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Recent advancements in single dose slow-release devices for prophylactic vaccines

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Abstract

Single dose slow-release vaccines herald a new era in vaccine administration. An ideal device for slow-release vaccine delivery would be minimally invasive and self-administered, making these approaches an attractive alternative for mass vaccination programs, particularly during the time of a pandemic. In this review article, we discuss the latest advances in this field, specifically for prophylactic vaccines able to prevent infectious diseases. Recent studies have found that slow-release vaccines elicit better immune responses and often do not require cold chain transportation and storage, thus drastically reducing the cost, streamlining distribution, and improving efficacy. This promise has attracted significant attention, especially when poor patient compliance of the standard multidose vaccine regimes is considered. Single dose slow-release vaccines are the next generation of vaccine tools that could overcome most of the shortcomings of present vaccination programs and be the next platform technology to combat future pandemics.

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AUTHOR CONTRIBUTIONS

Sayoni Ray: Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Armando Puente:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Nicole F. Steinmetz:** Funding acquisition (equal); writing – review and editing (equal). **Jonathan K. Pokorski**: Funding acquisition (equal); writing – review and editing (equal).

CONFLICT OF INTEREST

Drs. Steinmetz and Pokorski are co-founders of, have equity in, and have a financial interest with Mosaic ImmunoEngineering Inc. Dr. Pokorski serves as scientific advisor and paid consultant to Mosaic; Dr. Steinmetz serves as Director, Board Member, and Acting Chief Scientific Officer, and paid consultant to Mosaic. The other authors declare no potential conflicts of interest.

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Keywords

controlled release; immune cell kinetics; implant; microneedle; microparticle; PLGA; prophylactic vaccines; single dose; slow release; vaccine delivery; vaccine kinetics

1 | INTRODUCTION

Many infectious diseases are preventable using FDA-approved prophylactic vaccines, saving millions of lives. However, the high cost, poor patient compliance, needle-phobia, lack of proper healthcare services, effective supply chain and trained healthcare professionals on site have so far limited immunization in the United States, and have severely restricted immunization in developing countries (Arya & Prausnitz, 2016; Walters et al., 2015). Most vaccines require multiple doses to achieve a desired level of protection. The administration schedule demands multiple visits to healthcare centers, resulting in poor patient compliance and consequent vaccine wastage. Several vaccines require vaccine reconstitution prior to injection, increasing the challenges of administration and requirements for well-equipped healthcare facilities and skilled personnel. All of these factors accumulate to contribute to a higher cost of vaccination, improper immunization, slower vaccine distribution, and often times make certain vaccines unavailable to developing and underdeveloped countries (Arya & Prausnitz, 2016). A single dose vaccine could significantly improve patient compliance, reduce the need for sophisticated healthcare facilities and significantly lower the cost of vaccines (U. K. Jain & Shrivastava, 2010; Kalam et al., 2017; McHugh et al., 2015; Walters et al., 2015). These issues have been further magnified during the time of the COVID-19 pandemic, when mass vaccination had to be done urgently within a short period.

Single dose vaccine administration could be achieved by using slowly degrading polymeric devices, which contain multiple doses of the vaccine and release it slowly to mimic a prime-boost vaccination schedule. The most used vaccine delivery devices include polymeric microparticles, microneedles, and implants with controlled degradation rates that are dictated by the hydrolytic lability of the polymer (Figure 1; McHugh et al., 2015; Shao et al., 2021). The geometric constraints of the device (e.g., surface area), the chemical environment of the polymer, and the loading density of the device can all control the release kinetics of a particular delivery platform. The manufacturing of slow-release devices is not without challenge; one major hurdle is to ensure the stability and efficacy of the antigen during formulation and long-term storage (Lin et al., 2015; Sivakumar et al., 2011). Finally, vaccines require adjuvants to elicit the desired immune response and excipients to maintain stability (Sivakumar et al., 2011). The advantage of a vaccine delivery device is that it offers space for including different excipient and adjuvants, and the device itself can provide adjuvanticity (Dounighi et al., 2017; Guillaume & Mespouille, 2014).

In addition to overcoming known challenges, slow-release devices also show promise in improving the efficacy of vaccination by providing a continuous supply of the antigen over a sustained period (K. K. M. Cirelli et al., 2019; K. M. Cirelli & Crotty, 2017; Havenar-Daughton et al., 2019; Irvine et al., 2020; Tam et al., 2016). This strategy could be more effective in countering infectious diseases by suitably modulating antigen release kinetics compared with the sudden release of a large dose of antigen using traditional injection methods (Tam et al., 2016). A detailed understanding of the mechanism of action of vaccine delivery devices and how they modulate immunity will help improve the efficacy of the vaccine design for many fragile or complicated antigens (Irvine et al., 2020).

In this review, we have summarized the recent findings on slow-release delivery devices—with a focus on prophylactic vaccines targeting infectious diseases. The advantages and challenges of such polymeric devices are discussed.

2 | SLOW-RELEASE SYSTEMS

2.1 | Microparticles

Microparticles have been the most extensively studied delivery platform over the past 25 years as potential devices for slow-release vaccines. These are usually spherical particles having diameters in the range of ~1–1000 μm and are often referred to as microspheres or microcapsules (Figure 1; U. K. Jain & Shrivastava, 2010). They can be administered by traditional injection or orally. However, the preparation of the oral formulation is more challenging due to the instability of vaccine in the gastrointestinal (GI) tract, and has been discussed elsewhere (Shalaby, 1995). The most common method of producing microparticles is a single or double emulsion method (Lagreca et al., 2020; Peres et al., 2017; Rosca et al., 2004). A variety of polymeric materials are exploited for the preparation of microparticles, ranging from synthetic polymers such as polyesters, polyanhydrides, and polypeptides or natural polymers including chitosan, alginate, collagen, gelatin, and dextran (Allahyari & Mohit, 2016; Han et al., 2018; R. A. Jain, 2000; Lee & Pokorski, 2018; Peres et al., 2017). The choice of polymer is crucial to formulate a drug or vaccine delivery system considering its degradation properties. Depending on the chemical composition of the polymeric device, surface or bulk erosion can dominate the degradation process and can be manipulated by changing the surface area or monomer units of the system (Pillai & Panchagnula, 2001). Sometimes, a mixture of polymers is used to tune the release rate, for example, mixtures of chitosan, alginate, and poly(lactide-*co*-glycolide) (PLGA) were used to form a microparticle delivery system for the antigen Hepatitis B surface antigen (HBsAg) for vaccination against Hepatitis B (Zheng et al., 2010).

The most commonly used polymer for microparticle formulation is PLGA due to its biocompatibility, ease of production, and opportunity to fine-tune the release rate of the antigen (R. A. Jain, 2000; Lee & Pokorski, 2018; Peres et al., 2017). PLGA is composed of lactic acid and glycolic acid and degrades by ester hydrolysis to release the entrapped cargo over an extended period (McHugh et al., 2015). To provide a continuous supply of the antigen and to achieve the desired antigen release rates, either the molecular weight of PLGA or the ratio between lactic acid and glycolic acid can be altered (Makadia & Siegel, 2011; Richards Grayson et al., 2003). Further, the addition of a carboxylic acid end

group or porogens, like polyethylene glycol (PEG). blended into the device can change the release kinetics (Gentile et al., 2014; Y. P. Li et al., 2001). Figure 2 illustrates different types of the antigen release kinetics from the microparticles, which could be either an initial burst followed by a continuous release, multiple bursts, or a pulsatile release mimicking the traditional bolus doses. Single dose microparticles have been reviewed many times (Amorij et al., 2012; Lin et al., 2015; McHugh et al., 2015; Varde & Pack, 2004; Walters et al., 2015); however, we intend to summarize recent advancements in this field with the real antigens for prophylactic vaccines.

