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Review

Biodegradable polymers for modern vaccine development

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ABSTRACT

Most traditional vaccines are composed either of a whole pathogen or its parts; these vaccines, however, are not always effective and can even be harmful. As such, additional agents known as adjuvants are necessary to increase vaccine safety and efficacy. This review summarizes the potential of biodegradable materials, including synthetic and natural polymers, for vaccine delivery. These materials are highly biocompatible and have minimal toxicity, and most biomaterial-based vaccines delivering antigens or adjuvants have been shown to improve immune response, compared to formulations consisting of the antigen alone. Therefore, these materials can be applied in modern vaccine development.

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Introduction

Vaccines have significantly improved human health and have saved countless lives from various diseases [1]. Traditional vaccines are composed of either a whole pathogen, or its parts that induce a robust immune response [2]. However, they have not been effective against emerging pathogens such as *Mycobacterium tuberculosis*, human immunodeficiency virus (HIV), and hepatitis C virus [3] and instead have caused adverse effects. Such safety concerns have forced vaccine makers to shift their focus to subunit vaccines, composed of nucleic acids, recombinant proteins, or short peptide sequences, which favor safety over efficacy and may thus require additional immune stimulating agents, known as adjuvants [4]. These are essential components of modern vaccines, made of inorganic or polymeric materials, or macromolecular complexes, that are designed to enhance vaccine potency and the perdurance of immune responses [1]. Numerous compounds, including saponin, lecithin, mineral oils, and agar, have been investigated for their ability to enhance the effectiveness of antigens (Ags). Although most of these function as adjuvants in preclinical animal models, some have been identified as toxic and, therefore, unsuitable for use in humans. This has left aluminum compounds, used either alone or in combination with other compounds, as the major adjuvant in human vaccines [5]. However, it is becoming increasingly clear that these are ineffective against Ebola, influenza, tuberculosis, HIV infection, and malaria, which require complex immune responses, including the activation of CD4+ and CD8+ T cell-mediated immunity [3].

The recent outbreaks of Ebola and the Middle East respiratory syndrome have alarmed the medical community, demonstrating an urgent need for safe and effective vaccines to combat these infectious diseases [6]. To this end, novel vaccine delivery methods and adjuvants have been investigated [3]. The latter requires the development of complementary strategies to enhance the immune response toward a given Ag [7]. However, a critical challenge is to develop adjuvants with increased potency but minimal toxicity [8]. Only a few such compounds have reached clinical trial stages [9]. Biologics license applications for vaccines require evidence of strong safety/efficacy data from multi-center trials; purity, potency, and consistency between manufactured lots are also important. These stringent regulations pose an additional challenge to the development of new adjuvants [10].

Recently, polymeric biomaterial-based vaccine delivery systems and adjuvants have emerged as alternatives to classical alum-based adjuvants [11]. A summary of recent advancements in the applications of biomaterials as vaccine adjuvants or Ag carriers is presented in Fig. 1.

The most studied mechanism, triggered by polymeric biomaterials, relie on the activation of pathogenic pattern recognition receptors (PRRs), localized both at the surface and in the cytoplasm of immune cells, particularly dendritic cells (DCs) and macrophages. However, recent studies show that other cell types, like

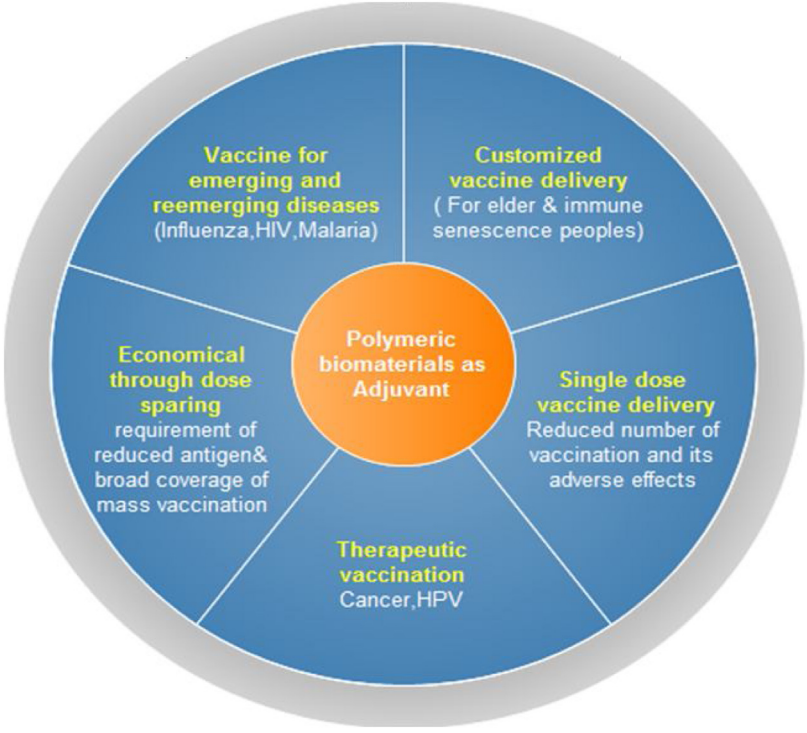

Fig. 1. Polymeric biomaterials as adjuvants.

mast cells (MCs), also have an important physiological role in the modulation of appropriate immune responses [12].

One of the most promising delivery strategies is the controlled release of vaccines from a biodegradable polymeric matrix [13]. The two major functions of biomaterial-based adjuvants are Ag delivery and immune stimulation [3]. Biomaterials are any biocompatible, natural or synthetic substance that can be engineered to interact with biological systems, and have favorable physiochemical properties, including high surface area for biofunctionalization (multivalent conjugation and ease of surface modification for specific cell targeting), flexible size (micro to nano scale), high Ag loading capacity and in vivo stability, and responsiveness to stimuli or to the environment.

Polymeric biomaterials can enhance immune responses in the following way: (1) They can increase the stability of an Ag by preventing its degradation through encapsulation or adsorption [14]; (2) they can incorporate multiple adjuvants and Ags, thereby enhancing the immunogenicity of weak Ags; (3) they are effectively taken and processed by Ag-presenting cells (APCs), which enhance cross-presentation through major histocompatibility complex class I (MHC I)-mediated cytotoxic (CD8+) T cell immune responses; and (4) their flexibility, in terms of surface functionalization, enables the active targeting of APCs through activation of endosomal TLRs or surface PRRs, which elicit robust immune responses (Fig. 2).

Collectively, these novel vaccine adjuvants or delivery systems have many advantages over classical vaccines including improved safety, higher immunogenicity through the stimulation of multiple immune response pathways, especially cell-mediated immunity (i.e., involving helper T cell [Th]-1), and greater flexibility in the route of administration (systemic, nasal, subcutaneous, or transdermal), making them more broadly applicable (e.g., to cancer, autoimmune diseases, and tuberculosis [TB]).

Numerous natural and synthetic biodegradable polymers have been investigated for vaccine development [3]. The development of controlled-release vaccines is hampered by the instability of Ags during encapsulation or processing, low encapsulation or adsorption efficiency, and colloidal instability [15], which can hinder their clinical translation [16]. Since modern vaccine formulations are tailored to current clinical requirements, careful selection of Ags and adjuvants is extremely important. Most biomaterials have complex immunological mechanisms that are not well understood.

In this review, we summarize the potential of various biodegradable materials as adjuvants and discuss the rational design of novel Ag carriers or adjuvants using representative, natural and synthetic biodegradable polymers (Fig. 3) as examples. We also critically evaluate the various factors contributing to immune stimulation and highlight recent applications of biodegradable material-based adjuvants and vaccines.

Techniques for adjuvants and vaccine delivery systems

Biodegradable polymers have been used as vaccine adjuvants to stimulate the immune system, and as carriers for vaccine delivery. Numerous forms of adjuvants and vaccine carriers have been developed, thus far.

