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The potential of porous silicon particles for multi-epitopic vaccine development

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Abstract: Vaccination is a crucial approach to eradicate and control a myriad of infectious and non-communicable diseases. Subunit vaccines are considered the most convenient approach for vaccine formulation; however, the development of new adjuvants and vaccine delivery vehicles to improve the immunogenicity of such formulations is needed. The authors of this review describe the recent application of porous silicon particles (PSiP) as both a potential vaccine delivery vehicle and adjuvant. PSiP are attractive for this application due to its safety, biodegradability and compatibility for functionalization. Herein, the development of multi-epitope cancer vaccines is discussed as an example on how PSiP are promising materials for the development of innovative vaccines.

Keywords: humoral response, cellular response, functionalization, adsorption

1 Introduction

Vaccination is a key invention for the biomedical field that had saved millions of deaths primarily associated to infectious diseases (WHO, 2016). In addition, vaccination against non-infectious diseases (e.g. cancer) is acquiring a great relevance. Multi-epitope vaccines are considered a relevant trend in vaccinology, since they comprise a set of specific protective epitopes and may achieve broad immunoprotective responses (Jafarpour et al., 2015). This is especially important for pathologies requiring wide immune responses against multiple targets to successfully combat the pathogen or malignant cells. A common approach for the formulation of such vaccines is based in mixtures of synthetic peptides; however, they tend to be poorly immunogenic. The use of chimeric recombinant proteins is an alternative to synthetic peptides with the advantage that they can be produced in recombinant organisms at the large scale and in general at a lower cost (Durántez et al., 2009). Nevertheless, the design of functional chimeric proteins faces the following challenges: 1) to achieve adequate expression of the protein in the host; 2) to retain the immunogenic properties of the individual epitopes and 3) to fulfill the uptake and processing by the antigen presenting cells (APC) and induce a proper immune response in terms of polarization and potency (Rosa et al., 2015).

Another approach to achieve the production of a highly immunogenic vaccine consists in the use of adjuvants, which enhance the immune response and influence immune polarization (Hirayama and Nishimura, 2016). The polarization of the immune system arms towards humoral or cytotoxic T cell responses is typically required to achieve immunoprotection to a specific pathology (Roitt et al., 2008). Although there are several adjuvants with proved efficacy such as Toll-like receptor (TLR) ligands (e.g. CpGs), bacterial toxins, silver salts, and interleukins (e.g. IL-12); common limitations in this group include high production cost and adverse effects (Apostólico et al., 2016; Reed et al., 2016). In addition, some peptides can exert adjuvant effects; for instance, the pan HLA DR-binding epitope (PADRE) is a peptide sequence that actives T helper lymphocytes (Th) and has been used and validated as an adjuvant in several vaccine formulations (Alexander et al., 1994; Jafarpour et al., 2015; Shukla et al., 2016; Juárez et al., 2016).

Moreover, a crucial need in vaccinology is the development of vaccines administered by attractive administration routes, such as the oral route; that allows an easy and painless administration not requiring sterile devices and trained personnel (Juárez et al., 2016). However, the

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induction of strong immune responses by oral vaccination is a challenge due to the tolerogenic nature of the gut associated lymphoid tissues (GALT), and the low antigen bioavailability derived from the antigen dilution and degradation in the gastrointestinal tract (Burbank et al., 2016). To address this challenge, several strategies have been explored to enhance the efficacy of oral vaccine formulations. For instance, the use of novel delivery vehicles has been assessed since they can increase antigen stability, particularly when it is administered by mucosal routes; and it can also activate mechanisms of the innate immunity, such as the antigen uptake and cytokine production by the APC (Xia et al., 2015). Therefore, the use of delivery vehicles could influence not only the potency but also the type of immune response (cellular or humoral).

Among the vaccine delivery vehicles assessed thus far, liposomes (Conniot et al., 2014, Schwendener et al., 2014), starch particles (Guillén et al., 2014), virus-like particles (VLPs) (Gilbert et al., 2001), and solid nanoparticles (Gregory et al., 2013; Salazar-González et al., 2015) have led to interesting findings. In parallel, recombinant cells expressing a particular antigen have also been used as the delivery vehicle, thus they serve as both antigen biofactory and delivery vehicles. In this sense, recombinant plant cells (Matić et al., 2016), yeast (King et al., 2016), and Gram-positive bacteria (Rosales-Mendoza et al., 2014) have achieved the induction of immune responses following oral immunization.

