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Review Article

Biodegradable Poly(D,L-lactic-co-glycolic acid)-Based Micro/Nanoparticles for Sustained Release of Protein Drugs - A Review

Rezaul H Ansary, Mohamed B Awang and Mokhlesur M Rahman*
Kulliyyah of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Malaysia

For correspondence:* ***Email:* *mdrahman@iium.edu.my*

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Abstract

Biodegradable poly(D, L-lactide-co-glycolide) (PLGA) and PLGA-based polymeric nanoparticles are widely used for sustained release of protein and peptide drugs. These formulations are usually prepared by water/oil/water (W/O/W) and solid/oil/water (S/O/W) double emulsion solvent evaporation method. Other methods of preparation are nanoprecipitation, emulsion solvent diffusion and salting-out. This review attempts to address the effects of PLGA molecular weight, lactide to glycolide ratio, crystallinity, hydrophilicity as well as nanoparticles preparation variables (e.g., homogenizer speed, surfactants nature and concentration) on the size, morphology, drug encapsulation efficiency and release profile of PLGA mico/nanoparticles. The current knowledge of protein instability during preparation, storage and release from PLGA micro/nanoparticles and protein stabilization approaches has also been discussed in this review.

Keywords: *Poly(D, L-lactic-co-glycolic acid), Nanoparticles, Microparticles, Protein/peptides, Sustained release, Protein instability*

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INTRODUCTION

Biodegradable polymeric nanoparticles are solid or semisolid colloidal particles ranging in size from 10 to 1000 nm [1]. They consist of both micro-and macromolecular substances and can be used as drug carriers. The drug can be adsorbed, dissolved, entrapped, or encapsulated into the nanoparticles matrix. In conventional administration, macromolecular drugs such as protein and peptide drugs are administered by daily, sometimes multiple injections via a parenteral route due to their short half life *in vivo*. To improve patient compliance and convenience, controlled release formulations of protein and peptide drugs have been developed [2-4].

Over the past three decades, poly(D, L-lactic-co-glycolic acid) (PLGA) has been investigated extensively for developing micro/nanoparticles for controlled release of protein and peptide drugs due its biodegradability and biocompatibility [5-7]. An ideal micro/nanoparticle formulation is one which should have high encapsulation efficiency, loading capacity, sustained and complete release of the encapsulated drug with retained structural integrity [8,9]. High protein loading and high encapsulation efficiency are very important due to the extremely high price of pharmaceutical proteins. Several protein and peptide -PLGA microspheres are currently available in the United States and European markets, although

they still present various challenges for researchers.

In general, the major drawbacks in the development of PLGA based micro/nanoparticle formulations in protein and peptide d delivery are the high initial burst, incomplete release and instability of the encapsulated proteins [10-12]. Initial burst release means the rapid release of a large amount of encapsulated protein. This phenomenon is commonly observed in PLGA based micro/nanoparticles delivery system. This is due to the rapid diffusion of protein molecules adsorbed or located at the surface of the PLGA micro/nanoparticles. A great amount of the loaded protein is not released after a certain period of release study either due to protein aggregation or adsorption to the strong hydrophobic PLGA surfaces resulting in incomplete protein release. As a result of the degradation of PLGA during the drug release process, lactic and glycolic acids are generated, thereby resulting in a significant reduction of the pH of the microenvironment and denaturation of the encapsulated proteins [13,14]. The release kinetics of encapsulated protein and peptide drugs from PLGA micro and nanoparticles depend on a number of factors such as PLGA molecular weight, lactide/glycolide ratio, PLGA end groups, particle morphology, drug distribution, etc [15,16]. This review addresses current knowledge on the preparation of PLGA-based micro/nanoparticles for parenteral and oral delivery of protein and peptide drugs with particular emphasis on the retention of biological activity of encapsulated drugs.

