Society For Biomaterials

Review Article

Optimization of PLGA formulation containing protein or peptide-based antigen: Recent advances

Jafar Hajavi,[1,2] Mahboubeh Ebrahimian,[3] Mojtaba Sankian,[2,4] Mohammad Reza Khakzad,[5] Maryam Hashemi[6]

[1]Department of Basic Sciences, Faculty of Allied Medicine, Gonabad University of Medical Sciences, Gonabad, Iran
[2]Immunology Research Center, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Division of Biotechnology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
[4]Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Innovated Medical Research Center & Department of Immunology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[6]Nanotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran

Received 6 November 2017; revised 25 February 2018; accepted 15 March 2018
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.36423

Abstract: Protein or peptide-based antigens are the most promising forms to generate custom protective immune responses for clinical applications. Over the last decades, poly(lactic-co-glycolic acid) (PLGA) as a biodegradable polymer has gained more attention for delivery of protein and peptide. Besides many appropriate characteristics, to improve its properties to overcome some obstacles such as release profile and it is important instability of antigen during both encapsulation and storage. Therefore, optimized procedures conditions require to be used to maintain the integrity of protein structure under several stress factors in formulation process. In this review article, the properties of PLGA particles, their preparation techniques and strategies for improvement of protein stability during encapsulation into PLGA, release from particle and storage as well as stabilization approaches were summarized. © 2018 Wiley Periodicals, Inc. J. Biomed. Mater. Res. Part A: 106A: 2540–2551, 2018.

Key Words: PLGA, Optimization, Formulation, Protein/peptide antigens

How to cite this article: Hajavi J, Ebrahimian M, Sankian M, Khakzad MR, Hashemi M. 2018. Optimization of PLGA formulation containing protein or peptide-based antigen: Recent advances. J. Biomed. Mater. Res. Part A 2018:106A:2540–2551.

INTRODUCTION

In the past few years, major efforts in the field of immunotherapy have led to the development of protein or peptide-based vaccines.[1] Potency of these vaccines can be significantly intensified via encapsulation of proteins into biodegradable particles by improvement of the antigen properties, targeting of antigen to defined cell types of the immune system and facilitating co-delivery of antigen and adjuvants.[2,3] Designing delivery systems for these agents depends on their biophysicochemical and physiological characteristics including molecular size, biological half-life, immunogenicity, conformational stability, dose requirement, site and rate of administration, pharmacokinetics, and pharmacodynamics properties.[4–8] Different carrier systems such as polymeric nanoparticles (NPs), liposomes, and solid lipid NPs have been designed for efficient delivery of proteins and peptides.[9] In this aspect, an increasing interest in applying of biodegradable polymers for antigen delivery has been observed.[10,11] These polymers could protect the encapsulated drug from enzymatic degradation and improve the efficiency of antigen delivery. Polymeric NPs also exhibit high stability in biological fluids compared to liposomes and solid lipid NPs. Many studies have been widely reported on the successful encapsulation of peptides and proteins into FDA approved synthetic polymer, poly (lactic-co-glycolic acid) (PLGA), with respect to loading and encapsulation efficiency, as well as nanoparticle size and morphology.[10,12] The insertion of antigen into PLGA nano or microparticles can also offer other advantages such as bioavailability and sustain release of antigens or adjuvants in order to enhance immune stimulation.[9,13,14] Table I presents some examples of peptide or protein-based antigens formulated into PLGA NPs.

Although PLGA is the most frequently used carriers in peptide or protein-based antigens formulations, it is essential to improve its properties to overcome some obstacles such as release profile and instability of antigen during

Correspondence to: M. Hashemi; e-mail: hashemim@mums.ac.ir

TABLE I. Some Examples of Peptide or Protein-Based Antigens Formulated into PLGA NPs

Antigen	Clinical Uses	Reference
Tetanus toxoid (TTxd)/diphtheria toxoid (DTxd)	Tetani and Diphtheria	15
HBsAg	Hepatitis B	16
SAG1/SAG2	Toxoplasmosis disease	17
Yersinia pestis F1 antigen	Pestis disease	18
Gp120 from HIV	Vaccine for acquired immunodeficiency syndrome (AIDS)	19
OVA/PA/HA	Influenza disease	20
Tetanus toxoid(TT)	Tetanus disease	21
Bee venom phospholipase A2 (PLA2)	Allergy vaccines	22
Ole e 1 allergen from olive	Allergy vaccines	23
Human interferon-α(IFN-α)	Treatments of hepatitis B, C, hairy cell leukemia, and Kaposi's sarcoma	24
E2 envelope glycoprotein of HCV type (HCV-E2)	Anti-HCV vaccines	25
Insulin	Antidiabetic	26
OVA24, a 24-residue long synthetic antigenic peptide covering a CTL epitope of ovalbumin (SIINFEKL)	Peptide-based therapeutic cancer vaccines	27
Recombinant caryota mitis profilin (rCmP)	Immunotherapy of allergic asthma	28
Chenopodium album (Che a 3)	Immunotherapy of allergic rhinitis	29

encapsulation procedure and storage.[30–32] In this review article, the properties of PLGA micro/nanoparticles, their preparation techniques, the strategies for improvement of antigen delivery, and stabilization approaches were summarized.

PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF PLGA

PLGA is one of the most commonly used polymers for encapsulation of therapeutic agents, especially peptides and proteins.[33–36] This polymer has been studied extensively for its remarkable characteristics such as the sustained delivery of proteins and therapeutic agents,[37] biocompatibility, biodegradability with minimal systemic toxicity, and good mechanical strength. PLGA contains two α-hydroxy acids, lactic acid, and glycolic acid, which could be hydrolyzed in aqueous solutions via the citric acid (Krebs) cycle to carbon dioxide and water (Fig. 1).[38]

The PLGA polymers that are commercially available usually characterized in terms of intrinsic viscosity, mechanical strength, swelling behavior, hydrolysis capacity, and biodegradation rate according to different stoichiometric ratios of lactic/glycolic acids and molecular mass.[10,11,39] Previous studies have been demonstrated that the encapsulation efficiency of drug into PLGA particles can be effected by factors such as polymer composition, particle size, surface charge, and drug content.[39,40] For drug and antigen delivery, mainly amorphous D,L-PLGA, has been used with different amount in LA:GA monomer ratio (50:50 up to 100:0), molecular weight of the polymer (*M*w of approximately 10–100 kDa), and end-group chemistry (free carboxylic acid or esterified carboxylic acid).[41,42]

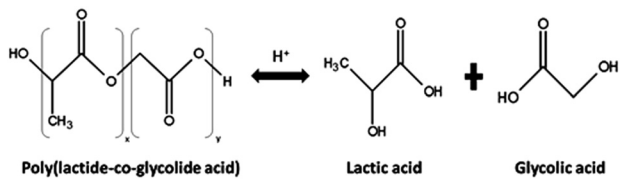

FIGURE 1. Hydrolysis of poly(lactic-co-glycolic acid).

COMMONLY USED METHODS FOR ANTIGEN ENCAPSULATION INTO PLGA PARTICLES

There are several methods used to prepare PLGA particles for drug delivery which could be affected on the size of particles, entrapment efficiency, biological activity, release profile, and stability of drugs in the polymer matrix. The most commonly used techniques for encapsulation of peptide or protein-based antigens into PLGA particles are spray-drying, double emulsion/solvent evaporation, phase separation, and also nanoprecipitation techniques[43] (Fig. 2). In double emulsion solvent evaporation technique (water/oil/water, W1/O/W2), the first step is emulsifying of drug in aqueous medium with a nonmiscible organic solution of the polymer such as dichloromethane (DCM) and ethyl acetate by homogenization or sonication to form a water/oil (W/O) emulsion. In the next step, this emulsion is dispersed by rapidly transferred into a larger aqueous volume containing a suitable stabilizer usually polyvinyl alcohol by high-speed homogenizer or sonicator to form the double emulsion.[10,44,45] In phase separation technique, an organic nonsolvent is added to the first W/O emulsion under stirring condition which gradually extracts the polymer solvent. After that the polymer is exposed to phase separation and it forms particles which encapsulate the drug. In spray-drying method, the first W/O emulsion is further atomized in a flow of drying air at a slightly increased temperature resulting in the formation of nanoparticles that are gathered from the airstream by a cyclone separator. The process is followed by the evaporation of the residual vaporizable organic solvent by magnetic stirring at room temperature or under reduced temperature and finally particles are separated by centrifugation, washing, and lyophilization.[10,42,45–51]

Nanoprecipitation technique was the first method for loading of protein or peptide-based antigen which was the one-step nanoprecipitation based on solvent displacement. In

