Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Platform technologies for modern vaccine manufacturing

Hayley K. Charlton Hume, Linda H.L. Lua*

The University of Queensland, Protein Expression Facility, St Lucia, QLD 4072, Australia

A R T I C L E   I N F O

Article history:
Available online 25 March 2017

Keywords:
Virus-like particle
Liposome
Vaccine design
Modular
Platform technology

A B S T R A C T

Improved understanding of antigenic components and their interaction with the immune system, as supported by computational tools, permits a sophisticated approach to modern vaccine design. Vaccine platforms provide an effective tool by which strategically designed peptide and protein antigens are modularized to enhance their immunogenicity. These modular vaccine platforms can overcome issues faced by traditional vaccine manufacturing and have the potential to generate safe vaccines, rapidly and at a low cost. This review introduces two promising platforms based on virus-like particle and liposome, and discusses the methodologies and challenges.

© 2017 Elsevier Ltd. All rights reserved.

1. Advancing from traditional vaccine production

Vaccination continues to be a leading defense strategy against infectious pathogens. Traditional vaccines that employ whole-cell antigens to raise an immune response have been irrefutably successful in the control or localized eradication of diseases such as poliomyelitis, measles, mumps, rubella, influenza and hepatitis A and B [1–3]. Eradication of smallpox was declared in 1980 after a global immunization effort by WHO [4]. Rinderpest was the second disease globally eradicated by traditional vaccine means as declared by the World Organization for Animal Health in 2011 [5]. Despite this success, live attenuated and inactivated vaccines possess several major drawbacks. Both live attenuated and inactivated vaccines require the production of large volumes of pathogens in the form of viruses and bacteria. This lengthy culturing process contributes to a considerable lag time between antigen production and vaccine delivery. Furthermore, it demands specialized containment facilities and poses considerable risk to the operators and environment due to the infectious nature of the material [6,7]. Despite adequate passaging to diminish virulence, live attenuated pathogens are capable of reverting to virulent strains as evidenced with simian immunodeficiency virus [8], African horse sickness [9] and infectious bronchitis virus vaccines [10]. The genuine threat of vaccine-derived polio associated with Sabin's oral polio vaccine has hindered immunization programs worldwide [11,12]. Inactivated polio vaccine has less of a biosafety risk to vaccine recipients as inactivated poliovirus is incapable of replication, thereby eliminating the possibility of vaccine-derived polio. However, inactivation of microorganisms can compromise the native conformation of antigenic epitopes resulting in reduced immunogenicity [13]. Pathogens that display high levels of antigenicity owing to high mutation rates (e.g. RNA viruses such as influenza and human immunodeficiency virus [14,15]) or existing as multiple genotypes and serotypes (e.g. rotavirus [16,17], enterovirus [18] and the Group A Streptococcus [19]) present a challenge for developing efficacious vaccines. While this is an important consideration for all vaccine manufacturing platforms, the current timescale of traditional vaccine manufacturing highlights their inadequacy.

Outbreaks of H1N1 influenza, Middle East Respiratory Syndrome, Ebola and Zika over the last decade, are timely reminders that improved modern vaccine technology is necessary to shorten the developmental and production time of vaccines. Vaccine platform technologies, the formulation of antigens of choice with a pre-defined platform base, have the potential to address vaccine manufacturing challenges such as speed, safety and efficacy. Platforms based on virus-like particle (VLP) and liposomes are discussed, with a focus on the challenges and opportunities offered by these vaccine platform technologies.

2. Modular vaccine approach

A tailorable platform that supports safe and simple manufacture of target antigens at high capacity has the potential to rapidly respond to an emerging disease. Most vaccine platform technologies consist of a platform base carrier (Fig. 1) that is amendable to modularization with target antigenic components of pathogens (known as modules). Independently, these components exhibit weak immunogenicity and poor stability. To harness the
Modularization of target epitopes onto VLP and liposome vaccine platforms. Antigenic modules from a variety of microorganisms may be modularized onto the surface of VLPs through electrostatic interaction, chemical conjugation or genetic fusion. In liposomes, these antigenic modules may be encapsulated into the aqueous core, adsorbed into the lipid bilayer or conjugated (both covalently or non-covalently) to the vesicle surface.