Among the solid nanoparticles used as delivery vehicles of vaccines, the following materials have been evaluated: polylactic acid (PLA), polyethylene glycol (PEG), poly(lactic-co-glycolic acid) (PLGA), chitosan, gold, silica and silicon (Salazar-González et al., 2015; Lin et al., 2015; Navarro-Tovar et al., 2016). This review is focused in the potential use of PSiP that may serve as delivery vehicles and adjuvants in vaccinology. A brief description of the synthesis and properties of PSiP is provided and the potential applications on vaccinology are discussed.

2 Nanoparticles as delivery vehicle in vaccines

Given the current challenges in vaccinology, new materials for obtaining formulations with the ability to attain proper immunogenicity and immune polarization are a need in the field. For instance, the immunity mediated by cytotoxic lymphocytes (Th1) plays a crucial role in the defense against cancer and intracellular pathogens. Since the induction of Th1 responses depends on the activation of components of the innate immune response leading to the production of specific cytokines and the type of antigen presentation into the APC, carriers or adjuvants that activate or influence such mechanisms have been explored (Bedoui et al., 2016). For more than a decade, nanoparticles have been applied for this purpose. A diverse group of nanoparticles varying in the nature of the material, shape, and size has been generated offering a myriad of possibilities for vaccine design. The efficacy of such particles is in part explained by their capability of being internalized by the APC through different mechanisms (Gregory et al., 2013).

The main factors that affect the immunostimulatory function of the nanoparticle comprise their size, shape and surface characteristics (Bachmann and Jennings, 2010). Therefore, nanomaterials can be optimized to achieve a proper uptake by APC with the subsequent delivery, processing and presentation of the antigen to lymphocytes (Paulis et al., 2013). In this scenario, an example is the use of nanocrystals and adjuvants based on aluminum salts, which are co-administered along with the antigen activating the inflammasome and promoting IL-1β production by dendritic cells (DC), therefore enhancing antigen presentation and strengthening the induced immune response (Sharp et al., 2009).

However, there are still several nanomaterials that might be developed and characterized in the vaccinology field as attractive delivery vehicles and adjuvants. In this context, PSiP have a potential use as both carrier and adjuvant. Interestingly, PSiP have been used in several biological applications, such as imaging diagnostic, biosensors (Márquez et al., 2014; Coffer et al., 2003), phototherapy (Lee et al., 2007) and drug delivery (Charnay et al., 2004; Vaccari et al., 2006; Salonen et al., 2008).

3 Synthesis and Properties of PSiP

The most popular synthesis method for PSiP consists on the electrochemical etching of single crystal silicon wafers using a fluorhydric acid (HF) electrolyte solution. Lehmann (2002) describes the electrochemistry and pore formation by the following chemical equations:

$$Si_{(s)} + 2H_2O_{(l)} + 4h^+ \rightarrow SiO_{2(s)} + 4H^+$$

$$SiO_{2(s)} + 6HF_{(l)} \rightarrow H_2SiF_{6(l)} + 2H_2O_{(l)}$$

Where h^+ stands for electron carrier (hole) injection into the valence band of the semiconductor (Si), as a result of the HF flow through the silicon-electrolyte interface.

Silicon is a semiconductor element that possesses four electrons in the outer band. The electric current takes

