Review Article:

An Overview of Novel Adjuvants and Delivery Platforms as a Model Approach to Current Vaccine Development

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Abstract

Vaccine development is one of the most significant achievements of humans in Biomedicine. These vaccines are usually supplemented with adjuvants to enhance immunity. Apart from alum which is recognized as the gold standard, one may increase the quality of the vaccine by adding other adjuvants or agents that boost the immune response with improved delivery vehicles such as liposomes, immune-stimulating complexes (ISCOMs), and micro/nanospheres. Adjuvants stimulate the effectiveness of a vaccine by activating the immune system to respond more strongly to the vaccine, resulting in enhanced immunity to a particular disease/antigen. Adjuvants do this by imitating specific sets of identical molecular sequences like lipopolysaccharides (LPS), bacterial cell wall basic constituents, and endocytosed nucleic acids such as double-stranded deoxyribonucleic acid (dsDNA), single-stranded ribonucleic acid (ssRNA), and unmethylated CpG-dinucleotide containing deoxyribonucleic acid (DNA). Apart from incorporating adjuvants to improve vaccine quality, the delivery system also is an important aspect of vaccine development. The development of next-generation technology has been explored to generate a vaccine with the same efficacy as the original live-attenuated and inactivated vaccines, but without the hazards and limitations that come with them, including their delivery methods. These novel vaccines have explored methods for targeted delivery of antigenic material, as well as for the control of release profiles so that dosing regimens can be matched to the timelines of immune system stimulation and the realities of healthcare delivery in dispersed populations. This review covers vaccination adjuvants and delivery vehicles that are currently in use/still developing.

Keywords:

Adjuvant, immunity, antigen, delivery systems, vaccines

Introduction:

Vaccines have significantly decreased disease morbidity and death, making them one of the important medical inventions created since Edward Jenner's smallpox vaccine was first introduced in 1798[1]. Although vaccines have unquestionably proved successful in reducing human mortality caused by many diseases, there is still a need for improvement in an earlier known paradigm of 'isolate, inactivate, inject' started by Louis Pasteur in vaccine development and usage. This improvement involves a more 'rational design' approach to optimizing novel adjuvant strategies and delivery systems through a deeper comprehension of immunological pathology and microbial studies. Presently, most immunizations are administered parenterally with the aid of a hypodermic needle and most of them elicit poor immune response. The base of vaccine delivery systems is using a hypodermic needle/syringe because it offers a direct, inexpensive means of delivery with immediate confirmation of the exact dosage being administered. However, the numerous difficulties and restrictions associated with needle and syringe distribution make it seem like a fairly antiquated strategy. One of these drawbacks is the impact that discomfort and needle phobia have on patient compliance and, ultimately, vaccination rates and success[2]. The spread of blood-borne pathogens as a result of needle reuse or needle-stick injuries is also another area of concern. Reports have shown that an alarming 40% of the 16 billion injections administered, were carried out with reused needles/syringes without sterilization. This resulted in an estimated 20·6 million new hepatitis B infections, 2 million new hepatitis C infections, and 260,000 new HIV infections in the United States[5]. Additionally, 77% of nurses and nearly half of all United States physicians sustained needle stick wounds from 2000-2019, which gave rise to 16,000 hepatitis C, 66,000 hepatitis B, and 1000 HIV infections[3]. The need for liquid formulations, which frequently necessitate costly cold-chain transport and storage, is another restriction to the reliance on needles[4]. Researchers have sought to create alternate strategies through the use of adjuvants and delivery systems to combat these challenges. Adjuvants play a vital role in human vaccinations. Adjuvants have a variety of uses, including enhancing immune response, supplying antigen-dose sparing, accelerating immunity, lowering the need for booster immunizations, extending the duration of protection, or improving efficacy in populations with poor immune response, such as newborns, elderly, and immune-compromised subjects[5]. In this review, we examined various new target adjuvants/delivery vehicles, each of

which has distinct physio-chemical and immunological characteristics, behaviors, relevance, and choices for vaccine formulation.