**Fig. 1.** Modularization of target epitopes onto VLP and liposome vaccine platforms. Antigenic modules from a variety of microorganisms may be modularized onto the surface of VLPs through electrostatic interaction, chemical conjugation or genetic fusion. In liposomes, these antigenic modules may be encapsulated into the aqueous core, adsorbed into the lipid bilayer or conjugated (both covalently or non-covalently) to the vesicle surface.

immunostimulatory properties of such antigens, platform carriers are engineered and developed to enhance the antigenicity but without the infectious trait of pathogens. Such engineering also allows the production of novel vaccine candidates that cannot be obtained through traditional methods (attenuation and inactivation). Basic research to determine suitable modules with antigenic potential is a prerequisite of this modular approach, yet the use of generic platforms supports streamlined and standardized vaccine development, potentially reducing the cost of development.

A well-exploited platform is based on VLP technology. VLPs are highly ordered structures, with varying degrees of complexity, which stimulate both innate and adaptive immune responses [20,21]. These intrinsic properties contributed to the commercialization of VLP-based vaccines against human papillomavirus (HPV), hepatitis B core and E [22–24]. The self-adjuvanting properties of VLPs, due to their particulate structure and optimal size for uptake by antigen presenting cells [20,25], makes them an attractive tool for increasing the immunogenicity of antigens. Antigens encapsulated within VLPs can also be used as vectors for drug delivery [26]. Well reported platforms based on self-assembling proteins include HPV L1 [27], Hepatitis B core [28] or surface antigen [29,30], murine polyomavirus VP1 [31,32] and bacteriophages MS2 [33], AP205 [34,35] and Q8 [36]. High antigen-specific antibody titers and protective efficacies have been demonstrated across a range of peptide epitopes and protein domains modularized onto these VLP platforms. As reported, a pre-existing immunity against the VLP proteins from previous exposure to the platform does not diminish the immune response against the antigenic modules [37,38]. Mosquirix™ (RTS,S/AS01, GlaxoSmithKline), a protein-based malaria vaccine comprising circumsporozoite protein and Hepatitis B surface antigen, has demonstrated safety and protection in children and infants in a Phase III trial [39], and WHO has recently announced the first pilot studies in sub-Saharan Africa [40].

Liposomes are another favorable vaccine platform owing to their natural ability to induce an immune response [41]. Composed of an aqueous core and a uni- or multilamellar phospholipid bilayer, these lipid-based vesicles have immense adaptability and parameters with relation to size, charge, lipid, adjuvant composition and antigen presentation are manipulable [42]. As a result of this versatility, liposomal-based platforms are less well-defined than VLP-based platforms. Surface charge of the vesicle is reported to be an important factor that influences the immune response [42–44]. Cationic formulations are considered the most effective tools in liposomal antigen delivery due to their ability to bind antigen presenting cells through electrostatic interactions and form antigen depot at the site of injection [45,46]. The combination of positively charged dimethyl季ocadecylammonium (DDA) with the immunostimulant, trehalose-6,6-dibehenate (TDB) was engineered for the delivery of the tuberculosis antigen, Ag85B-ESAT-6 [45] and is possibly the best characterized. DDA:TDB is also considered as a potential platform for Chlamydia vaccines [47].

3. Vaccine design

The strategy for modularizing antigenic peptide or protein module onto the platform base is the key driver for inducing the protective immune response. Maintaining both the native conformational structure of the antigenic module post modularization and the integrity of the immunostimulating platform base are of equal importance. The rules to guide vaccine design are still limited. Although computational simulation tools and structure-based vaccine design are still in their infancy, they offer alternative possibilities to traditional empirical vaccine development [48,49].

Modularization of chosen antigens onto VLPs is achieved through electrostatic interaction [50], chemical conjugation or genetic fusion [51]. Electrostatic interaction requires minimal processing but these non-covalent interactions can be weak and stability is questionable. A variety of linkage chemistries suitable for chemical conjugation result in a more permanent interaction albeit this requires more complex manufacturing processes under potentially harsh conditions that may alter protein structure. Permanent and regular modular display is afforded through genetic fusion, eliminating downstream processing yet insertion sites for modules
can place limitations on antigen size and may be incompatible with VLP assembly. Peptides are more amenable to VLP surface display than large protein domains although conformational structure can be compromised, ultimately affecting the quality of the immune response [52–54]. Displaying large protein domains has the added benefit of presenting multiple epitopes in the correct structure which may increase immunogenicity. However, expression of large genetically-fused antigens is a challenge owing to protein folding errors or compromised VLP formation through steric hindrance [55,56]. To overcome these issues, strategies such as linker designs [56], antigen titration [38,56,57], split-intein conjugation [34,58,59] and a tandem core fusion strategy [60] are implemented to enable ease of large antigen modularization.