APPLICATIONS OF PLGA IN CONTROLLED RELEASE FORMULATIONS

Method of preparation and properties

Among the different classes of biodegradable polymers, poly(D,L-lactide-co-glycolide) (PLGA) and its homopolymers poly(lactide) (PLA) and poly(glycolide) (PGA) are the most widely used biodegradable polymers. These polymers have been approved by the United States Food and Drug Administration (FDA) for drug delivery due to their excellent biocompatibility, biodegradability and mechanical strength [5]. They can degrade by non-enzymatic hydrolysis of the ester backbone in body fluid. The degradation products (i.e. lactic and glycolic acids) are metabolic compounds and readily eliminated from the body through Krebs cycle [6]. Due to these characteristics, these polymers have the advantage of not requiring surgery for removal from the body after they have served their purposes. They protect the entrapped drug against degradation and control its site specific release. PLGA is synthesized through ring-opening polymerization of two different monomers, the cyclic dimers (1, 4-dioxane-2, 5-diones) of glycolic acid and lactic acid. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding a linear, aliphatic polyester as a product [7]. The poly(D, L-lactide-co-glycolide) synthetic scheme is described in Figure 1.

Effect of PLGA properties on drug release

With varying the ratio of lactide to glycolide, amorphous and crystalline forms of PLGA can be obtained. It has been found that the time required for hydrolytic degradation of PLGA is related to the lactide to glycolide ratio, end group (ester or free carboxyl group) and molecular weight of polymer. As the most frequently used biodegradable polymer for drug loaded microsphere/nanospheres preparation, the effect of different PLGA properties such as molecular weight, lactide/glycolide ratio, and the end group capping on drug release have been extensively studied. Low molecular weight PLGA generally leads to a faster polymer degradation and a more rapid drug release [17,18]. As lactide is more hydrophobic than glycolide, an increase in lactide content in PLGA copolymers decreases the polymer degradation rate and results in a slower drug release [19]. The end group of PLGA is a factor that affects the hydrophilicity of the polymer. In general, PLGA copolymers carrying free carboxylic end groups caused a high initial burst and release rates compared to the end-capped polymer [20]. Uncapped PLGA with free carboxyl termini is more hydrophilic and absorb more water, leading to a higher degradation rate than its end-capped species with esterified carboxyl termini [21]. Thus, the physical and chemical characteristics of PLGA such as molecular weight, copolymer ratio, ester or free carboxyl end groups and glass transition temperature are important factors for the biodegradability of the polymers.

PLGA of varying molecular weight (ranging from 10 to > 100 kDa) and different lactide to glycolide molar ratios (50:50, 65:35, 75:25, and 85:15) is available on the market. Normally, 50:50 lactide/glycolide PLGA copolymers have the fastest half-life of degradation, around 50-60 days, whereas 65:35, 75:25, and 85:15 lactide/glycolide copolymers have progressively longer degradation half-lives *in vivo*.