Microparticles and nanoparticles

Synthetic or natural biodegradable polymer particles such as microcapsules, microspheres and nanospheres have been used as vaccine carriers and adjuvants, and function by encapsulating or through the adsorption of Ags [17]. Encapsulation within these particles can increase the stability of the Ags, and thereby deliver vaccines to APCs efficiently, to stimulate immune responses [14]. These particles can be used as immunostimulatory adjuvants via surface adsorption, or by coating the Ag membrane with the micro- and nanoparticles, in order to enhance immune response [18,19].

Nanometer-sized polymeric nanogels, or hydrogel nanoparticles are swollen networks of amphiphilic or hydrophilic polyionic

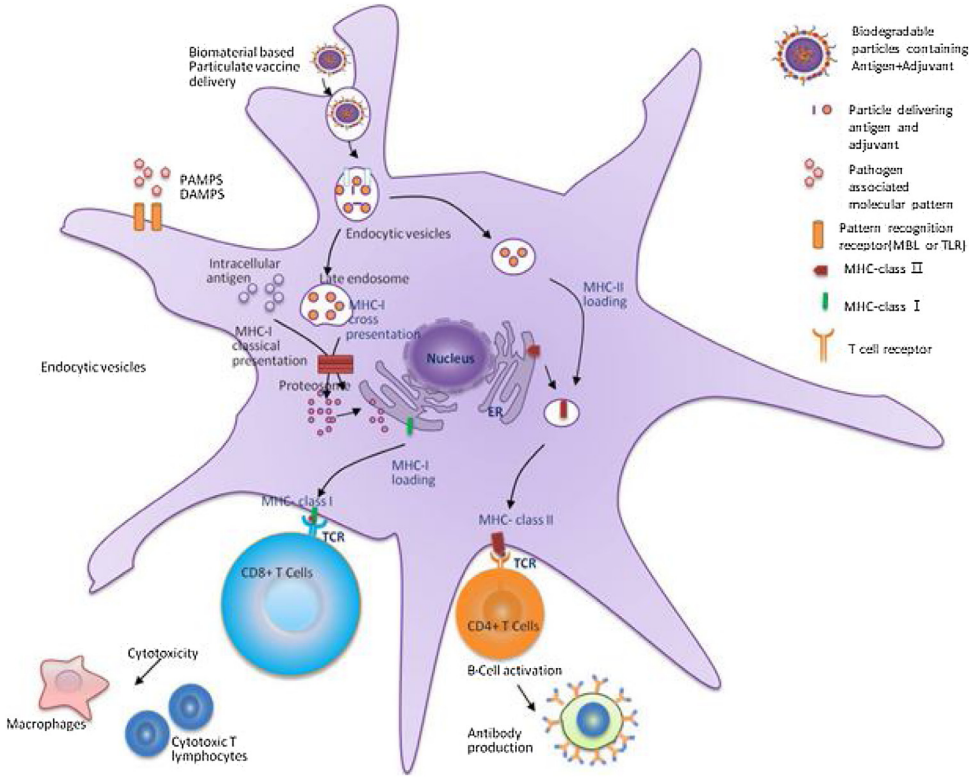

Fig. 2. Schematic illustration of MHC-1 mediated cytotoxic T cell immune system.

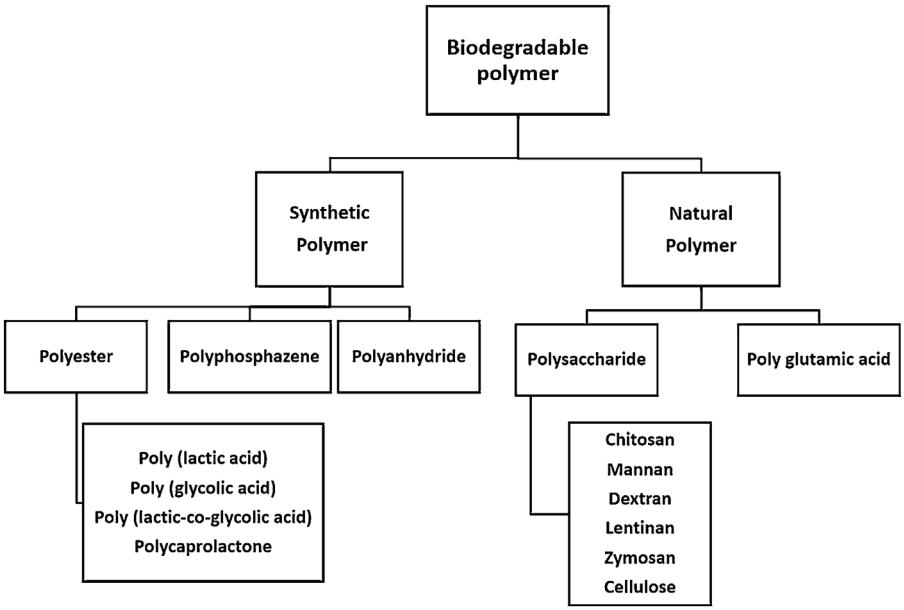

Fig. 3. Examples of natural and synthetic biodegradable polymers.

polymers. Nanogels are promising multifunctional polymeric nanoparticles, with the potential to be used as delivery systems because of their unique properties which include multivalent conjugation, high water content, and biocompatibility [20].

Nanogels have been designed using different approaches, such as physical self-assembly of interactive polymers, polymerization of monomers in a homogeneous phase or on a micro- or nanoscale heterogeneous environment, cross-linking of pre-formed polymers, and template-assisted nanofabrication [20]. Several synthetic and natural biodegradable polymers have been used to develop nanogels.

Microneedles

Microneedles are micronscale structures that can painlessly pierce through the skin to administer vaccines [21]. Ags are encapsulated within polymer microneedles and after insertion into the skin, the biocompatible polymer dissolves, within minutes, to release the vaccine [22]. Polyvinylpyrrolidone (PVP), which is used to make microneedles, has the chemical backbone structure of the vinyl pyrrolidone monomer ring, increasing intramolecular rigidity and providing mechanical strength to the polymer, which is important for microneedle insertion into the skin. PVP also has high water solubility, which facilitates rapid dissolution [22].

Nanofibers

The process of electrospinning provides a unique possibility to produce fibers of varying porosity, and thus, high surface area. The high surface area/volume ratio of these fibers, combined with their potential biocompatibility and biodegradable nature offers tremendous promise for biomedical applications such as targeted vaccine delivery [23]. In one study, the vaccine involved the delivery of *Shigella* antigens through a non-woven chitosan nanofibrous membrane [24]. These membranes which were produced through the process of electrospinning contained the N-terminal region of the IpaD Shigella subunit antigen [24]. An aqueous of chitosan (untreated and hydrolyzed)/ AcOH solution and the diluted recombinant antigen were used in all encapsulation processes, in a 1:1 volume ratio, to create a final recombinant antigen concentration of 0.5 mg/mL. This solution was used for electrospinning [24].

Hydrogel capsules

Layer-by-layer (LbL)-assembled nanoengineered hydrogel capsules are examples of a potent technology for the protection and delivery of labile vaccine candidates APCs [25]. Encapsulation in hydrogel capsules can protect the vaccines from degradation (ensuring the delivery of a high payload of antigen to APCs) and the particulate nature of the capsules makes them subject to phagocytosis by professional APCs and to macropinocytosis by DCs, targeting the delivery of the vaccine to key cells that initiate immune responses [25].

Chitosan hydrogels are a sustained-release vaccine delivery system, primarily consisting of the naturally occurring polymer chitosan [26]. Chitosan solutions can be altered by the addition of polyol salts to be thermosensitive, such that the hydrogels are only formed at body temperature. This provides an easily injectable subcutaneous depot system that can incorporate the adjuvant, while being a solution at room temperature [26].

Synthetic biodegradable polymers as vaccine adjuvants and delivery systems

Numerous synthetic biopolymers, including polyesters such as polylactides, polyglycolide, poly(lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL); polyphosphazene; and polyanhydrides have been investigated for their potential in vaccine delivery.

Here, we briefly discuss the factors that influence their potency as adjuvants.