For liposomal vaccine platform, antigens can be encapsulated into the hydrophilic aqueous core [61,62], intercalated into the lipid bilayer or surface attached [63]. Successful modularization of antigens up to 150 kDa have been reported [64–66], larger than those described for VLPs. Modularization with surface attached antigens often elicit superior immune responses in comparison to encapsulated antigens perhaps owing to intracellular processing which is possible for the latter [67]. Despite this, encapsulation protects antigens from protease degradation, facilitates longer circulation time and can generate effective immune responses [68–70]. Low encapsulation efficiency is common due to antigen loss from the vesicle during the manufacturing process which involves film extrusion and high shear methods [71]. Incubating antigens with pre-formed liposomes in the presence of 30% v/v ethanol improves encapsulation efficiency [71,72] and may aid a more streamlined manufacturing process whereby peptides can be encapsulated post-production. Unlike VLP technology, modules cannot be genetically fused to the carrier thus surface exposed antigens rely heavily upon bioconjugate technologies such as covalent conjugation (i.e. palmitoylation). Lipidation can compromise peptide conformation potentially resulting in altered immune responses [73]. Incorporating appropriate linkers between the module and the fatty acid to create spatial separation can address this [74–76]. As demonstrated by Lipotek Pty Ltd [77] and others [66,78], the use of nitriotrionic acid (NTA) - histidine conjugation is promising, yet this remains a relatively unexplored area of liposomal vaccines for modularization.

| Table 1 | Platform manufacturing technologies for modularization. |
|---------|----------------------------------------------------------|
| Mechanism of Modularization | Advantages and Challenges | Platform | Disease | References |
| VLP – Molecular insertion | Simple molecular cloning | Bacteriophage AP205 | Influenza (M2) | [64] |
| | Co-production of platform and module | Cucumber Mosaic Virus | Alzheimer’s disease (Amyloid β) | [85] |
| | Reproducible module display | Hepatitis B Core | Malaria (Circumsporozoite) | [28] |
| | Identification of insertion site | Dengue virus type 2 (Envelope domain III) | | [87] |
| | Determination of suitable linkers | Steric hindrance with large modules | | |
| | Limitations on module size | | | |
| | Human Papillomavirus L1 Capsid | | | |
| | Murine Polyomavirus | | | |
| | Tobacco mosaic virus | | | |
| VLP – Conjugation | Conjugation of large modules without affecting VLP assembly | Bacteriophage AP250 | Malaria (Circumsporozoite) | [34] |
| | Range of conjugation chemistries | | | [39] |
| | Quantification of conjugation efficiency | Bacteriophage Qi | | [58] |
| | Removal of unconjugated material | Hepatitis B Core | | [93] |
| | Location of module dependent upon method of conjugation | Rabbit Haemorrhagic | Disease Virus | [94] |
| | Harsh conditions alter epitope structure | | | |
| Liposome – Encapsulated | Module protected from proteases | Cationic liposome | | Leishmania | [61] |
| | Longer circulation time | | | | [65] |
| | Low encapsulation efficiency | | | | [62] |
| Liposome – Surface conjugation | Modularization possible on pre-formed liposomes | Cationic liposome | Human papillomavirus type 16 (E6) | [75] |
| | Range of conjugation chemistries | DMPC-DMPG-cholesterol-MPLc | Human immunodeficiency virus type 1 (gp120) | [95] |
| | Harsh conditions alter epitope structure | Metallochelating liposome | Candida albicans (Heat shock protein 90) | [66] |
| | Removal of unconjugated material | Neutral liposome | Group A Streptococcus | [76] |
| | Oleoyl liposome | Oligosaccharide (Ag85B-ESAT-6) | | | [96] |
| Liposome – Adsorbed | Minimal preparation | Cationic liposome | Tuberculosis | [97] |
| | Lacks control of module orientation or display | Cationic and neutral liposomes | | | [98] |
| | | | | | |

* DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-sn-glycero-3-phosphorylglycerol; MPL, monophosphoryl lipid A.

