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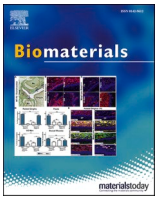

Recent advances in nano- and micro-scale carrier systems for controlled delivery of vaccines

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A B S T R A C T

Vaccines provide substantial safety against infectious diseases, saving millions of lives each year. The recent COVID-19 pandemic highlighted the importance of vaccination in providing mass-scale immunization against outbreaks. However, the delivery of vaccines imposes a unique set of challenges due to their large molecular size and low room temperature stability. Advanced biomaterials and delivery systems such as nano- and mciro-scale carriers are becoming critical components for successful vaccine development. In this review, we provide an updated overview of recent advances in the development of nano- and micro-scale carriers for controlled delivery of vaccines, focusing on carriers compatible with nucleic acid-based vaccines and therapeutics that emerged amid the recent pandemic. We start by detailing nano-scale delivery systems, focusing on nanoparticles, then move on to microscale systems including hydrogels, microparticles, and 3D printed microneedle patches. Additionally, we delve into emerging methods that move beyond traditional needle-based applications utilizing innovative delivery systems. Future challenges for clinical translation and manufacturing in this rapidly advancing field are also discussed.

1. Introduction

Vaccines are estimated to save approximately 8 million lives annually [1–3]. The implementation of the World Health Organization's (WHO) Expanded Program on Immunization (EPI) has substantially increased vaccination rates from 5% in 1974 to nearly 84% [4,5]. However, approximately one in six infants remains inadequately immunized annually, resulting in the deaths of approximately 1.5 million children under the age of 5 due to vaccine-preventable diseases each year [4,5]. Logistical barriers, such as the need for storage at low temperatures (i. e. −80 °C) or the need for multiple doses, weeks or months apart, remain a key contributor to underimmunization [6,7]. For example, almost half of under-immunized infants have received at least one dose of vaccines against diphtheria, tetanus, and pertussis but remain susceptible to these diseases due to the incomplete three-dose series [4]. While a single bolus vaccine dose can achieve seroprotection rates in the range of 75%–90% [8,9], full immunization entails scheduling multiple doses to ensure immunity levels reach nearly 100% [8,10]. Increasing convenience, vaccine stability, reducing dosing regimen, and enhancing dosing efficiency can potentially help eliminate these issues. Developing vaccine carriers with controlled release kinetics which can reduce the frequency of injections and mimic current vaccines regimens while maintaining vaccine stability at the physiological temperature would be an essential step towards this goal.

In a simplified form, vaccines function by prompting the adaptive immune system's immunological memory to identify and combat specific pathogens. Traditionally, vaccines contain weakened or inactivated components of the disease-causing antigen [11,12]. Upon vaccination, an individual's immune system generates antibodies against the antigen included in the vaccine. These antibodies collaborate with the rest of the immune system to eliminate an attenuated version of the pathogenic insult [11,12]. In the event of future exposures to the same pathogen, the immune system is trained to respond swiftly, providing long-term protection [11,12]. A comprehensive overview of the mechanism behind vaccine-mediated immunity against infectious diseases can be found in several review papers [12,13,14]. More recent vaccines are based on nucleic acid payloads (e. g. mRNA, DNA) [13–16]. In this approach, instead of delivering an inactivated antigen, a sequence of nucleic acids incorporating the genetic information is delivered to cells to encode the full structure or pathogenic subdomains of the antigen of interest. Nucleic acid-based vaccines outpaced conventional methods during the recent pandemic response due to their swift production, eliminating the extended cultivation and purification steps associated with egg- or cell-based vaccine manufacturing. Their synthesis is highly scalable, the

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purification process is straightforward, and the technology platforms are easily adaptable, allowing for rapid response and development when facing emerging infectious threats.

Developing effective vaccine delivery systems with desired kinetics has been a long-standing critical challenge. The recent COVID-19 pandemic has further drawn global attention to vaccine delivery systems. Even before, effective administration of vaccines globally was considered a powerful tool against widespread infectious diseases. Significant advances in biomedical technology, specifically in drug delivery systems, have substantially propelled the field of vaccine delivery by introducing novel technologies in nano- and micro-scales. This has further been accelerated by introduction of structurally complex vaccines such as mRNA vaccines encapsulated in lipid nanoparticles (LNPs) which currently require extremely low temperatures for storage [17,18 [19]]. These factors collectively motivate the establishment of a comprehensive review of the most recent advances in vaccine delivery, serving as a guide for future research. In this review, we focus on preclinical cutting-edge research in nano- and micro-scale for controlled delivery of vaccines. We aim to provide an overview of technologies used not only for traditional vaccines such as protein and peptide-based vaccines but also for recently developed nucleic acid-based vaccines, including DNA and mRNA. We first overview nanoscale vaccine delivery technologies developed to date, then describe microscale-based technologies. Exploring beyond traditional delivery modalities, we describe alternative, needle-free methodologies utilizing these carriers. Finally, we discuss key challenges for manufacturing and clinical translation of vaccine delivery systems. This review emphasizes delivery systems and platform technologies uniquely crafted for prophylactic vaccine administration. While we touch upon systems developed for other therapeutic applications, such as cancer vaccines, we highlight their potential relevance to vaccine delivery for infectious diseases. It's important to note that comprehensive discussions on general mRNA therapeutics are provided in-depth in other sources. [20,21].

Table 1 offers a concise summary of the various carrier systems discussed in this review, allowing for a concise comparison of their unique attributes, strengths, and limitations. Additionally, it highlights specific applications where these systems have shown promise, providing practical insights into their potential for controlled vaccine delivery.

2. Nanoparticle based systems for vaccine delivery

Nanoparticles are materials with overall dimensions in the nanoscale, ranging from 1 to 100 nm [22]. These minuscule carriers have led to a new generation of vaccine preparations, as well as a variety of clinically approved products and many more in the pipeline [23]. Nano-scaled vesicles can be classified into different classes based on their function, compositions, or sizes. Some of the recent breakthroughs in vaccine and therapeutic delivery using nanoparticles are detailed below.

2.1. Lipid-based nanoparticles

Lipid-based nanoparticles typically consist of phospholipid bilayers for the encapsulation of hydrophilic moieties in the aqueous compartment and hydrophobic moieties in the lipid bilayers. They can transport targeted cargo within the protective outer layer of lipids and are the most commonly used non-viral carrier for vaccine delivery [24]. Lipid-based nanoparticles have gained prominence in the past decades because of their high biocompatibility, low toxicity, and ease to manufacture. They can be loaded with cargos of choice, whether in the form of nucleic acids or protein subunits, and their surfaces can be functionalized to enable targeted delivery of therapeutics. Various lipid nanoparticles different in chemical composition and physical properties have been extensively studied [24,25].

2.1.1. Liposomes

Liposomes are closed lipid bilayer capsules that spontaneously form in water [26]. Liposomes are generally biologically inert and have low inherent toxicity, and have been widely recognized as the first nano-scale delivery platform to be successfully translated to clinical applications [26]. Their wide applications range from the delivery of the chemical inhibitor doxorubicin to treat ovarian cancer to the delivery of protein antigens as a hepatitis B vaccine [27,28]. Liposomes are generally composed of natural phospholipids possessing hydrophilic polar head groups and hydrophobic nonpolar tail groups [26]. Cationic lipids employed in formulating liposomes for vaccine delivery consist of a positively charged amine head group linked to a hydrocarbon chain or cholesterol derivative via glycerol. Their amphiphilic nature allows encapsulation of both hydrophilic and hydrophobic cargos, including mRNA or DNA vaccines.

There are commercially available vaccines against influenza, hepatitis A, and malaria that utilize liposomal formulations [25,26,28]. Furthermore, various liposomal delivery systems are currently in clinical trials for both preventive and therapeutic vaccine applications. However, liposomes have some inherent challenges. They can be sensitive to a variety of environmental conditions like temperature, pH, and mechanical stress. Such attributes not only make liposomes prone to degradation but also can cause premature release of the encapsulated payloads (Table 1).

To address this, various methods are being explored to enhance the stability and functionality of liposomes. Commonly investigated materials include polyethylene glycol (PEG) for prolonged immune stimulation, and polysaccharides, such as chitosan, for mucosal adhesion [25–27]. Additionally, crystalline coatings have emerged as promising candidates, offering protection against thermal and shear stress. For example, zeolitic imidazolate framework-8 (ZIF-8) is a promising candidate for liposomal coating due to its crystalline structure and biocompatibility, providing enhanced stability and protection against external stressors while maintaining the integrity of the encapsulated cargo [29]. In a recent study, liposomes of different surface charges were coated with ZIF-8 using different ligand/metal (L/M) molar ratios for encapsulation [29]. With the ZIF-8 coating, liposomes experienced reduced cargo leakage and showcased effective penetration in both agarose tissue models and porcine skin tissue (Fig. 1A). In essence, such coatings and modifications to liposomes can amplify their potential as efficient vaccine delivery systems by ensuring the stability and prolonged delivery of encapsulated antigens.

2.1.2. Lipid nanoparticles

Lipid nanoparticles and liposomes are similar in structural design. However, compared to conventional liposomes, lipid nanoparticles generally have a more complex lipid architecture and further enhanced physical stability [35,19]. LNPs have many advantageous features for nucleic acid delivery, including more robust encapsulation efficiency, improved tissue permeability, reduced cytotoxicity, and in some cases, adjuvant activity when compared to liposomes [18]. They also present enhanced mechanical stability, controlled morphology, and narrow size distribution [18,35,19]. Lipid nanoparticles are currently used for vaccine delivery, as they are used as carriers in both the Moderna vaccine and the Pfizer-BioNTech COVID-19 vaccines [19].

LNP formulations usually consist of an ionizable or cationic lipid to encapsulate the anionic nucleic acids, and an amphoteric lipid which is similar to the cellular lipid membrane to stabilize the LNP structure and facilitate cellular uptake and endosomal escape [19,35,36]. The incorporation of cholesterol into the LNP lipid bilayer enhances its rigidity and longevity [37]. Additionally, the inclusion of polyethylene glycol (PEG)-lipid offers improved colloidal stability by forming a protective layer, which reduces reticuloendothelial clearance, enhancing the overall performance of the vaccine [38].

Ionizable lipids are the critical components of LNPs as they allow the encapsulation of cargo into LNPs and subsequently the loaded cargo to

Table 1
Comparative summary of nano- and micro-scale delivery systems. Distinct strengths and limitations, specific applications, and references for representative works on each system are listed.