In a recent study, PLGA microspheres were utilized for the slow-release of diphtheria toxoid (DT) antigen; degradation rate was modulated through use of PLGA of different molecular weights and addition of stearic acid (SA) (Woo et al., 2018). It was found that PLGA having a lower molecular weight (M_n = 5000) showed a slow initial release followed by faster release, contrary to the rapid initial burst followed by an extended release for the case of the PLGA with a higher molecular weight (M_n = 50,000–75,000). Microspheres composed of lower molecular weight PLGA (LMW-MS) released <10% of the antigen in the initial 3 weeks, while the microspheres with high molecular weight PLGA (HMW-MS) released ~30% in the initial burst of the first week. However, the HMW-MS microparticles mixed with SA (HMW/SA-MS) exhibited pulsatile kinetics. The kinetic profiles were further tuned by mixing the LMW PLGA and HMW PLGA at a 1:1 ratio; SA was also added to obtain MIX-MS. The mixed microsphere (MIX-MS) exhibited an overall modified pulsatile release kinetics with ~30% in day 1 followed by a boost release in 3rd week (~20%) and continuous release in the increasing pattern for next 6 weeks. The detailed comparison data of different types of microparticles are shown in Figure 3. The immunization with a single dose of mixed microparticles MIX-MS led to a higher antibody production and complete protection against the toxin challenge, compared with the conventional alum-based bolus vaccination (Figure 4; Woo et al., 2018).

In some cases, to protect the antigen inside the microsphere for an extended duration, the use of an excipient is required (Tzeng et al., 2016; Wood et al., 1995). For example, immunization against polio requires multiple doses. To achieve a slow-release composition, the antigen is required to be stable inside the body for weeks to months to mimic the prime boost administration schedule (Tzeng et al., 2016). However, the inactivated polio vaccine (IPV) is unstable at 37°C (Tuladhar et al., 2012). Thus, IPV needs to be stabilized first at a high temperature for a controlled release platform. Jaklenec et al. developed a slow-release PLGA microsphere formulation (Tzeng et al., 2016) with the excipients, $MgCl_2$, carbohydrate and monosodium glutamate (MSG), to increase the thermostability of the IPV significantly. Additionally, the incorporation of a cationic copolymer Eudragit E improved the stability of IPV after the release of the antigen from the PLGA matrix. Through device and formulation optimization, this IPV slow-release device achieved antibody titers comparable to two bolus injections given at an interval of 10 weeks (prime-boost schedule). The efficacy was correlated with increased thermal stability of the active ingredient, IPV. Furthermore, the excipients also increased the pH stability of IPV (Tzeng et al., 2018). Since various types of excipients could be incorporated into a PLGA formulation along with the antigen to provide stability, this technique could be exploited for the controlled delivery of

oral vaccines as well, where the antigen suffers from degradation in the GI tract (Davis, 2006; Tan et al., 2017).

Tetanus toxoid (TT) is another antigen, where slow-release could help to reduce the burden on healthcare workers and increase patient compliance. Many earlier studies of slow-release microparticles explored this antigen (McHugh et al., 2015), however, the stability of the antigen during formulation and subsequent polymer degradation/release remained a challenge (Putney & Burke, 1998; Tamber et al., 2005; Van De Weert et al., 2000; Zhu et al., 2000). Natural polymers like chitosan are biocompatible and less susceptible to hydrolytic degradation, thus offering an attractive platform for enhancing antigen stability and controlled release. TT was formulated into preformed chitosan microspheres using an emulsion cross-linking procedure without introducing organic solvents (Varma & Sadasivan, 2014). Release rate was modulated by varying the concentrations of the chitosan gel; importantly the TT antigen was found to be stable in vitro at 37°C. Similarly, Acar et al. (2016) developed a simple crosslinking procedure using gelatin microspheres to load delicate antigens without introducing harsh chemicals. This technique was used for formulation of a single-dose vaccine delivery platform for a HIV-1 gp41-based protein for prevention and treatment of AIDS (Acar et al., 2016; Frey et al., 2010). This platform allows for antigen release over 1 month and allows the encapsulation of fragile antigens. In another study, hepatitis B antigen (HBsAg) was loaded into didodecyldimethylammonium bromide/poly(lactic acid) (DDAB/PLA) nanoparticles (NPs) and encapsulated in spherical microparticles composed of the natural polymer alginate by a modified spray-solidification technique (Yang et al., 2018). This single dose formulation resulted in significantly higher cellular immunity and an equal humoral immunity compared with that of conventional alum-based multiple dose immunization. Another interesting approach for the preparation of microspheres is an atomic layer deposition (ALD) method to coat microparticles with antigens and the desired adjuvant. A recent study showed that the microspheres prepared by the ALD technique containing HPV antigen mixed with alum demonstrate a slow-release profile and elicit better humoral antibody response compared with that of the traditional bolus injections (Garcea et al., 2020).

Recent studies explored recombinant protein/peptide vaccines due to their safety features. They are usually stable and can be easily manufactured in low cost. However, these often suffer from poor immunogenicity and need carriers for delivery. To improve the poor immunogenicity of such vaccines, adjuvants are essential (Sivakumar et al., 2011). In the case of a single dose formulation, microparticles offer an additional advantage of encapsulating the desired adjuvant together with the antigen, furthermore the polymer itself could act as an adjuvant. Anugraha et al. (2015) demonstrated that PLA microspheres containing a recombinant vaccine candidate against Lymphatic Filariasis (Gyapong et al., 2005; Hoerauf et al., 2011) demonstrated high immunogenicity compared with the vaccine alone or mixed with the commercial adjuvant alum and provided a high level of protection after a single dose administration (Anugraha et al., 2015). Similarly, high immunogenicity was observed when inactivated *B. pertussis* (Zepp et al., 2011) bacterial cells were entrapped inside microparticles made from alginate, compared with alum absorbed vaccines (Dounighi et al., 2017). Liu et al. showed that a stable formulation of a polycarbonate microsphere with the tetanus toxoid antigen by the water-in-oil-in-water evaporation technique exhibited

low toxicity and provided protection against the toxin challenge, indicating that the polycarbonate microsphere could act as an adjuvant (Guillaume & Mespouille, 2014).

2.2 | Implants

A useful choice of device geometries could be monolithic polymer implants, which are usually small cylindrical rod shaped (Figure 1) and subcutaneously implanted. Generally, the antigen is situated in a polymer matrix inside the implant or the antigen–matrix is polymer-coated. The degradation or swelling of the polymer results in a slow-release of the antigen (Engert, 2015). The choice of polymer primarily includes silicone, ethylene-vinyl acetate co-polymer, collagen, or PLGA (Engert, 2015). Furthermore, additives or excipients such as PEG are added as a pore-forming agent (or porogen) to fine-tune the release profile of the antigen (Herrmann et al., 2007; Schulze & Winter, 2009). Implants are prepared by different techniques like direct compression, melting/molding, extrusion and casting (Engert, 2015). Implantable systems have been extensively used for hormonal treatments, birth control, ocular treatment and controlled drug release (Engert, 2015; Palomba et al., 2012). To date the use of the implants for prophylactic vaccines has seen limited investigation. Some early works included tetanus toxoid in a collagen mini pellet, demonstrating the sustained release of the antigen (Higaki et al., 2001) or pulsatile release of the antigen mixed with the adjuvant alum in the excipients mix of the cellulose/lactose/magnesium stearate mix (Cardamone et al., 1997). Silicone matrices have shown promising results for different proteins and vaccines, such as *Clostridium tetani*, or *Clostridium novyi* toxoids (Kajihara et al., 2000, 2001; Kemp et al., 2002; S. Lofthouse et al., 2001; S. A. Lofthouse et al., 2002). However, it is not biodegradable and has to be removed surgically, risking patient incompliance and hence, might not be a good candidate for prophylactic vaccines.

