Mechanical Disruption of Skin Barrier for Vaccine Delivery

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Needles and syringes represent the most commonly used modality for delivering drugs and vaccines today. According to the WHO, about 12 billion injections are delivered worldwide annually of which approximately 600 million account for vaccinations1. A vast majority of vaccines including polio, hepatitis B, tetanus and flu are administered by injections2. In spite of their common use, needles have several drawbacks including pain, needle-phobia and improper use3. An estimated 20-30 million healthcare workers worldwide are potentially exposed to serious infections such as hepatitis B and C, or human immunodeficiency virus (HIV) annually4. The risk from accidental needle sticks is also high, especially during events of mass inoculations that could follow a bioterrorism emergency or a natural pandemic. Unsafe injection practices further add to the risk of transmission of infectious diseases. A WHO-sponsored review showed that at least 50% of all injections were unsafe in Asia, Sub-Saharan Africa and the former Soviet Republic, exposing patients to the risk of infection from hepatitis, HIV or other blood-borne pathogens5,6. Another issue with needle based administration is low compliance due to perceived and real pain, and the discomfort arising from injections7-9. Needle based immunizations also require some level of expertise and can pose a problem in many parts of the world.

Non-invasive vaccine delivery into skin by topical application offers a potential alternative to needles and syringes. In fact, an invasive version of vaccine delivery into skin has long been used for vaccination against small pox. The appeal of vaccine delivery into skin lies in the fact that the skin hosts a very
sophisticated immune surveillance network\textsuperscript{10}. The cutaneous immune network is the most accessible immune compartment of the body, making skin an attractive interface for administration of vaccines\textsuperscript{11}.

The primary challenge of skin-based vaccination, however, is that simple topical application of vaccines does not allow sufficient contact between the antigen and skin’s immune system. The primary transport barrier resides in stratum corneum which is 15-20 $\mu$m in thickness and is composed of corneocytes embedded in a lipid rich matrix. A majority of immune cells in the skin, particularly Langerhans Cells (LCs), reside in epidermis and superficial dermis\textsuperscript{10,12}. Immature LCs typically reside in the basal layers of the epidermis from where they survey and sample antigens and microbial pathogens entering the epidermis\textsuperscript{13}. Encounters with antigen (s) or other stimuli in the epidermis activate LCs priming them to capture the antigen (s), process them into immunogenic peptides, and present the peptides as complexes with major histocompatibility complex molecules on their surface. These cells then migrate via afferent lymphatics to the skin draining lymph nodes where they present the antigenic peptides to the resident lymphocytes\textsuperscript{14~16}. This forms the foundation of topical delivery of vaccines also known as transcutaneous immunization.

Numerous technologies have been developed to deliver vaccines into skin. Most of these strategies were originally developed for general drug delivery applications; however later translated for vaccine delivery. The approaches used can be classified into: (i) chemical- or formulation-based and (ii) physical- or device-based. Chemical-based approaches make use of permeation enhancers such as surfactants\textsuperscript{17}, liposomes\textsuperscript{18}, emulsions\textsuperscript{19}, and peptides\textsuperscript{20}, among others\textsuperscript{21}, to increase partitioning or diffusion of drugs through the stratum corneum. Several of these approaches have also been tested for vaccination\textsuperscript{22,23}. While permeation enhancement methods have been used for delivery of drugs as well as vaccines, these two applications demand different characteristics and not all technologies for drug delivery are easily translatable to vaccine delivery. Drugs of interest for transdermal applications are often chronic in nature and are often self-administered by patients. Vaccines, on the other hand, are always administered by healthcare providers. The required doses for vaccines are also lower than those for therapeutic drugs and the delivery target for vaccines is epidermis as opposed to systemic circulation for transdermal drugs. Cost considerations are also significantly different for drug and vaccine delivery applications. Delivery of vaccines in developing countries remains one of the greatest challenges in the field, which poses serious constraints on device cost. Nevertheless, the fundamental challenge in delivery, that is permeation across the stratum corneum, is the common ground between drug and vaccine delivery applications. This review focuses on vaccine delivery, in particular physical approaches that have been used to deliver vaccines into skin.
Liquid Jet Injectors

Liquid jet injectors are the oldest and the most commonly used needle-free method of vaccine delivery into skin. The origin of jet injection dates back to the late 1800’s when a technique called "aquapuncture" was reported in the literature. However, it was in 1950’s when this device was first used for widespread clinical applications in the form of multi-use-nozzle jet injectors (MUNJIs). MUNJIs were used for mass immunization against numerous diseases including poliomyelitis, influenza, typhoid, cholera, yellow fever, and smallpox all around the world. However, in the 1980s, reports showed person-to-person spread of Hepatitis B after the use of jet injections, which led to discontinuation of MUNJIs from mass use.

