Chapter

New Generation Peptide-Based Vaccine Prototype

Öznur Özge Özcan, Mesut Karahan, Palanirajan Vijayaraj Kumar, Shen Leng Tan and Yi Na Tee

Abstract

Synthetic peptide-based vaccine prototypes are the future potential vaccination. Antigens, which belong to minimal microbial component and produce antibodies such as peptides and polysaccharides, can promote long-term protection against pathogens that can cause infectious diseases. Production of peptides becomes simple with solid phase peptide synthesis and microwave-assisted solid phase peptide synthesis using automatic synthesizers. The use of synthetic peptides was approved by the health authorities for vaccine design. Peptides are themselves very weak immunogens and need adjuvants to provide an effective autoimmune response. For this reason, peptide antigens are conjugated with biopolymers and loaded with nanoparticles. The toxicity of vaccine prototypes is evaluated in cell culture, and non-toxic prototypes are selected for vaccinating experimental animals. The most effective peptide-based vaccine prototype is determined as the one with the highest antibody level. The goal of this book chapter is to illustrate the use of peptides vaccine systems and present their opportunities with their future development.

Keywords: biopolymers, nanoparticle systems, solid phase peptide synthesis (SPPS), synthetic peptide, peptide vaccine prototype

1. Introduction

The goal of this chapter is to review the importance of synthetic peptide-based vaccination, providing a brief knowledge about their new generation prototypes. In the first stage, relation to immunity and peptide vaccine with importance of using biopolymers was given under the title of solid-phase peptide synthesis including microwave system. After that, this review was focused on the established methods for peptide loaded nanoparticles or conjugated biopolymers preparation of peptide-based vaccine prototypes and nanotechnological particles as delivery system with touching on different methods. In addition, the impact of Contemporary Advancements in Peptide Based Vaccine like Liposome Based Subunit Vaccines was explained. In the last part, peptide-based vaccine prototypes studies *in vivo and in vitro* were given with their future perspective and development.

2. Peptide vaccines prototype and immunity

All vaccines generally are developed by using live or attenuated microorganisms. However, the use of whole microorganisms, their components or the biological

process for vaccine production has many weaknesses and a variety of approaches for synthetic peptide vaccination remain under investigation for the infectious diseases [1]. Peptides play an important role in a biological process, including the stimulate the immune response [2].

Peptide-based vaccination is an immunotherapy where a peptide is applied often with the use of an immunoadjuvant (nanoparticle or biopolymers) to stimulate T-cell and sometimes B-cell immunity. Peptide-based vaccinations are present in major histocompatibility complexes (MHC) the ultimate target for T cells in infection recognition and infection immune responses [3, 4]. Sometimes peptide-based vaccines play a role to stimulate innate and adaptive immunity both (**Figure 1**) and peptides are immunogen components of peptide-based vaccine and memory responses of peptide is weak in immune responses [1] without the biopolymer or nanoparticle system.

When producing a new generation of synthetic peptide vaccines, components of the pathogenic pathogen of interest are generally used. These components are linear peptides and produced by solid phase peptide synthesis (SPPS) method with high efficiency and purity [5–9]. When peptides are used in combination with a vaccine system, if they are used without a drug delivery system, there are risks of degradation by protease enzymes that break down proteins and phagocytosis by immune system cells such as antibodies [10]. In addition, drug delivery systems should be preferred as nanoparticles and biopolymers. A higher immune response