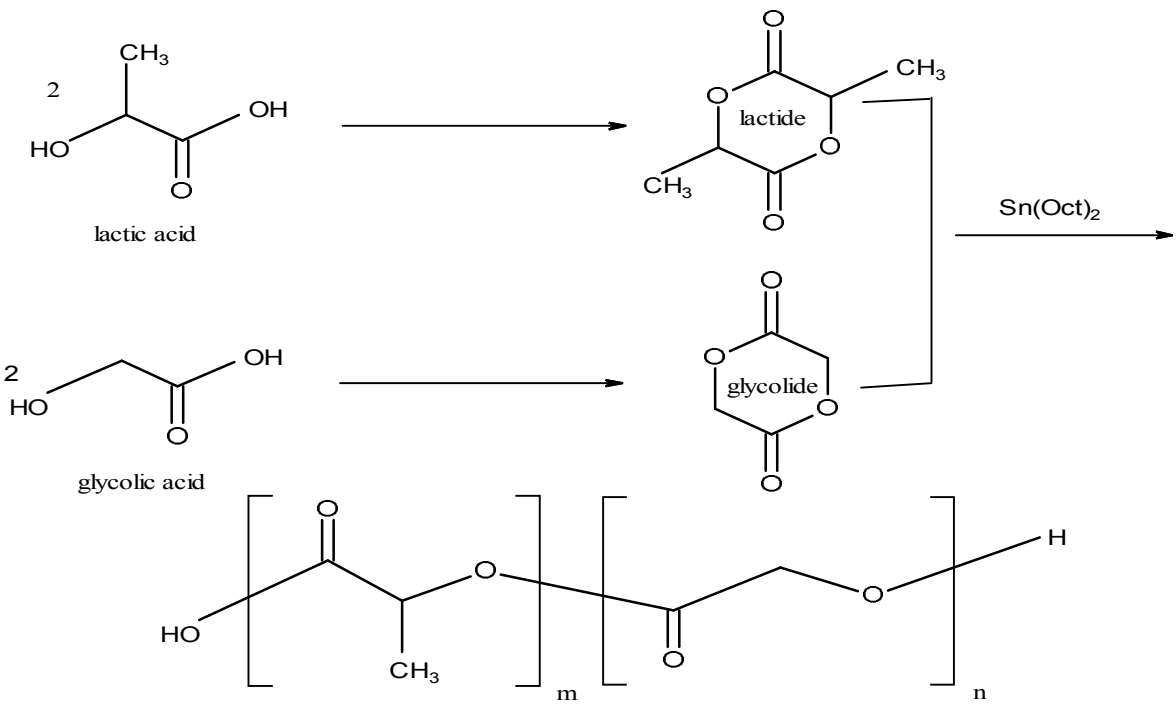

Fig 1: Pol(D, L-lactide-co-glycolide) (PLGA) synthetic schemes

Table 1 exhibits some physical properties of different PLGA. The half-life of these polymers can be decreased or increased by co-blending with more hydrophilic or hydrophobic compounds such as polyethylene glycol or polycaprolactone. Visscher *et al* studied the biodegradation of poly(D,L-lactide) and 50:50 poly(D,L-lactide-co-glycolide) in rat gastrocnemius muscles [22,23]. Poly(D,L-lactide) nanoparticles was completely degraded within 480 days, whereas the PLGA nanoparticles was degraded in 63 days. This happened due to more hydrophilic and semicrystalline nature of the glycolide part. Thus, PLGA with 50:50 lactide to glycolide ratio is more advantageous than other polymers due to its fastest degradation rate and thus resulting in a faster drug release from the nanoparticles.

Glass transition temperature (Tg) is the temperature at which the polymers change from glassy state to rubbery state. At this point, the mechanical behavior of the polymer changes from rigid and brittle to tough and leathery (plastic behavior). The Tg of PLGAs is commonly above the physiological temperature of 37 °C, which gives them enough mechanical strength to be fabricated into delivery devices. The Tg of the PLGA decreases with decrease of lactic acid content in copolymer and with decrease in their molecular weight [24].

Microspheres and nanospheres based on PLGA have been extensively investigated for sustained and targeted release of drugs such as anticancer drugs [25], antibiotics [26-28], peptide and protein drugs namely human growth hormone [29], leuprolide acetate [30], octreotide [31], lysozyme [32], bovine serum albumin (BSA) [33] and insulin [34].

Table 1: Physical properties of some PLGAs

Polymer (lactide to glycolide ratio)	Inherent viscosity (dL/g)	Physical state	Glass transition temp (ºC)	Solvent solubility*	Approx. degradation time (months)
PLGA (50:50)	0.55-0.75	Amorphous	45-50	1,2,3,4,5,6	1-2
PLGA (65:35)	0.55-0.75	Amorphous	45-50	1,2,3,4,5,6	3-4
PLGA (75:25)	0.55-0.75	Amorphous	50-55	1,2,3,4,5,6	4-5
PLGA (85:15)	0.55-0.75	Amorphous	50-55	1,2,3,4,5,6	5-6

** 1 = acetone, 2 = tetrahydrofuran, 3 = hexafluoroisopropanol, 4 = chloroform, 5 = ethylacetate, and 6 = dichloromethane*

FUNCTIONAL ALIPHATIC POLYESTERS FOR NANOPARTICLE PREPARATION

Most polyesters do not have significant number of functional groups that could enhance their potential applications. Recently, extensive work has been carried out towards functionalizing polyesters in order to enhance the drug delivery behavior of the synthesized nanoparticles. The introduction of functional pendant groups in side chains along the polymer backbone can be used to alter the key properties of these polyesters. Functional aliphatic polyesters can be synthesized by two different synthetic methods (1) the functionalization of pre-formed PLGA, and (2) the polymerization of functionalized monomers/dimers. Practically, the second protocol is preferred, due to the risk of side reactions (e.g. chain scission and racemization) associated with the first protocol. The polymerization of functionalized mono/dimers can generally be conducted via: (i) polycondensation, (ii) ring-opening polymerization (ROP), or (iii) enzymatic polymerization. Ring opening polymerization (ROP) is often preferred method. Since, high molecular weight polymers can be obtained easily in a controlled manner in contrast to the other two methods.