Toward vaccine delivery implants, we recently developed melt-manufacturing methods for the preparation of PLGA implants as vaccine delivery platforms to protect from human papilloma virus (HPV) (Lee et al., 2017); specifically, HPV antigens were formulated into PLGA implants using a hot melt extrusion process which can be readily scaled (Shao et al., 2021). Melt extrusion (Lee & Pokorski, 2018) is a versatile technology, which is commonly used in industrial settings and offers a high throughput and reproducibility. We chose peptide antigens of HPV and used virus-like-particles (VLP) (Chackerian, 2007; Chung, Cai, & Steinmetz, 2020; Frietze et al., 2016; Pokorski & Steinmetz, 2011) derived from the bacteriophage Qβ (Golmohammadi et al., 1996; Prasuhn et al., 2008) as adjuvant and delivery platform. The VLPs offer a high degree of thermal stability and thus are a suitable platform for hot melt extrusion. The single dose HPV-Qβ vaccines delivered by subcutaneous PLGA implant released the antigens over a period of 1 month and elicited an equivalent amount of anti-HPV IgG titer as that of three traditional doses administered via subcutaneous injection. The plasma collected from implant-based vaccination efficiently neutralized the HPV pseudovirus (Figure 5) (Shao et al., 2021). The VLP platform provides a unique opportunity to be adapted for other vaccines by simple conjugation or genetic recombination of the target epitope. An advantage of such systems is that multiple vaccines could be mixed in the desired ratio and administered together in a single dose. In a subsequent study (Ortega-Rivera, Pokorski, & Steinmetz, 2021), a similar PLGA implant was effective for the slow-release of a trivalent vaccine candidate targeting proprotein

convertase subtilisin/kexin-9, apolipoprotein B, and cholesteryl ester transfer protein for cardiovascular applications. Recently, covid vaccine candidates were also incorporated in a PLGA slow-release implant system and compared with traditional injection based and microneedle patches, which are described further in a later section (Ortega-Rivera, Shin, et al., 2021; Ortega-Rivera, Shukla, et al., 2021).

2.3 | Microneedles

Microneedle technology is a minimally invasive option and has the potential to be self-administered (Prausnitz, 2017). The typical form-factor is a square/rectangular patch with an array of micron-sized needles (Figure 1). The patch is applied with mild pressure to the skin, penetrating the dermis and releasing the contents of the needle. This is a painless procedure and does not require the presence of a trained professional. The procedure eliminates the risk of infection at injection site and needle phobia which would likely improve patient compliance. Due to these additional advantages, microneedles have garnered much interest in the research community for two decades (Arya & Prausnitz, 2016; Prausnitz, 2017; Sharma et al., 2019; Sullivan et al., 2008, 2010).

A recent focus has been on rapidly dissolvable patches that leave no biohazardous waste (Larrañeta et al., 2016; Park et al., 2006; Ray et al., 2022). To achieve slow-release in these systems, microparticles or implants are formulated to be incorporated inside the microneedles. After the microneedle dissolves, a slow-release implant or microparticle remains in the skin for subsequent release of vaccine. Many studies have shown such a delivery mechanism with model antigens and have been reviewed elsewhere (M. C. Chen et al., 2013; Z. Chen et al., 2020; Jamaledin et al., 2020; Larrañeta et al., 2016; Park et al., 2006; Raphael et al., 2016). However, one major challenge is to keep the antigen stable while formulating the patches. In this context, studies have investigated the potential of silk to stabilize antigen and load densely packed biomolecules in the hydrogel to obtain an extended-release profile (Pritchard & Kaplan, 2011; Wenk et al., 2011). Demuth et al. (2014) first applied this technology to entrap the cargos in a microneedle patch and demonstrated a slow-release profile for a model antigen OVA. Later Boopathy et al. extended this work and demonstrated the method for the HIV antigen in a more stable MN formulation with a PAA/silk matrix (Boopathy et al., 2019). They demonstrated that microneedles prepared using poly(acrylic acid) (PAA) backing and silk protein tips could stabilize the HIV trimer antigen, which is known to have a fragile and complex quaternary structure, for an extended period of release (Boopathy et al., 2019). The composite microneedle was made of PAA backing, which rapidly swelled to push the tip and dissolved in 5–10 min leaving the tip inside the skin. The tip of the microneedle was fabricated from regenerated silk fibroin protein (Raja et al., 2013). The silk protein provided a simple one-step loading process and created a hydrogel matrix, which subsequently released the entrapped antigen over an extended period. Upon drying the silk matrix, different degrees of β-sheet crystallinity were formed, and they were manipulated to obtain a slow-release profile (Boopathy et al., 2019). The crystallizable domains of silk fibroin provide control over physicochemical properties; like biodegradation/release profile by changing the content and nature of crystalline form. β-strands are formed by the hydrogen bonding of the crystallizable subdomains in water and further strengthened by

intermolecular silk fibroin self-assembly. This self-assembly process is another control point to achieve desired physical properties of silk-based formulations (Yucel et al., 2014). The crystalline nature of silk provided additional stability for the antigen at room temperature, thus eliminating the requirement of the cold chain (Zhang et al., 2012). To enhance the stability of the antigen and protect its integrity, a stabilizer MD39 (Kulp et al., 2017) was used and the antigens were released over a period of 2 weeks depending on the crystallinity of the silk (Boopathy et al., 2019). Approximately 1300-fold increase in the serum IgG titers was found from the GC B cells and a 16-fold increase was found in the bone marrow (BM) plasma cells compared with a bolus immunization method (Boopathy et al., 2019). This method of loading a microneedle patch with implantable tips (Boopathy et al., 2019) is a more practical approach to improve the immune response to construct a prophylactic HIV vaccine, compared with the use of mini-osmotic pump or dozens of injections to mimic continuous release of antigens (Tam et al., 2016). Along the same line, a recent study from Chen and coworkers has shown (Y. H. Chen et al., 2019) that vaccination against influenza is more effective to induce long-term immune memory when applied through a microneedle where a chitosan adjuvant is incorporated. This is another case, where the traditional bolus administration often fails to elicit a long-term immune memory, without the use of any adjuvant (Johansen et al., 2008). In this study (Y. H. Chen et al., 2019), the microneedle was made from a dissolving base with poly vinyl alcohol/poly vinyl pyrrolidone (PVA/PVP) and the implantable tips were prepared from chitosan. The microneedle base dissolved within a few minutes of application leaving behind the chitosan tips, which slowly degraded to release the entrapped antigens. The microneedle immunization led to a higher antibody titer compared with that of a traditional multiple dose vaccination and provided full protection without any weight loss or other side effects. The mice remained healthy, even after being challenged with influenza after 4 months of vaccine administration, indicating a durable immune memory while the control groups with the bolus doses showed weight loss and mortality.

Other interesting approaches include the incorporation of microparticles inside microneedles; for example, Mazzara et al. developed a PLGA microparticle loaded microneedle platform, which rapidly dissolved in the skin embedding the particles for slow-release (Mazzara et al., 2019). Efficacy was demonstrated for several antigens, including recombinant hepatitis B virus surface antigen (rHBsAg), anthrax antigen (rPA), plague antigen (Fl-V), and tetanus toxoid (TT). The antigens were loaded into the microparticles by an active self-healing encapsulation (ASE) (Desai & Schwendeman, 2013; Huang et al., 2015; Reinhold et al., 2012) technique that makes a complex of the antigen with a trehalose stabilized hydrogel. This new technique was highly efficient to encapsulate different antigens in a stable form inside the microparticles for the controlled delivery (Mazzara et al., 2019). These microparticles were loaded into microneedle patches composed of water-soluble materials PVA and sucrose. The microneedle patches exhibited a biphasic release pattern where the initial burst occurred due to the soluble antigen, followed by an extended release of (~2 month) of antigens complexed with the hydrogel. Preclinical testing in mice showed that highest antibody titers were elicited when the antigens were delivered via microneedle. Such design of long-lived microneedles with reservoir microstructures (Richards Grayson et al., 2003; Stevenson et al., 2012) could help to

modulate the release of antigen to mimic multiple bolus doses, supplying a continues pool of the antigens over an extended period. The recent advancements in lithographic techniques could create an assembly of layer-by-layer polymer stacking (SEAL; McHugh et al., 2017) to form microparticles, capable of multiple bursts of cargo release over an extended period of time. The layer-by-layer microneedle formulation technology holds promise of much quicker dissolution by changing the polymer composition. For example, Hammond et al. have shown that introducing a pH-induced charge invertible polymer could help the microneedles dissolve within a minute, rather than 15–90 min as required by preexisting technology (Regency & Regency, 2019). However, the manufacturing protocol for filling up microparticles is very complex in this technique and challenging to scale up for commercial use. To alleviate these issues, Nguyen and coworkers (Tran et al., 2020) have demonstrated a scalable and high-throughput 3D-manufacturing technique to prepare a core-shell microstructure for the microneedles having three different parts—a PLGA cap, a core loaded with vaccine and a shell made from PLGA. This unique structure allows researchers to tune the rate of burst release by fine-tuning the composition and molecular mass of the PLGA shell. The multiple microneedles could be applied at the same time with different PLGA compositions, which in turn degrade over different time intervals allowing a multi burst release at different time points (Figure 6). The microneedle was coated with water soluble PVP for the rapid dissolution into bodily fluid. This study was performed with three different compositions of PLGA with a clinical vaccine Prevnar-13, demonstrating that a precise control over the release kinetics could be achieved for the vaccines. The technique could be potentially translated as a patient friendly approach in a clinical setup and could be an alternative to traditional immunization.

