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Needle Free Immunobiologicals: A Perspective Role on Immunization for the Future

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Abstract

Vaccination is an extremely effective tool for controlling disease, and vaccination programs have significantly reduced morbidity and mortality associated with infectious diseases through mass immunisations. However, traditional needle-based vaccine delivery methods are negatively impacted by multiple disadvantages, including (but not limited to): pain, trypanophobia (fear of needles), needle-stick injury, waste from disposal of sharps, logistical challenges to manage mass immunisation programs, and social stigma of using needles. To address these disadvantages, new generation vaccine delivery methods known as needle-free immunobiologicals have been developed. Needle-free immunobiologicals provide improved safety, increased consumer acceptability and greater accessibility to vaccines around the world. Needle-free immunobiologicals represent a number of different vaccine technologies, including mucosal vaccines (for example: oral and nasal vaccines) as well as transdermal vaccines, including microneedles, electroporation, iontophoresis, sonoporation, laser-based microporation and advanced vaccine carrier technologies (e.g. nanoparticles & emulsion-based adjuvants). The majority of needle-free vaccine technologies target immunologically privileged sites (i.e. skin and mucosal surfaces), which are very effective in increasing the rate at which antigen-presenting cells capture antigens, resulting in antigen-sparing effects. Additionally, some needle-free vaccine technologies also offer the potential for decreased requirements for cold storage, ease of administration, and application to mass immunisation campaigns. However, there are still a number of challenges to overcome, including scalability, regulatory hurdles, high cost, device dependency & lack of clinical validation over time.

Keywords: *Needle-free immunization; vaccine delivery systems; microneedles; mucosal vaccines; nanoparticles; transdermal vaccination; vaccine adjuvants; immunization strategies.*

Introduction

Vaccines and vaccination remain two of the greatest success stories in public health, as they have drastically reduced the incidence and mortality rates due to infectious diseases on a worldwide scale. Vaccines are biologic preparations containing attenuated, inactivated, subunit, or recombinant components designed to stimulate an immune response to protect against any infectious diseases[1]. The vaccine provides the individual with an immune response to antigens, which include live-attenuated or inactivated pathogens, protein subunits, toxoids, viral vectors, or nucleic acids that stimulate both an innate and adaptive immune response, thus providing immunological memory[2]. The immunity generated from a vaccine activates both the innate and the adaptive systems of the body's immune system; when the innate system recognizes the antigen, it activates the antigen specific B and T lymphocytes to produce neutralizing antibodies and create the immunological memory required for long-term protection from disease [3], [4], [5].While conventional vaccines such as live-attenuated and inactivated vaccines will continue to be the cornerstone of the vaccination programme, the advances in molecular biology have enabled the use of vaccine technologies such as recombinant proteins/peptides, virus-like particles (VLPS), DNA, MRNA, and nanoparticle vaccines[6]. Protein and peptide vaccines tend to have low immunogenicity and require higher adjuvants and improved methods of delivery to achieve the required efficacy [7], [8].

In addition to safeguarding individuals, extensive vaccination fosters herd immunity, which in turn shields unvaccinated or immunocompromised people by disrupting the spread of pathogens in a community [9].

Vaccination has been responsible for many important achievements throughout history, such as the eradication of smallpox and the near eradication of other diseases, including polio, measles, diphtheria and tetanus throughout the world [10].In recent years, advances in molecular biology, immunology, and biotechnology have increased the speed at which next-generation vaccines developed. The most recent examples of this are recombinant, conjugate and mRNA-based vaccines, which were rapidly developed in response to emerging infectious disease threats such as SARS-CoV-2 [11], [12].

Despite the successes of vaccination to date, vaccine development and deployment continue to present many challenges, including variability of antigens, dependence on cold chain, hesitancy to vaccinate, and the need for safe, scalable and accessible means of delivering vaccines(Immunization Immunisation Agenda 2030: A Global Strategy To Leave No One Behind, 2020).As these challenges are addressed, they also spur continued innovation in vaccine design, adjuvant and delivery technologies that together reinforce vaccination as a key pillar of global public health and the prevention and control of diseases[13].

Needle-free immunobiologicals, or needle-free vaccines/biologic delivery systems, refer to immunogens such as vaccines, monoclonal antibodies, and other immunomodulators that are delivered without the conventional hypodermic needle. The interest in needle-free immunobiologicals has grown exponentially, especially because of their potential to eliminate or reduce needle pain and associated anxiety, reduce needle-stick injuries and sharps disposal, and, depending on the system, simplify logistics for mass immunisation campaigns[14]. Additionally, many needle-free delivery systems have been developed to allow for easy and effective delivery of immunogens to the skin and/or mucosal tissue, where there are many immune system cells. Some systems have been developed for this purpose with the understanding that by administering the immunogens to the immune-rich tissues, improved immune response can be achieved [15].

Significance Of Vaccines

Vaccines play an essential role in many ways; they save millions of lives every year, they are one of the least expensive methods to improve public health, and they help lessen the impact of many commonly occurring illnesses. Vaccines protect people from diseases by stimulating the body's immune system to produce certain antibodies and immune cells that help fight off future infections and related problems, such as hospitalizationor death, and by also helping to protect the community from the disease through the process of 'herd immunity' [9]. In many areas of the globe, large-scale immunisation programs have resulted in the complete elimination (or at least a very significant reduction) of a number of diseases including: Smallpox, Polio, Measles, and Tetanus [16][17]. Vaccination is critical for protecting the most vulnerable populations of individuals who may suffer severe complications from diseases caused by infections, such as infants, older adults and immunocompromised individuals [3], [18]. With the continuation of scientific investigation into vaccinology, it is clear that vaccinations will continue to be a primary element of ensuring global health security.

