Review

Poly-D,L-lactide–co-poly(ethylene glycol) microspheres as potential vaccine delivery systems

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Abstract

Adjuvants aimed at increasing the immunogenicity of recombinant antigens remain a focus in vaccine development. Worldwide, there is currently considerable care for the development of biodegradable microspheres as controlled release of vaccines, since the major disadvantage of several currently available vaccines is the need for repeated administration. Microspheres prepared from the biodegradable and biocompatible polymers, the polylactide (PLA) or polylactide–co-glycolide (PLGA), have been shown to be effective adjuvants for a number of antigens. This review mainly focuses on polylactide–co-poly(ethylene glycol) (PELA) microspheres adjuvant as vaccine delivery systems by summarizing our and other research groups’ investigation on properties of the microspheres formulation encapsulating several kinds of antigens. The results indicate that compared with the commonly used PLA and PLGA, PELA showed several potentials in vaccine delivery systems, which may be due to the block copolymer have its capability to provide a biomaterial having a broad range of amphiphilic structure. PELA microspheres can control the rate of release of entrapped antigens and therefore, offer potential for the development of single-dose vaccines. The PELA microspheres have shown great potential as a next generation adjuvant to replace or complement existing aluminum salts for vaccine potential. The review mainly aims to promote the investigation of PELA microspheres adjuvant for antigens for worldwide researcher.

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1. Introduction and background

Immunization has arguably been the most important way of protection against a number of devastating viral and bacterial infections [1]. Traditional vaccines are mainly composed of heat-inacti-vated bacteria or virus. These vaccines often generate many unwanted side effects. Recent advances in the understanding of the pathogenesis of infectious diseases and the underlying immunologic principles of immunization against them have inspired new approaches to vaccine development. Chief among these approaches are the development of many subunit antigens and peptides through recombinant DNA technology and chemical synthesis [2]. These subunit antigens are chemically well defined and free

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of the side effects associated with some traditional vaccines. However, they are, with rare exceptions, poorly immunogenic because of their small molecular structures and need potent adjuvants to induce an effective protective immunity. Aluminum phosphate and aluminum hydroxide, which are currently the approved adjuvants for human vaccination in the US, are widely used vaccine adjuvant for humans at present. However, the use of alum-type adjuvant for immunization has some disadvantages [3–7]. Although alum is efficient at increasing humoral immunity, cell-mediated immunity appears to be only slightly affected. Moreover, some viral antigens are poor immunogens in alum [4,8]. Also, alum is an undesirable adjuvant since it stimulates local production of granulomas and induces inflammation [7]. Conventional alum-type vaccines require multiple recall injections (e.g. diphtheria, pertussis, tetanus, hydrophobia and hepatitis B) at appropriately timed intervals in order to obtain long-lasting and optimal immune response. Therefore, development of more efficient and safe adjuvant/vaccine delivery systems requiring single administration to obtain high and long-lasting immune responses is of primary importance. A new generation of more effective adjuvants [2], including liposomes, muramyldipeptide, and ISCOMs (immunostimulatory compounds), have been proposed to replace alum.

In recent years there have been various attempts to demonstrate new immunization strategies to induce higher level and larger duration of immune responses following parenteral and/or oral administration. One of the means used to improve immunologic response has been to provide prolonged antigen release [5]. Controlled drug delivery technology using biodegradable polymers as carriers represents one of the most rapidly advancing areas of science. Controlled delivery systems consisting of biodegradable microspheres can potentially delivery either the antigens or adjuvants to the desired location at predetermined rates and durations to generate an optimal immune response. The carrier may also protect the vaccine from degradation until it is released. Other potential advantages of the controlled delivery approach include reduced systemic side effects and the possibility of coencapsulating multiple antigenic epitopes or both antigen and adjuvant in a single carrier. Biodegradable polymers provide sustained release of the encapsulated antigen and degrade in the body to nontoxic, low-molecular-weight products that are easily eliminated [5,9]. The microsphere vaccine delivery system based on biodegradable polylactide (PLA) and polylactide–co-glycolide (PLGA) has been extensively investigated due to the many advantages of the controlled-release delivery system [3,5,9–16].