3 | SINGLE-DOSE SLOW-RELEASE VACCINES IN A PANDEMIC SETTING

Single-dose slow-release vaccine technology could offer a significant improvement in vaccine administration for mass vaccination programs, especially in the scenario of a global pandemic. The current COVID-19 pandemic affects millions of lives worldwide (Nicola et al., 2020; Omer et al., 2020). It adversely impacted the economy since people were forced to stay at home leaving their regular livelihood. Although the vaccine against COVID-19 (Chung, Beiss, et al., 2020; Shin et al., 2020) was developed and manufactured in record time (less than a year) (Lurie et al., 2020), it is very difficult to complete a global mass vaccination program rapidly. Most of the vaccines now require three doses, must be administered by a qualified healthcare professional, and require ultra-low temperatures for transportation and storage. In this situation, a single dose vaccine could alleviate the need for multiple visits to health-care facilities; limit the requirement for the number of trained healthcare providers, thus significantly reducing the cost of the vaccine. Most of the single dose formulations are more stable than the regular two-dose vaccines, hence, limiting the requirement for cold chain storage. The most commonly used COVID-19 vaccines in the United States are from Pfizer-BioNTech (Centers for Disease Control and Prevention, 2021b) and Moderna (Centers for Disease Control and Prevention, 2021a), which have demonstrated very high efficacy, however, multiple doses of the vaccines have to be administered and the vaccines need cold chain transportation at −80°C/−20°C. These criteria undoubtedly slowed the distribution process, increased costs, and limited availability.

Another approved (February 2021) Janssen Ad26.COV2.S (COVID-19) vaccine (Centers for Disease Control and Prevention (CDC), 2021) was initially licensed as a single dose vaccine, which could be stored at 2°C–8°C. This vaccine is composed of Ad26 vector that expresses the SARS-CoV-2S protein and has been stabilized with excipients. However, later booster doses from Pfizer-BioNTech or Moderna become necessary to obtain protection from new strains of COVID-19. SARS-CoV-2 is still very young; however, studies are beginning to explore the field of single dose vaccines (Sanchez-Felipe et al., 2020; Yahalom-Ronen et al., 2020) and the current scenario indicates the importance of it in preparation for the future pandemics.

Several laboratories and companies have turned toward formulation of delivery devices to enable single dose administration of COVID19 vaccines (Counoupas et al., 2021). For example, we have developed implants and microneedles for delivery of COVID19 vaccine candidates (Ortega-Rivera, Shin, et al., 2021; Ortega-Rivera, Shukla, et al., 2021). We studied 13 peptide epitope vaccine candidates displayed on VLPs from bacteriophages and plant viruses and selected three neutralizing epitopes that are conserved among most variants of concerns. Trivalent vaccine candidates were then formulated by mixing VLPs displaying the three distinct epitopes. PLGA implant were prepared by hot melt extrusion, as described previously (Shao et al., 2021) and microneedle patches were prepared using a PVP solution-casting procedure either with or without Mg microparticles for active and passive deliveries, respectively (Figure 7) (Ortega-Rivera, Shin, et al., 2021). Both delivery via the implant or microneedle demonstrated efficacy—while the implant enables slow-release and efficacy after single dosing, the microneedle patch enables self-administration albeit requiring two doses. In another study we have shown slow-release hydrogel formulations with high molecular weight chitosan and glycerophosphate for COVID 19 vaccine candidate in a plant based VLP platform, The single dose subcutaneous injection of the hydrogel elicits equal (even higher at later timepoints) antibody titer, compared with that of multidose soluble bolus injections (Nkanga et al., 2022).

4 | EFFECT OF SLOW-RELEASE KINETICS ON THE IMMUNE SYSTEM

The reduced cost along with better compliance and availability of vaccines are certainly very good reasons to explore slow-release formulations. Moreover, the kinetics of a slow-release vaccine might provide new benefits (Irvine et al., 2020), such as improving the quality and quantity of antibody production due to the sustained release of an antigen over a period of time (K. K. M. Cirelli et al., 2019; K. M. Cirelli & Crotty, 2017; Havenar-Daughton et al., 2019; Schipper et al., 2016; Tam et al., 2016). This sustained release of antigen can mimic natural infection more closely, unlike the rapid surge and clearance of the antigen after vaccination with a bolus injection. A longer duration of antigen retention has been observed in the lymph nodes for slow-release formulations compared with bolus injections (Tam et al., 2016). It is particularly beneficial for complex antigens, such as HIV, where the maturation process of neutralizing antibody generation requires more steps. However, there should be a fine balance between tolerance and duration of release, which depends on the vaccine delivery design based on the modulation of vaccine kinetics. Recent studies have highlighted some possible mechanisms that help explain why slow-release technologies enable better antibody production (K. M. Cirelli & Crotty, 2017). During the course of

immunization, antigen fragments are carried to the lymph node, and there picked up and displayed by follicular dendritic cells to the B cell receptor (BCR) in a certain manner so the BCR could crosslink. Followed by recognition of the cognate antigen, B cells develop and proliferate in the activated lymphoid follicle which is termed the germinal center (GC). T helper cells stimulate B cells (and prevent apoptosis)—the expanding B cells create a dark zone in the GC where they undergo somatic hypermutation (SHM) to rearrange the genes and fine tune the affinity of the BCR for its cognate antigen. The ability of the mutated BCR to bind with the antigen of the follicular cells is evaluated and the high affinity B cells are selected, preserved and sent for mutation again. It acquires co-stimulation from the T follicular helper (Tfh) cells. The cycles of the mutation takes place in the dark zone, while, the affinity testing and selection happens in the light zone (Sompayrac, 2012). After the maturation of B cells they are converted either to a plasma B cell, producing immediate antibodies, or to a memory B cell that provides protection from the future attack by the same antigen (Sompayrac, 2012).

In the case of traditional bolus prime-boost injections, the availability of the antigen reaches its peak quite early compared with the GC peak, whereas in the case of the slow-release of antigen, the peak of antigen availability matches more closely with the timeline of the GC peak. The availability of the antigen over a longer period of time increases the binding probability of the higher affinity antibodies and the production of the number of the immune complexes, resulting in more significant GC response (Figure 8) and the retention of the rate of B cell affinity maturation (K. M. Cirelli & Crotty, 2017; Tam et al., 2016). Second, due to this time lag between antigen exposures and GC peak for the traditional bolus immunization, the antigens are exposed to proteases for a longer time before the GC peak. This increases the probability of antigen degradation. On the contrary, the continuous supply of the antigen over a much longer period (Figure 8) synchronizes with GC peak, limiting the time for proteolysis and increasing the probability of intact antigens to reach the GC. Therefore, GC exposure to degraded and thus distracting epitopes is avoided which is important, because processing of degraded epitopes often leads to the production of the irrelevant antibodies (K. M. Cirelli & Crotty, 2017). Third, the somatic hypermutation occurs in the GC and the enhanced GC with the increased Tfh cells are associated with the higher number of neutralizing antibodies in many cases (Tam et al., 2016). The increasing number of the Tfh helps to increase the BCR diversity, resulting in an enhanced antigen recognition feature (Figure 8). The higher number of the Tfh cells lead to the high affinity complex formation and enhance the response of the GC (K. M. Cirelli & Crotty, 2017). However, the mechanisms for the longer duration of the GC and the production of the higher number of Tfh cells by the slow-release vaccination have not yet been completely understood. Very recent studies (Tam et al., 2016) show that slow-release kinetics over a long period of time increases the production of the GC Tfh cells, resulting in a higher number of the neutralizing antibodies. Another hypothesis discusses (K. M. Cirelli & Crotty, 2017) the possibility that the memory B cells are maturing late resulting in a higher number of the mutations due to the longer availability of the antigens in the case of a sustained release compared with the traditional bolus injection.