4. Platform-based vaccine manufacturing

The long and complex vaccine development process (development, testing, regulatory) requires a huge investment of resources which includes time, facilities and money. Vaccine manufacturing
processes are often customized and conducted in dedicated facilities for separate vaccines due to the characteristics of vaccine antigens and safety issues. These factors pose barriers for a fast response that is critical for controlling modern-day disease outbreaks that spread rapidly, as observed for H1N1 influenza in 2009 [80] and most recently Zika [81]. A platform approach for vaccine manufacturing ideally streamlines the bioprocess, and shortens vaccine product development and delivery (time to market).

Platform technologies allow the standardization of upstream and downstream processes, given that the platform base remains unchanged. Certainly, processes will need optimization with modularization of different antigenic modules, but vaccine platform technologies provide flexibility and possibility for multi-product facilities. Prior knowledge, experience and production facility set-up is immensely beneficial. Merck Research Laboratories used their prior knowledge and know-hows from developing hepatitis B VLP vaccine (Recombivax) as the key decision factor when choosing to use the same host (Saccharomyces cerevisiae) for the production of HPV VLP vaccine (Gardasil) [82]. Similarly, the decision on the Joint of adjutant to formulae HPV vaccine was made based on Recombivax.

The desire to lower cost of goods, thus leading to cheaper vaccines in the market has been well discussed and debated in papers and at conferences. The largest vaccine market is in developing countries, where vaccines would have a significant impact on public health, but these low-income countries face vaccine accessibility and affordability challenges. In combination with modular single-use technologies [83], modern vaccine manufacturing based on platform technologies may potentially lower capital and operating costs, resulting in affordable vaccines.

Another benefit of platform technologies is the potential reduction of regulatory burden. The level of proof and documentation required for new antigenic module on the generic platform may lessen as regulatory authorities are well informed by regulatory track records on the platform base. In the scenario of a disease outbreak, a close collaboration with regulatory authorities may lead to fast-track development of a safe and effective vaccine for the public, against an emerging pathogen.

The benefits of VLP and liposome platform technologies are many but perhaps the most significant is their potential to generate multivalent vaccines. Vaccines designed for immunization against multiple strains of an antigenically diverse pathogen are possible through display of different modules on a single platform or formulation of multiple platform products. Future work is expected to optimize the methodologies by which modules are incorporated into each platform to ensure the success of modern vaccines.

Acknowledgement

We acknowledge the funding support from the Australian Research Council (ARC Discovery Project DP160102915).