Polyester

Biodegradable synthetic polymers that are commonly used in biomedical applications include aliphatic polyesters such as poly (lactic acid) (PLA), PLGA, and PCL, as well as their copolymers, a diverse family of synthetic biodegradable thermoplastic polymers that have been investigated as potential adjuvants and vaccine carriers [27,28]. Synthetic polyesters have excellent biocompatibility

and safety profiles and have been approved by the U.S. Food and Drug Administration (USFDA) and European Medicines Agency for biomedical applications [29].

Poly(glycolic acid) (PGA) and poly(lactic acid)(PLA)

PGA is a highly crystalline material that is susceptible to hydrolysis due to the ester bond present in the polymer backbone. As such, PGA is rapidly degraded, lacks mechanical strength, and produces an acidic environment. The hydrolysis of PGA to glycolide results in the activation of the classical complement pathway which leads to a strong, undesirable inflammatory response [30]. PLA is another important synthetic biopolymer that exist as different isomers; poly(L-lactic acid) and poly(D-lactic acid) have been studied in the context of vaccine development [17]. The additional methyl group in the PLA structure increases its hydrophobicity and stability against hydrolysis, as compared to PGA. PLA has been used for the preparation of microcapsules, microspheres, and nanospheres and is usually designed to achieve either controlled or pulsed release over a prolonged period [17]. For effective single vaccine delivery, pulsed release of Ag from the biopolymer matrix is essential [13]. An earlier study reported an enhancement in memory antibody response upon immunization with a single dose of tetanus toxin (TT)- or diphtheria toxin (DT)-loaded PLA particles [31]. Many studies since have highlighted the advantages of PLA–polyethylene glycol (PEG) nanocarriers [32]. For example, Verma et al. reported that the PLA–PEG-encapsulated M278 peptide (derived from the major outer membrane protein (MOMP) of Chlamydia) nanovaccine elicited strong $CD4^+$ T cell-mediated immune effector responses, mixed Th1 and Th2 responses, mucosal IgA responses and protected mice against chlamydial genital tract challenge [32]. The major advantages of this anionic PLA biopolymer is the possibility of encapsulation or adsorption of one or numerous antigens, together with adjuvant molecules like receptor ligands, to improve the immunogenic potential of Ags [33].

The surface adsorption of HIV type 1 (HIV-1) p24 Ag on surfactant-free anionic nanoparticles (NPs) has been shown to induce CD4+ T cell immune responses in experimental animals immunized subcutaneously [18]. Similarly, the co-adsorption of HIV-1 p24 and glycoprotein (gp)120 Ags on the PLA–NP surface showed strong immunogenicity [34], while co-encapsulation of the Toll-like receptor (TLR)7/8 and ovalbumin (OVA) Ag in PLA microparticles elicited an enhanced immune response [35]. A recent study comparing the adjuvant potential of PLA microparticles with alum, for H5N1 influenza split vaccines, revealed that PLA microparticles stimulated both humoral and cellular immune responses, whereas alum alone induced only the former [36]. These findings indicated that PLA biopolymer is a promising biomaterial for single-shot vaccines that can reduce the necessity for booster dosages and thereby reduce cost and the likelihood of adverse reactions.

PLGA

PLGA is a copolymer synthesized by the random polymerization of PLA and PGA [27]. The biodegradation of PLGA yields biocompatible lactic and glycolic acid, which are ultimately removed from the body through the citric acid cycle [37]. The composition of PLGA determines its Ag or adjuvant encapsulation and release profiles [13,27]. PLGA polymers with different stoichiometric ratios of lactic and glycolic acids, and different molecular weights, exhibit differences in Ag stability and release kinetics; for instance, PLGA with monomer ratios of 85:15, 75:25, and 50:50 have degradation times of 5–6, 4–5, and 1–2 months, respectively [27]. Thus, release kinetics can be predicted for different Ags or adjuvants, allowing long-term immune responses to be obtained from a single vaccination, without associated toxicity [13]. PLGA-based Ag carriers have been prepared as microspheres, microcapsules, and nanospheres to facilitate the controlled delivery of Ag payloads [11,37,38]. Poliomyelitis is a potentially fatal but vaccine-preventable infectious disease. However, 1 mL of the liquid inactivated polio vaccine (IPV) formulation is administered as two to three injections. While this is effective, it is infeasible in countries where patients may not have easy, or regular, access to healthcare. Interestingly, Tzeng et al. developed an injectable microparticle formulation of the inactivated polio vaccine (IPV) which released multiple pulses of stable antigen over time. This single vaccine technology is currently of particular interest as successful eradication will require an enormous coverage rate, to ensure that the disease cannot be carried or transmitted by unvaccinated individuals [15].

PLGA has numerous advantages such as dendritic cell (DC) targeting, high stability, ease of processing, and protracted release [13,39]. However, it also has limitations such as the production of an acidic microenvironment that can reduce Ag stability, and higher hydrophobicity, which decreases encapsulation efficiency and initial burst release, thereby altering the Ag release kinetics [13,37,40]. Nevertheless, such effects can be easily overcome by the inclusion of magnesium hydroxide or calcium carbonate [13]. Compared to alum adjuvants, PLGA biopolymers are inert carriers unless they have a specific composition or carry immunostimulants [41]. The adjuvant properties of PLGA particles are complex. In general, the uptake of Ags and adjuvant by APCs is facilitated by the delivery of the payload in particulate rather than soluble forms [42]. The cellular uptake of nano- and micro-sized PLGA particles is well documented [43]; these PLGA particles can protect Ag from premature proteolytic degradation and can function in vaccine delivery [44]. A variety of adjuvants and bacterial (hepatitis B virus [HBV] surface Ag [HBsAg] and Hip-B), viral (influenza), and parasite (malaria) Ags have been encapsulated or adsorbed onto PLGA nano-/microparticles [3,11,45,46]. However, the clinical development of PLGA-NPs presents several challenges, including their synthetic hydrophobic surface, low transfection efficiency (for DNA vaccines), short circulation half-life, and nonspecific tissue distribution. We, and others, have investigated various surface engineering strategies to overcome these problems [47–50]

PLGA biopolymers have considerable flexibility in terms of surface modification or functionalization for targeting [38,44]. PLGA particles functionalized with humanized targeting antibody hD1 and the encapsulating Ag was efficiently taken up by DCs, eliciting 10- to 100-fold more potent Ag-dependent T cell responses, even at low concentrations [44]. Similarly, surface modification of anionic PLGA with mucoadhesive polymers (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium, chitosan, *N*-trimethyl chitosan, and glycolchitosan) enhanced mucoadhesion, leading to a greater mucosal immune response (Fig. 4) [3,11,51,52].

The surface modification strategy has been applied for the development of oral mucosal vaccines against the HIV Ag. Oral vaccination with PLGA loaded with the HIV peptide Ag, together with poly(I:C) and CpG, which were designed to be released in the large intestine by surface coating with pH-responsive methacrylate-based Eudragit FS30D21 polymer, potently induced colorectal immunity and protected mice against rectal and vaginal viral challenges [53].