The concern of contamination arising from MUNJIs led to the development of single dose Disposable Cartridge jet injectors (DCJIs). Several DCJIs have been developed and commercialized for drug delivery applications. DCJIs are now available and have been used for delivering insulin, growth hormones, vaccines as well as many other drugs. Some DCJIs are only partly disposable (a disposable liquid reservoir in conjunction with a non-disposable actuation mechanism) while others are fully disposable. Current DCJIs are either spring or gas-powered. Advantages of spring-powered devices include compactness, low-cost and high durability. Their disadvantages include limited range of force and reduced versatility. Gas-powered devices offer the advantage of sustained force generation, greater flexibility and ability to deliver large volume. Their disadvantages include exhaustible energy source. Both types of injectors offer a significant advantage in that they can deliver formulations that are currently approved for injections, thus facilitating the regulatory path. They have been approved for delivery of Measles, Mumps and Rubella (MMR) vaccine and are specifically labeled for this purpose. While in principle the same injector can be used for the delivery of other vaccines, US FDA
issued a notice in Fall 2011 that they should not be used for inactivated influenza virus vaccine since they have not been approved for this purpose.

Regardless of the actuating mechanism, jet injectors deliver vaccines into skin by the same general principle\textsuperscript{30}, the high pressure created by the spring or the gas forces a vaccine solution through an orifice towards the skin. The impact of the jet on the skin surface produces high pressure, which leads to fracture and consequent penetration of the jet into the skin. Penetration of the jet into the skin is driven largely through an erosion-type mechanism. The jet continues to penetrate into the skin to a point where its residual energy is no longer sufficient to penetrate further, at which point, the jet is stagnated and the stagnation pressure delivers the liquid into deep layers of skin. Depending on the parameters, the jet can deliver most of its content into the dermis or subdermal tissues\textsuperscript{31}. Detailed fundamental studies and mathematical modeling of jet injections have also been performed to establish the mechanistic basis of the injections\textsuperscript{30,32}. Jet injections have also been shown to work synergistically with other enhancing methods, for example, iontophoresis\textsuperscript{33}.

While jet injectors in principle offer a needle-free alternative to injections, they have found limited acceptance due to occasional pain and bleeding. Current jet injectors can penetrate deep into the skin, which may be responsible for this behavior. To address this challenge, several newer types of jet injectors are being developed. These include the use of piezoelectric pulsed microjet injectors which deliver drugs through a series of microjets into epidermis of the skin\textsuperscript{34}, dynamically controlled microjets that provide real-time control of jet velocity to minimize variability\textsuperscript{35}, and Lorentz-actuator, feedback controlled injectors for precision delivery\textsuperscript{36}. These injectors, however, have not been tested for vaccine delivery.

### Powder Injection

Powder injections use accelerated vaccine powders that impact the skin and penetrate the stratum corneum by the virtue of their momentum and deposit the vaccine in the epidermis or superficial dermis\textsuperscript{37}. The use of accelerated particles to deliver drugs was first described in late 1980’s. This technique was initially developed for delivery of DNA-coated metal particles into plants. The concept was later adapted for delivery of DNA and other drugs in humans\textsuperscript{38}. Unlike liquid-jet injectors, which typically deliver the vaccine in the subcutaneous or intramuscular space, powder injectors deliver the vaccine mainly in the epidermis\textsuperscript{38}. The penetration depth of powder-injected drugs is largely determined by their mechanism of operation. Solid particles are accelerated by the gas until they impinge the skin. The momentum of impinging particles is largely responsible for their penetration. The momentum depends on the velocity, diameter and the density of the particles\textsuperscript{39}. Gold particles have been particularly used for this application owing to their high density. However, owing to the small size of the particles, deep penetration is prohibited. Computational fluid dynamic studies have also been performed to understand the fundamental mechanisms of powder injectors\textsuperscript{40}.