Category	Type of Carrier	Key Advantages	Main Limitations	Applications	Reference
Nanometer scale	Liposomes 	Biocompatible; safe for medical use	Prone to degradation; sensitive to environmental factors such as temperature, pH, and mechanical stress; specific storage conditions needed	Vaccines; cancer therapy; protein replacement therapy, etc	Kumari et al. PNAS. (2023)
Nanometer scale	Lipid nanoparticles 	Excellent for delivering nucleic acids, including mRNA; improved stability compared to liposomes	Size heterogeneity; specific storage conditions needed; potential off-target effect	Vaccines; mRNA therapeutics; cancer therapy; protein replacement therapies, etc	Patel et al. Nat. Commun. (2020)
Nanometer scale	Polymer nanoparticles 	Greater stability during storage; suitable for sustained release; versatile in drug encapsulation and feasible for higher specificity	Potential toxicity; lower cellular uptake efficiency and endosomal escape	Vaccines; cancer therapy; diagnosis and Imaging; antibacterial therapy, etc	Koerner et al. Nat. Commun. (2021)
Nanometer scale	Matallic nanoparticles 	Unique optical properties due to surface plasmon resonance; suitable for photothermal therapy; tend to have longer shelf lives	Limited biocompatibility; lower cellular uptake efficiency	Diagnostic imaging; photothermal therapy; cancer therapy; antimicrobial agents; dental applications, etc	Trabbic at al. ACS Bio & Med Chem Au. (2021)
Nanometer scale	Exosomes 	Natural origin; optimal cellular targeting and intercellular communication; biocompatibility and biodegradibility	Complex solation and purification; limited scalability; variable composition	Regenerative medicine; cancer therapy; cardiovascular disease; personalized medicine, etc	Wang at al. Nat. Biomed. Eng (2022)
Micrometer scale	Microspheres 	Design tunability; primarily sustained release; simplifying dosing regimen	Degredation of sensetive vaccines (especially upon release); acidic byproducts; need for process optimization to achieve monodisperse size; requires developing formulation with polymer	Vaccines; hormones; proteins; cancer therapy; opiod addiction treatment, etc	Tzeng et al. J. Contr. Release. (2016)
Micrometer scale	Non-spherical solid microparticles 	Precise and highly monodisperse design; design fine-tunability; scalability; simplifying dosing regimen	Poor understanding of compatibility with sensitive vaccines; absence of pulsatile release capability; constrained deliverable dose; inadequate comprehension of release kinetics; requires developing formulation with polymer	Vaccine delivery; cancer therapy, etc	Galloway et al. Nanomedicine. (2013)
Micrometer scale	Non-spherical core-shell microparticles 	Precise and highly monodisperse design; suitable for pulsatile release; design fine-tunability; compatibility with sensetive vaccines; formulation can be developed independent of shell	Deliverable dose; packing efficiency; generation of acidic byproducts with certain polymers	Vaccine delivery; cancer therapy; proteins; adjuvants, etc	McHugh et al. Science. (2017)
Micrometer scale	Microneedle patches (MNPs) 	Eliminates injection discomfort; minimal sharp hazzard; tunable design; can be adjusted to sensitive vaccines; dose-sparing effect; may be able to apply at home by patients	Deliverable dose; packing efficiency; requires developing formulation with polymer	Intradermal injection-free vaccine delivery; extending room temperature shelf-life stability of vaccines; medical record keeping, etc	McHugh et al. Sci. Transl. Med. (2019)
Nanometer to micrometer (nano- and micro-gel); Millimeter to centimeter scale (bulk application)	Hydrogel 	Tissue-like physicochemical properties; multiscale nature for versatile delivery strategies; tunable release kinetics	Mechanical weakness; potential for biofouling; swelling and dehydration issues; packing efficiency; requires developing formulation with polymer	Vaccine delivery; cancer therapy; regenerative medicine, etc	Roth et al. ACS Cent. Sci. (2020)
Millimeter to centimeter scale	Ingestible systems 	Less discomfort compare to injecitons; no biohazardous needle waste; require no trained professional for administration	High cost; restricted residence time; potential intolerance	Insuline delivery; vaccine delivery, etc	Abramson et al. Matter. (2022)

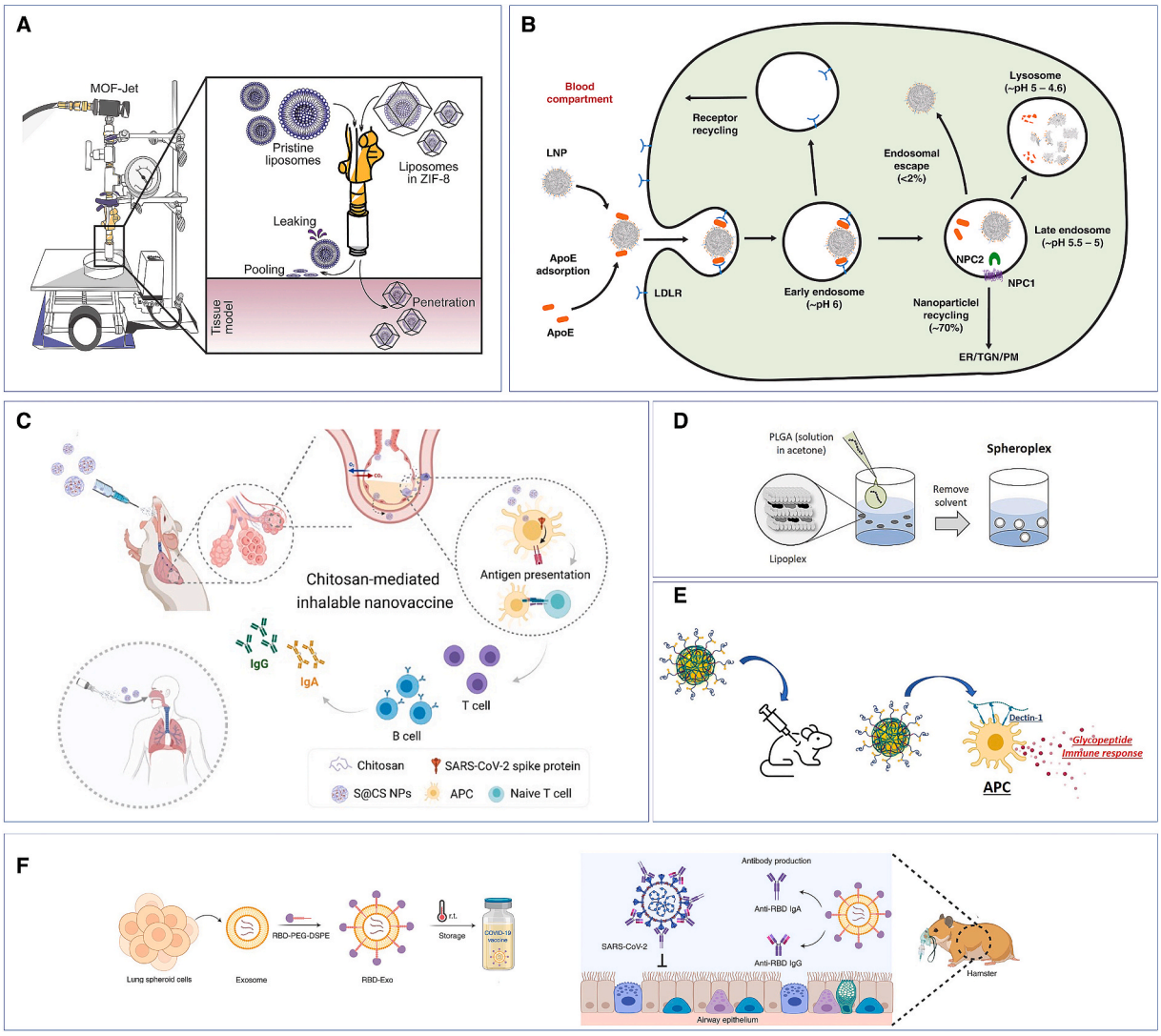

Fig. 1. Representative nanoparticle systems for vaccine delivery. (A) A Biolistic delivery system utilizing liposomes encapsulated within Nano-Sized ZIF-8 Shell [29]. **(B)** Cell uptake and nucleic acid endosomal escape of PEGylated LNPs after binding to apolipoprotein E (apoE), [30]. **(C)** An inhalable nanovaccine comprising chitosan and SARS-CoV-2 spike protein [31]. **(D)** The fabrication of hybrid nanoparticles from lipoplexes and PLGA, using a modified nanoprecipitation method [32]. **(E)** Polysaccharide-coated gold nanoparticles (AuNPs) synthesized using yeast-derived β-1,3-glucans (B13G) for targeting antigen-presenting cells (APCs) expressing Dectin-1 [33]. **(F)** A vaccine delivery system consists of a recombinant SARS-CoV-2 receptor-binding domain (RBD) conjugated to lung-derived exosomes [34]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cross the cell membrane [39]. Ionizable lipids have an important impact on the effectiveness of LNPs and the therapeutic effect of mRNA vaccines in particular. Therefore, the chemical structure of ionizable lipids plays a crucial role in optimizing cellular uptake and facilitating endosomal escape. Recent research efforts have focused on the multi-charged ionizable lipids, as they usually have higher N:P (nitrogen-to-phosphate) ratios which are conducive to the encapsulation, uptake, and lysosome escape of mRNA [39,40]. The increased positive charges, due of the N:P ratio, enhance efficient packaging within lipid nanoparticles, as they attract the negatively charged phosphate groups of mRNA [39,40]. Additionally, the high positive charge promotes enhanced cellular uptake due to the natural negative charge of cell surfaces. Once internalized, this charge plays a pivotal role in aiding the mRNA's escape from lysosomes, ensuring its delivery to the cytoplasm for protein translation. However, it is crucial to note that there is an optimal range for the N:P ratio that needs to be carefully optimized to balance efficient mRNA encapsulation with safety and stability concerns. As an illustrative example, in a recent paper, LNP containing multi-charged lipids with four tertiary amino nitrogen atoms (4N4T) showed higher delivery efficiency and triggered a more robust and durable immune response against the new variants of SARS-CoV-2, including Delta and Omicron compared to approved lipid-based vaccines[40].

In terms of optimizing lipid nanoparticle composition, helper lipids and their derivatives have also been extensively studied for their potential in enhancing LNP functionality [30,37,38]. Different forms of cholesterol can enhance the endosomal entrapment of LNPs. It was recently shown that the incorporation of cholesterol analogues, such as C-24 alkyl phytosterols has led to a remarkably enhanced *in vivo* delivery efficacy of lipid nanoparticle-mRNA formulations [30] (Fig. 1B). This work suggests that intracellular delivery can be significantly influenced by nanoparticle surface composition, structure, and interactions within subcellular environments.

LNPs have shown great promise as delivery systems for mRNA in vaccines and therapeutics. However, there are limitations that need to be addressed for their optimal performance and widespread application. Similar to liposomes, LNPs may be susceptible to aggregation, fusion, and degradation over time [19,35] (Table 1). Moreover, the

biodistribution of LNPs can vary depending on factors such as the lipid composition and the route of administration, necessitating efforts to enhance specific tissue-targeting while minimizing off-target effects [41, 38]. Ongoing research endeavors aim to address these challenges and refine the application of LNPs in the field of mRNA delivery.

2.2. Polymer-based nanomaterials

The use of polymeric biomaterials as nanocarriers is another emerging area in the development of novel vaccine formulations [42]. Due to their prolonged presentation to the immune system, these nanostructures can induce a long-lasting immune response, thereby offering the possibility of reducing the dosage required to achieve protective antibody levels [43]. Moreover, the formulation is tunable to avoid overactivation of the immune system and increase the specificity of the target site. Currently, a wide variety of natural and synthetic biodegradable polymer-based nanoparticles have been developed for vaccine delivery.

2.2.1. Chitosan-based nanoparticles

Chitosan (CS) is a natural polysaccharide derived from chitin, commonly found in the exoskeletons of crustaceans like shrimp and crabs. Owing to its bio-adhesive properties, this cationic polyelectrolyte can readily interact with the negatively charged mucosal surface, which is frequently the initial site of entry for many infectious disease pathogen [44]. While vaccines administered intramuscularly (IM) do not always prioritize biodistribution and specific targeting, the precision of CS-NPs becomes particularly pertinent for mucosal vaccines. Notably, their compatibility with needle-free administration methods further amplifies their appeal. CS carriers can enhance the immunogenicity of encapsulated vaccine antigens by targeting the cargo to mucosal microfold cells, dendritic cells, and macrophages, resulting in the induction of robust secretory and systemic antibodies, and cell-mediated immune response [45].

