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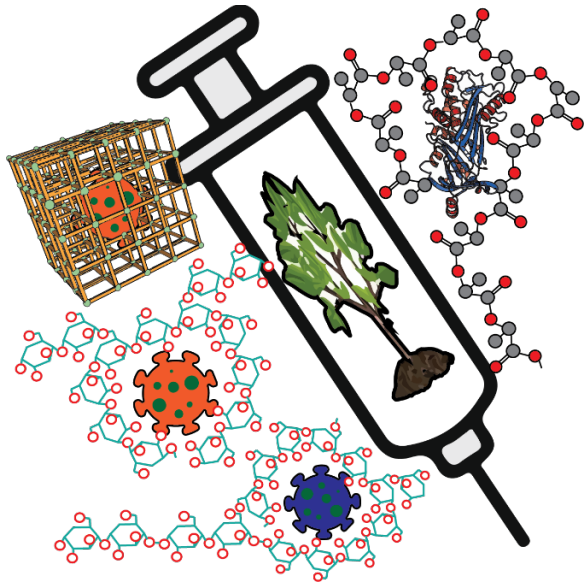
Abstract

Vaccines are considered one of the most significant medical advancements in human history, as they have prevented hundreds of millions of deaths since their discovery; however, modern travel permits disease spread at unprecedented rates, and vaccine shortcomings like thermal sensitivity and required booster shots have been made evident by the COVID-19 pandemic. Approaches to overcoming these issues appear promising via the integration of vaccine technology with biomaterials, which offer sustained-release properties and preserve proteins, prevent conformational changes, and enable storage at room temperature. Sustained release and thermal stabilization of therapeutic biomacromolecules is an emerging area that integrates material science, chemistry, immunology, nanotechnology, and pathology to investigate different biocompatible materials. Biomaterials, including natural sugar polymers, synthetic polyesters produced from biologically derived monomers, hydrogel blends, protein-polymer blends, and metal-organic frameworks, have emerged as early players in the field. This overview will focus on significant advances of sustained release biomaterial in the context of vaccines against infectious disease and the progress made towards thermally stable 'single-shot' formulations.

Graphical/Visual Abstract and Caption



Most vaccines require refrigeration and many need multiple-injections to exert their full therapeutic potential. In this overview, we discuss how combining vaccines with biomaterials are poised to help overcome both of these issues.

1. INTRODUCTION

Every year, infectious diseases cause millions of deaths worldwide, making it the third leading cause of death after cardiovascular disease (**Figure 1A-B**). It is likely that, because of the COVID-19 pandemic, death from infectious disease might emerge as the leading cause of death in the United States in 2020.(Woolf, Chapman, & Lee, 2021) Vaccine development has made remarkable progress since 1791 when Edward Jenner discovered he could inoculate patients against smallpox by infecting them with cowpox, a significantly milder disease.(Riedel, 2005) Since then, safer and more engineered vaccine designs have emerged. For example, inactivated/live-attenuated pathogen formulations,(Demento, Siefert, Bandyopadhyay, Sharp, & Fahmy, 2011) subunit vaccines,(Tsoras & Champion, 2019) immunogenic epitopes,(Black et al., 2012) and inclusion of different classes of adjuvants(Vajdy, 2011) have significantly improved long-term immunological memory. These new formulations generate higher antibody titers while reducing severe side effects.(L. Yang, Li, Kirberger, Liao, & Ren, 2016) However, 300 years after the first vaccination, many challenges remain in developing new vaccines – in particular, low stability, inefficient delivery, poor selectivity, and inability to translate into humans.(Irvine, Swartz, & Szeto, 2013; Welch, Lee, Luzuriaga, Brohlin, & Gassensmith, 2018) For the past three decades (**Figure 1C-D**), biomaterial-based technologies such as synthetic and natural polymers, lipids, scaffolds, microneedles, and other particle-carriers have emerged to improve vaccine efficacy, safety, and stability.(Shen, Hao, Ou, Hu, & Chen, 2018; Uppu et al., 2020; J. Yang et al., 2015) Biomaterials offer a unique design strategy of carrier/adjuvant for immune cargo loading, protection, modification, and administration to control targeted delivery, minimizing the number of injections, and reducing systemic and local toxicity.(Eric M. Bachelder et al., 2008; Elmowafy, Tiboni, & Soliman, 2019; Sahdev, Ochyl, & Moon, 2014)

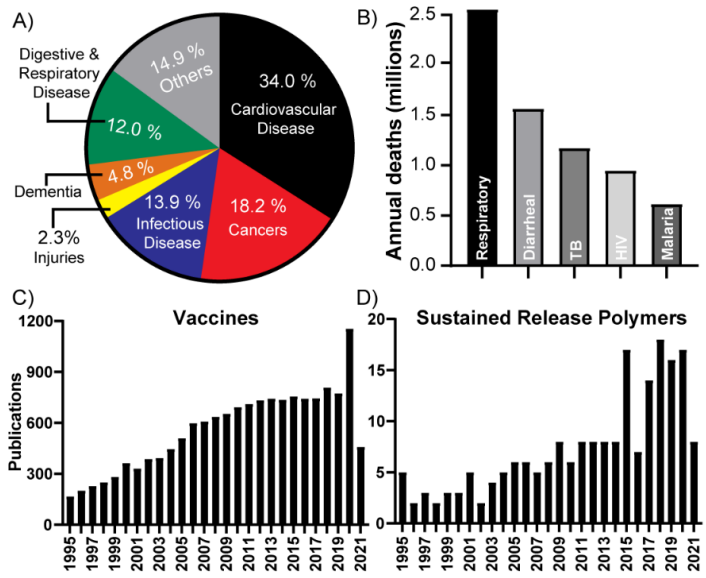


Figure 1: A pie chart showing in percentages the A) leading causes of death worldwide and B) the number of deaths by the leading causes of deaths by infectious diseases for 2017. The number of publications published from 1995 – 2021 with the keyword being C) vaccine refined to journals, letters, and reports, with additional refinement for *in vivo* and D) polymer vaccine or sustained release vaccine refined to journals, letters, and reports, explicitly regarding *in vivo* work. Note: 2021 publications were checked on April 25, 2021.

[1.1 The Interplay Between the Realities of the Immune System and Vaccine Technology]

Vaccines protect individuals by developing immunological memory, so when the body encounters a foreign pathogen, an immediate and proportional adaptive response against that pathogen begins before it can reproduce and cause systemic damage. The immune response to an infectious agent follows two broadly defined phases: an initial innate response followed by an adaptive response. The initial response occurs when the immune system's cellular vanguards—neutrophils, macrophages, monocytes, or immature dendritic cells—recognize pathogen-associated molecular patterns (PAMPs) on a foreign invader using pattern recognition receptors (PRRs) on their cellular surfaces. This recognition event allows these cells to verify that what it has encountered as potentially dangerous. When one of these "vanguards" encounters a foreign substance, the cell will consume the material via phagocytosis and release signaling chemicals called chemokines and cytokines that recruit other cells and induce the physical symptoms of local inflammation to indicate infection. Some of these cells will differentiate into antigen-presenting cells (APCs) and migrate to the T-cell region of the draining lymph node, where they will initiate the second phase of the immune response, the adaptive response.(Di Pasquale, Preiss, Tavares Da Silva, & Garçon, 2015; Plotkin, 2014)

The adaptive response and development of immune memory for rapid response to previously vaccinated antigen depends upon an essential interplay between T-cells and the rest of the immune system. CD4+ cells can only determine if an antigen is foreign or not if they are presented with the antigen by an APC that was activated in the initial infection or vaccination. From there, the activated CD4+ cells that have become memory CD4+ cells will no longer need activation from APCs during a second encounter with the infection, thus being able to mount a faster and more robust immune response. Clearing a pathogen, however, requires the immune system to respond in several different ways. Adaptive immunity is commonly divided into two major systems(Leleux & Roy, 2013): the first is the cellular-mediated response, which is the activation of cytotoxic CD8+ T-cells and other phagocytes to police human cells that might be harboring infectious pathogens. It is thought that vaccines that target tuberculosis, cancer, and HIV will need to focus primarily on developing a strong cellular response. The other system produces a humoral response, which is the activation of B-cells and plasma cells to secrete antibodies. How biomaterials affects these systems is discussed in section 4. There are several different types of antibodies, each serving specific purposes, but most vaccines focus on producing neutralizing IgG-type antibodies that can bind tightly to a pathogen's surface to either block it from entering cells and/or to flag it as a foreign invader that should be destroyed. This latter route has been the focus of most vaccines, including those for many viral infections excluding HIV; indeed, most successful vaccine development has primarily focused on activating a humoral response.

Differentiating between foreign and self-proteins is one of the most critical parts of adaptive immunity, and this differentiation is often made by assessing the surface antigens displayed on the outside of a pathogen. Therefore, vaccine development has historically involved identifying the most immune-stimulating aspect of a pathogen and presenting it to the immune system, with the hope that the immune

system will be able to mount the correct type of immune response. Four such formulations are employed clinically—live attenuated, inactivated, subunit, and nucleic acid-based vaccines. Live attenuated vaccines are “living” relatives of the infectious organism that produce no or only mild symptoms yet can instigate a protective immunity against a dangerous pathogen. However, at issue is that live attenuated vaccines might replicate too rapidly for elderly or immune-compromised patients and have the potential to mutate back to a virulent form, leading to severe complications.(Amanna, 2012; Cox, Baker, Nogales, Martínez-Sobrido, & Dewhurst, 2015; Kaufmann & McMichael, 2005; Ruprecht, 1999) The alternative to a live vaccine is to use an inactivated pathogen that has been rendered non-reproductive (or dead) through either heat or chemical crosslinking; however, these processes may damage the surface epitopes, and this can result in less effective vaccines. Alternatively, the exterior antigens can be removed, purified, or genetically engineered onto the surface of a nanoparticle to fool the immune system into producing antibodies that bind specifically to that antigen. In both cases, inactivation or subunit vaccines are considered safer than live attenuated vaccine systems(Clem, 2011; Foged, 2011; Gao et al., 2020) however, both approaches frequently fail to induce a strong immune response on their own, even after multiple injections. Consequently, subunit vaccines may not provide sufficient protection against the actual disease on their own(Bachmann et al., 1993; Vartak & Sucheck, 2016) The use of adjuvants has been a way to steer the type of response the immune system has as they can help promote strong and specific immune reactions; however, these reactions can be too strong, and thus, only a handful of adjuvants are approved. A distinct alternative to sourcing proteins or carbohydrates from the pathogen itself are nucleic acid-based vaccines, which deliver either DNA or RNA for *in situ* production of antigens. DNA vaccines, for example, contain synthetic constructs that encode for the expression of the antigen when successfully uptaken by cells after injection.(van Riel & de Wit, 2020) Similarly, mRNA vaccines induce protein production within a cell to induce potent immunogenic responses at lower dosages, comparable to high a number of antigens expressed per cell.(Vogel et al., 2018) Further, mRNA vaccines are non-infectious and have no inherent risk of insertional mutagenesis,(Pardi, Hogan, Porter, & Weissman, 2018) attributes that make them an appealing alternative to live-attenuated or inactivated vaccines. The use of this emerging technology, delivered by lipid nanoparticles (LNPs) to produce the antigen directly inside the cell,(Schlake, Thess, Fotin-Mleczek, & Kallen, 2012) has been approved to treat COVID-19 infection.(Oliver et al., 2020a, 2020b) This is particularly exciting since the results have so far shown that these vaccines are highly effective against preventing mild to severe COVID.(Mahase, 2020a, 2020b; Wendler, Ochoa, Millum, Grady, & Taylor, 2020) It is important to note that, in nearly all cases, these vaccines require constant refrigeration, and the LNP-based mRNA vaccines require even more stringent cooling with freezing temperatures required for shipping. This required cooling makes the delivery of vaccines expensive, and the cost of keeping them cold can account for as much as 80% of the total cost of the immunization.(Ashok, Brison, & LeTallec, 2017; Bogataj, Bogataj, & Vodopivec, 2005; Karishma, Donna, Timothy, & Neena, 2012; Setia et al., 2002; Welch et al., 2018) This instability has made controlled release strategies for delivery of LNP-based vaccines difficult, though emerging approaches using metal-organic frameworks have shown considerable promise.(Fabian C. et al., 2021) The ideal 21st-century vaccine should be safe,

patient-friendly, and stable enough such that it can be stored at ambient conditions and provide long-term immunity after one administration. Biomaterials have been investigated for the last three decades as strong candidates to eliminate the need for booster shots and improve proteins' stability. This overview will focus on biomaterials that provide sustained release *in vivo* for vaccines against infectious disease. Several reviews focus more specifically on cancer,(Abdou et al., 2020; J. Li et al., 2020; Yan et al., 2020; Yuan, Liu, Wang, Sun, & Chen, 2020; Zhang, Billingsley, & Mitchell, 2018; Y. Zhao, Guo, & Tang, 2018) which we will not discuss. Several excellent reviews are available that go into depth on particular types of biomaterials, and we have made an effort to direct readers to reviews as appropriate.

[2. TYPES OF BIOMATERIALS]

Biomaterial technologies offer many advantages including biocompatibility, tuneable immunogenicity, low reactogenicity and chemical stability over different classes of vaccine delivery systems. For the past three decades biomaterial-based technologies such as synthetic and natural polymers, lipids, crystalline scaffolds, microneedles, and other particle-carriers have rapidly emerged in order to improve vaccine efficacy, safety and stability.(Shen et al., 2018; Uppu et al., 2020; J. Yang et al., 2015) Biomaterials permit a design strategy that can combine an antigen, adjuvant, and growth factors into a single particle, which can provide protection, enhance immune activation, control targeted delivery, minimizing number of injection (dose), and reduce systemic and local toxicity.(Eric M. Bachelder et al., 2008; Elmowafy et al., 2019; Luzuriaga, Berry, Reagan, Smaldone, & Gassensmith, 2018; Sahdev et al., 2014) Several biomaterials have been developed in the nano and micron-size, but, from a long list of available biomaterials, only a few offer sustained release properties. In this section, we will discuss biomaterials with excellent biocompatibility, biodegradability, and easily functionalized that make them ideal candidates for sustained release vaccine delivery system.

[2.1 Synthetic biodegradable polymers]

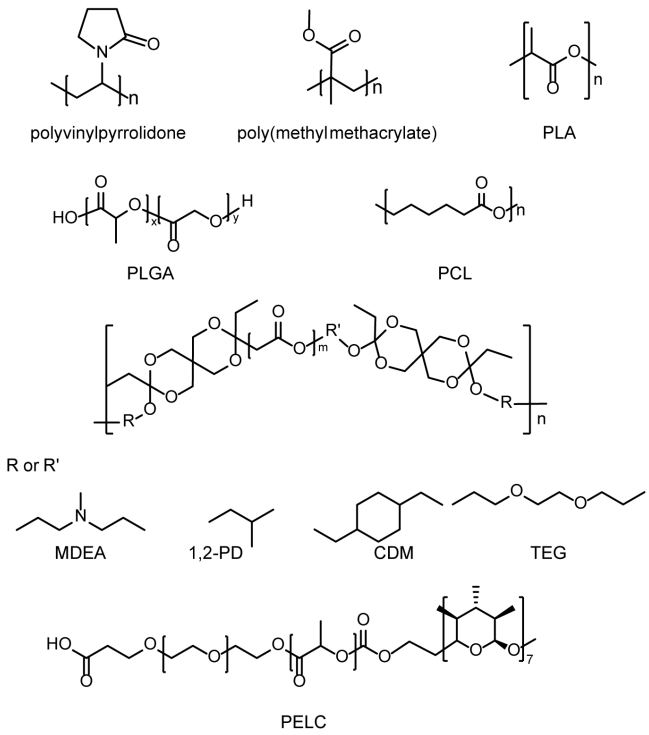


Figure 2: Chemical structures of commonly used synthetic biodegradable polymers used in vaccines.

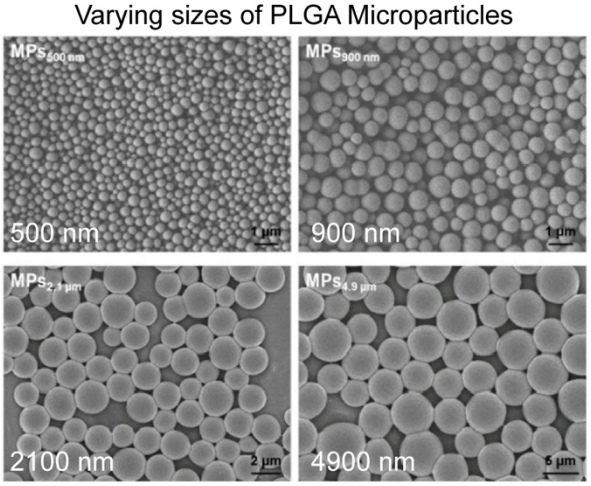


Figure 3: SEM images of different PLGA particle sizes tested by the Ma lab to determine the effects it has on activating the immune system. Reprinted from reference (Jia et al., 2017) with permission from American Chemical Society Copyright 2017.