place when electrons move through the material. This easily occurs in the holes of the material (h^+). The silicon wafers are doped with boron to add more h^+ (p-type material) or with phosphorus, which has an electron ready to be donated in the reaction (n-type material) (Lehmann, 2002). The layer of porous silicon obtained by electrochemical etching is subsequently fragmented in particles in a range of micro and nanosize with sonication and/or ultrasonication and finally, the particles are thermally oxydized to stabilize the surface (Si-OH and Si-O_x groups) (Kopermsub et al., 2011; Palestino et al., 2008). The set of experimental conditions such as time of electrochemical attack, potential (mV) and current density (mA/cm^2) are critical factors to tune the porous size. In this matter, Zhang (2004) compiled all variables affecting pore size in the electrochemical etching of silicon wafers and reported that pore size of PSiP ranged from 1-100 nm (Zhang, 2004). According with The International Unit of Pure and Applied Chemistry (IUPAC) recommendations, the PSiP are classified in terms of pore size as follows: microporous silicon ≤2 nm, mesoporous silicon 2–50 nm, and macroporous silicon ≥50 nm (Rouquerol et al., 1994). Moreover, the time of sonication and ultrasonication determines the particle size while the oxidation time and temperature are variables that modify the surface chemical characteristics of the silicon particle. The resulting material consists on particles with a high surface area/volume ratio and Si-H_x having Si-O_x chemical bonds on the surface and a Si-Si core. This typical surface allows for different types of interactions with the biomolecules being loaded (Ogata, 2014; Canham et al., 1994).

Furthermore, PSiP are degradable and compatible with biological systems (Tanaka et al., 2010). The final degradation product is silicic acid ($Si(OH)_4$), which is an inorganic component of bones (Anglin et al., 2008). Sailor's research group reported that PSiP are completely degraded in 24 h under *in vitro* physiological conditions (pH=7.4 and 37 °C). A study also conducted by this group showed that the silicon accumulated after intravenous injection of 20 mg/kg_{mouse} was cleared from the body within a period of 1 to 4 weeks (Park et al., 2009; Gu et al., 2012). Additional to the biological compatibility of PSiP, their potential for biological applications relies on the loading and controlled released of active molecules onto the surface, which is magnified by the presence of pores. These characteristics could be controlled by chemical surface modifications and by tunning the pore size. Particularly, there are evidences that PSiP of 1 μm in diameter and 400 nm in length can be loaded with nanometric size drugs and peptides. Thus, PSiP have been used as delivery vehicles for drugs with low molecular weight e.g. therapeutic agents against cancer (Shen et al., 2013; Xu et al., 2013; Chen et al., 2014; Dave et al., 2014). The pore size of PSiP is in the range of ~1–100 nm and is determined by the conditions of synthesis and doping percentage. Proteins of up to 150 kDa have been introduced into PSiP (Palestino et.al., 2008) and thus no limitations exist for the case of synthetic peptides that are much lower in molecular size.

4 Approaches for active biomolecule loading onto PSiP

There are different approaches to formulate nanoparticle-based delivery systems: (1) adsorption onto the particle surface; (2) chemical conjugation; and (3) encapsulation (Navarro-Tovar et al., 2016). Particularly, for PSiP only the first two approaches are achievable (since the material is not a capsule). In some applications loading may not be necessary; this is the case for vaccine formulations with PSiP along with antigens, in which the co-administered PSiP may exert an adjuvant effect not requiring specific interactions with the antigen (Seth et al., 2015). In this section, a brief description of each loading method is provided, emphasizing the PSiP cases.

4.1 Peptide Adsorption onto the particle surface

The simplest experimental method to load an active biomolecule, such as enzymes, antibodies or antigens, is placing directly the biomolecule and particles in a solution under specific pH and temperature. In the vaccinology area, it is well described by several authors that the range of antigenic peptides loading varies from ~4 to 30% depending on the testing system (Kaasalainen et al., 2015; Kilpeläinen et al., 2009; Kovalainen et al., 2012; Xia et al., 2015). The direct biomolecule-PSiP interaction is a physical type of interaction, which involves intermolecular forces. In this regard, the isoelectric point (PI) of PSiP is reported as 4.5 (Kaasalainen et al., 2015). Therefore, for most of the biological applications where the physiological pH is 7.34, the PSiP surface is negatively charged (Si-O_x^-). Thus, a direct interaction between a molecule, such as peptides, and PSiP surface at pH = 7.34 requires positively charged biomolecules (with a PI > 7.34) for strong intermolecular forces (electrostatic forces). Additionally, the interactions between biomolecules and PSiP surface could be through van der Waals forces between non-charged re-

gions on the particle surface (Si-Si and Si-H_x) and non-polar regions of the biomolecule.