PLA and PLGA are biodegradable and biocompatible polymers, which are nonimmunogenic and have a long history of safe use in humans as sutures and as controlled delivery systems [10,17–19]. The choice of PLA and PLGA as the matrix for vaccine formulations is based on its long-term safety in humans, its biodegradability, and the commercial availability of a variety of polymers of different molecular weights and monomer ratios [20]. Nevertheless, PLA and PLGA have some drawbacks, resulting from their hydrophobic nature. The difference in physico-chemical properties between hydrophilic antigens and hydrophobic polymer matrix leads to a lower antigen encapsulation efficiency within microspheres, and a higher burst effect of antigen release from microspheres. Furthermore, during the initial vaccine release phase in vivo, the hydrophobic PLA or PLGA prevents the penetration of water into the center of the microspheres, thus forming an acidic microenvironmnet due to the accumulated acidic breakdown products, such as lactic and glycolic acid end groups.

### 2. Synthesis of block copolymers (PELA) from poly(ethylene glycol) and lactide

To overcome the disadvantages resulting from the hydrophobic nature of PLA, a second hydrophilic component poly(ethylene glycol) (PEG), which has been widely used to improve the biocompatibility of the blood contacting materials, is introduced to form an ABA copolymer poly-D,L-lactide–PEG (PELA) [21–23] (shown in Scheme 1).

In recent years, the synthesis of polyester–polyether type block copolymer has attracted much attention, because they can be used in future medical applications in implantation and wound treatment, and as controlled-release drug carriers [24–30]. Both PEG and PLA are of great interest for temporary therapeutic applications, especially as matrix for
However, the number average molecular weight (mn) of resultant polymers was low (<10,000), and its distribution was wide (mw/mn = 2.0–3.0).

Other metal compounds have also been investigated as catalyst for PELA synthesis. With metal oxides, such as GeO2 and SnO2, only low conversions of lactide were obtained, regardless of the reaction temperature, whereas Sb2O3 caused partial racemization of L-lactide and only SnO gave satisfactory results [40]. Molecular weights of the ABA polymers were corresponded to the feed ratios of monomer and initiator by an equation based on a simple chain reaction model. However, the GPC trace showed a shoulder indicating that some homopolymerization of lactide occurred. Li et al. reported that the PELA copolymers were synthesized by polymerization of L-lactide in the presence of PEG, using zinc metal or CaH2 as catalyst [47]. In the system, residual calcium ions and Zn2+ ions were nontoxic at trace doses, and the residual Zn particles could be easily eliminated by filtration after polymerization. However, the polymerization time was long (4–7 days), and the mw of resultant polymers was low. Also aluminum triisopropoxide can be used as the catalyst in the copolymerization of L-lactide and PEG [48,49]. The polymerization was carried out in bulk at 150 °C and the conversion was over 90%. The GPC trace showed a narrow mw distribution and unimodal peaks. 1H-NMR also demonstrated the block structure of the copolymers. Deng et al. also reported that the Al(Bu)3–H3PO4–H2O complex catalyst system was also used to prepare triblock PLA–PEG–PLA copolymers [22]. The polymerization was carried out at 140–160 °C and the conversion was more than 90%. The feed ratio of PEG and the quality of the catalyst had a great influence on the molecular weight of the resultant polymer. The mw distribution was narrow (mw/mn = 1.29–1.83). Recently, rare earth metal alkoxides were used for the synthesis of ABA copolymers in solution. Deng et al. reported that the triblock copolymer PELA was synthesized with lanthanum acetate as the initiator, which was stable and easy to prepare [50]. The polymerization was carried out in bulk and the conversion was in range of 60–87%. 13C-NMR and 1H-NMR also confirmed the structure of triblock copolymer PELA.