Chitosan nanoparticles have been employed extensively in animal vaccine delivery, notably in poultry and pigs, demonstrating their effectiveness against both bacterial and viral infections [45,46]. This success in animals has paved the way for its exploration in human vaccine delivery. In recent years, these nanoparticles have been subjected to numerous preclinical studies and clinical trials for human vaccine delivery, showing their potential for various infectious diseases, including influenza, diphtheria, hepatitis B, and COVID-19 [31,44–47]. In a recent study by Zhuo et al., an inhalable nano-vaccine composed of chitosan and the SARS-CoV-2 spike protein was reported (Fig. 1C) [31]. This formulation not only triggered a potent spike-specific antibody immune response but also enhanced local mucosal immunity in the bronchoalveolar region and lungs. Inhalation of this nano-vaccine elicited an antibody response comparable with traditional intramuscular injection [31].

Chitosan nanoparticles, also possess several limitations as nanocarreirs for vaccines and other mRNA therapeutics. Specifically, chitosan is soluble only in acidic solutions at low concentrations and in organic solvents. This solubility issue restricts its effectiveness for encapsulating mRNA vaccines and solvent sensitive antigens (Table 1). Chemical modification of chitosan offers an important approach to improve its water solubility. For example, quaternized chitosan not only retains the excellent intrinsic properties of the polymer but also improves its water solubility, thus has been widely used in the biomedical field including vaccine delivery [48].

2.2.2. PLGA-based nanoparticles

Poly(lactic-co-glycolic acid) (PLGA) -based nanoparticles offer another attractive avenue for vaccine delivery principally due to their high biocompatibility, tunable biodegradability, and colloidal stability [49,50]. PLGA has been approved by the United States Food and Drug Administration (FDA) for various biomedical applications, including drug delivery and tissue engineering and has a proven record of safety in human [49,50]. It is widely available and easy to work with at the laboratory scale making it a common reference material for release formulations of both polymer nanoparticles and injectable microparticles.

The development of delivery systems compatible with both prophylactic vaccines and cancer vaccines holds significant importance. Cancer vaccines present a promising frontier in oncology, offering more precise, potentially safer, and often more effective alternatives to traditional cancer treatments. Cancer vaccines composed of biodegradable PLGA particles containing antigens and toll-like receptor ligands have been reported and showed vigorous antitumor immune responses *in vivo* [51]. Encapsulation of a recently established double-stranded (ds) RNA adjuvant Riboxxim together with antigens into PLGA particles potently activates murine and human dendritic cells and stimulates tumor-specific T cell responses [52]. In a murine model of solid tumor established by subcutaneous syngeneic cell injection, this PLGA-based nanoparticle vaccine affords primary tumor growth retardation, prevention of metastases, and improved survival rate in preclinical tumor models [52].

PLGA has been the most widely investigated copolymer that can be combined with lipids to produce hybrid nanoparticles [53,54]. In an investigation of the development of lipid-polymer hybrid nanoparticles, a construct containing complexes of cationic lipid and siRNAs combined with PLGA through a simple modified nanoprecipitation method was reported [32]. These particles present a hydrophobic PLGA matrix surrounded by a lipid envelope adopting a lamellar structure where the siRNA is complexed, and they exhibited superior stability and efficiency over siRNA lipoplexes for delivery to cultured cells (Fig. 1D).

However, like other polymer nanoparticles, PLGA nanoparticles have certain limitations that need to be considered. For example, the loading capacity of PLGA nanoparticles may vary depending on the characteristics of the cargo and the PLGA formulation, leading to suboptimal delivery efficiency for certain vaccines. Additionally, the encapsulation within PLGA nanoparticles may lead to incomplete release of certain cargos, potentially reducing therapeutic efficacy (Table 1).

2.2.3. Other biodegradable polymers

Alginate, derived from brown seaweed, is a typical anionic polymer with carboxyl groups in the molecular chain [55]. The alginate hydrogel exhibits biocompatibility and biodegradability in various biomedical applications such as cell encapsulation and drug delivery [55–57]. Recently, new vaccine delivery systems have been developed by using alginate nanogels as a carrier with adjuvant and prolonged release properties that enhance immunogenicity [55,57]. For example, mannose (MAN) modified alginate nanoparticles were conjugated to anti-Ovalbumin antibody (OVA) using a pH-sensitive Schiff base bond and used for cancer immunotherapy applications [57]. These pH-responsive nanoparticles promoted controlled antigen release in endosomes and cytosol delivery. In a solid tumor model induced by subcutaneously injecting syngeneic E.G7-OVA-luc + cells into the left flank of mice, the subcutaneous administration of these nanocarriers stimulated dendritic cell maturation, activated cytotoxic T lymphocytes, and ultimately resulted in the inhibition of E.G7 tumor growth [57].

The bacterial biopolymer poly (3-hydroxybutyric acid) (PHB) is another attractive material for the synthesis of vaccines [58]. This high molecular polymer produced by microorganisms has suitable biodegradability, biocompatibility, piezoelectricity, and optical activity for a wide spectrum of applications including tissue repair and vaccine delivery. Protein-coated PHB particles have been developed as a vaccine platform technology for the delivery of infectious-disease associated antigens originating from viruses (e.g. hepatitis C virus) and bacteria (e.g. Streptococcus pneumoniae) [58]. Other polymers such as polycaprolactone (PCL) and poly(D,L-lactic acid) (PLA), hyaluronic acid (HA), and albumin, have also been used for the delivery of

oligonucleotides, DNA, and proteins [58–60].

Biodegradable polymer nanoparticles hold potential for the delivery of vaccines and other therapeutics due to their ability to improve stability and controlled release. Advancements in nanoparticle engineering and surface modification techniques could address existing challenges, ensuring higher encapsulation consistency and reduced immunogenicity [60]. Furthermore, exploring synergistic combinations with other delivery strategies, such as lipid-based systems, may unlock novel avenues for enhancing mRNA delivery efficiency and therapeutic outcomes. As research in this field progresses, a deeper understanding of immune responses and long-term effects will be crucial for harnessing the full potential of biodegradable polymer nanoparticles in revolutionizing mRNA-based therapies.

2.3. Other nanocarriers

2.3.1. Metallic nanoparticles

Metallic nanoparticles have emerged as innovative theranostic agents for diagnostic and therapeutic applications [61]. Over the past few decades, magnetic nanoparticles, gold, and silver nanoparticles have been intensively studied for their application in imaging modalities [61]. In addition, metallic nanoparticles are emerging as new nanocarriers in vaccine development. These nanoparticles offer facile surface modification due to their chemistry, enabling the attachment of targeting ligands, protective coatings, and stabilizing agents. This enhances their specificity and stability especially for mRNA delivery. Metallic nanoparticles can be synthesized with precise control over size and shape, allowing optimization of delivery efficiency, cellular uptake, and biodistribution. Additionally, photothermal properties of metallic nanoparticles can trigger local heating under specific light conditions to induce programmed delivery.

Antigen delivery systems based on gold nanoparticles have been well studied. For example, polysaccharide-coated gold nanoparticles (AuNPs) have been developed and delivered to antigen-presenting cells [33]. These gold nanoparticles were synthesized de novo using yeast-derived β-1,3-glucans (B13G) as the reductant and passivating agent in a microwave-catalyzed procedure. These nanoparticles were further functionalized with both a peptide and a specific glycosylated form from the tandem repeat sequence of a glycoprotein overexpressed in pancreatic tumors. These particles are proven to be highly uniform and serum-stable, eliciting strong *in vivo* immune responses and cytokine expression [33] (Fig. 1E).

In another study, the combined use of iron oxide nanoparticles was reported as a vaccine delivery platform and immune potentiator [62]. Enhanced activation of immune cells and cytokine production was observed when OVA is formulated with these iron oxide nanoparticles compared to soluble OVA, suggesting that this nanoparticle-based delivery system has potential for cancer vaccine delivery [62].

Metallic nanoparticles, serving as delivery systems, offer distinct advantages, such as their plasmonic properties enabling controlled release via photothermal stimulation, tunable size and shape for optimized uptake, and versatile surface modification for enhanced stability. However, lipid and polymer nanoparticles generally exhibit superior biocompatibility, biodegradability, and targeting efficiency, and have well-established clinical translation pathways. The choice between these nanoparticle types hinges on specific application needs, weighing the unique benefits of metallic nanoparticles against the well-established advantages of lipid and polymer systems (Table 1).

2.3.2. Exosomes

Exosomes are nano-sized extracellular vesicles (EVs) generated within the endosomal compartment of all eukaryotic cells [63]. Exosomes carry nucleic acids, lipids, and proteins, and can be released into the surrounding body fluid as part of an intracellular communication system under various biological conditions [63]. Typically, exosomes can be isolated from body fluids or cell culture supernatants using methods like ultracentrifugation, size exclusion chromatography, and immunoaffinity capture [63,64]. Once isolated, they can be manufactured or engineered to contain specific cargos suitable for therapeutic applications [63,64]. Owing to their ability to transport a vast array of molecules, exosomes have gained traction as potential vehicles for vaccine delivery.

A recent study confirmed that mRNA-loaded exosomes can mediate the functional expression of heterologous proteins *in vitro* and *in vivo*, and have fewer adverse effects than comparable doses of lipid nanoparticles [64]. Further, exosomes derived from different origins are unique in function. A study showed that recombinant SARS-CoV-2 receptor-binding domain (RBD) can be conjugated to lung-derived exosomes and elicited RBD-specific IgG antibodies, mucosal responses, and cytokine expression profile in a mouse model, offering a promising new approach for targeted drug and vaccine delivery (Fig. 1F) [34].

Compared to traditional nanoparticles, exosomes provide a natural and biocompatible option with inherent intercellular communication capabilities, potentially enhancing targeted delivery and minimizing immunogenicity. They can protect mRNA cargo from degradation and carry endogenous molecules that aid in stability and cellular uptake. However, exosomes have a limited cargo capacity and their engineering can be complex, potentially affecting scalability. Additionally, their isolation and manufacturing processes present unique challenges compared to more conventional synthetic nanoparticles, such as LNPs. Obtaining exosomes in high purity and quantity requires meticulous and often labor-intensive methods. Moreover, ensuring the consistency and functionality of exosomes across batches can be challenging (Table 1). Balancing the benefits of exosomes' natural properties with the greater cargo capacity and engineering flexibility of synthetic nanoparticles is critical when considering mRNA delivery strategies.

3. Microscale systems for controlled delivery of vaccines

Microscale systems are those with dimensions in the range of 1–1000 μm that can be of various shapes and have a variety of mechanisms of action, typically administered subcutaneously to provide controlled release capability. Controlled release of active pharmaceutical ingredients (APIs) with an optimized release profile has been shown to be a promising approach to improve safety, efficacy and bioavailability of medications [65,66].

