EXPERT OPINION

Recent advances in polymeric microspheres for parenteral drug delivery—part 2

Shirui Mao, Chunqiang Guo, Yi Shi & Luk Chiu Li[†]
[†]Animal Health, Abbott Laboratories, Abbott Park, IL, USA

Introduction: Currently marketed microsphere products are manufactured with the use of organic solvents which have a negative impact on the environment and stability of biological molecules. With recent advances in fabrication technologies, solvent free methods have demonstrated potential for the preparation of microspheres.
Areas covered: New technical advances recently achieved in solvent based microsphere manufacturing processes have allowed for major improvement in product quality and properties. Novel solvent free fabrication methods combined with newly functionalized biodegradable polymers have been explored for their application in the preparation of microspheres containing biological molecules.
Expert opinion: Novel fabrication methods for microspheres have been recently reported but technical challenges and development risks remain high for scale up from bench to industrial commercialization. While the applications of microspheres for delivery of proteins, genes and vaccines have shown promise for clinical use, the approval of newly functionalized polymers as carriers may still face scrutiny on safety and biocompatibility, which can be key factors in securing the regulatory approval of the product.

Keywords: applications of microspheres, delivery of biologics, microsphere fabrication, polymeric microspheres

Expert Opin. Drug Deliv. (2012) 9(10):1209-1223

1. Introduction

In Part-1 of this review, biodegradable polymers commonly used for microsphere fabrication were reviewed with new research findings. The impact of formulation variables on the properties of microspheres prepared by emulsion methods was discussed with results from recently reported studies. In vitro characterization of microspheres using more advanced physical techniques was introduced. In vitro and in vivo drug release data as well as in vitro to in vivo correlation (IVIVC) were discussed with examples showing positive correlation and the challenges in establishing IVIVC for microspheres were also addressed. In Part-2 of this review, recent advances in microsphere fabrication methods and new applications of microspheres for parenteral delivery of biological molecules are presented.

Conventionally, microspheres are matrix systems with a drug embedded or loaded in a biodegradable polymer. Phase separation, emulsion-solvent evaporation, and spray drying/freeze drying are commonly used methods for the commercial manufacture of microsphere products. In the phase separation method, a solution or dispersion of the drug in an organic solvent containing the dissolved polymer is prepared followed by the addition of a non-solvent leading to the formation of soft coacervated particles that are further hardened with the addition of a second non-solvent. After removal of all organic solvents, lyophilization is generally used to produce the final product.

informa healthcare

10.1517/17425247.2012.717926 © 2012 Informa UK, Ltd. ISSN 1742-5247

Article highlights.

- New equipment and novel process design have led to major improvements of conventional solvent based methods in fabricating microspheres with enhanced properties and performance.
- New solvent free methods have also expanded their manufacturing capability for microspheres containing biological molecules.
- Parenteral applications of polymeric microspheres for delivery of biological molecules such as proteins, DNA and vaccines have been extensively studied in preclinical and early clinical phases with promising results.

This box summarizes key points contained in the article.

Depending on the solubility of the drug in the organic solvent, a single emulsion or double emulsion method can be used to fabricate microspheres (**Figure 1**). In the single emulsion method, the drug and polymer are dissolved in an organic solvent. An oil-in-water (o/w) emulsion is formed by emulsifying the organic phase with the aqueous phase containing an emulsifier. Solid microspheres are formed by evaporating the organic solvent via sparging with sterile filtered nitrogen under vacuum at elevated temperature. They can also be formed by extracting the organic solvent using a second solvent that is miscible with the organic solvent, but does not dissolve the polymer. Lyophilization or filtration followed by drying can be used to produce the finished product. The double emulsion method is generally applied to water soluble drugs such as peptides. A primary water-in-oil (w_1/o) emulsion is first formed by emulsifying an aqueous internal phase containing the drug in an organic external phase containing a dissolved polymer. A water-in-oil-in-water (w_1/o/w_2) double emulsion is formed when the primary w_1/o is further emulsified in a secondary aqueous phase. Solid microspheres are obtained by removing the organic phase and can be further processed into the finished product using processes similar to those used in the single emulsion method.