Adjuvants and their importance in vaccine development:

An adjuvant is any substance added to a vaccine to strengthen/enhance the immune response to a vaccine antigen. The word "adjuvant" is derived from the Latin word "adjuvare," meaning "to help" or "to enhance," and can be defined as any item or combination of items that heightens/ modifies the humoral or cellular immune response against an antigen. Adjuvants have historically been used to boost the immunity to a vaccine based on antibody titer or its ability to prevent infection according to some kinds of literature. So many groups of adjuvants have been used to augment immunogenicity. The major ones are Alum-based adjuvants (aluminum salts and other mineral salts adjuvants), Complete and incomplete Freund's adjuvant, adjuvant emulsions, and Adjuvant 65 amongst others. These adjuvants in addition to enhancing immune response also direct the type of adaptive response elicited in order to produce the most effective forms of immunity for each particular pathogen/antigen. Thus, adding an adjuvant to a vaccine has two separate benefits. Adjuvants are used in clinical settings to (i) increase the response to a vaccine in the general population by increasing mean antibody titers and/or the proportion of subjects who become protectively immunized (ii) increase seroconversion rates in populations with decreased responsiveness due to age (both infants and the elderly), disease, or therapeutic interventions. An example is the use of MF59 adjuvant to enhance the response of older people to influenza vaccine, (iii) make it easier to use smaller doses of antigen, since an adjuvant has the ability to elicit comparable immune responses with much lower doses of antigen, and (iv) allow immunization with fewer doses of vaccine. Furthermore, adjuvants are used more frequently in vaccine development to encourage some kinds of protection that are not efficiently produced by the non-adjuvanted antigens. Adjuvants have been used in both pre-clinical and clinical research to achieve several goals: (i) to induce immune responses that are functionally appropriate, such as promoting the activation of T helper 1 or T helper 2 cells, CD8+ or CD4+ T-cells, or producing specific antibody types; (ii) to enhance the development of memory immune response, especially T-cell memory; (iii) to accelerate the initial immune response, which is crucial during a pandemic outbreak; and (iv) to modify the rate of immune response, its specificity, or affinity to antigens[6].

Novel adjuvants target:

The goal of applying new technologies is the formulation of novel adjuvants that will assist the innate immune system to boost adaptive immunity and address the limitations of current adjuvants. Many studies have demonstrated the potential of cell-penetrating peptides and antimicrobial peptides to adjuvant vaccines by improving antigen delivery and immunogenicity, respectively [7,8]. The discovery of Toll-Like Receptors (TLRs) has initiated a revolution in adjuvant design. While TLR9 and TLR4 agonists are present in the clinical vaccines that are being developed, additional TLR agonists show promise as well because they can target distinct immune pathways. TLR5 is one of the most promising emerging adjuvant targets, which is a cell surface pattern recognition receptor that recognizes a highly conserved amino acid sequence on bacterial flagellin[9]. Stimulation of TLR5 leads to activation of dendritic cells and chemokine production, induces a robust humoral response but a minimal cellular immune response[10]. This specificity for humoral immunity makes it an attractive adjuvant for vaccines targeting pathogens that can be neutralized by antibodies but not CD8+ T cells. Flagellin has been formulated as an antigen fusion protein and conjugated onto the surface of nanoparticles and virus-like particles with promising pre-clinical results[11-13]. Clinical trials using flagellin as an adjuvant for influenza vaccines in healthy adults (NCT00921947) and people over the age of 65 (NCT00966238) have demonstrated its safety and efficacy[14]. Flagellin has also been clinically tested in a pneumonic plague vaccine (NCT01381744) with similar success[14]. Although antibodies can be generated against flagellin, thereby decreasing its efficacy, much progress has been made in designing epitope-deficient flagellin that retains its adjuvant activity without generating anti-flagellin antibodies[15]. Other promising emerging targets include TLR7 and TLR8, which are structurally similar, located in the endosome, and recognize RNA and small molecules, such as imiquimod[16]. When stimulated with imiquimod or similar small molecule agonists, TLR7/8 induces both humoral and cellular immunity. Unfortunately, these molecules also exhibit poor water solubility and high toxicity[17]. However, formulation with delivery vehicles such as liposomes, nanoparticles, or nanofibers can enhance the safety, solubility, and immunogenicity of these adjuvants[18]. Several clinical trials have investigated TLR7/8 agonists as adjuvants in vaccines for influenza (NCT01737580, NCT03472976) and hepatitis B (NCT00175435, NCT03307902) among other diseases. Although TLRs

have been the molecular target of choice for most rationally designed adjuvants, other Pattern recognition receptors (PRRs) are emerging targets for improving the immune response to co-delivered antigens, including C-type lectin receptors (CLRs), stimulators of interferon genes (STING), and others[19]. These different PRRs play important roles in a variety of innate immune pathways responsible for the effective response to a diversity of pathogens.