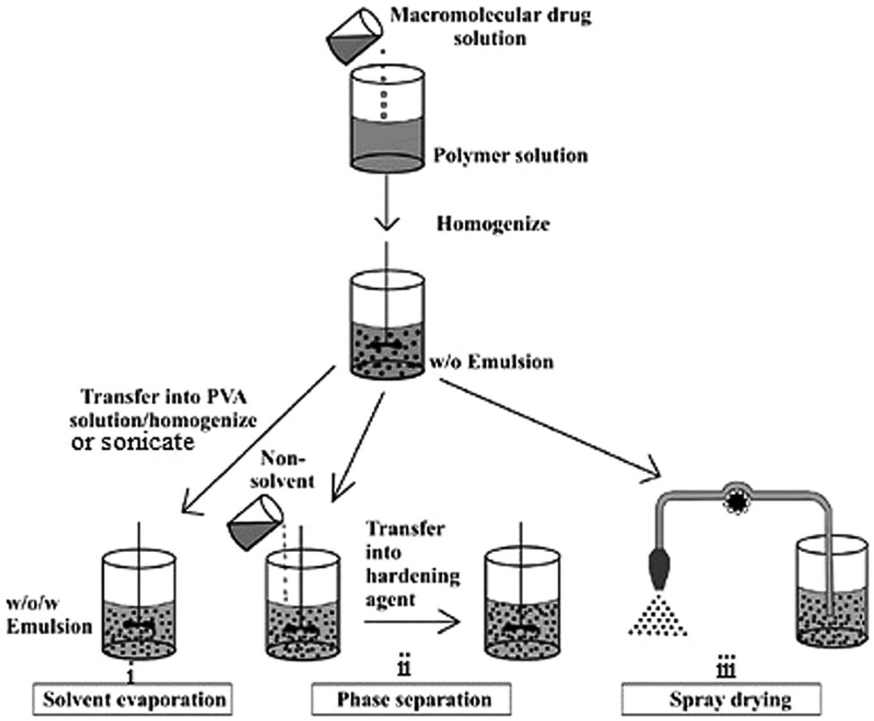

FIGURE 2. Comparison of encapsulation methods: (i) solvent evaporation, (ii) polymer phase separation, and (iii) spray-drying. Aqueous solution is dispersed in the organic polymer solution (W/O) emulsion; the W/O emulsion is processed further by specific methods to prepare the drug-loaded PLGA particles [37].

this method, the solution containing drug and the polymers dissolved in a water-miscible organic solvent and added into an aqueous solution containing surfactant. NPs are formed spontaneously due to precipitation of polymer in the aqueous environment. Proteins are poorly encapsulated within polymer nanoparticles using this method because of their limited solubility in organic solvents. Morales-Cruz et al. showed that protein could be loaded in PLGA NPs by two-step nanoprecipitation method with high efficiency. The first step in this new method consists in solvent-induced nanoprecipitation of the protein. Then, encapsulation was accomplished by a subsequent polymer nanoprecipitation step (Fig. 3).[52]

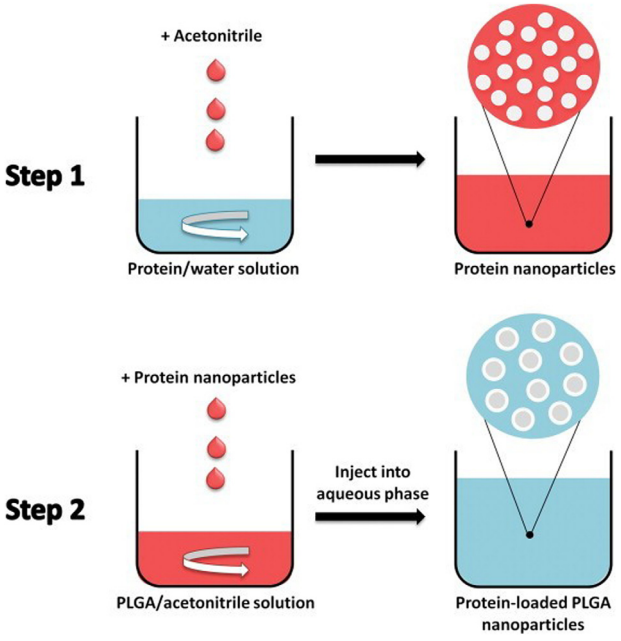

FIGURE 3. Two-step nanoprecipitation method for the encapsulation of proteins into PLGA particles [53].

The advantages and disadvantages of mentioned methods for encapsulation of peptide or protein-based antigens are summarized in Table II.

CHALLENGES IN LOADING OF PEPTIDE OR PROTEIN-BASED ANTIGENS INTO PLGA PARTICLES

One of the most important challenges in the synthesis of PLGA carriers is loss of structure and possible immunogenicity (antigenicity) of the encapsulated antigens.[68–70] Different factors could be affected on stability of proteins during formulation such as shear stresses or other physical forces, organic solvents, and lyophilization.[71–73] Instability has also been observed during storage and in *in vivo* environment.[69,74] Therefore, it is important to maintain the conformational structure of antigen in encapsulation process and storage.[58]

Physical instability

Physical instability often develops through conformational alterations leading to denaturation, surface adsorption, aggregation, or precipitation of the antigen which probably causes unwanted immunogenicity. It has been shown that the exposure of the protein to an organic solvent or aqueous/organic interfaces followed by the unfolding of protein

TABLE II. Some of Advantages and Disadvantages of PLGA Encapsulation Methods for Peptide or Protein

Methods of Peptide or Protein-Based Antigens Encapsulation into PLGA	Advantages	Disadvantages	Reference
Solvent evaporation	• Proteins used an aqueous solution • High yields • High encapsulation efficiencies • Size reduction by additives used for nanoparticle • Use of nonhighly toxic solvents (i.e., ethyl acetate). • Suitable for hydrophilic (double emulsions) and hydrophobic active components	• Complex process • High consumption of energy • Sensitive to polymer properties • Difficulties in modifying release profiles • Arguments related to the shelf life of antigens and stability • Large size of the nanoparticles formed • Leakage of the hydrophilic active component	44,53,58
Coacervation (polymer phase separation)	• Suitable to encapsulate both water soluble as well as water-insoluble drugs • Occur in the liquid phase	• Entirely sensitive to polymer properties • Common problem is accumulation • Lack of any emulsion stabilizer	10,11,43,59,62
Spray-drying	• Reproducibility • Well-defined control of particle size • Control of drug release	• High costly • Requires lyophilization of protein • Lover stability due to induce aggregation and denaturation	63,65
Nanoprecipitation	• Use of nonhighly toxic solvents (i.e., acetone) • Reduced energy consumption because it only requires regular stirring • Using additives for size reduction	• Time consuming of Solvent removed by evaporation • Suitable for drugs to be highly soluble in polar solvents (i.e., acetone) and slightly soluble in water • Lower loading efficiency • Polymer concentration effect on Nanoparticle size	53,66,67

finally leads to denaturation and aggregation.[11,75] The type of organic solvent and hydrophobic contacts between the protein and PLGA can also influence on protein instability and may lead to protein unfolding and aggregation.[76,77]

In addition, invariable aggregations and nonspecific adsorptions can occur due to hydrophilic interactions and also acidic microenvironment during degradation of PLGA polymers that result in a condition called incomplete release.[75,78,80] Previous studies have shown that proteins with positive charge could interact with the PLGA degradation components thus inhibiting its release.[56,69] Denaturation and aggregation of proteins are common phenomena during freezing and subsequent dehydration.[81,84] It was shown that the amorphous nature of PLGA may help in

TABLE III. Some Protein Stabilizers and Their Mechanism of Actions Used in PLGA Formulation

Stabilizer of Peptide or Protein-Based Antigen	Examples from Stabilizer	Antigen	Mechanism	Reference
Osmolytes	Cyclodextrins	Tetanus toxoid	Interactions between amino acids and the hydrophobic inner cavity of cyclodextrins	69
Other proteins	Albumin	Tetanus toxoid (Ttxd)	Cumulation at the water/organic solvent interface, shielding the interface from the protein of interest	107
	Bovine serum albumin	Diphtheria toxoid (Dtxd)	Cumulation at the water/organic solvent interface, shielding the interface from the protein of interest	108
Salts	$Mg(OH)_2$	Bovine serum albumin	Buffering agents to rectify acidic nanoenvironment	109
	Zinc oxide	Insulin	Buffering agents to rectify acidic nanoenvironment	110
Surfactants	Poly vinyl alcohol	Glutathione *S*-transferase of *Schistosoma mansoni* (rSm28GST)	A steric barrier between the W_1/O interface	74,111

TABLE IV. Some Surface Modification Agents of PLGA

Surface Modification Agents of PLGA	Examples	Mechanism	Reference
PEG	Bovine serum albumin	• Increases the hydrophilicity of PLGA • Enhance the affinity of protein for the matrix polymer and lead to better encapsulate efficiency	[138,139]
N-trimethyl chitosan (TMC)	HBsAg	• Carries a positive charge that can be utilized for cellular and anatomic targeting of NPs	[140]
Chitosan	Elcatonin	• Carries a positive charge that can be utilized for cellular and anatomic targeting of NPs	[141]
Protamine	Ovalbumin (OVA)	• Enhance the cross-presentation of encapsulated exogenous antigen by facilitating antigen uptake and lysosomal escape	[142]
Cationic emulsion stabilizer	• Poly(ethyleneimine) • Stearylamine	• Provide the necessary surface charge for ionic adsorption of counter-ions. • Alternatively, biodegradable polymers carrying ionic groups may be used to prepare unloaded particles	[143–145]
Targeting agents	Recombinant proteins	• Maintained its binding ability	[146]
Antibodies	Antibody recognizing Siglec-7 receptor	• With preservation of binding activity antibodies attachment and delivery of the entrapped payload directly into the cell	[147]
Peptides	RGD (Arg-Gly-Asp) as a β1 intregrin ligand	• Better accessibility of the ligand at the nanoparticle surface so increased the transport of the nanoparticles	[148]

preventing drying-induced harm to encapsulated proteins.[56,58,85]