In a spray drying process, the bulk drug is either dissolved or dispersed in an organic solvent containing the dissolved polymer. The resultant solution or dispersion is spray dried to form solid microspheres which are collected as a dry powder and filled into final containers. Spray freeze drying was successfully developed for the manufacture of a microsphere product of growth hormone [1]. In this case, a dispersion of the lyophilized protein is prepared in an organic solvent (i.e., methylene chloride) containing the dissolved polymer and sprayed into liquid nitrogen to form frozen solid microspheres. A second organic solvent (i.e., ethanol) is used to extract the organic solvent from the microspheres which are subsequently filtered, dried, and filled as a powder in the final containers.

In recent years, improvements in existing manufacturing processes have been reported and new processes with novel equipment design also have been developed. These technical advances are introduced to produce microspheres with improved product qualities such as more uniform particle-size distribution with better drug release control and enhanced drug encapsulation efficiency. In addition, new methods have been reported allowing the encapsulation and stability preservation of macromolecules such as proteins, genes, and vaccines in polymeric microspheres.

2. Microsphere fabrication

2.1 Advances in emulsion methods

2.1.1 Precision particle fabrication and ink-jet technology

The fabrication of microspheres with uniform size distribution using an emulsion-solvent evaporation method coupled with piezoelectric or acoustic excitation was reported [2-4].-**Figure 2** shows the design of a precision particle fabricator consisted of a specially designed dual-nozzle and an acoustic excitation device [5]. An organic phase containing the polymer and drug is pumped through the inner nozzle, while a carrier stream (aqueous phase) is flowed around the organic phase via the outer nozzle. The liquid stream is acoustically excited using an ultrasonic transducer controlled by a frequency generator. The size and morphology of the microspheres can be varied by controlling the process parameters such as the carrier phase composition, flow rates of the organic phase and the carrier phase, and the frequency and amplitude of vibration. Core-shell and double-wall microspheres can also be prepared with this method using a tri-nozzle setup, where the inner nozzle feeds the core, typically containing the drug, the middle nozzle delivers the polymeric phase, and the outer nozzle provides the carrier stream, which aids in particle formation and size control [6-8]. Uniform microspheres with particle size in the range of ~ 5 to 500 μm were successfully prepared.

The use of piezoelectric or acoustic excitation to generate uniform sized droplets from a liquid jet has been widely applied as the basic operation principle of ink-jet technology which has also found broad applications in the biomedical fields [9]. The SphereJet system developed by MicroFab offers drop-on-demand, pressure assisted drop-on-demand, and continuous ink-jet drop formation features and is capable of generating microspheres at rates of up to 50 kHz and with particle size of 20 μm to 100 μm. The technology has proven effective in producing paclitaxel-loaded monodisperse microspheres for localized chemotherapy delivery [10]. MicroFab also reported a system allowing the production of hollow microspheres [11]. The system consists of two concentric tubes. The outer tube is used to inject the liquid that forms the outer shell while air is injected in the inner tube. Air can be replaced with a second fluid thus generating the multilayer spheres.

2.1.2 Microsieve technology

The microsieve technology [12] developed by Nanomi allows for the production of monodispersed microspheres in a large scale. Microsieves are silicone-based membranes with uniform