Cyclic dinucleotides (CDNs) of both natural and synthetic origin have also been investigated as potential adjuvants. Preclinical studies of vaccines incorporated with these cyclic dinucleotide adjuvants have elicited cellular and humoral responses against influenza, tuberculosis, anthrax, Streptococcus pneumoniae, and methicillin-resistant Staphylococcus aureus[20]. The long-lasting cellular and humoral protection generated by these adjuvants makes them promising for use with vaccines targeting both intracellular and extracellular pathogens. There are several ongoing clinical trials (NCT02675439, NCT03010176, NCT03172936, and NCT03937141), investigating CDNs as adjuvants for infectious disease and cancer immunotherapy. CDNs have been formulated with liposomes, oil-in-water emulsions, nanoparticles, and hydrogels to enhance cellular uptake and presentation to immune cells[21,22].

C-type lectin receptors (CLRs) are efficient PRRs that interact and recognize an array of pathogen-associated carbohydrates. These receptors play an important role in infectious disease progression and protection, and using CLR agonists as adjuvants can help vaccines emulate natural infection[23,24]. These carbohydrates have been formulated as nanoparticles, gels, and antigen-fusion complexes to improve both humoral and cellular immune responses to vaccines. There are several ongoing and completed clinical trials investigating the safety and efficacy of carbohydrate adjuvants for infectious disease and cancer, such as chitosan (NCT00806962), β-glucan (NCT04936529), mannan[25], and crystalline δ-inulin under the trademark AdvaxTM (NCT05005559)[26]. These studies have thus far suggested that carbohydrate adjuvants can be well-tolerated and effective in humans at the doses necessary to confer an immunological benefit[25,27,28].

Major novel vaccine designs:

Virus-like particles: Virus-like particles (VLPs) are highly organized, repetitive structures that contain a high density of viral capsid proteins. This high density of capsid proteins provides copious amounts of conformational viral epitopes, capable of eliciting strong immune responses[29]. Significantly, VLPs are formed by the self-assembly of viral capsid proteins in the ab-

sence of any of the infectious nucleic acids from the virus. Thus, they are a potentially safer alternative to the attenuated viruses commonly used for vaccination due to their absolute inability to replicate. VLPs, due to their simpler makeup, enable speedier vaccine production than older methods, which is especially important for treating highly evolving viruses like influenza.

Conjugate vaccines: Vaccines that use either live-attenuated or inactivated pathogens contain a wide array of different antigens, both polysaccharide- and protein-based. However, only a small number of these may be required to induce protective immunity[30]. Computational modeling has been an effective method for finding and mapping the conformation of immunogenic epitopes in proteins[31]. Conjugate vaccines for Haemophilus influenza type B (Hib), pneumococcus (PCV), meningococcus (MenACWY) and malaria (Mosquirix) have been approved for use in humans. The RTS, S/AS01 (Mosquirix) vaccine, in particular, is a conjugate vaccine of a repeated region of the circumsporozoite protein from Plasmodium sporozoites conjugated to the hepatitis B surface antigen.

Peptide or polysaccharide-based vaccines: These vaccines tend to be less immunogenic when they are present on the surface of a pathogen, thus they require the inclusion of an adjuvant when being administered to enhance its immunogenicity[32]. The antigen can also be conjugated to a 'helper' protein or polysaccharide that is known to increase immunogenicity. Careful pairing and orientation of target and helper portions of the vaccine or spatial segregation of the two subunits by use of carrier systems, such as liposomes, are approaches to overcome this issue[33]. Synthetic peptides and polysaccharides are inexpensive and easy to make, eliminating the possibility of contamination with infectious material, which is a risk with traditional live-attenuated or inactivated vaccines.