A variety of functionalized polyesters containing different types of side chains and end groups have been synthesized in recent years [35-37]. An interesting type of functionalized polyester, poly(lactic-co-hydroxymethyl glycolic acid) (PLHMGA) has been synthesized by Hennink and co-workers [38,39]. This functionalized polymer (Figure 2) containing side chains with free OH groups are more hydrophilic than conventional PLGA. The introduction of OH groups provide polymers with tunable degradation behavior by suppression of crystallinity and enhanced hydrophilicity which improve cell adhesion. Thus, an increased hydrophilicity results in a greater water uptake capacity of the polymers, thereby increasing the degradation rate of the polymers.

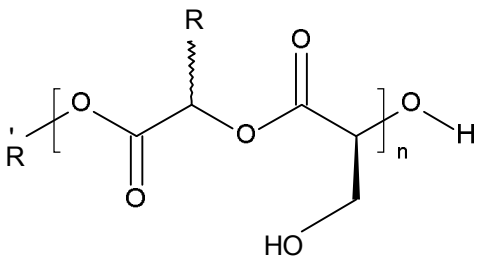

Figure 2: Functionalized poly(lactic-co-hydroxymethyl glycolic acid) (PLHMGA) polymer

PREPARATION OF DRUG LOADED POLY-MERIC NANOPARTICLES

Several methods have been developed to prepare drug loaded micro and nanoparticles with desired release characteristics from biodegradable polymers. These include: emulsification solvent evaporation [40], nanoprecipitation [41], emulsification solvent diffusion method [42] and salting out [43]. The use of a particular method in the preparation of drug loaded nanoparticles depends on the nature of the polymer employed, nature of the drug to be encapsulated, intended use of the system, and intended duration of the therapy. *In vitro* and *in vivo* responses from the nanoparticles are influenced by their various properties, such as the particle size and size distribution, surface morphology, porosity, surface chemistry, surface adhesion, zeta-potential, drug stability, drug encapsulation efficiency, surface/bulk erosion/degradation, diffusion of the drug, kinetics of drug release, and the thermodynamic properties of the nanoparticles.

Depending on the preparation methods drugs or antigens can either be entrapped in the polymer matrix, encapsulated in a liquid core, surrounded by a shell-like polymer membrane, or bound to the particle surface by adsorption [44]. For drug loading into nanoparticles, three major strategies can be employed: (1) covalent attachment of the drug to the particle surface or to the polymer prior to preparation, (2) adsorption of the drug to a preformed carrier system, and (3) incorporation of the drug into the particle matrix during particle preparation [45]. The release rates of nanoparticles depend upon: (i) desorption of the surface-bound/adsorbed drug, (ii) diffusion through the nanoparticle matrix, (iii) diffusion (in case of nanocapsules) through the polymer wall, (iv) nanoparticle matrix erosion, and (v) a combined erosion/diffusion process. During these preparation and release processes, the bioactivity of therapeutic agents must be remained in native form.

Emulsification-solvent evaporation method

There are single (O/W) and double (W/O/W) emulsion systems in this fabrication method. Single emulsion method is conducted for the formulation of hydrophobic drugs (oil soluble); while double emulsion is adopted for the encapsulation of hydrophilic drugs (peptide and protein drugs).

Water/oil/water (W/O/W) double emulsion solvent evaporation method

The W/O/W double emulsion solvent evaporation method has been widely used due to its relatively simple process, convenience in controlling process parameters, and ability to produce with inexpensive instrument [46]. In this method, the polymer is first dissolved in a water-immiscible, volatile, organic solvent. Usually, dichloromethane (DCM) is selected as organic solvent, but other solvents like chloroform, ethyl acetate or methylethyl ketone have also been investigated. An aqueous solution of hydrophilic drug is added to this polymer solution and the mixture is emulsified by a high speed homogenizer or a sonicator to form the first W/O emulsion. Then, the primary W/O emulsion is further added gently with stirring into a large volume of outer water phase containing surfactant, such as poly(vinyl alcohol) (PVA), resulting in a W/O/W double emulsion.