Basic Immunisation Coverage

The immunisation coverage of the basic immunisation schedule is how many children and adults, primarily infants and young children, are receiving vaccinations that are a routine part of the basic immunisation schedule, such as diphtheria, tetanus, pertussis, polio, measles, hepatitis B, and tuberculosis vaccines. High immunisation coverage of the basic immunisation schedule is important because it reduces childhood disease and death rates and prevents outbreaks of vaccine-preventable diseases through maintenance of herd immunity [16], [19].Global vaccination programs over the last several decades have resulted in increases in vaccine coverage; however, there continue to exist significant gaps in immunisation coverage for low- and middle-income countries due to barriers to health systems, socioeconomic status, conflict and hesitancy towards vaccines(Immunization Immunisation Agenda 2030: A Global Strategy To Leave No One Behind, 2020). The recent increase in the incidence of disease due to a decline or stagnation in routine immunization coverage demonstrates the importance of continuing to improve and strengthen basic immunization coverage, with the need for continued commitment of government officials and community organisations to provide immunization access for all people in all communities, as well as new ways to deliver vaccines to all people [20].

Universal Immunisation Schedule

The Universal Immunisation Schedule (UIS) is a structured framework to provide timely and equal access to vaccines as an individual progresses through their life. The UIS primarily focuses on infants, children, pregnant women, and high-risk groups. In India, the Universal Immunization Programme (UIP) (Table 1) is among the world's largest in terms of public health initiatives and provides free vaccinations for major vaccine-preventable diseases such as tuberculosis, poliomyelitis, diphtheria, pertussis (whooping cough), tetanus, measles, hepatitis B, *Haemophilus influenzae* type B, rotavirus infection, pneumococcal disease, rubella, and Japanese encephalitis in areas where they are endemic [21].The Indian UIS(Universal Immunization Immunisation Programme (UIP), n.d.)) is updated by the National Technical Advisory Group on Immunisation (NTAGI) periodically so that new vaccines can be added to the immunisation programme and to ensure they are in alignment with epidemiological priorities and global recommendations[23], [24].). India has made significant strides to improve the routine immunisation coverage of its most underserved and

hard-to-reach populations through initiatives such as Mission Indradhanush and Intensified Mission Indradhanush. As a result, there have been substantial decreases in child morbidity and mortality[23], [25]. The immunisation delivery system in India continues to face challenges, including regional disparities, urban slum populations, migratory populations, and constraints within the health delivery systems. Therefore, policies and strategies that continue to develop and support creative and innovative solutions are needed going forward.

Table 1: Universal Immunisation Schedule (India): Vaccines, Timing, Indications and Administration

Vaccine / Antigen	Target Disease(s)	Recommended Age / Schedule	Route of Administration	Anatomical Site
BCG	Tuberculosis	At birth	Intradermal	Left upper arm
Hepatitis B (Birth dose + primary series)	Hepatitis B	At birth; 6, 10 and 14 weeks	Intramuscular	Anterolateral aspect of mid-thigh
Oral Polio Vaccine (OPV)	Poliomyelitis	At birth (within 72 hours); 6, 10 and 14 weeks	Oral (drops)	Oral cavity
Pentavalent Vaccine (DPT-HepB-Hib)	Diphtheria, Pertussis, Tetanus, Hepatitis B, *H. influenzae* type b	6, 10 and 14 weeks	Intramuscular	Anterolateral aspect of mid-thigh
Rotavirus Vaccine	Rotaviral diarrhoea	6, 10 and 14 weeks	Oral	Oral cavity
Pneumococcal Conjugate Vaccine (PCV)	Pneumococcal pneumonia and invasive disease	6 and 14 weeks; booster at 9–12 months	Intramuscular	Anterolateral aspect of mid-thigh
Fractional Inactivated Polio Vaccine (fIPV)	Poliomyelitis	6 and 14 weeks	Intradermal	Right upper arm
Measles / Measles-Rubella (MR)	Measles ± Rubella	9–12 months; booster at 16–24 months	Subcutaneous	Right upper arm
Japanese Encephalitis (JE)	Japanese encephalitis (endemic areas)	9–12 months; booster at 16–24 months	Subcutaneous (live) / Intramuscular (killed)	Left upper arm (SC) / mid-thigh (IM)
DPT / DTaP / Tdap (Booster)	Diphtheria, Pertussis, Tetanus	16–24 months; 5–6 years	Intramuscular	Mid-thigh (1st booster); left upper arm (subsequent)
Td (Adolescents & Pregnancy)	Tetanus, Diphtheria	10–16 years; during pregnancy	Intramuscular	Upper arm

In certain countries,the pentavalent vaccine (Hepatitis B, Diphtheria, Pertussis, Tetanus and Haemophilus influenzae type B) is given instead of DPT and Hep B.

Japanese Encephalitis (JE) is administered in identified endemic districts.