Nucleic acid vaccines: Long-term gene expression from plasmid DNA has been demonstrated to be achieved by intramuscular injection in mice[34]. Such plasmids can be used to encode a viral antigen, which can lead to both humoral and cellular antigen-specific immune responses[35]. These discoveries prompted further research into DNA-based vaccines for a wide range of diseases, including influenza, human immunodeficiency virus (HIV), and lymphocytic choriomeningitis virus (LCMV)[36]. Because DNA vaccines might be produced in large quantities in bacteria or with simple, scalable chemistry, they are relatively affordable. However, the main drawback of DNA vaccines is their low immunogenicity due to the very low transfection rate

they achieve. This limitation resulted in the development of RNA-based vaccines. RNA-based vaccines, like DNA-based vaccines, are less expensive and can be mass-produced rapidly. However, their use has traditionally been limited due to RNA instability and entry barriers into the nucleus. The entry barrier of RNA vaccine can be addressed by utilizing polycationic carrier molecules to compress and protect the RNA while still allowing for rapid cellular absorption[37]. Two main types of RNA vaccines have been used for infectious pathogens: non-replicating and self-amplifying RNA vaccines. Non-replicating RNA vaccines are simpler and less expensive to create, but their lifespan and level of expression are limited. Self-amplifying RNA systems can use sequences and concepts from single-positive strand RNA viruses, such as Alphavirus (Alphavax, United States). These vectors contain both the nonstructural genes and the immunogen. As a result of intracellular amplification of the antigen-encoding RNA, a small dose of vaccine can create a significant amount of antigen. While RNA appears to be the more appealing of the nucleotide-based options, it should be highlighted that DNA has the potential to provide advantages in terms of coding capacity as well as the volume and duration of immunogenic protein production.

Cellular vaccines: Immunization with live attenuated viruses, inactivated viruses, or bacteria, has recorded some numbers of efficacy and so, attempts have been made to utilize a similar strategy to prevent cancer. To generate an immune response against specific forms of cancer, attenuated tumor cells were delivered. Whole-cell vaccines have been classified as either autologous or allogenic. Autologous cell vaccines have been studied for a range of cancers, including lung, colorectal, melanoma, kidney, and prostate cancer[38-40]. Autologous cell vaccines are confined to specific types and stages of tumors since they require a significant amount of the patient's tumor to prepare. Allogenic cell vaccines, on the other hand, are often made up of a combination of existing human cancer cell lines, and while they are not patient-specific, they do not have the manufacturing constraints that autologous cells do. Many whole-cell vaccines have been genetically engineered to improve immune activation by promoting the expression of cytokines, chemokines, and co-stimulatory molecules[41]. Another type of cellular-based vaccine uses the patient's immune cells mainly dendritic cells. Dendritic cell vaccines can be created by loading tumor-associated antigens or genetic material encoding for tumor-associated antigens into a patient's autologous dendritic cells, which are then

treated with immunoadjuvants. The freshly matured, antigen-loaded dendritic cells are then reintroduced into the patient to promote anti-tumor immunity. Clinical trials have used dendritic cell vaccines to treat prostate, melanoma, kidney, and glioma tumors[42]. The first therapeutic cancer vaccine approved by the Food and Drug Administration was a dendritic cell-based vaccine for prostate cancer, Sipuleucel-T (Provenge)[43].

Novel vaccine delivery platforms:

(a) Liposomes:

Liposomes are spherical vesicles with a biocompatible phospholipid bilayer that serves as a delivery vehicle or as an adjuvant[44,45]. Liposomes' flexibility and adaptability are major advantages; the choice of lipids and formulation process permits control over their charge, size, and antigen inclusion position[46]. Liposomes are often made up of four key components: a charged lipid, which influences how liposomes behave in vivo; a lipid-linked polyethylene glycol (PEG), which enhances in vivo stability; cholesterol that improves structural stability; and a phospholipid, which aids in the formation of a lipid bilayer. Unlike their anionic complements, cationic liposomes can skip the endosomal-lysosomal breakdown pathway in cells.[47] Additionally, their net positive charge allows for condensing nucleic acid constructions (DNA or RNA) into distinct entities capable of entering target cells. Antigens can be encapsulated within, conjugated to the surface of, or embedded within the lipid bilayer determining the sort of immunological response induced by the vaccine[33,48]. Liposomes' carrying capacity enables immunostimulatory substances such as cytokines or Toll-like receptor agonists to be co-delivered to immune cells[33,44]. Liposomes were first used in the development of diphtheria toxin vaccine in 1974[49]. Since then, liposome-based vaccines for hepatitis A (Epaxal) and influenza (Inflexal V) have been approved and used for human vaccination[50].