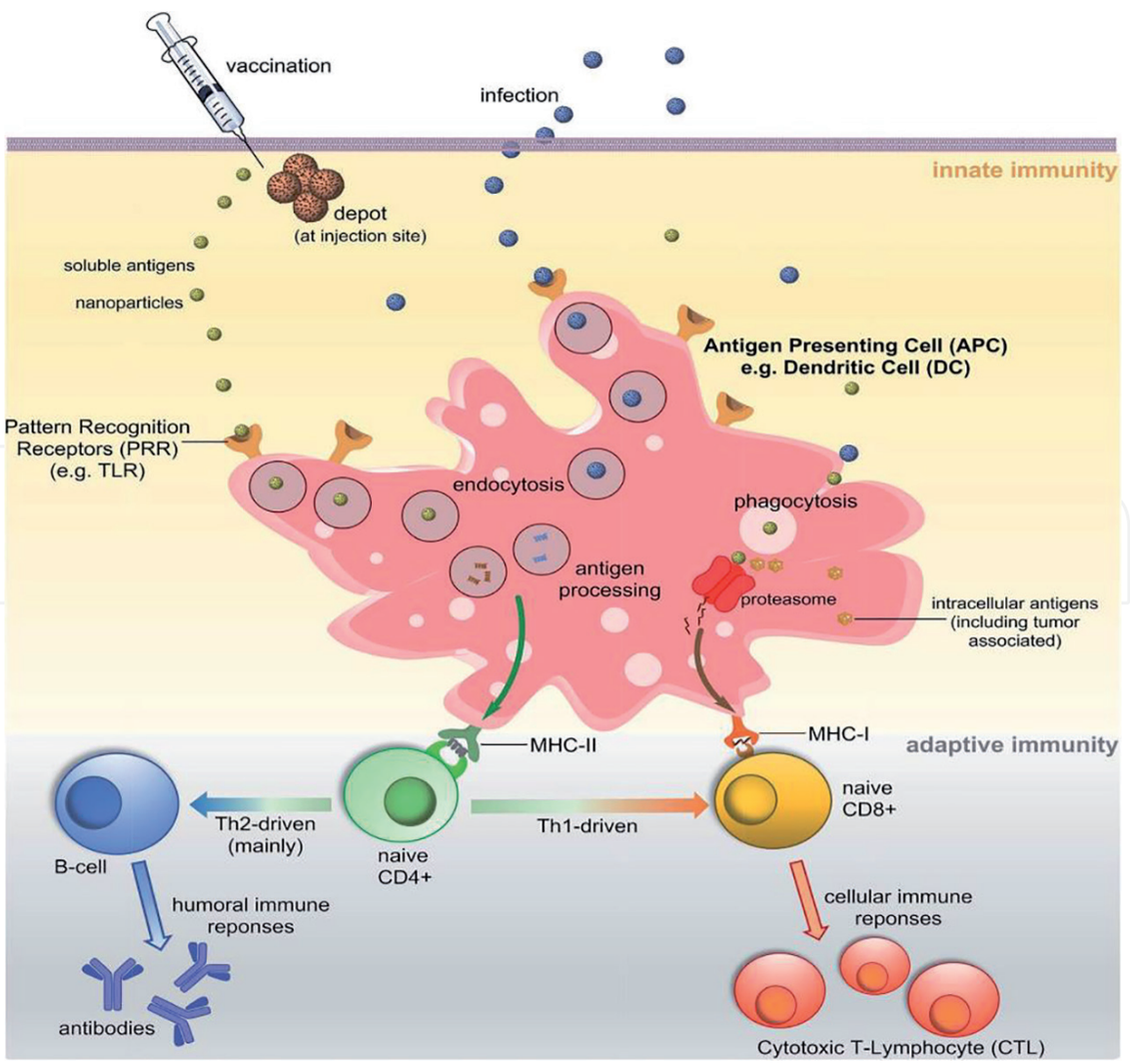

Figure 1.
Cellular representation of immune cells after vaccination [1].

and to protect peptides from harmful effects of degrading enzymes and aggressive antibodies, it is generally necessary to use nanosystems such as protein, biopolymer conjugations or nanoparticles (NPs). Peptide delivery systems based on nanoparticles are developing more and more for the development of peptide-based vaccines. Especially, biodegradable polymers offer very popular and patented vaccines [11]. For instance, poly(amino acid) and polylactic acid (PLA) are used for NPs and when antigenic peptides are encapsulated by them in order to vaccinate mice can provide significantly higher levels of total the antibodies like immunoglobulins (IgGs); IgG, IgG1, IgG2. This means they can able to stimulate humoral immune responses and also CD4+ and CD8+, T and B cell activation and for the cellular immune responses; interferon γ (IFNγ) which induce Ig class switching to IgG2a [12–14]. In another study, Murine model was used in an immunization study. An antigen of Hepatitis B disease loaded on Poly (lactic-co-glycolic acid) (PLGA) NPs (300 nm) provided better immune responses compared to the antigen alone. Immunization with PLA NPs (200–600 nm) can also provide higher levels of IFNγ production related to a Th1 response. In contrast, immunization of PLA microparticles (2–8 μm) promoted IL-4 secretion due to Th2 response [15]. Both PLGA NPs and liposomes are phagocytosed efficiently by cells to localize intracellular localizations and produce an immune response [16, 17]. Carbon NPs are promising in oral vaccine administration for the use of synthetic peptides [18].

Different approaches are available to develop synthetic peptide-based vaccines, using metal ions in combination with peptide sequences. In particular, the investigation of the complex formation biopolymer by peptide in the presence of metal ions contributes greatly to the technological development of peptide-based vaccine prototypes [19]. The contact of the peptides with the polyelectrolyte (PE) is found at the interface. Solubility of polyplexes and complexes with NPs and peptides; it depends on the structure of the peptides (such as hydrophilic and lipophilic) and correlates with the isoelectric points in this system. Metal ions such as copper (Cu^{+2}) generally promote two effects: (1) conjugation of polyelectrolyte to peptide molecules and (2) aggregation of polyplex particles in the intermolecular region. Some of these polyplexes exhibit strong immunogenicity and provide a high level of immunological protection for peptide vaccine prototypes, making them more efficient, but the solubility, composition and stability of these polycomplexes depend on pH, metal/PE and protein/PE ratios. These systems are based on conjugation of PE and antigen molecules with covalent bonds to NPs or biopolymers, which induce an immune response to the immunizing agent. The hydrophobic interactions in such a complex create an adjuvant effect for prototyping technology in vaccination. [19–22]. In the studies on the development of peptide vaccine prototypes previously made by our study group, it was observed that the purification of characterization of binding of synthetic peptides to various adjuvants and subsequent high immune response was obtained in BALB/c mice from experimental animals [23].