Synthetic polyesters, including poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polyurethane (PU), and poly(ε-caprolactone) (PCL) are the most widely studied biodegradable polymers in the biomedical and vaccine field—examples of biodegradable polyesters are shown in **Figure 2**.(C.-H. Huang et al., 2016; Lou et al., 2009; Men, Tamber, Audran, Gander, & Corradin, 1997; Sah, Toddywala, & Chien, 1995; J. Singh, Pandit, Bramwell, & Alpar, 2006; Su, Kim, Kim, Hammond, & Irvine, 2009; C. Wang et al., 2004) These polyesters are synthesized either by condensation or ring-opening polymerization and are degraded by hydrolysis of their ester backbones *in vivo* over a period

that is determined by the polymer's composition. Several research reports focusing on these polymers in vaccine delivery are listed in **Table 1**. These polymers have high biocompatibility, tunable hydro/lipophilicity, high antigen loading, and sustained cargo release for *in vivo* applications(Allahyari & Mohit, 2016; Bose et al., 2019; Sahdev et al., 2014). Further, they can be synthesized from nano to micron sized and with various surface chemistries to provide selective cell targeting delivery (*e.g.* to APCs) and showing switchable and stimuli-responsive behavior in cargo release.(Gu et al., 2019; Morachis, Mahmoud, & Almutairi, 2012; Pawar, Mangal, Goswami, & Jaganathan, 2013) The synthetic flexibility of polyester-based vaccine platforms for antigen encapsulation (*e.g.* single and double emulsion solvent evaporation, nanoprecipitation, and spray drying) and administration route (*e.g.* dermal, intranasal and subcutaneous) provides a selection of formulations that can enhance the immune response activation. Among the polyester materials, PLGA copolymers are very well represented—generally recognized as safe by the FDA—sustained release vaccine delivery vehicles for both antigen and adjuvant thanks to their excellent safety profile.(Lü et al., 2009; Silva, Soema, Slütter, Ossendorp, & Jiskoot, 2016) PLGA is one of the most studied biomaterials effects of surface charge,(Avgoustakis, 2004; Oyewumi, Kumar, & Cui, 2010) injection interval,(Shi et al., 2002) and administration route,(Igartua et al., 1998) have been well characterized. Therefore, it is interesting that that particle size was comprehensively investigated only recently by the Ma lab.(Jia et al., 2017) PLGA (molar ratio for lactide/glycolide = 75:25, Mw ≈ 13000 Da) particles were formulated using an oil in water technique to obtain particle sizes at 500 nm, 900 nm, 2.1 μm and 4.9 μm, all having a similar negative surface charge (**Figure 3**). Each particle was mixed with 25 μg of ovalbumin and injected intramuscularly twice at two-week intervals. While each size showed good cell viability, uptake, and overall sound immune activation compared to the antigen alone, the 900 nm particle proved to perform the best in producing antibodies and cytokines compared to the other particle sizes. These studies show that many variables contribute to immune activation, including surface charge, dosage, the interval of injections, molecular weight, polymer ratio, and particle size. Despite the successful immunization seen *in vivo*, PLGA particles and other polyesters face several significant limitations, including the production of a local acidic environment following hydrolysis and synthetic conditions that use organic solvents and high temperatures. These limitations can lead to protein denaturation, reduction in encapsulation efficiency, and potential formulation bottlenecks if organic solvent must be removed from the formulated polymer. To overcome these limitations, the Pokorski lab used melt-extrusion—a technique mainly used by the plastic industry to melt and form thermoplastics.(Repka et al., 2012) In the last two decades melt extrusion has been used in the pharmaceutical industry to form slow release formulations of small molecule drugs. In 2017, the Pokorski lab demonstrated that mix powdered PLGA could be co-extruded with a virus called Qβ to make pellets that could be implanted.(Parker W. Lee et al., 2017) A key to this is that the group selected an especially robust virus that could survive the high temperature and sheer stress. In 2021, the same group went a step further and covalently attached antigens against human papillomavirus (HPC) on the surface of Qβ to create a slow-release PLGA implant as an alternative for vaccination.(Shao, Ortega-Rivera, Ray, Pokorski, & Steinmetz, 2021) In these experiments, they found a strong humoral response from a single administration compared to mice receiving three

injections.(Ortega-Rivera, Pokorski, & Steinmetz, 2021) While this technique is limited to vaccine platforms that are capable of lyophilization and elevated temperature without losing structure, the results were nevertheless very promising.

Table 1: Common sustained release biodegradable biopolymers. N/D means the study did not discuss.

Material	Antigen	Properties	Ref
poly(ortho ester) microspheres	DNA plasmid (pCIneo-hsp65-p1) and β-galactosidase	**Particle Size**: 5 μm **Antigen Dose**: 45 μg of DNA per μg of polymer. **Adjuvant**: None **Key Result**: Promotion of primary/secondary cellular and humoral immunogenicity as well as tumor growth suppression.	(C. Wang et al., 2004)
PLGA–PCL blend and copolymer	Diphtheria toxoid (DT)	**Particle Size**: 250-270 nm **Antigen Dose**: 5 μg of DT either intramuscular or intranasal immunization **Adjuvant**: Polymer hydrophobicity is considered to play a pivotal role. **Key Result**: Increased DT specific IFN-γ production compared to free polymer constituents.	(J. Singh et al., 2006)
Poly(methyl methacrylate) (PMMA)	CRT-E7 DNA	**Particle Size**: 460±160 nm **Antigen Dose**: 2 μg of CRT-E7 plasmid **Adjuvant**: None **Key Result**: High level stimulation of TNF-α production and antitumor protection.	(Lou et al., 2009)
Layer-by-layer (LbL) of cationic poly(β - amino ester) films	Ovalbumin	**Particle Size**: N/D (not discussed) **Antigen Dose**: 5 μg/cm² of film **Adjuvant**: CpG **Key Result**: Up-regulation of the cell-surface activation markers CD40, CD86, and MHC II	(Su et al., 2009)
PELC	Ovalbumin	**Particle Size**: ~150 nm **Antigen Dose**: 10 μg of OVA **Adjuvant**: Considered PELC as adjuvant **Key Result**: Enhanced antigen-specific T-cell responses and enhanced APC recruiting at the injection site.	(C.-H. Huang et al., 2016)
Glycol chitosan coated PLGA (GC-PLGA)	Hepatitis B surface Antigen (HBsAg)	**Particle Size**: 164-181 nm **Antigen Dose**: 10 μg of HBsAg **Adjuvant**: Considered PLGA derivatives as adjuvants **Key Result**: Augmented systemic and mucosal immune response	(Pawar et al., 2013)
PLA microspheres coated with cationic polymers (*e.g.* chitosan)	HBsAg	**Particle Size**: 800-835 nm **Antigen**: 4 μg of HBsAg **Adjuvant**: Polymer matrix considered as immunopotentiator **Key Result**: Enhanced antigen uptake, adsorption, and augmented humoral/cellular immunogenicity	(X. Chen et al., 2014)
Crosslinked poly(methacrylic acid) hydrogel capsules	Ovalbumin	**Particle size**: 1 μm and 500 nm capsules **Antigen Dose**: 7 μg of OVA **Adjuvant**: None **Key Result**: Increased CD4 and CD8 proliferation in vivo	(Sexton et al., 2009)
PELC	Ovalbumin	**Particle Size**: 300-500 nm **Antigen Dose**: 10 μg of OVA **Adjuvant**: PELC double emulsion considered as immunopotentiator **Key Result**: Enhanced $CD11c^+$ LN cell uptake and augmented antigen-specific IgG antibody production.	(C.-H. Huang, Huang, & Huang, 2019)
PELC-bioresorbable polymer/Span®85/squalene	Inactivated H5N1+CpG	**Particle Size**: 400-500 nm **Antigen Dose**: 0.5 or 5 μg of inactivated H5N1 **Adjuvant**: CpG **Key Result**: Augmented antibody count compared to alum-adjuvanted counterpart.	(M.-H. Huang et al., 2010)
Hydrophilic polymer solutions	Influenza strains (*e.g.* A/H1N1)	**Particle Size**: N/D **Antigen Dose**: 4.5 μg of hemagglutinin **Adjuvant**: Xanthan gum, cationic lipid, and poly-$_L$-arginine. **Key Result**: Incorporation of influenza vaccine into nasal inserts while conserving intact hemagglutinin-specific activity.	(Bertram, Bernard, Haensler, Maincent, & Bodmeier, 2010)
Cationic pentablock copolymers	Ovalbumin	**Particle Size**: N/D **Antigen Dose**: 100 μg of OVA **Adjuvant**: Pentablock copolymers considered as adjuvants. **Key Result**: Sustained antigen release in vivo (Depot effect) and high antibody titers compared to controls.	(Adams, Haughney, & Mallapragada, 2015)

PVP based hydrogen bonded polymeric microparticles	Ovalbumin	**Particle Size**: 1 μm in diameter **Antigen Dose**: 50 μg of OVA **Adjuvant**: None. **Key Result**: Induction of humoral and antigen-specific immunogenicity	(Dierendonck et al., 2014)
PLGA/ polylactide blend particles	Tetanus toxoid (TT)	**Particle Size**: mean size of 3 μm and 630 nm **Antigen Dose**: 30 μg of TT **Adjuvant**: Considered polymer blend as adjuvant **Key Result**: Anti-TT antibody titer presence in immunized rats for > 5 months after injection	(Raghuvanshi, Singh, & Panda, 2001)
Polymeric bioresorbable amphiphiles	Ovalbumin	**Particle Size**: 100 nm **Antigen Dose**: 10 μg of OVA **Adjuvant**: Polymeric matrix considered as adjuvant **Key Result**: Depot effect generated from slow polymer degradation in vivo and enhanced antigen-specific antibody titer count when compared to naked OVA	(C.-Y. Huang et al., 2018)
Acid-degradable protein-loaded polymer particles	Ovalbumin	**Particle Size**: 250-500 nm **Antigen Dose**: 50 μg of OVA **Adjuvant**: Hydrophilicity of polymer matrix considered as immunopotentiator. **Key Result**: Enhanced MHC class I presentation, tumor immunity in murine models, and prolonged mice survival rate after challenge experiment.	(Standley et al., 2004)
Redox-responsive hyperbranched poly(amido amine) and polymer dots	Ovalbumin	**Particle Size**: ~ 180 nm **Antigen Dose**: ~ 30 μg of ovalbumin **Adjuvant**: None **Key Result**: The polymer systems produce higher OVA IgG2a/IgG1 antibody ratio, increase production of cytokine, and improved activation of CD4+/CD8+ T cells levels compared to ova alone	(Lv et al., 2017)
PLGA	Ovalbumin	**Particle Size**: 200 – 300 nm **Antigen Dose**: ~ 50 μg of ovalbumin **Adjuvant**: Freund complete adjuvant **Key Result**: Goal of the study was to determine how charge and antigen loading mode affects the immune response. Antibodies and the activation of immune cells were investigated	(Gu et al., 2019)
	Bovine serum albumin (model antigen)	**Particle Size**: 10 μm **Antigen Dose**: ~ 70 or 400 μg of BSA depending on formulation **Adjuvant**: Mentions PLGA is considered an adjuvant **Key Result**: Determined PLGA formulations with pulsatile release that could mimic common vaccine dosage regimen. Single injections of PLGA formulation Similar antibody production was generated compared to three bolus injections.	(Guarecuco et al., 2018)
	Hepatitis B surface antigen (HBsAg)	**Particle Size**: 25 – 45 μm **Antigen Dose**: 3 – 12 μg of HBsAg **Adjuvant**: Alum **Key Result**: Single injection can provide similar antibody response compared to two injections	(Shi et al., 2002)
	OVA and influenza split vaccine antigen (H5N1)	**Particle Size**: 500, 900, 2100, and 4900 nm **Antigen Dose**: 25 μg of OVA and 3 μg Influenza Adjuvant: IMQ **Key Result**: They showed that different sizes of PLGA can affect the the immune response. Particle size of 900 nm had a stronger humoral response based on antibodies and cellular response based on cytokines	(Jia et al., 2017)
	Denatured insulin	**Particle Size**: ~ 2 μm **Antigen Dose**: 20 mg of insulin **Adjuvant**: CpG **Key Result**: Used the immune system to prevent Type 1 Diabetes. PLGA was used to release antigens slowly and a commercial hydrogel (PuraMatrix) was used to recruit immune cells. The vaccine protected 40% of mice from becoming diabetic.	(Yoon et al., 2015)
	MVFMF2 (peptide vaccine for human T-lymphotropic virus type 1)	**Particle Size**: ~ 10 μm **Antigen Dose**: 1 mg of peptide in rabbits **Adjuvant**: nor-MDP **Key Result**: The peptide encapsulated within PLGA produced high antibody titers after a single injection without the use of an adjuvant	(Frangione-Beebe, Rose, Kaumaya, & Schwendeman, 2001)

	Plasmid expressing HBV small envelope antigen	**Particle Size**: ~ 2 – 6.5 μm **Antigen Dose**: 20 or 100 µg of PLGA containing plasmid **Adjuvant**: CpG **Key Result**: Mice immunized with PLGA containing plasmid had an increase in $CD11c^+$ cells, higher levels of antibodies, IFN-γ secretion, and cytotoxic T lymphocytes, which provided protection against HBsAg-expressing tumors compared to naked DNA.	(X. He et al., 2005)
	Inactivated AIV	**Particle Size**: ~ 750 nm **Antigen Dose**: 20 µg of AIV **Adjuvant**: CpG **Key Result**: Promotion of Mucosal and systemic immunogenicity from PLGA nanoparticles measured by the increase in antibody production	(Alkie, Yitbarek, Taha-Abdelaziz, Astill, & Sharif, 2018)
	Ovalbumin	**Particle Size**: ~ 250 nm **Antigen Dose**: 100 µg of ovalbumin **Adjuvant**: alum **Key Result**: PLGA is compared to Lipsome at the same size. PLGA formulation that released antigens slower produced a strong cellular response and reduced CFU compared to Liposome and alum	(Demento et al., 2012)
	HIV-1 peptide immunogen (200M)	**Particle Size**: ~ 1 – 3 μm **Antigen Dose**: 300 – 750 μg peptide in guinea pigs **Adjuvant**: alum **Key Result**: To determine the safety, toxicity, and pyrogenicity of the PLGA microparticle. The microparticles showed high levels of serum IgG and neutralizing antibodies against HIV.	(O'Hagan et al., 1995)
	Tetanus toxoid (TT)	**Particle Size**: ~ 10 – 50 μm **Antigen Dose**: 15 µg TT **Adjuvant**: alum **Key Result**: TT was radiolabeled (^{14}C) to monitor the release from PLGA or alum. They showed that alum did not act as a depot when injected, whereas PLGA forms a depot for approximately 1 month at the injection site	(Gupta, Chang, Griffin, Rivera, & Siber, 1996)
	rgp120 (subunit protein for HIV-1)	**Particle Size**: ~ 40 – 50 μm **Antigen Dose**: 60 µg guinea pigs & 150 – 300 µg for baboons **Adjuvant**: QS-21 or alum **Key Result**: Different PLGA ratios were developed to deliver antigens as a pulse release to eliminate the need for multiple immunizations. They showed that continuous release had less antibody production and decayed more rapidly than pulsatile	(Cleland et al., 1998)
	rCDPK6 & rROP18 (recombinant protein in *Toxoplasma gondii*)	**Particle Size**: N/D **Antigen Dose**: 10 µg of each protein **Adjuvant**: Montanide™ ISA 206 VG **Key Result**: The recombinant proteins induced a Th1 biased immune response, higher antibodies, and high levels of IFNγ against the intracellular parasite *T. gondii*. This led to an improvement in survival for mice injected with rROP18 + PLGA	(N.-Z. Zhang et al., 2016)
	Hepatitus B antigen (HBsAg)	**Particle Size**: ~ 4 μm **Antigen Dose**: 7.5 μg of HBsAg **Adjuvant**: Alum **Key Result**: The characteristics and degradation of the polymer was considered in the study for developing a single dose vaccine against hepatitis B. A single injection produced serum antibodies comparable to three injections of alum with HBsAg	(Feng et al., 2006)
	CAMP factor (conserved virulent protein on *streptococcus agalactiae*)	**Particle Size**: 1 – 5 μm **Antigen Dose**: 10, 100, or 1,000 μg of CAMP factor **Adjuvant**: None, but consider PLGA to be adjuvant **Key Result**: Developed a single dose vaccine against S. agalactiae. The mice injected with CAMP encapsulated within PLGA had a greater antibody response and higher survival rate when challenged	(G. Liu et al., 2017)
	Qβ	**Particle Size**: Implant **Antigen Dose**: 150 μg for implant and 50 ug for each injection **Adjuvant**: None	(Parker W. Lee et al., 2017)

		Key Result: Developed a single dose vaccine using melt extrusion. The mice injected with Qβ produced antibodies similar to three subcutaneous injected mice.	
	Qβ-L2 (a peptide epitope from HPV)	**Particle Size**: Implant **Antigen Dose**: 100 μg for implant, 30 μg for each injection **Adjuvant**: None **Key Result**: Conjugated L2 to the surface of Qβ and lyophilized. The lyophilized Qβ-L2 was mixed with powdered PLGA for melt extrusion to produce pellets. The pellets were implanted into mice and produced antibodies similar to mice receiving three subcutaneous injections. In addition, they did an *in vitro* study to show that the sera of the implanted mice prevented infection more efficiently compared to mice implanted with the peptide alone	(Shao et al., 2021)
	Recent PLGA in vaccine delivery reviews		(Lagreca et al., 2020; P. W. Lee & Pokorski, 2018; Lofano, Mallett, Bertholet, & O'Hagan, 2020)

[2.2 Polysaccharides]

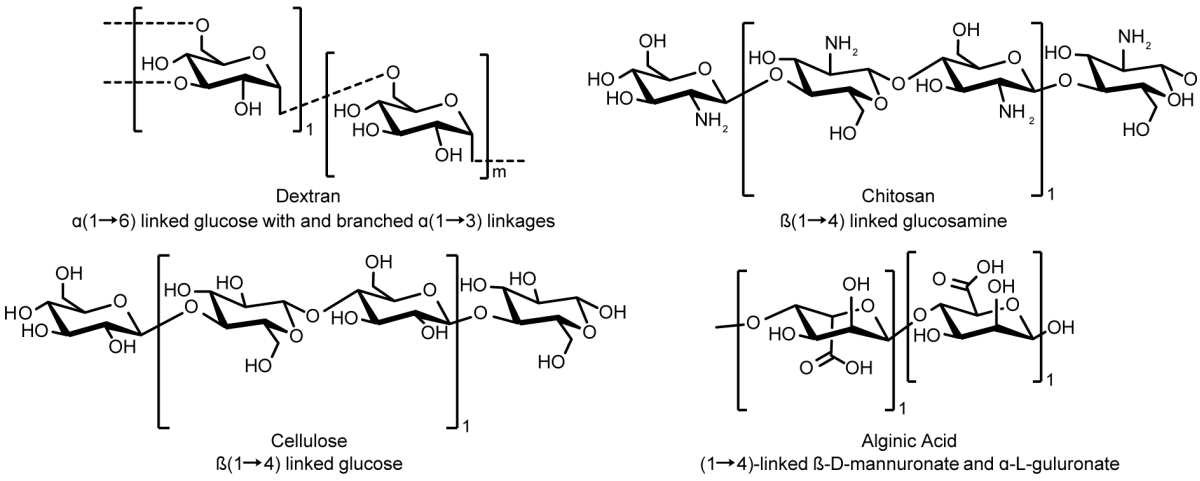


Figure 4: Chemical structures of commonly used polysaccharides used in vaccines.