Indeed, recent studies (K. K. M. Cirelli et al., 2019; Havenar-Daughton et al., 2019; Schipper et al., 2016; Tam et al., 2016) demonstrated the efficacy of sustained release and

immune cell kinetics using a repeated fractional injection method or introducing a mini osmotic pump. The repeated fractional injection method provided the supply of the vaccine in three different scenarios: (1) at a constant rate, (2) at an exponentially increasing rate or (3) at an exponentially decreasing rate. Schipper et al. (2016) tested inactivated polio vaccine serotype I (IPV1) in 1/4th or 1/8th doses for four or eight consecutive days under these three scenarios and the results were compared with the same total amount of the polio vaccine given by a bolus injection. It was observed that a 10-fold higher IPV1 specific IgG titer was produced in the fractional dosing scheme compared with the bolus injection.

HIV is a tough human pathogen, where sufficient production of a long term broadly neutralizing antibody (bnAb) against the HIV envelope has not yet been achieved by traditional vaccination primarily due to the requirement of very complicated and high degrees of somatic hypermutation (SHM; Burton & Mascola, 2015). The researchers explored HIV vaccine strategies with a continuous release of the antigen over a longer period of time to sustain SHM for a longer time to achieve the required complex affinity maturation (K. K. M. Cirelli et al., 2019; Havenar-Daughton et al., 2019; Tam et al., 2016). Tam et al. (2016) introduced a mini osmotic pump to administer continuous, exponentially increasing or decreasing (Figure 9) prime and boost doses and compared these to traditional immunization. Higher antigen retention in the lymph nodes, enhancement of the Tfh cells and more than 10-fold increase of neutralizing antibodies were observed in the case of the exponentially increasing dosage in comparison with the bolus injection. This result is consistent with the computational GC kinetics modeling. This idea was further explored by administering the HIV vaccine (K. K. M. Cirelli et al., 2019; Havenar-Daughton et al., 2019) to rhesus monkeys and a 20-fold higher autologous neutralizing antibody titer along with the increased production of the Tfh cells and high affinity B cells toward the HIV envelope (K. K. M. Cirelli et al., 2019) was observed for the sustained-delivery immunization compared with the traditional injection method. This slow delivery approach could modulate the formation of non-neutralizing irrelevant antibody production toward the development of the antibodies with binding affinity to the diversified epitopes (K. K. M. Cirelli et al., 2019). Although the slow-release delivery appears to be very promising, the fractional dosing of dozens of injections or introduction of the mini-osmotic pumps are impractical for a mass immunization program. Thus, recent efforts in this direction includes a feasible design for a slow-release delivery device would have to better tune and improve the vaccine efficacy as well as must be cost-effective and safe to handle, as discussed previously in section B.

5 | CONCLUSION AND FUTURE DIRECTION

Single-dose slow-release vaccine devices hold the promise of lower cost and improved patient compliance, thus facilitating easier mass vaccination. Additionally, slow-release kinetics of antigen delivery have demonstrated improved efficacy. However, to achieve slow-release of the antigens over an extended period, patient friendly devices are required. In this context, we have discussed three state-of-the-art primary designs—microparticles, implants, and microneedles. The key challenges are to retain the stability of the antigen and provide the same level of protection as that of conventional immunization without any adverse effects due to foreign materials. Microparticles have been widely studied in this field and they show promising results for many antigens. Several researchers have shown

new directions with model antigens (Chiu et al., 2018; Du et al., 2017; Sinha et al., 2019). However, the usual process of preparation demands the presence of harsh chemicals and pH changes which often lead to the degradation of the antigens during the formulation. Recent studies have developed new methods (Acar et al., 2016) of loading antigens in a softer manner to protect the integrity of the delicate antigens. However, extensive studies are required to understand the exact pharmacokinetics of complex microparticles, and data thus far are limited. Scalable PLGA implant formulation via a melt extrusion technique (Shao et al., 2021) holds promise and the VLP platform offers versatility to manufacture different vaccines and slow-release formulations (Czapar et al., 2018) against a number of diseases. Still, both the microparticles and implants require trained healthcare professionals for their administration. Thus, at this point, microneedle technology clearly stands out. Microneedles could be self-administered just like pills and may be as effective as traditional injections. Microneedles are minimally invasive, thus eliminating needle phobia among patients, decreasing the chances of infection at the injection site, and removing the need for a biohazardous sharp disposal system. Previous studies have shown that any person could vaccinate himself/herself using microneedles without any prior training (Donnelly et al., 2014; Norman et al., 2014). The self-administration of vaccines would certainly improve the rate of vaccination (Norman et al., 2014) and would be a major milestone for the success of any urgent mass vaccination program. Apart from promoting patient compliance, the studies discussed in the previous sections have pointed out that microneedles could provide higher antibody response compared with traditional injections, because microneedles inherently target the most densely populated antigen presenting cells (APC) of the skin (Zaric et al., 2013). Zaric et al. have shown that PLGA nanoparticles with an encapsulated antigen could be loaded into microneedles to selectively target the dendritic cells of the skin to generate a robust antigen specific cellular response (Zaric et al., 2013). The authors induced OVA specific CD8+, IFN-y, and CD4+, which were sufficient to provide protection against the parainfluenza and have shown reduced tumor growth in B16 murine melanoma models, thus demonstrating another advantage of the application of the microneedles to enhance the activation of the immune response. This is a promising direction to explore a self-administered single-dose patch for administering vaccines. However, more studies are required to obtain the detailed immunological reasoning for the significantly higher immune response. Certain hydrogel scaffold formulations that have shown efficacy for model antigens (Nishiguchi & Taguchi, 2020; Roth et al., 2020) could be useful in this context as well.

Several recent studies have explored the idea of the release of vaccine over an extended time period with nucleic acid vaccines or a combination of the protein and nucleic acid vaccines that could induce both humoral and cellular immunity (K. K. M. Cirelli et al., 2019; Jalah et al., 2014; J. Li et al., 2013). This approach has been tested for Zika, Ebola, H1N1 Influenza, *Toxoplasma gondii*, and simian immunodeficiency viruses (SIV) (Chahal et al., 2016; Erasmus et al., 2018; Patel et al., 2013) and reviewed elsewhere (Irvine et al., 2020). Another important application of such a slow-release single dose vaccine could be in the field of oncology, where a single-dose cancer vaccine would have long-term benefits such as the improved patient compliance, the quality of the care, and greater affordability than the traditional immunotherapies, requiring multiple and frequent administrations. However,

the mechanisms for such vaccine kinetics are different and recently reviewed (Briquez et al., 2020; Cheung & Mooney, 2015; Graciotti et al., 2017; Saung et al., 2019).

Slow-release technology opens the possibility for vaccine distribution to many places having poor medical infrastructure such as to the developing and underdeveloped countries with high populations. New studies have started to explore the immunological aspect of this slow-release mechanisms and more investigation in this area would be required to design new devices for tuning the kinetics to achieve superior immune responses. Biocompatible formulations are being used and they hold the promise to be easily translated to the clinic as the next generation of the immunization tools.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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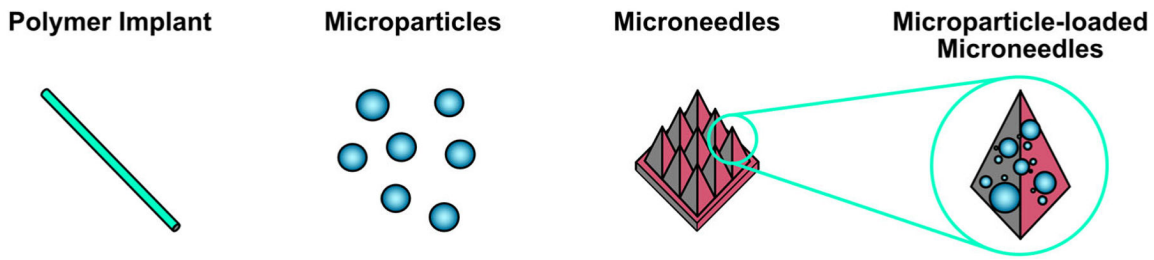

FIGURE 1.
Schematic illustration of major types of vaccine delivery devices; polymer implant, microparticles, and microparticle-loaded microneedles.