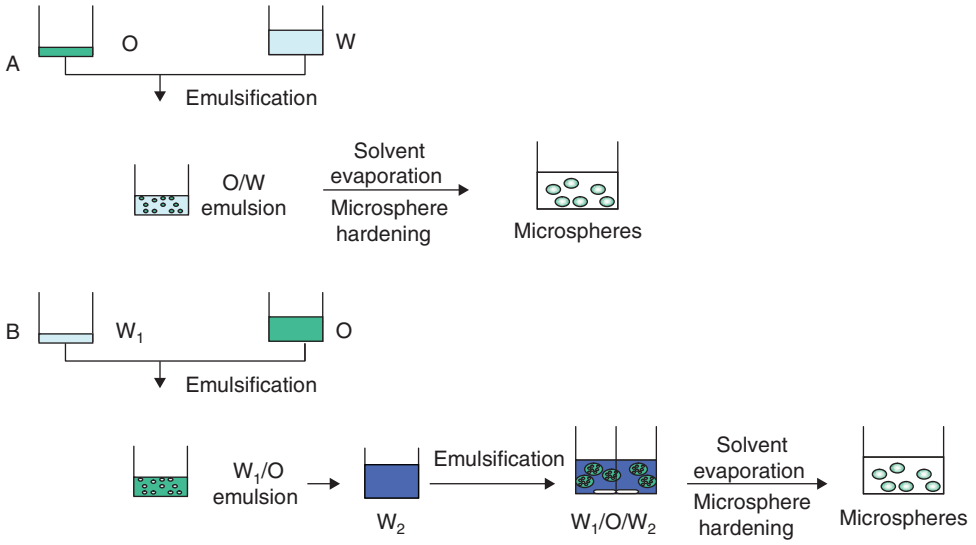

Figure 1. Schematic illustration of conventional emulsion methods for microsphere fabrication. (A) single oil-in-water (O/W) emulsion method; (B) double water-in-oil-in-water (W1/O/W2) emulsion method.

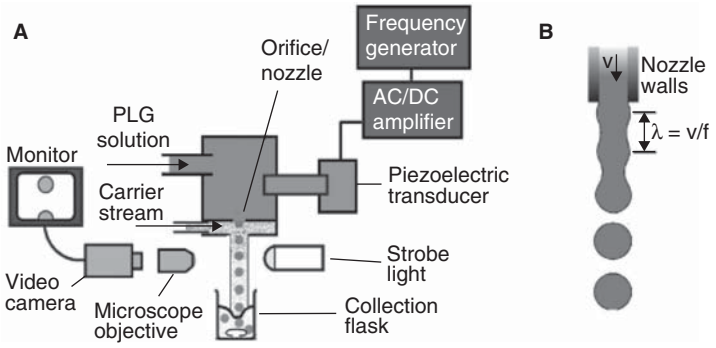

Figure 2. Schematic of the Precision Particle Fabricator for production of narrow sizedistribution of microparticles.
Reproduced from [5] with permission of Elsevier © 2001.
PLG: Poly(lactide-co-glycolide).

pore size and shape, which are fabricated using a photolithographic technique widely utilized in the semiconductor industry. A monodispersed emulsion is generated by forcing an organic solvent containing the dissolved polymer and drug through the microsieve into an aqueous solution. The droplet size of the emulsion is solely controlled by the membrane and independent of the formulation. The solvent is subsequently removed by evaporation resulting in uniformly sized solid microspheres. The process is capable of producing microspheres in the size range of 1-50 µm. It is also compatible with both single and double emulsion methods for microsphere manufacture. Using this process, monodispersed microspheres of goserelin acetate and insulin growth factor-1 (IGF-1) were prepared respectively for in vitro characterization including drug release evaluation [13,14]. The microsieve emulsification process is readily scaled up by increasing the number and surface area of the membranes. The technology also allows continuous and close operations which make aseptic processing feasible.

2.1.3 *Microfluidics*

Microfluidic technologies have attracted great interests for preparing microspheres because of their capability of generating monodispersed droplets [15]. A microfluidic device was used for producing narrow size ranged polymeric microspheres for controlled drug delivery [16]. The microfluidic channels composed of polydimethylsiloxane (PMDS) were fabricated using soft lithograph and sealed against an oxidized glass slide. An example of a microfluidic device is shown in **Figure 3**. The channels were filled with an aqueous solution of PVA. Both the aqueous phase and the organic phase containing the polymer and drug were delivered to the microfluidic device at