Routes of Immunization

The route of immunisation describes the method bywhich the immune system recognisesthe vaccine. It describes how a vaccine is given and is the factor that determines the type, intensity and the time-frame in which the body responds to the vaccine. The most used types of vaccines are delivered by Intramuscular, Subcutaneous, Intradermal and Oral Immunization routes (the choice of which depends on the vaccine formulation, antigen type, and desired immunological outcome). Intramuscular Vaccines (IMV) are the most commonly used route for both Inactivated Vaccines and Subunit Vaccines, since this route promotes efficient uptake by Antigen Presenting Cells (APCs) and results in a strong systemic immune response[26]. Subcutaneous (SC) and Intradermal (ID) immunizations targets the skin and associated tissue (under the skin), where there are large numbers of Dendritic Cells and Macrophages, which provide for enhanced antigen presentation (thereby requiring lower amounts of antigen)[27].

Mucosal routes of administration (Oral and Intranasal) provide the potential for both systemic immunity and Mucosal Immunity to be induced at the same time (at the sites of entry of pathogens). Oral Vaccines (OPV and Rotavirus) have all been successfully administered orally and provide for a simplified route of administration, improving compliance, especially in mass immunisation programs[28], but problems would remain with degradation and variability in the immune response to the antigen. Therefore, the selection of the route for immunisation is a critical part of the design and administration of Vaccines that may result in safe administration[29].

Problems Pertaining To Needle Based Vaccination

Needle-use in immunisations is plagued by many issues limiting overall vaccination reach and effectiveness. A key issue associated with meeting childhood and needle-fearing individuals'vaccination needs is the pain and anxiety associated with a needle. The result is that these individuals experience delays in receiving their vaccines and consequently delay herd immunity and any reduction in disease levels[30]. Needle-stick accidents and injury to healthcare personnel area significant source of occupational health risks due to the risk of spreading blood-borne diseases [31]associated with failing to maintain proper sterile procedures for needle use.

With respect to a public health perspective, the conventional needle-based immunisation generates an abundance of biomedical pyramidically sharp waste that requires special disposal methods. Many of the places in the world where the most frequent immunisation programs take place do not have adequate disposal systems, creating a high risk for reusing and/or improperly disposing of sharps [32]. Also, the conventional method of administering intramuscular immunisations is to use certified and trained personnel who require sterile needles and supplies that are kept in a cold-storage chain until used. The supplies may be "doctored" in the case of a non-sterile needle or injection[33], such as washing the needle with bleach to make it appear sterile. Each of these issues are contributing factors to the growing interest in acceptable and safer needle-free immunization solutions that will improve patient safety, improve patient acceptance of vaccinations provided by others and ultimately improve access to vaccinations, while at the same time maintaining, if not improving, the immunogenicity of the immunization.

Trypanophobia, or extreme fear of needles and needles-based vaccinations, is one of the greatest psychological barriers hindering individuals from receiving vaccinations.

Around the world, a significant number of both children and adults suffer from trypanophobia, which has emerged as a strong psychological factor contributing to individuals' choices to avoid vaccinations[30]. The physiological response(s) to fear such as anxiety, dizziness, vasovagal syncope, etc., can have a long-lasting impact on a person's choice to receive a vaccine as well as to have their children vaccinated, and the resulting effects of these fears can become magnified throughout families and communities as a result of an individual's response to fears [34].). As a child, having a negative experience with receiving an injection may create long-term fear of needles, thus resulting in trust issues with vaccine delivery programs[35]. Therefore, proper attention to trypanophobia is essential to increasing vaccination rates, and, as such, it is the driving force behind the development and implementation of needle-free and minimally invasive vaccine administration systems.

Available Alternative Methods of Immunization

A. Nasal Vaccine

The Intranasal Vaccination Method (Nasal vaccines); are a form of mucosal immunisation that is administered using the intranasal route, targeting the nasal-associated lymphoid tissue (NALT) where most mucosal and systemic immune responses begin. Using this route allows secretory immunoglobulin A (sIgA) to be produced at mucosal surfaces along with serum IgG responses, therefore providing the first line of defence against respiratory pathogens entering through the nose[36]. The nasal route allows vaccines to avoid the gastrointestinal tract as well as bypass the hepatic first-pass effect, allowing direct interaction between antigens and mucosal immune cells including dendritic cells and M-cells. Examples include the successful use of live-attenuated influenza viruses and several experimental vaccines against respiratory viruses[37].

The primary benefit of nasal vaccination is that they are given without injections (needles) therefore it is also painless to administer; therefore increasing patient acceptance and compliance, especially in children and individuals who are needle phobic [38]. Stronger induction of mucosal immunity with nasal vaccines provides better protection from respiratory infections compared to parenterally administered (not via the mouth or nose) vaccines, because the mucosal immune system helps prevent pathogens from colonising at their intended site of entry. Nasal vaccines have the additional advantage of being self-administered, with a reduced requirement for trained healthcare personnel and suitability for mass immunisation events (e.g., pandemic). Furthermore, nasal vaccination requires lower volumes/doses of vaccine antigens than do parenteral vaccines[36].

Although these nasal vaccines have many benefits, they also have numerous disadvantages associated with them. For example, antigens may rapidly be eliminated from the nasal cavity through a process referred to as mucociliary clearance, thereby limiting their residency time as well as their likelihood of eliciting an immune response[37]. In addition, antigen uptake could be further limited by enzymatic degradation, and barriers like mucus.

Another area of concern is that the vaccine components could reach the CNS (central nervous system) through the transport of the olfactory nerve. Hence, it is crucial to choose the correct antigen and adjuvant[38]. Furthermore, the variability of nasal physiology caused by infection, allergies, or age-related issues could affect the ability to create an effective formulation therefore creating difficulties in regulatory approvals.