(b) Polymeric particles:

Polymeric particles have drawn a lot of attention in the area of vaccine delivery due to their potential biocompatibility and biodegradability. Polysaccharides[51], poly D, L-lactic-coglycolic acid (PLGA)[52], polylactic acid (PLA)[52], and poly D, L-lactide-co-glycolide (PLG) are examples of natural and synthetic polymers used to create vaccine delivery particles[53]. Because of their delayed biodegradation rate, these particles can entrap or adsorb antigen and transport it to specific cells, or they can allow for prolonged antigen release[54,55]. Polymeric particles are the primary target for developing a single-dose delayed-release vaccine that could obviate the need for

booster doses in many current vaccines. The antigen-releasing pattern of a PLGA particle can be altered for periods ranging from a few days to over a year[56]. Many studies have used polymeric particles as adjuvants rather than antigen-release profiles. An insulin-derived microparticle, Advax has been tested in human trials as an adjuvant for hepatitis B, influenza, and insect sting allergy vaccinations[57,58]. They are divided into biodegradable (D, L-Lactide-co-glycolide) (PLG), polylactide (PLA), and non-biodegradable (Gold, latex, silica, and polystyrene) nanoparticles.

(c) Inorganic Particles:

Many vaccines that were formulated from inorganic particles have been investigated. Despite their low biodegradability, they have a key advantage of a high degree of control over the synthesis process[58]. To increase immune response, inorganic particles have been utilized as adjuvants and antigen delivery vehicles[59]. The four most prevalent types of inorganic particles found in vaccines are gold, aluminum, calcium phosphate, and silica including structures built of pure carbon. Gold particles are stable and can easily be produced in a range of forms and sizes with a flexible surface for easy conjugation of antigen[60,61]. Gold particles have been utilized in clinical trials for a variety of disorders, including melanoma, influenza, and hepatitis B[62]. Aluminum as a widely used adjuvant in vaccines has sparked some research on the conjugation of antigens to aluminum nanoparticles[63,64]. These studies demonstrated that aluminum particles can act as both a transporter and adjuvant to boost the immune system.

Calcium phosphate particles are promising candidate for vaccine applications as they are bioresorbable, nontoxic, have adjuvant properties, and can easily be loaded with antigen[65]. Calcium phosphate was once used as an adjuvant in commercialized diphtheria, tetanus, pertussis, and poliomyelitis vaccine, but was replaced with aluminum salts in the 1980s[66]. Due to the toxicity and side effects associated with aluminum adjuvants, it has been recommended that calcium phosphate be used as an adjuvant in currently available vaccines[66,67].

Silica-based particles are a popular form of inorganic particle in vaccine development, as they are biocompatible and their interactions with cells can be modified by altering their size and shape[68]. Their surface can also be modified to enhance cellular targeting and uptake[69]. Mesoporus silica particles are efficient antigen transporters for prolonged antigen exposure in vivo while carbon nanoparticles have also been widely studied for drugs and vaccine delivery[70]. Carbon nanoparticles that respond to magnetic force were also created and utilized to track, target, and modulate

dendritic cells, together with a model antigen[71]. Carbon mesoporous spheres, encapsulating antigen, were used as an oral vaccination method for bovine serum albumin as a model antigen[72].

(d) Plant-like Material:

Plant cells are a promising vaccine delivery platform for oral administration because of the ease and low cost with which large populations could be vaccinated. Furthermore, its thick cell wall can protect intracellular material from the harmful conditions found within the stomach[73]. Transgenic plant cells were created to express antigenic material for use in vaccination[74], and may subsequently be able to transport antigenic material to the intestines, where it can interact with the gut's lymphoid tissue. Crops like rice and maize have been employed as expression vectors. These crops are staple foods in target immunization areas. They are cheap to produce and easy to grow in large quantities[75,76]. Plant cell-based vaccines have been produced for a variety of infections, including influenza, hepatitis B, and anthrax[76-78]. One possible restriction to this strategy is the effect of the complexity and variety of the microbiome on the reliability of the response.

Similar to plant cells, single-cellular algae also have a tough cell wall, making them another attractive delivery platform. Algae-based vaccines are easier to genetically modify and can be grown in bioreactors not requiring large areas of land[79]. Several pathogens have been targeted pre-clinically by algae-based vaccines such as foot-and-mouth disease, malaria, and staphylococcus[80,81].