Anionic polymerization was also used in the
preparation of the block copolymer of PEO and lactone. Jedlinshi et al. [51] and Kricheldorf et al. [52] reported that PELA was synthesized by using macroinitiators, such as potassium poly(ethylene glycol)ate and sodium poly(ethylene glycol)ate. With the initiator of potassium poly(ethylene glycol)ate, Zhu et al. synthesized PELA triblock copolymers [53]. The anionic polymerization proceeds quickly and the lactide was almost entirely consumed within 5 min. The resultant polymer exhibits a mw higher than that of the prepolymer and a unimodal mw distribution. However, the mw distribution was wide (mw/mn=3.0–4.2), and the PEG content in the ABA copolymer was very limited [53]. In recent years, the low-molecular-weight PEG–PLLA multiblock copolymers were synthesized by multistep condensation polymerization of PEG and PLLA [54]. The polymer yields were ranged from 80 to 90%. The mw distribution was narrow (mw/mn=1.25–1.74).

### 3. Properties of PELA copolymers

PEG segment can be quantitatively inserted between PLA backbones to adjust the properties of PELA [46,53,55]. Deng et al. reported PLEA copolymers with different contents (0–50%) of PEG were synthesized and the PEG content was estimated according to the integral height of hydrogen in 1H-NMR [55] (shown in Table 1). Then, five kinds of PELA block copolymers were synthesized with the same content (10%) and different molecular weight of PEG, such as 6, 4, 2, 1.54 and 0.8 kDa [23] (shown in Table 2). It is indicated that PELA shows several potentials in protein delivery systems [56], compared with the commonly used PLA and PLGA. Li et al. also confirmed that the PEG component introduced into PLA could decrease the amount of emulsifier used in microsphere preparation [56]. The reason may be that PELA copolymers possess surfactant properties because the PEG block is very hydrophilic and the PLA block is hydrophobic. When PELA is employed in a fabrication process that uses an aqueous external phase, e.g. microspheres fabrication by the double emulsion technique, PEG enriches the surface. The hydrophilic domains of PELA copolymers acting as a protein stabilizer or surface modifier of hydrophobic PLA networks could promote the stability of proteins and increase the drug and protein loading efficiency [21]. Li et al. investigated the effect of process parameters on the struc-

### Table 1

Characteristics of PELA [55]

<table>
<thead>
<tr>
<th>Sample</th>
<th>PELA-5</th>
<th>PELA-10</th>
<th>PELA-13</th>
<th>PELA-20</th>
<th>PELA-30</th>
<th>PELA-50</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG (% added)</td>
<td>5.0</td>
<td>10.0</td>
<td>13.5</td>
<td>20.0</td>
<td>30.0</td>
<td>50.0</td>
</tr>
<tr>
<td>PEG (% calculated)</td>
<td>6.1</td>
<td>11.5</td>
<td>13.0</td>
<td>23.3</td>
<td>32.9</td>
<td>59.7</td>
</tr>
<tr>
<td>mw (kDa)</td>
<td>98.4</td>
<td>52.2</td>
<td>46.2</td>
<td>25.8</td>
<td>18.2</td>
<td>10.1</td>
</tr>
<tr>
<td>[η] (dl/g)</td>
<td>0.543</td>
<td>0.359</td>
<td>0.323</td>
<td>0.228</td>
<td>0.181</td>
<td>0.124</td>
</tr>
</tbody>
</table>

* Estimated from the integral height of hydrogen shown in 1H-NMR spectrum.
* Weight average molecular weight measured by GPC (calibrated with polystyrene standards).
* Intrinsic viscosity determined by Ubbelohde viscometer at 30 °C.

