Development of liposome-based freeze-dried rods for vaginal vaccine delivery against HIV-1


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The aminolysis effect on thermal behaviour of PLLAsc was investigated by DSC analysis (Fig. 2). Pristine PLLAsc thermogram shows no glass transition and a complex thermal behaviour between 150 and 190 °C, due to the fusion of the original crystalline phase at 168 °C, followed by recrystallization at 172 °C and melting of the just-formed thicker crystals at 183 °C. As far as the determination of the protein absorbed onto the PLLAsc surface is concerned, increasing amounts of the conjugated PLLA-E7 were solubilised in SDS-loading buffer (50 mM Tris-HCl pH 6.8, 3% SDS, 5% b-Mercaptoethanol, 10% glycerol), heated at 95 °C for 5 min and loaded on a 15% polyacrylamide gel. After the electrophoresis the protein was stained on the gel by Coomassie blue and the amount of E7 was determined by comparing the bands in the gel with standard quantity of BSA (Fig. 3). A value of 0.1 mg of E7 per mg of PLLAsc was obtained. In order to verify the immunogenic activity of PLLA-E7 system, in vivo experiment on mice is in progress.

Fig. 3. SDS-PAGE results. Lane 1: molecular weight markers. Lane 2, 3, 4: increasing amount of conjugated PLLA-E7. Lane 5, 6, 7: BSA. Lane 8: free E7.

Conclusion

The E7 protein of the Human Papillomavirus 16 (HPV16) was adsorbed on 2D-micro-1D-nano-sized poly(l-lactide) lamellar single crystals grown from dilute solution. In order to increase the interaction of the protein with polymer substrate, amino groups were introduced on the lamellae surface by aminolysis reaction. The high adsorbed protein amount opens interesting perspectives in a possible use of the developed system as single-dose vaccine.

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Abstract summary

The present investigation deals with development and characterization of the liposomes-based freeze-dried rods for the vaginal delivery of gp140 antigen in mice. Positively charged, negatively charged and neutral liposomes were prepared and characterized for various parameters e.g. morphology, size, polydispersity index, zeta potential and antigen encapsulation efficiency. To further improve the efficacy of vaccine delivery, antigen encapsulated liposomes were formulated as polymer gel-based freeze-dried rods, which were then characterized for moisture content. The redispersibility of the liposomes-based freeze-dried rods was determined in simulated vaginal fluid and liposome gel was investigated for mucoadhesion. The developed liposome-based freeze-dried rods systems could offer potential as stable and practical dosage form for the mucosal immunization against HIV-1 infection.

Introduction

The HIV epidemic has become one of the main global health problems. Vaginal vaccination approaches are well documented in the literature; most involve the administration of antigen in simple buffer solutions rather than delivery modalities specifically designed for vaginal administration [1–2]. From a formulation perspective, inducing effective antigen specific immune responses by cervicovaginal instillation of buffer solution containing solubilized antigen is far from ideal owing to the potential for leakage at the administration site, rapid enzymatic degradation of the antigen, the influence of the menstrual cycle, and inadequate exposure of antigen to the mucosal associated lymphoid tissue [3].

There is a strong rationale for expanding the formulation options for effective vaginal administration of vaccine candidates. Liposomes are known to be effective as immuno-adjuvant and vaccine carriers. A number of approaches have been made to improve the immunoadjuvant action of the liposomes including the modification of the structure of the vesicles. Positively charged liposomes are shown to be preferentially taken up by the liposomes including the modification of the structure of the vesicles. Positively charged liposomes are shown to be preferentially taken up by macrophages [4]. Thus an attempt has been made to investigate whether such positively charged liposomes could efficiently deliver soluble protein antigen (gp140) to macrophages/APC and function to induce antigen specific immune responses. Additionally, negatively charged and neutral liposomes were considered for the comparative studies. Despite the variety of the formulations for the intravaginal therapy their efficacy is often limited by a self-cleansing action of the vaginal tract.

To overcome these limitations liposomes in hydroxyethyl cellulose (HEC) based-freeze dried rods and liposomes in HEC mixed Gan-trez®MS-955 based freeze-dried rods were developed for vaginal immunization in mice.

Experimental methods

Preparation of liposomes

Antigen encapsulated liposomes were prepared by dehydration-rehydration method [5]. Briefly, lipid composition for positively charged (EPC:Chol:SA; 2:1:1 molar ratio), negatively charged (EPC:Chol:DMPG; 2:1:1) and neutral liposomes (EPC: Chol; 2:1), were taken in chloroform in a 50 ml round-bottomed flask and dried to a lipid film with a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) under vacuum.

The lipid film was kept in desiccators to remove traces of organic solvent. The lipid film was rehydrated with sucrose buffer. The flask

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was shaken vigorously until the lipid film has been transformed into a milky dispersion. The milky liposomal dispersion was sonicated (Vibra-Cell™ Jencons Scientific Ltd., USA) for 5 min (cycle of 30 s run and 30 s pause) which were then centrifuged for 2 min at 1700 g to remove titanium fragments and supernatant is allowed to rest for 1 h.