Polysaccharides, or glycans, are carbohydrate polymers composed of monosaccharide subunits linked via glycosidic bonds, some examples are shown in **Figure 4**. Polysaccharides are a very broad class of compounds found in plants, bacteria, fungi, and even mammalian cells. They are typically extracted and processed from biomass as complex polymers differing in the type of saccharides used and the nature of their linkages. Polysaccharides, such as alginate,(Nagpal, Kesarwani, Sahu, & Upadhyay, 2019; Sarei, Dounighi, Zolfagharian, Khaki, & Bidhendi, 2013) cellulose,(H. Wang & Roman, 2016) chitosan,(Harde, Agrawal, & Jain, 2014; Van Der Lubben, Verhoef, van Aelst, Borchard, & Junginger, 2001) dextran,(E. M. Bachelder, Beaudette, Broaders, Dashe, & Frechet, 2008; N. H. Chen et al., 2018; Gallovic et al., 2016; Moore et al., 2020) hyaluronic acid,(Bussio, Molina-Perea, & González-Aramundiz, 2019) and starch,(Rydell, Stertman, & Sjöholm, 2005) have been explored in controlled

vaccine delivery system and several examples of these efforts are provided in **Table 2**. Significant properties that have attracted researchers to natural materials are their good water solubility, ease of preparation, and simple chemical modification. Additionally, these biologically sourced particles have led to effective oral and intranasal vaccine administration that have advantages over parenteral injection, such as patient compliance and convenience of at-home self-care vaccination.(Harde, Agrawal, & Jain, 2015; Walke et al., 2018) Among the natural materials, chitosan has been studied heavily and evaluated in humans for the potential use in vaccination against infectious diseases owing to its high safety and ease of clearance.(Jabbal-Gill, Watts, & Smith, 2012; B. Singh et al., 2018; Xing et al., 2018) Twenty years of research has shown that chitosan significantly enhances APC uptake through electrostatic interaction owing to its positively charged nitrogens and bendability with other biomaterials (e.g. PLGA), synergistically enhance immune activation.(Bobbala, Gibson, Gamble, McDowell, & Hook, 2018; Gordon et al., 2012; Highton, Kojarunchitt, Girardin, Hook, & Kemp, 2015) The mechanism by which chitosan enhances an immune response was comprehensively explored in 2016 by the Xu Lab.(Z.-B. Wang et al., 2016) For this study, they used hepatitis B antigen and mixed it with acid-soluble chitosan for *in vitro* and *in vivo* adjuvant mechanistic evaluation. Their study showed that the intranasal delivery of chitosan mixed with antigens involves a depot effect produced by insoluble chitosan at physiological pH found in the extracellular fluid. In contrast, chitosan can facilitate endosomal escape of the encapsulated antigen, which is schematically shown in **Figure 5**. This was corroborated *in vitro* with higher dendritic cell activation and *in vivo* with improved cell mediated activation. Again, this study shows the importance of understanding the biomaterial properties—as it is possible that adjuvants may not be needed for some of these biomaterials, and some are starting to consider biomaterials to have adjuvant properties.

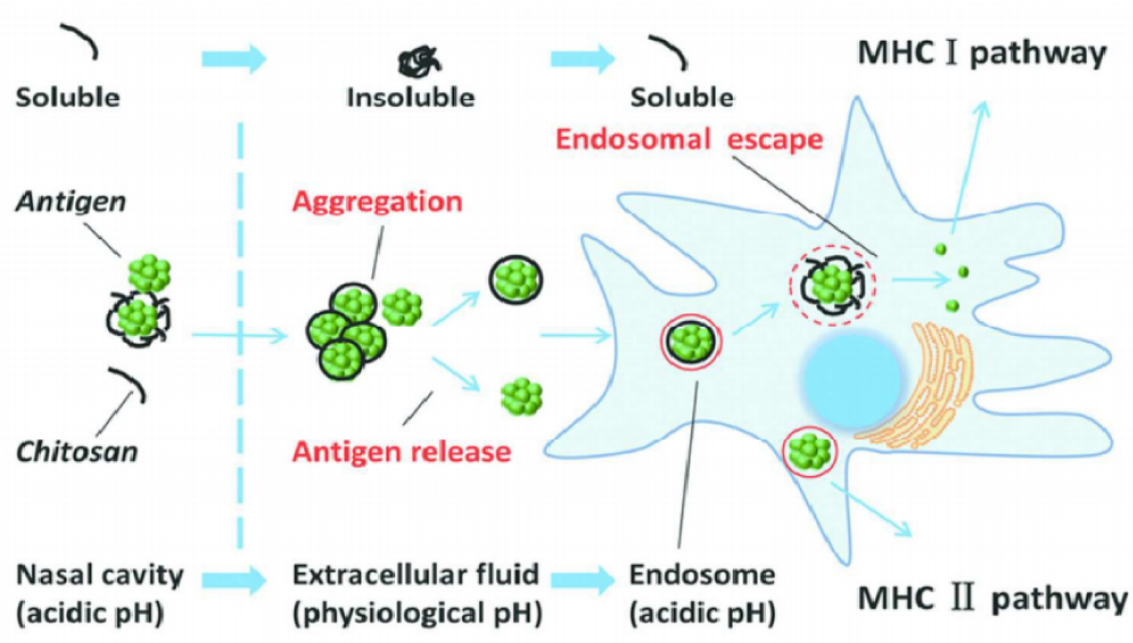


Figure 5: The Xu lab investigate chitosan with antigen to evaluate it as a potential adjuvant and determine the mechanism of immune activation. Republished with permission from Royal Society of Chemistry for reference (Z.-B. Wang et al., 2016); permission conveyed through Copyright Clearance Center, Inc.

Table 2: Common sustained release polysaccharide biopolymers. N/D means the study did not discuss.

Material	Antigen	Properties	Ref
Alginate particles	Diphtheria toxoid (DT)	**Particle Size** :< 100nm **Antigen Dose**: Two dose of 10Lf/ml DT (0.5 ml) **Adjuvant**: Toxoid **Key Result**: Extended sustained release of Diphtheria toxoid *in vitro* and invoke highest humoral immune response in guinea pig model than conventional counterpart	(Sarei et al., 2013)
	Mycobacterium particles (Bacille Calmette–Guérin)	**Particle Size**: 2-4 µm **Antigen Dose**: 2000-3000 bacilli **Adjuvant**: None/ but considered BCG/MIP provide adjuavant eddect **Key Result**: Developed superior immune response and higher protection in mice than the liquid aerosol against H37Rv infection	(Nagpal et al., 2019)
	Pasteurella multocida	**Particle Size**: 6 µm **Antigen Dose**: 2.08 × 10^7 cells **Adjuvant**: None/ used oil adjuvant as a control **Key Result**: high antigen loading efficiency and long physiochemical stability along with sustained release profile over 30 days in vivo	(Nimtrakul, Atthi, Limpeanchob, & Tiyaboonchai, 2015)
Chitosan particles	Hepatitis B surface antigen (HBsAg)	**Particle Size**: 397.1 nm **Antigen Dose**: 2µg of HBaAg **Adjuvant**: None **Key Result**: Formulation enhanced uptake of antigen by DC and promoted their maturation , also showed an augmentation of cellular and mucosal immunity	(Z.-B. Wang et al., 2016)
	BSA	**Particle Size**: between 150 – 200 nm **Antigen Dose**: 50 µg/mice **Adjuvant**: None **Key Result**: High systemic (serum IgG titer), mucosal (secretory IgA) and cell-mediated (IL-2 and IFN-g) immune responses	(Harde et al., 2014)
	Tetanus Toxoids (TT)	**Particle Size**: 123 nm **Antigen Dose**: immunized twice with 5 Lf TT **Adjuvant**: None but stable glucomannosylated chitosan NPs considered as an adjuvant **Key Result**: High cellular uptake and in vitro stability, Higher humoral, mucosal, and cellular immune response compare to commercial TT vaccine	(Harde et al., 2015)
	Ovalbumin	**Particle Size**: 280 nm **Antigen Dose**: 20 µg OVA **Adjuvant**: N-trimethyl chitosan **Key Result**: Increased *in vitro* DC uptake and *in vivo* IgG antigen specific titers	(Slütter et al., 2010)
	GRA-1 pDNA	**Particle Size:** 400 nm **Antigen Dose**: 50 µg **Adjuvant**: None **Key Result**: High antigen specific antibody counts *in vivo*	(Bivas-Benita et al., 2003)
	rHBsAg	**Particle Size**: 200 nm **Antigen**: 10 µg **Adjuvant**: None but polysaccharide chitosan considered as an adjuvant **Key Result**: High stability and sustained release , Enhanced anti-HBsAg IgG count compared to alum-absorbed counterpart	(Prego et al., 2010)
	Tetanus, diphtheria, and divalent toxoids	**Particle Size**: 1-20 µm **Antigen Dose**: 0.5 Lf/6 µL **Adjuvant**: None **Key Result**: High antibody count after subcutaneous administration of antigen loaded microspheres	(Hashem, Fahmy, El-Sayed, & Al-Sawahli, 2013)
	Bovine serum albumin	**Particle Size**: 125-203 nm **Antigen Dose**: immunized twice with 20 µg **Adjuvant**: Alum and CpG as a control groups **Key Result**: Antigen loaded aminated and aminated plus thiolated chitosan showed a high protein loading efficiency and biocompatibility, high levels of systemic antibodies (IgG, IgG1 and IgG2a) and Th1/Th2 immune response	(Sinani et al., 2019)

	viz. Diphtheria toxoid, whole cell pertussis antigens and tetanus toxoid	**Particle Size**: < 2 µm **Antigen Dose**: 30 unit/ml **Adjuvant**: None **Key Result**: high cellular uptake and negligible *in vitro* toxicity. Enhanced systemic (IgG) and mucosal (sIgA) immune response	(Walke et al., 2018)
	Ovalbumin loaded cationic nanosized liposomes and cubosome	**Particle Size**: 200 or 700 nm **Antigen Dose**: 20 µg OVA **Adjuvant**: Quil A **Key Result**: Induction of cluster of differentiation $C8^+$ and $CD4^+$ T-cell proliferation and the production of interferon (IFN)-γ and OVA-specific antibody	(Gordon et al., 2012)
Chitosan gel-based formulations	Ovalbumin	**Particle Size**: N/D **Antigen Dose**: 20 µg of OVA **Adjuvant**: Quil-A **Key Result**: Production of ovalbumin-specific memory $CD8^+$ T cells and protection from subcutaneous melanoma challenge 30 days later	(Highton et al., 2015)
	Ovalbumin	**Particle Size**: 325 nm **Antigen Dose**: 20 µg of OVA **Adjuvant**: MPL, QA **Key Result**: Strong, long lasting, cellular and humoral responses and significantly longer survival time for tumor bearing mice.	(Bobbala et al., 2018)
Poloxamer 407-chitosan (CP) grafted copolymer loaded in PLGA	Matrix protein 2 (M2e)	**Particle Size**: 0.2 – 3 µm **Antigen Dose**: M2e (10 µg) **Adjuvant**: cGAMP **Key Result**: Induced robust humoral and cellular immune response, cross reactivity against multiple flu viral strains and tunable delivery profiles of antigen and adjuvant with different formulations	(N. H. Chen et al., 2018)
Acetalated dextran	Ovalbumin	**Particle Size**: small (0.67 × 10.2 μm^2), medium (1.28 × 20.7 μm^2), and large (5.67 × 90.2 μm^2) **Antigen Dose**: (0, 0.02, 0.1, 1, and 10 µg/mouse) **Adjuvant**: resiquimod **Key Result**: Stimulated humoral response for blank MCand effective Th1 -skewed immune response for small and medium sized MC along with the adjuvant	(Moore et al., 2020)
Dextran	Ovalbumin	**Particle Size**: 200 – 300 nm **Antigen Dose**: 200 µL using 50 µg OVA **Adjuvant**: Alum **Key Result**: Increased cellular and humoral response compared to alum-adjuvanted counterpart	(Gallovic et al., 2016)
Acid-sensitive silylated polysaccharides (dextran)	Ovalbumin	**Particle Size**: 236 nm **Antigen Dose**: 30 µg of OVA **Adjuvant**: None **Key Result**: Both MHC-I and MHC-II antigen presentation. Up-regulation of MHC, co-stimulatory molecules and cytokines. Antigen-specific CD4+ and CD8+ T-cell responses, the production of antigen-specific IgG antibodies and the generation of memory T cells	(L. Liu et al., 2016)
Hyaluronic acid-decorated cationic lipid–PLGA hybrid nanoparticles	HBsAg	**Particle Size**: 100 nm **Antigen Dose**: 1 µg of HBsAg **Adjuvant**: considers delta inulin an adjuvant **Key Result**: Enhanced the production of anti-HBsAg immunoglobulin compared to HBsAg alone or with HBsAg combined with GI. Induced antigen specific CD4 and CD8 T-cell responses	(Cooper & Petrovsky, 2011)
	Recent polysaccharides in vaccines reviews		(Eric M. Bachelder, Pino, & Ainslie, 2017; Moran, Turley, Andersson, & Lavelle, 2018; Sun et al., 2018; D. Y. Zhao et al., 2018)

[2.3 Others]

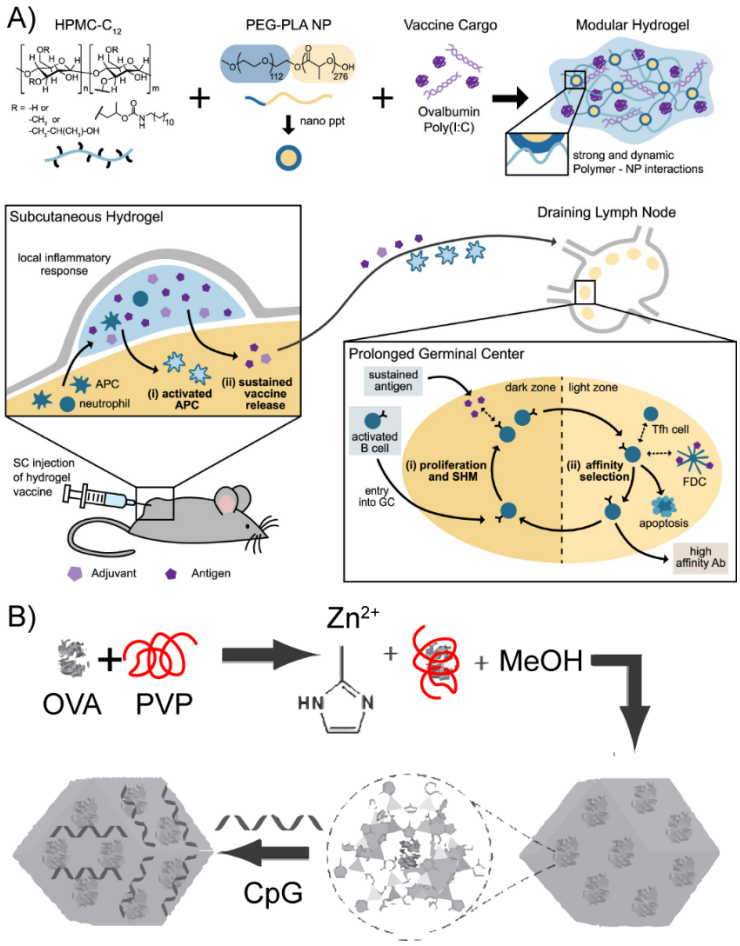


Figure 6: A) The Appel Lab formulated different hydrogels to tune the release and determine the activation of the germinal center in the draining lymph node. Reprinted from reference (Roth et al., 2020) with permission from American Chemical Society Copyright 2020. B) Coprecipitation begins by adding ovalbumin into water containing PVP. Zinc and 2-methylimidazole in methanol are mixed together and placed in an ultrasonic bath for 10 min to react. After 10 minutes, PVP + ovalbumin is added and sonicated for an additional 3 minutes. The resulting solution is washed with methanol and centrifuged—the washing is done three times. CPG is later mixed and adsorbs to the surface to make ova@ZIF-8-CPG. Reprinted with permission from John Wiley and Sons for reference (Y. Zhang et al., 2016) Copyright 2016.

Biocompatible hydrogels, proteins, and more recently metal-organic frameworks (MOFs) are other biomaterials that have been used for drug delivery.(A. L. Z. Lee, Yang, Gao, Hedrick, & Yang, 2019; L. Liu et al., 2016; Maghrebi, Jambhrunkar, Joyce, & Prestidge, 2020; Yoon et al., 2015) Several research reports focusing on these biomaterials in vaccine delivery are listed in **Table 3**. A significant advantage of these materials is their ability to self-assemble with antigens under aqueous conditions and their availability for multiple and site-specific post-synthetic modifications.(Corthésy & Bioley, 2018; Salatin et al., 2016; N. Wang, Chen, & Wang, 2019) An advantage of hybrid or blended biomaterials is that formulations can be tuned beyond nano or microparticles or solid implants. For example, an excellent study by the Appel Lab investigated the encapsulation of ovalbumin and Poly(I:C), a toll-like receptor-3 agonist, using differently formulated hydrogels.(Roth et al., 2020) The hydrogels were composed of PEG-PLA nanoparticles and hydroxypropylmethylcellulose derivatives at different ratios. The focus of the paper was to show an improve immunogenic response compared to free ovalbumin and adjuvant,

showing the 2:10 ratio had slower delivery, prolonged germinal center activation (**Figure 6A**), and produced a better overall humoral response compared to the bolus and 1:5 ratio injections. While the toxicity, dosage, and length between each injection remain open questions, this work elegantly tied recent discoveries in formation of germinal centers with persistent immunity(Boopathy et al., 2019; Tam et al., 2016) and compellingly demonstrated a proof-of-principle approach.