3. Solid-phase peptide synthesis (SPPS)

A historical overview of peptide chemistry from T. Curtius (who achieved the first synthesis of peptide in 1882) and Fischer (who synthesized the first dipeptide in 1901) to M. Bergmann and L. Zervas is first in presenting the Solid-Phase peptide synthesis. Next, the fundamentals of peptide synthesis with a focus on SPPS by R. B. Merrifeld are described. Although the peptides can be synthesized in three methods: in a solution medium, on a solid support, or as a combination of the solid and the solution synthesis, this chapter emphasizes an overview of peptide synthesis giving importance on SPPS. Currently, most of the peptides for research,

vaccination or therapeutic drugs for cancer and brain diseases are synthesized by SPPS methods. Successful peptide synthesis depends on the appropriate selection of suitable resins, linkers, amino acid derivatives and coupling reagents, as well as the side chain (de) protection and cleavage conditions, and the correct synthesis of the assay. In the SPPS method, the solid support is attached at the end of the first amino acid-COOH at the carboxyl end a polymeric support insoluble in the newly formed peptide chain is referred to as resin. A covalent binding step that binds the resin is important for the reaction [24]. The peptides may be gradually joined between the C and N terminus using N-protected amino acids. The Nα protecting group (Boc) is unstable in the presence of intermediate acid (trifluoroacetic acid; TFA), the side chain protecting benzyl (Bzl) based groups and the peptide/resin linkage are stable in the presence of intermediate acid and are variable in the presence of strong acid (HF). Fmoc group is important for solid-phase applications. Fmoc-based strategies are also available, and hydroxymethylphenoxy-based binders are used to add peptide to the resin with t-butyl (tBu) based side chain protection [25]. The solid phase peptide synthesis method consists of three basic steps. According to this, deprotection of the carboxyl group activation and peptide bond formation (Coupling). Following this procedure, the final deprotection of the last added amino acid is removed and the N- terminal is released. Cleavage and deprotection of the resin-bound peptide from the solid support [26].

The stepwise representation of solid phase peptide synthesis is illustrated in **Figure 2**. The starting amino acid masked by a non-persistent protecting group at the N-α terminus is loaded from the C-terminus to the resin. A semi-permanent protection group can also be used to mask the side chain if necessary (**Figure 3**, Step 1). The synthesis of the peptide, repeated deprotection of the N-α-transient protecting group, and binding of the next protected amino acid (**Figure 3**, Step 3).