The vesicles thus formed were mixed with antigen solution (gp140) followed by lyophilisation. The lyophilized formulation was then subjected to controlled rehydration to obtain antigen encapsulated liposomes.

**Characterization of the liposomes**

Morphology of the developed liposomes was investigated by transmission electron microscopy (TEM, Philip CM100). Size, polydispersity index and zeta potential of the liposomes were determined by using Malvern Instrument (Malvern Zetasizer 3000HSA). Diluted 100 μl of the liposome suspension upto 5 ml and recorded measurement at 25 °C. Percent encapsulation efficiency of gp140 encapsulated liposomes was estimated by ELISA method.

**Development and characterization of liposome-based freeze-dried rods**

HEC (Natrosol®250HHX; 6%w/w) was dispersed in liposome dispersion by using Speed mixer™ (DAC150, FVZ-K, FlackTek Inc.). It was then kept overnight at 4 °C. Similarly, a mixture of Natrosol®250HHX (2%w/w) and Gantrez® MS-955(4%w/w) was used to develop liposome gel. Liposome gel was filled in the Silicon tube (OD 2.41 mm, ID 1.57 mm) which was then centrifuged to remove any air bubbles. Finally, liposome gel filled tube was cut in 5 mm size and then lyophilized. Freeze-dried liposome rods were subjected to visual inspection under sectioning microscope (Pyser-SCI XE series). Redispersibility of the liposome-rod was determined in simulated vaginal fluid. Moisture content of the lyophilized liposomes and liposome based lyophilized rods was estimated by thermo gravimetric analysis (TGA) using TGA Q500 instrument. Also, the mucoadhesive properties of gel formulations were determined using a TA-XT2 Texture Analyzer (Stable Microsystems, Surrey, England) as reported previously [6].

**Results and discussion**

Antigen encapsulated liposomes were prepared by dehydration-rehydration method which is well documented for the preparation of vaccine loaded liposomes [5]. The method does not involve exposure of antigen to the harsh conditions i.e. organic solvents, sonication and hence is suitable for preparation of antigen bearing liposomes. However, for the protection of the antigen during freeze drying cryoprotectant is required. Sucrose was used to stabilize the antigen during freeze drying. Different lipid compositions were used to formulate neutral, positively charged and negatively charged liposomes. Spherical shaped liposomes were observed under TEM (Fig. 1). The size of the neutral, positively charged and negatively charged liposomes was found to be 151.86 ± 0.76, 161.63 ± 1.56 and 118.43 ± 0.32 respectively. These liposomes exhibited good polydispersity index (Fig. 2). Zeta potential of neutral, positively charged liposomes and negatively charged liposomes was recorded to be −0.33 ± 0.15, 14.36 ± 1.06 and −30.33 ± 0.94 mV respectively. The entrapment efficiency of gp140 encapsulated liposomes is shown in Fig. 3. Positively charged liposomes exhibited higher encapsulation efficiency as compared to negatively charged and neutral liposomes.

The liposomes were then formulated in HEC based freeze-dried rods. Additionally, a mixture of HEC and Gantrez was also used to develop freeze-dried rods because Gantrez is reported to enhance both Th1 and Th2 markers [7], and may result in effective HIV-1 elimination. The freeze-dried liposome rods were investigated for moisture content which was found to be less in case of freeze-dried liposomes as compared to freeze-dried liposomes rods (Fig. 4). Liposome-rod when incubated in simulated vaginal fluid resulted in liposomal dispersion in case of positively charged and negatively charged liposomes, however slight aggregation was observed in case of neutral liposomes. Mucoadhesion behaviour of the formulations was confirmed by the mucin-disc based bioadhesion test. In case of positively charged liposomes most of the antigens are probably delivered into processing pathways for MHC class II presentation of peptides, but some antigens might escape from phagosomes into the cytoplasmic compartments and be delivered into MHC class I presentation pathways [4], this may result in induction of both humoral and cellular immunity.

**Conclusion**

The developed freeze-dried liposome-based rods are novel formulation strategy for mucosal immunization against HIV-1 infection. The positively charged liposome-based freeze-dried rods could be a good option for the induction of systemic, mucosal and cellular immune response against HIV-1, however its immune stimulating efficacy is yet to be assessed in animal model. For vaginal immunization these rods are advantageous in terms of ease of...
administration, mucoadhesion, needle-free systems and could offer potential as stable and practical dosage form.

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References


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Supramolecular triblock copolymers controlled by the coiled-coil motif: A new tool for drug delivery

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Abstract summary

Here we present a new class of peptide, polypeptide-b-designed peptides. The formation of a noncovalent triblock copolymer based on a coiled-coil peptide motif is shown. These nanostructures could be formed in water at neutral pH over the entire compositional range. It was found that the size, morphology (polymersomes or bicontinuous), and surface functionality could be specified by combining the appropriate modular building blocks. Applications in the field of drug delivery are discussed.

