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Biodegradable polymeric microsphere-based vaccines and their applications in infectious diseases

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Abbreviations and acronyms: APC, antigen-presenting cell; DEN-1–DEN-4, dengue virus serotypes 1–4; DC, dendritic cell; DT, diphtheria toxoid; DT or TD, diphtheria + tetanus vaccine; DTP, diphtheria + tetanus + pertussis vaccine; NS1, nonstructural protein 1; PEG, poly (ethylene glycol); PLA, poly (lactide); PLGA, Poly (lactic-co-glycolic acid); TT, tetanus-toxoid; VC, *Vibrio cholera*; WHO, World Health Organization.

Vaccination, which provides effective, safe infectious disease protection, is among the most important recent public health and immunological achievements. However, infectious disease remains the leading cause of death in developing countries because several vaccines require repeated administrations and children are often incompletely immunized. Microsphere-based systems, providing controlled release delivery, can obviate the need for repeat immunizations. Here, we review the function of sustained and pulsatile release of biodegradable polymeric microspheres in parenteral and mucosal single-dose vaccine administration. We also review the active-targeting function of polymeric particles. With their shield and co-delivery functions, polymeric particles are applied to develop single-dose and mucosally administered vaccines as well as to improve subunit vaccines. Because polymeric particles are easily surface-modified, they have been recently used in vaccine development for cancers and many infectious diseases without effective vaccines (e.g., human immunodeficiency virus infection). These polymeric particle functions yield important vaccine carriers and multiple benefits.

Introduction

Infectious diseases, caused by pathogenic microorganisms, are among leading global health problems. Millions of people die of infectious diseases annually. One approach toward preventing infectious diseases is vaccination, which helps an individual develop resistance to an infectious disease.

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The first vaccine targeted smallpox and was developed by Edward Jenner in 1796. Jenner initially inoculated a boy with cowpox virus and later reinoculated the boy again with smallpox virus after the boy had suffered from cowpox. The boy exhibited no symptoms of smallpox after the second inoculation. Thus, Jenner concluded that inoculation with the cowpox virus had protected the boy from smallpox. In 1980, after the achievement of global smallpox vaccination, the World Health Assembly endorsed the worldwide eradication of smallpox.

The earliest vaccines, such as smallpox vaccines, comprised inactivated or live attenuated viruses or bacteria. Bacterial toxoids, virus-like particles, and purified viral proteins and their subunits were introduced as knowledge about pathogens increased. These toxoid and subunit vaccines are considered safer and are not infectious.[1]

Today, several vaccines against infectious diseases are recommended for children. The recommended routine immunization schedule for children in Taiwan is listed in **Table 1**. Although vaccine immunization has succeeded in controlling several vaccine-preventable diseases in the developed countries, many children in developing countries continue to contract such diseases.[2] A complete immunization schedule for infants and children typically includes repeated vaccine administrations over the course of several years.[3] However, many rural children in developing countries have poor living conditions and medical care and therefore do not receive complete immunization.[4] To improve patient accessibility and maintain long-lasting protection, single-dose vaccines that mimic repeated injections administered via vaccination schedules may provide a promising solution.[5-10]

To mimic repeated injections of conventional vaccinations, single-dose vaccines have been used to release entrapped antigens over periods lasting weeks or months.[10] Several controlled release technology materials, including liposomes, polymers, and virus-like particles, have been tested to improve the efficacy of conventional vaccination.[6,11] The different types of polymers that can be used in controlled release delivery systems to encapsulate antigens and thus protect and control antigen release are listed in **Table 2**.[12-14]

Table 1. Immunization schedule for children in Taiwan

Immunization schedule Vaccine	<24 h after birth	≥24 h after birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	27 months	>5 years old
Hepatitis B	First dose		Boost 1			Boost 2					
BCG (Bacillus Calmette-Guérin)		One dose									
DTaP–Hib–IPV*				First dose	Boost 1	Boost 2			Boost 3		
Varicella							One dose				
MMR (Measles, mumps and rubella)							First dose				Boost 1
JE (Japanese encephalitis)								First dose Boost 1**		Boost 2	Boost 3
Influenza						First dose and boost 1 with an interval of 1 month and one boost every year after boost 1					
Hepatitis A							First dose		Boost 1		

*DTaP-Hib-IPV: Diphtheria, Tetanus, acellular Pertussis, Haemophilus influenzae type b conjugate and poliovirus vaccine.
**An interval of 2 weeks between first dose and boost 1.