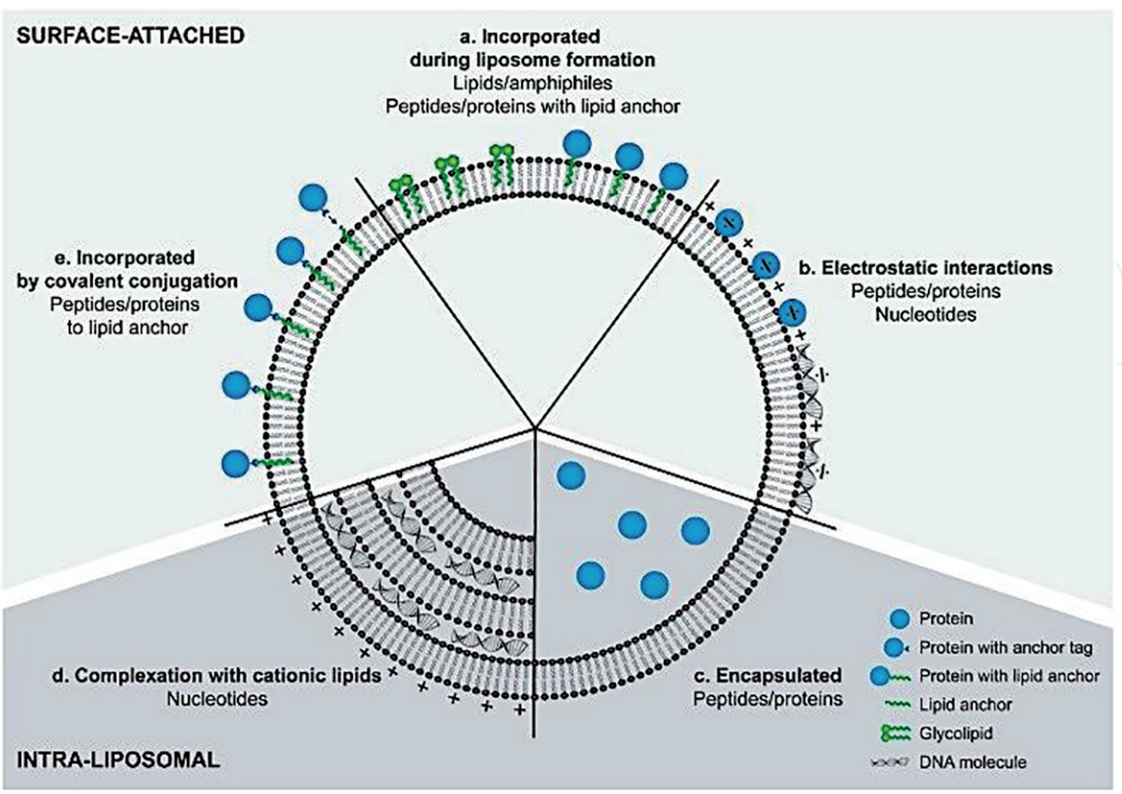

Figure 2.
The antigens and immunomodulators that can be used for inclusion in liposomes; it is shown in different strategies depending on the target and structure of the molecule [28].

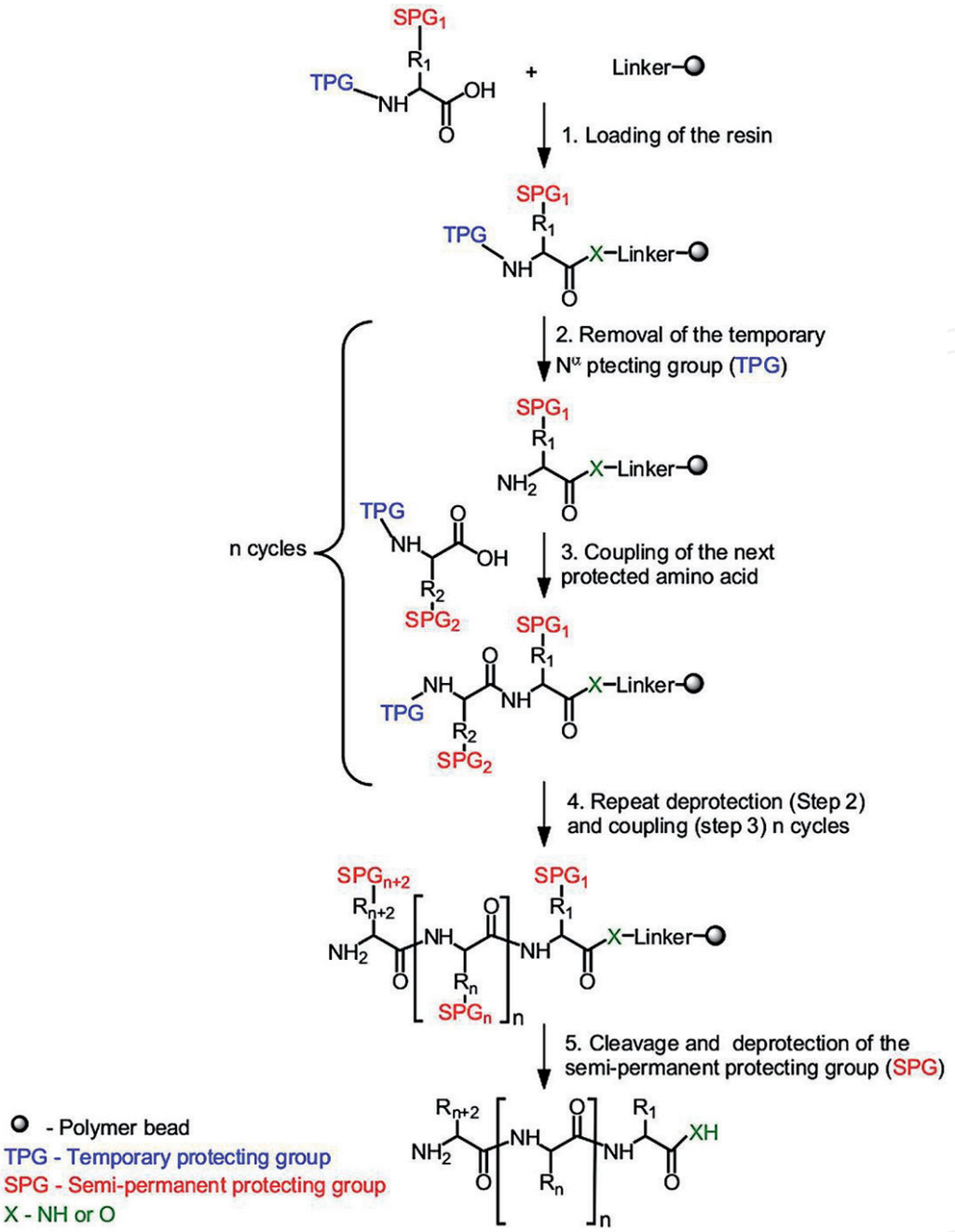

Figure 3.
Stepwise representation of solid phase peptide synthesis [25].