On the other hand, a negatively charged biomolecule at physiological pH (PI < 7.34) is unable to interact with the negatively charged PSiP by electrostatic forces. Then, to assure a strong interaction, the porous material can be functionalized with organic molecules to turn its negative charge into a positive charge. The most common organosilane used for silica and silicon nanoparticle functionalization is 3-aminopropyltriethoxysilane (APTES) (see Figure 1), which possesses three hydrophobic chains that interact with the surface of the inorganic material. The fourth chain of APTES has an NH_2-terminal exposed onto the surface and it is easily charged at physiological conditions (Navarro-Tovar et al., 2016; Digigow et al., 2014; Hak-Sung et al., 2012; Márquez et al., 2014). The modification of the zeta potential on the silicon particles not only favors the interaction with negatively charged active biomolecule, but also, could enhance the internalization of PSiP into the cell. In this regard, Graf et al. (2012) described the modification of silica nanoparticles with several NH_2-terminal organocompounds: L-Lysine, L-Arginine, N-Trimethoxysilylpropyl-N,N,N- trimethylammonium chloride (NPC), APS subsequently modified by N-guanylpyrazole (GP), (3-Aminopropyl)- trimethoxysiliane (APS), N-(6-Aminohexyl)- aminopropyltrimethoxysilane (AHAPS) and PEG-silane. It is relevant that the authors found out that AHAPS-modified particles are readily taken up by HeLa cancer cells in an *in vitro* study. Thus, the positive charged particles favour the association with the negatively charged cell membrane. Furthermore, positively charged particles stabilized by short alkyl chain aminosilanes are adsorbed on the cell membrane; however, they are weakly taken up due to aggregation (Graf et al., 2012).

Other surface modifications could be performed to achieve PSiP with hydrophobic surface. In this sense, the hydrosilylation involves the reaction of a terminal alkene or alkyne with Si-H bond under thermal, photochemical or Lewis acid catalysis. Moreover, the covalent binding of Grignard and alkyl reagents and also, the electrochemical oxidation of methyl-Grignard reactants are an alternative when hydrosilylations are not possible. Thus, the addition of alkyl chains to the structure provides a hydrophobic region that also stabilizes the PSiP and facilitates the loading of hydrophobic drugs and proteins (Anglin et al., 2008).

The possible intermolecular forces between peptides and the silicon surface are discussed by Ramakrishnan et al. (2014) by an interesting modeling study on the interaction of various peptides onto a silicon surface, where electrostatic forces and interactions between non polar regions are determined by quantum mechanistic calculations (Ramakrishnan et al., 2014).

It is important to mention at this point that none of the surface modifications have a 100% surface coverage, and experimental analysis have shown that remants of Si-H are present after modifications. However, the modifications offer high stability and increase the interaction with the biomolecules (Lee et al., 2003).

4.2 Chemical conjugation of a peptide onto the PSiP surface

The chemical conjugation consists on covalent attachment of the biomolecule to the previously modified PSiP surface. This approach is well reported for biosensors (de la Mora et al., 2013) and drug molecules such as doxorubicin (Haidary et al., 2012). Several reagents can be used for the hydrosilylation of functional PSiP. In this context, Buriak (2002) summarized the chemistry involved in the modification of porous silicon surface. Anglin et al. (2008) pointed out that linking an organic molecule to functionalize PSiP can be done through either Si-C or Si-O covalent bond, being Si-C the bond that provides more stability, since the Si-O union is vulnerable to nucleophilic attack. Moreover, the linked molecule that modified the PSiP surface requires a reactive group if a covalent union with the biomolecule is intended. The chemical conjugation with proteins or peptides requires a linker termination of any of the following organic groups: carboxyl, aldehyde, thiol, alkene, epoxy, ester, amine and others (Aslam and Dent, 1998). Each group reacts with different amino acids to form a covalent union with the protein or peptide. For instance, a linker with thiol easily reacts with cysteine and methionine in a disulfide bond. Furthermore, glutaraldehyde (GTA) is one of the most reported linkers for crosslink biomolecules such as single-stranded DNA (ssDNA) (Ashtary et al., 2005) and enzymes (Kishore et al., 2012). The aldehyde termination of GTA rapidly reacts with terminal amines of peptides and proteins (Aslam and Dent., 1998; see Figure 1).