References

[1] Cutts FT, Lesher J, Metcalf CJG. Measles elimination: progress, challenges and implications for subela control. Expert Rev Vaccines 2013;12:217–22.
[2] Roush SW, Trudy V. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. J Am Med Assoc 2007;298:215–2.
[3] Van Panhuis WGB, Shawn, Zadorozhny Vladimir, Lee Bruce Y, Eng Heather, Cross Anne, et al. Contagious diseases in the United States from 188 to the present. New Engl J Med 2013;369:2153. 6.
[4] Henderson DA. The eradication of smallpox—an overview of the past, present, and future. Vaccine 2011;29(Suppl 4):97–10.
[5] Mariner JH, House JA, Mebus CA, Sollod AE, Chibeau D, Jones BA, et al. Rinderpest eradication: appropriate technology and social innovations. Sci Transl Med 2012;373:1309–12.
[6] Uddowala S, Hullister J, Pacheco JM, Rodriguez LR, Rieder E. A safe foot-and-mouth disease vaccine platform with two negative markers for differentiating infected from vaccinated animals. J Virol 2012;86:11675–85.
[7] Steele J, Lowen AC, Pena L, Angel M, Solorzeto J, Albrecht R, et al. Live attenuated influenza viruses containing NS1 truncations as vaccine candidates against H5N1 highly pathogenic avian influenza. J Virol 2009;83:1742–53.
[8] Whatmore AC, Cook N, Hall CA, Sharpe S, Rud EW, Cranage MP. Repair and evaluation of nef in vivo modulates simian immunodeficiency virus virulence. J Virol 1995;69:5117.
[9] Weyer CT, Grewar JD, Burger P, Rossouw E, Lourens C, Joone C, et al. African horse sickness caused by genome reassortment and reversion to virulence of live, attenuated vaccine viruses, South Africa, 2004–2014. Emerg Infect Dis 2016;22:807–96.
[10] Zhang Y, Wang HN, Wang F, Fan WQ, Zhang AY, Wei K, et al. Complete genome sequence and recombination analysis of infectious bronchitis virus attenuated vaccine strain H120. Virus Genes 2010;41:377–88.
[11] Nathanson N, Kew GM. From emergence to eradication: the epidemiology of polioviruses. Front Immunol 2011;2:1213–29.
[12] Bandypadhyoy AC, Caron J, Sibb K, Orenstein WA. Polio vaccination: past, present and future. Future Microbiol 2015;10:791–808.
[13] Fan YC, Chiu HC, Chen HK, Chang GJ, Chiou SS. Formalin inactivation of Japanese encephalitis virus vaccine alters the antigenicity and immunogenicity of a neutralization epitope in envelope protein domain III. PLoS Negl Trop Dis 2015;9:e0001676.
[14] Treanor J. Influenza vaccine — outmaneuvering antigenic shift and drift. New Engl J Med 2004;350:218–20.
[15] Lipsitch M, O’Hagan B. Patterns of antigenic diversity and the mechanisms that maintain them. J Nat Soc Interface 2007;4:787–802.
[16] Miles MG, Lewis KD, Kang G, Parashar UD, Steele AE. A systematic review of rotavirus strain diversity in India, Bangladesh, and Pakistan. Vaccine 2012;30(Suppl 1):A131–9.
[17] Chung JY, Kim MS, Jung TW, Kim SJ, Kang JH, Han SB, et al. Detection of rotavirus genotypes in Korea 5 years after the introduction of rotavirus vaccines. J Korean Med Sci 2015;30:1471–5.
[18] Xu MI, Si L, Cao L, Zhang H, Dong N, Dong Z, et al. Genotypes of the enterovirus causing hand, foot and mouth disease in Shanghain, China, 2012–2013. PLoS ONE 2015;10:e0138514.
[19] Steer AC, Carapetis JR, Dale JB, Fraser JD, Good MF, Guilherme L, et al. Status of research and development of vaccines for Streptococcus pyogenes. Vaccine 2016;34:2953–9.
[20] Keller SAB, Bauer Monika, Manolova Vania, Muntwiler Simone, Saund Philosoph, Bachmann Tilman, Cutting edge: limited specialization of dendritic cell subsets for MHC class II-associated presentation of viral particles. J Immunol 2010;184:26–9.
[21] Bossaert LF, Monor G, Leclerc C. Virus-like particles: a new family of delivery systems. Expert Rev Vaccines 2002;1:101–9.
[22] Kushnir N, Streafeld SJ, Yusibov V. Virus-like particles as highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. Vaccine 2012;31:58–83.
[23] Pili S, Joura EA. From the monovalent to the nine-valent HPV vaccine. Clin Microbiol Infect 2015;21:827–33.
[24] Lua LH, Conners NK, Sainsbury F, Chuan YP, Wibowo N, Middelberg AP. Bioengineering virus-like particles as vaccines. Biotechnol Bioeng 2014;111:425–40.
[25] Manolova VF, Flace Anna, Bauer Monika, Schwarz Katrin, Saund Philipp, Bachmann Martin F. Nanoparticles: Cutting edge: limited specialization of dendritic cell subsets for MHC class II-associated presentation of viral particles. J Immunol 2008;181:1404–13.
[26] Zdanowicz M, Chroboczek J. Virus-like particles as drug delivery vectors. Acta Biochim Pol 2016;63:469–73.
[27] Murata Y, Lightfoot PM, Rose RC, Walsh EE. Antigenic presentation of heterologous epitopes engineered into the outer surface-exposed helix 4 loop region of human papillomavirus 13 capsomeres. Virol J 2009;6:81.
[28] Sallberg M, Hughes J, Jones J, Phillips TR, Milich DR. A malaria vaccine candidate based on a Hepatitis B virus core platform. Intervirology 2003;45:350–61.
[29] Stuchelkunov SN, Salayev RK, Pozdnaykov SG, Rekoslavskaya NI, Nesterov AE, Ryzhova TS, et al. Immunogenicity of a novel, bivalent, plant-based oral vaccine against hepatitis B and human immunodeficiency viruses. Biotechnol Lett 2012;34:2899–57.
[30] Ballou WR. The development of the RTS, S malaria vaccine candidate: challenges and lessons. Parasite Immunol 2009;31:492–500.
[31] Middelberg AP, Rivero-Hernandez T, Wibowo N, Lua LH, Fan Y, Magor G, et al. A microbial platform for rapid and low-cost virus-like particle and capsomere vaccines. Vaccine 2011;29:7154–62.
[32] Wibowo N, Chuan YP, Lua LH, Middelberg AP. Modular engineering of a microbe-produced viral capsomere vaccine for influenza. Chem Eng Sci 2010;65:1031–20.
[33] Fu Y, Li J. A novel delivery platform based on Bacteriophage MS2 virus-like particles. Virus Res 2016;211:9–16.
[34] Jainzetz CM, Matondo S, Thrane S, Nielsen MA, Kavisev R, Rwakaliga SB, et al. Bacterial superlue generates full-length circumsporozoite protein virus-like particle vaccine capable of inducing high and durable antibody responses. Malar J 2016;15:545.
[35] Pastori C, Tudor D, Diomedes L, Drillit AS, Jegerlehner A, Rohn TA, et al. Virus-like particle based strategy to elicit HIV-protective antibodies to the alphaherpesvirinae gpl41. Virology 2012:431–11.
imunized with liposome encapsulated recombinant NE protein based on Hepatitis E vaccine candidate. Vaccine 2016;34:5895–902.