Synthetic cycle	Reagents	Time & conditions
Deprotections	Trifluoroacetic acid (Boc) and 20% piperidine in DMF (Fmoc)	1–5 min (70°C Fmoc and Boc)
Couplings	Amino acids, HBTU/HATU/HOBt/HOAt/DIC, DIPEA	5–15 min 50–70°C

Table 1.
Synthetic cycle and important reagents with time and conditions in microwave-assisted SPPS [27].

After the last amino acid is loaded (**Figure 3**, Step 4), the peptide is separated from the resin support and the Fmoc or Boc groups are removed [25].

The development of microwave-assisted solid-phase peptide synthesis has been developed by the synthesis of linear and complex peptide sequences and long

peptide sequences in a shorter time and high throughput. Time and temperature conditions, reagents and synthesis cycle for microwave assisted SPPS with using Boc or Fmoc are represented below in **Table 1**. The disadvantage of this technique could be the cost of resin (the binding procedure of the first binding amino acid to the resin in peptide synthesis can requires different and complex processes) and equipment [27, 29].

4. Peptide-based vaccine for nanotechnological prototypes

Synthetic peptides alone are not sufficient to develop vaccine prototypes because they cannot stimulate the cellular and humoral protection system sufficiently. So, different adjuvant systems are formed by conjugating the peptides biopolymers or loading them into NPs, resulting in a high immune response [30]. The use of peptide-polymer complexes and peptide loaded nanoparticles are the best way for the developing the peptide-based vaccine prototypes.

4.1 Type of nanoparticles

The binding of the antigenic peptides with the water-soluble polymer has multiple effects. Some of those are as follows:

- to provide modification of peptides,
- to increase the water solubility of those with hydrophobic properties,
- to raise regional impact,
- to increase immunogenic effects and immunoreactivity, and
- to be more effective in the living organism [31].

NPs are spherical polymeric carriers. The particles that are below 1000 nanometers (nm) are called nanoparticles. These particles with superior properties are used in many fields such as electricity, electronics, biotechnology, automotive, medical. NPs are morphologically and physicochemically influenced by the physical and chemical properties of the starting material used. The nanoparticles used as polymeric carriers are solid colloidal structure. The active substance can be encapsulated, absorbed or dissolved in the particle. Polysaccharides, polyanhydride, polycaprolactone, polyacrylic acid and polylactic-co-glycolic acid is also used for producing an effective nanoparticles and produce a co-polymer system such as poly(ethylene glycol) (PEG)-Nps and poly(ethylene glycol)-poly(ε-caprolactone) copolymers (PEG-PCL) copolymers. The copolymers of N-vinyl-2-pyrrolidone with acrylic acid (P(VP-co-AA)), PLGA, NPs loaded with the antigenic peptide can be used for future vaccine prototypes [32]. PLGA NPs is approved by U.S. Food and Drug Administration (FDA) and using for peptide carrier in vivo because of strong immune response [33].

Polymeric NPs are used for therapeutic applications and some of popular NPs are as follows:

1. Pluronics®
2. PEG–PLA

3. PEG-PCL
4. PEG-Lipid
5. PEG-PLGA
6. PEG-poly (amino acids)
7. Stimuli-sensitive polymeric micelles
8. Endogenous stimuli-sensitive polymeric micelles
9. pH-sensitive polymeric micelles
10. Reduction sensitive polymeric micelles
11. Thermo-sensitive polymeric micelles
12. Exogenous stimuli-sensitive polymeric micelles
13. Light-sensitive polymeric micelles
14. Magnetic field-sensitive polymeric micelles
15. Ultra-sound sensitive polymeric micelles
16. Margination of micro/NPs: Requirement for optimum drug delivery

5. Established methods for peptide loaded NPs or conjugated biopolymers preparation

We have mentioned that the peptides alone cannot produce an adequate immune response and also have poor stability with the internalization problem while crossing cell membranes. To solve all these limitations, peptides are loaded nanoparticle systems or conjugated biopolymers. Biopolymers are generally nontoxic products are generally preferred for producing continuously release systems with long term effect [34]. Here, the applicable and most common strategies for the synthesis of peptide-based NPs and encapsulation or conjugated methods of biopolymers are shown.

5.1 Emulsification-solvent evaporation method

The emulsion solvent evaporation technique is known as the most successful and useful method in the preparation of peptide loaded NPs and this technique is studied under two groups as single and double emulsion solvent evaporation methods [35].