The physicochemical properties of PLGA-based particulate vaccines can be rationally optimized to allow targeted delivery of Ag for the modulation of local immune responses [54,55]. Micron-sized PLGA particles can efficiently stimulate humoral (Th2) responses, whereas nano-sized particles (<1000 nm) promote cellular (Th1) responses [54,55]. Thus, surface modification and target functionalization can enhance the immunogenic properties of PLGA biopolymer-based nano-/microparticles, allowing more rapid clinical translation [3,56,57]

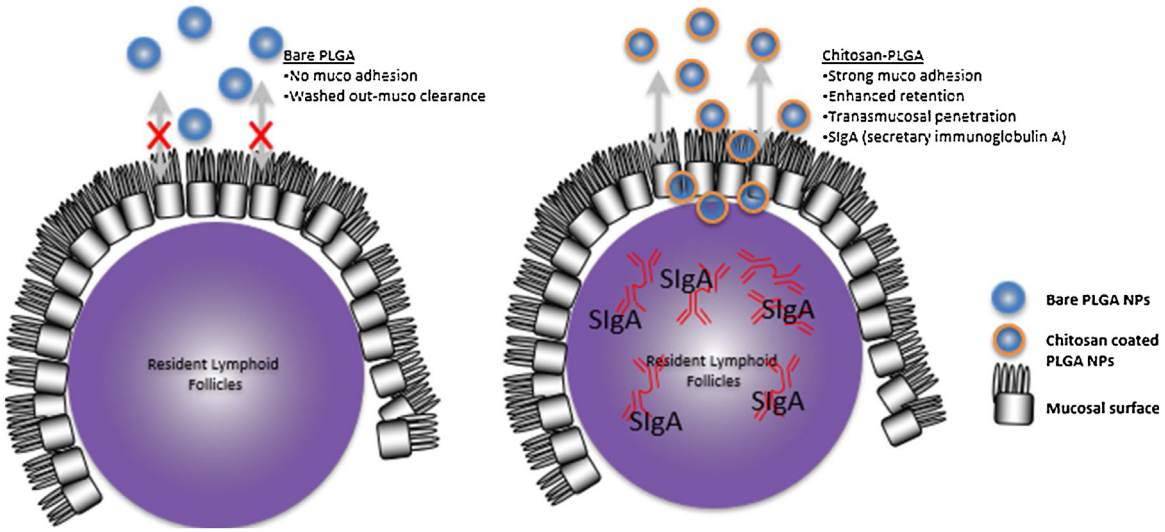

Fig. 4. Enhanced mucosal immune responses by surface modification of anionic PLGA with mucoadhesive polymers.

In addition, PLGA nanoparticles have been used as major core materials in the development of biomimetic nanovaccine platforms [19]. This new generation of biomimetic nano vaccines (BMNVs) prepared using cell membrane coating technology, in which whole cancer cell membranes (CCM) or CCM-derived vesicles can be used as a cell mimicking source [58]. These BMNV platforms that mimin the key features of surface molecular organization and physicochemical properties, such as the size and shape of biological entities, have emerged as a new concept in cancer vaccine development. Integrating synthetic PLGA nanoparticles wrapped with cancer cell derived membranes (CCMC) holds tremendous potential for cancer immunotherapy. CCMC offers a full array of tumor-associated antigens (TAAs) to immune cells, thereby stimulating robust tumor-specific immune responses [19]. We previously carried out extensive research on the preparation of cell membrane coated PLGA nanoparticles and demonstrated their potential biomedical applications [59]. In a recent study, Jin et al. demonstrated the potential application of human primary glioblastoma CCM (U87)-coated PLGA-NPs in cancer immunotherapy. Subcutaneous injection of these U87-CCMC NPs triggered a tumor-specific immune response by inducing CD4+ and, CD8+ T lymphocytes in the lymph nodes and spleens of a BALB/c mouse model [60].

PCL

PCL is a semi-crystalline polyester with a melting temperature of 55 °C – 60 °C and glass transition temperature of −54 °C that has been investigated as vaccine carrier and adjuvant. In contrast to PLGA, PCL degrades very slowly and does not generate an acidic environment [61]. PCL also has excellent biocompatibility [62]. The adjuvant potential of PCL-based microparticles with *Schistosoma mansoni* Ag for oral vaccine delivery has been tested [63]. Another study compared the immunogenicity of a nasally or orally administered recombinant *S. mansoni* (rSm28GST) Ag encapsulated in PLGA or PCL microparticles [64]. Other researchers tested hot saline extracts of *Brucella ovis*, encapsulated in PCL microparticles, as a vaccine against brucellosis infection in mice and found that the PCL–hardystonite extract of the *B. ovis* vaccine enhanced Ag-specific immunogenicity, and that oral administration of these particles activated interferon (IFN)-γ, interleukin (IL)-2, and—to a lesser degree—IL-4, indicating a mixed Th1/Th2 cellular immune response [65]. In a study comparing PCL, PLGA, and PLGA–PCL blended polymer NPs, loaded with DT Ag for mucosal vaccine delivery, PCL NPs induced a more potent DT-specific IgG antibody response than PLGA, which was positively correlated with polymer hydrophobicity [66]. Intranasal administration of *Streptococcus equi* Ag encapsulated in PCL NPs generated a strong mucosal immune response (secreted [s]IgA) in mice [67]. Similarly, HBsAg-loaded PCL microspheres administered intramuscularly evoked greater cellular (IFN-γ and IL-2) and humoral (IgG) responses in BALB/c mice than an alum-adjuvanted vaccine [68]. Chitosan-based surface modification of PCL NPs loaded with H1N1 hemagglutinin protein generated strong cellular and systemic/mucosal immune responses upon nasal administration [69]. A recent study investigating the long-term Ag release potential of PCL NPs encapsulating TT Ag and comparing the effect of PCL NP size (small, 61.2 nm and large, 467.6 nm) on immunological responses showed that particles <100 nm induced M1/M2-type macrophage polarization, Th1/Th2 polarization of autologous CD4 + T cells, and a CD8+ T cell response, whereas larger (450 nm) particles failed to induce polarization [70]. PCL and reduced aluminum adjuvant systems with TT as a model Ag induced higher levels of IFN-γ and IL-4 than conventional aluminum-adjuvanted formulations [71].

Polyphosphazene

Polyphosphazene is a synthetic, biodegradable macromolecular polymer, with phosphorous and nitrogen linearly attached through alternating single and double bonds [72]. The two phosphorous side groups are open to conjugation via esterification, etherification, or amidification [72,73]. Many studies have demonstrated the immunological adjuvant property of polyphosphazenes, and polyphosphazene-based polyelectrolytes can be used to prepare nano/micro-Ag carriers for Ag delivery [74]. Poly(di[sodium carboxlatophenoxy]phosphazene) (PCPP) salt is a potential mucosal adjuvant which can be combined with various Ags such as pertussis toxin, pneumococcal surface protein A, formalin-inactivated PR8 influenza virus, trivalent influenza virus, HBsAg, and HIV-1 LAI [73,75–77]. The incorporation of an additional polyphosphazene immune modulator showed superior immunogenicity [74]. The multicarrier efficiency of PCPP microparticles has been demonstrated using different Ags and adjuvants (Fig. 5) [78]. For instance, the immunogenicity of respiratory syncytial virus (RSV) F protein Ag and CpG oligodeoxynucleotide (ODN), and immunomodulatory innate defense regulator peptide adjuvant encapsulated in polyphosphazene microparticles, stimulated strong immune responses and conferred complete protection in

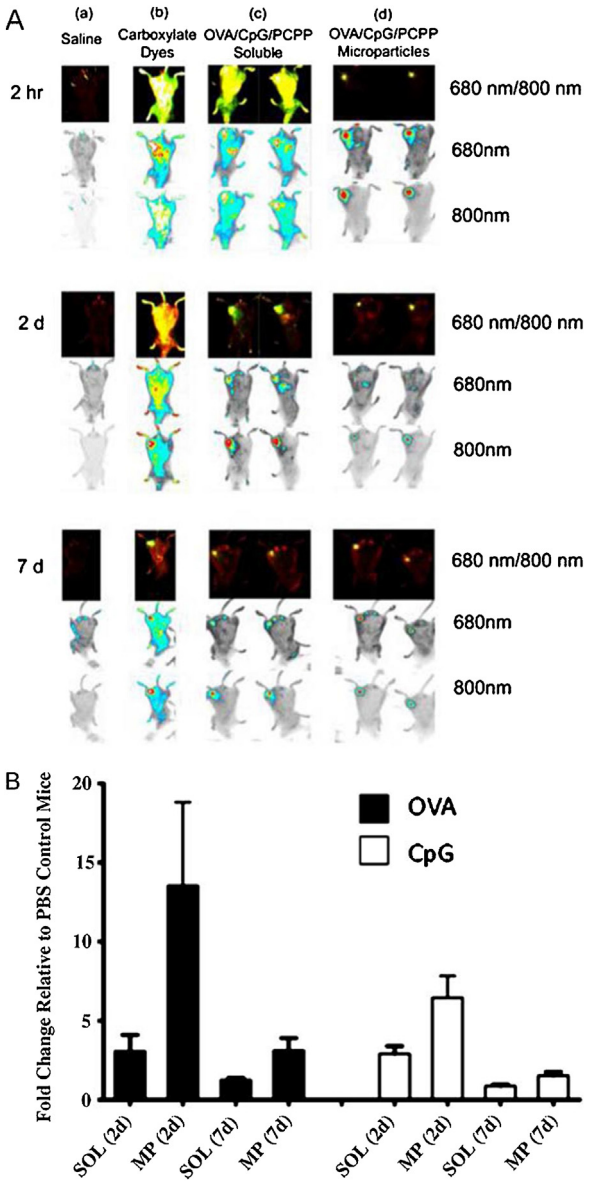

Fig. 5. Multicarrier efficiency of PCPP microparticles with different Ags and adjuvants (from Ref. [78]).