It is reported that the biomolecule, also called payload, is only released in the biological system when the covalent bonds are broken or the PSiP is degraded. However, every single study must incorporate activity assays to ensure that the biomolecule retains its biological activity (Kilian et al., 2007; Anglin et al., 2008).

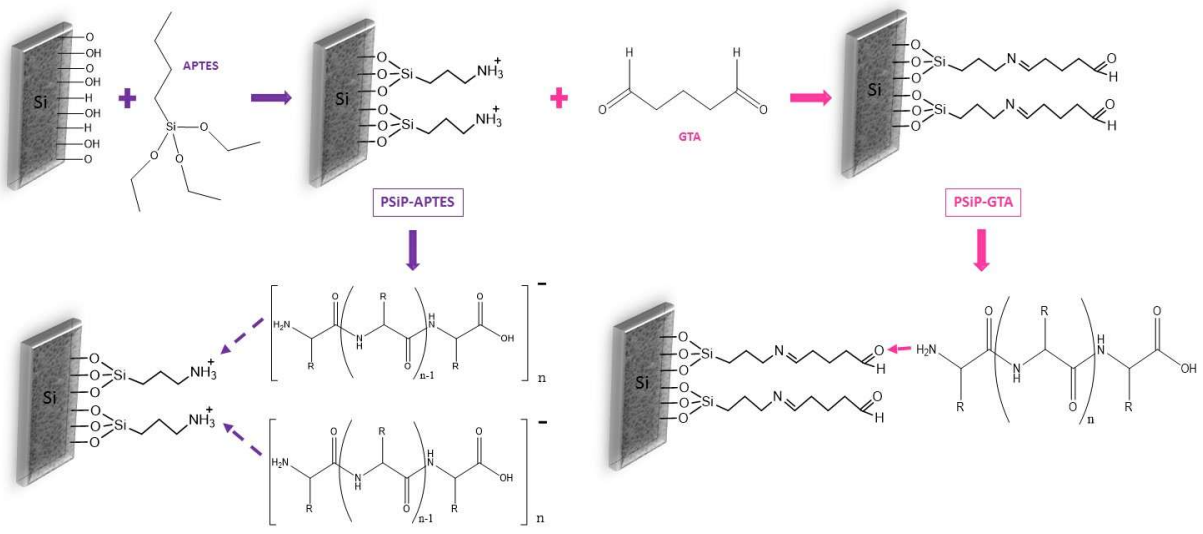

Figure 1: Representative scheme of the APTES- and GTA-based PSiP functionalization and their electrostatic interaction (APTES-PSiP) or imine bond (GTA-PSiP) with a peptide chain.

5 Effects of PSiP in immune system cells

To the date, only preliminary reports regarding the use of PSiP to deliver antigens have been reported. In this aspect, a study reports the use of PSiP as a strategy to deliver antigens and enhance the antigen presentation through major histocompatibility complex class I (MHC-I) molecules (Jiménez-Periáñez et al., 2013). The authors supported this notion by loading PSiP with a panel of 23 different peptides (from 8 to 11 amino acids). All peptides were viral-specific CD8 T cell epitopes from influenza virus, cytomegalovirus and Epstein-Barr virus. Peptides were loaded onto the PSiP surface by shaking solutions for 24 h at 4 °C. According with indirect measurements, peptide loading in all cases was in a range of 20 to 30%. Then, the PSiP-peptide conjugates were added to DC cultures that were subsequently co-cultivated with mononuclear cells. The results point out to further investigation to evaluate the *in vivo* efficacy of the cytotoxic lymphocytes response. Another report by Xia et al. (2015) was focused on the use of PSiP as the delivery vehicle of a peptide related with breast cancer expressing the human epidermal growth factor receptor 2 (HER2). The PSiP vehicle outstandingly increased the antigen presentation and also activated interferon type 1 (IFN-1) production by DC. The antigen associated to PSiP showed a higher residence time in early endosomes and higher cross presentation through proteasome and lysosome paths. Moreover, phagocytosis of the conjugated PSiP induced IFN-1 responses through two dependent pathways: the Toll/4ll-1 Receptor-domain-containing adapter-inducing interferon-beta (TRIF) and the mitochondrial antiviral signaling protein (MAVS). Later, DCs loaded with the antigen were inoculated in mice bearing tumors induced with TUBO cells and a therapeutic effect was observed. The therapeutic effect correlated with $CD8^+$ responses (Xia et al., 2015). These findings highlight the potential use of PSiP as adjuvant in the immunotherapy against cancer based in DC transference.

