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Platform technologies for modern vaccine manufacturing

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ABSTRACT

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.

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

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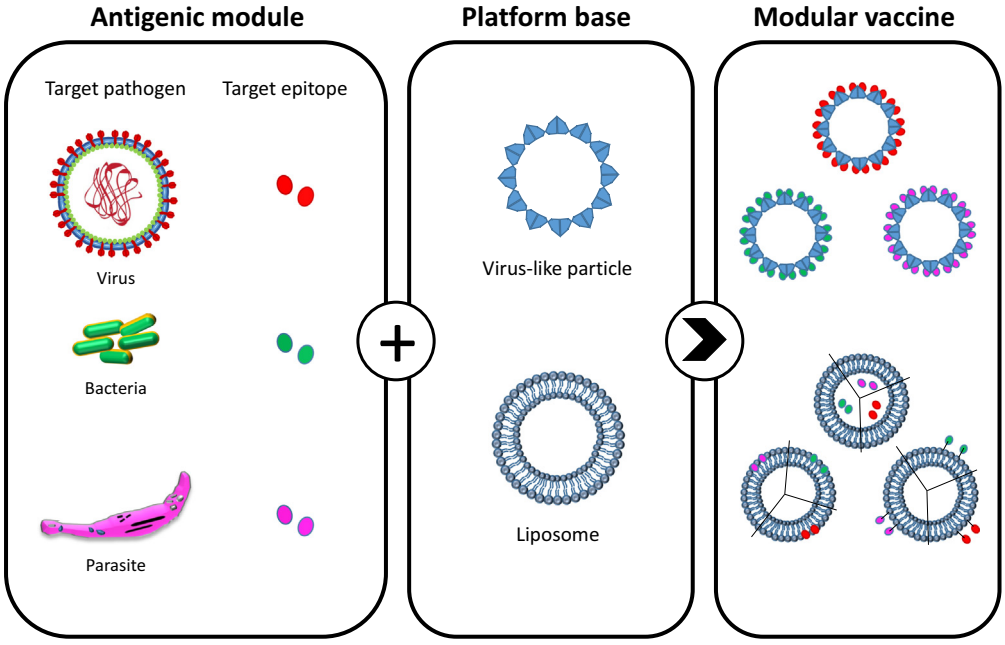

Fig. 1. Modularization of target epitopes onto VLP and liposome vaccine platforms. Antigenic modules from a variety of microorganisms may be modularized onto the surface of VLPs through electrostatic interaction, chemical conjugation or genetic fusion. In liposomes, these antigenic modules may be encapsulated 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 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 Qβ [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/ASO1, 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 depots at the site of injection [45,46]. The combination of positively charged dimethyldioctadecylammonium (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 module display is afforded through genetic fusion, eliminating downstream processing yet insertion sites for modules

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)	[84]
	Co-production of platform and module	Cucumber Mosaic Virus	Alzheimer's disease (Amyloid β)	[85]
	Reproducible module display		Newcastle disease virus	[86]
	Identification of insertion site	Hepatitis B Core	Malaria (Circumsporozite)	[28]
	Determination of suitable linkers		Dengue virus type 2 (Envelope domain III)	[87]
	Limitations on module size			
	Steric hindrance with large modules		Influenza (M2e)	[88]
			Tuberculosis (CFP-10)	[89]
		Human Papillomavirus L1 Capsid	Human respiratory syncytial virus	[27]
		Murine Polyomavirus	Influenza (M2e)	[32]
			Group A Streptococcus (J8)	[31]
			Rotavirus (VP8*)	[38]
		Tobacco mosaic virus	Poliovirus (type 3)	[90]
			Foot-and-mouth disease	[91]
VLP – Conjugation	Conjugation of large modules without affecting VLP assembly	Bacteriophage AP250	Malaria (Circumsporozite)	[34]
	Range of conjugation chemistries		Malaria (Pfs25 / VAR2CSA), Tuberculosis (Ag58A)	[59]
	Quantification of conjugation efficiency		Malaria (Pfs25 / CIDR)	[58]
	Removal of unconjugated material	Bacteriophage Qβ	Influenza (Hemagglutinin)	[92]
	Location of module dependent upon method of conjugation	Hepatitis B Core	Influenza A (M2e)	[93]
	Harsh conditions alter epitope structure	Rabbit Haemorrhagic Disease Virus	Human papillomavirus type 16 (E6)	[94]
Liposome – Encapsulated	Module protected from proteases	Cationic liposome	Leishmania	[61] [65]
	Longer circulation time		Hepatitis E	[62]
	Low encapsulation efficiency		Duck Tembusu virus	[70]
Liposome – Surface conjugation	Modularization possible on pre-formed liposomes	Cationic liposome	Human papillomavirus type 16 (E7)	[75]
	Range of conjugation chemistries	DMPC-DMPG-cholesterol-MPL[a]	Human immunodeficiency virus type 1 (gp41)	[95]
	Harsh conditions alter epitope structure			
	Determination of suitable linkers	Metallochelating liposome	*Candida albicans* (Heat shock protein 90)	[66]
	Removal of unconjugated material	Neutral liposome	Group A Streptococcus	[76]
		Oleoyl liposome	Hepatitis C virus	[96]
Liposome – Adsorbed	Minimal preparation	Cationic liposome	Tuberculosis (Ag85B-ESAT-6)	[97]
	Lacks control of module orientation or display	Cationic and neutral liposomes	Influenza (Hemagglutinin)	[98]

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

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 sheer 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 nitrilotriacetic acid (NTA) - histidine conjugation is promising, yet this remains a relatively unexplored area of liposome technology. NTA conjugation offers the opportunity of assembling entire protein domains [66] onto pre-formed liposomes whilst removing costly purification processes. Novel liposomal platforms encapsulate immunostimulants (including diphtheria toxoid and TLR9 agonists) independent of surface attached target antigen [76,79]. This spatial segregation of antigenic components (with the immunostimulant exposed only upon intracellular processing) has been shown to enhance target specific immune responses. Table 1 summarizes manufacturing technologies for modularization.

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 streamline the bioprocess, and shorten 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 choice of adjuvant to formulate HPV VLP 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.

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