RSV-challenged animals [79]. Several studies investigating the mechanism of action of polyphosphazenes have suggested that poly(di[sodium carboxylatoethylphenoxy]phosphazene) (PCEP) activated the inflammasome through a caspase-dependent process, resulting in the secretion of IL-1β and -18. Furthermore, PCEP directly activated B cells to produce IgM and increased the production of Ag-specific IFN-γ by T cells [80]. These findings demonstrate that polyphosphazene-based synthetic biopolymers have immunological potential as adjuvants in modern vaccines.

Polyanhydrides

Polyanhydrides are surface erodible biopolymers that have been evaluated as possible adjuvants, or for Ag delivery. The metabolites of polyanhydride are carboxylic acids. These are biocompatible, non-mutagenic and have been approved by the USFDA [7]. Since the first report of polyanhydride-based carriers, the capacity of polyanhydrides for immune stimulation, Ag sparing (single-dose vaccination), prolongation of Ag exposure, and other immunological activities have been explored [81–84]. The degradation kinetics of polyanhydrides can be controlled by mixing rapidly degrading aliphatic polyanhydrides with their more slowly degrading aromatic counterparts to obtain polymers that encapsulate Ags in a stable manner. In contrast to the bulk erosion of PLGA polymers, polyanhydrides degrade by surface erosion, which ensures maximizes Ag release [81]. Polyanhydrides have numerous advantages over other synthetic polymers, such as high immunogenicity and capacity for Ag stabilization [7]. Sebacic acid, 1,6-bis (*p*-carboxyphenoxy) hexane, and 1,8-bis-(*p*-carboxyphenoxy)-3, 6-dioxaoctane are some of the hydrophobic moieties of polyanhydride that have been investigated for their adjuvant properties with diverse Ags [7,85,86]. Biofunctionalization of polyanhydride particles resulted in enhanced immune responses [87,88]; and bioadhesive polymer (mannose or flagellin)-modified polyanhydride NPs, with OVA as the Ag, elicited robust systemic and mucosal immune responses in an animal model, after oral immunization [87]. In a similar study, functionalization of polyanhydride NPs with pathogen-mimicking carbohydrate structures, (galactose and di-mannose) to target C-type lectin receptors expressed by alveolar macrophages, increased particle uptake, production of the pro-inflammatory cytokines IL-1β and IL-6, and tumor necrosis factor (TNF)-α [88]. It was also found that the internalization of nanoparticles enhanced expression of CD40 which activates CD4+ T cells (Fig. 6) [88]. Interestingly, TLR-9 agonist–CpG ODN-functionalized polyanhydride, with OVA as the Ag, had antitumor effects and enhanced CD8+ T cell and Ag-specific IgG_1 antibody responses in mice [89]. Thus, polyanhydrides are potential vaccine adjuvants that can modulate the immune response, induce long-term immunity with a single administration, and deliver Ags in a safe and continuous manner via multiple routes of administration.

Natural biodegradable polymers as vaccine adjuvants and delivery systems

Natural biopolymers have attracted attention as controlled delivery systems for peptide, protein, and DNA Ags. In addition to chitosan, protein assemblies and other naturally occurring biopolymers, such as β-glucans, dextran, and mannan, have been proposed for different vaccine applications. Interestingly, these biopolymers are present in the cell wall of various pathogens including bacteria and yeast and are thus inherently target APCs and potentiate the immune response to the associated Ags. Glucans were shown to enhance humoral and cellular immunity and exert their adjuvant effects by binding to specific carbohydrate receptors (lectins) such as mannan, β-glucan, and dectin-1 receptor, expressed on monocytes and other APCs, resulting in the activation of nuclear factor (NF)-κB, maturation of monocytes, and production of pro-inflammatory cytokines [5]. In addition, natural biopolymers are biocompatible with minimal toxicity, making them ideal candidates for modern vaccine development [90]. Natural biopolymer-based nanovaccines can be prepared by simple methods such as solvent displacement, complexation, and ionic gelation, which reduce the use of organic solvents, thereby improving the stability of the biomolecules [90,91]. Above all, the biopolymer selected as an adjuvant or for vaccine development should be of high purity and safe for human use [5,16]. The major natural biopolymers with demonstrated adjuvant properties and their application in modern vaccine development are described in the following sections.

Polysaccharides

Advances in biological and microbiological technologies have increased our understanding of pathogens and have led to the development of newer and safer subunit Ags. Nevertheless, these

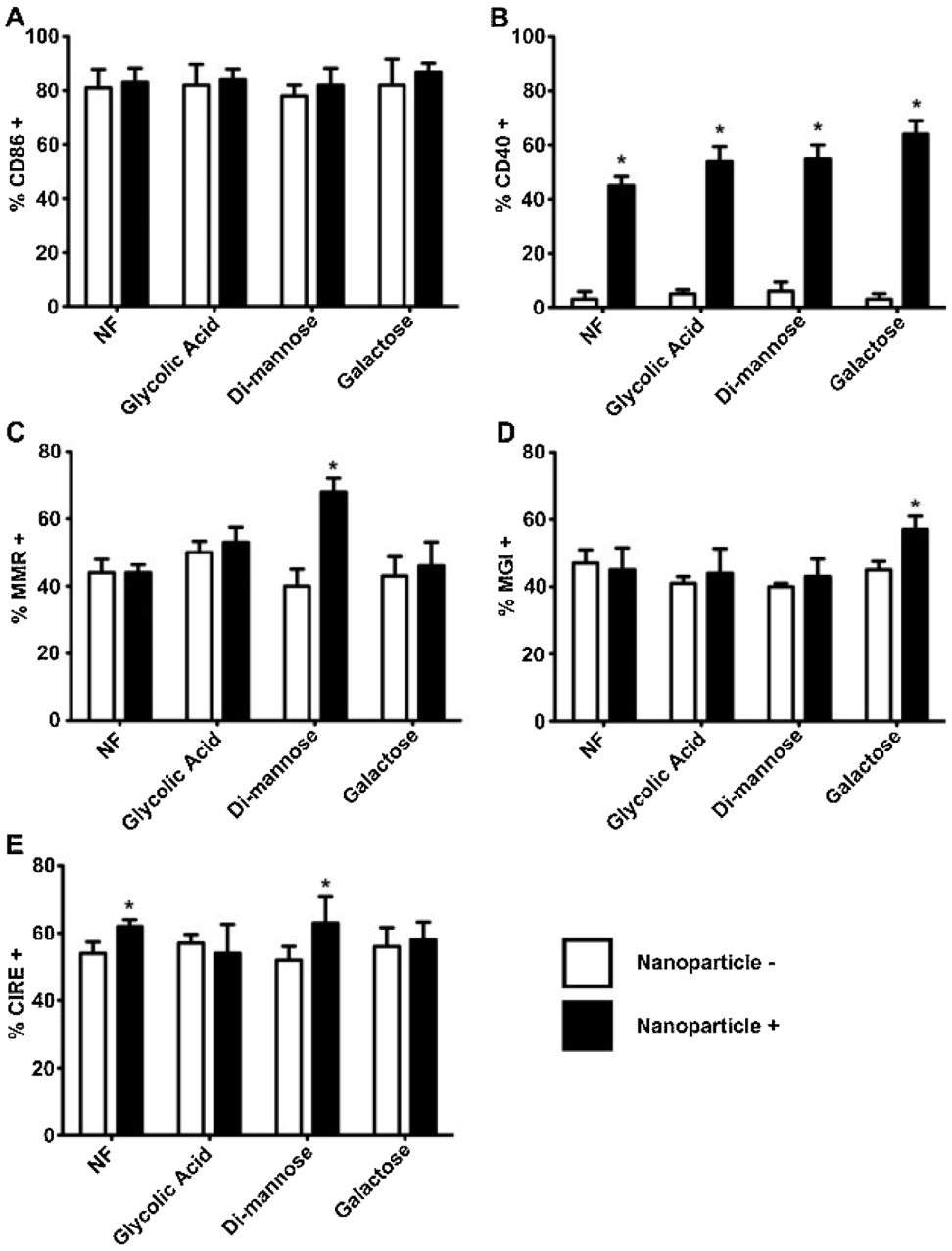

Fig. 6. Internalization of functionalized polyanhydride NPs is required for upregulation of CD40 expression on the surface of alveolar macrophages (from Ref. [88]).