### Table 2

Characteristics of PELA [23]

<table>
<thead>
<tr>
<th>Sample</th>
<th>PELA-1</th>
<th>PELA-2</th>
<th>PELA-3</th>
<th>PELA-4</th>
<th>PELA-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG (% added)</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>PEG (% calculated)</td>
<td>11.0</td>
<td>10.1</td>
<td>8.9</td>
<td>9.9</td>
<td>9.7</td>
</tr>
<tr>
<td>mw (kDa)</td>
<td>82.3</td>
<td>42.0</td>
<td>26.7</td>
<td>25.9</td>
<td>12.8</td>
</tr>
<tr>
<td>[η] (dl/g)</td>
<td>0.56</td>
<td>0.33</td>
<td>0.26</td>
<td>0.23</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* Estimated from the integral height of hydrogen shown in the 1H-NMR spectrum.
* Weight average molecular weight measured by GPC (calibrated with polystyrene standards).
* Intrinsic viscosity determined by Ubbelohde viscometer at 30 °C.
tural integrity and activity retention of encapsulated protein [57]. The SDS–PAGE results showed that the activity loss was detected, no rough changes of molecular weight of protein were observed during the encapsulation procedure and the initial days of incubation into the in vitro release medium. Deng and Li and co-workers also reported that the protein-loaded microspheres prepared from PELA copolymer achieved the highest loading efficiency among PELA copolymers and PLA [23,55,56]. It is possible to control the degradation and delivery of the materials by changing the compositions. The mw and hydrophilicity of PELA copolymers could be adjusted by changing the mw of PEG segment [23] and/or changing the PEG content in the copolymer chain [55]. The PELA copolymers exhibited a faster degradation rate in vitro than PLA, which may be that the hydrophilicity of PELA increases [46,55].

In addition, the PELA copolymer displays a good biocompatibility in vivo. Younes et al. determined the biological response of PLA–PEO block copolymers under in vivo conditions. The studies compared the tissue reaction elicited by various PLA–PEO ABA polymers to that evoked by PLA homopolymer after implantation in rabbits. The results showed a nonspecific foreign body, granulomatous reaction and chronic inflammation being apparent for all tested samples [58].

4. Characteristics of PELA microspheres

Compared with PLA and PLGA, PELA is a new biomaterial and fewer reports are published on its use as a drug delivery carrier. In the last 10 years, our research group has focused on studying the preparation of PELA microspheres as a drug delivery system, the factors influencing the in vitro degradation profiles and antigen in vitro release profiles, and the effect on immunization by oral and subcutaneous administration, etc. All results indicate PELA as a potential biomaterial for protein, peptide and gene delivery system. PELA microspheres were prepared by a double emulsion w/o/w based on solvent extraction methods [23,59]. The antigen is homogeneously dispersed within the copolymeric matrix. The microspheres have smooth and spherical surface (shown in Fig. 1). The size and distribution can be controlled by adjusting the mw of the matrix polymer, the dispersion strength and the components of phases of the double emulsion system.

There are several potentials in protein delivery systems for PELA, compared with commonly used PLA and PLGA [56]. Firstly, PELA microspheres could increase the protein encapsulation efficiency. The microspheres containing human serum albumin (HSA) prepared from PELA copolymer achieved the highest loading efficiency among PELA copolymers and PLA [23,55,56]. Secondly, PELA microspheres could promote the stability of protein. A higher specific activity retention of glucose oxidase (GOD) was achieved for microspheres prepared from copolymer PELA than that from PLA and PLGA microspheres [57]. A core-coated microspheres delivery system composed of alginate microcores surrounded by copolymer PELA was also effective to improve the loading efficiency and stability of proteins [60]. Perez et al. also verified that higher activities of tetanus toxoid as well other proteins were preserved in PELA nanoparticles [61,62]. Lucke et al. confirmed that the combination of PEG with biodegradable poly(ε-caprolactide) was an effective approach to prevent or reduce peptide acylation [63]. Jiang et al. reported that BSA could be stabilized when it was encapsulated in PLA and PEG microspheres blends [64]. Thirdly, PELA microspheres could decrease initial burst release of proteins. As seen from Table 3 [65], the burst release of protein within 24 h from the PELA microspheres was much
lower than that from PLA microspheres. Finally, the PELA matrix degradation and protein release rate were highly mw dependent, which could be controlled by adjusting the mw of PEG and/or the two monomers ratio of PEG and lactide [23]. It is indicated in our further research work that the PELA copolymer is also more suitable as a carrier for the development of control delivery other water-soluble compounds, such as peptides and DNA, than the PLA and PLGA polymers. PELA nanoparticles with sizes <300 nm were prepared containing a high loading of plasmid DNA in a free form or co-encapsulated with either poly(vinyl alcohol) or poly-(vinylpyrrolidone) [61]. Tobio et al. reported the PELA nanoparticles are used as protein carriers for nasal administration [66].