Chemical instability

Several internal chemical degradations have been reported such as oxidation, deamination, amide bond hydrolysis, or acylation which lead to protein degradation in PLGA particles.[85–88] Although the storage stability may be a tiny issue when compared to the stability problems encountered during preparation and release steps, it has become clear that proteins are not necessarily stable in the solid state.[72,85,89] In this condition, hydrolysis of polymer can be initiated in the presence of small amount of moisture caused pH drop. Polymer and encapsulated proteins can have some interactions such as formation of amide linkage between amines of protein and carboxyl group of the degraded polymer.[90] The sugars as lyoprotectants cause the reduction of these interactions by the formation of covalent bonds with lysine residue of proteins.[75,90]

STRATEGIES FOR IMPROVEMENT OF ANTIGEN DELIVERY USING PLGA PARTICLES

Denatured or aggregated peptide or protein-based antigen will not only be therapeutically inactive but also may cause unpredictable side effects, such as immunogenicity or toxicity.[91] It is hence important to devise the improvement of formulation strategies for protein stability.

It was shown that the size of particles containing antigen could influence the immune response, therefore, the optimization of the preparation method to obtain the different size of NPs is an important parameter.[92–94] In our previous study, encapsulated rChe a 3 allergen (Chenopodium album) into PLGA NPs with different sizes (about 200, 400, and 800 nm) showed different encapsulation efficacy and release patterns.[95] Gutierro et al. observed significant differences in serum IgG2a/IgG1 ratios after using the different size of bovine serum albumin (BSA)-PLGA NPs compared to either free antigen or free antigen adsorbed to alum. However, these ratios were similar for each particle size (200, 500, and 1000 nm) among the subcutaneous, oral, and intranasal routes.[92] Recently, it was shown that PLGA-encapsulated rChe a 3 allergen with 400 nm size could significantly enhance systemic T regulatory (Treg) and T helper 1 (Th1) immune responses by sublingual administration.[29] Some factors which could be affected on the size, encapsulation efficiency, release, and stability of antigen-encapsulated PLGA particles include solvent, sonication time, polymer properties, stabilizers, osmolytes, and insoluble metal complexes, addition of several proteins, salts, surfactants, and chemical modification.

EFFECT OF SOLVENT

Solvent type in the synthesis of PLGA NPs could effect on size, antigen encapsulation efficiency, and stability.[51,96] For example, it was shown that in encapsulation of BSA into poly(lactide) (PLA) NPs using various solvents, BSA contents were comparable when both DCM and ethyl acetate were used as polymer solvents.[80] In our previous study, PLGA NPs containing rChe a 3 allergen showed smaller size and higher encapsulation efficiency when DCM and acetone (ACE) (4:1, v/v) was used as the solvent system compared to DCM alone. Since acetone has more polarity index than DCM, it causes an increase in miscibility of organic phase in the drug-loading process (W/O/W).[95] Conversely, the lower encapsulation efficiency of rChe a 3 was observed, when the DCM/ACE volume ratio was decreased, because more protein molecules were carried into the aqueous phase or at the organic solvent/water interface by a considerable amount of acetone.[95]

Similarly, aggregate formation increased and antigenicity corrupted following exposure of tetanus toxoid (Ttxd) to DCM whereas ethyl acetate exerted little effect.[68] During preparation of W_1/O emulsions, the Diphtheria toxoid (Dtxd) precipitated in contact with PLA and PLGA in ethyl formate but remained soluble when replaced with DCM. Also, residual solvents in the NPs can have detrimental influences on both antigen and PLGA properties.[42] In another study, Ttxd or insulin was encapsulated into PLGA NPs by solid-in oil-in-water and double emulsion methods in the present of different solvents. The results showed that in encapsulation of Ttxd into PLGA, the highest entrapment efficiency (about 95%) could be obtained both for NPs prepared by the double emulsion technique and with using both ethyl acetate and methylene chloride as organic solvents. Conversely, ethyl acetate leads to smaller mean size than methylene chloride. For loading of insulin in PLGA NPs, methylene chloride was able to improve entrapment efficiency more than ethyl acetate during the double emulsion procedure (13).

EFFECT OF SONICATION PROCESS

It was demonstrated that the duration and intensity of sonication could modify the size and distribution of the PLGA particles. These factors have the greatest effect in the final mean particle size in second step than the first step of sonication (water in oil emulsion) so that the mean size decrease when the sonication time increase, until reaching a plateau.[97,98] In our previous study, an increase in the sonication time could lead to the reduction of size and polydispersity index of PLGA nanoparticle containing rChe a 3 allergen.[95]

To minimize the deleterious effect of shearing and sonication stress on protein stability and encapsulation efficiency during PLGA formulation, different strategies have been developed such as reducing the exposure time or the intensity of the stress and the use of additives or appropriate organic solvent systems.[99] In different researches, a decrease in entrapment efficiency was observed by increasing sonication time, may be due to leaching of entrapped drug during higher sonication.[99,100]

EFFECTS OF POLYMER PROPERTIES

Since PLGA polymers are different in their properties such as the degradation rate, the acidity of the degradation products, and hydrophobicity, so, the type of polymer used for the synthesis of PLGA particles can effect on the stability and release of encapsulated proteins.[101] In general, hydrophilic polymer with faster degradation and higher release rate can be obtained by low lactic/glycolic acid ratio, low M_W, and uncapped of PLGA polymer.[40,102,103] Thomasin et al. showed two antigens, Ttxd, and a weakly immunogenic synthetic branched malaria peptide (P30B2), encapsulated into PLGA50:50–14 kDa and PLGA75:25–17 kDa by spray-drying had different kinetic release and immune response. They had burst release during the first 24 h and an additional release pulse toward the end of polymer degradation. The maximum release for both P30B2-PLGA50:50–14 kDa and Ttxd-PLGA50:50–14 kDa appeared around day 28 while for PLGA75:25–17 kDa, this time point was shifted to day 43 and day 70 for P30B2 and Ttxd, respectively. The difference between the release pattern of two antigens may be due to higher M_w of Ttxd (150 kDa) than P30B2 (16 kDa) which exhibit stronger interaction with the polymeric carboxyl groups. After immunization, P30B2-PLGA50:50–14 kDa with the fast degradation rate and small-sized microspheres could produce an immediate and strong antibody response compared to PLGA75:25–17 kDa containing P30B2 with more gradual increase in antibody titers[104]

The introduction of hydrophobic groups (e.g., fatty alcohol or acid moieties) into the polymer chain may serve to sustain antigen stability by decaying water uptake and subsequent moisture-induced antigen instability.[28] For example, BSA encapsulated into stearyl-poly(L-lactide)-stearate (10–20 kDa) and oleylpoly(L-lactide)-oleate showed slow release over 15 weeks (20–40%) due to the slow polymer degradation.[105,106]

EFFECT OF STABILIZERS

Different protein stabilizers have been used in PLGA formulations including osmolytes, insoluble metal complexes, other proteins, salts, and surfactants.

Osmolytes

The most commonly used protein stabilizers in formulations are osmolytes such as polyols and carbohydrates.[68,112] The stabilizing properties of osmolytes appear to be equilibrium between their binding to (deteriorating effect) and exclusion from (stabilizing effect) the antigen surface with hydrophobic or electrostatic interactions and hydrogen bonding.[113,114]

Some examples of osmolytes agents include cyclodextrin, trehalose, manitol, dextran, carboxymethyl cellulose[4] and hydrophobic compounds such as ethyl stearate, sodium acetate, and sodium glutamate.[75,115] Cyclodextrins used for encapsulating tetanus toxoid (Ttxd) in PLGA NPs increased Ttxd encapsulation efficacy, also prevented aggregation during encapsulation may be due to interactions between amino acids and the hydrophobic inner cavity of cyclodextrins.[69,116]

In the emulsion method, trehalose and other sugars covers the proteins in the organic solvent by preferential hydration and act as a water substitute, thereby prevent organic solvent-protein contacts.[117,118] Lyoprotectants such as dextran, glycol, glycerol, and cyclodextrin have been found to minimize instability of proteins in freeze-dried formulations.[86,87] The protective influence of these compounds maybe attribute to their amorphous, glass-forming characteristics, and their role as a water substitute in the solid state.[7,37,82,119,121]