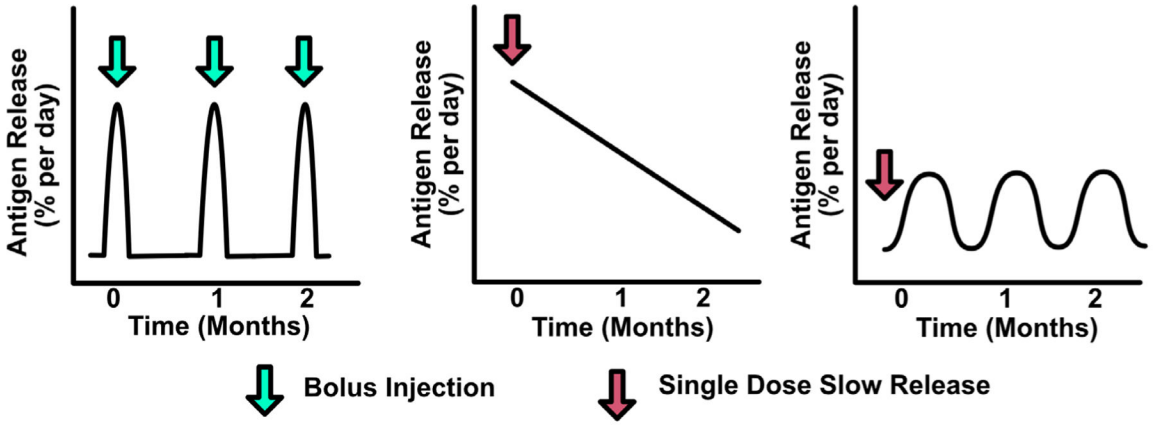

FIGURE 2.

Different antigen release profiles. Left panel illustrates the antigen release profile for conventional bolus injection, where three doses at a specific interval are usually given. Middle and right panel demonstrate the antigen profile for extended release with microparticles. Microparticles can show an initial burst followed by a slow continuous release (middle), or pulsatile (right), where several bursts happen at different times, however the bursts are broader compared with the traditional bolus injection. This figure is recreated from reference McHugh et al. (2015).

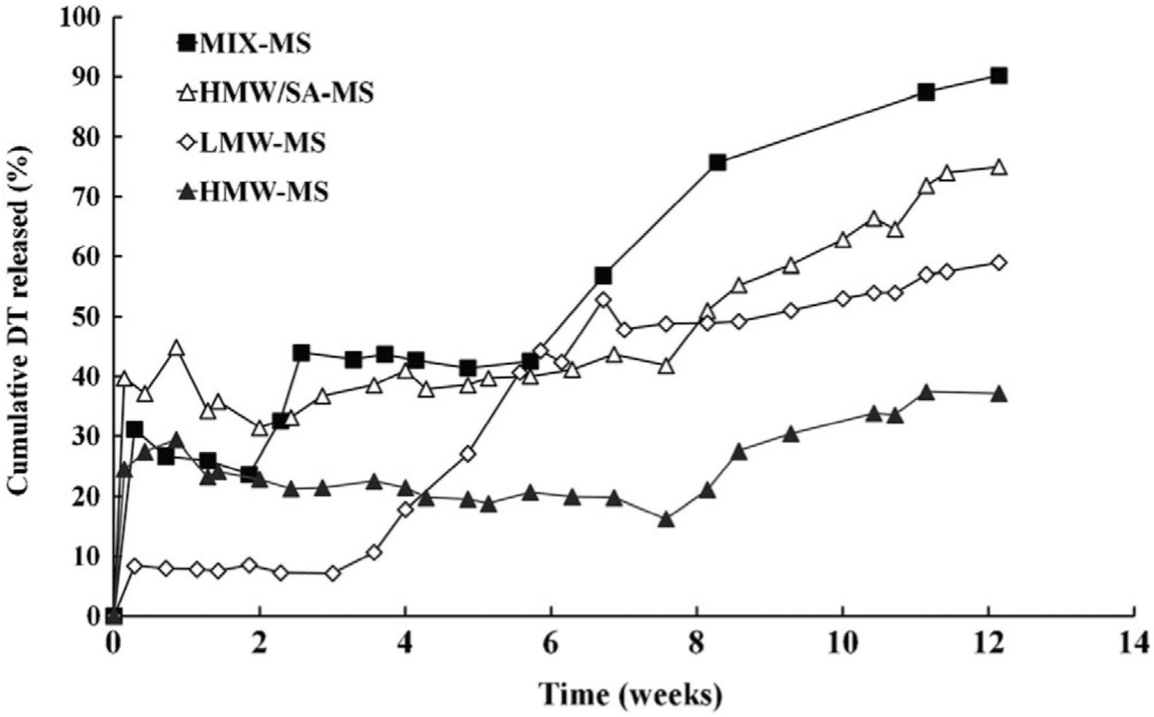

FIGURE 3.
Cumulative release profile of DT-loaded PLGA microspheres. Reprinted figure 1 with permission from reference Woo et al. (2018).

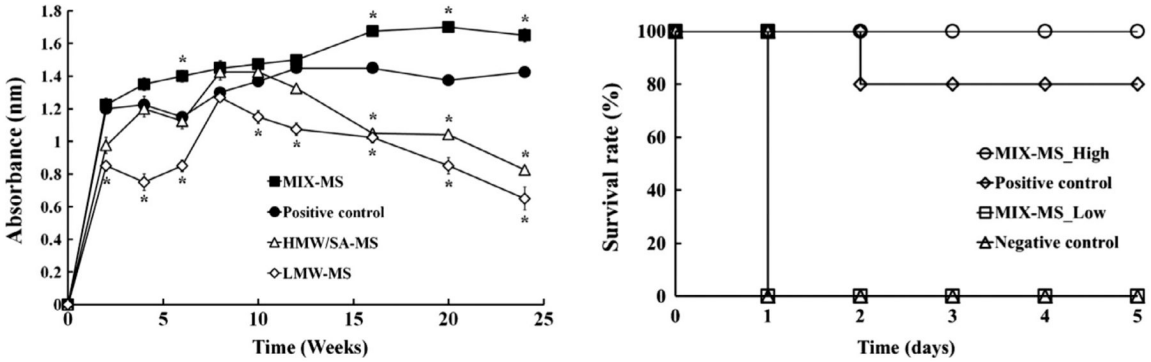

FIGURE 4.

Left panel. Serum antibody levels measured by ELISA after DT immunization using Balb/c mice. Data are expressed as mean ± SD ($n = 5$). Statistical analysis was performed using the two-sample Student's t test (*$p < .05$ vs. positive control). Right panel. Kaplan–Meier survival curves for direct toxin challenge test in Balb/c mice (n = 5 for each group). The formulations were subcutaneously injected to the mice as follows: MIX-MS Low and MIX-MS high were the combined formulations of LMW-MS and HMW/SA-MS at a 1:1 ratio, and administered once at 6 and 18 Lf (limes flocculation) doses, respectively. The positive control group received 6 Lf of alum-adsorbed DT three times at 2-week intervals. The negative control group received saline. Reprinted with permission from figures 2 and 3 from reference Woo et al. (2018).