B. Oral Vaccines

Oral vaccines are immunobiologicals that are given through the gastrointestinal tract and allow for antigens to interact with gut-associated lymphoid tissue (GALT); specifically, Peyer's patches and microfold (M) cells. The interaction of an antigen on GALT can activate both mucosal and system immune response. The oral route of immunization is particularly effective at stimulating secretory immunoglobulin A (sIgA) responses in the intestinal mucosa. SIgA is critical to preventing the attachment of pathogens to and invading through mucosal surfaces[36]. Oral vaccines have also been used successfully to prevent enteric pathogens, with classical examples being the oral poliovirus vaccine, oral rotavirus vaccine, and oral typhoid vaccines. These three examples attest to the efficacy of the use of oral vaccines in large-scale immunization programs [37].

The major benefit of oral vaccines is that they can be given without a needle, and as such, are pain-free. Moreover, because they do not require a needle, oral vaccines greatly increase compliance and acceptance of vaccination by patients, especially among children [38]. By using this route of immunization, strong mucosal immune responses are generated at the principal site of entry of the pathogens, resulting in both local and systemic immune protection. Because of their ease of use, oral vaccines are well suited to mass vaccination campaigns since they reduce the need for trained health care professionals to administer vaccinations, reduce the production of biohazardous waste, and decrease the number of needle-stick injuries and blood-borne infections [36]. Oral vaccines can also help contribute to the achievement of herd immunity through decreased shedding of pathogens and transmission of diseases; examples of this have been noted with the administration of the oral poliovirus vaccine [37].

Despite these advantages, oral vaccines face several challenges that limit their broader application. Antigen degradation due to gastric acid, digestive enzymes, and bile salts can significantly reduce vaccine stability and immunogenicity[38] . Oral tolerance, a phenomenon where repeated exposure to antigens via the gut leads to immune hyporesponsiveness, poses another major limitation and necessitates careful antigen and adjuvant selection [36]. Furthermore, variability in gut microbiota, nutritional status, age, and enteric infections—particularly in low-income settings—can result in inconsistent immune responses and reduced vaccine efficacy [37]. Formulation complexity and the need for protective delivery systems such as encapsulation or live vectors further complicate development and regulatory approval.

C. Microneedles

A newer vaccination method, microneedle vaccination, uses a series of tiny needles with lengths of under 1mm to introduce vaccine into two layers of skin, the epidermis and dermis. Both of these areas contain many antigen presenting cells, where the immune system is activated more effectively than if the pain receptors deeper in the tissue were to be used[27], [39]. Microneedles can be made in four configurations; solid, coated, hollow and dissolving. Of the four, dissolving microneedles are of major interest because they are very safe and produce no sharps waste [29], [40].

The benefits of immunizing by microneedles include extremely low or eliminated pain for all patients who receive a vaccine, especially children and phobic patients. The removal of the risk of needle stick injuries and the potential to administer vaccinations with lower doses, which could lead to enhanced immunogenicity[27], [41]. In addition, many microneedle vaccines are stable at higher temperatures which allows them to be stockpiled and may also reduce reliance on cold-chain transportation for vaccination campaigns in countries with limited resources [15].

While the benefits of microneedle vaccination provide attractive opportunities, there are a few challenges that need to be addressed including the ability to manufacture large quantities, successful delivery of a uniform dose to each patient, the variability in the depth that the microneedles penetrate through the skin for people of different ages, and the regulatory issues regarding how microneedles should be classified when used in conjunction with drugs [42]. Microneedles are a great way to deliver vaccines with little or no pain, and they will be an integral part of the development of the next generation of simply administered vaccines.

D. Transdermal Immunization

Transdermal immunization is a new type of vaccine delivery system that uses the skin to administer an antigen and trigger an immune response. Immunizing through the skin is an attractive method because of the high density of antigen presenting cells present, such as Langerhans cells and dermal dendritic cells, that capture and present antigens in an efficient manner and initiate both the humoral and cellular immune responses[27]. Furthermore, unlike the conventional method of intramuscular injection, transdermal immunization can be administrated through minimally invasive methods such as microneedle patches, jet injectors or skin abrasion techniques, usually with little to no pain or bleeding associated with the delivery of the antigen.

Transdermal immunization has a major benefit of potentially being able to induce a greater immune response with a smaller amount of antigen through dose sparing. When using transdermal immunization, more of the delivered antigen is targeted directly to the skin layers that are rich in immune cells[15]. In addition, transdermal vaccination has been demonstrated to be well accepted by patients, significantly lowers the incidence of needle-stick injuries and sharps, has the potential to simplify administration of the vaccine, and may provide improved stability for the vaccine [43]. For these reasons, transdermal vaccination may be a particularly attractive delivery device for mass immunization campaigns and for individuals that have needle phobia.

However, challenges of transdermal immunization exist, including variability in the thickness and barrier properties of the skin, limitations in the ability to deliver large molecules without specialized devices, and the regulatory complexity associated with drug/device combination products [27]. Above and beyond these barriers, there may be a lack of awareness about this form of vaccination, and/or lack of interest in developing new or innovative products that use such delivery methods. More work continues to be required in demonstrating that transdermal immunization is as effective as, or better than, conventional intramuscular vaccination, and can be used in conjunction with other vaccination methods.

E. Electroporation

Electroporation is a physical method of delivering vaccines, where the brief application of controlled electrical pulses is used to temporarily make little holes in the cell membrane thus allowing for more efficient delivery of vaccine antigens (most commonly DNA and RNA) into the interior of cells, which increases the expression of vaccine antigens and therefore enhances the activation of both humoral and cellular immune responses when compared to the traditional method of injections[44], [45].