MOFs have also recently emerged as effective antigen depots that impart thermal stability to entrapped proteins. A unique feature of MOFs is that, they are entirely crystalline metal-coordination polymers. The use of a metal center to bridge multiple ligands that connect to other metals and those in turn connects to yet more ligands creates a thermodynamically stable framework that can protect proteins from thermal denaturation. These metal-ligand interactions also offer kinetic lability, which permits complete dissolution into monomers in the presence of intensely competitive biological anionic metal binders like phosphate. While many MOFs use transition metals that would be difficult to translate, iron and zinc-based MOFs have found success in drug delivery and have recently been employed for vaccine delivery. (Miller et al., 2010)

In 2016, the Qu lab encapsulated ovalbumin within polyvinylpyrrolidone (PVP) and co-precipitated this composite material in methanol with zinc and methyl-imidazole, forming the MOF ZIF-8 around the PVP encapsulated ovalbumin (**Figure 6B** and **Figure 7A-B**).(Y. Zhang et al., 2016) They showed that less than 20% of antigen was released when incubated in pH 7.4 PBS buffer; however, they found that ~90 % of the antigen would be released after 2 h when the pH was lowered to 6.0, stating the antigen would only release after endocytosis. They injected mice subcutaneously and showed their system combined with the adjuvant CpG increased total anti-ova IgG and produced a balanced Th1/Th2 response

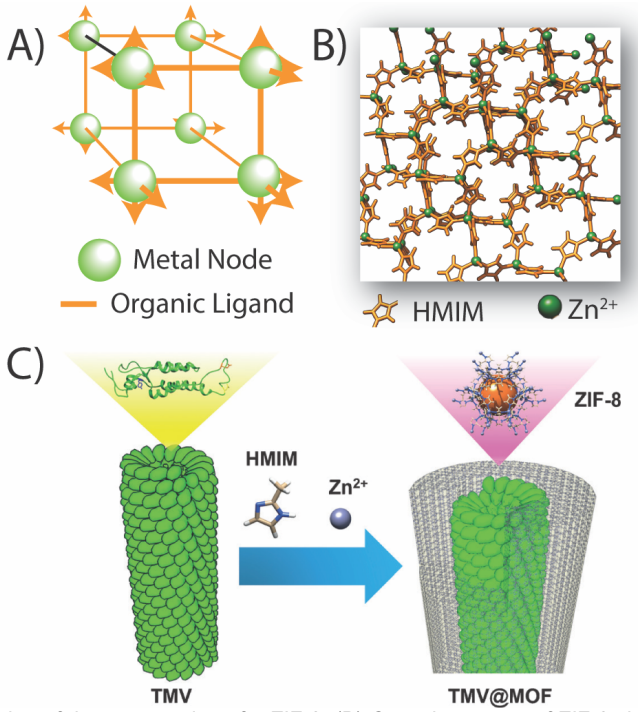


Figure 7: (A) Cartoon illustration of the construction of a ZIF-8. (B) Crystal structure of ZIF-8 showing cage and pores in the extended lattice and how they are connected by zinc and imidazole. (C) Conceptualization of the synthesis and product of a biomimetic mineralization process where the viral proteins from tobacco mosaic virus (TMV) triggers the growth and then results in its entrapment inside the framework (TMV@MOF).

compared to free ovalbumin + CpG. This was quite a groundbreaking discovery at the time, but the use of methanol to create ZIF-8 presents numerous issues as most proteins denature in methanol, which means proteins would have to be pre-encapsulated in a different polymer before encapsulation in ZIF. Our group overcame these drawbacks by using a method to grow ZIF-8 directly on the surface of viral nanoparticles in a 'biomimetic mineralization' process (**Figure 7C**).(Ellis et al., 2019; S. Li et al., 2018; S. Li et al., 2016; Riccò et al., 2018)

Biomimetic mineralization differs from coprecipitation used with PVP coated antigens because the polarized protein backbone and sidechains induce direct growth of the MOF under purely aqueous conditions. Further work by us and the Falcaro group found that endosomal uptake is not needed to degrade the ZIF coating, instead, the coating is degraded when incubated in serum because metal ions are extracted by serum proteins and biological inorganic salts (*e.g.* phosphate and acetate).(Luzuriaga, Benjamin, et al., 2019; Luzuriaga, Welch, et al., 2019) (Velásquez-Hernández et al., 2019) Luzuriaga *et al* showed that subcutaneous injection of ZIF coated viral nanoparticles indeed slowly release for 10 days and promote a robust humoral response in the absence of adjuvant.(Luzuriaga, Welch, et al., 2019) This study was interesting because they showed a more stable method to encapsulate antigens and demonstrated that the biomaterial could enhance the immune system without the need of the adjuvant. A more recent development has shown that biomimetic coating of ZIFs on lipid nanoformulations promote their stability for many months in the mail.(Fabian C. et al., 2021) This could have potential applications for stabilizing the newest lipid-based vaccines. However, ZIF-8 formulations have yet to be studied for how particle size, dosage, and surface modification affect immune activation. The dissection of these biomaterials and newly developed polymer systems that offer sustained release attributes needs to be further investigated, specifically on the tuning of size, charge, dosage, and frequency of injections the biomaterial itself has activation of the immune system.

Table 3: Other common sustained release biomaterials. N/D means the study did not discuss.

Material	Antigen	Properties	Ref
Self-healing polymer nanoparticle (PNP) hydrogel	Ovalbumin	**Particle Size**: N/D **Antigen Dose**: 100 µg of OVA **Adjuvant**: Poly(I:C) **Key Result**: 1000-fold increase in antigen-specific antibody affinity. Increased potency and durability of the humoral immune response	(Roth et al., 2020)
self-adjuvanted hydrogel	Ovalbumin	**Particle Size**: N/D **Antigen Dose**: 3 subcutaneous injection of 20 µg of OVA **Adjuvant**: considers the hydrogel to be an adjuvant **Key Result**: Did an *in vitro* study to show an increase in uptake by dendritic cells and the cytokines produced in the supernatant. They also showed an enhanced production of antibodies compared to mice injected with just ova or alum/ova.	(T. He et al., 2021)
Zeolitic imidazolate framework-8 (ZIF-8) over PVP composite	Ovalbumin	**Particle Size**: 200 nm **Antigen Dose**: 312.5 µg of OVA@ZIF-8-CpG **Adjuvant**: CpG **Key Result**: Induced strong humoral and cellular immune response by facilitating the co-delivery of OVA and CpG ODNs to the same APCs	(Y. Zhang et al., 2016)

ZIF-8	Tobacco Mosaic Virus, RNA plant virus	**Particle Size**: rod shape ~ 100 x 350 nm (Diameter x Length) **Antigen Dose**: 10 µg of TMV **Adjuvant**: None **Key Result**: Enhanced thermal and chemical stability of TMV within ZIF-8 (TMV@ZIF) and sustained release of TMV over the course of 14 days. Mice injected with TMV@ZIF produced more antibodies compared to TMV group	(Luzuriaga, Welch, et al., 2019)
Vitamin E-PEG-Vitamin E triblock 'ABA' hydrogel	Ovalbumin	**Particle Size**: N/D **Antigen Dose**: 200 µg of OVA **Adjuvant**: Alum **Key Result**: Increased survival (66.7%) compared to other formulations (12.5-50%) over 100 days in lymphoma metastasis mouse model. Mice vaccinated with hydrogel formulations showed an increased quantity of antibodies compared to solution formulations	(A. L. Z. Lee et al., 2019)
Ceramic nanoporous microneedle arrays(npMNA)	Ovalbumin specific peptides	**Microneedle Size**: 170 µm long, 36 needles per array for mice or 729 needles per array for human example **Antigen Dose**: 40 nmol $OVA_{257-264}$ **Adjuvant**: Agonistic anti-CD40 antibodies **Key Result**: Triggered antigen specific $CD8^+$ effector T cell response in *vivo* and the frequencies of induced IFN-g-specific effector CD8+ T cells were found to be comparable with those induced via conventional needle-syringe injection	(Boks et al., 2015)
Lyophilized RTA recombinant protein (RiVax)	Mutated ricin	**Particle Size**: N/D **Antigen Dose**: Volume corresponding to 1% of mice body mass (10 µL/g) **Adjuvant**: Alum **Key Result**: The vaccine on alum was as protective as 10-fold more vaccine without alum. Proven antigen protection for 12 months storage without refrigeration and efficacy with or without alum	(Smallshaw & Vitetta, 2010)
Metal microneedle patches	Seasonal influenza strains	**Microneedle Size**: 700 µm in length and 160 µm in width **Antigen Dose**: Microneedles coated with 0.4 µg of inactivated influenza virus. **Adjuvant**: None **Key Result**: Triggered a robust systemic and functional antibodies and provided complete survival after lethal dose challenging experiments similar to those from conventional intramuscular injection.	(Kim, Quan, Compans, Kang, & Prausnitz, 2010)
Lipid-based cubosomes	Ovalbumin	**Particle Size**: 260 to 350 nm **Antigen Dose**: 15 µg of Ova **Adjuvant**: Imiquimod and monophosphoryl lipid A (MPL) **Key Result**: Enhanced $CD4^+$, and $CD8^+$ T cell proliferation. Produced antigen specific IgG antibodies to alum and increased the production of production of Th1 type cytokine IFNγ. Also, efficient in antigen-specific cellular responses and equally as effective in generating humoral responses compared to the liposomes containing the same adjuvants.	(Rizwan et al., 2013)
Calcium phosphate nanoparticles	HSV-2 & EBV	**Particle size**: Less than 1000 nm **Antigen Dose**: 60 mg of HSV-2 protein **Adjuvant**: calcium phosphate (CAP) and Alum **Key Result**: Augmented a higher IgG2a antibody titers and a lower IgE response relative to the alum adjuvant and protection against live HSV-2 infection	(Q. He et al., 2000)
Silk Fibroin	CFT073 (epitopes for FimH & IutA)	**Particle size**: 180 nm **Antigen Dose**: three varying doses from 10 – 50 µg **Adjuvant**: Alum **Key Result**: The mice receiving epitopes within hydrogel produced the most IgG1, IgG2a, and IgA in sera. In addition, there was higher antibodies found in the urine and higher cytokine levels measured from splenocyte restimulation.	(Hasanzadeh et al., 2020)
Silk fibroin based solid pyramidal microneedle (MN) arrays	HIV-1 Env trimer	**Microneedle Size**: 250 µm at the base, 650 µm in height with a pitch of 250 µm **Antigen Dose**: 5 µg of trimer **Adjuvant**: TLR2 agonist pam3CSK4 and the TLR3 agonist polyI:C **Key Result**: Enhanced humoral immunogenicity. 1,300-fold higher serum MD39-specific IgG titer than the equivalent intradermal injections at week 13	(Boopathy et al., 2019)

[3. THE INTERACTION BETWEEN ANTIGEN AND BIOMATERIAL]

Weak immunogenicity and short-term stability are some limitations associated with subunit antigens, where a standard solution to overcome these is to use biomaterials as delivery/protection vehicles.(Reddy, Swartz, & Hubbell, 2006; Y. Wang, Deng, Kang, & Wang, 2018; Yenkoidiok-Douti & Jewell, 2020) Biomaterials can be a host that stabilizes antigens against denaturing conditions (*e.g.* absence of refrigeration) and can be tailored to achieve different release profiles (*e.g.* sustained and pulsatile), which can augment the desired immune activation, and in some cases, can provide lifetime immunity from a single dose.(Frangione-Beebe et al., 2001) However, a significant challenge associated with their usage is the formulation of antigen/biomaterial to achieve optimal therapeutic efficacy. **Figure 8** illustrates several antigen/biomaterial interaction approaches developed over the years. In this section, we discuss some of the forces driving such interactions, the methods to test the protein stability, and briefly mention their performance when injected in murine models.

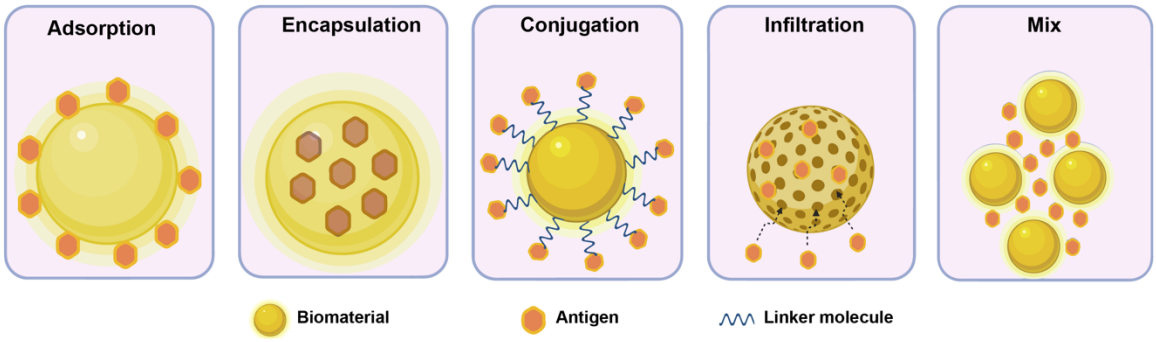


Figure 8: A schematic of different ways biomaterials interact with antigens. Each approach has focused to improve delivery of the antigens in order to enhance the immune response. Encapsulation and infiltration have been more heavily investigated owing to the potential stability they offer to the antigens trapped within. Created with BioRender.com

[3.1 Interaction and Release]

The interaction between the antigen and biomaterial can be broadly classified into five categories; surface adsorption, mixing, encapsulation, conjugation, and infiltration.(L. Zhao et al., 2014) Surface adsorption for example, is entirely driven by electrostatic or hydrophilic/hydrophobic interactions leading to the weak attachment of the antigen to the surface of the biomaterial. Thus, when tested *in vivo,* the composite readily dissociates, exhibiting a burst release kinetics profile.(Mody et al., 2013) Differently, conjugation relies on chemical crosslinking of the antigen to the biomaterial, and release is achieved through biomaterial decomposition either intracellularly or extracellularly and this interaction can be used to improve immunogenicity.(Slütter et al., 2010) Encapsulation is achieved through mixing the antigen and biomaterial precursors during synthesis.(Q. He et al., 2000). Antigens encapsulated are gradually released *in vivo* through biomaterial degradation or when taken up by cells and digested in low pH compartments. For sustained-release kinetic profiles, adsorption and encapsulation interactions are currently the most investigated interactions for improving vaccines.(Alkie et al., 2018; Bivas-Benita et al., 2003; Boks et al., 2015; X. He et al., 2005) While adsorption studies have focused more on the

uptake of the biomaterial rather than a slow release of antigen,(X. Chen et al., 2014) most *in vivo* studies for sustained release use encapsulation as it offers both long-term stability and slow release for single injection vaccinations.(Pawar et al., 2013; Sexton et al., 2009) For the development of single injection vaccinations, slow or consistent release has been most studied *in vivo* and has shown significant immune system stimulation. (C.-H. Huang et al., 2019; M.-H. Huang et al., 2010) A concern articulated by some researchers has been that antigen persistence can lead to immune cell exhaustion and lower antibody affinity to antigens.(Han, Asoyan, Rabenstein, Nakano, & Obst, 2010; Mueller & Ahmed, 2009; Tam et al., 2016; S. Wang et al., 2015) Instead, pulsatile release seems to be a better alternative as it mimics a single injection followed by several booster shots.(Cleland, 1998) For example, in 2018 the Langer Lab investigated 16 different formulations of PLGA and took three formulations that exhibited pulsatile release profiles.(Guarecuco et al., 2018) The goal of this study was to show that pulsatile release could develop a humoral response identical to a single injection, followed by two booster shots. They focused on the biomaterial and IgG production and future studies will need to consider cytokine production and look at further tuning the material to elongate the pulse from two weeks to two months to mimic better the injections schedule of current vaccines.

[3.2 Antigenicity and Long-term Stability]

Biomaterials play a pivotal role in vaccine development, as they can improve the stability of the antigens encapsulated within. Since one aspect of thermal stabilization is to enable stockpiling of vaccines, antigenicity and epitope stability must be monitored for many months. Stability *in vitro* is typically investigated for shorter periods (several weeks to months) using circular dichroism, western blot, or enzyme-linked immunosorbent assay (ELISA) to determine the stability of model proteins—typically ovalbumin.(Shi et al., 2002; Smallshaw & Vitetta, 2010) While ovalbumin is exceptionally well characterized and a cottage industry of antibodies, antigen-specific cell lines, and assays are available to study how formulations of ovalbumin affect the immune system, ovalbumin is relatively stable and studies that include ovalbumin should include other antigens as well.(Adams et al., 2015; Bertram et al., 2010; Dierendonck et al., 2014; Kim et al., 2010; Prego et al., 2010) The Tiyaboonchai lab encapsulated *Pasteurella multocida* within alginate microparticles for subcutaneous injections. Key to this study was a six-month storage test, where antigenicity was investigated in samples kept either at 4 or 37 °C.(Nimtrakul et al., 2015) Results revealed that mice injected with either formulation elicited an immune response similar to freshly encapsulated antigens. This study provides an ideal experimental template for single-dose vaccination investigations. Future investigations should focus on long-term stability. Researchers should test the antigenicity at room temperature for at least six months or longer to remove the cold chain's financial burden and expand these resources to developing areas without this infrastructure.(Clénet, 2018; Dumpa et al., 2019)

[4. BIOMATERIAL IMMUNE ACTIVATION]

The investigation of biomaterials to deliver vaccines against infectious diseases offers new strategies to engineer specific immune responses. As discussed in section 3, determining the antigenicity of proteins within a biomaterial will ensure that the immune activation will be produced for the correct epitope. To further verify this, the biomaterials discussed above need to be studied *in vivo* to understand their role for long-term immunity from single or multi shots to any associated toxicity. Since most biomaterials investigations tend to be short-term (*i.e.* less than two months),(Cooper & Petrovsky, 2011; Walke et al., 2018) experiments need to be developed to ensure long-term issues are being addressed. The following section focuses on biomaterials studied *in vivo* and analyzes some examples in the literature, which focus primarily on the production of antibodies, cytokines, immune cells, and survival studies.(Hashem et al., 2013)

[4.1 Antibodies]

From 1995 to 2005 the main experiment conducted to determine the immune response of an antigen combined with biomaterial was based on the production of antibodies. The main biomaterial investigated was PLGA with a plethora of antigens, such as diptheria, tetanus toxoid, and hepatitis B, used to determine its effectiveness in animal models.(Cleland et al., 1998; Gupta et al., 1996; O'Hagan et al., 1995; Raghuvanshi et al., 2001) Those studies' goal was to show that a single dose of PLGA slowly releases the antigens and produces a humoral response similar to 3 injections of the antigen alone.(Feng et al., 2006) In 2013, the Hook Lab showed that cubosomes, a lipid based nanocarrier, could prime T cells more efficiently because they can encapsulate a higher amount of antigen compared to liposomes.(Rizwan et al., 2013) In this study, they use ovalbumin as their subunit antigen and toll-like receptor-7 (TLR7) agonists imiquimod and monophosphoryl lipid A as adjuvants. As shown in **Figure 9A**, the cubosome with adjuvant produced the highest amount of antibodies compared to liposome with adjuvant and antigen and alum. More Recently in 2019, the Irvine Lab fabricated microneedles (MN) with silk fibroin protein tips and encapsulated HIV envelope trimer as shown in **Figure 9B**.(Boopathy et al., 2019) When the timer was mixed within the tips they added TLR2 agonist pam_3CSK_4 and TLR3 agonist Poly I:C as adjuvants to recruit immune cells to the skin. They showed that the mice vaccinated with MNs containing the trimer and adjuvant had the highest anti-trimer IgG titers compared to the mice receiving bolus injections (**Figure 9C**). Though the studies showed that the biomaterial developed an immune response without the need of an adjuvant, they found the inclusion of an adjuvant provided a higher response.