Compared with conventional vaccines, a benefit of particle-based controlled release systems is the ability to simultaneously co-deliver antigens and adjuvants to the same antigen-presenting cells (APCs). Another advantage is the ability to protect antigens and adjuvants from degradation before reaching the target cells. Other advantages include site-directed delivery and the ability to induce cell-mediated immune responses.[15] Further, of the different materials used in particle-based controlled release systems, liposome delivery systems are less stable than polymer particle systems.[16] Although virus-like particles are stable as polymeric particles, they introduced the issue of capsid component immunogenicity.[17]

Biodegradable polymers are superior to non-degradable polymers because the later may require additional removal procedures. For reliability and reproducibility, synthetic biodegradable polymers are the best choice for antigen encapsulation in single-dose vaccine production. Poly (lactic-co-glycolic acid) (PLGA) is among the most widely used synthetic biodegradable polymers.[18] Biodegradable PLGA microspheres have been widely used because of their safety and ability to provide long-term controlled vaccine antigen release.[5,19] The microspheres have not only been used for controlled release vaccines but also in delivery systems for other drugs, such as cancer therapies and birth control.[20-22]

To mimic repeated immunizations, 2 types of vaccine antigen release are possible with biodegradable PLGA microspheres: sustained release and pulsatile release. Sustained release, or continued vaccine antigen diffusion after the initial release, mimics the administration of several small boosters. Pulsatile release, or a second vaccine antigen diffusion distinct from the first release, mimics the current immunization schedule.[6] In previous studies, microsphere-based vaccines were developed using sustained antigen releases; however, this method could not mimic clinical vaccine administration. Therefore, pulsatile antigen release was developed to improve the antigen release pattern.[6,23]

Here, we review the use of biodegradable polymeric microspheres for single-dose vaccines with parenteral and mucosal administrations. In addition to the function of sustained and pulsatile release of antigens, we also review the active-targeting function of polymeric particles. With their shield function and their ability to co-deliver antigens and adjuvants to the same target cells (e.g., dendritic cells), polymeric particles are applied to develop various vaccines. For examples, because they can prevent antigens from degradation and control release of antigens, they were applied to single-dose and mucosal administered vaccines. Furthermore, their ability to co-deliver antigens and adjuvants to the same target cells make them suitable to improve subunit vaccines, which are safer but sometimes fail to induce potent immune responses. Recently, polymeric particles have been used in the development of vaccines for cancers and many infectious diseases for which there are not currently effective vaccines (e.g., human immunodeficiency virus infection, malaria, and tuberculosis) because they are easy to be surface-modified. These functions of polymeric particles maintain their importance in the development of various vaccines and bring multiple benefits into vaccination.

Vaccines Encapsulated in Biodegradable Polymeric Microspheres—Parenteral Routes

To date, most vaccines are parenterally administered via intramuscular, subcutaneous, and intradermal injections. Several parenteral vaccines have been encapsulated in biodegradable polymeric microspheres, including the tetanus and diphtheria vaccine.

Tetanus vaccine

Tetanus, also known as lockjaw, is caused by the bacterium *Clostridium tetani* and is characterized by severe muscle spasms that initially occur in the jaw muscles. The administration of tetanus toxoid (TT) containing vaccines can prevent tetanus. Furthermore, a global initiative to eliminate neonatal tetanus was

Table 2. Polymer microsphere materials used in microencapsulation

	Types	Materials
Natural Polymers	Carbohydrates	Agarose
		Alginate
		Chitosan
		Polydextran
		Polystarch
		Starch
	Proteins	Albumin
		Collagen
		Gelatin
	Others	Calcium carbonate
		Lipids
		Tricalcium phosphate
Synthetic Polymers	Non biodegradable	Acrolein
		Glycidyl methacrylate
		Lactides
		Polyanhydride
		Polymethylmetharylate
		Polyiminocarbonates
	Biodegradable	Glycolides
		Epoxy polymers
		Hydrogels
		Paraffin
		Pegylated poly(lactide)
		Poly(lactide-co-glycolide)
		Polyacrylates
		Polyacrylonitrile
		Polyamide
		Polyamino acids
		Polycaprolactones
		Polyelectrolytes
		Polyester
		Polyethylene glycol
		Polyphosphazenes
		Polyurea
		Polyurethane

launched in 1989, and the World Health Organization (WHO) selected TT as the first single-dose vaccine to be administered via biodegradable polymeric microspheres.[24,25]

The sustained release of TT from biodegradable PLGA microspheres has been widely studied.[19,26] A pattern of constant release with a decreasing release rate after the initial burst of TT has been identified. Small-sized TT-PLA microspheres with rapid release kinetics induced an earlier release compared with larger TT-PLGA 50:50 microspheres with slow release kinetics.[26] A continuously increasing release rate after the initial burst was observed with low-molecular-weight TT-PLGA microspheres.[19]