Genetic vaccinations are an area demonstrating particular promise for electroporation as the successful transfer of immunogenic material to muscle and skin cells is critical for strong immune response. When intradermal and intramuscular approaches to electroporation take advantage of the abundant immune cells found in the skin and muscle, respectively, strong CD4+ and CD8+ T-cell responses are observed, making them appealing for the development of vaccines against infectious disease and cancer[46], [47]. Electroporation-based vaccine delivery has been assessed in a variety of clinical trials and has generally produced improved immunogenicity. In addition, the clinical trials conducted show that electroporation has an acceptable safety profile; however, the potential for transient pain at the site of application is commonly reported.

Despite advantages over traditional methods of vaccination, electroporation is limited by the need for a unique delivery device, trained personnel who can use the device and a power source. Therefore, electroporation's impact will be limited when it comes to large-scale or resource-limited implementation. Technological advancements that seek to reduce the pain associated with electroporation, simplify electroporation delivery devices and combine electroporation with needle-free administration methods should lead to a more widespread utilization of electroporation as a means of delivering vaccines [45].

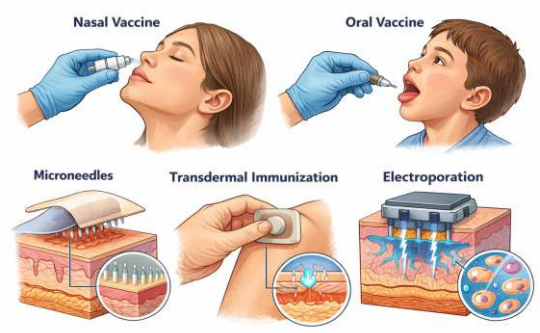

Figure 1: Diagrammatic representation of a. Mucosal Vaccne (Nasal) b. Oral vaccine c. Microneedles d. Transdermal Immunization e. Electroporation technique for vaccine delivery.

F. Iontophoration

Iontophoresis is a physical method of delivering drugs through the skin that does not involve the use of needles. With iontophoresis, a small amount of current is passed through a previously charged electrode to help push the drug across the stratum corneum facilitating access to the viable epidermis and dermis—skin compartments rich in antigen-presenting cells such as Langerhans cells and dermal dendritic cells [27], [48].

When the drug is delivered via an iontophoresis, its effectiveness may be enhanced by the fact that it is being transported with other charged materials in combination with electrical forces. In addition, when receiving a vaccine via the iontophoresis method, the transportation of the vaccine to the immune cells will occur much faster than if the vaccine were delivered without the iontophoresis technique.

Research has been performed to validate the effectiveness of using iontophoresis as a painless and needleless vaccine administration route for many types of vaccinations including, but not limited to, protein antigens, peptide vaccines and DNA vaccines that induce humoral and cellular immunity. Preliminary research has demonstrated that the administration of antigens to the skin using iontophoresis may result in higher than normal levels of antigenic uptake and enhance the immune activation of lymphocytes.[49].

The advantages of using iontophoresis for vaccine delivery are that it is non-invasive and does not cause pain, so it may help to alleviate anxiety associated with needles especially for young children and people with needle phobias[50]. In addition, the iontophoresis delivery system provides controlled and localized delivery of a therapeutic agent by changing the amount of electrical current used to deliver the vaccine[51]. The use of iontophoresis avoids generation of sharps waste and may allow for self administration or simplified clinical workflow; therefore, it has potential for mass immunization or when give booster vaccinations.

While iontophoresis has many advantages, it has limitations. It has been shown to be mainly useful for delivering small charged molecules, whereas delivering macromolecular antigens or whole vaccines can be difficult; in some cases, formulation or other types of enhancers must be used [48]. It has also been documented that improper or prolonged application of electrical current can lead to skin irritations, erythema and burns. Therefore, it is essential that device application and patient monitoring be carried out closely[27]. In addition, the requirement for electrical devices and power sources limits use of iontophoresis in low income resource or field settings, so there are limited clinical data that establish long term immunogenicity of vaccines delivered iontophoretically[50]. In conclusion, although iontophoresis presents a promising aid or alternative to traditional methods of vaccination, continued clinical testing and technology refinement is needed.

G. Microporation Or Thermal Ablation

Microporation, particularly laser-based microporation, and related thermal ablation techniques are increasingly explored as innovative strategies for vaccine delivery through the skin. Micropores are created in the stratum corneum by means of controlled, short bursts of laser energy or heat that cause a temporary disruption to the skin barrier. The microchannels that are created allow the vaccine antigens to diffuse down into the epidermis and upper dermis, where they come into contact with a high concentration of antigen presenting cells (APC's), such as Langerhans cells and dermal dendritic cells, thereby targeting the cutaneous immune system. Research has shown that microporation-assisted transcutaneous immunization will induce effective immune responses at lower antigen doses than conventional intramuscular vaccination, indicating that this could be a viable alternative to needles[52], [53].

One of the benefits of microporation and thermal vaccine are that they are minimally invasive and do not require needles; therefore they may increase patient compliance, decrease pain and distress associated with the use of needles, and eliminate sharp disposal issues. As a result, there is an increase in antigen uptake and presentation when antigens are delivered directly to the immunologically active layers of the skin; this may lead to a stronger immune response both humoral and cellular. Laser microporation also enables precise pore depth and density control.[54], [55].