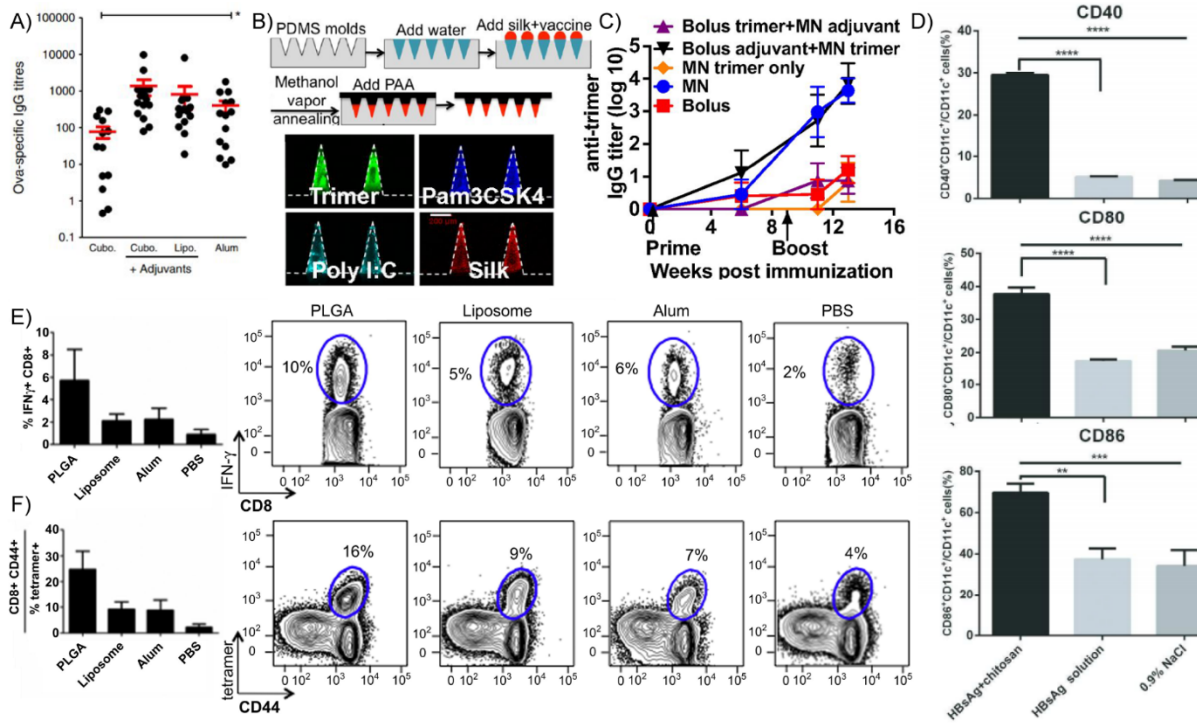


Figure 9: A) The comparison of ovalbumin antibodies that have been encapsulated inside of a cubosome polymer with and without adjuvant has similar production compared to alum. Reprinted from reference(Rizwan et al., 2013), with permission from Elsevier. An image of a B) the steps to develop silk loaded microneedle tips, containing HIV trimer along with adjuvants and when delivered into mice models, C) the microneedle containing trimer and adjuvant produced the highest amount of antibodies. Reprinted from reference,(Boopathy et al., 2019) with permission from PNAS. D) The Xu lab shows that chitosan nanoparticles have a higher upregulation in CD40, CD80, and CD86 in bone marrow dendritic cells compared to a bolus shot of the hepatitis B antigen. Republished with permission from Royal Society of Chemistry for reference;(Z.-B. Wang et al., 2016) permission conveyed through Copyright Clearance Center, Inc. The Fahmy lab compares the slow release of PLGA, Liposome, and alum for release of ovalbumin and show that PLGA stimulates a higher population of cytotoxic T cells producing E) IFN-γ and F) activated specifically to ovalbumin. Reprinted from reference,(Demento et al., 2012) with permission from Elsevier.

[4.2 Immune Cell Activation]

It is understandable to just look at antibody production, as the current method to determine if a vaccine is still active is to test whether a patient is still producing antibodies. However, to better understand how well a formulation works, it is necessary to investigate the activation of immune cells. For APCs, researchers mainly focus on macrophage and dendritic cells, and for the activation of the adaptive immune system, they look at T-cells and B-cells found in secondary lymphoid organs—the lymph nodes and spleens. For example, the Xu lab investigated the mechanism of chitosan as an adjuvant, with hepatitis B as the model antigen.(Z.-B. Wang et al., 2016) They showed that the insolubility of the chitosan particles enabled the formation of a sustained-release depot and enhanced the uptake of antigens by bone marrow dendritic cells. It was clear that the chitosan particles containing hepatitis B improved dendritic cells' maturation as can be seen from the upregulation of CD40, CD80, and CD86 (**Figure 9D**). However, an interesting study would have been to test if the chitosan particle improved T cell activation. More recent studies to determine if a biomaterial can activate immune system is by directly looking at the draining lymph nodes and spleen for activation markers on dendritic cells and T-cells.(C.-Y. Huang et al., 2018) The Fahmy lab(Demento et al., 2012) showed that liposome and PLGA nanoparticles improve the T cell activation owing to sustained release of antigens. They mention that

most vaccines focus on developing neutralizing antibodies, even though a defense against viruses and intracellular bacteria would benefit more from a cellular immune response. Thus, they show that the PLGA and liposome nanoparticle not only enhance antibodies but also enhance the cytotoxic immune cells (CD8+) and these activated T cells (CD8+, CD44+) are specific to ovalbumin (tetramer) as seen in (**Figure 9E-F**).

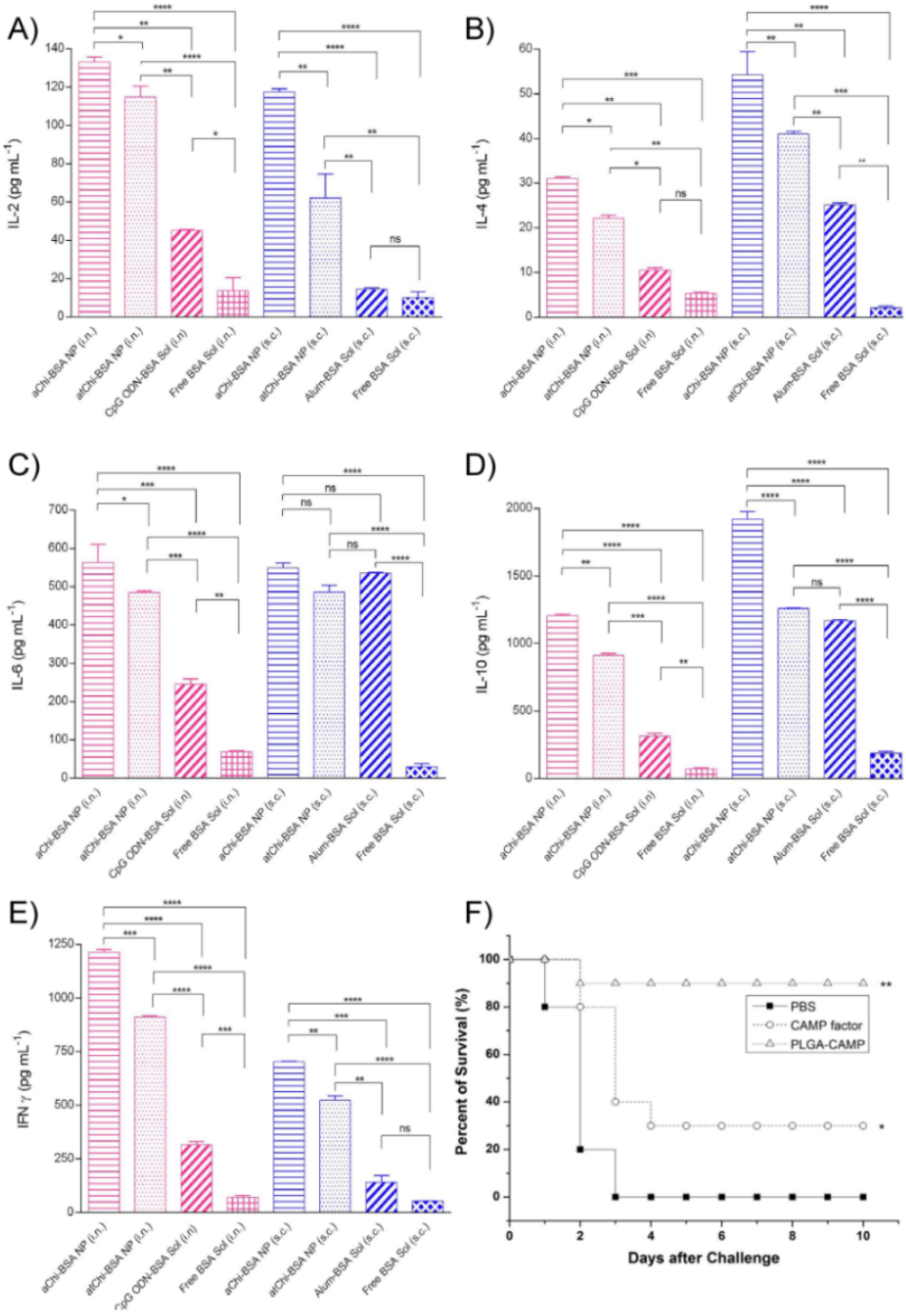


Figure 10: The Cevhar lab investigates chitosan activation of the immune system when different administrations are used and compare the cytokine production of A) IL-2, B) IL-4, C) IL-6, D) IL-10, and E) IFN-γ. Reprinted from reference,(Sinani et al., 2019) with permission from Elsevier. F) The Han Lab vaccinated mice with PLGA containing a subunit protein CAMP found in Toxoplasma *gondii*, which improve the survival rate of mice compared to a bolus shot of the subunit protein alone. Reprinted from reference,(G. Liu et al., 2017) with permission from Elsevier.

[4.3 Cytokines (Type of Response)]

Cytokines as additional experiments to test the biomaterial ability to activate the immune system *in vivo* did not start to pick up until 2006. In addition to immune cell activation, the cytokines produced can indicate whether the immune response activates CD4+ T helper 1 (Th1), T helper 2 (Th2), or both and can distinguish how size, adjuvant, dosage, and other attributes can affect this response.(Jia et al., 2017) Th1 is an intracellular or cell-mediated response combined with an antibody response, and Th2

tends to focus on extracellular pathogens and is a predominantly humoral response.(Rosenthal & Zimmerman, 2006; Spellberg & Edwards, 2001) In 2016 the Ainslie lab chemically modified a naturally occurring polysaccharide to have acid-cleavable acetal and silyl groups.(Gallovic et al., 2016) They showed that extending the alkyl chain or mixing the inulin and dextran polysaccharides at different ratios could shorten or extend antigens' release from within. They mention an ongoing challenge with subunit vaccines is incorporating adjuvants that safely stimulate and activate both a Th1 and Th2 immune response. They used a solvent evaporation technique to encapsulate ovalbumin within dextran microparticles to show that their particle could more efficiently target APCs and obtain a more balanced cellular and humoral response. They vaccinated mice subcutaneously with 50 μg of ovalbumin and showed that their biomaterial produced antibodies like ova+alum. Additionally, they looked at the amount of TNF-α produced by macrophages *in vitro* and the IFN-γ produced by splenocytes restimulated with SIINFEKL—a peptide sequence of ovalbumin. In 2019 the Cevhar lab did a thorough investigation(Sinani et al., 2019) to show that their chitosan particles could activate a balanced Th1/Th2 immune response. They found that their aminated chitosan and aminated/thiolated chitosan provides a balanced Th1/Th2 response when delivered intranasally and compared this to mice vaccinated subcutaneously (**Figure 10A**). The results obtained from spleens harvested at day 253 show that intranasal injections of 20 μg of BSA encapsulated within the chitosan formulations have a similar Th2 response compared to mice vaccinated subcutaneously based on the IL-6, IL-4, and IL-10 cytokine productions (**Figure 10B-D**). The cell-mediated response for mice vaccinated with chitosan was higher than subcutaneous injections and controls using CpG as an adjuvant based on the IFN-γ and IgG2a antibodies (**Figure 10E**). This is a great study that compared injection routes and compared their chitosan formulation to current adjuvants used in vaccinations. To truly understand the type of immune response, intracellular staining of CD4+ T cells for different cytokines, such as IL-4 and IFN-γ, would clarify the activation these biomaterials produce. This staining method along with an antigen-specific marker for the T-cell receptor would without a doubt determine how balanced of an immune response these biomaterials are creating.

[4.4 Survival Studies]

Survival studies for biomaterials with an encapsulated antigen against infectious disease are uncommon, though it has been used in cancer vaccines studies routinely starting around 2004.(Standley et al., 2004) Most survival studies with biomaterials tend to focus on how long their biomaterial can keep mice alive after inoculation with tumor cells.(Foster, Duvall, Crownover, Hoffman, & Stayton, 2010; X. He et al., 2005; A. L. Z. Lee et al., 2019; Lv et al., 2017) One of the earliest experiments that look at survival against infectious diseases using a sustained-release polymer was conducted by the Zhu lab in 2016 using PLGA as the biomaterial.(N.-Z. Zhang et al., 2016) They encapsulated subunit proteins, rROP18 and rCDPK6 from an intracellular parasite called *Toxoplasma gondii* (*T. gondii*) within PLGA microparticles to generate a long-lasting immune response. The mice were vaccinated subcutaneously, and immune response was measured by lymphocyte proliferation,

cytokine expression, and antibody production. In addition, they challenged the vaccinated mice six weeks after the last injection with live *T. gondii* and their survival was recorded daily until all mice were dead. The mice vaccinated with PLGA and rROP18 subunit protein survived the longest.

Similarly, the Han lab entrapped(G. Liu et al., 2017) a subunit protein, CAMP factor, from *streptococcus agalactiae* within PLGA to develop single-dose vaccines to protect against diseases. Mice were vaccinated by intraperitoneal injection and six weeks later were challenged with a lethal dose of *S. agalactiae* (LD_{50} = 2×10^8 CFU). Mice were monitored for 10 days for mortality and the CAMP-PLGA vaccinated group had the highest chance of survival as shown in **Figure 10F**. As researchers continue to investigate biomaterials for vaccination, survival experiments provide an important tool to directly demonstrate efficacy.

Conclusion and Perspective

This overview summarizes how far we have come in understanding the use of sustained-release biomaterials as a tool to improve thermal stability and vaccine performance; however, there are still questions that need to be addressed. Many reports do not discuss protein loss during integration with these polymer systems, along with the performance of their antigen-biomaterial composite in the absence of adjuvant. The binding affinity between the polymer system and antigen needs to be investigated to understand the antibody production *in vivo*. Further, antibody-antigen affinity should be assessed as some studies have shown that the continuous release of antigens can reduce the binding affinity of the antibodies produced against it and induce immune exhaustion.(Han et al., 2010; Mueller & Ahmed, 2009; S. Wang et al., 2015) Additionally, by looking at T-cell and B-cell receptors that are antigen-specific and determining the population of effector cells and memory cells, we can better understand how biomaterials promote an antigen-specific immune response. Correlating all this with survival studies, whenever possible, can give us a more quantitative answer on the level and type of response from the immune system needed to protect against infectious diseases. Finally, protein-based delivery strategies, as discussed here, are emerging as well-tread ground even if some details still need to be filled in. On the horizon, however, are biomaterial-based methods to control the delivery and thermally protect next-generation vaccines based on lipid nanoparticles, RNA, and DNA—an area still in its infancy given that lipid nanosystems tend to be even more unstable than proteins.(Lu et al., 2020) The application of sustained-release biomaterials to infectious diseases will require strong collaborative efforts between researchers in diverse fields. The research conducted so far has shown—with relative consistency—that biomaterials that span the size regimes from nano- to micron-sized can generate an immune response after a single injection that is on par with current parental routes that require three injections. As this field progresses forward, priority should be given to materials that induce long-lasting immunity, instigate the production of memory cells, and can provide protection against infectious diseases without requiring expensive cold-chain infrastructure.