Pollen grains are naturally occurring plant-based materials that have been studied as a vaccine delivery platform[82]. The thick outer shell can withstand the severe conditions of the stomach, making it a viable delivery platform for oral immunization[83]. Studies have shown that when the model antigen, ovalbumin, was encapsulated within pollen grains, systemic and mucosal antibody responses could be elicited. Also, the bacterial or viral strains employed in this kind of vaccination are generally thought to be safe, either due to a natural lack of pathogenicity or attenuation, but they can nevertheless closely resemble a natural infection and hence stimulate an immune response. Immunostimulating complexes (ISCOMs), 40nm cage-like particles that develop spontaneously when cholesterol is combined with Quil-A can also serve as an adjuvant. Antigens are incorporated into ISCOMs via hydrophobic interactions and they can elicit a robust response across all immunoglobulin classes. These also promote cellular immunity, as evidenced by T-cell responses and delayed-type hypersensitivity. Immunostimulating complexes

may be unique in their capacity to trigger CD8+ specific cytotoxic responses. Research showed that when delivered intranasally, influenza ISCOM vaccines elicited robust mucosal IgG and IgA responses[84].

(e) Outer Membrane Vesicles:

Outer membrane vesicles are naturally occurring, non-replicating vesicles produced by Gram-negative bacteria. They are made up of bacterial phospholipids, lipopolysaccharides, outer membrane proteins, and entrapped periplasmic components[85,86]. This offers them inherent immunostimulatory properties and adjuvant activity since they naturally have numerous pathogen-associated molecular patterns on their surface.

Vaccine administration routes:

(a)Transdermal route:

Using a needle and syringe is an effective method of delivering a substance into the body since a needle can easily penetrate the skin's barrier. However, using needles and syringes has many disadvantages such as pain, needle phobia, the risk of needle-stick injuries, and the spread of infections, all of which contribute to increased costs and poor patient compliance[2]. Studies have explored the use of passive delivery technologies through the use of microneedles to administer vaccines transdermally[87,88]. The main drawback of passive delivery is the long lag time to induce an immune response. Nevertheless, the most explored method of transdermal delivery is the use of microneedles, which consist of 10s to 1000s of pointed microsized projections fabricated onto a surface. Several microneedles have been developed, which include solid, hollow, coated, and dissolving microneedles to carry out transdermal delivery. Solid microneedles made of a variety of materials, such as silicon, polymers, water-soluble chemicals, metals, and ceramics, can be used to permeabilize the skin before topical vaccine delivery. Hollow microneedles, like hypodermic needles, allow for pressure-driven injection of a liquid, giving the user control over the injection rate. Coated microneedles use solid microneedles as vehicles to deliver drugs or vaccines put on their surfaces into the dermal layers thereby administering the desired amount rapidly. Dissolving microneedles are an excellent alternative to hypodermic needles since they are engineered to disintegrate completely when injected into the skin, leaving no harmful sharps waste[89]. Microneedles have been used for both transdermal and mucosal immunization, and they can be self-administered without the need for professional training, reducing the workload on medical personnel. Electricity has also been used to administer drugs and vaccines transdermally via two different methods: iontophoresis and electropora-

tion. Iontophoresis uses electrical current to push charged particles into the skin via electrostatic forces[90]. Electroporation disrupts cellular membranes with electrical pulses of hundreds of volts lasting 10 μs to 10 ms[91]. Due to the short pulse length, electroporation mostly relies on weakening the stratum corneum to facilitate passive diffusion to the layers below, while the capacity to rupture target cell membranes also aids in the administration of nucleic acid-based vaccines[92,93].

Sonophoresis is the use of ultrasound to improve transdermal drug and vaccine delivery. The main mechanism that enhances the delivery of sonophoresis is cavitation. This process involves the application of focused ultrasound to achieve expansion and collapse of gas bubbles which, in turn, creates microstreaming and shockwaves[94,95]. Sonophoresis has been used to either increase skin permeability before topical vaccine application or as a method of concurrently applying both cavitation and vaccine, thereby actively pushing the vaccine particles into the skin[96,97]. The invention of nano-sized polymeric cavitation nuclei (nano cups), capable of sustaining and enhancing cavitation activity allows for long-term delivery[97,98].