Lasers and radiofrequency devices are expensive, require specialized training to use, may be more challenging to implement in large scale mass vaccination programs and restricted resources to deploy in low-resource countries. Also, it is critical to understand the varying amounts of antigen penetrating through micropores based on the formulation characteristics, skin type, and application technique which may affect dosing to be delivered(Zhao et al., 2023). Some adjuvants or particulated formulations may not be compatible withs effective delivery through the skin (transcutaneous) using thermal microporation. In addition, the implementation of these technologies into clinical practice is currently constrained by regulatory requirements associated with combination products containing both medical devices and vaccines and the need for extensive clinical validation(Parhi&Mandru, 2021; Zhao et al., 2023).

H. Sonophoration

Vaccine delivery using sonoporation is an immunization technique that employs ultrasound energy. The ultrasound temporarily increases the permeability of biological boundaries (like the stratum corneum of the skin) to allow for transdermal delivery of DNA, RNA or proteins associated with the vaccine to reach target tissues. An additional benefit of using ultrasound in this fashion to accomplish needle-free immunization is that those techniques utilize the same method and techniques that ultrasound agents are used to facilitate drug administration (i.e., sonophoresis). Low- frequency ultrasound (20-100 kHz) to transdermally deliver vaccine antigens through the disruption of the stratum corneum is one example of sonoporation. This is advantageous because the skin has a large number of antigen-presenting cells, including Langerhans cells and dermal dendritic cells, giving the skin a primary role in the stimulation of immune systems[58], [59].

Sonoporation is achieved primarily via the mechanism of acoustic cavitation; a process by which ultrasound waves generate oscillating microbubbles in the coupling medium or tissue. Microbubbles caused by ultrasound, particularly at low frequencies, collapse violently creating microjets and shockwaves that disrupt lipid bilayer and intercellular junctions, thereby resulting in the formation of transient aqueous pores that provide entry for vaccine antigens and nucleic acids to cross non-permeable barriers. Further, these aqueous pores typically reseal within minutes to hours and provide integrity to the tissue. Importantly, these pores typically reseal within minutes to hours, preserving tissue integrity. The typical time for closing of these pores is between 5 minutes and many hours, so tissue integrity remains intact. Transient permeability improves uptake of antigen into the tissue and promotes presentation of the antigen by local immune cells, which ultimately increases the immune response to the vaccine [58], [60].

Ultrasound has also been investigated as a physical adjuvant for vaccine delivery via transcutaneous administration. Studies have shown that application of ultrasound to the skin activates Langerhans cells in the epidermis and enhances the presentation of antigen to these cells, resulting in a stronger (antigen-specific) antibody response. Kost has shown that the application of low frequency ultrasound significantly improves the immune response to topically administered antigens in animals; therefore, ultrasound increases both delivery of antigen to the immune system and enhances the activation of these immune cells [61]. Further supporting the potential of ultrasound in needle-free vaccine delivery, a recent study has shown that ultrasound-treated skin has elevated levels of immune signaling[62].

Sonoporation is being investigated as a possible solution to the challenge of efficiently delivering DNA vaccines (as well as other nucleic-acid based vaccines) into cells. Researchers have found that when using microbubbles to assist sonoporation, the result has been an increase in gene transfection rates due to the enhanced localization of cavitation effects at the focus of the ultrasonic waves.

Optimization of both the microbubbles and ultrasound parameters has resulted in increases in antigen expression and improved immune responses in vivo. These findings suggest that sonoporation could be a viable solution for developing future DNA and RNA vaccine platforms[60], [63].

Despite its benefits, there are several limitations to the use of sonoporation for vaccine delivery. For example, ultrasound-mediated delivery is dependent on accurate control of various parameters such as frequency, intensity, duty cycle, and duration of exposure. The use of suboptimal settings can lead to non-delivery of vaccines. However, using excessive ultrasonic energy could result in skin dermatological damage, local heating of the dermis, and cavitation damage to the tissue surrounding the vacuoles created from the sonorestrictive phenomenon. In addition to the difficulty in obtaining specialized ultrasound equipment and a lack of standardization for protocols for large-scale vaccination programs in low-resource settings, the variability in delivering consistent and reproducible dosing through the skin is more complicated than with typical needle injection methods [58], [61], [64].

In conclusion, sonoporation is a very exciting method of delivering a vaccine without the use of needles. It can enhance the amount of antigen that enters your body and stimulates your immune system to respond to the vaccine. While laboratory studies and early human trials have demonstrated that it has the ability to deliver both protein and nucleic acid vaccines, more work needs to be done to improve the effectiveness and safety of sonoporation at higher doses (greater than 18mg/ml) and also create standardization among devices before we will see widespread clinical use.

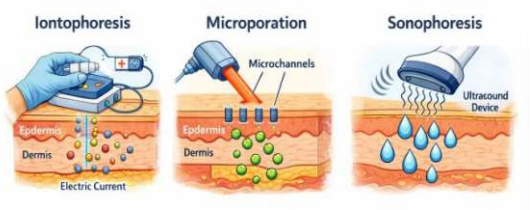

Figure 2: Diagrammatic representation of vaccine delivery via Iontophoresis, Microporation and Sonophoration technique.