Several vaccines including influenza and diphtheria toxoid have been delivered using powder injectors at a pre-clinical level\textsuperscript{41~44}. Powder injectors have also been used to deliver DNA vaccines in animals. Small (1-3 μm) DNA-coated gold or tungsten particles delivered by powder injectors directly penetrate into epidermal keratinocytes or LCs, where they express the antigens\textsuperscript{45}. There are fewer reports on vaccine powder injections in humans. In one study, powder injection efficiently delivered influenza vaccine in humans\textsuperscript{46}. IgG titers against all
influenza-virus strains were equivalent or higher in powder injection groups compared to needle-injections. Clinical studies of DNA vaccination for hepatitis B using powder injections have also yielded encouraging results. Powder injection devices have been approved for topical delivery of lidocaine for local anesthesia.

Powder injectors offer several advantages as a mode of vaccine delivery. They use dry powders, which simplifies handling and storage compared with liquid formulations. Powder injectors deliver the vaccine in the epidermis, so that the vaccine is naturally delivered into the LC rich epidermis. Safety studies of powder injections seem to be satisfactory, although occasional bleeding was observed in some cases. Newer designs of powder injection devices continue to be developed to address the limitations of current versions.

**Ultrasound**

The use of ultrasound for delivering drugs into skin dates back to early 1950’s when ultrasound-based physiotherapy devices were used to deliver topical pain medications. Use of ultrasound for delivering macromolecules, however, was reported much later in 1990’s. Ultrasound at various frequencies in the range of 20 kHz-16 MHz has been used to enhance skin permeability. However, transdermal transport enhancement induced by low-frequency ultrasound (f <100 kHz) has been found to be more significant than that induced by high frequency ultrasound. The transport enhancement induced by ultrasound is mediated by acoustic cavitation. Cavitation bubbles induce shock waves that disrupt the skin structure, thereby enhancing its permeability. Effect of ultrasound on skin microstructure has been studied using electron microscopy. These studies revealed that ultrasound induced heterogeneous and significant distention within the lipid regions of the SC, creating several hundreds of nanometer wide voids referred to as lacunar domains. Incorporation of excessive water and surfactants further promotes bilayer disruption in the SC, thereby opening pathways for solute permeation. Mathematical models of ultrasound-induced skin permeabilization have also been developed. These models indicated that ultrasound increases the pore-density in the skin and macromolecules can be delivered through ultrasound-permeabilized skin.

Ultrasound has been used to deliver various small and high molecular weight drugs. Ultrasound has also been shown to induce convective flow across the skin and impart directionality to transport. Ultrasound has been shown to work synergistically with various other enhancers including iontophoresis, chemical enhancers and electroporation.

Low-frequency ultrasound has been used to deliver vaccines into the skin in mice. Ultrasonic delivery of tetanus toxoid generated a strong systemic immune response in animals. Two possible mechanisms were proposed to explain why pretreatment of skin with low-frequency ultrasound prior to contact with the antigen vaccine may enhance the immune response. One possible mechanism is that ultrasound pretreatment results in increased delivery of the vaccine compared to control, thus enabling sufficient amount of vaccine to enter the skin in order to activate the skins immune response. Second, application of ultrasound may activate LCs in the skin. Mechanisms responsible for ultrasound-induced activation of LCs are not clear, although barrier disruption or release of pro-inflammatory signals by the keratinocytes are possible candidates.

Ultrasound offers certain advantages for vaccine delivery. Ultrasound-based skin permeabilization have already been approved by the FDA and...
have been used in the clinic for drug delivery\(^6\)). Ultrasound activates LCs, which is likely to increase the efficacy of vaccines. Ultrasound devices, however, are typically large and expensive, thus limiting their utility.

**Microneedles**

Microneedles are micron-scale needles that are employed for transdermal vaccination and drug delivery\(^6\). The idea that very small needles may be sufficient to transport drugs across the stratum corneum was first proposed in 1970’s\(^6\), but demonstration of this concept is a recent phenomenon, little over a decade old\(^6\). To date, a large number of microneedle designs have been developed and tested by numerous academic and industrial groups\(^70,71\). These designs can be divided into four groups\(^72\): (i) solid microneedles that pierce the skin to porate the skin followed by placement of a patch or a formulation for drug delivery, (ii) solid microneedles coated with dry powder vaccines, which upon penetration into skin allow the vaccine to get solubilized and retained in the epidermis, (iii) microneedles prepared from dissolvable materials which not only serve to pierce the skin but also as an instantaneous\(^73\) or controlled release\(^74\) depot in the skin, and (iv) hollow microneedles which, when connected to an infusion pump, can actively deliver drugs into the skin. A variety of materials including stainless steel, titanium and nickel-iron, polycarbonate, polylactic-coglycolic acid, polyvinyl pyrrolidone, maltose, and carboxymethyl-cellulose have been used to design microneedles.