Addition of proteins or amino acids

BSA is commonly used as a stabilizer in protein formulations. The protective effect of BSA can may be related to cumulation at the water/organic solvent interface, thereby covering the interface from the protein of interest.[122] Johansen et al. showed that albumin or a mixture of albumin and trehalose enhanced the encapsulation efficacy and stability of tetanus toxoid (Ttxd) into PLGA during preparation by spray-

drying and *in vitro* release.[107] In another study, the effect of different excipients on the stability of Tetanus Toxoid (TT) Encapsulated in PLGA Microspheres was evaluated. The results showed lysine or histidine significantly improved TT antigenicity upon exposure to moisture in the solid state and heat in solution may be due to the presence of side chains derived from the incorporation of lysine into toxoid.[123]

Salts (antacids)

PLGA hydrolysis results in the decrease of the pH inside the nanospheres due to the release of acidic degradation components. Therefore stimulate the denaturation or aggregation of encapsulated protein leading to loss of antigenicity.[75,124,125] Several techniques have been used to overcome this obstacle such as inserting poorly soluble bases or pore-forming factors, increasing loading of antigen or water-soluble additive, and reducing the level of PLGA hydrolysis.[109,126] Basic salts such as sodium bicarbonate and magnesium hydroxide may be incorporated as buffering agents into the matrix to rectify acidic nanoenvironment. Furthermore, since the acidic microclimate resulting from accumulation of polymer degradation products[127,128] is a major factor causing protein instability, then, many studies showed that addition of buffering salts can stabilize encapsulated proteins. For example, $Mg(OH)_2$ increased the microclimate pH in PLGA polymer containing BSA resulting in protein stability.[109]

Metal ions such as zinc could act as antigen stabilizers by formation of reversible complex with protein, then this complex encapsulated in nanoparticles. Antigen physicochemical integrity can be preserved for prolonged periods of time, until protein dissociated from the complex and release from the NP.[42] It was shown that zinc salts could prevent the changes of α-helix and β-sheet values of insulin in exposure to the oil–water interface during the primary emulsification step subsequently resulting in the preservation of the secondary structure of insulin.[129]

Surfactants

The addition of surfactants to PLGA formulation stabilizes proteins against denaturation during several steps from insertion to release at the site of delivery.[130] The protection offered by surfactants is primarily a function of their surface activity including decreasing the mass transfer rate of antigen to the W_1/O interface, thus reducing encapsulation of interface-denatured antigen. Therefore, surfactants provide additional protection against unalterable aggregation of partially denatured antigens.[131] However, surfactant should be restricted to the minimum level required to avoid possible toxic and hypersensitivity reactions.[8,132] Poly vinyl alcohol (PVA) was historically used to stabilize proteins during emulsification and these remains until today, especially for stabilization of the secondary emulsion.[59] PVA also was used as a steric barrier between the W_1/O interfaces to preserve the integrity of the recombinant 28 kDa glutathione *S*-transferase of *Schistosoma mansoni* (rSm28GST).[74,96] It has been demonstrated that using PVA in lower molecular weight and lower degree of hydrolysis resulted in the PLGA NPs with smaller particle sizes.[133,134] The concentration of PVA could effect on the particle size of PLGA NPs. PVA in 1 and 5% concentrations was applied in the synthetize of PLGA NPs containing rChe a 3 allergen. It was shown that the mean diameter of the NPs with PVA 5% was lower than formulation using PVA 1%.[95]

Azizi et al. utilized Sorbitan monostearate (Span 60) and Tween 80 as surfactant in different concentrations in organic (internal) and aqueous (external) phase, respectively, for encapsulation of BSA into PLGA NPs. Lower mean particle sizes were obtained with higher concentration of Span 60 (14% (w/w)) and lower concentration of Tween 80 (4% (w/v)) in the present of PVA 1%. The results showed that the role of Span 60 on NPs size is more important than Tween 80 because the higher emulsifier concentration in outer phase resulted in higher viscosity, leading to highly aggregated droplet and resistance toward shear forces in the second emulsion.[134]

In another study, BSA-loaded PLGA nanoparticle or microparticles fabricated by W/O/W method using different emulsifiers such as PVA, poloxamer, or polyvinylpyrrolidone (PVP). The results showed for preparation both submicrons sized- and microparticles, PVA and poloxamer are efficient, but for nanoparticle smaller than 220 nm only PVA are useful. Also PVP was efficient in microparticle synthesis.[135]

Increasing concentrations of PVA had more inhibition effect on coalescence of the inner aqueous-phase droplets (144). Also, common surfactants such as Tweens and Pluronics F68 have been investigated[38] but have not been very successful as a stabilizer in the emulsification step.[68,74] The poor protection by surfactants may be explained by inadequate competition with the protein for the water/organic solvent interface, or improvement of organic solvent/protein contacts through hydrophobic contacts with both components.[68,74,136] Interestingly, poloxamer 188 decreased the percentage of BSA encapsulated in PLGA, aggregates from 31 to 5%, as estimated from the difference between BSA total and BSA monomer.[137] This stabilizer have been shown to stabilize the primary emulsion and to reduce protein–polymer interactions.[32,137]

SURFACE MODIFICATIONS OF PLGA

The surface of PLGA particles could be modified in order to improve physicochemical properties and enhance immunogenicity of antigen with different agents (Table IV).

Poly ethylene glycol (PEG) is the most commonly copolymer for encapsulation of peptide or protein-based antigen into PLGA particles.[149,151] It is thought that PEG by increases the hydrophilicity of PLGA polymers may enhance the affinity of protein for the matrix polymer and lead to better encapsulate efficiency.[138,139]

Ya-Ping et al. fabricated PEG–PLGA-BSA nanoparticles by double emulsion method. Intravenous administration assesses over 24 h in rats. The entrapment efficiency, particle size and zeta potential were 48.6%, about 200 nm and −16.1 mV, respectively. This nanoparticle in compared with that of PLGA nanoparticles could increase BSA half-life from BSA release from 13.6 min to 4.5 h, and also they alter biodistribution in rats.[55] When used *N*-trimethyl chitosan (TMC)-coated HBsAg-

PLGA particles for nasal immunization of mice in compare to loaded only HBsAg loaded PLGA particles, resulted to strong antibody production.[152] Elcatonin-PLGA-chitosan (CS) complex were fabricated by emulsion solvent diffusion method. These surface modification resulted to slowly eliminated from the lungs, prolonged the pharmacological action to 24 h, retention of nanospheres adhered to the bronchial mucus and lung tissue and sustained drug release at the adherence site (enhanced the absorption of drug). The absorption-enhancing effect may have been caused by opening the intercellular tight junctions.[153] In another study, PLGA-OVA were coated with cationic and arginine-rich protamine. These coated particles, produce higher antibodies and T-cell responses in mice. *In vitro* studies suggested that the improved immunological performance was mediated by an increased uptake.[142] Charged PLGA particles prepared by solvent evaporation/extraction methods could be obtained using cationic emulsion stabilizer [poly(ethyleneimine); stearylamine] or anionic emulsifier (sodium dioctylsulfosuccintate; sodium dodecyl sulfate [SDS]) located in second water phase.[118–120] Previous studies showed adsorbed protein antigens and DNA on PLGA surface nanoparticle there have been highly efficient in stimulation of strong immune responses.[154]

Targeting of antigen-loaded PLGA NPs has been developed to enhance cellular uptake by defined cell types of the immune system such as DCs or increase the immunogenicity of NPs.[155] For example, recombinant proteins with C-terminal region incorporated into PLGA NPs showed high affinity to Claudin-4 resulted in enhanced uptake by upper airway and intestinal M-cells on mice.[156] PLGA conjugated to antibodies recognizing sialic acid-binding immunoglobulin-like lectins (Siglec-7) receptor could successfully internalize into fibroblasts expressing this receptor.[147] Garinot et al. demonstrated that the conjugation of RGD (Arg-Gly-Asp) as a β1 intregrin ligand, into PEGylated PLGA NPs significantly increased uptake of particles in M-cells and induced an IgG response in *in vitro* and *in vivo* studies.[148]