Introduction

For materials scientists polypeptides are a fertile area for investigation as they can be programmed with the ability to adopt specific intra- and intermolecular conformations, which may allow heightened levels of control over the morphologies and properties of the self-assembled structures. The structure and functional properties of proteins and peptides are determined by their primary sequence of amino acids. Materials scientists are still unable to design the complex structures found in nature. Yet there has been some progress, particularly in understanding the folding of silks, elastins, collagens, and coiled-coil motifs [1].

Experimental methods

Details of the synthesis of the peptides and the self-assembly studies can be found in Ref [2].

Result and discussion

In the present study we created a new class of polypeptide-b-designed peptides (Table 1), which unites the useful qualities of the two constituent peptide types. We demonstrate the synthesis and self-assembly possibilities of this class of peptide chimera with a series of amphiphilic polypeptide-b-designed peptides in which the hydrophobic block is poly(γ-benzyl l-glutamate) (PBLG), and the hydrophilic block is a coiled-coil forming peptide (denoted E) [3]. The synthetic approach was to synthesize the coiled-coil forming peptide on the solid phase, followed by the ring-opening polymerization of γ-benzyl l-glutamate N-carboxyanhydride, initiated from the N-terminal amine of the peptide E on the solid support. The polypeptide-b-peptide was then cleaved from the resin, requiring no further purification. Peptide E contains 22 amino acids, while the average length of the PBLG block ranged from 36 to 250 residues. This new class of peptide was applied to create a modular system, which relied on juxtaposing the properties of the component peptide types, namely the broad size range and structure-inducing characteristics of the polypeptide PBLG blocks, and the complex functionality of the sequence-designed peptide.

Table 1. Molecular characteristics of the compounds used in this study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>Mn (g/mol)</th>
<th>PDI³</th>
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<tr>
<td>K</td>
<td>Ac-(K I A A L K E)₃G-NH₂</td>
<td>~ 40</td>
<td>2378.0⁴</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Ac-G(E I A A L E K)₁₂-NH₂</td>
<td>~ 40</td>
<td>2380.6⁴</td>
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</tr>
<tr>
<td>PEG-K</td>
<td>Ac-(K I A A L K E)₁₂-G-PFG₇</td>
<td>~ 10</td>
<td>582.6⁴</td>
<td></td>
</tr>
<tr>
<td>PBLG₃₆E</td>
<td>PBLG₃₆-G(E I A A L E K)₁₂-NH₂</td>
<td>28</td>
<td>10,230.3⁴</td>
<td></td>
</tr>
<tr>
<td>PBLG₅₅E</td>
<td>PBLG₅₅-G(E I A A L E K)₁₂-NH₂</td>
<td>30</td>
<td>14,396.1³</td>
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<tr>
<td>PBLG₇₇E</td>
<td>PBLG₇₇-G(E I A A L E K)₁₂-NH₂</td>
<td>56</td>
<td>19,877.4³</td>
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<tr>
<td>PBLG₉₉E</td>
<td>PBLG₉₉-G(E I A A L E K)₁₂-NH₂</td>
<td>69</td>
<td>24,262.4³</td>
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<tr>
<td>PBLG₁₂₀E</td>
<td>PBLG₁₂₀-G(E I A A L E K)₁₂-NH₂</td>
<td>74</td>
<td>57,148.4³</td>
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</table>

⁷GPC calibrated with polystyrene standards. PEG: poly(ethylene glycol). PBLG: poly(γ-benzyl l-glutamate), amino acids in the designed peptides are represented by their one letter codes, Ac: acetyl.

⁸Obtained from MALDI-TOF MS.

Specifically, the different PBLG block lengths could be connected noncovalently with various hydrophilic blocks via the specific coiled-coil folding of peptide E with peptide K or K-poly(ethylene glycol), where K is a peptide of complementary amino acid sequence to E [3]. In this way nanostructures could be formed in water at neutral pH over the entire compositional range, which has not been demonstrated previously with such large PBLG blocks. It was found that the size, morphology (polymersomes or bicontinuous), and surface functionality could be specified by combining the appropriate modular building blocks. The self-assembled structures were characterized by dynamic light scattering, circular dichroism, scanning electron microscopy, cryogenic-transmission electron microscopy, fluorescence spectroscopy, and zeta-potential measurements.

Conclusion

Vaccination has been the greatest medical success in the fight against infectious diseases. Nevertheless, there are many suboptimal vaccines (e.g. influenza) and against numerous diseases (e.g. malaria) there is no effective vaccine available at all. While many classical vaccines consist of attenuated or inactivated pathogens, current approaches focus on making safer vaccines from isolated or recombinant antigens derived from the disease-causing pathogen. A serious drawback of such subunit vaccines is their poor immunogenicity.

Common vaccine delivery systems currently under investigation are colloidal carrier systems of nano- or microparticles in which the antigen is incorporated. Immune stimulators are either added or