Specifically, in vaccine delivery research, simultaneous administration of prime and booster shots is an attractive concept as it can dramatically reduce the burden of multiple visits to healthcare providers, which are often months apart [66]. Osmotic pumps can serve as efficient systems for sustained release of vaccines, enabling vaccine kinetics to mimic natural pathogen infections. These pumps utilize osmotic pressure to steadily dispense medication over extended periods, optimizing treatment outcomes and reducing dosing frequency. In a study by Cirelli et al. slow release of neutralizing antibody (bnAb) vaccine to HIV using nonmechanical osmotic pumps in rhesus monkeys led to stronger immune responses and better antigen-binding in germinal center (GC) B cells, resulting in significantly higher autologous neutralizing antibody (nAb) titers compared to bolus immunization [67].

Microscale carriers offer several advantages over osmotic pumps. They are more versatile in design, can be easily tailored to specific drug release profiles, and eliminate the need for external devices or surgical implantations, thus offering a more patient-friendly and cost-effective solution for sustained drug delivery [68]. In this section, we overview three major classes of microscale systems developed and used for vaccine delivery applications in the literature, namely, hydrogel-based systems, polymeric microparticles (in a form of microsphere or microfabricated) and microneedle patches (with single or multiple delivery applications).

3.1. Hydrogel-based vaccine delivery system

Hydrogels are crosslinked polymer networks composed of a large amount of water (70–99%) [69,70]. The high-water content in hydrogels provides tissue-like physicochemical properties and good biocompatibility. Hydrogel delivery systems can enable the encapsulation for prolonged release of therapeutic agents, used in many branches of medicine, such as cancer immunotherapy, and infectious disease treatment [71–79]. Owing to the sustained release properties of hydrogels, they have the potential to extend the duration of vaccine efficacy, which could enhance immune responses and reduce the need for frequent booster doses. Hydrogels can deliver vaccines through various approaches due to their multiscale nature. They can be designed into microgelsand nanogels for needle injection, pulmonary inhalation, and intravenous infusion [74–77]. Therefore, hydrogels serve as versatile platforms to meet specific clinical requirements through optimizing overall efficacy and patient compliance. In this section, we focus on reviewing injectable and inhalable hydrogel-based vaccines (Fig. 2A).

3.1.1. Shear-thinning and shape-memory hydrogels

Shear-thinning hydrogels, which are initially formed or "pre-gelled" outside the body, can be injected because they act like fluids when subjected to the force of being pushed through a needle. Once inside the body and no longer under that force, they return to a more solid, gel-like state [74]. The shear-thinning behavior is due to reversible physical crosslinks, such as hydrophobic interactions, electrostatic interactions, hydrogen bonding, and dynamic covalent bonds [74]. Representative shear-thinning hydrogels include alginate, peptide, hyaluronic acid and many others [73,76,77]. For example, Roth et al. reported a supramolecular polymer–nanoparticle (PNP) hydrogel that can be subcutaneously administered (Fig. 2B) due to its shear thinning and self-healing properties [73]. The polymeric constituents of PNP hydrogels are held together by dynamic, multi-valent noncovalent interactions between polymers and nanoparticles. This PNP hydrogel platform enables co-delivery of subunit vaccines (e.g. antibody, antigen, and adjuvant) to the immune system for a prolonged period (weeks to months) in mice model. A single administration of PNP hydrogel-based vaccines could

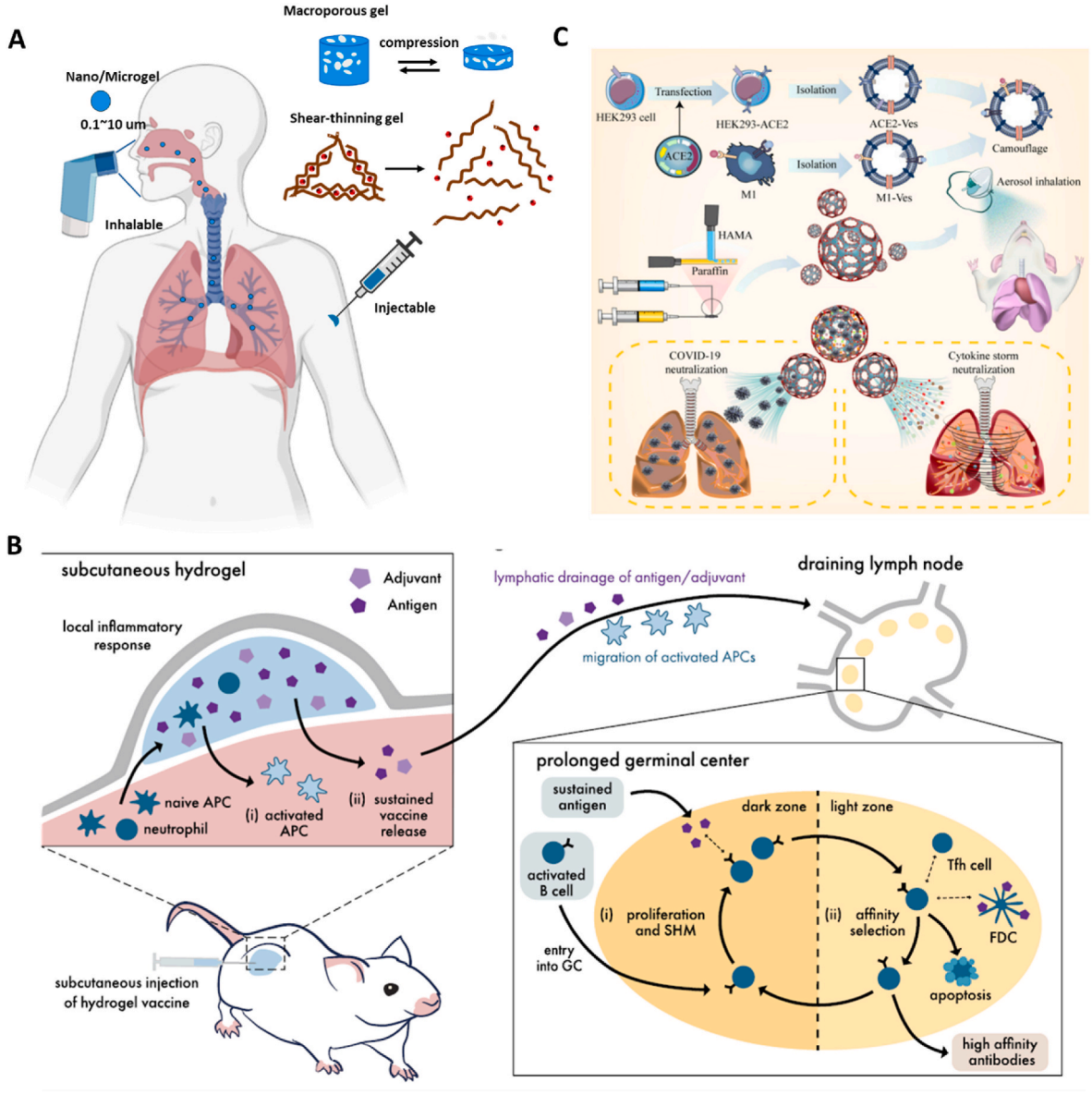

Fig. 2. An overview of hydrogel-based vaccine delivery systems. (A) Injectable and inhalable hydrogel-based vaccine delivery systems. (**B**) Representative shear-thinning supramolecular polymer-nanoparticle (PNP) hydrogel subcutaneously administered for prolonged vaccine delivery [73]. **(C)** Representative inhalable hydrogel vaccine particles to achieve protection from SAR-CoV-2 [80].

enhance the magnitude and duration of the humoral response through sustained vaccine exposure. For example, the peak concentration of antigen specific antibodies in the serum was 2–3 times higher for mice receiving PNP hydrogel-based vaccines than mice receiving the same vaccine in a standard PBS bolus administration [73]. This platform can be used to induce a potent humoral response to cancer and the COVID virus [73,77].

Shape-memory hydrogels that are gelatinized outside the body can be collapsed during needle injection and recover to the original shape after mechanical constraint removal in the body [81]. Shape-memory behavior is typically due to interconnected macropores filled with shape-memory hydrogels. Macroporous structures in hydrogels can be fabricated by cryogelation, gas foaming, microemulsion formation, freeze drying and porogen leaching [79,81–85]. For example, Shih et al. developed a tough covalently crosslinked methacrylated-alginate cryogel that can be subcutaneously injected into mice through an 18G needle. This cryogel-based vaccine induces strong antigen-specific cytotoxic T-lymphocyte and humoral responses [86].

Compared with shear-thinning systems, shape-memory systems can create pre-defined geometries and volumes for vaccine payloads. However, the diffusion length of shape-memory hydrogels for vaccine release is significantly reduced, which can potentially lead to the rapid release of vaccine.

3.1.2. Vaccine delivery systems using microgels

Hydrogels, given their versatile nature, can be tailored into both nanogels and microgels, each with its distinct characteristics and production methods [87]. Nanogels are typically synthesized using techniques like emulsion and nanomolding, leading to structures at the nanometer scale that offer advantages in terms of cellular uptake and diffusion through biological barriers, covered in the last section. In contrast, microgels are molded at the micron scale and can be crafted using microfluidics and micromolding methods [87]. These micro-scale structures are advantageous for applications requiring a larger payload, prolonged release, or specific targeting within the body.

Highlighting the potential of microgels, Wang et al. developed a microsphere-based inhaled aerosol through microfluidics [80] (Fig. 2C). The inhaled aerosol is fabricated using dual camouflaged FDA-approved methacrylate hyaluronic acid hydrogel microspheres with bioactive membranes from angiotensin-converting enzyme II receptor-overexpressing cells and proinflammatory macrophages [80]. The inhaled microspheres significantly reduce SARS-CoV-2 infectivity over respiratory system *in vivo*. When evaluated in an acute pneumonia model, these inhaled microspheres exhibited significant therapeutic prowess by modulating the multisystem inflammatory syndrome, leading to reduced mortality rates [80]. Therefore, these inhaled aerosols could potentially be a powerful synergic strategy for the treatment of patients with severe COVID-19 via non-invasive administration.

Hydrogels, despite their potential as carriers for vaccine delivery, present certain challenges and limitations. Their mechanical properties, especially in high water content gels, can sometimes be too weak to provide sustained release or protection to the encapsulated vaccine. The swelling or dehydration behavior of hydrogels [88,89] can also affect the consistent release of the vaccine. Additionally, the potential for biofouling in biological environments might interfere with the intended immune response, hindering the efficacy of the vaccine (Table 1). Moving forward, continued research in the design and applications of hydrogels will be imperative, focusing on addressing these limitations and harnessing their full potential for more effective and efficient vaccine delivery systems.

3.2. Microparticles

3.2.1. Microspheres made from biodegradable polymers

Microspheres made from biodegradable polymers are the first generation of microparticles used for delivery of vaccines [90]. Design, mechanism of action, and optimization of microspheres have been extensively studied in the literature [90,91]. Microspheres provide many advantages such as design tunability, customizable release kinetics (pulsatile or sustained), protection of the cargo in the body over the course of release against degradation, etc. [90]. Cargo protection enabled by a polymeric matrix has made microspheres an effective candidate for oral or nasal delivery of vaccines. Furthermore, it has been shown that the presence of microspheres provides an additional adjuvant effect that can enhance immune response to the encapsulated antigen [92–95]. Finally, microspheres can provide a co-delivery capability in which vaccine release is supplemented by release of a complementary adjuvant (e. g. cytokine), attached to the polymer backbone or co-encapsulated inside the microspheres [90–92].