OTHER APPROACHES TO IMPROVE THE DELIVERY OF PROTEIN OR PEPTIDE-BASED ANTIGEN USING PLGA PARTICLES

Common nanoencapsulation methods involve relatively rough conditions that are not totally tolerated by antigens without stabilization. Therefore, new and improved procedures covering the antigen from disadvantaged conditions have been proposed and evaluated. One approach included of dispersing the antigen in a mineral oil before insertion into PLGA NP by an $O_1/O_2/W$ method. The mineral oil (O_1) was predesignated as a barrier to protect the antigen during emulsification with the polymer solution and from exposure to moisture during release.[157] Atomization of PLA and PLGA solutions using gases, for example, CO2, in the supercritical or near-supercritical state has been proposed as an alternative route to prepare NPs.[158,159] The ProLeaseR technology was developed to ensure maximum stability of proteins or peptides during and after nanoencapsulation. The method relies on the use of stabilizing and release-controlling agents, low processing temperature, and nonaqueous nanoencapsulation.[160,161] The main benefits of ultrasonic atomization of W_1/O dispersions techniques encompass the possibility of easy particle size control and scale-up, processing at ambient or reduced temperature, and the suitability for aseptic manufacturing in a small containment chamber such as an isolator.[162] Exposure of antigen to potentially detrimental aqueous conditions or W_1/O liquid interfaces can be avoiding by nonaqueous nanoencapsulation methods, typically administrating dried antigen powders. The dry mood proposes increased stability owing to the decreased conformational flexibility and, therefore, less potential for structural disorderliness. Nanoencapsulation of solid antigen powders may involve the first step of either spray-drying or freeze-drying aqueous antigen, or locating the aqueous antigen into water-soluble excipients which act as protective barriers against the organic solvent; in a second step, the dry antigen powder or incorporated antigen is then diffused in the organic polymer solution.[42] During BSA encapsulation into PLGA microparticle by a solid-in-oil-in-water (S/O/W) method, the protein secondary structure was less changed as compared to encapsulation by an aqueous $W_1/O/W_2$ method.[124] Coating the antigen in a stabilizing matrix has also proved to be effective against denaturation. HBsAg pre-embedded into hydroxypropyl cellulose (HPC) and then further encapsulated, as solid particles, into PLGA microshper remained 90% antigenicity properties.[30,163] Using of protein- or peptide-counter-ion complexes showed the decrease of aqueous solubility of the protein or peptide, The improvement of its disperse in nonaqueous environment, such as organic (polymer) solvents and the increased structural stability due to restricted chain mobility of the protein in the complex.[164] Particle Replication in Non-wetting Templates (PRINT) technology is a high-resolution method for producing monodisperse nano/micro particles. In one study, cylindrical cationic PLGA-based NPs prepared by PRINT technology through surface were electrostatically bound to Hemagglutinin (HA) antigens in influenza vaccine (TIV).[165]

CONCLUSION

Nanoparticles are useful for delivering several types of compounds, including small molecule drugs, therapeutic proteins, vaccines, and gene agents. Many studies have been widely reported on the successful encapsulation of protein or peptide-based antigen into PLGA; however, it is essential to improve its properties to overcome some obstacles such as release profile and instability of antigen during formulation procedure and storage. In this review, different types of protein instability in PLGA formulation process were discussed. It was shown that selective modification and design of a new generation of polymers as well as enhanced manufacturing processes can be equally applied to ensure nanoencapsulation and delivery of stable antigens.

REFERENCES

1. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity 2008;29:372–383.

2. Tran KK, Zhan X, Shen H. Polymer blend particles with defined compositions for targeting antigen to both class I and II antigen presentation pathways. Adv Healthc Mater 2014;3:690–702.
3. Waeckerle-Men Y, Allmen EU, Gander B, Scandella E, Schlosser E, Schmidtke G, Merkle HP, Groettrup M. Encapsulation of proteins and peptides into biodegradable poly (D, L-lactide-co-glycolide) microspheres prolongs and enhances antigen presentation by human dendritic cells. Vaccine 2006;24:1847–1857.
4. Frokjaer S, Otzen DE. Protein drug stability: A formulation challenge. Nat Rev Drug Discov 2005;4:298.
5. Lee VH. Peptide and protein drug delivery-opportunities and challenges. Pharm Int 1986;7:208–212.
6. LoPresti C, Vetri V, Ricca M, Foderà V, Tripodo G, Spadaro G, Dispenza C. Pulsatile protein release and protection using radiation-crosslinked polypeptide hydrogel delivery devices. React Funct Polym 2011;71:155–167.
7. Shire SJ, Shahrokh Z, Liu J. Challenges in the development of high protein concentration formulations. J Pharm Sci 2004;93: 1390–1402.
8. Singh R, Singh S, Lillard JW. Past, present, and future technologies for oral delivery of therapeutic proteins. J Pharm Sci 2008;97: 2497–2523.
9. Sahdev P, Ochyl LJ, Moon JJ. Biomaterials for nanoparticle vaccine delivery systems. Pharm Res 2014;31:2563–2582.
10. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide) (PLGA) devices. Biomaterials 2000;21:2475–2490.
11. Wu X. Preparation, Characterization, and Drug Delivery Applications of Microspheres Based on Biodegradable Lactic/Glycolic Acid Polymers. Encyclopedic Handbook of Biomaterials and Bioengineering. New York: Marcel Dekker; 1995. p. 1151–1200.
12. Silva A, Rosalia R, Varypataki E, Sibuea S, Ossendorp F, Jiskoot W. Poly-(lactic-co-glycolic-acid)-based particulate vaccines: Particle uptake by dendritic cells is a key parameter for immune activation. Vaccine 2015;33:847–854.
13. Jain S, Harde H, Indulkar A, Agrawal AK. Improved stability and immunological potential of tetanus toxoid containing surface engineered bilosomes following oral administration. Nanomedicine: Nanotechnology, Biology and Medicine. 2014;10(2):431–440.
14. Sarti F, Perera G, Hintzen F, Kotti K, Karageorgiou V, Kammona O, et al. In vivo evidence of oral vaccination with PLGA nanoparticles containing the immunostimulant monophosphoryl lipid A. Biomaterials. 2011;32(16):4052–4057.
15. Quintilio W, Takata CS, Sant'Anna OA, da Costa MHB, Raw I. Evaluation of a diphtheria and tetanus PLGA microencapsulated vaccine formulation without stabilizers. Curr Drug Deliv. 2009;6(3): 297–304.
16. Feng L, Qi XR, Zhou XJ, Maitani Y, Wang SC, Jiang Y, et al. Pharmaceutical and immunological evaluation of a single-dose hepatitis B vaccine using PLGA microspheres. J Control Release. 2006; 112(1):35–42.
17. Chuang S-C, Ko J-C, Chen C-P, Du J-T, Yang C-D. Encapsulation of chimeric protein rSAG1/2 into poly (lactide-co-glycolide) microparticles induces long-term protective immunity against Toxoplasma gondii in mice. Exp Parasitol. 2013;134(4):430–437.
18. Huang S-s, Li I-H, Po-da Hong M-kY. Development of Yersinia pestis F1 antigen-loaded microspheres vaccine against plague. Int J Mol Med. 2014);9:813.
19. Cleland JL, Lim A, Barr□n L, Duenas ET, Powell MF. Development of a single-shot subunit vaccine for HIV-1: Part 4. Optimizing microencapsulation and pulsatile release of MN rgp120 from biodegradable microspheres. J Control Release. 1997;47(2):135–150.
20. Kasturi SP, Skountzou I, Albrecht RA, Koutsonanos D, Hua T, Nakaya HI, et al. Programming the magnitude and persistence of antibody responses with innate immunity. Nature. 2011;470(7335): 543–547.
21. Raghuvanshi RS, Katare YK, Lalwani K, Ali MM, Singh O, Panda AK. Improved immune response from biodegradable polymer particles entrapping tetanus toxoid by use of different immunization protocol and adjuvants. Int J Pharm. 2002;245(1):109–121.
22. Guerin V, Dubarryr M, Robic D, Brachet F, Rautureau M, Andre C, et al. Microsphere entrapped bee-venom phospholipase A2 retains specific IgE binding capacity: a possible use for oral specific immunotherapy. J Microencapsul 2002;19(6):761–765.
23. Batanero E, Barral P, Villalba M. Rodriguez Ra. Encapsulation of Ole e 1 in biodegradable microparticles induces Th1 response in mice: a potential vaccine for allergy. J Control Release. 2003;92(3): 395–398.
24. Diwan M, Park TG. Stabilization of recombinant interferon-α by pegylation for encapsulation in PLGA microspheres. Int J Pharm. 2003;252(1):111–122.
25. Roopngam P, Liu K, Mei L, Zheng Y, Zhu X, Tsai H-I, et al. Hepatitis C virus E2 protein encapsulation into poly d, l-lactic-co-glycolide microspheres could induce mice cytotoxic T-cell response. Int J Nanomedicine. 2016;11:5361.
26. Sun S, Cui F, Kawashima Y, Liang N, Zhang L, Shi K, et al. A novel insulin-sodium oleate complex for oral administration: preparation, characterization and in vivo evaluation. J Drug Deliv Sci Technol. 2008;18(4):239–243.
27. Silva AL, Rosalia RA, Sazak A, Carstens MG, Ossendorp F, Oostendorp J, et al. Optimization of encapsulation of a synthetic long peptide in PLGA nanoparticles: low-burst release is crucial for efficient CD8+ T cell activation. Eur J Pharm Biopharm. 2013;83 (3):338–345.
28. Xiao X, Zeng X, Zhang X, Ma L, Liu X, Yu H, et al. Effects of Caryota mitis profilin-loaded PLGA nanoparticles in a murine model of allergic asthma. Int J Nanomedicine. 2013;8:4553.
29. Salari F, Varasteh A-R, Vahedi F, Hashemi M, Sankian M. Down-regulation of Th2 immune responses by sublingual administration of poly (lactic-co-glycolic) acid (PLGA)-encapsulated allergen in BALB/c mice. Int Immunopharmacol. 2015;29(2):672–678.
30. Lee HJ. Protein drug oral delivery: the recent progress. Arch Pharm Res. 2002;25(5):572–584.
31. Schwendeman S, Cardamone M, Klibanov A, Langer R. Stability of proteins and their delivery from biodegradable polymer microspheres. Drugs Pharm Sci. 1996;77:1–49.
32. Ye M, Kim S, Park K. Issues in long-term protein delivery using biodegradable microparticles. J Control Release. 2010;146(2): 241–260.
33. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids and Surfaces B: Biointerfaces. 2010;75(1):1–18.
34. Manchanda R, Fernandez-Fernandez A, Nagesetti A, McGoron AJ. Preparation and characterization of a polymeric (PLGA) nanoparticulate drug delivery system with simultaneous incorporation of chemotherapeutic and thermo-optical agents. Colloids and Surfaces B: Biointerfaces. 2010;75(1):260–267.
35. Muthu M. Nanoparticles based on PLGA and its co-polymer: An overview. Asian J Pharm. 2009;3(4):266.
36. Vandervoort J, Ludwig A. Biocompatible stabilizers in the preparation of PLGA nanoparticles: a factorial design study. Int J Pharm. 2002;238(1):77–92.
37. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly (D, L-lactide-co-glycolide) and its derivatives. J Control Release. 2008;125(3):193–209.
38. G□pferich A. Mechanisms of polymer degradation and erosion. Biomaterials. 1996);17(2):103–114.
39. Semete B, Booysen L, Lemmer Y, Kalombo L, Katata L, Verschoor J, et al. In vivo evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems. Nanomedicine: Nanotechnology, Biology and Medicine. 2010;6(5):662–671.
40. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev. 2003;55 (3):329–347.
41. Prior S, Gander B, Blarer N, Merkle HP, Subir□ ML, Irache JM, et al. In vitro phagocytosis and monocyte-macrophage activation with poly (lactide) and poly (lactide-co-glycolide) microspheres. Eur J Pharm Sci. 2002;15(2):197–207.
42. Tamber H, Johansen P, Merkle HP, Gander B. Formulation aspects of biodegradable polymeric microspheres for antigen delivery. Adv Drug Deliv Rev. 2005;57(3):357–376.
43. Freitas S, Merkle HP, Gander B. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of