Biolistics is the use of high pressures to accelerate vaccines to velocities high enough to penetrate the stratum corneum and epidermal cell membranes. It is capable of delivering a jet of liquid or particle vaccines. Jet injections of liquid vaccine have been tested for smallpox immunization, as well as measles, BCG, and influenza vaccinations[99]. A study indicated that particulate vaccine distribution by biolistics requires particles of identical size (less than 70 μm), density over 1 g/ml, and physical stability during the process[100].

(b)Mucosal administration:

The majority of infections enter through mucosal surfaces in the respiratory, gastrointestinal, and reproductive tracts. These surfaces are in direct touch with the air, water, and food in our surroundings, providing a first point of contact for opportunistic infections and their colonization. Conventional systemic vaccination approaches involving a needle and syringe are usually thought to be ineffective in inducing significant mucosal immune responses[101]. However, vaccine administration over mucosal surfaces has the potential to stimulate such responses, resulting in the neutralization of invading pathogens before they may cause widespread disease. Oral vaccination is a recommended method of vaccine administration since it is painless, safe, inexpensive, and does not require skilled workers to administer. The oral approach typically includes swallowing the vaccine, which then goes through the gastrointestinal tract. Al-

ternatively, the vaccines can enter the oral cavity, where conditions are significantly less hostile, however, this route has not been studied thoroughly. In order to induce immunity through the oral gastrointestinal route, a relatively large amount of vaccine must be administered due to factors such as dilution while passing through the gastrointestinal tract, degradation within the stomach, or failure to breach the epithelial tight junctions[102]. One commonly employed method of reducing the severity of the conditions in the stomach is to include basic substances such as sodium bicarbonate to neutralize the conditions[82]. There are currently oral vaccines licensed for human use for cholera (Dukoral, Vaxchora, and Shanchol), poliomyelitis (OPV), rotavirus (Rotarix, RotaTeq, Rotavac, Rotavin-M1, Lanzhou lamb and Rotasiil) and typhoid (Vivotif). Intranasal vaccination is a popular alternative choice for vaccine delivery, as it uses a site that is easily accessible and has the potential for self-administration.

The nasal cavity is likewise well-vascularized, with a wide surface area for antigen uptake. Because nasal vaccination delivers the antigenic material directly to the target region, a lower dose is required as compared to other types of vaccination[103]. Currently, the commonly licensed intranasal vaccine for use in humans is for influenza (Flumist), which consists of a live attenuated virus delivered by nasal spray. Many pathogens are transmitted sexually via the genital tract, such as Human Immunodeficiency Virus, Human Papillomavirus, and chlamydia. Therefore, intravaginal vaccination has been explored as an option to prevent sexually transmitted infections[104]. This is a challenging way of eliciting an immune response since the immunological properties of the female reproductive system change considerably in response to hormone variations throughout the menstrual cycle105.

Intrarectal vaccination is another method of administration explored to prevent diseases such as enteric pathogens, sexually transmitted diseases, and cancer[105,106]. In general, an immune response is more strongly induced at the site of vaccination and in nearby mucosal sites; as such, it is possible to generate both rectal and genital tract immunity in response to an intrarectalvaccination[107].

Conclusions:

Research on novel vectors and techniques of vaccine development in the field of vaccinology is being explored at a rapid pace. The old 'three Is' strategy of vaccine development (isolate, inactivate, inject) is being phased out in favor of a more logical design paradigm. Along with the advancement of these new rationally designed vaccines comes the development of enhanced and more patient-acceptable

delivery mechanisms. These mechanisms ensure a more efficient vaccine target and sustained pain-free antigen administration. By making vaccines more self-administrable, the frequency of needle-stick injuries can be reduced as well as strains on medical professionals. Understanding the specific mechanism of action of new vaccines and vaccine delivery platforms through the use of novel technologies can be enhanced to reduce the limitations of conventional methods. Vaccines are the most effective, life-saving medical therapy available today. According to this review, the variety of complementary techniques and technologies coming from research and clinical testing will allow the vaccine's beneficial influence to continue to rise. Potential impediments to wider adoption of these techniques will include the costs involved and the complexities of some of the technology necessary, thus these issues, together with the scientific and technological problems, warrant careful attention throughout the development phase.

Authors' Contributions:

Akunna Perpetua Emeruwa wrote the paper; Angus Nnamdi Oli and Malachy Chigozie Ugwu edited the paper.

Conflict of interest statement:

The authors declare that they have no conflict of interest.

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