The selection of surfactants in the outer water phase is an important factor for successful nano or microparticles fabrication. The aggregation of PLGA particles during the process of particle formation is a major problem regardless of the fabrication method. In order to prevent the aggregation of PLGA particles, polymer stabilizers are often used. Furthermore, the size and shape of the particles can also be influenced by the stabilizer used.

Surfactants or stabilizers are amphiphilic molecules that posses both hydrophilic and hydrophobic parts. The hydrophilic moiety is called the head and the hydrophobic part the tail. The head can be charged or uncharged polar group. Depending on the nature of head groups, different kinds of surfactants such as non-ionic surfactant poly(vinyl) alcohol (PVA) [47], anionic surfactant sodium dodecyl sulphate (SDS) [48], cationic surfactant didodecyl dimethyl ammonium bromide (DMAB) [49], and amphiphilic surfactant d-α-tocopheryl polyethylene glycol 100 succinate vitamin E (TPGS) [50] are commonly applied based on emulsion systems.

To harden the nanoemulsion droplets into solid nanoparticles, the organic solvent is removed by either solvent extraction or solvent evaporation and the nanoparticles are collected by filtration or centrifugation. For the removal of solvent, the stirring process may be continued for several hours at high-temperature/low-pressure conditions. The properties of nanoparticles such as drug loading capacity (LC), encapsulation efficiency (EE), release profiles and morphology (shape and size) depend on various parameters such as drug (type and concentration), polymer (composition, molecular weight, and concentration), volume ratio between drug and polymer solution, emulsification method (homogenization time and speed), surfactant (types and concentration) additives in the internal water phase and external water phase (e.g., NaCl, $NaHCO_3$, sucrose) [51-53].

Bilati *et al* [15] investigated the effect of polymer type on drug encapsulation efficiency (EE) and mean size of protein loaded PLGA nanoparticles prepared by a W/O/W double emulsion method. From the Table 2 it was found that when the molecular weight of PLGA was higher the EE was enhanced and the particles were larger. The effect of high molecular weight PLGA on particle size seems to be independent of protein loadings but might be due to longer polymeric chains and their higher inherent viscosities. The use of PLGA with uncapped carboxylic end groups having a molecular weight of 34 kDa, The EE was 97 %, while the size was 271 nm. This increase in EE is due to an ionic interaction between the positively charged amino groups of proteins and the negative charges of the copolymer carboxylic acids [54]. A large particle size 1090 nm (i.e. so-called microparticles) was observed when the polymer concentration was doubled. This effect of polymer concentration on particle size might be due to the increased viscosity of the organic phase.

In W/O/W technique, proteins encapsulated into PLGA nano or microparticles are susceptible to denaturation or aggregation, oxidation, deamidation and cleavage, while creating the W/O primary emusion [55]. A large interface between the aqueous and organic phase is formed in this process, at which the protein may adsorb and denature. Protein stability may be enhanced if the protein is encapsulated as a solid rather than in solution. So, the modification of this method has been developed to solve those problems.

Solid/oil/water (S/O/W) double emulsion solvent evaporation method

Protein adsorption and denaturation at the aqueous/organic solvent interface is one of the major limitations for decreased protein bioactivity occurring during the encapsulation process. To protect protein from denaturation during formation of W/O emulsion, S/O/W method has been developed. This is because, proteins in the

solid state are believed to maintain their bioactivity by drastically reducing conformational change in comparison to the large structural change found in the dissolved state [56]. In the S/O/W method, dehydrated protein powders are dispersed in the polymer solution to form the primary emulsion. Then the solid dispersion is introduced into a large volume of aqueous solution containing surfacting agent, such as PVA. It can be noted that making dispersion of protein particles in organic solvent is not so easy. Protein particle micronization is one of the major challenges in the S/O/W method which include lyophilization, spray drying, and spray freeze-drying [57]. Spray freeze-drying collects atomized protein microdroplets in a frozen form and followed with the ice sublimation under reduced pressure. Thus, the temperature dependent protein denaturation and deactivation experienced in spray drying is circumvented. Nevertheless, one of the problems hampering the use of S/O/W technique is the low encapsulation efficiency of proteins. It is notable that encapsulation yield is an important parameter for cost-efficient production of microspheres containing expensive pharmaceutical proteins.