microsphere preparation process technology. J Control Release. 2005;102(2):313–332.
44. Han K, Lee K-D, Gao Z-G, Park J-S. Preparation and evaluation of poly (L-lactic acid) microspheres containing rhEGF for chronic gastric ulcer healing. J Control Release. 2001;75(3):259–269.
45. Hans M, Lowman A. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci. 2002;6(4):319–327.
46. Arshady R. Preparation of biodegradable microspheres and microcapsules: 2. Polyactides and related polyesters. J Control Release. 1991;17(1):1–21.
47. Mao S, Xu J, Cai C, Germershaus O, Schaper A, Kissel T. Effect of WOW process parameters on morphology and burst release of FITC-dextran loaded PLGA microspheres. Int J Pharm. 2007;334(1): 137–148.
48. Ramezani M, Ebrahimian M, Hashemi M. Current Strategies in the Modification of PLGA-based Gene Delivery System. Curr Med Chem. 2017;24(7):728–739.
49. Rosca ID, Watari F, Uo M. Microparticle formation and its mechanism in single and double emulsion solvent evaporation. J Control Release. 2004;99(2):271–280.
50. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release. 2001;70(1):1–20.
51. Wischke C, Schwendeman SP. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. Int J Pharm. 2008;364 (2):298–327.
52. Morales-Cruz M, Flores-Fern□ndez GM, Morales-Cruz M, Orellano EA, Rodriguez-Martinez JA, Ruiz M, et al. Two-step nanoprecipitation for the production of protein-loaded PLGA nanospheres. Results Pharma Sci. 2012;2:79–85.
53. Astete CE, Sabliov CM. Synthesis and characterization of PLGA nanoparticles. Journal of Biomaterials Science, Polymer Edition. 2006;17(3):247–289.
54. Ficheux M-F, Bonakdar L, Leal-Calderon F, Bibette J. Some stability criteria for double emulsions. Langmuir. 1998;14(10):2702–2706.
55. Li Y-P, Pei Y-Y, Zhang X-Y, Gu Z-H, Zhou Z-H, Yuan W-F, et al. PEGylated PLGA nanoparticles as protein carriers: synthesis, preparation and biodistribution in rats. J Control Release. 2001;71(2): 203–211.
56. Morlock M, Koll H, Winter G, Kissel T. Microencapsulation of rh-erythropoietin, using biodegradable poly (D, L-lactide-co-glycolide): protein stability and the effects of stabilizing excipients. Eur J Pharm Biopharm. 1997;43(1):29–36.
57. Saez V, Hern□ndez JR, Peniche C. Microspheres as delivery systems for the controlled release of peptides and proteins. Biotecnologia Aplicada. 2007;24:108–116.
58. Sinha V, Trehan A. Biodegradable microspheres for protein delivery. J Control Release. 2003;90(3):261–280.
59. Edelman R, Russell RG, Losonsky G, Tall BD, Tacket CO, Levine MM, et al. Immunization of rabbits with enterotoxigenic E. coli colonization factor antigen (CFA/I) encapsulated in biodegradable microspheres of poly (lactide-co-glycolide). Vaccine. 1993;11(2): 155–158.
60. Johansen P, Moon L, Tamber H, Merkle HP, Gander B, Sesardic D. Immunogenicity of single-dose diphtheria vaccines based on PLA/PLGA microspheres in guinea pigs. Vaccine. 1999;18(3): 209–215.
61. Pisal DS, Kosloski MP, SV B-I. Delivery of therapeutic proteins. J Pharm Sci. 2010;99(6):2557–2575.
62. Yeh M-K, Liu Y-T, Chen J-L, Chiang C-H. Oral immunogenicity of the inactivated Vibrio cholerae whole-cell vaccine encapsulated in biodegradable microparticles. J Control Release. 2002;82(2): 237–247.
63. Bittner B, Kissel T. Ultrasonic atomization for spray drying: a versatile technique for the preparation of protein loaded biodegradable microspheres. J Microencapsul. 1999;16(3):325–341.
64. Kissel T, Brich Z, Bantle S, Lancranjan I, Nimmerfall F, Vit P. Parenteral depot-systems on the basis of biodegradable polyesters. J Control Release. 1991;16(1-2):27–41.
65. Takada S, Yamagata Y, Misaki M, Taira K, Kurokawa T. Sustained release of human growth hormone from microcapsules prepared by a solvent evaporation technique. J Control Release. 2003;88(2): 229–242.
66. Bala I, Hariharan S, Kumar MR. PLGA nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst. 2004; 21(5).
67. Mathiowitz E. Encyclopedia of controlled drug delivery. Wiley; 1999.
68. Alonso MJ, Gupta RK, Min C, Siber GR, Langer R. Biodegradable microspheres as controlled-release tetanus toxoid delivery systems. Vaccine. 1994;12(4):299–306.
69. Schwendeman SP. Recent advances in the stabilization of proteins encapsulated in injectable PLGA delivery systems. Crit Rev Ther Drug Carrier Syst. 2002;19(1).
70. Xing DK-L, Crane DT, Bolgiano B, Corbel MJ, Jones C, Sesardic D. Physicochemical and immunological studies on the stability of free and microsphere-encapsulated tetanus toxoid in vitro. Vaccine. 1996;14(13):1205–1213.
71. Barrow EL, Winchester GA, Staas JK, Quenelle DC, Barrow WW. Use of microsphere technology for targeted delivery of rifampin to Mycobacterium tuberculosis-infected macrophages. Antimicrob Agents Chemother. 1998;42(10):2682–2689.
72. Castellanos IJ, Cruz G, Crespo R, Griebenow K. Encapsulation-induced aggregation and loss in activity of γ-chymotrypsin and their prevention. J Control Release. 2002;81(3):307–319.
73. Walsh MC, Banas JA, Mudzinski SP, Preissler MT, Graziano RF, Gosselin EJ. A two-component modular approach for enhancing T-cell activation utilizing a unique anti-FcγRI-streptavidin construct and microspheres coated with biotinylated-antigen. Biomol Eng. 2003;20(1):21–33.
74. Jiang W, Gupta RK, Deshpande MC, Schwendeman SP. Biodegradable poly (lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. Adv Drug Deliv Rev. 2005;57(3): 391–410.
75. van de Weert M, Hennink WE, Jiskoot W. Protein instability in poly (lactic-co-glycolic acid) microparticles. Pharm Res. 2000;17(10): 1159–1167.
76. Sah H. Protein instability toward organic solvent/water emulsification: implications for protein microencapsulation into microspheres. PDA J Pharm Sci Technol. 1999;53(1):3–10.
77. van de Weert M, Hoechstetter J, Hennink WE, Crommelin DJ. The effect of a water/organic solvent interface on the structural stability of lysozyme. J Control Release. 2000;68(3):351–359.
78. Brunner A, M□der K, G□pferich A. pH and osmotic pressure inside biodegradable microspheres during erosion. Pharm Res. 1999;16 (6):847–853.
79. Crotts G, Park TG. Protein delivery from poly (lactic-co-glycolic acid) biodegradable microspheres: release kinetics and stability issues. J Microencapsul. 1998;15(6):699–713.
80. Gander B, Wehrli E, Alder R, Merkle H. Quality improvement of spray-dried, protein-loaded D, L-PLA microspheres by appropriate polymer solvent selection. J Microencapsul. 1995;12(1): 83–97.
81. Carpenter JF, Chang BS, Garzon-Rodriguez W, Randolph TW. Rational design of stable lyophilized protein formulations: theory and practice. Rational design of stable protein formulations. 2002; 109–133.
82. Carpenter JF, Pikal MJ, Chang BS, Randolph TW. Rational design of stable lyophilized protein formulations: some practical advice. Pharm Res. 1997;14(8):969–975.
83. Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. Pharm Res. 2010; 27(4):544–575.
84. Wang W. Protein aggregation and its inhibition in biopharmaceutics. Int J Pharm. 2005;289(1):1–30.
85. Chang LL, Pikal MJ. Mechanisms of protein stabilization in the solid state. J Pharm Sci. 2009;98(9):2886–2908.
86. Domb AJ, Turovsky L, Nudelman R. Chemical interactions between drugs containing reactive amines with hydrolyzable insoluble biopolymers in aqueous solutions. Pharm Res. 1994;11(6): 865–868.
87. Houchin M, Topp E. Chemical degradation of peptides and proteins in PLGA: a review of reactions and mechanisms. J Pharm Sci. 2008;97(7):2395–2404.
88. Shenoy B, Wang Y, Shan W, Margolin AL. Stability of crystalline proteins. Biotechnol Bioeng. 2001;73(5):358–369.