Ags are less effective in inducing protective immune responses and, therefore, require parallel development of adjuvants such as immunomodulatory molecules and particle delivery systems. Polysaccharide-based nanosystems are potentially useful for the development of vaccine formulations (Fig. 7) [16,92].

Chitosan

Chitosan is a natural linear poly(amino saccharide) composed of α (1–4) 2-amino 2-deoxy β-D glucan—a copolymer of glucosamine and *N*-acetyl glucosamine, obtained by the alkaline deacetylation of chitin—that shows promise as an immunological adjuvant [93,94]. Chitosans are of special interest for mucosal vaccine delivery owing to their bioadhesivity, biocompatibility, and low toxicity [16]. The mucoadhesive nature of chitosans is attributed to the electrostatic interactions between positively charged chitosan and negatively charged sialic acid residues on the mucosal surface [94]. Chitosan can induce the redistribution of cytoskeletal F-actin and the tight junction protein zona occludens-1, thus promoting paracellular permeability to hydrophilic macromolecules [95]. The mucoadhesive properties of chitosan prolong the residence time of formulations in mucosa associated lymphoid tissues (MALTs) [96]. Chitosan and its derivatives have been formulated as solutions, gels, powders, and microparticles/NPs to deliver Ags via various routes of administration [97]. Chitosan was shown to activate rat macrophages in vitro and exert local and systemic effects through the activation of cytokines such as TNF α, IL-12, IL-4, and IL-10, as well as transforming growth factor-β [98].

Chitosans are increasingly being used in nanovaccine development [96]. Chitosan-based nanovaccine–DNA NPs are taken up by M cells in MALT [94], and chitosan DNA nanospheres stimulate Th1-dependent cytokine and cytotoxic T lymphocyte (CTL) responses, when co-administered with Ags [99]. As an adjuvant for H5 inactivated influenza vaccines, administered intramuscularly in mice, chitosan increased antibody titers and conferred

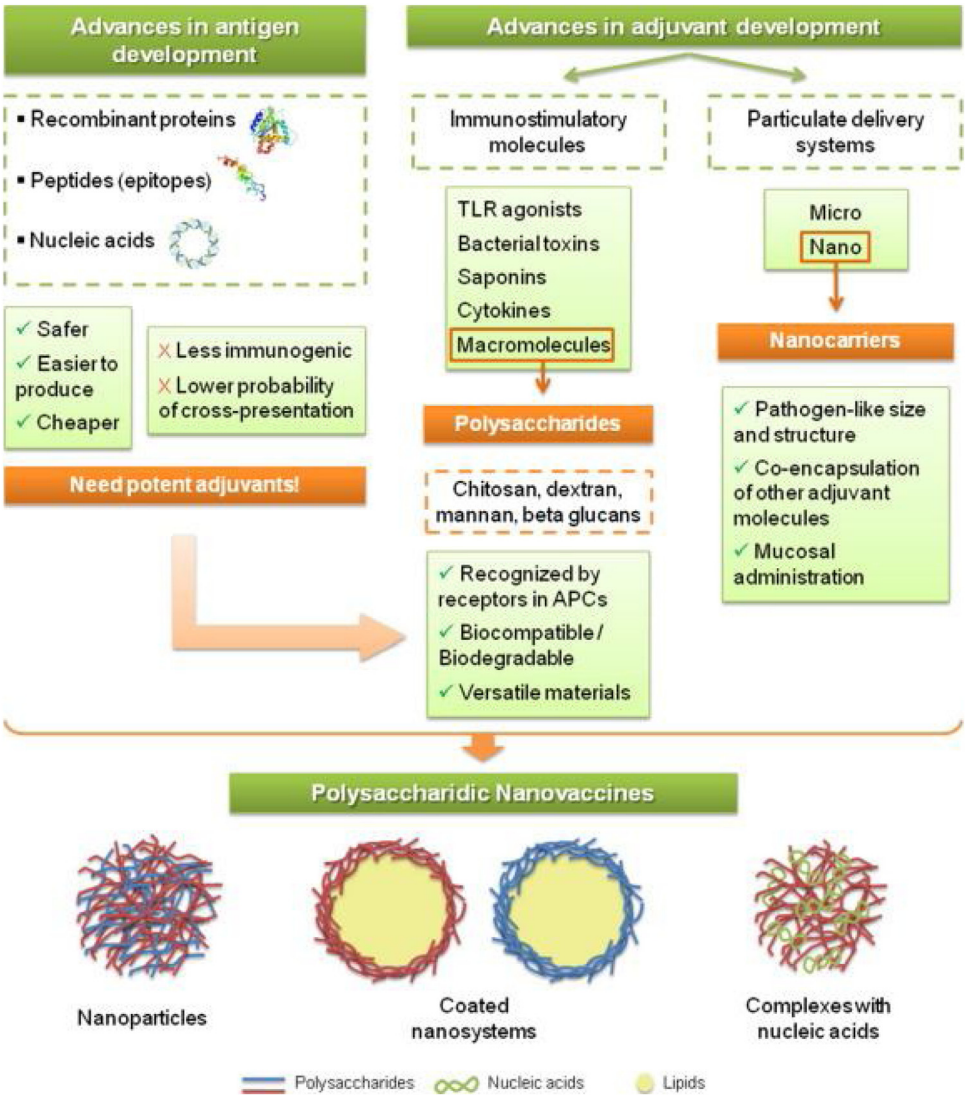

Fig. 7. Advances in Ag and adjuvant development and schematic illustrations of polysaccharide-based nanosystems (from Ref. [16]).

protection against homologous as well as drift variants of the influenza virus [100]. Furthermore, Ag-specific humoral and T (CD4+) cell-mediated immune responses to chitosan, delivered subcutaneously, along with β-galactosidase as the Ag were higher than responses to non-adjuvanted and alum-adjuvanted groups [101]. In another study, PLA–chitosan microparticles encapsulating HBsAg stimulated serum-specific IgG to a greater extent than PLA/HBsAg microparticles and free HBsAg [102]. The greater adjuvant potency of chitosan, relative to alum, with the inactivated H5N1 influenza virus vaccine, was also demonstrated in mice [103].

The major challenge for the clinical translation of chitosan-based mucosal vaccines is their uncontrolled precipitation at physiological pH [16]. However, a few studies have been conducted in human subjects. CRM_{197} formulated with chitosan showed promising results as a protective mucosal vaccine against diphtheria in humans [104], and a chitosan-based nasal delivery system with inactivated, subunit influenza vaccine was also tested in humans [2]. Another study showed that chitosan-adjuvanted Ag had higher immunogenicity than alum-adjuvanted vaccine in healthy human volunteers [105]. The Swedish company ViscoGel AB recently developed the chitosan-based adjuvant ViscoGel, which was found to be safe and effective in preclinical studies [106]. In a clinical trial, chitosan co-administered with Act-HIB vaccine in healthy volunteers showed good safety profiles and was more effective than Act-HIB vaccine alone [107].