The adjuvant behavior of microparticles is mediated by multiple mechanisms. First, according to the depot theory of adjuvant action [92], controlled release delivery systems enhance the immune response by serving as a sustained reservoir for the antigen. PLGA microspheres, for instance, can maintain antigen release for extended periods ranging from weeks to months, surpassing the duration of the depot effect observed with substances like aluminum salts or water/oil emulsions [90]. These microspheres can administer the antigen continuously or in pulses over several months [92,94]. Moreover, design of the microparticle system can be fine-tuned for enhanced antigen processing to target phagocytosis by antigen presenting cells (APC's). Microspheres less than 10 μm in diameter are readily engulfed by macrophages, leading to direct intracellular delivery of the antigen for processing via the major histocompatibility complex (MHC) class II pathway, which handles exogenous antigens [96,97]. Recent studies have also demonstrated that encapsulating the antigen within particulates or placing it on their surface can lead to antigen presentation via the MHC class I pathway, dealing with endogenous antigens [96,97]. Through this process, the antigen fragments are presented on the cell surface, priming the immune system to recognize and respond to the specific pathogen from which the antigen was derived. This is a fundamental step in generating an adaptive immune response, underscoring the pivotal role of these delivery systems in vaccine efficacy [96,97]. Lastly, microspheres can either deliver adjuvants themselves or be constructed from polymers that degrade into adjuvant-active molecules [92,94]. This feature enables the prolonged delivery of antigen, coupled with a vaccine adjuvant, thereby further enhancing the immune system's potency.

In the literature, microspheres made from FDA-approved biodegradable polymers such as PLGA, PLLA, and PLA have been utilized for delivery of vaccine subunits, DNA vaccines, and some complex biologics. For example, PLGA and PLA microspheres were employed to develop a single-injection delivery system to provide a second burst of gp120 (a subunit vaccine) to mimic immunizations at 1 to 6 months [98]. Microspheres provided two bursts of the encapsulated cargo, the first on day 1, and the second one (mimicking a booster) customizable based on the composition of the microsphere, ranging from several weeks to months [98].

Further, microspheres can be synthesized from materials with less acidity upon degradation than PLGA to enable controlled delivery of more sensitive cargos such as DNA vaccines [95]. Poly(ortho ester), POE, microspheres were developed to protect DNA from degradation *in vivo*, and to enable uptake by antigen-presenting cells. Unlike PLGA which follows bulk degradation, POE exhibits surface erosion, therefore protecting the encapsulated DNA from acidic products upon degradation of microspheres. Experiments in mice demonstrated that POE microspheres can successfully provide distinct primary and secondary humoral and cellular immune responses [95].

In another study, PLGA microspheres were mixed with various excipients, such as Eudragit E, to deliver a structurally complex and thermosensitive vaccine, inactivated poliovirus (IPV) in a single shot [99] (Fig. 3A). Authors utilized PLGA microspheres to encapsulate two IPV doses along with additional excipients to deliver a single administration of the vaccine with the booster already built into it [99]. They

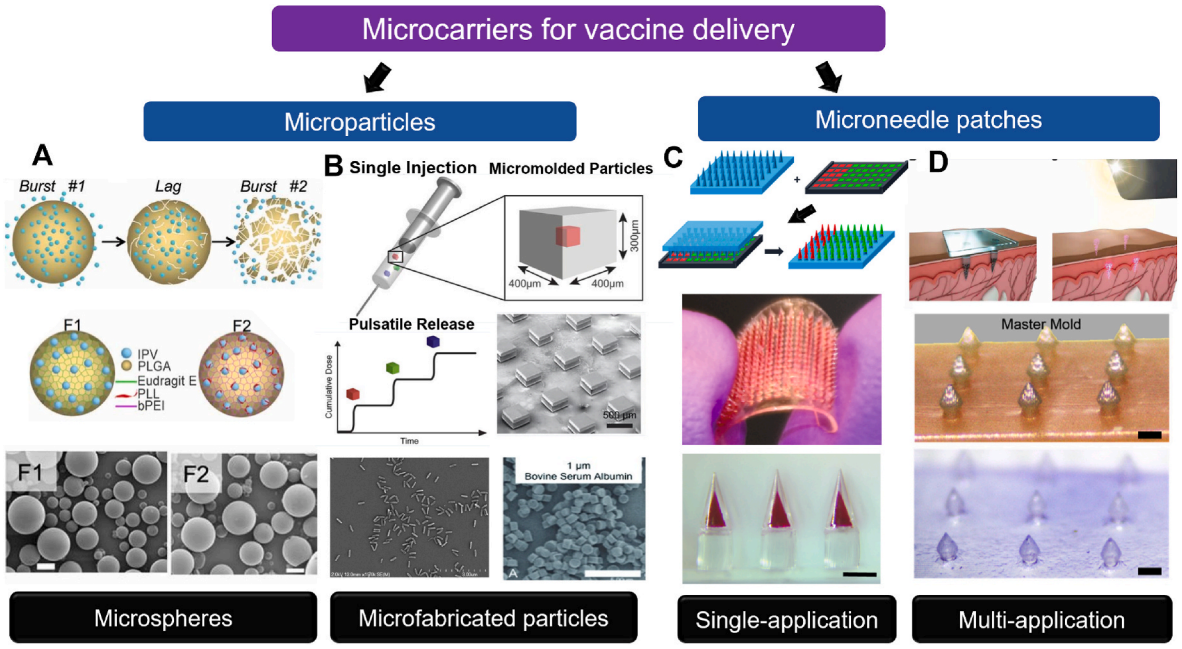

Fig. 3. Overview of various vaccine delivery technologies in micro-scales. (A) PLGA microspheres with optimized formulations to deliver encapsulated IPV in two bursts (pulses) mimicking two doses of vaccine delivered one month apart [99]. Illustrated is the mechanism of pulsatile release (top), formulation optimization (middle), and SEM image of microspheres (bottom) [99] **(B)** Representative microfabricated particle platforms.Core-shell particles for single-injection pulsatile release of self-boosting vaccine at predetermined timepoints (top and middle) [100], and PRINT-fabricated microparticles for extended delivery of encapsulated vaccines (bottom) [101]. **(C)** Representative microneedle-based vaccine delivery systems with a single application in design. CLIP 3D-printing technology for mold-freerapid fabrication of microneedles (top) [102], and core-shell microneedles for single-administration delivery of multiple doses of vaccine through transdermal patch (middle and bottom) [103]. **(D)** Representative MNPs for vaccine delivery. MNPs loaded with quantum dots as long-term photostable dye for on-patient vaccine record tracking capability (top) [104]. 3D-printed mushroom-like microneedle patches for simultaneous delivery of COVID-19 vaccine and vaccination tracking (middle and bottom) [105].(For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

demonstrated that a single shot of PLGA microspheres can simultaneously provide prime and booster shots months apart similar to two boluses of IPV. A key aspect of stabilization was the utilization of pH-sensitive, cationic dopants such as Eudragit E polymer to maintain biological activity and therefore immunogenicity of IPV upon microsphere degradation [99]. Immunogenicity studies in rats showed that the release of IPV encapsulated in these microspheres leads to a strong neutralizing immune response, similar to two injections of soluble IPV.

Demonstrating a more complex microsphere formulation, authors developed a dual-particulate delivery system for controlled release of DNA vaccines [106]. In this system, first, DNA was encapsulated in nanospheres made from polyethylene glycol-graft-polyethylenimine (PEG-g-PEI)/DNA polyplexes [106]. These nanoparticles were then encapsulated into PLGA microspheres forming a dual component system termed “nanoparticle-in-microsphere (NIM)”. The microparticle system was loaded with NIM encoding HIV proteins administered by intramuscular injection to mice, eliciting both humoral and cellular immune response [106].

The main purpose of using microspheres is to generate a local depot in the body to provide booster shot(s) without the need for repeated injections. A key challenge related to microspheres for vaccine delivery, fabricated using single-or double emulsion techniques, is the exposure of the vaccine to organic solvents which, in some cases, can reduce the bioactivity of encapsulated API from the surface of the particles or lead to vaccine denaturation in case of structurally sensitive vaccines, such as mRNA-based ones. For mRNA delivery, particularly when using LNPs, this encapsulation approach is even more challenging. LNPs are sensitive to alterations in their environment, and exposure to organic solvents can disrupt their lipid bilayers, potentially compromising the integrity and function of the encapsulated mRNA. Additionally, various washing steps involved in fabrication of microspheres can also remove unencapsulated API, further reducing the yield in the resulting microspheres. Lastly, acidic products caused by degradation of PLGA can further lower the bioactivity of the encapsulated vaccine (Table 1). The next generation of microparticles prepared using microfabrication techniques aim to address some of these challenges.

3.2.2. *Microfabricated particles*

Microfabricated or micromolded particles provide excellent monodispersity and tunability over design and can be fabricated with high throughput which is necessary for vaccine delivery applications [101,107,108]. Application of microfabricated particles for vaccine delivery is rather new and still in development. In a study led by Galloway et al, particle replication in nonwetting templates (PRINT) method, a novel micromolding technique, was utilized [101]. Cylindrical particles with a solid microstructure and about half a micron in size were fabricated from PLGA and subsequently coated with influenza vaccine using electrostatic binding. Vaccine-deliverable particles, tested in murine models, were found to be safe and elicited a superior immune response at two weeks post-boost when compared to soluble administration [101] (Fig. 3B).

A more recent microfabrication technique called SEAL enables fabrication of microparticles with 3D features, which unlike PRINT-fabricated particles, have a hollow internal structure [100] (Fig. 3B). These particles, have a core-shell structure and are fabricated with a unique microstructure which provides pulsatile release of antigens at predetermined, programmed timepoints, mimicking bolus releases (Fig. 3B). In these particles, first, the API is dispensed into the core located in the first layer of the microparticle, and subsequently covered by a secondary layer of polymeric material [100,109]. Unlike microspheres, steps involved in fabrication of core-shell particles imposes no thermal, chemical or mechanical stress on the compound, therefore making it a safe platform for delivery of structurally sensitive vaccines [100,109]. Core-shell particles were utilized to simplify two injections

of OVA albumin, as a model vaccine, one month apart, into a single shot [100]. The antibody titers achieved from a single injection of core-shell particles were superior to two boluses of soluble OVA-albumin administered at the same release timepoints from the particles [100].

Microfabricated core-shell particles also provide a distinct advantage over traditional emulsion methods when encapsulating mRNA and other nano-vaccines. Unlike emulsion techniques that expose vaccines to harsh solvents, microfabrication offers a gentle, protective environment, ensuring mRNA stability. This method also allows for precise control over encapsulation conditions and efficient loading of mRNA-LNPs, making it particularly ideal for preserving and delivering sensitive mRNA structures in a more controlled manner (Table 1).

3.3. Microneedle patches

Microneedle patches (MNPs) are another microscale drug delivery platform widely researched in industry and academia for vaccine delivery applications [110]. MNPs induce only minimal pain, can be self-administered, reduce hazardous waste, extend shelf-life stability of API, and are potentially easier to ship/handle due to being solid rather than in liquid form [110,111]. They are fabricated using a wide range of techniques, and in different forms (water-dissolvable, rigid, multi-component, etc.) [112,113].