Nanoprecipitation method

Nanoprecipitation or solvent displacement method was introduced by Fessi and co-workers and has become a popular technique to prepare nanoparticles due to narrow size distribution, absence of shear stress, and absence of surfactants for amphiphilic polymers [41]. In this method, particles are formed spontaneously by precipitation and subsequent solidification of the polymer upon rapid solvent diffusion. The polymer and drug are dissolved in a water miscible organic solvent for example acetone or methanol. The solution is then poured under magnetic stirring into an aqueous solution which contains surfactant. Through rapid solvent diffusion, the nanoparticles are formed immediately. After that, the solvents are removed under reduced pressure. The mechanism of formation of NPs by this technique has been explained by the interfacial turbulence generated at the interface of the solvent and non-solvent. Thus, the process is often called solvent displacement or interfacial deposition.

Emulsification solvent diffusion method

In this technique, the organic solvent containing the dissolved polymer and the drug is emulsified in an aqueous surfactant solution (usually with PVA as a stabilizing agent) by using a high-speed homogenizer. Water is subsequently added under constant stirring to the O/W emulsion system, thus causing phase transformation and outward diffusion of the solvent from the internal phase, leading to the nanoprecipitation of the polymer and the formation of colloidal nanoparticles. Finally, the solvent can be eliminated by vacuum steam distillation or evaporation. The most important fabrication step is solvent diffusion, in which the organic phase diffuses from the oil phase to outer water phase and the formed particles become hardened. The selection of the surfactants in the outer water phase is also crucial for successful fabrication.

Salting-out method

Salting out is another fabrication method for the preparation of PLGA nanoparticles. In this method, firstly PLGA is dissolved in a water miscible organic solvent such as tetrahydrofuran (THF) or acetone. Then, the oil phase is emulsified in an aqueous phase consisting of surfactant and salt of high concentration under strong shearing force by an overhead mechanical stirrer. Typically, the most commonly used salts are magnesium chloride hexahydrate or magnesium acetate tetrahydrate with a ratio of

Table 2: Effect of polymer type on encapsulation efficiency (EE) and mean size of protein-loaded PLGA nanoparticles[a] [15]

PLGA[b]	Polymer Mw by GPC (kDa)	Amount of polymer (mg)	Volume of inner phase(w) (µL)	EE (%)	Size (nm)
PLGA	34	200	100	81	288
PLGA	12	200	100	89	207
PLGA	80	200	100	98	408
PLGA-H	34	200	100	97	271
PLGA	34	200	200	94	378
PLGA	34	400	200	84	1090

[a] All batches were produced with ethyl acetate as organic solvent and at 1% of nominal DL; [b] PLGA = Resomer® RG 502, RG 503 or RG 505; PLGA-H = Resomer® RG 503 with uncapped carboxylic end groups

Table 3: Some marketed formulations of proteins based PLGA micro/nanooparticles [59]

Product name	Active ingredient	Company	Application
Lupron Depot®	Leuprolide acetate	TAP	Prostate cancer
Nutropin Depot®	Growth hormone	Genetech	Pediatric growth hormone deficiency
Suprecur® MP	Buserelin acetate	Aventis	Prostate cancer
Decapeptyl®	Triptorelin pamoate	Ferring	Prostate cancer
Sandostatin LAR® Depot	Octreotide acetate	Novartis	Acromegaly
Somatuline® LA	Lanreotide	Ipsen	Acromegaly
Trelstar™ Depot	Triptorelin pamoate	Pfizer	Prostate cancer
Arestin®	Minocycline	Orapharma	Periodontal disease
Risperidal® Consta™	Risperidone	Johnson & Johnson	Antipsychotic

1:3 (polymer to salt) [58]. The main difference between the emulsion diffusion and salting out method is that for the second one there is no solvent diffusion due to presence of salts. This is because, addition of pure water into the formed O/W emulsion under magnetic stirring reduced the ionic strength of salt. At the same time, the hydrophilic organic solvents migrate from the oil phase to the aqueous phase resulting in the formation of nanoparticles. Finally, the salting out agent is removed by centrifugation.