A pulsatile release pattern that mimics the current vaccine regimen has also been investigated. The time between the first and second pulsed TT release is determined by the degradation rate of biodegradable polymers. The degradation rates of these polymers depend on the composition and molecular weight.[27] Moreover, the time between the first and second TT release increases from 21 d to 52 d as the lactic acid ratio increases from 50:50 to 75:25 and the molecular weight increases from 0.33 dl/g to 0.80 dl/g. The pulsatile release pattern was achieved with a combination of different TT-biodegradable PLGA microspheres. A single administration of a combination of PLGA 50:50 and PLGA 75:25 microspheres yielded a pulsed release pattern with second and third releases after the initial release.[28] The second release occurred between 3 and 5 weeks and the third release occurred between 9 and 11 weeks after the initial release. The antibody responses induced by single administrations of mixtures of TT-biodegradable PLGA microspheres with different particle sizes were similar to those obtained following 3 administrations of TT- aluminum.

In a mouse model, the in vivo induction of tetanus-specific antibodies following a single administration of TT-biodegradable PLGA microspheres with different compositions (PLGA 50:50 and PLGA 75:25) was compared with that following the conventional multiple administration of aluminum-adsorbed TT.[29] The abilities of the TT-biodegradable PLGA microsphere combination (PLGA 50:50 and PLGA 75:25) and of aluminum-adsorbed TT to elicit antibody titers were similar. In addition, protection against a subcutaneous TT challenge following immunization with this TT-biodegradable PLGA microsphere combination (PLGA 50:50 and PLGA 75:25) or aluminum-adsorbed TT was compared in this same mouse model. Both preparations protected the pre-immunized mice from a TT challenge.[29] These results demonstrated that a single administration of TT-biodegradable PLGA microspheres provided similar protective immunity against TT as did conventionally administered aluminum-adsorbed TT. A comparison between a single dose of TT-biodegradable PLGA microspheres and multiple doses of conventional aluminum-adsorbed TT was also studied in a rat model.[30] The antibody responses induced by a single dose of TT-biodegradable PLGA microspheres were similar to those induced by multiple doses of aluminum-adsorbed TT.[30] These results revealed that antigen encapsulation by microspheres reduced the number of required vaccinations while yielding a performance similar to that of conventional vaccines administered in multiple shots.

Diphtheria vaccine

Diphtheria is caused by the bacterium *Corynebacterium diphtheriae* and is characterized by the presence of pseudomembranes (adherent membranes) in the upper respiratory tract. Diphtheria can be prevented by the administration of diphtheria vaccines, which are based on diphtheria toxoid (DT). Diphtheria vaccines are usually combined with tetanus and pertussis vaccines to yield combination vaccines against diphtheria, tetanus, and pertussis (DTP vaccines). Combination vaccines against diphtheria and tetanus are also available as DT or TD vaccines.

Studies of the in vitro release and in vivo induction of DT-specific antibodies following a single administration of DT-biodegradable PLGA microspheres have been reported.[31] Sustained DT-specific antibodies were elicited in guinea pigs immunized with DT-biodegradable PLGA 50:50 microspheres. These antibody responses were comparable with those elicited by DT plus aluminum adjuvant.

Combination vaccines based on biodegradable PLGA microspheres have also been studied for tetanus and diphtheria.[32]

Guinea pigs were immunized with a single subcutaneous injection of a combination of TT-biodegradable PLGA microspheres and DT-biodegradable PLGA microspheres. The specific antibody titers following that immunization were comparable with those obtained from guinea pigs immunized with the licensed divalent vaccine. The protective immunity provided by immunization from the combined of TT- and DT-biodegradable PLGA microspheres was comparable with that induced by the licensed vaccine. Guinea pigs preimmunized with the combined of TT- and DT-biodegradable PLGA microspheres were also protected from tetanus and diphtheria toxins challenges 6 weeks after immunization.[32]

Vaccines Encapsulated in Biodegradable Polymeric Microspheres—Mucosal Routes

Although mucosal administration routes have the advantage of being needle-free, vaccine antigens are easily degraded during delivery through a mucosal route. The protection of vaccine antigens delivered via mucosal administration may be provided by encapsulation in biodegradable polymer microspheres.[33]

