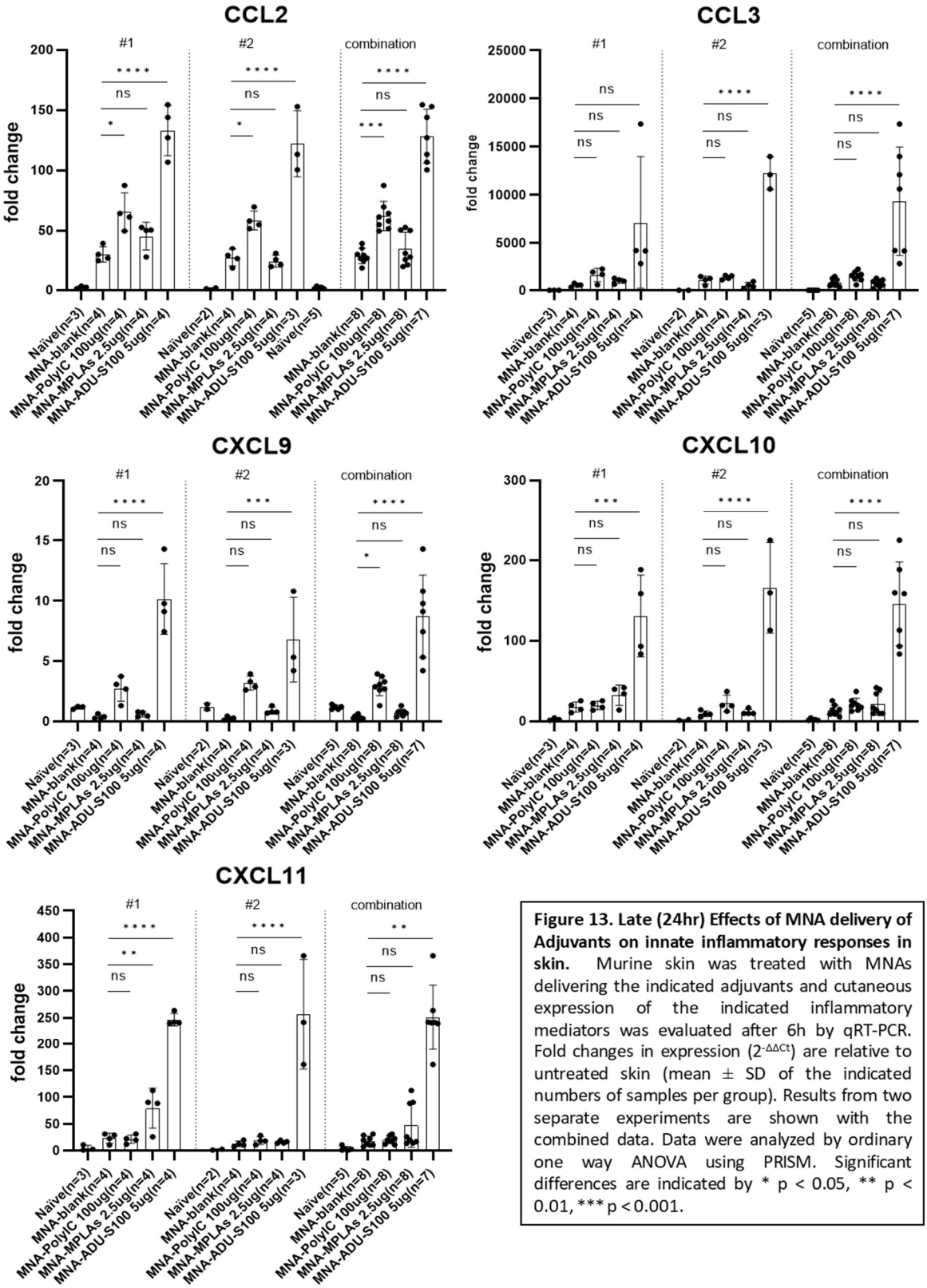



**Figure 13. Late (24hr) Effects of MNA delivery of Adjuvants on innate inflammatory responses in skin.** Murine skin was treated with MNAs delivering the indicated adjuvants and cutaneous expression of the indicated inflammatory mediators was evaluated after 6h by qRT-PCR. Fold changes in expression ($2^{-\Delta\Delta Ct}$) are relative to untreated skin (mean ± SD of the indicated numbers of samples per group). Results from two separate experiments are shown with the combined data. Data were analyzed by ordinary one way ANOVA using PRISM. Significant differences are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Figure 13. Late (24hr) Effects of MNA delivery of adjuvants on innate inflamatory responses in skin.**

## 3.5 Discussion

We found that both ADU-S100 and Poly(I:C) induced significant increases in this panel of innate immune indicators at both early and late time points. MNA-ADU-S100 induced significant increases in IL1α, IL6, OAS1a, and TNFα, and all 3 of the C-X-C motif chemokines (CXCL9, CXCL10, and CXCL11) tested at 6hrs. MNA-Poly(I:C) induced significant increases in IL1α, OAS1a, TNFα, CCL3, and all 3 C-X-C motif cytokines. For both, expression of all other mediators tested trended upward. Similarly, both adjuvants induced significant increases of multiple mediators at 24h. Delivery of ADU-S100 by MNAs resulted in statistically significant increases in gene expression of all mediators tested. MNA-Poly(I:C) resulted in significant increases in expression of IL1β, OAS1a, CCL2, and CXCL9 and trended upward for several others. Thus, MNA delivery of either of these adjuvants resulted in activation of the skin's innate immune response and created a skin environment with features consistent with induction of effective systemic immunity. Interestingly, MNA-delivered MPLA appeared to be the least inflammatory of the adjuvants tested. It is possible that the dose delivered in these studies was below the optimal dose. Alternatively, the structural differences from LPS that were introduced to reduce toxicity may have reduced the binding of this ligand to receptors on skin cells. Additional dose optimization with correlative antibody response analysis will need to be done to further evaluate MPLA as a skin adjuvant. Taken together, these preliminary results provide feasibility for in vivo skin immune engineering and support the development of the emerging MNA-technology platform for the development of MNA-delivered skin-targeted vaccines.