BIODEGRADABLE PLGA MICRO/ NANOPARTICLES AS PROTEIN DELIVERY SYSTEM

Various therapeutic peptides and proteins encapsulated PLGA micro and nanoparticles have recently received much attention for their application of sustained release over an extended period. Since this technology provides unique advantages over traditional delivery approaches (e.g., improved drug efficacy and patient compliance), several formulations of proteins based on biodegradable micro/nanoparticles have already been marketed, as shown in Table 3 [59]. Since proteins are hydrophilic, high molecular weight macromolecules, and unstable to various detrimental environments, low encapsulation efficiency, incomplete and erratic release profiles are the most common features of controlled release protein delivery system using PLGA [32]. Denatured or aggregated protein species will not only be therapeutically inactive, but also may cause unpredictable side effects, such as immunogenicity or toxicity [60]. In the past two decades, many strategies have been investigated for the sustained, complete and native form of protein release from PLGA based micro and nanoparticles.

Approaches to retain protein integrity in PLGA-based micro/nanoparticles

The presence of water/solvent interface is one of the major factors for decreased protein bioactivity occurring during the emulsion method. In this emulsion preparing step, protein is exposed to large extent of water/organic interface, resulting in protein aggregation. The extent of protein aggregation depends on the nature of solvents. Dichlorometane (DCM) induces more protein aggregation than ethyl acetate [61]. The extent of protein aggregation during emulsification also depends on the methods of emulsification. It has been found from the study on the effect of different emulsification methods that sonication and vortex mixing generated slightly increased aggregation than homogenization [62].

Role of viscous microenvironment in reducing protein denaturation

A variety of stabilizing interface-active excipients such as sugars (e.g., trehalose, sorbitol) [61,63], polyethylene glycol (PEG) [64] are added into the internal protein solution to protect protein from aggregation as well as denaturation during emulsification. These excipients either reduce the protein adsorption at the water/organic solvent interface by competitive adsorption from additives (PEG, carrier proteins), or to accumulate at the water/organic solvent interface, thereby shielding proteins from degrading environment [9].

Jintian *et al* [65] prepared recombinant human erythropoietin (rhEPO) loaded PLGA microspheres using human serum albumin (HSA) as a stabilizer by a modified S/O/W technique. They claimed that the integrity of rhEPO was protected during the encapsulation process and 33 days release period from the polymeric

matrices. Insulin encapsulated PLGA microspheres were prepared by a modified O/O solvent evaporation method [66]. In this single was involved, and high sheering mixers (homogenizer or sonicator/or freeze-drying techniques) were not applied.

Role of basic salts in reducing pH-induced protein denaturation

The degradation of biodegradable PLGA delivery matrices leads to generation of acidic oligomers (lactic/glycolic acids) which results in increase in acidity of microenvironment. This drop of pH in the microenvironment induces protein degradation. To overcome acidic microclimate within the device, basic salts such as $NaHCO_3$, $ZnCO_3$, $MgCO_3$, or $Mg(OH)_2$ has been found to be used as buffering agents into matrix to counteract acidic microenvironment. Zhu *et al* [67] reported that polymer microclimate has an acidic pH (less than 3) and it triggers unfolding of proteins like bovine serum albumin (BSA). To neutralize the acids liberated by the biodegradable lactic/glycolic acid polymers, they incorporated antacid $Mg(OH)_2$ which increased the microclimate pH and prevented bovine serum albumin (BSA) structural losses and aggregation for over 1 month.

Rafi *et al* [68] reported that incorporation of HSA and $NaHCO_3$ during particle formulation stabilized the recombinant human growth hormone (r-hGH) inside the PLGA microparticles. Three different zinc salts (i.e., zinc oxide, zinc carbonate, and zinc acetate) were incorporated in the preparation of insulin loaded PLGA microspheres [34]. Insulin secondary structure was unaltered due to the addition of zinc salts as compared to the formulation prepared without a zinc salt. They reported that formation of hexamers in presence of zinc salt was the major reason of improved insulin stability. In most cases, the buffering approach increased the stability of the released protein *in vitro*. Nevertheless, protein release profiles from PLGA microspheres were not fully controlled. So, the addition of other excipients or incorporation a salt in a different way is very important for maintaining protein stability and release kinetics.