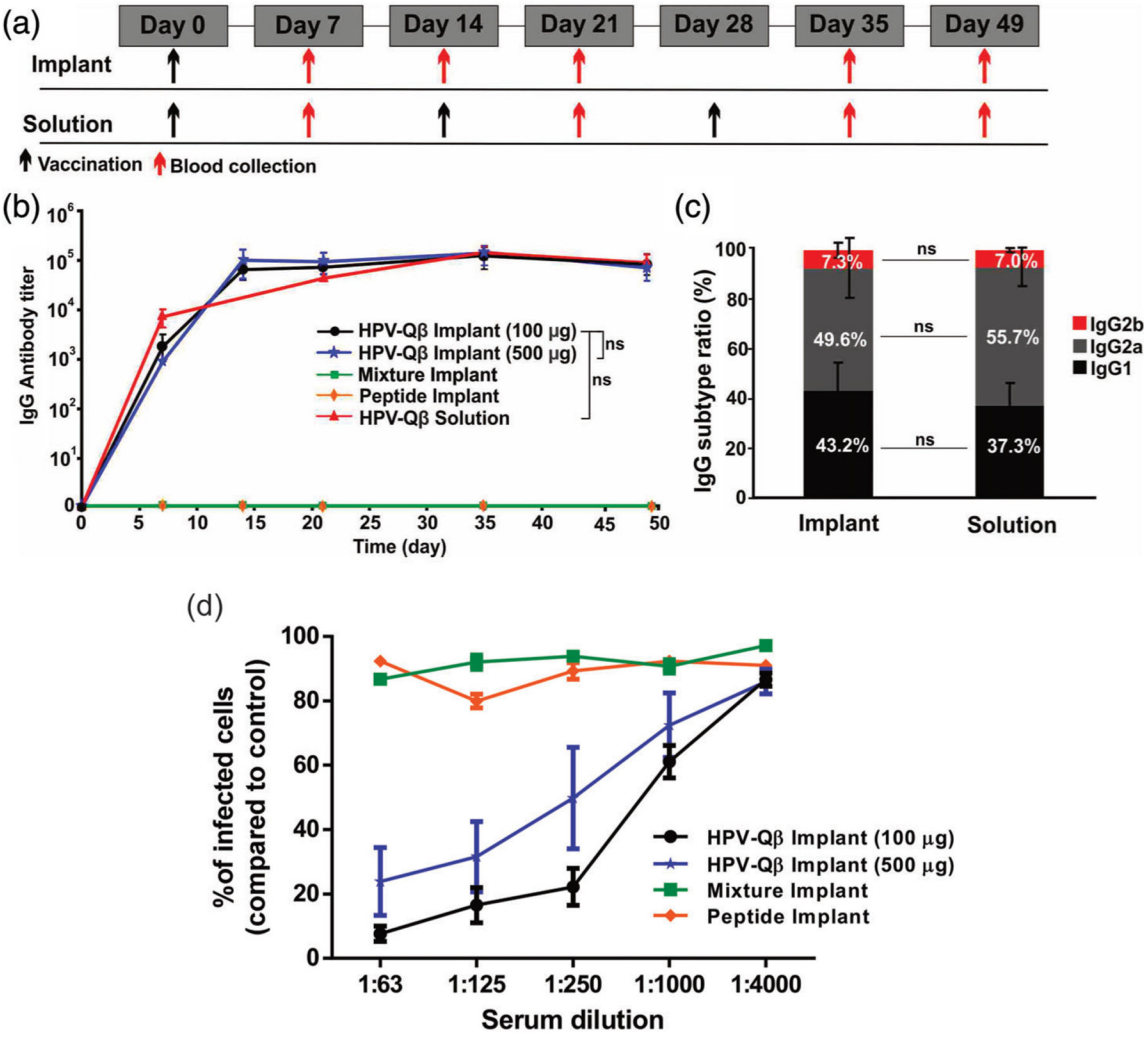

FIGURE 5.

Single-dose vaccination using PLGA implants loaded with HPV-Qβ. (a) Vaccination and bleeding schedule for mice with subcutaneous PLGA implants or equivalent HPV-Qβ injections. (b) Serum titers of HPV-specific IgG for mice vaccinated with three subcutaneous injections of 30 μg HPV-Qβ or a single-dose PLGA implant loaded with 100 μg HPV-Qβ, 500 μg HPV-Qβ, a mixture of 100 μg Qβ and 20 μg HPV peptide, or 20 μg HPV peptide alone. Data are means ± SD for $n = 6$ mice per group). Statistical significance was determined by unpaired two-tailed student's *t*-test: ns, not significant with $p > 0.05$. (c) HPV-specific IgG subtype ratio for mice vaccinated with a single-dose PLGA implant containing 100 μg HPV-Qβ, or three subcutaneous doses of 35 μg HPV-Qβ. Blood was collected on day 35 (data are means ± *SD* for $n = 6$ mice per group). (d) Serum (day 35) from three mice immunized with various vaccine formulations tested in duplicate for neutralization against HPV16 pseudovirus at ID_{60} (pseudovirus infectious dose that infects 60%–70% of control cells). Infected cells (expressing GFP) were identified by flow cytometry (data are means ± standard errors based on the relative percentage of infected

cells in wells exposed to serum compared with non-exposed controls). Reprinted figure 5 with permission from reference Shao et al. (2021).

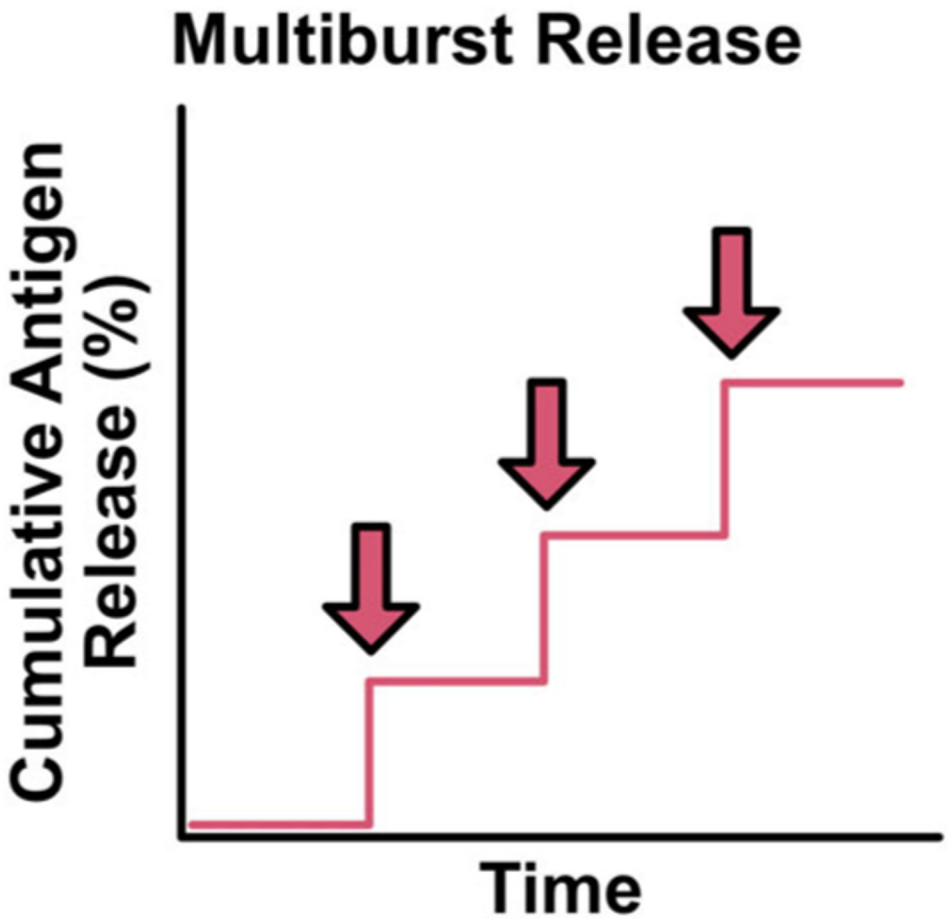

FIGURE 6.
Schematic illustration of multiburst release. Antigen release at a specific interval leading to stepwise increase of total antigen as done in reference (Tran et al., 2020). This figure is recreated from the reference 2020