## 4.0 Putting It All Together

The pandemic has demonstrated a critical need for developing effective and rapidly deployable vaccines against a broad range of pathogens. Importantly, experience gained through the pandemic has defined the strengths and limitations of current vaccine technologies. Ethical and public health questions, including important considerations of vaccine equity, have never been more prevalent in the public eye than now as a result of the COVID pandemic.

Today's standard approach for intramuscular vaccine distribution and administration creates a large gap in vaccine equity on an international scale that must be addressed. In chapter one we present the "capability approach" analysis supporting the development of new technologies to address vaccine equity. We concluded that an alternative method for vaccine development and distribution must be considered to propel our society forward toward achieving vaccine equity in every country and for every life.

We propose that the highly adaptive skin immune system coupled with its easy accessibility provides strong rationale for targeting the skin for more effective vaccination. In chapter two we introduced the immunological advantages of targeting the skin for vaccine delivery. The skin is not only rich in professional antigen presenting cells (e.g. dendritic cells, macrophages), but having evolved for self-defense, it has a uniquely plastic and adaptable innate immune system. This innate immune system includes several types of immune cells, but also cells previously not considered as immune cells, such as keratinocytes and fibroblasts, that play critical roles in shaping the skin's immune environment. The skin is also uniquely "connected" to systemic immunity by a high concentration of resident dendritic cells that serve as the "sentinels" of the immune system. These DCs themselves are functionally plastic, altering their function based on conditions in the skin

environment. Skin resident DCs internalize foreign antigens from skin invaders, are functionally programmed by the innate immune responses in the environment in which they encounter the antigen, and then in response to these signals, migrate through the draining lymphatics to program T-cells and B-cells in the draining lymph nodes.