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References

Abdou, P., Wang, Z. J., Chen, Q., Chan, A. M., Zhou, D. J. R., Gunadhi, V., & Gu, Z. (2020). Advances in Engineering Local Drug Delivery Systems for Cancer Immunotherapy. *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology*, *12*, e1632. https://doi:10.1002/wnan.1632

Adams, J. R., Haughney, S. L., & Mallapragada, S. K. (2015). Effective Polymer Adjuvants for Sustained Delivery of Protein Subunit Vaccines. *Acta Biomaterialia*, *14*, 104-114. https://doi.org/10.1016/j.actbio.2014.11.050

Alkie, T. N., Yitbarek, A., Taha-Abdelaziz, K., Astill, J., & Sharif, S. (2018). Characterization of Immunogenicity of Avian Influenza Antigens Encapsulated in PLGA Nanoparticles Following Mucosal and Subcutaneous Delivery in Chickens. *PloS One* , *13*, e0206324. https://doi.org/10.1371/journal.pone.0206324

Allahyari, M., & Mohit, E. (2016). Peptide/Protein Vaccine Delivery System Based on PLGA Particles. *Human Vaccines & Immunotherapeutics*, *12*, 806-828. https://doi.org/10.1080/21645515.2015.1102804

Amanna, I. J. (2012). Balancing the Efficacy and Safety of Vaccines in the Elderly. *Open Longevity Science*, *6*, 64-72. https://doi:10.2174/1876326X01206010064

Ashok, A., Brison, M., & LeTallec, Y. (2017). Improving Cold Chain Systems: Challenges and Solutions. *Vaccine*, *35*, 2217-2223. https://doi: 10.1016/j.vaccine.2016.08.045

Avgoustakis, K. (2004). Pegylated Poly(Lactide) and Poly(Lactide-Co-Glycolide) Nanoparticles: Preparation, Properties and Possible Applications in Drug Delivery. *Current Drug Delivery*, *1*, 321-333. https://doi.org/10.2174/1567201043334605

Bachelder, E. M., Beaudette, T. T., Broaders, K. E., Dashe, J., & Frechet, J. M. J. (2008). Acetal-Derivatized Dextran: An Acid-Responsive Biodegradable Material for Therapeutic Applications. *Journal of the American Chemical Society*, *130*, 10494-10495. https://doi.org/10.1021/ja803947s

Bachelder, E. M., Beaudette, T. T., Broaders, K. E., Paramonov, S. E., Dashe, J., & Fréchet, J. M. J. (2008). Acid-Degradable Polyurethane Particles for Protein-Based Vaccines: Biological Evaluation and in Vitro Analysis of Particle Degradation Products. *Molecular Pharmaceutics*, *5*, 876-884. https://doi:10.1021/mp800068x

Bachelder, E. M., Pino, E. N., & Ainslie, K. M. (2017). Acetalated Dextran: A Tunable and Acid-Labile Biopolymer with Facile Synthesis and a Range of Applications. *Chemical Reviews*, 117,1915-1926. https://doi:10.1021/acs.chemrev.6b00532

Bachmann, M. F., Rohrer, U. H., Kundig, T. M., Burki, K., Hengartner, H., & Zinkernagel, R. M. (1993). The Influence of Antigen Organization on B Cell Responsiveness. *Science* , 262, 1448-1451. https://doi:10.1126/science.8248784

Bertram, U., Bernard, M-C., Haensler, J., Maincent, P., & Bodmeier, R. (2010). In Situ Gelling Nasal Inserts for Influenza Vaccine Delivery. *Drug Development and Industrial Pharmacy*, *36*, 581-593. https://doi.org/10.3109/03639040903382673

Bivas-Benita, M., Laloup, M., Versteyhe, S., Dewit, J., Braekeleer, J. D., Jongert, E., & Borchard, G. (2003). Generation of Toxoplasma Gondii GRA1 Protein and DNA Vaccine Loaded Chitosan Particles: Preparation, Characterization, and Preliminary in Vivo Studies. *International Journal of Pharmaceutics*, 266, 17-27. https://doi.org/10.1016/S0378-5173(03)00377-6

Black, M., Trent, A., Kostenko, Y., Lee, J. S., Olive, C., & Tirrell, M. (2012). Self-Assembled Peptide Amphiphile Micelles Containing a Cytotoxic T-Cell Epitope Promote a Protective Immune Response *in Vivo. Advanced Materials*, *24*, 3845-3849. https://doi:10.1002/adma.201200209

Bobbala, S., Gibson, B., Gamble, A.B., McDowell, A., & Hook, S. (2018). Poloxamer 407-Chitosan Grafted Thermoresponsive Hydrogels Achieve Synchronous and Sustained Release of Antigen and Adjuvant from Single-Shot Vaccines. *Immunology and Cell Biology*, 96, 656-665. https://doi:10.1111/imcb.12031

Bogataj, M., Bogataj, L., & Vodopivec, R. (2005). Stability of Perishable Goods in Cold Logistic Chains. *International Journal of Production Economics*, *93-94*, 345-356. https://doi:10.1016/j.ijpe.2004.06.032

Boks, M. A., Unger, W. W. J., Engels, S., Ambrosini, M., Kooyk, Y. V., & Luttge, R. (2015). Controlled Release of a Model Vaccine by Nanoporous Ceramic Microneedle Arrays. *International Journal of Pharmaceutics*, *491*, 375-383. https://doi.org/10.1016/j.ijpharm.2015.06.025

Boopathy, A. V., Mandal, A., Kulp, D. W., Menis, S., Bennett, N. R., Watkins, H. C., Wang, W., Martin, J. T., Thai, N. T., He, Y., *et al*. (2019). Enhancing Humoral Immunity Via Sustained-Release Implantable Microneedle Patch Vaccination. *Proceedings of the National Academy of Sciences*, *116*, 16473. https://doi:10.1073/pnas.1902179116

Bose, R. J. C., Kim, M., Chang, J. H., Paulmurugan, R., Moon, J. J., Koh, W-G., Lee, S-H., & Park, H. (2019). Biodegradable Polymers for Modern Vaccine Development. *Journal of Industrial and Engineering Chemistry (Seoul, Korea)*, *77*, 12-24. https://doi.org/10.1016/j.jiec.2019.04.044

Bussio, J. I., Molina-Perea, C., & González-Aramundiz, J. V. (2019). Hyaluronic Acid Nanocapsules as a Platform for Needle-Free Vaccination. *Pharmaceutics*, 11, 246. https://doi:10.3390/pharmaceutics11050246

Chen, N.H., Gallovic, M.D., Tiet, P., Ting J.P.Y, Ainslie, K.M., & Bachelder, E.M. (2018). Investigation of Tunable Acetalated Dextran Microparticle Platform to Optimize M2e-Based Influenza Vaccine Efficacy. *Journal of Controlled Release*, 289, 114-124. https://doi:10.1016/j.jconrel.2018.09.020

Chen, X., Liu, Y., Wang, L., Liu, Y., Zhang, W., Fan, B., Ma, X., Yuan, Q., Ma, G., & Su, Z. (2014). Enhanced Humoral and Cell-Mediated Immune Responses Generated by Cationic Polymer-Coated PLA Microspheres with Adsorbed Hbsag. *Molecular Pharmaceutics*, *11*, 1772-1784. https://doi.org/10.1021/mp400597z

Cleland, J-L. (1998). Solvent Evaporation Processes for the Production of Controlled Release Biodegradable Microsphere Formulations for Therapeutics and Vaccines. *Biotechnology Progress*, 14, 102-107. https://doi:10.1021/bp970128t

Cleland, J. L., Lim, A., Daugherty, A., Barron, L., Desjardin, N., Duenas, E. T., *et al*. (1998). Development of a Single-Shot Subunit Vaccine for Hiv-1. 5. Programmable in Vivo Autoboost and Long Lasting Neutralizing Response. *Journal of Pharmaceutical Sciences*, *87*, 1489-1495. https://doi.org/10.1021/js980263f

Clem, A. (2011). Fundamentals of Vaccine Immunology. *Journal of Global Infectious Diseases*, *3*, 73-78. https://doi:10.4103/0974-777x.77299

Clénet, D. (2018). Accurate Prediction of Vaccine Stability under Real Storage Conditions and During Temperature Excursions. *European Journal of Pharmaceutics and Biopharmaceutics*, 125, 76-84. https://doi.org/10.1016/j.ejpb.2018.01.005

Cooper, P. D., & Petrovsky, N. (2011). Delta Inulin: A Novel, Immunologically Active, Stable Packing Structure Comprising B-D-[2 → 1] Poly(Fructo-Furanosyl) α-D-Glucose Polymers. *Glycobiology*, 21, 595-606. doi:10.1093/glycob/cwq201

Corthésy, B., & Bioley, G. (2018). Lipid-Based Particles: Versatile Delivery Systems for Mucosal Vaccination against Infection. *Frontiers in Immunology*, *9*, 431-431. https://doi:10.3389/fimmu.2018.00431

Cox, A., Baker, S. F., Nogales, A., Martínez-Sobrido, L., & Dewhurst, S. (2015). Development of a Mouse-Adapted Live Attenuated Influenza Virus That Permits in Vivo Analysis of Enhancements to the Safety of Live Attenuated Influenza Virus Vaccine. *Journal of Virology*, *89*, 3421-3426. https://doi:10.1128/JVI.02636-14

Demento, S. L., Cui, W., Criscione, J. M., Stern, E., Tulipan, J., Kaech, S. M., & Fahmy, T. M. (2012). Role of Sustained Antigen Release from Nanoparticle Vaccines in Shaping the T Cell Memory Phenotype. *Biomaterials*, *33*, 4957-4964. https://doi.org/10.1016/j.biomaterials.2012.03.041

Demento, S. L., Siefert, A. L., Bandyopadhyay, A., Sharp, F. A., & Fahmy, T. M. (2011). Pathogen-Associated Molecular Patterns on Biomaterials: A Paradigm for Engineering New Vaccines. *Trends in Biotechnology*, *29*, 294-306. https://doi:10.1016/j.tibtech.2011.02.004

Di Pasquale, A., Preiss, S., Da Silva, F. T., & Garçon, N. (2015). Vaccine Adjuvants: From 1920 to 2015 and Beyond. *Vaccines*, *3*, 320-343. https://doi:10.3390/vaccines3020320

Dierendonck, M., Fierens, K., De Rycke, R., Lybaert, L., Maji, S., Zhang, Z., Zhang, Q., Hoogenboom, R., Lambrecht, B. N., Grooten, J., *et al*. (2014). Nanoporous Hydrogen Bonded Polymeric Microparticles: Facile and Economic Production of Cross Presentation Promoting Vaccine Carriers. *Advanced Functional Materials*, *24*, 4634-4644. https://doi.org/10.1002/adfm.201400763

Dumpa, N., Goel, K., Guo, Y., McFall, H., Pillai, A-R., Shukla, A., Repka, M-A., & Murthy, S-N. (2019). Stability of Vaccines. *AAPS PharmSciTech*, 20, 42. https://doi:10.1208/s12249-018-1254-2

Ellis, J. E., Zeng, Z., Hwang, S. I., Li, S., Luo, T-Y., Burkert, S. C., White, D. L., Rosi, N. L., Gassensmith, J. J., & Star, A. (2019). Growth of Zif-8 on Molecularly Ordered 2-Methylimidazole/Single-Walled Carbon Nanotubes to Form Highly Porous, Electrically Conductive Composites. *Chemical Science*, *10,* 737-742. https://doi:10.1039/C8SC03987A

Elmowafy, E. M., Tiboni, M., & Soliman, M. E. (2019). Biocompatibility, Biodegradation and Biomedical Applications of Poly(Lactic Acid)/Poly(Lactic-Co-Glycolic Acid) Micro and Nanoparticles. *Journal of Pharmaceutical Investigation*, *49*, 347-380. https://doi:10.1007/s40005-019-00439-x

Feng, L., Qi, X. R., Zhou, X. J., Maitani, Y., Wang S. C., Jiang, Y., & Nagai, T. (2006). Pharmaceutical and Immunological Evaluation of a Single-Dose Hepatitis B Vaccine Using PLGA Microspheres. *Journal of Controlled Release*, *112*, 35-42. https://doi.org/10.1016/j.jconrel.2006.01.012

Foged, C. (2011). Subunit Vaccines of the Future: The Need for Safe, Customized and Optimized Particulate Delivery Systems. *Therapeutic Delivery*, *2*, 1057-1077. https://doi:10.4155/tde.11.68

Foster, S., Duvall, C. L., Crownover, E. F., Hoffman, A. S., & Stayton, P. S. (2010). Intracellular Delivery of a Protein Antigen with an Endosomal-Releasing Polymer Enhances CD8 T-Cell Production and Prophylactic Vaccine Efficacy. *Bioconjugate Chemistry*, *21*, 2205-2212. https://doi.org/10.1021/bc100204m

Frangione-Beebe, M., Rose, R. T., Kaumaya, P. T. P., & Schwendeman, S. P. (2001). Microencapsulation of a Synthetic Peptide Epitope for Htlv-1 in Biodegradable Poly (D, L-Lactide-Co-Glycolide) Microspheres Using a Novel Encapsulation Technique. *Journal of Microencapsulation*, *18*, 663-677. https://doi.org/10.1080/02652040110055216

Gallovic, M.D., Bandyopadhyay, S., Borteh, H., Montjoy, D.G., Collier, M.A., Peine, K.J., Wyslouzil, B.E., Bachelder, E.M., & Ainslie, K.M. (2016). Microparticles Formulated from a Family of Novel Silylated Polysaccharides Demonstrate Inherent Immunostimulatory Properties and Tunable Hydrolytic Degradability. *Journal of Materials Chemistry B,* 4, 4302-4312. https://doi:10.1039/C6TB00745G

Gao, Q., Bao, L., Mao, H., Wang, L., Xu, K., Yang, M., Li, Y., Zhu, L., Wang, N., Lv, Z., *et al*. (2020). Development of an Inactivated Vaccine Candidate for SARS-CoV-2. *Science*, *369*, 77-81. https://doi:10.1126/science.abc1932

Gordon, S., Young, K., Wilson, R., Rizwan, S., Kemp, R., Rades, T., & Hook, S. (2012). Chitosan Hydrogels Containing Liposomes and Cubosomes as Particulate Sustained Release Vaccine Delivery Systems. *Journal of Liposome Research*, 22:193-204. https://doi:10.3109/08982104.2011.637502

Gu, P., Wusiman, A., Zhang, Y., Liu, Z., Bo, R., Hu, Y., Liu, J., & Wang, D. (2019). Rational Design of PLGA Nanoparticle Vaccine Delivery Systems to Improve Immune Responses. *Molecular Pharmaceutics*, *16*, 5000-5012. https://doi.org/10.1021/acs.molpharmaceut.9b00860

Guarecuco, R., Lu, J., McHugh, K. J., Norman, J. J., Thapa, L. S., Lydon, E., Langer, R., & Jaklenec, A. (2018). Immunogenicity of Pulsatile-Release PLGA Microspheres for Single-Injection Vaccination. *Vaccine*, *36*, 3161-3168. https://doi.org/10.1016/j.vaccine.2017.05.094

Gupta, R. K., Chang, A-C., Griffin, P., Rivera, R., & Siber, G. R. (1996). In Vivo Distribution of Radioactivity in Mice after Injection of Biodegradable Polymer Microspheres Containing 14c-Labeled Tetanus Toxoid. *Vaccine*, *14*, 1412-1416. https://doi.org/10.1016/S0264-410X(96)00073-4

Han, S., Asoyan, A., Rabenstein, H., Nakano, N., & Obst, R. (2010). Role of Antigen Persistence and Dose for Cd4+ T-Cell Exhaustion and Recovery. *Proceedings of the National Academy of Sciences*, 107, 20453-20458. https://doi:10.1073/pnas.1008437107

Harde, H., Agrawal, A. K., & Jain, S. (2014). Development of Stabilized Glucomannosylated Chitosan Nanoparticles Using Tandem Crosslinking Method for Oral Vaccine Delivery. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *9*, 2511-2529. https://doi.org/10.2217/nnm.13.225

Harde, H., Agrawal, A.K., & Jain, S. (2015). Tetanus Toxoids Loaded Glucomannosylated Chitosan Based Nanohoming Vaccine Adjuvant with Improved Oral Stability and Immunostimulatory Response. *Pharmaceutical Research*, 32, 122-134. https://doi:10.1007/s11095-014-1449-5

Hasanzadeh, S., Farokhi, M., Habibi, M., Shokrgozar, M. A., Cohan, A. R., Rezaei, F., Karam, M. R. A., Bouzari, S. (2020). Silk Fibroin Nanoadjuvant as a Promising Vaccine Carrier to Deliver the FimH-IutA Antigen for Urinary Tract Infection. *ACS Biomaterials Science & Engineering*, *6*, 4573-4582. https://doi.org/10.1021/acsbiomaterials.0c00736

Hashem, F. M., Fahmy, S. A., El-Sayed, A. M., & Al-Sawahli. M. M. (2013). Development and Evaluation of Chitosan Microspheres for Tetanus, Diphtheria and Divalent Vaccines: A Comparative Study of Subcutaneous and Intranasal Administration in Mice. *Pharmaceutical Development and Technology*, 18, 1175-1185. https://doi:10.3109/10837450.2011.618949

He, Q., Mitchell, A.R., Johnson, S.L., Wagner-Bartak, C., Morcol, T., & Bell, S.J.D. (2000). Calcium Phosphate Nanoparticle Adjuvant. *Clinical and Diagnostic Laboratory Immunology*, *7*, 899-903. https://doi:10.1128/CDLI.7.6.899-903.2000

He, T., Liang, X., Li, L., Gong, S., Li, X., Zhang, M., Zhu, S., Xiao, H., Wu, Q., & Gong, C. (2021). A spontaneously formed and self-adjuvanted hydrogel vaccine triggers strong immune responses. *Materials & Design*, *197*, 109232. https://doi.org/10.1016/j.matdes.2020.109232

He, X., Jiang, L., Wang, F., Xiao, Z., Li, J., Liu, L. S., *et al*. (2005). Augmented Humoral and Cellular Immune Responses to Hepatitis B DNA Vaccine Adsorbed onto Cationic Microparticles. *Journal of Controlled Release*, *107*, 357-372. https://doi.org/10.1016/j.jconrel.2005.06.020

Herbert, F. C., Abeyrathna, S., Abeyrathna, N., Wijesundara, Y., Brohlin, O., Luzuriaga, M. A., Durand-Silva, A., Diwakara, S. D., Smaldone, R. A., Meloni, G., & Gassensmith, J. J. (2021). Stabilization of Supramolecular Membrane Protein-Lipid Bilayer Assemblies Through Immobilization in a Crystalline Exoskeleton. *Nature Communications*, *12*, 2202. https://doi.org/10.1038/s41467-021-22285-y

Highton, A.J., Kojarunchitt, T., Girardin, A., Hook, S., & Kemp, R.A. (2015). Chitosan Hydrogel Vaccine Generates Protective CD8 T Cell Memory against Mouse Melanoma. *Immunology and Cell Biology*, 93, 634-640. https://doi:10.1038/icb.2015.14