Role of functionalized hydrophilic polymers in reducing protein denaturation

Due to the hydrophobic nature of PLGA, a slower release rate of recombinant human insulin-like growth factor-I (rhIGF) and of a somatostatin analogue have been observed [69,70]. So, the phase O/O method insulin instability problems were minimized as no aqueous organic interface

use of promising new polymers that are more hydrophilic than PLGA have emerged. These polymers are more compatible with proteins; especially since they reduce protein absorption and favour homogeneous distribution within the matrix. Moreover, they increase water uptake within the microspheres. To enable a better water uptake and an easier diffusion of the protein and of the polymer degradation products out of the polymer, porous microspheres were prepared by a range of groups.

Biodegradable PLA or PLGA have been co-dissolved with various biocompatible hydrophilic or amphiphilic compounds in the encapsulation procedure to prevent acidic microclimate-induced instability reactions of proteins in degrading polymers. For instance, PLGA was blended with pore-forming PEG in order to enhance the release of EPO and insulin [71,72]. But, a burst and a steady rate of protein release in vitro were observed over 1 month. Complete release was not reached due to the presence of protein aggregates. Ghassemi *et al* [73] recently formulated microspheres from a novel functionalized hydrophilic aliphatic polyester, PLHMGA for reducing the pH induced aggregation of therapeutic proteins. From the in vitro release studies, they reported that the release of lysozyme was incomplete, likely due to aggregation of part of the encapsulated protein.

ORAL DELIVERY FORMULATIONS OF PROTEIN/ PEPTIDE-LOADED PLGA NANOPARTICLES

For the treatment of Type 1 diabetic patients, three subcutaneous injections are required daily to maintain the blood glucose level. The daily injection routine is inconvenient for patients due to pain, tenderness, local tissue necrosis, microbial infection and fear of hypoglycemia. Oral delivery of insulin is expected to overcome these problems. For many years various unsuccessful approaches have been developed for oral delivery of insulin [74,75]. The reasons for failure of oral delivery of bioactive macromolecules include the acidic environment of the stomach, rapid enzymatic degradation and the poor intestinal absorption [76]. Recently, Zhi *et al* [77] developed a two-stage delivery system for oral delivery of insulin. The system composed of pH-sensitive hydroxypropyl methyl cellulose phthalate (HP 55) coated capsule containing insulin loaded PLGA and Eurdragit® RS (RS)

cationic nanoparticles. In this study, insulin was protected from rapid enzymatic degradation in gastrointestinal (GI) tract due to the coating of hard gelatin capsules by pH sensitive HP 55. Whereas, PLGA/RS cationic nanoparticles were adhered to the intestinal mucosa resulting in a improved absorption of insulin across the intestinal epithelial cells. In another study, Sharma *et al* [78] developed antacid-insulin co-encapsulated PLGA nanoparticles for oral delivery of insulin. They investigated both *in vitro*/*in vivo* studies of the prepared nanoparticles. From the *in vitro* test, they claimed that encapsulated insulin was well protected under simulated gastric and intestinal fluids. From the *in vivo* test, they showed that in diabetic rats, a 120 IU/Kg oral dose of insulin nanoparticles achieved an equivalent blood glucose lowering effect to a 20 IU/Kg subcutaneous dose of insulin solution. From this studies, they concluded that although oral delivery of insulin was achieved, further improvements is required to increase the C_{max} (maximum concentration attained in plasma) and to reduce the T_{max} (time to attain maximum plasma concentration) for successful oral delivery of protein drugs.

CONCLUSION

This review outlines the present research and development activities on PLGA and PLGA-based micro/nanoparticles as protein and peptide drug delivery devices. A deep understanding of the effects of fabrication methods, PLGA, surfactants and excipients nature is necessary for the formulation of protein loaded micro/nanoparticles with desired protein release profiles and structural integrity (i.e., bioactivity). Another important factor has to be considered in drug loading is protein encapsulation efficiency (EE). Since protein drugs are usually extremely expensive, achieving the high EE is very essential to reduce the loss of expensive drug. The method of making protein drugs stable for extended period of time in the body condition has to be incorporated into the micro/nanoparticle preparation method. All the above mentioned factors need to be understood for successful development of long-term protein delivery systems using biodegradable PLGA micro or nanoparticles. Although many advances have been made for formulation of successful protein delivery devices, much work still remains before proteins can be used as a therapeutic molecule in PLGA based drug delivery systems.

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