Mannan-based biopolymers

Mannan is a natural biopolymer of mannose with a 1, 4-linkage that selectively binds to mannan-binding lectin (MBL), a C-type lectin of the mannose receptor family, that is involved in phagocytosis, complement activation, and supplementary opsonization-based immune responses [5,108,109]. These MBL interactions have been correlated with DC trafficking and induction of both humoral and cellular responses [110]. Mannan derivatives such as the reduced and oxidized forms have dual activity towards APCs—namely, inducing phenotypic variation and functional maturation of DC cells. These derivatives also induce the upregulation of inflammatory cytokines such as IL-1β and TNF-α, as well as Th1/Th2 cytokines. The oxidized form of mannan stimulated a Th1-type CTL response with high IFN-γ secretion, no IL-4 release, and a predominant IgG2 antibody response, whereas the reduced form stimulated Th2-type responses with IL-4 production and a predominant IgG1 antibody response, but no IFN-γ [5]. Intranasal administration of oxidized mannan with recombinant protein Ags induced a more potent immunological response than cholera toxin through the production of sIgA, in

various mucosal secretions, and Ag-specific IgG1 and IgG2a in serum [111].

Aloe polymannose combined with coxsackievirus B3 in a murine model increased Ag-specific antibody production against capsid protein epitopes of non-enveloped picornavirus, compared to the control [112]. The adjuvant effect of mannan-coated liposomes on HIV-1 DNA vaccine was evidenced by the enhanced stimulation of Th1-mediated cellular immune responses [113]. Similarly, *O*-palmitoyl mannan-coated niosomes, as a carrier and adjuvant system for HBV DNA vaccine delivered orally, elicited mucosal, systemic, and cellular immune responses [114].

Dextran and biopolymer combinations

Dextran, a highly hydrophilic neutral polysaccharide produced by certain acid lactic bacteria, such as *Leuconostoc mesenteroides*, consists of a main chain of α-1,6-linked glucose and α-1,3 branches with a large number of hydroxyl groups, which make it a good target for modification of its solubility pattern [115]. This microbial polysaccharide, and its sulfated derivative, have immunogenic and pro-inflammatory effects in mice. Diethylaminoethyl (DEAE)-dextran, a polycationic derivative containing DEAE groups linked to glucose, has been evaluated as a veterinary adjuvant for inactivated virus vaccines since the 1970s. Although its mechanism of action is not clear, its effects on the humoral response are recognized and suggest that DEAE-dextran stimulates and polarizes the response to Th2 for antibody synthesis as detected when administered with formalin-inactivated *Venezuelan equine* encephalomyelitis virus [5,92,116]. Dextran nanospheres with entrapped Ag and DEAE have also been used as adjuvants [117]. Whole cell cholera Ag and DEAE–dextran were shown to enhance immune responses [118], while DEAE–dextran adjuvant, combined with whole-cell typhoid vaccine, was more effective by the active mouse protection test than TT-adjuvanted vaccine or Ag alone [119]. Acetylated dextran (Ac-DEX) is a dextran derivative that can be synthesized by reversible modification of dextran hydroxyl groups with acetal motifs [120]. Modifying the properties of Ac-DEX based particles was shown to alter Ag presentation in MHC I and II presentation assays [121].

Ac-DEX is a water-insoluble biopolymer that is relatively stable at pH 7.4 but degrades rapidly under the acidic conditions of the lysosomal compartment (pH 5.0) of APCs. These properties of Ac-DEX have been used for the delivery of the TLR-7 agonist imiquimod to intracellular TLR-7 receptors. Encapsulation of imiquimod in Ac-DEX microparticles increased immune stimulation by IL-1, IL-6, and TNF-α [122]. Ac-DEX was similarly used to deliver poly(I:C) and CpG adjuvants to macrophage phagosomes [123]. Another study used Ac-DEX microcarrier encapsulating recombinant protective Ag and the TLR-7/8 agonist resiquimod, for anthrax vaccine development [124]. DEAE–dextran with *Haemonchus contortus* larva-specific Ag (HcsL3) in sheep conferred greater protection than alum or Quil A-adjuvanted vaccine [125]. Microspheres composed of a blend of PLGA and dextran have also been developed for the intramuscular delivery of HBsAg [126].

Dextran-based nanovaccines induce robust immune responses. Incorporation of OVA and lipopolysaccharide in dextran NPs stimulated APCs in a mannose receptor-dependent manner as well as strong cellular (OVA peptide-specific CD4+ and CD8+ T cell) and humoral immune responses in mice [127]. Dextran and chitosan-based polyelectrolyte complexes have been used to deliver p24, and HIV-1 capsid protein [128].

Lentinan and its synthetic analogue

Lentinan is a glucohexose-based fungal β-glucan that is known for its anti-tumor properties. Lentinan was shown to stimulate the proliferation of blood mononuclear cells such as lymphocytes, monocytes, and macrophages and increase resistance to malignant transformation, and was demonstrated as an effective adjuvant for T cells [129]. DNA vaccination can induce specific CD8+ T cell immune responses, but at a relatively low level in humans, due to their large size. Lentinan has been evaluated for its potential to maximize the immunity elicited by DNA vaccines [130]. Lentinan has shown immunomodulatory abilities against respiratory influenza virus infection [131], and acted synergistically with Bacillus Calmette–Guérin Ag in activating alveolar macrophages [132]. Furthermore, lentinan showed immunotherapeutic effects along with DC vaccines in the treatment of B16 melanoma. The presence of lentinan enhanced the efficiency of gp100+ DCs through activation of T and natural killer cell populations via stimulation of IL-2 and IFN-γ production [133]. The adjuvant potency of the synthetic β-(1 → 6)-branched β-(1 → 3) glucohexose analog of lentinan (β-glu6) was demonstrated in conjunction with HBV core Ag ([HBcAg]-pB144)-encoding DNA vaccines in mice. Lentinan (β-glu6) enhanced the recruitment and maturation of APCs and CD8+ and CD4+ T cell activation and increased the size of the T cell population [130]. Finally, in BALB/c mice, β-glu6-adjuvanted HBsAg vaccine elicited both humoral and cellular immune responses by promoting APC maturation, Ag presentation, and activation of CD4+ T cells, leading to IL-4 expression and anti-HBV antibody production [134].

Zymosan

Zymosan (β-1,3-glucan) is an insoluble preparation of *Saccharomyces cerevisiae* cell wall that stimulates immune cells such as APCs through TLR-2 and Dectin-1, a phagocyte receptor expressed on DCs and macrophages that induces inflammatory cytokine production via NF-κB activation and also stimulates different complement pathways [135]. In BALB/c mice, HIV-1 DNA vaccine combined with zymosan enhanced the humoral and cellular immune responses via an alternative pathway that promoted the recruitment and activation of APCs [136]. Zymosan activates the inflammasome of macrophages and DCs through cryopyrin inflammasome and apoptosis-associated speck-like protein containing a caspase recruitment domain [137]. In addition, zymosan acted synergistically with mucosal adjuvant poly(I:C) in influenza vaccines. Zymosan may enhance the adjuvant activity of poly(I:C) by increasing the production of cytokines (i.e., IL-6, IL-10, IFN-β and TNF-α) by DCs, resulting in simultaneous activation of poly(I:C)-induced TLR3-mediated and zymosan-induced TLR2-mediated signaling pathways [138]. Interestingly, the latter could provide a basis for developing a neonatal vaccine adjuvant since the immaturity of TLR-mediated innate immunity in neonates depends on monocytes rather than on DCs [139].