6 Perspectives for PSiP use in cancer immunotherapy

Cancer is a medical terminology used for the groups of pathologies typified by the presence of abnormal cells characterized by alterations in the cell division control and the capability of invading other tissues as well as inducing angiogenesis (WHO, 2016). There are more than 100 types of cancers and any part of the body can be affected by this pathology. In 2008, 7.6 million people died of cancer, accounting for 13% of all deaths worldwide. The most common therapies against cancer are surgery, chemotherapy and radiotherapy. However, these thera-

pies are limited due to their low specificity and/or ineffectiveness for the control of the minimal residual disease (Subiza et al., 1994). It is known that the immune system plays a key role on the vigilance and destruction of malignant cells. Nonetheless, the malignant cells also possess mechanisms to evade the immune system (Duharte, 2003). An important trend in the fight against cancer consists on immunotherapies targeting tumor-associated antigens (TAAs), which are proteins or other compounds over expressed in cancer cells. To the date, several TAAs have been reported (Cheever et al., 2009). Immunotherapies can rely on passive immunization where monoclonal antibodies are administered, or active immunization that comprises the activation of T and B cells, both intended to induce malignant cell clearance.

In this respect, some cancer immunotherapies have been approved by the FDA; for instance, the use of a dendritic cells (DCs)-based vaccine against metastatic prostatic cancer (Provenge) and therapies based on CTLA4 or PD1 antibodies (Hodi et al., 2010; Sharma et al., 2011; Postow et al., 2015).

Two relevant TAAs that exemplify such wide group of molecules are the receptor tyrosine-protein kinase erbB-2 (HER2/neu/ErbB2) and Wilms tumor protein (WT1), which are associated to therapeutic effects against several cancer types. HER2/neu/ErbB2 is a 185 kDa membrane glycoprotein, which belongs to the ErbB2 family that groups the EGFR 1 (HER1), HER3 and HER4 receptors (Yarden and Sliwkowski, 2001). Despite the HER2 does not possess a specific ligand, it is an important inductor of a heterodimerization process that leads to transduction of several signals that promote mitosis, apoptosis, angiogenesis and cellular differentiation. The extracellular domain of HER2 comprises 630 amino acids in four domains (I-IV). It is known that the dimerization loop in domain II is permanently exposed. This could explain the predominant role of HER2 from its family receptors in the dimerization process (Menard et al., 2000).

The company Genentech Inc. (San Francisco, CA) developed the most known therapy against HER2. The commercial drug is Herceptin, which is constituted by a monoclonal antibody called Trastuzumab. Herceptin increases the survival rate (Popat and Smith, 2008) and it has been proven as the first treatment election in patients diagnosed with HER2 positive breast cancer and metastasis. Trastuzumab targets the terminal portion of the domain IV, where the binding site with the loop II of HER1 and HER3 receptors is located (Cho et al., 2003). However, the therapies based in passive immunity have limitations. This is the case of Trastuzumab, for which most of the treated patients have a positive initial response; however a subsequent progression of the cancer is frequently observed (Nahta et al., 2006). Therefore, major efforts are needed to improve the efficacy of immunotherapies. In this sense, the use of monoclonal antibodies against different TAAs (Sharon et al. 2005) has been proposed as well as the use of antibody-mimicking agents targeting several epitopes (Nahta et al., 2006; Pal and Pegram, 2007).