In summary, PELA microspheres as protein delivery systems have clear advantages over PLA and PLGA as regards drug loading, control of protein release rate and protein compatibility with the PELA copolymer matrix. The preservation of protein stability during encapsulation and release is essential for the development of controlled release formulations of protein drugs. One of great advantages of PELA is that it preserves the protein stability during the microsphere preparation and in the polymer matrix during release conditions.

5. PELA microspheres as vaccine carriers

Controlled release vaccine delivery systems are an important research area where new approaches and new biomaterials with improved properties enhancing protein stability in devices might be of critical importance [67]. ABA block polymers consist of d,L-PLA A blocks and PEG B blocks (content: 10%; mw: 6 kDa) designated as PELA were used to encapsulate outer membrane proteins (OMP) of *Vibrio cholera* and *Leptospira interrogans* antigens using a w/o/w double emulsion technique [50,68]. A small volume of internal w phase and organic phase were favorable to prepare microspheres with a size of 1–2 μm and high antigen loading efficiency (70–80%). In vitro OMP release profiles from PELA matrix consist of a small burst release (20%) followed by a gradual release phase up to 60% after 30 days [68]. OMP of *Vibrio cholera* was encapsulated in PELA polymer with smooth surface and a suitable size (0.5–5.0 μm) for oral targeting delivery system [50,55,69,70]. High loading efficiency (about 60%) and low level of residual solvent (<20 ppm) were obtained [55]. The microspheres distribution tests in rabbits and mice through scanning electronic micrograph and fluorescence microscope indicated microspheres have successfully reached the immunization-related tissues, such as the liver, spleen and intestinal peyer’s patches, following oral administration [71]. PELA microspheres showed enhanced immune responses compared with soluble antigens after either oral or subcutaneous inoculation [50]. PELA microspheres were also evaluated as an efficient antigen delivery system by enhancing a higher protective ratio against live *Vibrio cholera* (Table 4) [50].

Another vaccine candidate is hepatitis B surface antigen (HBsAg), which has been chosen to investigate the effect of the PELA microspheres formulation on humoral immunities and cellular immunities in recent years. It aimed to use the designed biodegradable polymer as vaccine adjuvant instead of the conventional aluminum hydroxide. Hepatitis B is a significant health problem affecting about 5% of the world population. It is known that 7–10% of acute infection cases becomes chronic and that there is no specific treatment for hepatitis. Hence immuni-

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<tbody>
<tr>
<td>SP (%)</td>
<td>53.6</td>
<td>44.7</td>
<td>23.3</td>
<td>17.4</td>
<td>9.3</td>
</tr>
<tr>
<td>Release in 12 h (%)</td>
<td>33.8</td>
<td>30.0</td>
<td>17.4</td>
<td>14.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Release in 24 h (%)</td>
<td>51.1</td>
<td>42.2</td>
<td>22.7</td>
<td>21.7</td>
<td>17.8</td>
</tr>
</tbody>
</table>

*PELA-5, PELA-10, PELA-20 and PELA-30 were prepared from dilactide and PEG with content of 5, 10, 20 and 30%, respectively.

*Surface-associated protein removed from microspheres surface and determined by Bradford’s method.*
Table 4 Immunization protective tests in vivo [50]

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice no.</th>
<th>Death/survival</th>
<th>Protective ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c. TCP/PELA MS</td>
<td>10</td>
<td>0/10 1/9 1/9 1/9 90</td>
<td></td>
</tr>
<tr>
<td>p.o. TCP/PELA MS</td>
<td>10</td>
<td>2/8 4/6 6/4 40</td>
<td></td>
</tr>
<tr>
<td>s.c. PELA MS+TCP</td>
<td>10</td>
<td>1/9 2/8 4/6 4/6 60</td>
<td></td>
</tr>
<tr>
<td>s.c. TCP+AL(OH)3</td>
<td>10</td>
<td>0/10 1/9 3/7 70</td>
<td></td>
</tr>
<tr>
<td>p.o. TCP</td>
<td>10</td>
<td>3/7 5/5 7/3 30</td>
<td></td>
</tr>
</tbody>
</table>

*Challenged i.p. with O139 biotype M045 strain.