Microneedles in principle should be less than 200 micron long to avoid penetration into the dermis and hence avoid pain. Studies have shown that microneedle length affects pain; a three-fold increase in needle length (i.e. 500-1500 µm) increased the pain seven fold (i.e. from 5% to 35% of the pain caused by a hypodermic needle)\(^75\). Typical microneedles vary from 150-1500 µm in length, 50-250 µm in base width and 1-25 µm in tip diameter\(^72\). The design of microneedles is governed by avoidance of pain, deposition of sufficient drug quantities, no fracture during insertion and biocompatibility.

Microneedles have received significant attention for vaccine delivery. They offer several advantages as immunization agents: (i) they are relatively inexpensive and do not require large devices or power to operate, (ii) they can target the vaccine to the epidermis, where the contact with LCs can be maximized, (iii) vaccines can be deposited on microneedles in the dry form, thus facilitating storage and handling, and (iv) they can be used relatively easily, thus eliminating high degree of training for the administrator. Microneedles have also been combined with other methods such as iontophoresis\(^76\) or electroporation\(^77\) to further enhance transdermal drug delivery.

Several studies document the success of microneedles in vaccine delivery. Various vaccines including influenza\(^78\) and hepatitis B\(^79\), among many others, have been delivered at an experimental level using microneedles. In all these studies, microneedles generated immune responses at least as strong as those generated by subcutaneous or intramuscular injections. Studies also demonstrated dose sparing ability of microneedles, where lower antigen dosage via microneedles elicited immune response comparable to higher antigen doses via alternate routes, i.e. subcutaneous and intramuscular injections\(^80,81\).

**Removal, poration, and ablation of Stratum Corneum**

Several strategies have been developed to completely or partially remove the stratum corneum for enhanced delivery of drugs\(^82\). These
include strategies such as the use of an abrasive pad to break the stratum corneum barrier or scotch tape to peel off stratum corneum in a layer by layer mode. While both methods are effective in eliminating the stratum corneum barrier, their use in practical devices that can be used in a controlled manner in patients is challenging. Several advanced technologies for removing stratum corneum have been proposed. These include the use of microdermabrasion where controlled bombardment of skin with crystals can be used to remove the stratum corneum. The momentum of impinging particles dislodges pieces of stratum corneum at a controlled rate. The area of stratum corneum that is removed is controlled by using a metallic mask. Macromolecular drugs including insulin have been delivered through microdermabraded skin. Skin barrier properties have been shown to recover within 24 hours after microdermabrasion in rodents.

Thermal energy has also been used to remove stratum corneum. Skin surface is briefly heated for a fraction of a second beyond the boiling point of water to instantaneously vaporize tissue fluid, thus leading to its ablation. Temperature of the underlying tissue is largely left unchanged, thus avoiding thermal injury. This process has been used to create well-defined pores of tens of micron in diameter and depth. Both dimensions can be controlled via controlling the duration and localization of thermal energy. Heating has been controlled by using a two dimensional grid of wires with micron-scale resistors. Application of electric currents through this network is used to briefly heat the skin. In another approach, an array of electrodes is heated using radiofrequency (RF) current. The resulting heat generated within the stratum corneum selectively heats the tissue for localized ablation. These devices have been tested for delivery of various drugs including testosterone, granisetron hydrochloride, diclofenac sodium and plasmid DNA in various in vitro and in vivo models as well as in human clinical studies. Safety of these methods has also been demonstrated in clinical studies.

Lasers have also been used to disrupt stratum corneum to enhance delivery of drugs. In one study, Erbium-YAG laser was used to enhance transdermal delivery of antibodies. Exposure to lasers has also been used to induce pressure waves, which can disrupt the skin in a mechanism similar to that induced by ultrasound. These pressure waves are characterized by peak pressure, rise time, pulse and the decay. The number of pulses applied can also be varied, which typically range from 1 to 20 pulses; however, even a single pulse has been shown to be sufficient to permeabilize the stratum corneum. The permeabilization of the stratum corneum can last for several minutes. Microscopic evaluations of human skin exposed to pressure waves showed expansion of lacunar domains within the intercellular lamellae of the stratum corneum. It was indicated that in the skin exposed to pressure waves, these lacunae for three-dimensional connected network that enhances skin delivery.