MNPs can be applied into the skin to deliver the vaccine intradermally. A key benefit of this method is an effect called "dose-sparing" observed in some studies [114,115]. Accordingly, studies in human and animal models have shown that a lower dose of vaccine delivered through intradermal route elicits titers that are non-inferior or significantly higher than comparable or higher doses administered by intramuscular injection [114,115]. The key purpose of using MNPs for vaccines is to 1) extend the shelf-life provided by the solid-state matrix, 2) reduce injection pain (especially in children), and 3) improve the immune response due to dose sparing effect. Depending on their design, MNPs can be used to either only improve the vaccine delivery aspect (single-application) or to provide additional features such as on-patient medical record keeping (multi-application).

3.3.1. Microneedles for attenuated virus vaccination

In multiple studies led by Prausnitz and co-workers, inactivated influenza vaccines were delivered to humans or animal models using dissolvable MNPs [116,117]. Collectively, these studies concluded that most participants were accepting of the vaccination applied by MNP and preferred it over conventional injection of the vaccine [116,117]. The same group also developed MNPs coated with inactivated rotavirus vaccine (IRV) for skin vaccination [118]. Authors demonstrated that intradermal vaccine delivery by MNPs can reduce the IRV dose needed to elicit a robust immune response in mice compared to IM injection [118].

In another study, a combination of measles and rubella vaccines was delivered using dissolving MNPs for improved immune response [119]. The immunogenicity of patches was evaluated in rhesus macaques, showing that both vaccines provide a superior performance in microneedle delivered groups compared to soluble injections. Similarly, hepatitis B vaccine is another API of interest for delivery by MNPs intradermally to the skin [120,121]. Experiments in mice and rhesus macaques demonstrated that delivery of hepatitis B vaccination using MNPs elicited strong immune responses in which hepatitis B surface antibody levels were comparable to human seroprotection in mice and/or rhesus macaques [120].

Several innovations in fabrication and micromolding of MNPs have further optimized this technology for vaccine delivery applications. Using advanced microfabrication techniques, authors fabricated microneedles with core-shell structure with therapeutic cargo encapsulated inside the core [103] (Fig. 3C). Fabricated microneedles provided a tunable degradation rate for the MNP matrix to achieve multiple bursts by a single application of MNPs. As a proof of concept, core-shell MNP loaded with Prevnar-13 was studied, generating immunity against the bacterium Streptococcus pneumoniae in rats, equivalent to multiple bolus injections of the vaccine [103]. This approach is especially attractive since the vaccine is released in multiple pulses which could elicit long-term immunogenicity. A new MNP fabrication approach termed CLIP (continuous liquid interface production) 3D printing technology enabled mold-free, rapid fabrication of microneedles in various geometries driven from a computer design (Fig. 3C) [102]. CLIP-fabricated MNPs were loaded with CpG and OVA vaccines and tested in mice, demonstrating successful dose sparing effect and potent generation of OVA-specific IgG titers after 30 days, superior to most control groups studied [102].

In more recent work on MNPs, the design of MNPs was modified to provide additional functionalities complementing the vaccine delivery aspect. In an interesting demonstration of such a capability, authors utilized dissolvable microneedle patches to co-deliver IPV and a highly photo-stable fluorescent dye based on biocompatible quantum dots [104] (Fig. 3D). The fluorescent dye was invisible under the naked eye but detectable with a near infrared camera and embedded in MNPs for on-patient tracking of vaccination status. As a proof-of-concept, authors fabricated three different patterns of MNPs each corresponding to a certain dose/type of vaccines and applied to rats for 5 simulated years of skin exposure to sunlight [104]. Results demonstrated that patterns delivered with MNPs remained photostable for at least nine months [104]. Further MNPs co-delivering dye and type-2 IPV elicited a strong immune response in rats above the threshold required for full immunity. This approach could reduce the need for healthcare infrastructure and potentially provide a more reliable vaccination tracking system in areas with poor data storage infrastructure. In a similar study, authors developed a MNP platform, inspired by the design of mushrooms (Fig. 3D), imprinted by a fluorescent dye that could act as a tattoo when applied to skin to transfer a pattern (as a vaccine signature) to the patient [105]. Authors tested this device for delivery of COVID-19 in mice, and demonstrated that after 6 weeks, the antibody titers for SARS-CoV-2 RBD delivered by MNPs is non-inferior to that of soluble vaccine [105].

3.3.2. Microneedles for nucleic acid-based vaccination

The high design tunability of MNPs makes them an attractive platform for delivery of more sensitive cargo such as DNA/RNA-based vaccines. In a proof-of-concept study, authors developed an MNP to deliver a polymer-encapsulated spike protein encoding DNA vaccine supplemented with an immune adjuvant for immunity against COVID-19 virus [122]. Experiments in mice demonstrated successful generation of T-cells or virus specific IgG after vaccination, providing immunogenicity even after 30 days of storage at room temperature [122]. In another study, MNPs were used to deliver coated polyplex-based DNA vaccines using a pH-responsive polyelectrolyte multilayer assembly [123]. Microneedles successfully delivered a DNA vaccine encoding a secretable fusion protein containing amyloid beta monomer (Aβ1–42), leading to a robust immune response in mice [123].

Recent work by Prausnitz's group further demonstrated the potential of MNPs to deliver SARS-CoV-2 DNA vaccine through skin electroporation [124]. Comparisons were made between IgG titer against SARS-CoV-2 spike surface protein, showing a dose sparing effect in the MNP group which provided a non-inferior response compared to a ten-fold higher IM dose [124].

MNP-based delivery of mRNA-based vaccines, especially encapsulated in LNPs, can be particularly challenging due to structural sensitivity of mRNA. Delivery of thermostable mRNA vaccines (especially with LNP) can be highly attractive for future pandemics and is still an active area of research. In one study, hollow MNPs were utilized to deliver synthetic mRNA to reduce injection pain [125]. Results demonstrated that the MNP-delivered vaccine led to high levels of secretable humanized Gaussia luciferase (hGLuc) protein encoded by the synthetic mRNA [125]. In another study, a hyaluronic acid-based MNP design was utilized to test immunogenicity of COVID-19 Comirnaty

mRNA vaccine [126]. MNP-delivered mRNA produced S-RBD IgG titer response in mice inferior to subcutaneous injection but significantly higher than empty MNP as the control group [126]. In a recent study towards developing shelf-stable mRNA-LNP vaccines, researchers developed a novel device called "microneedle vaccine printer" (MVP) for on-demand fabrication of MNPs loaded with LNP-encapsulated mRNA vaccines [127]. It was shown that an optimized vaccine ink incorporating two polymers, namely, PVP (polyvinylpyrrolidone) and PVA (polyvinyl alcohol) combined with LNP-mRNA can be used to stabilize mRNA vaccines in a dried polymeric matrix at room temperature. Notably, MNPs fabricated with the process in this study were found shelf-stable even after six months at room temperature and successfully activated long-term immune response in mice against COVID-19 virus [127].

4. Beyond the needles: unconventional vaccine delivery methods via nano- and micro-carriers

Traditional vaccine injections, primarily administered via intramuscular or subcutaneous routes, have been the mainstay of immunization for decades. While effective in many cases, these methods come with a range of challenges and limitations. The creation of sharp waste, including needles and syringes, requires meticulous disposal to prevent health and environmental hazards. Additionally, many vaccines require a stringent cold chain for preservation, making their distribution, especially to remote areas, both challenging and costly. Moreover, the proper administration of these vaccines demands trained healthcare personnel, who are not always available in resource-limited settings. Furthermore, certain vaccines require multiple doses for efficacy, posing additional logistical and compliance issues. Lastly, traditional injections, while inducing systemic immunity, may fall short in generating the optimal mucosal immunity crucial for defending against many pathogens. Given these factors, there is a growing interest in alternative vaccine delivery methods that can overcome these barriers. Innovations likenano- and micro-carriers pave the way for delivery routes that can be more efficient and effective, ultimately aiming to increase global immunization outcomes.

4.1. Inhalable vaccine delivery using nano- and micro- systems

Inhalable vaccine delivery offers an attractive strategy of needle-free immunization, specifically targeting the respiratory system. As discussed earlier, hydrogels serve as a versatile material in inhalable delivery systems. Specifically, hydrogels with particle sizes ranging from 0.1 to 10 μm, encompassing both microgels and nanogels, are poised for inhalation via the pulmonary tract, presenting a defense against viruses, including SARS-CoV-2 [80]. The particle dimensions of these inhalable hydrogels can be fine-tuned by modulating gelation factors like polymer concentration, surfactant levels, or fabrication variables such as nozzle size and flow rate [74].

Inhalable aerosols may emerge as a potent, non-invasive therapeutic strategy, especially for patients grappling with severe cases of COVID-19. Building upon the example mentioned in the previous section, where inhalable microspheres demonstrated potential for reducing SARS-CoV-2 infectivity and improving therapeutic outcomes in acute pneumonia models [80], it is essential to acknowledge some limitations and consider future directions for inhalable vaccines. These limitations include challenges in achieving precise dosing, stability during storage, device design, and safety concerns. To advance this innovative approach, future directions should focus on developing improved inhalation devicesand implementing long-term safety monitoring. Addressing these considerations will be crucial in realizing the full potential of inhalable vaccines and ensuring their safe and effective use on a global scale.

4.2. Ingestible devices facilitated vaccine delivery

Compared to parenteral injection, oral dosage forms of vaccines may be preferred because they generate less discomfort and no biohazardous needle waste, and require no trained professional for administration. In addition, oral administration of vaccines could potentially enable local cell transfection in the gastrointestinal tract that are not easily targeted via parenteral administration [128]. However, it is challenging to orally deliver vaccine-like macromolecules due to the acidic gastric pH, digestive enzymes, and poor absorption across the GI epithelium [129,130]. In addition, vaccine efficacy may be reduced due to the direct interference between the attenuated virus from other pathogens or substances present in the gut. As such, ingestible devices that aim to protect the encapsulated vaccines are crucial for oral administration. In this section, we summarize emerging ingestible devices for oral delivery of macromolecules, including oral mRNA delivery using capsule-mediated gastrointestinal tissue injections. Additionally, we propose potential strategies for long-term vaccine release in the GI tract.

Incorporation of injection capability into ingestible devices, such as capsule endoscopy, microdevices, and pills, enables macromolecules administration orally. Ingestible devices with micrometer- and millimeter-sized needles have been developed for macromolecular drug delivery in the GI tract. For example, Rani Therapeutics ® designed an ingestible robotic pill, a commercial product, capable of therapeutics delivery through the intestinal wall [131]. With an enteric coating, the therapeutic payload can bypass the gastric environment safely. Upon exposure to the fluid in the small intestine, the microinjectors can inject into the intestinal wall after the dissolving of enteric coating and chemical-induced inflation of the balloon housed in the pill. Clinical studies demonstrated the safety, tolerability, and performance of the robotic pill in healthy volunteers [131]. In addition, multiple platforms have been developed to orally deliver therapeutic doses of macromolecular drugs. For example, the recently reported ingestible capsule known as the luminal unfolding microneedle injector (LUMI, Fig. 4A). LUMI enables oral macromolecule delivery by rapidly propelling drug-loaded dissolvable microneedles into intestinal tissues using a set of unfolding arms [132]. Through a compressed spring and pH responsive polymer coating, LUMI system can deploy upon exposure to pH 5.5 in the duodenum and can degrade after drug deployment to reduce the possibility of intestinal obstruction. Using insulin as a model drug in swine, LUMI showed a faster pharmacokinetic uptake profile compared to administration of an insulin solution placed into the small intestine [132]. A self-orienting millimeter-scale applicator (SOMA) device was also developed, capable of self-orientation to the gastric wall using a leopard tortoise-mimic design (Fig. 4B) [133]. By incorporating a solid drug formulation of insulin, SOMA devices can use a mechanical spring to inject the insulin into the gastric wall resulting in a drop in blood glucose in the swine. In addition, the SOMA device showed the capacity for oral delivery of systemic monoclonal antibodies, peptides and small molecules [134]. Recently, a SOMA pill was developed for GI-targeted oral mRNA delivery, opening a new avenue for oral mRNA medicines, including vaccines (Fig. 4C) [135].