Mucosal immunity provides major protection against pathogenic microorganisms. Mucosal surfaces include the respiratory, alimentary, and urogenital tracts. Pathogenic microorganisms caused several infectious diseases adhering to mucosal surfaces. The induction of mucosal immunization through respiratory tracts by pathogenic antigens promotes the secretion of antigen-specific antibodies in various human body fluids.[34-38] This finding suggests that the mucosal route may be a good option for vaccine inoculation.[39]

Biodegradable polymeric microspheres are suitable for mucosal administration because the spheres can prevent antigens from low pH, bile salts, and digestive enzymes present in the gastrointestinal tracts.[40] PLGA and poly (lactide) (PLA) have been developed for vaccine delivery through the gastrointestinal tract and nasal cavity.[41,42] The delivery routes are Peyer's patches in the intestinal cavity and mucosa-associated lymphoid tissues in the nasal cavity.[43,44] The biodegradable polymeric microspheres have been used to encapsulate oral vaccines (e.g., cholera vaccine).

Cholera vaccine

Cholera, caused by the food- and water-borne bacterium *Vibrio cholera* (VC), is an acute intestinal infection. The main symptoms are copious, painless, watery diarrhea, and vomiting. Severe diarrhea and vomiting can lead to acute dehydration, and occasionally death. Cholera can be prevented by the administration of cholera vaccines.

In vivo induction of Vibrio-specific serum antibody titers following the oral immunization of VC-loaded PLG microspheres has been studied.[45] Compared with the administration of a VC solution, significantly higher serum titers of Vibrio-specific immunoglobulin (Ig) G and IgM antibodies were elicited in mice immunized with VC-loaded microspheres. These VC-loaded microspheres were also prepared in different combinations: 50:50 PLG, 75:25 PLG, and PLA/poly (ethylene glycol) (PEG) blended microspheres.[46] The serum titers of Vibrio-specific antibodies were examined in mice immunized with VC-loaded 50:50 PLG, 75:25 PLG, and PLA/PEG-blended microspheres. Higher antibody responses were elicited in mice immunized with VC-loaded 75:25 PLG microspheres, and the highest antibody responses were obtained in mice immunized with VC-loaded PLA/PEG-blended microspheres. The administration of VC-loaded PLA/PEG-blended microspheres protected preimmunized mice from a VC challenge with a survival rate of 92%.[46]

Vaccines Encapsulated in Biodegradable Polymeric Microspheres—Nonvaccine-Preventable Diseases

Vaccine-preventable diseases, such as tetanus and diphtheria, are infectious diseases, for which effective vaccines are available. In addition to these vaccine-preventable diseases, biodegradable polymeric microspheres have been studied in terms of encapsulation of antigens from diseases without effective vaccines.[47,48]

Dengue vaccine

Dengue, caused by dengue virus, is a mosquito-borne tropical disease. The main symptoms are mild or high fever, headache, muscle and joint pains, pain behind the eyes, and rash. No licensed dengue vaccine is currently available.

There are 4 distinct serotypes of the dengue virus, DEN-1–DEN-4. A study of the effectiveness of nonstructural protein 1 (NS1) protein-loaded microspheres against dengue 2 virus (DEN-2) has been reported.[48] The NS1 protein of DEN-2 was encapsulated in PLGA/PEG microspheres. Strong antibody responses were elicited in mice immunized with these NS1 protein-loaded PLGA/PEG microspheres. In a dengue virus challenge test in mice, an increased survival was observed in mice immunized with NS1 protein-loaded PLGA/PEG microspheres compared with mice immunized with NS1 protein plus an aluminum adjuvant or PBS solution.[48]

Active-Targeting Polymeric System

Most vaccines are believed to block the spread of infection primarily through the induction of protective antibodies.[49] Despite the importance of antibodies, in many infectious diseases for which no effective vaccines exist, such as human immunodeficiency virus infection, malaria, and tuberculosis, T-cell responses are believed to be required for protection.[50-52] In addition, to induce both cellular and humoral immunity, dendritic cells (DCs), the APCs that initiate adaptive immunity, have become a key target of vaccine design.[53]

The first attempt to use DC as a vaccination target initiated from ex vivo antigen-loaded DC.[54] In addition to cell-based immunotherapy involving *in vitro*-cultured, antigen-loaded DCs, another promising approach for designing DC-targeted vaccines is the selective targeting of DC-specific receptors by coupling the desired antigen to an antibody or ligand.[55] Several

studies have revealed that these direct-conjugate approaches efficiently induce antigen-specific CD4+ and CD8+ T-cell responses.[56,57] However, direct antigen-antibody conjugation may alter the antigen conformation. Furthermore, besides antigens, additional stimulating signals, usually provided by adjuvants, are often required to induce an effective immune response, particularly for subunit vaccines.[58] In the absence of an adjuvant, T cell tolerance may be induced instead of T-cell immunity.[59] Therefore, particulate carriers such as polymeric particles have been studied because of their abilities to co-deliver antigens and adjuvants to target cells (e.g. DCs).[60-62] In addition to protecting antigens and adjuvants from degradation, the selection of flexible combinations of target cells and adjuvants, which is important for inducing appropriate immune responses, is another advantage of the co-delivery of antigens and adjuvants by polymeric particles.