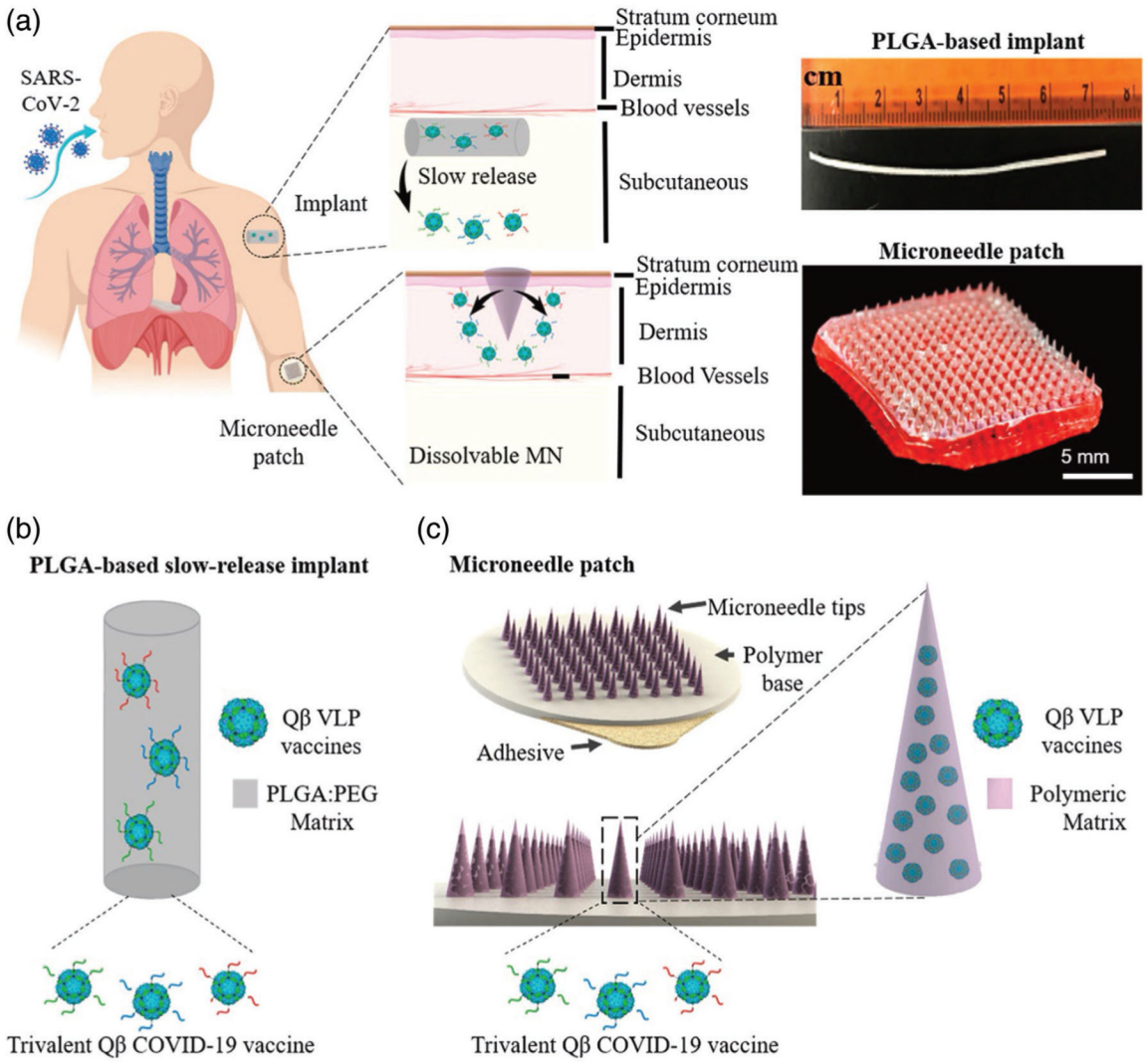

FIGURE 7.

Trivalent Qβ COVID-19 vaccine candidate delivery strategies. (a) Concept of implant application subcutaneous highlighting the sustained and slow-release and passive microneedle (MN) patches showing the release of the vaccine after the MN dissolves in the dermis. Position of the devices in the arm is for illustration only. Photographs of an implant and a MN patch are shown. Detailed illustration of the PLGA-based implant (b) and MN patch (c) with the trivalent Qβ COVID-19 vaccine (mixture of three selected vaccines in equivalent ratio). Reprinted figure 5 with permission from reference Ortega-Rivera, Shin, et al. (2021).

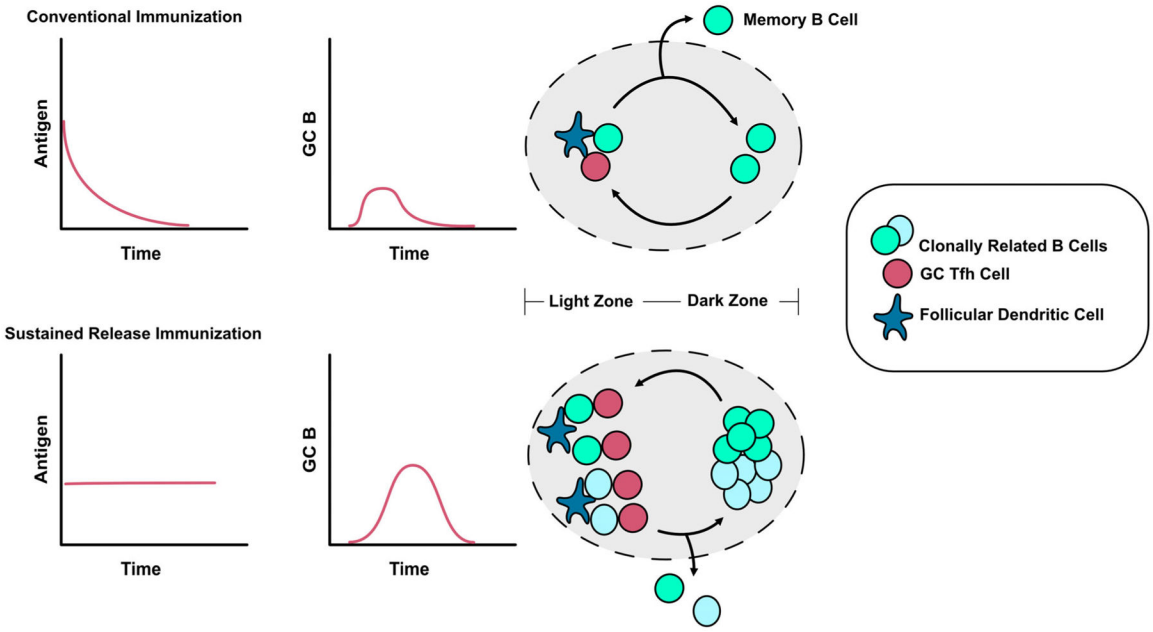

FIGURE 8.

Enhancement of germinal center kinetics by slow-release. Left panel shows the antigen availability pattern; antigen availability decreases over time for conventional immunization, whereas sustained release immunization maintain a constant supply of intact antigen in the later time points during immune response. Middle panel illustrates the germinal center (GC) response. During conventional immunization, a significant amount of antigen presentation happens relatively early to the GC peak, resulting in a weaker GC response (top image), whereas; in the case of slow-release immunization the antigen presentation time window sustain during the peak of the GC response inducing a larger GC response (bottom). Right panel shows the role of Tfh cells; sustained immunogen release increases the number of the Tfh cells that increases the B cell receptor (BCR) diversity resulting in lowering down the B cell competition and allows better antigen recognition feature compared with that of conventional immunization (described in reference K. M. Cirelli & Crotty [2017]). This figure is recreated from reference 2017

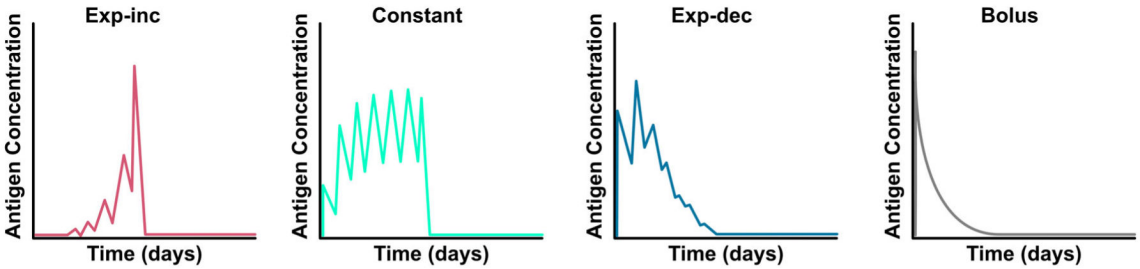

FIGURE 9.

Schematic illustration of various kinetic profile of antigen release, exponentially increasing, constant release, exponentially decreasing and conventional bolus doses (from left to right respectively), as tested in reference Tam et al. (2016). This figure is recreated from reference 2016