Given this understanding of how the skin functions in immunity, the critical challenge for skin vaccination is to deliver antigen to the skin with appropriate immunostimulatory agents (commonly referred to as "danger signals" or "adjuvants"). This requires 1) an effective delivery technology capable of delivering both antigen and adjuvant to the same skin microenvironment; and 2) the identification of "adjuvants" that induce immunoresponsive cells in the skin to produce an effective innate proinflammatory microenvironment.

Toward this end, in chapter two we also presented an example of an emerging vaccine technology platform, the microneedle array patch (MAP). This delivery technology platform can target the skin's immune system and provide unique advantages in vaccine efficacy, safety, economics, production, storage, distribution, administration, and patient acceptance. MNAs can effectively integrate and co-deliver protein antigens (peptides, proteins, or pathogen fragments) and small molecule adjuvants to the skin microenvironment.

In chapter three, we turned to the second challenge that must be addressed if we are to advance these technologies from concept to clinical reality. Specifically, we began to explore the capability of specific adjuvants to stimulate proinflammatory innate immune responses in the skin after MNA delivery. Currently, very few "adjuvants" have been approved for clinical use. However, several immunostimulatory small molecules are under investigation as cancer therapeutics. Of these, we focused on agents that stimulated innate immune pathways through receptors and pathways known to be active in the skin. This includes MPLA (monophosphoryl

lipid A) which is synthetic form of the lipopolysaccharide (LPS) molecule, a component of the outer membrane of Gram-negative bacteria. MPLA binds to and activates TLR4, which is expressed on the surface of several skin cell types. MPLA is less inflammatory and more selective than the native LPS, making it less toxic. Thus, MPLA is an example of an adjuvant appropriate for clinical use. Poly(I:C) and ADU-S100 are novel small molecules that activate innate immune responses, as previously discussed, through the TLR3 and STING pathways respectively. Each of these 3 adjuvants can be readily incorporated into the substrate of MNAs, enabling effective co-delivery of protein antigen and adjuvant in the same MNAs.

In chapter three we describe studies we conducted studies to evaluate the effect of these antigens on skin immunity using a mouse model. Though mouse and human skin have anatomical and functional differences, the mouse presents an established model for these types of immune analysis. After MNA delivery we evaluated innate immune responses in the skin using a panel of known cytokines and chemokines associated with an inflammatory skin microenvironment. These representative markers have both complementary and overlapping functions in skin immunity. Expression of these molecules may differ under different conditions, and the specific contributions of each to the overall innate immune response are not well established. However, considering the activity of the panel overall provides a reasonable surrogate marker of innate immune activation relevant to vaccine efficacy. We examined responses at both early (6hr) and late (24hr) time points. At early time points, we would expect most of the inflammatory mediator expression would come from cells that are typically skin resident. At the later time point, sufficient time would have elapsed for the migration of other immunologically active cells into the inflamed skin, while other skin cells may have migrated out. While the kinetics of these events has not yet been addressed, we believe that 6hr vs. 24hr timepoints would begin to enable discrimination of these events. An

adjuvant that resulted in effective systemic immunity would likely result in significant skin inflammation at both time points. Our preliminary studies show that MNAs can deliver at least two emerging small molecule adjuvants to the skin to induce innate skin immune responses. This was true at both 6hr and 24hr time points. Further, no clinically visible toxicity occurred at the site of delivery (erythema, edema, ulceration, scaling). Thus, these preliminary studies support the feasibility of MNA vaccines, and the further development of this technology platform for both efficacious vaccination and the opportunity to improve vaccine equity globally.