5.2 Conjugation methods

Conjugation is a technique for achieving peptide and biopolymer complexes. The covalently linked peptide biopolymer conjugates can be linked using the water-soluble carbodiimide method as a cross-linker and synthesis with microwave

energy methods [36]. Peptides conjugates biopolymers can be synthesized in organic media using microwave energy. Also, there are another methods, including complex formation of biopolymers and peptides and electrostatic complex formation and metal coordination via ion coordination [35]. Specific antibody titers were observed in mouse experiments against peptides containing polymeric conjugates and complexes. The molecular weights of these conjugates are also very important. Biopolymer conjugation is crucial to obtain a high immune response to antigens at low molecular weights [37–39].

5.3 Nanoprecipitation

Nanoprecipitation is the most strategic method for the preparing of vaccine prototypes. Reducing the pH is very important to stabilization of system. Also, salt concentration under the solubility conditions is another important thing for the encapsulation method [70]. If the experiments cannot move on then adding a non-solvent phase in the quality of the solvent technique in which the parent compound of the NPs is dissolved can help [40]. Nanoprecipitation is frequently used in encapsulation of peptides. A pH-controlled precipitation rather than a non-solvent precipitation is a more preferred approach for passing the polymer to a non-dissolved phase with a simple pH change in the medium. For NPs or biopolymers prepared by nanoprecipitation, these solvents are known as the organic phase of acetone and ethanol [41].

5.4 Encapsulation of peptide

Encapsulation is carried out simultaneously by synthesizing NPs and biopolymers in all of the methods mentioned the encapsulation method for peptides should be selected based on the hydrophobic or hydrophilic facilities of peptide. Using of peptide encapsulation is important because of

- peptide release controlling,
- modeling of targeted delivery systems,
- mask unfavorable organoleptic properties (taste, odor, color),
- protection of peptide from immune attacks and enzyme degradation,
- insurance of bioconjugate molecules stability,
- decrease toxicity, and
- design of new dosage forms [42].

5.5 Peptide characterization

After purification of the peptides, they are commonly characterized by liquid chromatography-electrospray ionization-mass spectrometry (LS-ESI-MS), fluorescence spectroscopy and possible three-dimensional structures of the synthetic peptide (PEP-FOLD) server. It has validation since the chromatographic method has positive properties in terms of linearity, accuracy, precision and repeatability. Synthetic peptide vaccines are immunogens that can be used when creating vaccine

prototypes, especially because of their lipophilic structure (which also allows cell permeability to pass easily) [43]. Methods such as Fourier transform infrared (FT-IR) and nuclear magnetic resonance (1H- and 31P-NMR) are frequently used to visualize the physical structure of the copolymer and peptide biopolymer conjugates and to perform characterization studies. The conjugation of molecular weights is measured via size-exclusion chromatography (SEC) [44].

5.6 Characterization of peptide vaccine prototype

Ultraviolet (UV) and FT-IR Spectrophotometers and ZetaSizer are used for studying the nanoparticles and Scanning Electron Microscope (SEM) is used for morphological examination of Polymers or bioconjugate [45].

5.7 Toxicity studies

Peptide-based vaccine prototypes need to be tested in a cell culture medium to be feasible because they may have physiological, biological and chemical effects, causing cytotoxicity. The method used to investigate the cytotoxic profiles of peptide-based vaccines is also called *in vitro* cytotoxicity assays or cell culture-based measurement methods [46, 47]. Tetrazolium salts are compounds used in cell lines to measure the metabolic pathways of cells of microbial origin. Tetrazolium salts are the heterocyclic organic structure of these compounds and their reduction to colorless or weak colored aqueous solutions known as formazans has been the basis of their use as vital dyes in redox chemistry, biological and chemical applications [46, 47]. The tetrazolium ring can only be broken by active mitochondria, so viable cells and dead cells can be distinguished by discoloration. The fact that this change can be made only by living cells in vitro has made tetrazolium compounds a highly biologically important to measure toxicity of peptide-based vaccine formulas. The mechanism of toxicity assays, such as 3-(4,5-dimethylthiazolyl)-2,5 diphenyltetrazolium bromide (MTT) [19], 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT), sodium 5-(2,4-disulfophenyl)-2-(4-iodophenyl)-3-(4-nitrophenyl)-2Htetrazolium inner salt (WST), 5-methyl-phenazinium methyl sulfate(PMS), 5-[3-(carboxymethoxy)phenyl]-3-(4,5-dimethyl-2-thiazolyl)-2-(4- sulfophenyl)-2H-tetrazolium inner salt; (MTS) are used [49, 50] and in our studies, we generally use MTT analysis. For example, our technological vaccine prototype example is Zika peptide loaded PLGA nanoparticles which were determined on ECV304 human epithelial cells via MTT assay, which is the cytotoxicity test, was performed to determine the cytotoxic effects of the peptide, peptide loaded NPs [45]. The importance of toxicity studies is to determine the non-toxic vaccine prototype and to switch to in vivo animal studies.