Ablation of stratum corneum has been shown to induce delivery of vaccines into skin. Abrasion of stratum corneum using simpler means, for example, sandpaper is often performed before the placement of topical vaccines in experimental and clinical systems. Devices have also been developed to obtain controlled disruption of stratum corneum using abrasives devices. In this device, abrasive strips of various lengths were used and tested for efficacy by placement of heat-labile enterotoxin from Escherichia coli on abraded skin. In other studies, laser-induced disruption of SC has been shown to enhance delivery of a lysosomal antigen and ovalbumin.
Summary and Future Prospects

Transcutaneous immunization offers several advantages over conventional needle based vaccination methods. A skin-patch based vaccine is painless and therefore patient compliant. Compliance is critical for success in immunization programs especially among children. Skin-patch based vaccines offer self-administration capabilities reducing the need for a clinical setting or medical supervision, which is especially critical during an epidemic. The primary challenge of transcutaneous vaccination is delivery of sufficient quantities of vaccines into skin. Various techniques have been developed to overcome this challenge and this review summarizes some of them. Several additional physical methods have been developed to enhance drug delivery into skin including iontophoresis which utilizes application of small electric currents\(^{103, 104}\) and electroporation which used high voltage-short duration pulses to increase skin permeability\(^{105}\). These methods are non-mechanical in nature and hence are not discussed in this review.

A review of the techniques discussed here makes it clear that numerous options exist to deliver vaccines into skin. These techniques make use of various means to mechanically disrupt the stratum corneum for vaccine delivery. While these techniques exhibit various stages of development, they share some common features; they have undergone significant in vitro and in vivo preclinical studies. Many of these techniques also benefit from significant fundamental studies and mathematical models. These models, in particular, have proved very valuable in understanding the fundamental basis of their operation and dependence on parameters. Most techniques described here have been advanced to human studies and provided indications of safety and efficacy. Significantly more clinical studies, however, are needed to better understand the full range of capabilities of these techniques. With further research into mechanisms and device designs supplemented by larger scale clinical studies, these techniques are poised to make a strong impact on medicine.

Biography: Professor Samir Mitragotri

Professor Samir Mitragotri is a Professor of Chemical Engineering at the University of California, Santa Barbara (UCSB). He also serves as the founding director of UCSB’s Center for Bioengineering. He received his Ph.D. from MIT in 1996 and B.S. from Institute of Chemical Technology, Mumbai in 1992. Professor Mitragotri’s research is focused on drug delivery. His major research areas include transdermal drug delivery using ultrasound, needle-free liquid jet injectors, chemical penetration enhancers and peptide transporters as well as oral delivery of macromolecules and nanoparticles for drug delivery. Prof. Mitragotri serves on editorial boards of several journals including Journal of Pharmaceutical Science, European Journal of Pharmaceutical Sciences, Therapeutic Delivery, Drug Delivery and Translational Research, and Experimental Biology and Medicine. Professor Mitragotri also serves as an Associate Editor of the Journal of Controlled Release.


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84) Benfeldt, E., In vivo microdialysis for the investigation of drug levels in the dermis and the effect of barrier perturbation on cutaneous drug penetration. Studies in
無針注射器（needle-free injection system, jet injector）

無針注射器は、液体や粉末の薬剤を高圧によって皮膚に噴射することで、皮内、皮下および筋肉内に薬物を直接送達させるデバイスである。無針注射は、局所麻酔剤を目的としたリドカイン、成長ホルモンやインスリンなどのペプチド医薬、ワクチン、遺伝子などの経皮送達方法として注目されている。この方法は注射針を用いないことから、医療従事者の針刺し事故または廃棄処理における感染症の危険性の回避、針に対する恐怖のある患者のコンプライアンス向上などの利点がある。また、ノズルの形状や薬剤の噴射速度などデバイスの最適化により、従来の注射投与に比べて痛みを軽減しながら薬物を目的部位に送達できるという利点がある。現在、薬剤噴射の動力源として圧縮ガスや圧縮バネなどが利用されており、さまざまなデバイスが開発されている。

液体を噴射する liquid injector は、すでにインスリンや成長ホルモンの投与に実用化されている。Powder injector は、遺伝子導入に用いられる gene gun の原理を利用したもので、粉末の薬剤を高圧ヘリウムガスによって皮膚に噴射することで薬物を体内に送達するデバイスである。また、ワクチン投与のための無針注射器としては、すでに Biojector® 2000 (Bioject Medical Technologies, Inc.) や Injex® (Injex Pharma AG) が FDA により承認されており、注射投与と同等の免疫応答を誘導できることが報告されている。