These studies are introductory and have several limitations that suggest future experiments. We did not specifically address which cell types respond to each of the studied adjuvants. This could be accomplished using single cell RNAseq, which would provide sufficient gene expression data to identify both cell types and their cytokine/chemokine expression patterns. These preliminary results provide the rationale for doing those experiments. From a kinetic perspective, these studies were exploratory. We have not directly addressed cytokine/chemokine expression changes across several time intervals. Further, we did not compare the identity of the cells in the immune infiltrates stimulated by these adjuvants. This could readily be accomplished by flow cytometry analysis of single cell suspensions of treated skin at various time points. Also, correlates between skin immune events and systemic immune responses must be determined. Immune responses from combinations of antigen/adjuvant MNA must be characterized, including the nature, neutralizing activity, and longevity of the antibody and T-cell responses stimulated. Only then will we be able to match "cause with effect" and have sufficient understanding to engineer vaccine responses by purposefully engineering the immune microenvironment in the skin.

Another limitation to be considered is the potential for the needles to break upon patch application. Despite engineering advances designed to stabilize the needles, applying insertion

pressure off the 90-degree vertical angle results in high levels of stress along the length of the needle, including at the site where the needles attach to the backing. Broken microneedles reduce insertion efficiency and thus result in lower doses of drug delivery than intended. Manufacturing improvements are being developed to ensure the needles of microneedle arrays remain intact during initial application and contact with the skin. This will be especially important to enable widespread self-application.

It should also be taken into consideration that microneedle arrays cannot be effectively applied to all skin surface areas. The solid, flat backing material limits application to areas of the body that have a relatively flat surface. Further, because microneedle lengths are optimized to target the cargo to specific skin strata, applying microneedle arrays to hair-bearing areas can be problematic. While low-density, vellus hair does not pose problems, thick hair can act as a cushion, preventing full needle insertion. This constraint typically makes the inner arm or shoulder preferred areas for delivery.

Fortunately, there is considerable accumulating data supporting solutions to each of these obstacles. Until results from large-scale clinical trials are in, we will not know with certainty that microneedle array vaccines will be effective. However, given the enormous potential advantages for global vaccine equity, and the rapidly accumulating supporting experimental evidence, microneedle array vaccines present an attractive opportunity to change the vaccine paradigm and achieve the goal of vaccine availability for all.

In addition to these limitations, it is important to keep in mind that there are considerable differences between mouse and human skin, and between vaccine responses in mice and humans in general. Ultimately, clinical trials will be required to validate the efficacy of these approaches. Given the considerable expense and safety considerations for clinical studies, intermediate

translational models could be developed to reduce the risks and cost of skin targeted vaccine development. Taken together, the preliminary efforts described here support the further development of skin targeted vaccines, and the novel skin delivery systems and immune modifiers necessary to engineer the skin immune response for effective global immunization campaigns.

In summary, we have reviewed the generational problem of global vaccine inequity and ultimately why this is an issue that must be addressed. Although our current models for vaccine delivery may serve as a reliable source for effective vaccination in developed countries, pivoting to an alternative vaccine strategy is essential if we are to make progress in implementing a global vaccination strategy that values human need over economic status. It is for this reason that exploring a novel approach to developing and distributing vaccines is an action worth taking. This is why we do what we do.

The answer to this global vaccine inequity crisis could be new technology, specifically the emerging microneedle array technology platform. Beyond the possibility of creating an immune response more effective than intramuscular injection by unlocking the potential of our skin's immune system, key attributes associated with microneedle arrays, like temperature stability, and the facilitation of distribution and storage (stockpiling), and more favorable economics suggest that MNA technology presents an opportunity for a major step forward towards global vaccine equity.

Taken together, it is possible that the answer to a problem big enough to be felt around the world may come through a device the size of our fingertip. The emerging biotechnology of microneedle arrays could enable painless, needle-free, and efficacious vaccination. Thus emerging technology could provide the answer to the ongoing vaccine inequity crisis, thus making vaccine inequity a problem of the past.

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