5.8 Contemporary advancements in peptide based vaccine

5.8.1 Liposome based subunit vaccine

Live attenuated vaccine is highly immunogenic and considered as well-tolerant for healthy individuals. However, live attenuated vaccine should not be administered to immunocompromised individual as it would cause systemic infection. An alternative vaccine technology, subunit vaccine, is safer and more suitable for immunocompromised individual. It uses fragment of a pathogen (antigen) to trigger an immune response and stimulate immunity against the pathogen. However,

Figure 4.
General features of virus-like particle [48].

subunit vaccine has low immunogenicity and often combined with adjuvants to induce protective immunity. The adjuvants are capable to enhance vaccine effectiveness and stimulate immune responses [28].

Human alphaherpesvirus 3 (HHV-3) is known as Varicella-zoster virus (VZV), which is the causative agent of varicella (chicken pox) and herpes zoster (shingles). In 1978, the first commercial Varicella-zoster immune globulin, Vzig™ (Massachusetts Public Health Biologic Laboratories, Boston, Massachusetts) became available. However, the supply was removed from the U.S. market in 2006. The alternative preparation, Varizig® (Cangene Corporation, Winnipeg, Canada) was licensed by FDA in 2012 and has shown to be comparable to Vzig™ [Saol Therapeutics Inc. 2012]. Varizig® is supplied as a sterile solution containing human Varicella-zoster immune globulin (IgG) which showed for post-exposure prophylaxis in high-risk patients [51].

Zostavax® is the vaccine licensed for herpes zoster prevention in individuals above the age of 50. It is a lyophilized preparation which is given as subcutaneous injection [52]. A non-replicating liposome-based subunit vaccine (HZ/su) is the new development for zoster prevention. The HZ/su is a non-live recombinant VZV glycoproteins E with the adjuvant ASO1B [53]. A randomized placebo-controlled study has shown that HU/su poses age-independent defense against HZ and has better efficacy compared to Zostavax® in reducing the risk of HZ for immunocompromised adults with the age above 50. Unlike HZ/su, Zostavax® lose efficacy as age increase [54]. HZ/su vaccine is not yet approved by FDA.

Liposomes are nano-carriers and they are useful in delivering vaccine antigen by forming liposome-based vaccine delivery systems. It is advantageous over other carriers due to its biocompatibility, non-toxic and biodegradable features [55]. Besides, liposomes can be customized to achieve desired immune profiles by optimizing their composition, antigen-loading strategies and the use of adjuvants system [28, 56].

5.8.2 Virus-like particle

Eculizumab (Soliris®) is a humanized monoclonal antibody (mAb) which function as a terminal complement inhibitor [57]. It was the first therapeutic agent approved by the FDA for atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH)-associated with thrombotic microangiopathy (TMA) in 2007 and 2011 respectively [58]. Soliris® (Alexion Pharmaceuticals, New Haven, Connecticut, USA) is in the form of sterile solution for i.v. injection. Eculizumab increases the patient's susceptibility to meningococcal infection (Neisseria meningitidis), all patients must be vaccinated against meningococcal infections prior to or at the time of initiating Eculizumab.

Virus-like particles (VLPs) is one of the alternative types of nanoparticles delivery system [59]. A recent research has shown that the development of autologous C5 vaccine in nanoparticle form is able to elicit strong humoral responses [60]. A peptide epitope (PADRE peptide) in the C5 vaccine is used to create a recombinant virus-like particles (VLPs). It showed a reduction in hemolytic activity and protect the mice from complement-mediated intravascular hemolysis [60]. Based on the study's result, it is showed that the recombinant VLPs could be used as an alternative or supplement for Eculizumab.

VLPs is known as an emerging class of targeted delivery vehicles with potential of overcoming the limitations of other nanoparticles [48]. VLPs is a potential delivery system due to their immunogenic nature, well-defined structure, ability to present a wide variety of potential epitopes, and ease of production [60]. They lack natural genome thus it is non-infectious. Besides, it can turn as self-adjuvant which is proficient in breaking the immune tolerance.

One of the limitations of VLPs are phagocyte-mediated clearance [59]. Besides, a recent study showed that ellipsoid nanoparticles can extravasate from the blood vessel more effectively than spherical nanoparticles. Meanwhile, the ellipsoid shape is possible for conventional polymeric NPs, but is not feasible for icosahedral VLPs. However, this limitation can be overcome through the modification of VLP surface by adding a variety of useful ligands [61]. VLPs may be able to efficiently extravasate from the vasculature of the blood vessels by showing multiple ligands with high affinity for the tight connections between endothelial cells [59].