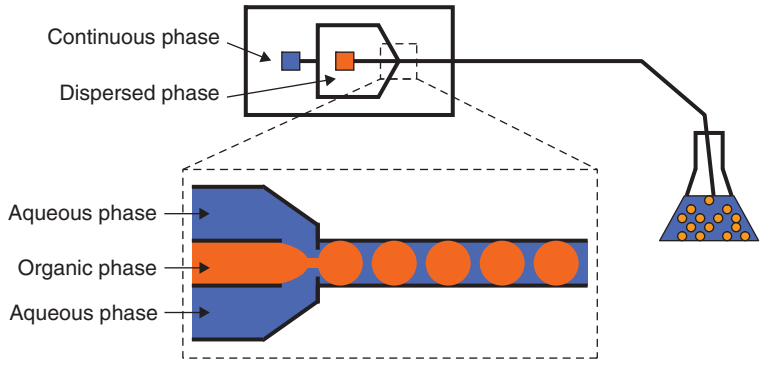

Figure 3. Schematic illustration of microfluidic flow-focusing device for preparation of monodisperse polymer microparticles.

a constant rate of flow by a digitally controlled syringe pump. The aqueous phase was introduced into the two side channels and the organic phase was directed into the central channel of the device and broken up at the junction of three inlets to generate monodispersed emulsion droplets which were collected at the outlet of the device (**Figure 3**). After removal of the organic solvent by evaporation under reduced pressures, solid microspheres were obtained. Microspheres with different sizes were produced by varying the flow rate of each liquid stream.

Using microfluidic technology, Q Chip has developed a platform technology called Q-Sphera for the manufacture of monodispersed microspheres [17,18]. Unlike traditional emulsion/solvent evaporation process, the production of microspheres using Q-Sphera is at ambient temperature and only Class-III organic solvents (e.g., dimethyl sulfoxide) and water are used. The mild processing conditions make this technology more suitable for manufacture of microspheres containing biopharmaceuticals. The individual process time of a microsphere from the formation of a droplet to a solid microsphere is less than 10 sec. Due to the short process time, high drug entrapment efficiency (more than 90%) with final drug loading up to 15% in the microspheres can be achieved. The technology permits aseptic production of microspheres and can be scale up production with a throughput of 20,000 microspheres per second.

2.1.4 *Porous microsphere formation*

The formation of PLGA microspheres with a porous core and nonporous surface was reported by Han et al. [19] using hydrogen peroxide as an effervescent agent in a double emulsion method. Hydrogen peroxide is added in the internal aqueous phase of the primary emulsion. When the double emulsion is formed, a catalytic enzyme, catalase was added and the entrapped hydrogen peroxide decomposed to form water and oxygen creating an internal porous structure within the microspheres when the microspheres were hardened upon evaporation of the solvent. Using dexamethasone as a model drug, microspheres with a porous core and non-porous surface were prepared showing very low initial drug burst and one-month in vitro sustained drug release.

2.2 Advances in spray drying

Microspheres produced by spray drying usually exhibit a broad particle-size distribution. The relatively high process temperature also makes spray drying less desirable for thermally unstable biological molecules such as proteins. The recent advances in spray drying technology mainly relate to better particle size control, improved process conditions suitable for biological molecules and high product yield. The use of additives to protect proteins from denaturation during spray drying has been reported. Park et al. [20] demonstrated that polyethylene glycol (PEG) was able to solubilize BSA (bovine serum albumin) and rhGH (recombinant human growth hormone) in methylene chloride solution of PLGA by forming complexes and protect the integrity of proteins during spray drying. The spray dried PLGA microspheres showed no significant aggregation and structural changes of the encapsulated protein.

A monodisperse spray drying technology that combines conventional spray drying with novel microsieve nozzle technology has been introduced by Nanomi [21]. The technology allows the production of well-defined micro- or nanoparticles with predictable and uniform particle size. Nanomi's data show that PLGA and maltodextrin microparticles produced by the monodisperse spray drying technology have significantly narrower particle size distribution (span < 0.6) than those produced by conventional spray drying (typical span ~ 1.5). Significant losses of product during spray drying of a small sample size are very common using conventional spray drying technology. Van der Gucht [22] reported a new lab scale micro-spray dryer (ProCept) that is capable of spray drying small samples with very high yields (90% for 0.25 mL). The micro-spray dryer utilizes laminar air flow to minimize product losses and ultrasonic nozzles to create a uniform spray resulting in microspheres with a narrow size distribution. The use of the micro-spray dryer for preparing PLG microspheres has been demonstrated [23]. Specially designed nozzle has also been developed to expand the manufacturing capability of spray dryers; a three-fluid nozzle was used with a spray dryer (Buchi, Mini spray dryer B-290) for generating polymer coated microparticles of lysozyme [24].