H. Nanoparticles

Nanoparticles (NPs) are an important part of vaccine delivery because of their small size (usually nanometres), surface chemistry can be modified to protect Antigens/Nucleic Acids or determine where they will go in within the body, and the immune response they elicit can be modified by the composition of the NP. In practice, NP vaccine encompasses LNPs used to deliver mRNA/saRNA, polymeric NPs (e.g. PLGA or other polymers), liposomes, inorganic NPs, and self-assembling proteins (e.g. VLPs) that act as both a delivery mechanism and often a means of enhancing the efficacy of the vaccine by facilitating the presentation of Antigens to the innate immune system and by activating the innate immune system [65], [66], [67].

Nanoparticle-based delivery has one major benefit: it enables the delivery of unstable cargo in a protected form.

The encapsulation or complexation of mRNA/DNA or protein antigens provides protection against enzymatic destruction, and also enhances the amount of antigen/nucleic acid that can enter cells compared to an unencapsulated or uncomplexed antigen/nucleic acid[65], [68]. Additionally, nanoparticles can also be designed to target lymphatic drainage and lymph-node entry. This enhanced targeting is critical for promoting robust adaptive immune responses. Particles that are approximately 10 to 100 nm in size have a greater ability to reach draining lymph nodes where they are taken up by antigen-presenting cells (APCs) and enhance the processing and presentation of antigens to T lymphocytes[67]. NPs can be engineered for immune bias by determining the size, charge, surface ligands, and the inclusion of innate immunity agonists to drive stronger neutralising antibody and/or potent T cell immune responses in recipients[56], [66]. In addition, NPs can support dose-sparing through improved antigen usage. There are many NP systems that allow for the controlled or sustained release of antigens, thereby improving the availability of antigens to immune cells and possibly extending the duration of the immune response[66], [69]. Finally, LNPs and other NP platforms have also made possible the rapid design and production of vaccines against novel pathogens because the basic carrier structure can remain unchanged while the sequence of the encoded antigen varies considerably[65].

Despite having these benefits, there are a lot of negatives and challenges to bringing a nanoparticle-based vaccine into use as well. There may be reactogenicity and safety-related issues associated with the components of the carrier used in addition to activation of the innate immune system. For example, the composition of the lipid nanoparticles (ionizable lipids, PEG-lipids, etc.) can impact tolerance and inflammation-related signalling, and there are limited data regarding hypersensitivity reactions in relation to the composition of the nanoparticles; thus, the formulation of LNPs will need to be carefully optimised for intramuscular administration[65], [68]. In addition to this, biodistribution and off-target uptake (e.g. hepatic/splenic phagocytes) may potentially reduce the efficiency of delivering the nanoparticle(s) to the desired subsets of antigen-presenting cells and make it more difficult to assess the safety profile of the nanoparticle when delivered systemically[68], [69]. Additionally, the manufacturing and quality control of the nanoparticles are also difficult; the reproducible manufacture of large quantities requires a high degree of control over particle size distribution, the degree of encapsulation, residual solvents, and stability of the particles during storage and transport; and in the case of some mRNA-LNP products, there is a reliance upon cold-chain; thus, there are still many practical barriers to commercialisation in different parts of the world [68]. For other types of nanoparticles, such as nanoparticle vaccine delivery via polymeric nanoparticles or virus-like particles, the complexity involved in formulating the nanoparticles and conjugating the antigens may lead to increased costs, longer times for commercialisation, and influence the rate of success in commercialisation[66], [67], [69]. Whether or not a nanoparticle-based vaccine will be successful and able to achieve approval for use as a vaccine will be contingent upon the level of anti-carrier immunity developed following immunisation with a nanoparticle platform and the rate of clearance of nanoparticle-based vaccines (based on platform) following initial dosing[69], [70].ere are several limitations to the use of sonoporation for vaccine delivery. For example, ultrasound-mediated delivery is dependent on accurate control of various parameters such as frequency, intensity, duty cycle, and duration of exposure. The use of suboptimal settings can lead to non-delivery of vaccines. However, using excessive ultrasonic energy could result in skin dermatological damage, local heating of the dermis, and cavitation damage to the tissue surrounding the vacuoles created from the sonorestrictive phenomenon. In addition to the difficulty in obtaining specialized ultrasound equipment and a lack of standardization for protocols for large-scale vaccination programs in low-resource settings, the variability in delivering consistent and reproducible dosing through the skin is more complicated than with typical needle injection methods [58], [61], [64].

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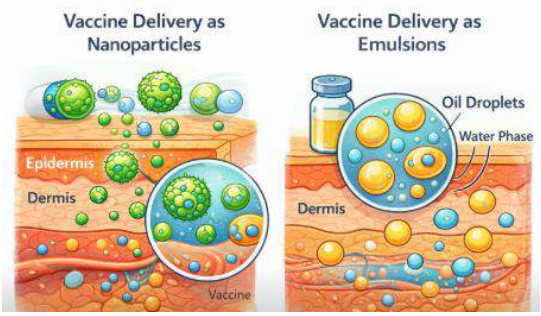

Figure 3: Pictorial representation of the delivery of vaccine as Nanoparticles and Emulsions.

I. Emulsions

Vaccine emulsions are two-phase (water and oil) liquid mixtures composed of 2 non-mixable liquids (water and oil) emulsified by surfactants to create either oil-in-water or water-in-oil emulsions. Oil-in-water emulsions, like MF59, AS03 and AF03, are highly researched and developed in today's vaccine world as adjuvant systems with proven clinical efficacy. Emulsions provide immunostimulating delivery systems instead of simple repositories for an antigen, thus improving immune responses by increasing the uptake of the antigen, enhancing the

recruitment of antigen-presenting cells to the injection site and improving the transport of antigens to the draining lymph nodes[71], [72]. The nanoscale diameters of the droplets of emulsion formulations will cause localisedactivation of the innate immune system (via cytokine and chemokine production), which in turn helps to generate strong humoral and cellular immune responses[73].