Huang, C-H., Huang, C-Y., Cheng, C-P., Dai, S-H., Chen, H-W., Leng, C-H., Chong, P., Liu, S-J., & Huang, M-H. (2016). Degradable Emulsion as Vaccine Adjuvant Reshapes Antigen-Specific Immunity and Thereby Ameliorates Vaccine Efficacy. *Scientific Reports*, *6*, 36732. https://doi.org/10.1038/srep36732

Huang, C-H., Huang, C-Y., & Huang, M-H. (2019). Impact of Antigen-Adjuvant Associations on Antigen Uptake and Antigen-Specific Humoral Immunity in Mice Following Intramuscular Injection. *Biomedicine & Pharmacotherapy*, *118*, 109373. https://doi.org/10.1016/j.biopha.2019.109373

Huang, C-Y., Huang, C-H., Liu, S-J., Chen, H-W., Leng, C-H., Chong, P., & Huang, M-H. (2018). Polysorbasome: A Colloidal Vesicle Contoured by Polymeric Bioresorbable Amphiphiles as an Immunogenic Depot for Vaccine Delivery. *ACS Applied Materials & Interfaces*, *10*, 12553-12561. https://doi.org/10.1021/acsami.8b03044

Huang, M-H., Lin, S-C., Hsiao, C-H., Chao, H-J., Yang, H-R., Liao, C-C., Chuang, P-W., Wu, H-P., Huang, C-Y., Leng, C-H., *et al*. (2010). Emulsified Nanoparticles Containing Inactivated Influenza Virus and CpG Oligodeoxynucleotides Critically Influences the Host Immune Responses in Mice. *PloS One*, *5*, e12279. https://doi.org/10.1371/journal.pone.0012279

Igartua, M., Hernández, R. M., Esquisabel, A., Gascón, A. R., Calvo, M. B., & Pedraz, J. L. (1998). Enhanced Immune Response after Subcutaneous and Oral Immunization with Biodegradable PLGA Microspheres. *Journal of Controlled Release*, *56*, 63-73. https://doi.org/10.1016/S0168-3659(98)00077-7

Irvine, D. J., Swartz, M. A., & Szeto, G. L. (2013). Engineering Synthetic Vaccines Using Cues from Natural Immunity. *Nature Materials*, *12*, 978-990. https://doi:10.1038/nmat3775

Jabbal-Gill, I., Watts, P., & Smith, A. (2012). Chitosan-Based Delivery Systems for Mucosal Vaccines. *Expert Opinion on Drug Delivery*, 9, 1051-1067. https://doi:10.1517/17425247.2012.697455

Jia, J., Zhang, W., Liu, Q., Yang, T., Wang, L., & Ma, G. (2017). Adjuvanticity Regulation by Biodegradable Polymeric Nano/Microparticle Size. *Molecular Pharmaceutics*, *14*, 14-22. https://doi.org/10.1021/acs.molpharmaceut.6b00434

Karishma, M., Donna, M., Timothy, J. M., & Neena, M. (2012). Freeze-Drying of Protein-Loaded Nanoparticles for Vaccine Delivery. *Drug Delivery Letters*, 2, 83-91. https://doi:10.2174/2210304x11202020083

Kaufmann, S. H. E., & McMichael, A. J. (2005). Annulling a Dangerous Liaison: Vaccination Strategies Against AIDS and Tuberculosis. *Nature Medicine*, *11*, S33-S44. https://doi:10.1038/nm1221

Kim, Y-C., Quan, F-S., Compans, R.W., Kang, S-M., & Prausnitz, M.R. (2010). Formulation and Coating of Microneedles with Inactivated Influenza Virus to Improve Vaccine Stability and Immunogenicity. *Journal of Controlled Release*, 1*42*, 187-195. https://doi.org/10.1016/j.jconrel.2009.10.013

Lagreca, E., Onesto, V., Di Natale, C., La Manna, S., Netti, P. A., & Vecchione, R. (2020). Recent Advances in the Formulation of PLGA Microparticles for Controlled Drug Delivery. *Progress in Biomaterials*, *9*, 153-174. https://doi.org/10.1007/s40204-020-00139-y

Lee, A. L. Z., Yang, C., Gao, S., Hedrick, J. L., & Yang, Y. Y. (2019). Subcutaneous Vaccination Using Injectable Biodegradable Hydrogels for Long-Term Immune Response. *Nanomedicine: Nanotechnology, Biology and Medicine*, 21, 102056. https://doi.org/10.1016/j.nano.2019.102056

Lee, P. W., & Pokorski, J. K. (2018). Poly(Lactic-Co-Glycolic Acid) Devices: Production and Applications for Sustained Protein Delivery. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. https://doi.org/10.1002/wnan.1516

Lee, P. W., Shukla, S., Wallat, J. D., Danda, C., Steinmetz, N. F., Maia, J., & Pokorski, J. K. (2017). Biodegradable Viral Nanoparticle/Polymer Implants Prepared via Melt-Processing. *ACS Nano*, *11*, 8777-8789. https://doi.org/10.1021/acsnano.7b02786

Leleux, J., & Roy, K. (2013). Micro and Nanoparticle-Based Delivery Systems for Vaccine Immunotherapy: An Immunological and Materials Perspective. *Advanced Healthcare Materials*, *2*, 72-94. https://doi.org/10.1002/adhm.201200268

Li, J., Luo, Y. Q., Li, B. Q., Xia, Y. L., Wang, H. Y., & Fu, C. F. (2020). Implantable and Injectable Biomaterial Scaffolds for Cancer Immunotherapy. *Frontiers in Bioengineering and Biotechnology*, *8*, 612950. https://doi:10.3389/fbioe.2020.612950

Li, S., Dharmarwardana, M., Welch, R. P., Benjamin, C. E., Shamir, A. M., Nielsen, S. O., & Gassensmith, J. J. (2018). Investigation of Controlled Growth of Metal–Organic Frameworks on Anisotropic Virus Particles. *ACS Appl. Mater. Interfaces*, *10*, 18161-18169. https://doi:10.1021/acsami.8b01369

Li, S., Dharmarwardana, M., Welch, R. P., Ren, Y., Thompson, C. M., Smaldone, R. A., & Gassensmith, J. J. (2016). Template-Directed Synthesis of Porous and Protective Core–Shell Bionanoparticles. *Angewandte Chemie International Edition*, *55*, 10691-10696. https://doi:10.1002/anie.201604879

Liu, G., Yin, J., Barkema, H. W., Chen, L., Shahid, M., Szenci, O., De Buck, J., Kastelic, J. P., & Han, B. (2017). Development of a Single-Dose Recombinant Camp Factor Entrapping Poly(Lactide-Co-Glycolide) Microspheres-Based Vaccine against Streptococcus Agalactiae. *Vaccine*, *35*, 1246-1253. https://doi.org/10.1016/j.vaccine.2017.01.041

Liu, L., Cao, F., Liu, X., Wang, H., Zhang, C., Sun, H., Wang, C., Leng, X., Song, C., Kong, D., & Ma, G. (2016). Hyaluronic Acid-Modified Cationic Lipid–PLGA Hybrid Nanoparticles as a Nanovaccine Induce Robust Humoral and Cellular Immune Responses. *ACS Applied Materials & Interfaces*, 8, 11969-11979. https://doi:10.1021/acsami.6b01135

Lofano, G., Mallett, C. P., Bertholet, S., & O'Hagan, D. T. (2020). Technological Approaches to Streamline Vaccination Schedules, Progressing Towards Single-Dose Vaccines. *Npj Vaccines*, *5*, 88. https://doi.org/10.1038/s41541-020-00238-8

Lou, P-J., Cheng, W-F., Chung, Y-C., Cheng, C-Y., Chiu, L-H., & Young, T-H. (2009). PMMA Particle-Mediated DNA Vaccine for Cervical Cancer. *Journal of Biomedical Materials Research Part A*, *88A*, 849-857. https://doi.org/10.1002/jbm.a.31919

Lü, J-M., Wang, X., Marin-Muller, C., Wang, H., Lin, P. H., Yao, Q., & Chen, C. (2009). Current Advances in Research and Clinical Applications of PLGA-Based Nanotechnology. *Expert Review of Molecular Diagnostics*, *9*, 325-341. https://doi.org/10.1586/erm.09.15

Lu, Y., Wu, F., Duan, W., Mu, X., Fang, S., Lu, N., Zhou, X., & Kong, W. (2020). Engineering a "PEG-g-PEI/DNA nanoparticle-in- PLGA microsphere" hybrid controlled release system to enhance immunogenicity of DNA vaccine. *Materials Science and Engineering: C*, *106*, 110294. https://doi.org/10.1016/j.msec.2019.110294

Luzuriaga, M. A., Benjamin, C. E., Gaertner, M. W., Lee, H., Herbert, F. C., Mallick, S., & Gassensmith, J. J. (2019). Zif-8 Degrades in Cell Media, Serum, and Some—but Not All—Common Laboratory Buffers. *Supramolecular Chemistry*, *31*, 485-490. https://doi:10.1080/10610278.2019.1616089

Luzuriaga, M. A., Berry, D. R., Reagan, J. C., Smaldone, R. A., & Gassensmith, J. J. (2018). Biodegradable 3D printed polymer microneedles for transdermal drug delivery. *Lab on a Chip*, *18*, 1223–1230. https://doi.org/10.1039/C8LC00098K

Luzuriaga, M. A., Herbert, F. C., Brohlin, O. R., Shahrivarkevishahi, A., Wijesundara, Y. H., Veera, K., . . . Gassensmith, J. J. (2020). A Whole Cell Metal-Organic Framework Encapsulated Vaccine Against Septicemic UPEC Infection. *bioRxiv*. doi:10.1101/2020.06.14.148452

Luzuriaga, M. A., Welch, R. P., Dharmarwardana, M., Benjamin, C. E., Li, S., Shahrivarkevishahi, A., Popal, S., Tuong, L. H., Creswell, C. T., & Gassensmith, J. J. (2019). Enhanced Stability and Controlled Delivery of Mof-Encapsulated Vaccines and Their Immunogenic Response in Vivo. *ACS Applied Materials & Interfaces*, *11*, 9740-9746. https://doi:10.1021/acsami.8b20504

Lv, M., Li, S., Zhao, H., Wang, K., Chen, Q., Guo, Z., Liu, Z., & Xue, W. (2017). Redox-Responsive Hyperbranched Poly(Amido Amine) and Polymer Dots as a Vaccine Delivery System for Cancer Immunotherapy. *Journal of Materials Chemistry B*, *5*, 9532-9545. https://doi.org/10.1039/C7TB02334K

Maghrebi, S., Jambhrunkar, M., Joyce, P., & Prestidge C. A. (2020). Engineering PLGA–Lipid Hybrid Microparticles for Enhanced Macrophage Uptake. *ACS Applied Bio Materials*, 3, 4159-4167. https://doi:10.1021/acsabm.0c00251

Mahase, E. (2020). Covid-19: Moderna Vaccine Is Nearly 95% Effective, Trial Involving High Risk and Elderly People Shows. *BMJ*, 371, m4471 https://doi:10.1136/bmh.m4471

Mahase, E. (2020). COVID-19: Pfizer Vaccine Efficacy Was 52% after First Dose and 95% after Second Dose, Paper Shows. *BMJ*, 371, m4826. https://doi:10.1136/bmj.m4826

Men, Y., Tamber, H., Audran, R., Gander, B., & Corradin, G. (1997). Induction of a Cytotoxic T Lymphocyte Response by Immunization with a Malaria Specific Ctl Peptide Entrapped in Biodegradable Polymer Microspheres. *Vaccine*, *15*, 1405-1412. https://doi.org/10.1016/S0264-410X(97)00047-9

Miller, S. R., Heurtaux, D., Baati, T., Horcajada, P., Grenèche, J-M., & Serre, C. (2010). Biodegradable Therapeutic Mofs for the Delivery of Bioactive Molecules. *Chemical Communications*, *46*, 4526-4528. https://doi:10.1039/C001181A

Mody, K-T., Popat, A., Mahony, D., Cavallaro, A-S., Yu, C., & Mitter, N. (2013). Mesoporous Silica Nanoparticles as Antigen Carriers and Adjuvants for Vaccine Delivery. *Nanoscale*, 5, 5167-5179. https://doi:10.1039/C3NR00357D

Moore, K.M., Batty, C.J., Stiepel, R.T., Genito, C.J., Bachelder, E.M., & Ainslie, K.M. (2020). Injectable, Ribbon-Like Microconfetti Biopolymer Platform for Vaccine Applications. *ACS Applied Materials & Interfaces*, 12, 38950-38961. https://doi:10.1021/acsami.0c10276

Morachis, J. M., Mahmoud, E. A., & Almutairi, A. (2012). Physical and Chemical Strategies for Therapeutic Delivery by Using Polymeric Nanoparticles. *Pharmacological Reviews*, *64*, 505-519. https://doi.org/10.1124/pr.111.005363

Moran, H. B. T., Turley, J. L., Andersson, M., & Lavelle, E. C. (2018). Immunomodulatory Properties of Chitosan Polymers. *Biomaterials,* 184, 1-9. https://doi:10.1016/j.biomaterials.2018.08.054

Mueller, S-N., & Ahmed, R. (2009). High Antigen Levels Are the Cause of T Cell Exhaustion During Chronic Viral Infection. *Proceedings of the National Academy of Sciences*, 106, 8623-8628. https://doi:10.1073/pnas.0809818106

Nagpal, P. S., Kesarwani, A., Sahu, P., & Upadhyay, P. (2019). Aerosol Immunization by Alginate Coated Mycobacterium (Bcg/Mip) Particles Provide Enhanced Immune Response and Protective Efficacy Than Aerosol of Plain Mycobacterium against M.Tb. H37rv Infection in Mice. *BMC Infectious Diseases*, *19*, 568. https://doi.org/10.1186/s12879-019-4157-2

Nimtrakul, P., Atthi, R., Limpeanchob, N., & Tiyaboonchai, W. (2015). Development of Pasteurella Multocida-Loaded Microparticles for Hemorrhagic Septicemia Vaccine. *Drug Development and Industrial Pharmacy,* 41, 423-429. https://doi:10.3109/03639045.2013.873448

O'Hagan, D. T., McGee, J. P., Boyle, R., Gumaer, D., Li, X. M., Potts, B., Wang, C. Y., & Koff, W. C. (1995). The Preparation, Characterization and Pre-Clinical Evaluation of an Orally Administered Hiv-I Vaccine, Consisting of a Branched Peptide Immunogen Entrapped in Controlled Release Microparticles. *Journal of Controlled Release*, *36*, 75-84. https://doi.org/10.1016/0168-3659(95)00052-A

Oliver, S. E., Gargano, J. W., Marin, M., Wallace, M., Curran, K. G., Chamberland, M., McClung, N., Campos-Outcalt, D., Morgan, R. L., Mbaeyi, S., *et al*. (2020). The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Pfizer-Biontech COVID-19

Vaccine - United States, *Morbidity and Mortality Weekly Report (MMWR)*, 69, 1922-1924. https://doi:10.15585/mmwr.mm6950e2

Oliver, S. E., Gargano, J. W., Marin, M., Wallace, M., Curran, K. G., Chamberland, M., McClung, N., Campos-Outcalt, D., Morgan, R. L., Mbaeyi, S., *et al*. (2020). The Advisory Committee on Immunization Practices' Interim Recommendation for Use of Moderna Covid-19 Vaccine—United States, *Morbidity and Mortality Weekly Report (MMWR)*, *69*, 1653-1656. https://dx.doi.org/10.15585/mmwr.mm695152e1

Ortega-Rivera, O. A., Pokorski, J. K., & Steinmetz, N. F. (2021). A Single-Dose, Implant-Based, Trivalent Virus-like Particle Vaccine against "Cholesterol Checkpoint" Proteins. *Advanced Therapeutics*, https://doi.org/10.1002/adtp.202100014

Oyewumi, M. O., Kumar, A., & Cui, Z. (2010). Nano-Microparticles as Immune Adjuvants: Correlating Particle Sizes and the Resultant Immune Responses. *Expert review of vaccines*, *9*, 1095-1107. https://doi.org/10.1586/erv.10.89

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines — a new era in vaccinology. *Nature Reviews Drug Discovery*, *17*, 261-279. https://doi.org/10.1038/nrd.2017.243

Pawar, D., Mangal, S., Goswami, R., & Jaganathan, K. S. (2013). Development and Characterization of Surface Modified PLGA Nanoparticles for Nasal Vaccine Delivery: Effect of Mucoadhesive Coating on Antigen Uptake and Immune Adjuvant Activity. *European Journal of Pharmaceutics and Biopharmaceutics*, *85*, 550-559. https://doi.org/10.1016/j.ejpb.2013.06.017

Plotkin, S. (2014). History of Vaccination. *Proceedings of the National Academy of Sciences*, *111*, 12283. https://doi:10.1073/pnas.1400472111

Prego, C., Paolicelli, P., Díaz, B., Vicente, S., Sánchez, A., González-Fernández, Á., & Alonso, M. J. (2010). Chitosan-Based Nanoparticles for Improving Immunization against Hepatitis B Infection. *Vaccine*, 28, 2607-2614. https://doi.org/10.1016/j.vaccine.2010.01.011

Raghuvanshi, R. S., Singh, O., & Panda, A. K. (2001). Formulation and Characterization of Immunoreactive Tetanus Toxoid Biodegradable Polymer Particles. *Drug Delivery*, *8*, 99-106. https://doi.org/10.1080/10717540175017708 9Reddy, S-T., Swartz, M-A., & Hubbell, J-A. (2006). Targeting Dendritic Cells with Biomaterials: Developing the Next Generation of Vaccines. *Trends in Immunology*, 27, 573-579. https://doi.org/10.1016/j.it.2006.10.005

Repka, M. A., Shah, S., Lu, J., Maddineni, S., Morott, J., Patwardhan, K., & Mohammed, N. N. (2012). Melt extrusion: process to product. *Expert Opinion on Drug Delivery*, *9*, 105-125. https://doi.org/10.1517/17425247.2012.642365

Riccò, R., Liang, W., Li, S., Gassensmith, J. J., Caruso, F., Doonan, C., & Falcaro, P. (2018). Metal–Organic Frameworks for Cell and Virus Biology: A Perspective. *ACS Nano*, *12*, 13-23. https://doi:10.1021/acsnano.7b08056