6. Importance *in vitro* and *in vivo* experiments using peptide-based vaccine prototypes

After forming a synthetic vaccine prototype, the cytotoxicity of the bioconjugate of the peptides and biopolymers is first determined (generally we use MTT analysis). After the apoptotic effect of the prototype on living cells is measured by flow cytometric detection, the vaccine prototype with the most viable cell number should be selected for further study [62]. After all these methods, immunization is the next step. We immunize BALB/c mice with each one of the peptides biopolymer conjugates or peptides loaded nanoparticles following conventional immunization protocol. The goal is to identify the most antigenic vaccine prototype. The

antibodies are measured in blood (for humeral response such as; T and B lymphocytes, IgGs) or splenic (cellular response like ILs and IFNs) samples from the immunized BALB/c mice via the indirect enzyme-linked immunosorbent assay (ELISA) to determinate the highest antibody level. Thus, the most suitable peptide-based vaccine prototypes will be identified for future clinical phase studies. In brief, cell culture and toxicity studies are important before the analyses the effect of vaccine prototype in vivo [62].

7. Current situation and future perspective

Peptides can affect important brain regions that are essential for life-sustaining functions. There are studies about Peptide drug and Peptide-Polymer Vaccines and drugs using for brain disorders. In this future perspective we will explain the using of peptides in common brain disorders such as Alzheimer Disease, Parkinson Disease (PD), Multiple Sclerosis. In a study using an interference-inducing peptide (TAT-DATNT) to elute a protein complex consisting of interaction between DAT and the dopamine D2 receptor (D2R), it was determined that locomotor behaviors were induced in Sprague–Dawley (SD) rats. This peptide can provide potential therapy for regulating the activity of DAT and dopaminergic neurotransmission of Attention Hyperactivity Deficit Disorder (ADHD) therapies [63]. Alpha Synuclein (A-syn) aggregate is very important for the PD. Against of this aggregate, an immunogenic peptide the sequence of CGGVDPDN [64] is developed with solid phase peptide synthesis method as a vaccine in PD. Peptides can also be neuroprotection for the PD. TFP5 peptide, FITCGGGKEAFWDRCLSVINLMSSKMLQINAYARAARRAARR; TP5 peptide, KEAFWDRCLSVINLMSSKMLQINAYARAARRAARR; SCP peptide, FITCGGGGGGFWDRCLSGKGKMSSKGGGINAYARAARRAARR are reduction in neuroinflammation and apoptosis. **MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)** is a neurotoxin drug of MPP+, which causes permanent symptoms of PD by destroying dopaminergic neurons in the substantia nigra of the brain for mouse modeling [65]. PD01A is a Phase 1 epitope vaccine in experimental — a synthetic a-syn mimicking peptide-polymer based on a-syn aggregate by inducing an immune response that generates antibodies specifically against it [66]. In general, vaccines are developed according to the T cell response, but a peptide epitope with a three-celled peptide epitope with a three-cell universal peptide such as Syn85-99 (AGSIAAATGFVKKDD), α-Syn109-126 (QEGILEDMPVDPDNEAYE), α-Syn126-140 (EMPSEEGYQDYEPEA) and P30 (*FNNFTVSFWLRVPKVSASHLE*) epitope vaccines comprising three peptide-based epitope vaccines comprising different α-Syn peptides, but consisting of different B cell epitopes as follows, are noted for their high immunogenicity [67]. So peptides can have different immune cell response in brain disorders. Peptides can also protect cell biological agents such as microtubules activity. This is very important for cell stability while the diseases are seen in the cells. The dysregulation of ADNP / ADNP2 expression in the relevant brain tissue and animal model may improve the prognosis of schizophrenia because these genes are responsible for the regulation of interacting microtubules. The microtubule-interacting drug candidate, NAP (davunetide) is a small peptide and belong to activity-dependent neuroprotective protein (ADNP) which contains a small peptide motif, *NAPVSIPQ* sequence that provides potent neuroprotection for tau pathology, neuronal cell death as well as social and cognitive dysfunctions [68]. Especially in Amyloid beta pathology, *DAEFRHDSGY* peptide, Wang et al. synthesized peptide immunogens, A1-14 peptide immunogens for *UBITh*® AD immunotherapeutic vaccine by using automated SPPS for the Alzheimer Disease.

Wang's group has developed a synthetic peptide vaccine prototype for the prevention and treatment of AD and is conducting *phase II* clinical trials. The occurrence of Alzheimer's disease constitutes a strong immune response to Amyloid Beta (Ab) Plaques; *UB-311* was constructed with two synthetic Ab1-14 targeting peptides (B cell epitopes), each bound to different helper T cell peptide epitopes and formulated in a Th2 delivery system [69].

8. Conclusion

Consequently, this chapter provides a brief manual for anyone in the fields of solid-phase peptide synthesis, peptide vaccines, Nanotechnological importance for effective vaccine prototypes, and their future perspective for other diseases such as brain disorders.

Conflict of interest

The authors declare no conflict of interest.

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