Immunization represents the only known method to prevent the spread of virus. The commercially available vaccines for hepatitis B in China are obtained from yeast based on recombinant DNA technique, whose safety, immunogenicity and efficiency have been demonstrated on animal trials and clinic studies. But the commonly used immunization schedule is three injections given at 0, 1 and 6 months to provide protective antibody levels. The multiple injection schedules often lead to dropouts among subjects to be immunized causing failure of protection. This is truer in developing countries where the population at highest risk lives mainly in isolated rural areas with poor access to health services, the target population is large and healthy facilities are unavailable. The development of a delivery system for hepatitis B virus, which could induce the desired antibody response from a single injection and oral administration, would be of enormous benefit. Concerning with the potential development of single-dose vaccine for HBsAg, previous studies have investigated using controlled release PLA, PLGA and PGA microspheres [9,72,73]. These studies indicated the feasibility of converting the present three-injection schedule for HBsAg into a single-shot therapy.

Recently, PELA microspheres were reported from our previous work as a hepatitis B surface antigen (HBsAg) delivery system following subcutaneous (s.c.) or oral immunization over the current injection of an alum-absorbed antigen [74–77]. The antigen-loading microspheres were elaborated by the solvent-extraction method based on the formation of modified multiple w/o/w emulsion. HBsAg-loaded microspheres with particle distribution 0.5–10.0 μm had high antigen encapsulation efficiency (~78.6%) were prepared [73,74,77,78]. The SDS–PAGE results showed that HBsAg kept its structural integrity during encapsulation (Fig. 2). Balb/c mice were immunized with an s.c. injection of a single dose and oral administration of two doses of a microspheres formulation. For comparison, the alum-absorbed HBsAg-immunized mice had a following intramuscular (i.m.) injection at weeks 0 and 4. At weeks 8, 10, 14 and 24 postadministration, blood and saliva samples were collected and detected by the enzyme-linked immunosorbed assay (ELISA) method. A single injection of HBsAg–PELA microspheres could induce a serum IgG antibody level comparable to the two-injection alum-absorbed HBsAg at the 14th week and higher than that at the 24th week. The saliva IgA of peroral groups was significantly higher than that of the s.c. injection of a microspheres formulation and i.m. injection of soluble antigen. These preliminary results demonstrated the potential of oral administration of antigen-loading microspheres in the induction of a secretory response.
immune response, and it is suggested that a single-dose s.c. injection of antigen-loading microspheres would be an efficient immunization schedule to elicit a protective response [74,75].

The biological study of PELA copolymer indicated that PELA had the effect of improving immune response by itself. The blank PELA microspheres could significantly improve sheep red blood cells (SRBC)-induced delayed-type hypersensitivity (DTH) response of mice (P<0.05) and could also enhance Con A-induced IL-2 and IFN-γ production. As regards humoral immunity, the anti-HBs of alum-vaccine and HBsAg-loaded microspheres formulation were detected by solid radioimmunoassay [77]. The anti-HBs titer of HBsAg-loaded microspheres formulation was 85 mIU/ml, which was significantly higher than that of HBsAg with alum (36 mIU/ml). The IgG subclass was estimated by ELISA [77]. The mean OD of IgG1 in HBsAg-loaded microspheres was significantly lower than that of HBsAg with alum. The results of IgG1 showed that HBsAg-loaded PELA microspheres inhibited the immune response of the Th2 cell. The results of IgG2a showed no significant differences between HBsAg-loaded PELA microspheres formulation and HBsAg with alum, but the mean OD of HBsAg-loaded PELA microspheres formulation was 1.23 while the mean OD of HBsAg with alum was 0.24. The above results indicated that HBsAg-loaded PELA microspheres formulation could possibly improve the immune response of the Th1 cell. In a word, the PELA polymer could improve HBsAg-induced cellular immunities.