The direct incorporation of ligands, such as avidin–fatty acid conjugates, in the polymeric matrices of biodegradable PLGA polymeric particles has been reported.[63] However, PLGA polymeric particles are often PEGylated by anchoring a layer of PEG chains to which the targeting ligands or antibodies are attached.[64,65] The advantages of active-targeting polymeric particles are the protection of antigens from degradation; delivery of antigens to APCs, such as DCs, in a targeted and prolonged manner; prevention of antigen spread to the systemic circulation; co-delivery of antigens and adjuvants to DCs; lower required doses of antigens and adjuvants; and stability. However, antigen and adjuvant destabilization may occur during the preparation of polymeric particles.[17]

Conclusions

Biodegradable polymeric particles have been shown to be effective for the development of single-dose and mucosal vaccines and have been applied to the development of active-targeting delivery systems. These polymeric particles have been shown to release antigens in either a sustained or pulsatile pattern to provide long-lasting protection without repeated immunizations. The particles are also capable of delivering antigens via mucosal administration routes. Furthermore, the targeting functions of polymeric particles and their ability to co-deliver antigens and adjuvants make them important carriers in vaccine development. These functions permit the use of biodegradable polymeric particle-based vaccines not only for infectious diseases but also for cancers and chronic diseases. The development of such vaccines is expected to greatly improve protection against infectious diseases and cancers.

Particle-based delivery systems are used for mucosal vaccinations because they can protect antigens during gastrointestinal tract delivery. However, the uptake of particles by cells of the mucosal system is highly size-dependent.[66] Other challenges of polymeric microspheres include the problem of production scale-up and remnants of unacceptable solvents in the final products.[67] Moreover, the requirement of the microencapsulated polymeric microsphere solubility under harsh conditions, such as organic solvents or high temperatures, is problematic. Vaccine antigens and adjuvants may be degraded in such harsh conditions. Regarding the aspect of size, nanoparticles, which feature smaller sizes in the nanoscale range, can better penetrate mucosal barriers. Nanoparticles have gained considerable attention in recent years because of their broad applications for several uses, including industry, agriculture, medicine, cosmetics, and clothing.[68] Although there have been some concerns about their safety and the toxicities of various nanomaterials have been reported,[69-75] nanoparticle application for vaccines and immunotherapies remains very attractive. For example, vaccine delivery using nanoparticles has been recently reviewed.[67] Nanoparticles have also been discussed in the context of cancer immunotherapy.[76] Because the 2 size classes of polymeric particles have different properties,[77] different applications of microspheres and nanoparticles should be considered according to their advantages and disadvantages. For example, DCs preferentially take up nanoparticles over microparticles.[60] Nanoparticles also induce stronger humoral immune responses than microparticles.[78] Smaller nanoparticles traffic to lymph nodes because they can penetrate tissue barriers, whereas larger particles are usually retained at the site of injection.[79] In addition to size, surface charge is another factor that may affect the performance of polymeric particles. Positively charged particles induce stronger humoral immune responses than do negatively charged particles.[80] In another study, immunization with positively charged liposomes induced stronger antibody responses than did antigen alone.[81]

In this review, we have summarized the functions of biodegradable polymeric particles, including their shield, controlled-release, targeting, and co-delivery functions, as well as their applications in vaccines for several infectious diseases. These functions promote the importance of these particles as carriers in vaccine development. As infectious diseases still cause the deaths of many children in developing countries, the shield and controlled release functions of biodegradable polymeric particles make them useful in developing single-shot vaccines. Considering the discovery of DCs and further great achievements in this field, recently, the targeting and co-delivery functions of polymeric particles maintain their importance in the development of vaccines for cancers and many infectious diseases for which there are not currently effective vaccines, such as human immunodeficiency virus infection, malaria, and tuberculosis. In addition to the functions listed herein, the development of new materials; improvements in encapsulation procedures; understanding of the immune responses elicited by these polymeric particles; and improvements in the co-encapsulation of adjuvants, additives, and stabilizers in these polymeric particles will further improve and extend the applications of these particles to additional vaccines and therapies, resulting in the improvement of human health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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