Riedel, S. (2005). Edward Jenner and the History of Smallpox and Vaccination. *Baylor University Medical Center Proceedings*, *18*, 21-25. https://doi:10.1080/08998280.2005.11928028

Rizwan, S.B., McBurney, W.T., Young, K., Hanley, T., Boyd, B.J., Rades, T., & Hook, S. (2013). Cubosomes Containing the Adjuvants Imiquimod and Monophosphoryl Lipid a Stimulate Robust Cellular and Humoral Immune Responses. *Journal of Controlled Release*,*165*, 16-21. https://doi.org/10.1016/j.jconrel.2012.10.020

Rosenthal, K-S., & Zimmerman, D-H. (2006). Vaccines: All Things Considered. *Clinical and Vaccine Immunology*, 13, 821. https://doi:10.1128/CVI.00152-06

Roth, G. A., Gale, E. C, Alcántara-Hernández, M., Luo, W., Axpe, E., Verma, R., Yin, Q., Yu, A. C., Lopez Hernandez, H., Maikawa, C. L., Smith, A. A. A., Davis, M. M., Pulendran, B., Idoyaga, J., & Appel, E. A. (2020). Injectable Hydrogels for Sustained Codelivery of Subunit Vaccines Enhance Humoral Immunity. *ACS Central Science*, 6,1800-1812. https://doi:10.1021/acscentsci.0c00732

Ruprecht, R. M. (1999). Live Attenuated Aids Viruses as Vaccines: Promise or Peril? *Immunological Reviews*, *170*, 135-149. https://doi:10.1111/j.1600-065X.1999.tb01335.x

Rydell, N., Stertman, L., & Sjöholm, I. (2005). Starch Microparticles as Vaccine Adjuvant. *Expert Opinion on Drug Delivery*, 2, 807-828. doi:10.1517/17425247.2.5.807

Sah, H., Toddywala, R., & Chien, Y.W. (1995). Continuous Release of Proteins from Biodegradable Microcapsules and in Vivo Evaluation of Their Potential as a Vaccine Adjuvant. *Journal of Controlled Release*, *35*, 137-144. https://doi.org/10.1016/0168-3659(95)00029-8

Sahdev, P., Ochyl, L. J., & Moon, J. J. (2014). Biomaterials for Nanoparticle Vaccine Delivery Systems. *Pharmaceutical Research*, *31*, 2563-2582. https://doi:10.1007/s11095-014-1419-y

Salatin, S., Barar, J., Barzegar-Jalali, M., Adibkia, K., Milani, M. A., & Jelvehgari, M. (2016). Hydrogel Nanoparticles and Nanocomposites for Nasal Drug/Vaccine Delivery. *Archives of Pharmacal Research*, *39*, 1181-1192. https://doi:10.1007/s12272-016-0782-0

Sarei, F., Dounighi, N. M., Zolfagharian, H., Khaki, P., & Bidhendi, S. M. (2013). Alginate Nanoparticles as a Promising Adjuvant and Vaccine Delivery System. *Indian Journal of Pharmaceutical Sciences*, *75*, 442-449. https://doi:10.4103/0250-474X.119829

Schlake, T., Thess, A., Fotin-Mleczek, M., & Kallen, K.-J. (2012). Developing mRNA-vaccine technologies. *RNA Biology*, *9*, 1319-1330. https://doi.org/10.4161/rna.22269

Setia, S., Mainzer, H., Washington, M. L., Coil, G., Snyder, R., & Weniger, B. G. (2002). Frequency and Causes of Vaccine Wastage. *Vaccine*, *20*, 1148-1156. https://doi: 10.1016/S0264-410X(01)00433-9

Sexton, A., Whitney, P. G., Chong, S-F., Zelikin, A. N., Johnston, A. P. R., De Rose, R., Brooks, A. G., Caruso, F., & Kent, S. J. (2009). A Protective Vaccine Delivery System for in Vivo T Cell Stimulation Using Nanoengineered Polymer Hydrogel Capsules. *ACS Nano*, *3*, 3391-3400. https://doi.org/10.1021/nn900715g

Shao, S., Ortega-Rivera, O. A., Ray, S., Pokorski, J. K., & Steinmetz, N. F. (2021). A Scalable Manufacturing Approach to Single Dose Vaccination against HPV. *Vaccines*, *9*, 66. https://doi.org/10.3390/vaccines9010066

Shen, Y., Hao, T., Ou, S., Hu, C., & Chen, L. (2018). Applications and Perspectives of Nanomaterials in Novel Vaccine Development. *MedChemComm*, 9, 226-238. https://doi:10.1039/C7MD00158D

Shi, L., Caulfield, M. J., Chern, R. T., Wilson, R. A., Sanyal, G., & Volkin, D. B. (2002). Pharmaceutical and Immunological Evaluation of a Single-Shot Hepatitis B Vaccine Formulated with PLGA Microspheres. *Journal of Pharmaceutical Sciences*, *91*, 1019-1035. https://doi.org/10.1002/jps.10042

Silva, A. L., Soema, P. C., Slütter, B., Ossendorp, F., & Jiskoot, W. (2016). PLGA Particulate Delivery Systems for Subunit Vaccines: Linking Particle Properties to Immunogenicity. *Human Vaccines & Immunotherapeutics*, *12*, 1056-1069. https://doi.org/10.1080/21645515.2015.1117714

Sinani, G., Sessevmez, M., Gök, M. K., Özgümüş, S., Alpar, H. O., & Cevher, E. (2019). Modified Chitosan-Based Nanoadjuvants Enhance Immunogenicity of Protein Antigens after Mucosal Vaccination. *International Journal of Pharmaceutics*, 569, 118592. https://doi.org/10.1016/j.ijpharm.2019.118592

Singh, B., Maharjan, S., Sindurakar, P., Cho, K.H., Choi, Y.J., & Cho, C.S. (2018). Needle-Free Immunization with Chitosan-Based Systems. *International Journal of Molecular Sciences*, 19, 3639. https://doi:10.3390/ijms19113639

Singh, J., Pandit, S., Bramwell, V.W., & Alpar, H.O. (2006). Diphtheria Toxoid Loaded Poly-(E-Caprolactone) Nanoparticles as Mucosal Vaccine Delivery Systems. *Methods*, *38*, 96-105. https://doi.org/10.1016/j.ymeth.2005.11.003

Slütter, B., Soema, P. C., Ding, Z., Verheul, R., Hennink, W., & Jiskoot, W. (2010). Conjugation of Ovalbumin to Trimethyl Chitosan Improves Immunogenicity of the Antigen. *Journal of Controlled Release*, 143, 207-214. https://doi.org/10.1016/j.jconrel.2010.01.007

Smallshaw, J.E., & Vitetta, E.S. (2010). A Lyophilized Formulation of Rivax, a Recombinant Ricin Subunit Vaccine, Retains Immunogenicity. *Vaccine*, *28*, 2428-2435. https://doi.org/10.1016/j.vaccine.2009.12.081

Spellberg, B., & Edwards, J-E., Jr. (2001). Type 1/Type 2 Immunity in Infectious Diseases. *Clinical Infectious Diseases*, 32, 76-102. https://doi:10.1086/317537

Standley, S. M., Kwon, Y. J., Murthy, N., Kunisawa, J., Shastri, N., Guillaudeu, S. J., Lau, L., & Fréchet, J. M. J. (2004). Acid-Degradable Particles for Protein-Based Vaccines: Enhanced Survival Rate for Tumor-Challenged Mice Using Ovalbumin Model. *Bioconjugate Chemistry*, *15*, 1281-1288. https://doi.org/10.1021/bc049956f

Su, X., Kim, B-S., Kim, S. R., Hammond, P. T., & Irvine, D. J. (2009). Layer-by-Layer-Assembled Multilayer Films for Transcutaneous Drug and Vaccine Delivery. *ACS Nano*, *3*, 3719-3729. https://doi.org/10.1021/nn900928u

Sun, B. N., Yu, S., Zhao, D. Y., Guo, S. H., Wang, X. H., & Zhao, K. (2018). Polysaccharides as Vaccine Adjuvants. *Vaccine*, 36, 5226-5234. https://doi:10.1016/j.vaccine.2018.07.040

Tam, H. H., Melo, M. B., Kang, M., Pelet, J. M., Ruda, V. M., Foley, M. H., Hu, J. K., Kumari, S., Crampton, J., Baldeon, A. D., *et al*. (2016). Sustained Antigen Availability During Germinal

Center Initiation Enhances Antibody Responses to Vaccination. *Proceedings of the National Academy of Sciences*, *113*, E6639. https://doi:10.1073/pnas.1606050113

Tsoras, A. N., & Champion, J. A. (2019). Protein and Peptide Biomaterials for Engineered Subunit Vaccines and Immunotherapeutic Applications. *Annual Review of Chemical and Biomolecular Engineering*, *10*, 337-359. https://doi:10.1146/annurev-chembioeng-060718-030347

Uppu, D. S. S. M., Turvey, M. E., Sharif, A. R. M., Bidet, K., He, Y., Ho, V., Tambe, A. D., Lescar, J., Tan, E. Y., Fink, K., Chen, J., & Hammond, P. T. (2020). Temporal release of a three-component protein subunit vaccine from polymer multilayers. *Journal of Controlled Release*, *317*, 130-141. https://doi.org/10.1016/j.jconrel.2019.11.022

Vajdy, M. (2011). Immunomodulatory Properties of Vitamins, Flavonoids and Plant Oils and Their Potential as Vaccine Adjuvants and Delivery Systems. *Expert Opinion on Biological Therapy*, *11*, 1501-1513. https://doi:10.1517/14712598.2011.623695

Van Der Lubben, I. M., Verhoef, J. C., van Aelst, A. C., Borchard, G., & Junginger, H. E. (2001). Chitosan Microparticles for Oral Vaccination: Preparation, Characterization and Preliminary in Vivo Uptake Studies in Murine Peyer's Patches. *Biomaterials*, *22*, 687-694. https://doi.org/10.1016/S0142-9612(00)00231-3

van Riel, D., & de Wit, E. (2020). Next-generation vaccine platforms for COVID-19. *Nature Materials*, *19*, 810-812. https://doi.org/10.1038/s41563-020-0746-0

Vartak, A., & Sucheck, S. J. (2016). Recent Advances in Subunit Vaccine Carriers. *Vaccines*, *4*, 12. https://doi:10.3390/vaccines4020012

Velásquez-Hernández, Md.J., Ricco, R., Carraro, F., Limpoco, F. T., Linares-Moreau, M., Leitner, E., Wiltsche, H., Rattenberger, J., Schröttner, H., Frühwirt, P., *et a*l. (2019). Degradation of Zif-8 in Phosphate Buffered Saline Media. *CrystEngComm*, *21*, 4538-4544. https://doi:10.1039/C9CE00757A

Vogel, A. B., Lambert, L., Kinnear, E., Busse, D., Erbar, S., Reuter, K. C., Wicke, L., Perkovic, M., Beissert, T., & Haas, H. J. M. T. (2018). Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. *Molecular Therapy*, *26*, 446-455. https://doi.org/10.1016/j.ymthe.2017.11.017

Walke, S., Srivastava, G., Routaray, C.B., Dhavale, D., Pai, K., Doshi, J., Kumar, R., & Doshi, P. (2018). Preparation and Characterization of Microencapsulated DwPT Trivalent Vaccine Using Water Soluble Chitosan and Its in-Vitro and in-Vivo Immunological Properties. *International Journal of Biological Macromolecules*, 107, 2044-2056. https://doi.org/10.1016/j.ijbiomac.2017.10.073

Wang, C., Ge, Q., Ting, D., Nguyen, D., Shen, H-R., Chen, J., Eisen, H.N., Heller, J., Langer, R., & Putnam, D. (2004). Molecularly Engineered Poly(Ortho Ester) Microspheres for Enhanced Delivery of DNA Vaccines. *Nature Materials*, 3, 190-196. https://doi.org/10.1038/nmat1075

Wang, H., & Roman, M. (2016). Cellulose-Based Oral Vaccine Formulation for Influenza Virus. *Nanomedicine: Nanotechnology, Biology and Medicine*, *12*, 489. https://doi.org/10.1016/j.nano.2015.12.123

Wang, N., Chen, M., & Wang, T. (2019). Liposomes Used as a Vaccine Adjuvant-Delivery System: From Basics to Clinical Immunization. *Journal of Controlled Release*, *303*, 130-150. https://doi:10.1016/j.jconrel.2019.04.025

Wang, S., Mata-Fink, J., Kriegsman, B., Hanson, M., Irvine Darrell, J., Eisen Herman, N., Burton Dennis, R., Wittrup, K-D., Kardar, M., & Chakraborty, A-K. (2015). Manipulating the Selection Forces During Affinity Maturation to Generate Cross-Reactive Hiv Antibodies. *Cell*, 160, 785-797. https://doi.org/10.1016/j.cell.2015.01.027

Wang, Y., Deng L., Kang, S-M., & Wang, B-Z. (2018). Universal Influenza Vaccines: From Viruses to Nanoparticles. *Expert Review of Vaccines*, 17, 967-976. https://doi:10.1080/14760584.2018.1541408

Wang, Z.-B., Shan, P., Li, S.-Z., Zhou, Y., Deng, X., Li, J.-L., Zhang, Y., Gao, J.-S., & Xu, J. (2016). The Mechanism of Action of Acid-Soluble Chitosan as an Adjuvant in the Formulation of Nasally Administered Vaccine against HBV. *RSC Advances*, 6, 96785-96797. https://doi:10.1039/C6RA14419E

Welch, R. P., Lee, H., Luzuriaga, M. A., Brohlin, O. R., & Gassensmith, J. J. (2018). Protein–Polymer Delivery: Chemistry from the Cold Chain to the Clinic. *Bioconjugate Chemistry*, *29*, 2867-2883. https://doi:10.1021/acs.bioconjchem.8b00483

Wendler, D., Ochoa, J., Millum, J., Grady, C., & Taylor, H. A. (2020). COVID-19 Vaccine Trial Ethics Once We Have Efficacious Vaccines. *Science*, *370*, 1277. https://doi:10.1126/science.abf5084

Woolf, S. H., Chapman, D. A., & Lee, J. H. (2021). Covid-19 as the Leading Cause of Death in the United States. *The Journal of the American Medical Association*, *325*, 123-124. https://doi:10.1001/jama.2020.24865

Xing, L., Fan, Y.T., Zhou, T.J., Gong, J.H., Cui, L.H., Cho, K.H., Choi, Y.J., Jiang, H.L., & Cho, C.S. (2018). Chemical Modification of Chitosan for Efficient Vaccine Delivery. *Molecules*, 23, 229. https://doi:10.3390/molecules23020229

Yan, S. Q., Luo, Z. C., Li, Z. L., Wang, Y., Tao, J., Gong, C. Y., & Liu, X. G. (2020). Improving Cancer Immunotherapy Outcomes Using Biomaterials. *Angewandte Chemie-International Edition*, *59*, 17332-17343. https://doi:10.1002/anie.202002780

Yang, J., Li, Y., Jin, S., Xu, J., Wang, P. C., Liang, X-J., & Zhang, X. (2015). Engineered Biomaterials for Development of Nucleic Acid Vaccines. *Biomaterials Research*, *19*, 5. https://doi:10.1186/s40824-014-0025-8

Yang, L., Li, W., Kirberger, M., Liao, W., & Ren, J. (2016). Design of Nanomaterial Based Systems for Novel Vaccine Development. *Biomaterials Science*, *4*, 785-802. https://doi:10.1039/c5bm00507h

Yenkoidiok-Douti, L., & Jewell, C-M. (2020). Integrating Biomaterials and Immunology to Improve Vaccines against Infectious Diseases. *ACS Biomaterials Science & Engineering*, 6, 759-778. https://doi:10.1021/acsbiomaterials.9b01255

Yoon, Y. M., Lewis, J. S., Carstens, M. R., Campbell-Thompson, M., Wasserfall, C. H., Atkinson, M. A., & Keselowsky, B. G. A. (2015). Combination Hydrogel Microparticle-Based Vaccine Prevents Type 1 Diabetes in Non-Obese Diabetic Mice. *Scientific Reports*, *5*, 13155. https://doi.org/10.1038/srep13155

Yuan, C., Liu, Y., Wang, T., Sun, M., & Chen, X. (2020). Nanomaterials as Smart Immunomodulator Delivery System for Enhanced Cancer Therapy. *Acs Biomaterials Science & Engineering*, *6*, 4774-4798. https://doi:10.1021/acsbiomaterials.0c00804

Zhang, N-Z., Xu, Y., Wang, M., Chen, J., Huang, S-Y., Gao, Q., & Zhu, X-Q. (2016). Vaccination with Toxoplasma Gondii Calcium-Dependent Protein Kinase 6 and Rhoptry Protein 18 Encapsulated in Poly(Lactide-Co-Glycolide) Microspheres Induces Long-Term Protective Immunity in Mice. *BMC Infectious Diseases*, *16*, 168. https://doi.org/10.1186/s12879-016-1496-0

Zhang, R., Billingsley, M. M., & Mitchell, M. J. (2018). Biomaterials for Vaccine-Based Cancer Immunotherapy. *Journal of Controlled Release*, *292*, 256-276. https://doi:10.1016/j.jconrel.2018.10.008

Zhang, Y., Wang, F., Ju, E., Liu, Z., Chen, Z., Ren, J., & Qu, X. (2016). Metal-Organic-Framework-Based Vaccine Platforms for Enhanced Systemic Immune and Memory Response. *Advanced Functional Materials*, 26, 6454-6461. https://doi.org/10.1002/adfm.201600650

Zhao, D. Y., Yu, S., Sun, B. N., Gao, S., Guo, S. H., & Zhao, K. (2018). Biomedical Applications of Chitosan and Its Derivative Nanoparticles. *Polymers*, 10, 462. doi:10.3390/polym10040462

Zhao, L., Seth, A., Wibowo, N., Zhao, C-X., Mitter, N., Yu, C., & Middelberg, A-P-J. (2013). Nanoparticle Vaccines. *Vaccine*, 32, 327-337. https://doi.org/10.1016/j.vaccine.2013.11.069

Zhao, Y., Guo, Y. G., & Tang, L. (2018). Engineering Cancer Vaccines Using Stimuli-Responsive Biomaterials. *Nano Research*, *11*, 5355-5371. https://doi:10.1007/s12274-018-2162-1