63. Bobbala S, Hook S. Is there an optimal formulation and delivery strategy for liposome vaccines? Pharm Res 2008;25:978–987.

64. Davis D, Gregoriadis G. Liposomes as adjuvants with immunoprotected tetanus toxoid: Influence of liposomal characteristics. Immunology 1987;61:229–34.

65. Nagui R, Kaur S. Enhanced efficacy and immunogenicity of 7B4 antigen recombinant in various adjuvants against murine visceral leishmaniasis. Vaccine 2010;28:4002–12.

66. Mašek J, Bartheldyová E, Turánek-Knotigová P, Škrabalová M, Korvasová Z, Kuchalkova J, Metelka M. Liposomes with associated lipoprotein norA minibody as a bioconjugate platform for co-delivery of vaccines with recombinant His-tagged antigens: Preparation, structural study and immune response towards His90. J Control Release 2011;151:193–201.

67. Tan M, Wessel NM, Ahrens P, Krzych U. Intracellular processing of liposome-encapsulated antigens by macrophages depends upon the antigen. Infect Immun 1995;63:2396–402.

68. Taki A, Simooker P. Small-wonders-the use of nanoparticles for delivering antigen. Vaccines (Basel) 2015;3:638–61.

69. Teng X, Tian M, Li J, Tan S, Yuan X, Yu Q, et al. Immunogenicity and protective efficacy of DMT liposome-adjuvanted tuberculosis subunit CTTH vaccine. Hum Vaccin Immunother 2015;11:1456–64.

70. Ma T, Liu Y, Cheng J, Liu Y, Fan W, Cheng Z, et al. Liposomes containing recombinant E protein vaccine against duck Trembusu virus in ducks. Vaccine 2016;34:2157–63.

71. Shanbat SB, Badree Ali, Jaafari Mahmoud Reza, Moretazayi Sayed Alireza. Optimization of a murine model to prepare liposomes containing HER2/Neu- derived peptide as a vaccine delivery system for breast cancer. Iran J Pharm Res 2014;13:15–25.

72. Wang CH, Huang YJ. Encapsulating protein into preformed liposomes by a self-assembled nanodroplet method. Artif Cells Blood Substit Biotechnol 2003;31:303–12.

73. Hickman DT, Lopez-Deber MP, Ndao DM, Silva AB, Nand D, Pilgrim M, et al. Sequence-independent control of peptide conformation in liposomal vaccines: Bacteriophage protein multimerization. J Biol Chem 2011;286:12966–71.

74. Mubs AH, Hickman David T, Pilgrigen Maria, Chuaard Nathalie, Giuresi Valeria, Meerschman Carine, et al. Liposomal vaccines with conformation-specific myeloid antigen peptides define immune response and efficacy in APP transgenic mice. Proc Nat Acad Sci USA 2007;104:10332–6.

75. Chen W, Huang L. Induction of cytotoxic T-lymphocytes and antitumor activity by a liposomal lipopeptide vaccine. Mol Pharm 2008;5:464–71.

76. Wills NA, Oosterveld G, Lavigne EL, McPhun V, Powell JL, Phillips ZN, et al. Novel platform technology for modular mucosal vaccine that protects against streptococcus. Sci Rep 2016;6:39274.

77. Tyne AS, Chan JG, Shanahan ER, Armstrong KO, Chan KH, Britton WJ, et al. TLR2-targeted secreted proteins from Mycobacterium tuberculosis are protective as powdered pulmonary vaccines. Vaccine 2013;31:4322–9.