Based on above results, further studies have investigated PELA as an immune response enhancer of cellular immunities induced by HBsAg [77], which has not been reported on to our knowledge. With respect to the cellular immunity, on the 25th day after injection, the SI of Con A induced proliferation of MNC from mice immunized with HBsAg-loaded PELA microspheres reached the highest level (133.59) which was significantly higher than that of HBsAg with alum (P<0.05). The LPS reactivity of mice was enhanced after the immunization with HBsAg-loaded PELA microspheres and with an increasing tendency. On the 25th day after injection, the SI of LPS induced proliferation of MNC from mice immunized reached the highest level (47.48), which was significantly different compared with other groups (P<0.05). T lymphocyte proliferation tests of the specific antigens proved a further verification on the effects of T cell activation and proliferation induced by HBsAg with PELA. The delayed-type hypersensitivity (DTH), as determined by footpad swelling using sheep red blood cells (SRBC) as sensitinogens, was also studied in these immunized mice. The response level of SRBC-induced DTH response of mice immunized with HBsAg-loaded PELA microspheres was significantly higher than that of HBsAg with alum. Through the determinations of cytokine and lymphocyte subpopulations, further study was made on the influence of HBsAg cellular immunity by HBsAg encapsulated by PELA. From the test on CTL activity in 51Cr release assay against P815-HBsAg, the specific cytolitic activity of splenocyte of mice immunized with HBsAg-loaded PELA microspheres was significantly higher than that of HBsAg with alum [77]. The tests indicated that the CTL of mice immunized with the formulation was improved. This clearly indicated that PELA copolymer had a function in improving the HBsAg-induced cellular immunities and also played a role in control release of the immune effects of the antigens. It is well known that alum adjuvant lacks these functions. The result is very significant, since the HBsAg-loaded microspheres formulation can not only prevent the infection of hepatitis B virus but also may be an effective method to treat hepatitis. Furthermore, the function that PELA can increase the HBsAg-induced cellular immunities may be very important to treat other infectious diseases caused by virus, such as AIDS, cholera, and hydrophobia.

The outburst of human immunodeficiency virus (HIV) since the early 1980s, and the re-emergence of antibiotic-resistant strains of tuberculosis serve as powerful reminders that infectious diseases are an ever-present threat to the human race. There is thus a pressing need to develop effective single-dose vaccines that would reduce the cost by eliminating the booster shots while broadening the coverage. Polymeric controlled-release technology, which has been extensively developed for a wide range of pharmaceuticals, offers the potential of meeting this need. Other than prophylactic vaccines that target the healthy population, there is also a need to develop therapeutic vaccines for people who are at risk of
contracting or are inflicted by diseases such as cancers and AIDS [2]. Therefore, the PELA microspheres may be a competent candidate for developing therapeutic vaccines. The results, although highly qualitative in nature and in the absence of a relevant small animal challenge model for hepatitis B virus, suggests potential for protection with a microspheres formulation in vivo.

6. Conclusion

Biodegradable PELA microspheres are extremely flexible delivery systems capable of encapsulating a wide range of antigens. A single injection or oral administration of PELA microspheres could provide a sustained and higher antibody titer than an alum absorbed vaccine. Furthermore, the PELA polymer improves cellular immunity, a property which alum lacks. Finally, the polymer microspheres formulation can be lyophilized into a solid powder, which is more easily transported and placed in cold storage than liquid. All these advantages make it more qualified for applications in developing countries where the population at highest risk lives mainly in isolated rural areas with poor access to health services. Therefore, PELA copolymer has potential use as a new adjuvant instead of alum, and, the microspheres formulation is very suitable to be as vaccine delivery system. PELA copolymer is a novel biomaterial, and it is very worth studying.

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References