In resource-limited areas, these ingestible mRNA systems could simplify administration of vaccines that often need multiple doses [135]. One step further, multiple vaccine doses loaded into a single ingestible pill with long-term GI retention strategies (e.g., star devices, unfolding retentive systems [136,137], and swelling hydrogel devices [138]) could even enable the sustained vaccine delivery in the GI tract. To safely exit the body, these devices can be constructed using bioresorbable materials such as poly(lactic-co-glycolic acid) and polycaprolactone that have controllable dissolution rates, or using materials that can be disintegrated using optical [139] or chemical [138] triggers.

4.3. Needle-free systems for cutaneous and mucosal delivery

Needle-free vaccination was first applied for the delivery of live

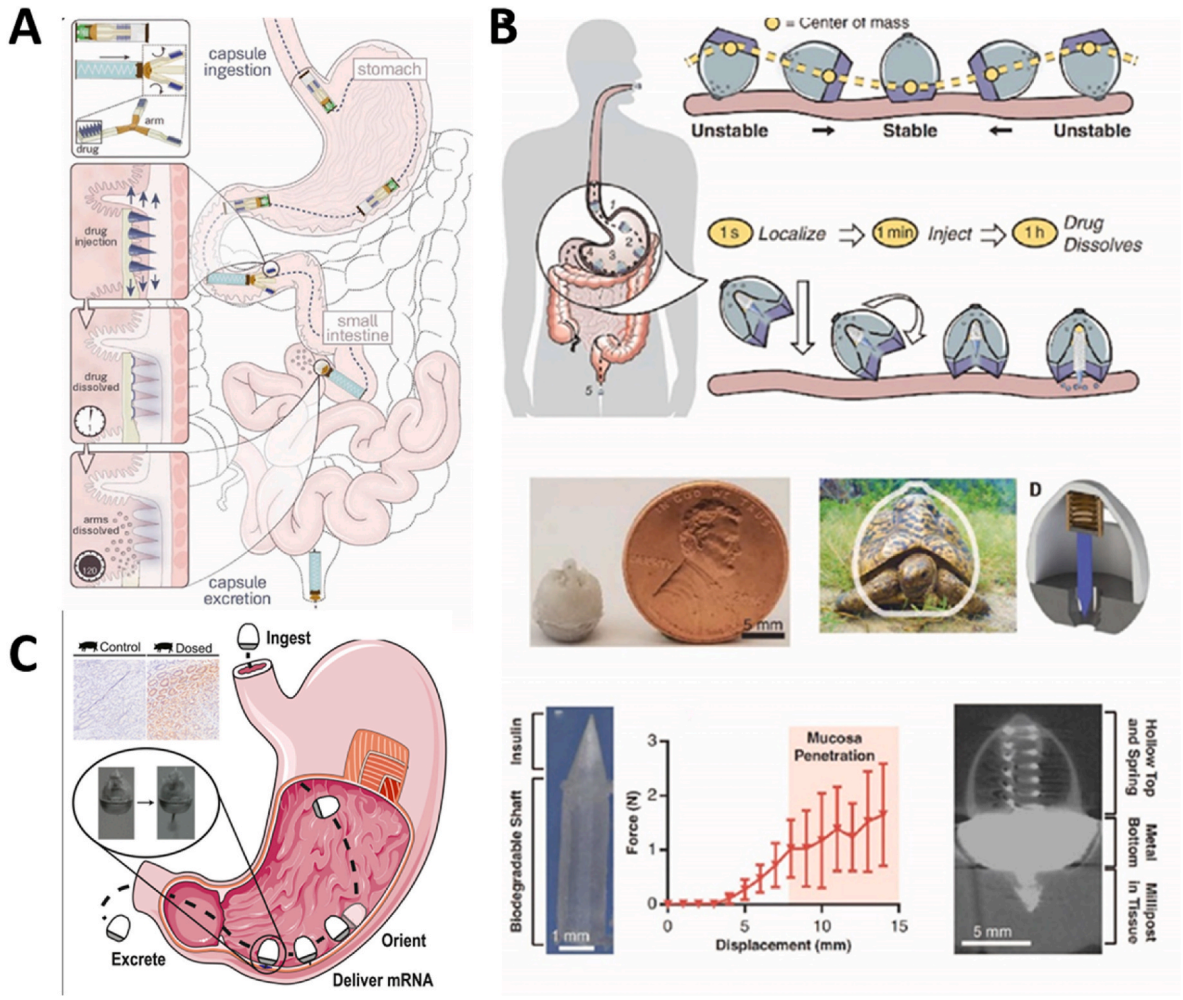

Fig. 4. Macromolecular oral delivery devices. (**A**) LUMI device that injects microneedles into the small intestine and disassembled for excretion [132]. (**B**) SOMA device that self-reorients from any starting position to attach to the gastric wall for the injection of insulin [133], and (**C**) SOMA device for oral mRNA delivery through the gastric wall [135].

attenuated poliovirus in the 1940s [140]. With the rapid evolution, current injection-free immunization systems can be mainly categorized into cutaneous and mucosal immunization in terms of the administration sites. Cutaneous immunization methods include microneedle patches, liquid-jet injection, ballistic methods, and topical application methods [140]. Liquid-jet injection uses a high-velocity jet (typically >100 m·s^{-1}) to deliver a vaccine stream across the skin into the subcutaneous or intramuscular region. Ballistic methods accelerate particulate vaccine material and deposit it in the skin. Topical methods deliver the vaccine into or across the skin through passive diffusion or facilitated transdermal transport. On the other hand, mucosal immunization methods can deliver vaccines to a mucosal membrane, such as the ocular, oral, nasal, pulmonary, vaginal, and rectal membrane. Mucosal vaccines can initiate strong protective immune responses at key infection sites, potentially preventing establishment of infections by inducing adaptive immunity through secretory antibodies and tissue-resident T cells, going beyond simply controlling infections and symptoms [141]. Readers can refer to these reviews [140–144] for more details. Several injection-free vaccination technologies have been translated into clinical settings. As we covered microneedle patches in the previous section, here we focus on injection-free vaccination systems through nasal or GI mucosal routes, particularly systems that are in clinical development.

4.3.1. *Injection-free vaccine delivery through nasal mucosa*

Nasal vaccination is non-invasive, easy for administration, and generally well-tolerated, a convenient and patient-friendly option for immunization. Unlike inhalable vaccines that target the respiratory tract, nasal vaccination delivers the vaccine directly to the nasal mucosa using techniques such as nasal spray, droplet delivery, and intranasal devices [145]. The liquid in the nasal spray typically contains the vaccine component, and other ingredients such as preservatives or stabilizers. The nasal mucosa has a large surface area and is rich in immune cells, such as dendritic cells and lymphocytes. Therefore, nasal vaccine allows for efficient immune stimulation and induction of both systemic and mucosal immune responses at the site of entry. Particularly, nasal vaccines are beneficial in the context of respiratory diseases through the production of specific antibodies and immune cells that can neutralize pathogens and provide protection against respiratory infections [141]. For instance, live attenuated influenza vaccine (LAIV) is a type of influenza vaccine in the form of a nasal spray that is recommended for the prevention of influenza [146]. They contain weakened influenza viruses that stimulate an immune response without causing illness. Nasal spray influenza vaccines are available under various brand names, such as FluMist® Quadrivalent. On the other hand, several COVID-19 nasal vaccines have been developed and are undergoing clinical trials. These vaccines utilize different platforms, such as live attenuated viruses, viral vectors, or protein subunits, to induce a protective immune response against the SARS-CoV-2 virus [145].

However, challenges are associated with nasal vaccine delivery because the nasal mucosa is a dynamic and complex barrier that can

limit the uptake and delivery of vaccines. Factors such as the vaccine formulation, dosage, and nasal clearance can affect the efficacy of nasal vaccines. Additionally, individual variations in nasal anatomy and nasal conditions may impact the effectiveness of this delivery method. Nevertheless, ongoing research and development aim to optimize nasal vaccine formulations and delivery techniques to enhance their immunogenicity and protective efficacy. Nasal vaccine delivery holds promise as a needle-free alternative for vaccination, particularly for respiratory infections and diseases.

4.3.2. Injection-free vaccine delivery through GI mucosa

Injection-free vaccine delivery through the gastrointestinal (GI) mucosa holds potential as an alternative emerging technology for non-invasive immunization. Various methods such as jetting, iontophoresis, ultrasound, hyperthermia, and magnetic approaches have demonstrated the ability to enable macromolecule delivery in the GI tract [147]. Ultrasound with a low frequency range of 20–100 kHz, in particular, has shown efficacy in enhancing transdermal and *trans*--mucosal macromolecule delivery by inducing transient cavitation in localized regions [148]. For example, a recently developed handheld low frequency ultrasound delivery system has proven successful in the rectal delivery of substances such as glucose, insulin, mesalamine, and hydrocortisone [149]. In swine models, a mere 1-min application of ultrasound using this system resulted in a remarkable enhancement in mesalamine delivery, along with the successful systemic delivery of insulin, leading to the expected hypoglycemic response [149]. This ultrasound delivery system has even entered clinical trials for the localized treatment of inflammatory bowel disease (IBD) [150]. While the application of this technology for vaccine delivery has not yet been reported, the successful delivery of small interfering RNA and messenger RNA in mice models [151] indicates future potential for ultrasound-assisted vaccine delivery through the GI mucosa.

5. Manufacturing of nano- and micro- carriers for vaccine delivery

Design and synthesis of delivery systems are crucial steps for clinical translation and downstream applications. Manufacturing nano- and micro-carriers for vaccine delivery, especially those housing mRNA, requires sophisticated processes that capitalize on advancements in nanotechnology and material science.

5.1. Manufacturing of nano-carriers

Typically, mRNA vaccines with lipid nanoparticle encapsulation are manufactured through a process involving several key steps. After mRNA encoding the target antigen is synthesized in the laboratory, lipid nanoparticles are formulated using selected lipids, creating a lipid bilayer structure around an aqueous core [7,18,22,24]. The synthesized mRNA is encapsulated within these lipid nanoparticles, providing protection from degradation and facilitating its delivery into cells.

Traditional NP manufacturing methods rely on batch mode synthesis of mixing in a bulk solution [16,18,19,24]. This method is not ideal for scaled-up production due to batch-to-batch variables caused by heterogeneous mixing or NP aggregation [16,19,152–154]. To streamline production and ensure consistency, industrial manufacturing facilities often require specialized equipment designed for large-scale production, such as high-pressure homogenizers, continuous flow systems, and large-scale filtration units. These industrial-scale machines allow for efficient and consistent production of LNPs. In contrast, many research labs still use smaller-scale equipment, such as sonicators or benchtop homogenizers, which may not have the same level of throughput or efficiency as industrial machinery [152–154].