On the other hand, WT1 was described from etiologic studies of Wilms tumor, a type of renal paediatric cancer. This protein is a transcription factor carrying zinc fingers, and is involved in the regulation of proliferation, cellular differentiation, apoptosis and organs development (Drummond et al., 1992; Englert et al., 1995; Godyer et al., 1995; Hewitt et al., 1995). Although WT1 was first considered as a tumor gene suppressor, further evidence indicated that this gene has a role as oncogene. The main evidence relied on its overexpression in leukemia and solid tumors (Brieger et al., 1994; Inoue et al., 1994) as well as the inhibition of leukemia progress and tumor growth after the administration of an antisense oligonucleotide targeting the WT1 gene (Oji et al., 1999). Therefore, WT1 is considered a TAA and a therapeutic target for WT1+ breast cancer cases (Call et al., 1990; Gessler et al., 1990). In this sense, several clinical evaluations have explored the therapeutic effects and safety of a WT1-based vaccine consisting in a peptide (9 amino acids) and the Montanide ISA51 adjuvant. The evaluations have been conducted in different cancer cases with promising results, including leukemia and breast cancer (Morita et al., 2006; Rein et al., 2014; Di et al., 2015).

Considering that passive immunotherapies require frequent dosage and are highly expensive, active immunization is considered the ideal approach to direct the immune system against cancer. Despite the extensive knowledge on these targets, the challenges in cancer vaccinology are achieving immune responses whose potency and broadness led to cancer remission. This requires not only defining the proper combination of targets but also developing formulations with appropriate carriers/adjuvants that enhance the immune response and favor the desired Th-response polarization (Tagliamonte et al., 2014). There is still a need to develop effective immunotherapies targeting several TAAs to achieve synergic immune responses (cellular and humoral) leading to improved efficacy (Pal y Pegram, 2006). Vaccine formulations containing a mix of multiple synthetic peptides offer opportunities to target several TAAs at lower costs.

In this context, the perspective of using PSiP for the delivery of TAAs (such as synthetic peptides or multiepitopic proteins) is of great relevance for the field. The avenues to be explored could include: (i) evaluating the effi-

cacy of PSiP for promoting the induction of CTL responses; (ii) assessing the efficacy of formulations of multicomponent vaccines comprising peptides from several TAAs along with adjuvant peptides. Since peptides vary in their physicochemical properties, it is proposed a methodology comprising individual adsorption/conjugation reactions under the corresponding optimal conditions followed by mixing the different PSiP-peptide complexes in a single formulation; (iii) the design of hybrid peptides carrying sequences from TAAs along with PSiP binding peptides; (iv) studying the adjuvant effect of the co-administration of TAAs and PSiP and (v) performing loading optimization studies for TAAs by testing the effects of concentration, pH and temperature.

7 Concluding remarks

Nanotechnology is opening new avenues for improving the vaccine development field. Surprisingly, PSiP have been narrowly explored for vaccination despite their advantageous characteristics in terms of safety, versatility for functionalization, adjuvant effects, and stability. Then, multi-target vaccines based in the use of PSiP as carriers represent a key future prospect for vaccinology. Research efforts in such directions will have a positive impact in the development of vaccines, particularly those requiring strong cellular responses, such as cancer and infections caused by intracellular pathogens, for which a limited number of effective adjuvants are in advanced developmental stages.

Acronyms

AHAPS	N-(6-Aminohexyl)- aminopropyltrimethoxysilane
APC	antigen presenting cells
APS	(3-Aminopropyl)- trimethoxysiliane
APTES	3-aminopropyltriethoxysilane
DC	dendritic cells
EGFR	epidermal growth receptor factor receptor
GALT	gut associated lymphoid tissues
GP	N-guanylpyrazole
GTA	glutaraldehyde
HER/neu/erbB-2	receptor tyrosine-protein kinase erbB-2
HER2	human epidermal growth factor receptor 2
HLA DR	human leukocyte antigen - antigen D related
IFN-1	interferon type 1
IL	interleukin
IP	isoelectric point
IUPAC	The International Unit of Pure and Applied Chemistry
MAVS	mitochondrial antiviral signaling protein MAVS
MHC-I	major histocompatibility complex class I
NPC	N-Trimethoxysilylpropyl-N,N,N- trimethylammonium chloride
PADRE	pan HLA DR-binding epitope
PEG	polyethylene glycol
PLA	polylactic acid
PLGA	poly(lactic-co-glycolic acid)
PSiP	porous silicon particles
ssDNA	single-stranded DNA
TAA	tumor-associated antigen
Th	helper T lymphocytes
TLR	Toll-like receptor
TRIF	Toll/4ll-1 Receptor-domain-containing adapter-inducing interferon-beta
VLP	virus-like particles
WT1	Wilms tumor protein

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