89. Griebenow K, Klibanov AM. Lyophilization-induced reversible changes in the secondary structure of proteins. Proc Natl Acad Sci. 1995;92(24):10969–10976.
90. Wu F, Jin T. Polymer-based sustained-release dosage forms for protein drugs, challenges, and recent advances. AAPS PharmSciTech. 2008;9(4):1218–1229.
91. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011;3(3):1377–1397.
92. Gutierro I, Hernandez R, Igartua M, Gascon A, Pedraz J. Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. Vaccine. 2002;21(1):67–77.
93. Igartua M, Hernandez R, Esquisabel A, Gascon A, Calvo M, Pedraz J. Enhanced immune response after subcutaneous and oral immunization with biodegradable PLGA microspheres. J Control Release. 1998;56(1):63–73.
94. Win KY, Feng S-S. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. Biomaterials. 2005;26(15):2713–2722.
95. Hajavi J, Sankian M, Varasteh A-R, Hashemi M. Synthesis strategies for optimizing sizes of PLGA nanoparticles containing recombinant Chenopodium album (rChe a 3) allergen. Int J Polym Mater. 2017;66(12):603–608.
96. Gander B, Johansen P, Nam-Trân H, Merkle H. Thermodynamic approach to protein microencapsulation into poly (D, L-lactide) by spray drying. Int J Pharm. 1996;129(1-2):51–61.
97. Bilati U, Allémann E, Doelker E. Sonication parameters for the preparation of biodegradable nanocapsulesof controlled size by the double emulsion method. Pharm Dev Technol. 2003;8(1):1–9.
98. Mohamed F, van der Walle CF. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. J Pharm Sci. 2008;97(1):71–87.
99. Bilati U, Allémann E, Doelker E. Strategic approaches for overcoming peptide and protein instability within biodegradable nano-and microparticles. Eur J Pharm Biopharm. 2005;59(3):375–388.
100. Dangi R, Shakya S. Preparation, optimization and characterization of PLGA nanoparticle. International Journal of Pharmacy & Life Sciences. 2013;4(7).
101. Varde NK, Pack DW. Microspheres for controlled release drug delivery. Expert Opin Biol Ther. 2004;4(1):35–51.
102. Panyam J, Dali MM, Sahoo SK, Ma W, Chakravarthi SS, Amidon GL, et al. Polymer degradation and in vitro release of a model protein from poly (D, L-lactide-co-glycolide) nano-and microparticles. J Control Release. 2003;92(1):173–187.
103. Tracy M, Ward K, Firouzabadian L, Wang Y, Dong N, Qian R, et al. Factors affecting the degradation rate of poly (lactide-co-glycolide) microspheres in vivo and in vitro. Biomaterials. 1999;20(11):1057–1062.
104. Thomasin C, Corradin G, Men Y, Merkle HP, Gander B. Tetanus toxoid and synthetic malaria antigen containing poly (lactide)/poly (lactide-co-glycolide) microspheres: importance of polymer degradation and antigen release for immune response. J Control Release. 1996;41(1-2):131–145.
105. Rafler G, Jobmann M. Controlled release systems of biodegradable polymers: 4th communication: Hydrophobic and hydrophilic polyactides for drug delivery systems. Pharmazeutische Industrie. 1996;58(12):1147–1151.
106. Tamber H. Development of biodegradable polymer microspheres for peptide/protein antigen delivery 2002.
107. Johansen P, Men Y, Audran R, Corradin G, Merkle HP, Gander B. Improving stability and release kinetics of microencapsulated tetanus toxoid by co-encapsulation of additives. Pharm Res. 1998;15(7):1103–1110.
108. Johansen P, Tamber H, Merkle HP, Gander B. Diphtheria and tetanus toxoid microencapsulation into conventional and end-group alkylated PLA/PLGAs. Eur J Pharm Biopharm. 1999;47(3):193–201.
109. Zhu G, Mallery SR, Schwendeman SP. Stabilization of proteins encapsulated in injectable poly (lactide-co-glycolide). Nat Biotechnol. 2000;18(1):52–57.
110. Takenaga M, Yamaguchi Y, Kitagawa A, Ogawa Y, Kawai S, Mizushima Y, et al. Optimum formulation for sustained-release insulin. Int J Pharm. 2004;271(1):85–94.
111. Baras Bt BM-A, Poulain-Godefroy O, Schacht A-M, Capron A, Gillard J, et al. Vaccine properties of antigens entrapped in microparticles produced by spray-drying technique and using various polyester polymers. Vaccine. 2000;18(15):1495–1505.
112. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process and storage considerations. Adv Drug Deliv Rev. 2006;58(15):1688–1713.
113. Surve M, Pryamitsyn V, Ganesan V. Depletion and pair interactions of proteins in polymer solutions. J Chem Phys. 2005;122(15):154901.
114. Zhou Y, Hall CK. Solute excluded-volume effects on the stability of globular proteins: A statistical thermodynamic theory. Biopolymers. 1996;38(2):273–284.
115. Chaisri W, Hennink WE, Okonogi S. Preparation and characterization of cephalexin loaded PLGA microspheres. Curr Drug Deliv. 2009;6(1):69–75.
116. Jalil R, Nixon J. Biodegradable poly (lactic acid) and poly (lactide--co-glycolide) microcapsules: problems associated with preparative techniques and release properties. J Microencapsul. 1990;7(3):297–325.
117. Hora MS, Rana RK, Smith FW. Lyophilized formulations of recombinant tumor necrosis factor. Pharm Res. 1992;9(1):33–36.
118. Ohtake S, Kita Y, Arakawa T. Interactions of formulation excipients with proteins in solution and in the dried state. Adv Drug Deliv Rev. 2011;63(13):1053–1073.
119. Bhatnagar BS, Bogner RH, Pikal MJ. Protein stability during freezing: separation of stresses and mechanisms of protein stabilization. Pharm Dev Technol. 2007;12(5):505–523.
120. Cleland JL, Powell MF, Shire SJ. The development of stable protein formulations: a close look at protein aggregation, deamidation, and oxidation. Crit Rev Ther Drug Carrier Syst. 1992;10(4):307–377.
121. S□nchez A, Villamayor B, Guo Y, McIver J, Alonso MJ. Formulation strategies for the stabilization of tetanus toxoid in poly (lactide-co-glycolide) microspheres. Int J Pharm. 1999;185(2):255–266.
122. Betancourt T, Brown B, Brannon-Peppas L. Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and in vitro evaluation. Nanomedicine (London, England). (2007);2(2):219-32.10.2217/17435889.2.2.219
123. Jiang W, Schwendeman SP. Stabilization of tetanus toxoid encapsulated in PLGA microspheres. Molecular pharmaceutics. 2008;5(5):808–817.
124. Carrasquillo KG, Stanley AM, Aponte-Carro JC, De Jésus P, Costantino HR, Bosques CJ, et al. Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly (lactide-co--glycolide) microspheres results in release of native protein. J Control Release. 2001;76(3):199–208.
125. Kersten GF, Donders D, Akkermans A, Beuvery EC. Single shot with tetanus toxoid in biodegradable microspheres protects mice despite acid-induced denaturation of the antigen. Vaccine. 1996;14(17-18):1627–1632.
126. Kang J, Schwendeman SP. Comparison of the effects of Mg (OH) 2 and sucrose on the stability of bovine serum albumin encapsulated in injectable poly (D, L-lactide-co-glycolide) implants. Biomaterials. 2002;23(1):239–245.
127. Fu K, Pack DW, Klibanov AM, Langer R. Visual evidence of acidic environment within degrading poly (lactic-co-glycolic acid)(PLGA) microspheres. Pharm Res. 2000;17(1):100–106.
128. Shenderova A, Burke TG, Schwendeman SP. The acidic microclimate in poly (lactide-co-glycolide) microspheres stabilizes camptothecins. Pharm Res. 1999;16(2):241–248.
129. Manoharan C, Singh J. Insulin loaded PLGA microspheres: effect of zinc salts on encapsulation, release, and stability. J Pharm Sci. 2009;98(2):529–542.
130. Chi EY, Krishnan S, Randolph TW, Carpenter JF. Physical stability of proteins in aqueous solution: mechanism and driving forces in nonnative protein aggregation. Pharm Res. 2003;20(9):1325–1336.
131. Arakawa T, Kita Y. Protection of bovine serum albumin from aggregation by Tween 80. J Pharm Sci. 2000;89(5):646–651.
132. Hanson MA, Rouan SE. Introduction to formulation of protein pharmaceuticals. Stability of Protein Pharmaceuticals, Part B: In Vivo Pathways of Degradation and Strategies for Protein Stabilization. New York: Plenum Press; 1992. p 209–233.