78. Marques-Gallego P, de Kroon AI. Ligation strategies for targeting liposomal delivery. J Liposome Res 2010;20:197–204.

79. Anggraeni MR, Connors NK, Wu Y, Chuan YP, Lua LH, Middelberg AP. Bacterial superglue enables easy development of efficient virus-like particle carriers. PLoS ONE 2015;10:e0120751.

80. De Filette M, Martens W, Smet A, Schotsaert M, Birkett A, Londono-Arcila P, et al. Sequence-independent control of peptide conformation in liposomal vaccines: Bacteriophage protein multimerization. J Biol Chem 2011;286:12966–71.

81. Nakagawa Shinsaku, et al. Positively charged liposome functions as an efficient delivery system for antigen vaccination in cancer. Proc Nat Acad Sci USA 2007;104:10332–6.

82. Plocková J, et al. Metallochelating liposomes with associated lipophilised hormones TDM) confers long-term protection against visceral leishmaniasis through a host immune response and participation of CD4+ central and effector memory T cells in mice. J Control Release 2011;151:193–201.

83. Bachmann Martin F. Efficient induction of mucosal and systemic immune responses to liposomal antigens. Nature 1997;324:252–2.

84. Watson DS, Endesley AN, Huang L. Design considerations for liposomal vaccines: influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens. Vaccine 2012;30:2256–72.

85. Kraaijveeld CAS, Schilham M, Jansen J, Benaisa-Trouw B, Harmsen M, Van Houte AJ, et al. The effect of liposomal charge on the neutralizing antibody response against hepatitis B virus (HBV) and encephalomyocarditis virus and Semliki Forest viruses. Clin Exp Immunol 1984;56:509–14.

86. Nakamisrawa Jun, Hayashi Akira, Tsutsuni Yasuo, Kubo Kazuyoshi, Nakagawa Shinshu, et al. Positively charged liposome functions as an efficient immunoadjuvant in inducing cell-mediated immune response to soluble proteins. J Control Release 1999;63:231–40.

87. Christensen AC, Gregoriadis G. Liposomes as immunological adjuvants. Nature 1974;242:252–2.

88. Peacey M, Wilson S, Baird MA, Ward VK. Versatile RHDV virus-like particles: a modular platform technology for modular mucosal vaccine that protects against streptococcus. Sci Rep 2016;6:39274.

89. Tyne AS, Chan JG, Shanahan ER, Armstrong KO, Chan KH, Britton WJ, et al. TLR2-targeted secreted proteins from Mycobacterium tuberculosis are protective as powdered pulmonary vaccines. Vaccine 2013;31:4322–9.

90. Marques-Gallego P, de Kroon AI. Ligation strategies for targeting liposomal delivery. J Liposome Res 2010;20:197–204.
Jegerlehner A, Zabel F, Langer A, Dietmeier K, Jennings GT, Saudan P, et al. Bacterially produced recombinant influenza vaccines based on virus-like particles. PLoS ONE 2013;8:e78947.

Jegerlehner A, Schmitz N, Storni T, Bachmann MF. Influenza a vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. J Immunol 2004;172:5598–605.

Jemon K, Young V, Wilson M, McKee S, Ward V, Baird M, et al. An enhanced heterologous virus-like particle for human papillomavirus type 16 tumour immunotherapy. PLoS ONE 2013;8:e66866.

Watson DS, Platt VM, Cao L, Venditto VJ, Szoka Jr FC. Antibody response to polyhistidine-tagged peptide and protein antigens attached to liposomes via lipid-linked nitrilotriacetic acid in mice. Clin Vaccine Immunol 2011;18:289–97.

Takagi A, Kobayashi N, Taneichi M, Uchida T, Akatsuka T. Coupling to the surface of liposomes alters the immunogenicity of hepatitis C virus-derived peptides and confers sterile immunity. Biochem Biophys Res Commun 2013;430:183–9.

Hamborg M, Kramer R, Schante CE, Agger EM, Christensen D, Jorgensen L, et al. The physical stability of the recombinant tuberculosis fusion antigens h1 and h56. J Pharm Sci 2013;102:3567–78.

Barnier-Quer C, Esharkawy A, Romeijn S, Kros A, Jiskoot W. Adjuvant effect of cationic liposomes for subunit influenza vaccine: influence of antigen loading method, cholesterol and immune modulators. Pharmaceutics 2013;5:392–410.