Emulsion-based vaccine delivery systems such as aquasomes, emulsion minigels and other types of emulsion formulations can provide a number of benefits to the vaccine recipient and public health, including greater antimicrobial activity, increased vaccine stability and improved safety. One of the key advantages of emulsion-based vaccine delivery systems is that they will enhance the immunogenicity of poorly immunogenic antigens. For example, adjuvanted emulsion-based vaccines have been shown to significantly increase antibody titres, enhance the affinity maturation of antibodies, and broaden the recognition of epitope with improved magnitude and quality of the immune response[74]. One particularly beneficial aspect of oil-in-water emulsions is that they are able to decrease the amount of antigen needed to generate protective immunity (i.e. Antigen Dose Sparing), particularly during pandemic preparedness and large scale mass vaccination campaigns[72]. Emulsion-based vaccine delivery systems have been shown to elicit a balanced Th1/Th2 response and facilitate cross-protection, particularly against influenza virus strains where there is frequent antigenic drift[73]. Emulsion-based adjuvants such as MF59 have also been demonstrated to be safe for all age groups (including older adults with compromised immune systems)[75].

Despite these advantages, emulsion-based vaccine delivery systems can also have some limitations. For example, there is a higher incidence of local reactogenicity (e.g. injection site pain, erythema and swelling) associated with the use of adjuvanted emulsion based vaccines when compared to the use of non-adjuvanted emulsion formulations; however, the local reactions associated with the use of adjuvanted emulsion formulations typically resolve themselves quickly and without intervention[75]. The most notable formulation constraints associated with emulsion-based vaccine delivery systems are that they can be difficult to manufacture and require specialisedformulation development in order to achieve stable, effective and safe formulations. Therefore, a better understanding of how to develop emulsions for use as vaccine delivery systems will allow for the further development of novel vaccine formulations for global application as well as potential use in veterinary and animal health products.[76]. Another obstacle is the dependence on the cold chain for the ideal temperature storage and shipping of emulsions, as temperature deviation during shipment could lead to decreased stability of emulsion products. While emulsions aid in the generation of antibody-mediated immunity, their efficacy at producing strong T-cell cytotoxicity may be lower than newer adjuvant technologies, such as those involving pattern recognition receptor agonists, due to the multicomponent nature of emulsions and the multitude of data necessary for regulatory acceptance, i.e. safety, consistency and post-marketing data[72][73].

Overall, emulsions have demonstrated their place as an established, validated delivery method for vaccines, providing an appropriate balance of immunogenicity and safety. Ongoing research will continue to advance the development of emulsions by providing a better understanding of their thermostability; decreasing reactogenicity; and developing novel immunostimulatory molecules in combination to optimize immune response for the next generation of vaccines[77].

Future Thrust

The future of needle-free bio-immunization will be improved by the development of more efficient, accurately targeted, and globally accessible delivery methods. Advances in nano-materials, nano-technology, and formulation science will facilitate the creation of vaccine systems that can be self-administered, stored at room temperature for longer periods of time, and are less reliant on cold-chain distribution systems or trained medical personnel. The integration of a needle-free delivery platform with the use of novel adjuvants, nucleic acid vaccines, and virus-like particles is anticipated to result in the development of systems that can induce robust cellular and mucosal immune responses, which are crucial for protecting against both old and new infectious agents. Smart delivery technologies that incorporate controlled release mechanisms, immune-targeting ligands, and digital monitoring devices may enable personalisedand evidence-based immunisationprograms to be developed. The creation of a common set of regulatory standards, large-scale clinical trials, and cost-effective manufacturing processes will be necessary to support the widespread use of needle-free bio-immunisation worldwide. As vaccine research increasingly aligns with equity, safety, and rapid deployment, needle-free bio-immunisations will play a major role in the development of new immunisation programs.

Conclusion

Immunobiologicals using needle-free administration area new breakthrough in vaccine as well as therapy delivery and will help to overcome many of the barriers that have historically existed. By eliminating the need to use hypodermic needles to administer vaccines, these techniques will also significantly reduce the amount of pain, anxiety related to needles, risk of needle-stick injuries, and transmission of blood-borne pathogens; thus improving patient compliance and vaccination coverage. Currently, there are a number of needle-free platforms for vaccine delivery, including transdermal systems (microneedles), jet-injecting devices, electroporation, iontophoresis and mucosal delivery routes, that have been shown to enhance the presentation of the antigen in the immune system because the antigen is presented to the immunological tissues that are in abundance, such as the skin and mucosa. These technologies frequently also lead to dose sparing, better immunogenicity, and a more balanced immune response, which are

extremely important attributes for mass immunisation programs, pandemic preparedness, and vaccination programs in resource-limited settings. There is a growing amount of clinical and pre-clinical data supporting that needle-free immunobiologicals are safe, effective, and patient-centred alternatives to traditional vaccine delivery systems. As research into these methodologies continues, it is critically important for researchers as well as practitioners to work toward solving the existing challenges, allowing for a new time in vaccinations that will occur without needles.

Author's Contributions

All the authors contributed significantly in developing the manuscript. All the authors have reviewed and approved the final draft for submission.

Conflict of Interest

There is no potential conflict of interest.

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