The development of microfluidic devices that can precisely control the size and dispersity of NPs, and show high encapsulation efficiency could provide substantial improvement in vaccine development [16,19]. Microfluidics mixer devices are now commonly employed as a powerful tool for reproducible and scalable production of nano-structured particles [153,154]. For example, LNP-based-mRNA formulations can be manufactured by rapid mixing using microfluidics mixers. An ethanol phase with lipid components and an aqueous phase with nucleic acids are mixed under specific conditions [154,155]. Such microfluidic reactors enable the rapid mixing of reagents, temperature control, and the precise spatial-temporal manipulation of reactions. As a result, the controlled and homogeneous mixing results in smaller and uniform nanoparticle sizes, and a higher yield. Synthesis of other classes of nanosized particles, such as semiconductor nanoparticles, metal nanoparticles, colloidal nanoparticles, and polymer nanoparticles, have also been demonstrated in microfluidic devices in a homogeneous and well-controlled fashion. The mechanochemistry properties of nanoparticles can be precisely controlled in a reproducible manner by tuning the reaction and fluid dynamic parameters such as flow rates and pressure/flow ratio [153,154].

5.2. Manufacturing of micro-carriers

Polymer-based microcarriers are typically produced using one of two primary methods: 1) emulsion-based microsphere fabrication and 2) soft-lithography. The selection of the appropriate fabrication technique hinges on various considerations. These include the desired throughput, the stability of the vaccine, the specific design and composition of the microcarrier, the intended release kinetics, and the chosen route of administration.

The emulsion-based technique, specifically double emulsion solvent evaporation, is a favored method for drug encapsulation [156,157]. It creates a double emulsion where larger droplets enclose smaller ones. This straightforward method allows control over process parameters and can encapsulate both hydrophobic and hydrophilic payloads. Initially, a primary water-in-oil emulsion is formed, where the active ingredient is mixed with an oil phase. This primary emulsion is combined with a second aqueous phase, typically with a surfactant like PVA, creating a double emulsion. Upon solvent evaporation, adjustable solid particles emerge.

The main challenge in using double emulsions is controlling homogeneous droplet size by optimizing different process variables [156,157]. Other manufacturing processes such as spray-drying have been developed to increase microsphere monodispersity and scalability. However, in case of vaccines process should be chosen with regard to maintaining physiochemical stability of vaccines on a case-by-case basis. For example, spray drying incorporates applying hot air or nitrogen flow to the active pharmaceutical ingredient, which can be detrimental to thermostability of vaccines. Different variations of double emulsion based microparticles fabrication technique are described in detail in other publications [95,156,157].

Soft-lithography is a bottom-up approach that allows precise control over the feature size in the microcarrier. This technique is mostly utilized when a specific design feature is of interest (e. g. shape, precise release kinetics, encapsulation strategy etc.), more commonly in polymeric microcarriers [101,100,104,158]. The process typically starts with master mold development, where a master mold (often on a silicon wafer) is crafted using soft lithography, followed by creating a replica with polydimethylsiloxane (PDMS) to capture the master mold's negative design. Subsequently, the vaccine is combined with a biocompatible polymer, forming the microcarrier material. This composite is cast onto the PDMS replica using methods like spin or dip coating, ensuring the microdevices mimic the negative pattern of the PDMS mold. Upon solidification, the microcarrier is detached from the mold, either manually or with vacuum apparatus [100,103,158].

A common drawback in soft-lithography is low throughput. More advanced techniques such as PRINT (roll-to-roll processing) and SEAL (multi-layer microfabrication) aim to increase the microparticle yield while controlling the feature size and vaccine stability [101,100,158].

More variations are also introduced for fabricating complex microcarriers such as hollow microneedle patches which might require an extension of the steps mentioned above [103].

6. Opportunities and challenges for clinical translation of vaccine delivery systems

As the landscape of vaccine delivery systems evolves, several pivotal factors need to be considered for successful clinical translation. Scalable Good Manufacturing Practice (GMP) manufacturing plays a crucial role in widespread distribution, while understanding the *in vivo* mechanism of action and its interplay with the immune system is vital for efficacy. Additionally, considerations such as ease of administration, vaccine loading capacity, stability, and shelf-life further delineate the opportunities and challenges in this field.

6.1. Scalable GMP manufacturing

As highlighted in the last section, one of the primary hurdles in the clinical translation of nano- and micro- carriers for mRNA vaccine delivery is scalable manufacturing. GMP standards ensure that products are consistently produced and controlled according to quality standards, vital for any clinical application. As these carriers transition from research settings to widespread use, the production needs to accommodate large-scale vaccine campaigns and sudden outbreak demands. Present manufacturing technologies are laden with numerous processing steps, which not only pose scalability concerns but also introduce complexities that can hinder regulatory approval. Adhering to stringent GMP standards, especially ensuring aseptic handling, is paramount. Aseptic handling ensures that the vaccine remains free from contamination by microorganisms throughout the manufacturing process. Any breach in this sterile environment can compromise the safety and efficacy of the vaccine. The challenges arise due to the intricate nature of the carriers and the numerous processing steps involved, each of which presents potential points of contamination and degradation. It becomes imperative to foster collaborations between academic research institutions and industrial partners. Such partnerships can facilitate the shift from laboratory-scale techniques to scalable manufacturing processes that pharmaceutical companies can seamlessly integrate and uphold, aligning with both GMP and aseptic handling requirements.

6.2. Understanding the mechanism of action in vivo and its impact on the immune system

Often drug delivery systems have to undergo design or formulation modifications (e. g. co-encapsulation of excipients) to be compatible with a vaccine of interest. These modifications could lead to alter performance of the drug delivery systems *in vivo*. In such cases, a comprehensive understanding of the design-function relationship for the system of interest can save time and costs for additional experimentations. *In vivo* behavior of emerging technologies such as mRNA-deliverable MNPs or oral vaccine delivery devices, especially for repeated use, need further investigation. A key aspect in understanding the behavior of vaccine delivery systems is vaccine release kinetics and its impact on the immune response. Some areas for future studies can be related to optimizing the release profile, dose and timing of vaccine delivery for optimized immune response [159].

6.3. Ease of administration

This aspect is especially critical in injectable drug delivery systems [160]. Injectable vaccine carriers should be small enough to be injected by narrow hypodermic needles to minimize injection pain. Furthermore, the viscosity of the injectable solution should be optimized to deliver the highest concentration of microparticles while not blocking the needle. Finding the right combination of concentration, solution viscosity, and needle gauge can impose additional considerations when optimizing the formulation of the vaccine delivery system [160].

6.4. Vaccine loading, in vivo stability, and shelf-life

Many vaccines might be structurally sensitive to thermal, chemical, or physical stresses which could destabilize them, hence ineffective [99,161]. Drying of vaccines in various steps during preparation of the drug delivery system can potentially damage vaccine stability and cause aggregation. Moreover, maintaining long-term stability of vaccines, once inside the body, is challenging and requires potential modifications to the drug delivery system [99,161]. Incorporation of excipients to the vaccine formulation upon encapsulation into the drug delivery system, can be an important strategy for improving shelf-life of the vaccine cargos in dried, solid form. Moreover, using freeze-drying processes to extend shelf-life of vaccines, before or after encapsulation in the drug delivery system, can be another area for further investigation [19,160,162]. Freeze-drying can be a potential solution in case of LNP-mRNA vaccines which suffer from short shelf-life at room temperature and require extremely low temperatures for storage [162].

6.5. Vaccine delivery schedule and dosing

Current vaccines have to be administered in multiple shots to provide prime and boosters shots that can be separated from a few weeks to a few years. Tracking vaccination history and administering the second vaccine dose can place a considerable burden on healthcare systems, particularly in regions with limited access to advanced healthcare infrastructure. As a result, development of vaccines with simplified dosing schedule, delivering prime and booster shots with a single administration, can be critical. Additionally, optimizing the dosing schedule for delivery of prime and booster shots (i. e. the best time window to deliver the boosters, the optimal dosing per shot, sustained vs pulsatile delivery, etc.) to achieve robust immunity remains elusive. More studies are needed to comprehend the optimal vaccine delivery schedule.

7. Future studies

Further studies are necessary to fully understand the mechanism of action provided by vaccines and how nano- and micro-carriers can improve immunization. Currently, there are several clinical trials underway investigating the feasibility of using microneedles patches for vaccine delivery [163,]. These clinical investigations in addition to FDA-approval of LNP-mRNA nanocarriers (Moderna and Pfizer-BioNTech) for COVID-19 vaccination highlights the potential for clinical translation of nano- and micro-carrier systems.

However, as with all promising medical interventions, there are challenges to be addressed. Future studies can prioritize the design of carriers with enhanced precision, ensuring targeted delivery of therapeutics only to intended cells or tissues, thus minimizing off-target effects. By manipulating the size, surface chemistry, and payload release kinetics of these carriers, we can modulate the immune system's response, striking a balance between therapeutic efficacy and potential toxicity. Additionally, research avenues that could substantially advance the clinical translation of these carrier systems for vaccine delivery include elucidating the impact of vaccine release kinetics on the immune response, such as pulsed versus sustained release, and variations in dosing regimens or intensities. There's also a need to develop continuous production of shelf-stable LNP-based vaccine formulations. Enhancing the specificity and adjuvanticity of nano- and micro-carriers through advanced conjugation techniques with novel formulations, such as antibodies, will be crucial. Furthermore, innovations in single-injection, self-boosting vaccine delivery systems, and leveraging computational modeling to map the design spectrum for vaccine carrier systems can provide insights into how distinct nano- andmicro-carrier designs

impact release kinetics. An exploration into the immune responses elicited by different administration routes for these carriers , such as intramuscular versus subcutaneous delivery is poised to be highly informative.

Finally, establishing scalable and GMP-compliant manufacturing processes for the production of nano- andmicro-carriers is pivotal to the progression of the field. Research into state-of-the-art fabrication methods, including one step multi-material 3D printing and microfluidics, can pave the way for more scalable manufacturing techniques. Additionally, incorporating automation and robotics into these processes can significantly minimize human error and enhance production efficiency, thereby propelling their utility in various biomedical applications.

8. Conclusions

In this review, we explored the latest advancements in technologies tailored for controlled delivery of vaccines with a focus on prophylactic vaccines. We covered a comprehensive spectrum of carriers from nanoparticles to microparticles to emerging technologies such as hydrogels, microneedle patches, and ingestable devices. Furthermore, we probed the primary challenges that stand before the clinical translation of these innovative vaccine delivery systems. Overall, this review highlights the transformative potential of nano- and micro-carriers. If harnessed to their fullest, these carriers promise a paradigm shift towards more precise, safe, and efficacious vaccine delivery systems.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: R. L. reports a relationship with Moderna that includes: board membership and equity or stocks. R.L. has patent pending to Massachusetts Institute of Technology. R.L. is a co-founder of Moderna and other biotech companies. For a list of entities with which R.L. is involved, compensated or uncompensated, see www.dropbox.com/s/yc3xqb5s8s94v7x/Rev. For a list of entities with which A.J. is involved, compensated or uncompensated, see https://www.dropbox.com/s/wre16lh7jr7bvoi/230410%20Jaklenec%20COI.pdf?dl=0.

Data availability

Data will be made available on request.

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