Cellulose-based biopolymers

Cellulose is a polysaccharide composed of β (1 → 4) linked D-glucose units, components of the cell wall of plants. Microcrystalline cellulose and cellulose derivatives, such as carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and hydroxypropylmethyl cellulose (HPMC), are recognized as the popular polymeric materials routinely used in medical and pharmaceutical applications [140]. Especially, the novel CMC–QC (quaternized cellulose) nanoparticles have proven to be promising vehicles for controlled delivery of protein drugs [140]. Both CMC and QC come from cellulose, which is a natural material with good tolerance by the body [140]. Moreover, the formation of CMC–QC nanoparticles is through physical crosslinking by electrostatic interactions instead of chemical bonds, therefore possible toxicity or deactivation caused by chemical crosslinking reagents is avoided [140]. These attractive features make CMC–QC nanoparticles potential carriers for prophylactic and therapeutic active agents, 28 such as DNA vaccines [140].

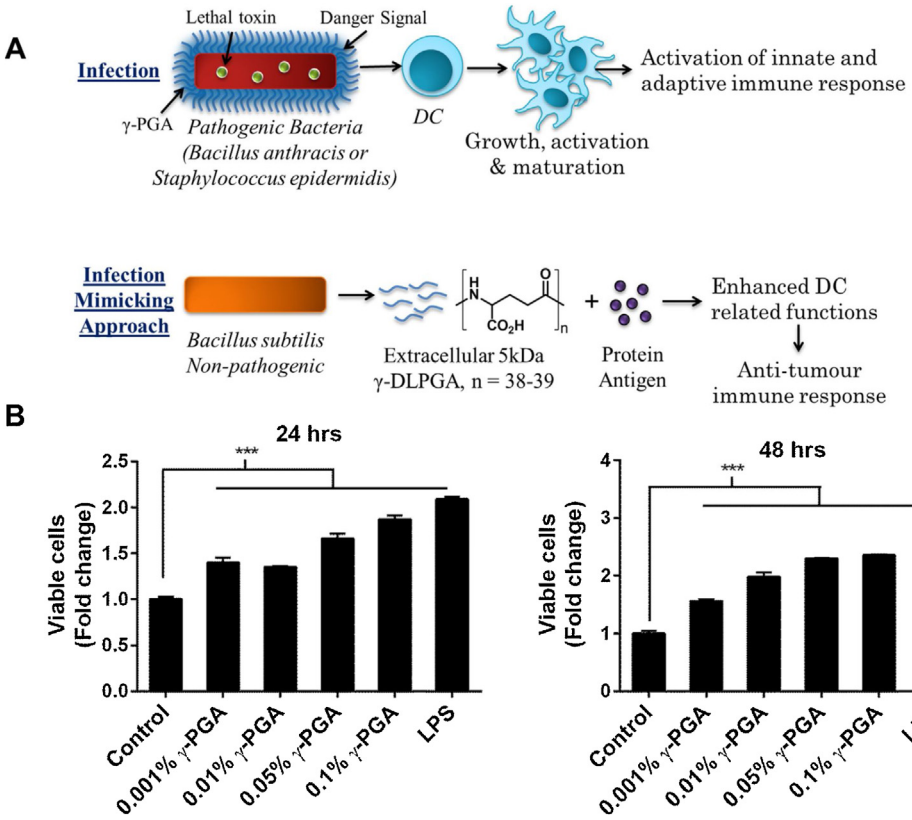

Fig. 8. (A) Schematic illustrating immune-stimulation upon encounter with pathogenic bacteria. (B) In vitro proliferation of BMDCs in the presence of various concentration of 5 kDa γ-PGA and the positive control, LPS(0.1 μg/ml). $^{***}p < 0.001$ by one-way ANOVA, with Vonferroni posttest (from Ref. [155]).

Cellulose acetate phthalate (CAP) is and anionic polymer composed of phthalic acid and acetate [141]. Thiolated polymers have been intensively used as mucoadhesive polymers because these polymers can form covalent bond between thiol groups of thiolated polymer and cysteine rich subdomain of mucus glycoproteins through disulfide bonds [141]. M5BT protein as a subunit vaccine for the FMD was encapsulated into T-CAP microparticles (MPs) [141]. It is important to develop vaccine delivery carrier to prevent disease infected through mucosa [141]. CAP also has anti-HIV-1 activity. CAP in micronized form and formulated into a cream, is a broad spectrum microbicide inactivating several sexually transmitted disease (STD) pathogens, including HIV-1 [142].

PGA

γ-PGA is a water-soluble, non-toxic poly (amino acid) produced by certain strains of *Bacillus* [143]. γ-PGA is composed of D- and L-glutamic acid units linked via an α-carboxylate side group that can be chemically modified with various bioactive ligands to modulate overall polymer behavior [144]. Biodegradable PGA biopolymer NPs prepared by self-assembly of amphiphilic biopolymers of hydrophilic γ-PGA and hydrophobic L-phenylalanine ethyl ester were shown to induce Ag-specific cellular and humoral immune responses following immunization with various Ags [145–147]. Ags including OVA, HIV-1 p24, and HIV-1 gp120 have been combined with the γ-PGA-NP system to activate immunity [146,148]. In allergen-specific immunotherapy, γ-PGA-NPs have been used as activators of human monocyte-derived DCs to divert Th2 immunity to efficient T cell priming [149]. In addition, next-generation nanovaccines containing Japanese encephalitis virus (JEV)-like-particles encapsulated in γ-PGA-NPs provided 90% protection against JEV infection in mice [150]. It was also shown that γ-PGA-NPs induced immune responses through TLR-4 and myeloid differentiation primary response gene 88 signaling pathways [151]. Interestingly, γ-PGA-NPs and CpG-ODN adjuvants were found to act synergistically via TLR-4 and TLR-9 in macrophages and potently induced Ag-specific cellular immunity [152].

γ-PGA-NPs have been investigated for their anti-cancer effects; for instance, OVA/γ-PGA-NPs suppressed the growth of EG7-OVA tumors, and intranasal immunization with OVA-loaded γ-PGA-NPs stimulated the production of CD8+ (CTL) and IFN-γ-secreting cells in the spleen and lymph nodes. In addition, OVA/γ-PGA nanovaccines conferred resistance to tumor challenge by EG7-OVA cells and inhibited lung metastasis by B16-OVA cells [153], and the effects were confirmed in a separate study [154]. Infection-mimicking OVA/γ-PGA-NPs enhanced the functions of DCs such as Ag uptake, migration to lymph nodes, and cross-presentation, and vaccination with OVA/γ-PGA-NPs protected mice against EG7-OVA tumor challenge, suppressing tumor growth and generating a long-lasting immune response (Fig. 8) [155]. Interestingly, tailored immune responses can be induced by γ-PGA-nanovaccines, for instance by fine-tuning hydrophobic amino acid ethyl esters (AAE) in the γ-PGA-AAE-/OVA nanocomplexes. Finally, a γ-PGA-graft-L-phenylalanine ethyl ester-based polyion complex is a potential Ag carrier and adjuvant [156]. Thus, γ-PGA-NPs have excellent adjuvant potential and act through TLR-4 by stimulating both arms of the immune response.

Conclusions

Biodegradable materials can serve as a basis for the development of subunit Ag-based prophylactic or nucleic acid-based therapeutic vaccines owing to their biocompatibility and minimal

toxicity, which can ensure safety and therapeutic efficacy. In this review, we discussed representative biodegradable polymers and the immune responses that they elicit. In most cases, biomaterial-based particulate vaccines delivering Ags or acting as adjuvants have been shown to enhance immune responses as compared to formulations comprising the Ag alone. In particular, mucoadhesive polymers and polymers that have the intrinsic ability to increase cross-presentation of Ags and induce CTL-mediated immune response are a significant improvement over classical alum-adjuvanted vaccines. These biomaterials can accelerate the clinical translation of new vaccines against Ebola, TB, and HIV infection and cancer. However, a greater understanding of the physiochemical properties (e.g., size, shape, and charge) of biomaterials and the mechanisms by which they stimulate immunity is needed to develop more effective vaccines. In addition, recent advances in materials engineering can be applied to customize vaccines to meet specific immunological needs. For instance, the recent development of artificial Ag-presenting cells and cell membrane-mediated biofunctionalization approaches are useful tools in the rapidly growing field of immunotherapy.

Author contributions

All authors helped to research, write, and proofread the review articles.

Conflicts of interest

The authors declare no conflict of interest.

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