133. Feczkó T, Tóth J, Dósa G, Gyenis J. Optimization of protein encapsulation in PLGA nanoparticles. Chemical Engineering and Processing: Process Intensification. 2011;50(8):757–765.
134. Azizi M, Farahmandghavi F, Joghataei M, Zandi M, Imani M, Bakhtiary M, et al. Fabrication of protein-loaded PLGA nanoparticles: effect of selected formulation variables on particle size and release profile. Journal of Polymer Research. (2013);20(4): 110.10.1007/s10965-013-0110-z
135. Feczkó T, Tóth J, Gyenis J. Comparison of the preparation of PLGA–BSA nano-and microparticles by PVA, poloxamer and PVP. Colloids Surf A Physicochem Eng Asp. 2008;319(1):188–195.
136. Cleland JL, Jones AJ. Stable formulations of recombinant human growth hormone and interferon-γ for microencapsulation in biodegradable mircospheres. Pharm Res. 1996;13(10):1464–1475.
137. Nihant N, Schugens C, Grandfils C, Jérôme R, Teyssié P. Polylactide microparticles prepared by double emulsion/evaporation technique. I. Effect of primary emulsion stability. Pharm Res. 1994; 11(10):1479–1484.
138. Choi S-W, Kim J-H. Design of surface-modified poly (D, L-lactide--co-glycolide) nanoparticles for targeted drug delivery to bone. J Control Release. 2007;122(1):24–30.
139. Jain RA, Rhodes CT, Railkar AM, Malick AW, Shah NH. Controlled release of drugs from injectable in situ formed biodegradable PLGA microspheres: effect of various formulation variables. Eur J Pharm Biopharm. 2000;50(2):257–262.
140. Makita-Chingombe F, Kutscher HL, DiTursi SL, Morse GD, Maponga CC. Poly(lactic-co-glycolic) Acid-Chitosan Dual Loaded Nanoparticles for Antiretroviral Nanoformulations. J Drug Deliv. 2016;2016:10. https://doi.org/10.1155/2016/3810175.
141. Han R, Zhu J, Yang X, Xu H. Surface modification of poly(D,L-lactic-co-glycolic acid) nanoparticles with protamine enhanced cross-presentation of encapsulated ovalbumin by bone marrow--derived dendritic cells. J Biomed Mater Res A. 2011;96A(1):142–149. https://doi.org/10.1002/jbm.a.32860.
142. Gómez JMM, Csaba N, Fischer S, Sichelstiel A, Kündig TM, Gander B, et al. Surface coating of PLGA microparticles with protamine enhances their immunological performance through facilitated phagocytosis. J Control Release. 2008;130(2):161–167.
143. Jung T, Breitenbach A, Kissel T. Sulfobutylated poly (vinyl alcohol)-graft-poly (lactide-co-glycolide) s facilitate the preparation of small negatively charged biodegradable nanospheres. J Control Release. 2000;67(2):157–169.
144. Jung T, Kamm W, Breitenbach A, Klebe G, Kissel T. Loading of tetanus toxoid to biodegradable nanoparticles from branched poly (sulfobutyl-polyvinyl alcohol)-g-(lactide-co-glycolide) nanoparticles by protein adsorption: a mechanistic study. Pharm Res. 2002;19(8):1105–1113.
145. Li XW, Lee DKL, Chan ASC, Alpar HO. Sustained expression in mammalian cells with DNA complexed with chitosan nanoparticles. Biochim Biophys Acta, Gene Struct Expr. 2003;1630(1):7–18.
146. Rajapaksa TE, Stover-Hamer M, Fernandez X, Eckelhoefer HA, Lo DD. Claudin 4-targeted protein incorporated into PLGA nanoparticles can mediate M cell targeted delivery. J Control Release. 2010; 142(2):196–205. https://doi.org/10.1016/j.jconrel.2009.10.033.
147. Scott CJ, Marouf WM, Quinn DJ, Buick RJ, Orr SJ, Donnelly RF, et al. Immunocolloidal targeting of the endocytotic siglec-7 receptor using peripheral attachment of siglec-7 antibodies to poly (lactide-co-glycolide) nanoparticles. Pharm Res. 2008;25(1): 135–146.
148. Garinot M, Fiévez V, Pourcelle V, Stoffelbach F, des Rieux A, Plapied L, et al. PEGylated PLGA-based nanoparticles targeting M cells for oral vaccination. J Control Release. 2007;120(3):195–204.
149. Lam XM, Duenas ET, Cleland JL. Encapsulation and stabilization of nerve growth factor into poly (lactic-co-glycolic) acid microspheres. J Pharm Sci. 2001;90(9):1356–1365.
150. Sturesson C, Carlfors J. Incorporation of protein in PLG-microspheres with retention of bioactivity. J Control Release. 2000;67 (2):171–178.
151. Wolf M, Wirth M, Pittner F, Gabor F. Stabilisation and determination of the biological activity of L-asparaginase in poly (D, L-lactide-co-glycolide) nanospheres. Int J Pharm. 2003;256(1):141–152.
152. Pawar D, Goyal AK, Mangal S, Mishra N, Vaidya B, Tiwari S, et al. Evaluation of mucoadhesive PLGA microparticles for nasal immunization. The AAPS journal. 2010;12(2):130–137.
153. Yamamoto H, Kuno Y, Sugimoto S, Takeuchi H, Kawashima Y. Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions. J Control Release. 2005;102(2): 373–381.
154. Singh M, Kazzaz J, Ugozzoli M, Chesko J, O'Hagan DT. Charged polylactide co-glycolide microparticles as antigen delivery systems. Expert Opin Biol Ther. 2004;4(4):483–491.
155. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012;161(2):505–522.
156. Rajapaksa TE, Stover-Hamer M, Fernandez X, Eckelhoefer HA, Lo DD. Claudin 4-targeted protein incorporated into PLGA nanoparticles can mediate M cell targeted delivery. J Control Release. 2010; 142(2):196–205.
157. Sanchez A, Gupta RK, Alonso MJ, Siber GR, Langer R. Pulsed controlled-release system for potential use in vaccine delivery. J Pharm Sci. 1996;85(6):547–552.
158. Bleich J, Müller B. Production of drug loaded microparticles by the use of supercritical gases with the aerosol solvent extraction system (ASES) process. J Microencapsul. 1996;13(2):131–139.
159. Randolph TW, Randolph AD, Mebes M, Yeung S. Sub-micrometer-sized biodegradable particles of poly (L-lactic acid) via the gas antisolvent spray precipitation process. Biotechnol Prog. 1993;9 (4):429–435.
160. Bartus RT, Tracy MA, Emerich DF, Zale SE. Sustained delivery of proteins for novel therapeutic products. Science. 1998;281(5380): 1161–1162.
161. Cleland JL, Daugherty A, Mrsny R. Emerging protein delivery methods. Curr Opin Biotechnol. 2001;12(2):212–219.
162. Felder CB, Blanco-Prieto MJ, Heizmann J, Merkle HP, Gander B. Ultrasonic atomization and subsequent polymer desolvation for peptide and protein microencapsulation into biodegradable polyesters. J Microencapsul. 2003;20(5):553–567.
163. Lee HK, Park JH, Kwon KC. Double-walled microparticles for single shot vaccine. J Control Release. 1997;44(2):283–293.
164. Yoo HS, Choi HK, Park TG. Protein–fatty acid complex for enhanced loading and stability within biodegradable nanoparticles. J Pharm Sci. 2001;90(2):194–201.
165. Galloway AL, Murphy A, DeSimone JM, Di J, Herrmann JP, Hunter ME, et al. Development of a nanoparticle-based influenza vaccine using the PRINT® technology. Nanomedicine: NBM. 2013; 9(4):523–531.