2.3 Other new technologies

2.3.1 *Electrospray*

Electrospray is a process utilizing a high electrostatic force (Coulomb force) created within a liquid in an electric field to break the liquid into fine charged droplets. The application of electrospray in fabrication of drug loaded nanoparticles or microspheres has generated great interest recently [25-27]. The technique allows better particle size control, high recovery, and mild processing conditions. An electrospray setup includes a liquid delivery system (e.g., pump), a tip with high electric potential, a collector which is grounded and placed a short distance from the tip. This technique not only can be used to prepare drug-loaded microspheres with a matrix structure, but also microspheres with a co-shell structure. Wang et al. [27] reported the use of a co-axial electrospray process to encapsulate protein drugs in polymeric microspheres, where a protein aqueous solution was introduced in the inner capillary of a co-axial nozzle and an immiscible polymer solution was fed through a larger outer capillary of the nozzle.

2.3.2 *Supercritical fluid technology*

Supercritical carbon dioxide ($scCO_2$) with its unique ability to plasticize polymers and diffuse through solids has served as an excellent alternative to organic solvents in the fabrication of microspheres containing biological molecules. Furthermore, its low critical point (31.1°C at 73.8 bar) makes it a very attractive processing medium for heat-labile drugs [28]. Rapid expansion of supercritical solutions (RESS) process is an organic solvent-free method that has been used to produce microspheres for drug delivery applications [29-31]. The RESS process uses $scCO_2$ to plasticize polymers (e.g., PLGA or PLA) allowing effective incorporation of solid drug particles into the liquefied polymer at near ambient temperature without the use of an organic solvent. The subsequent spraying of the drug polymer mixture through a nozzle results in rapid expansion of the CO_2 and formation of drug loaded microspheres.

Supercritical carbon dioxide can also be used as an antisolvent for the preparation of microspheres [32,33]. In a typical process with $scCO_2$ as an antisolvent, the polymer and drug are dissolved in an organic solvent followed by atomizing the solution through a nozzle into a vessel containing $scCO_2$. Rapid extraction of the organic solvent into $scCO_2$ causes precipitation and formation of drug-loaded microspheres. Greater drug entrapment is feasible with this method. Various ways of introducing the solution into the supercritical fluid have been explored, including the use of an ultrasonic component to the spray to produce monodisperse particles [34] and ultrasonic vibration [35] to produce smaller particles leading to increased mass transfer rate between the solvent and the $scCO_2$. Supercritical fluid processes have been successfully used to encapsulate both small molecules and macromolecules such as paclitaxel [36], insulin, lovastatin, and human growth hormone (hGH) in polymeric microspheres.

2.3.3 *Soft lithography-based techniques*

Soft lithography-based techniques [37,38] offer many attractive features for fabrication of microspheres with a highly uniform size, precisely well-defined structures, surface functionality, and mechanical characteristics. The technology uses an elastomeric stamp (e.g., PDMS) with topological microfeatures to generate nano or microspheres. The process typically involves coating the elastomeric stamp with a polymer which is then transferred to a substrate. The substrate is coated with a sacrificial layer (release layer) to release the microspheres from the substrate (**Figure 4**) [39]. Particle Replication in Non-wetting Templates (PRINT) technology developed by Liquidia [40,41] uses a perfluoropolyether (PFPE)-based mold and has demonstrated the feasibility of manufacturing polymeric microspheres with desired geometry and size ranging from nanometer to several hundred microns in size. Gelatin has also been proposed to be used as a template [42]. Its sol-gel transition with temperature allows efficient production of template. The good aqueous solubility of gelatin also makes particle collection easier by simply dissolving the template in aqueous solutions. The gelatin template can be used to make microspheres ranging from 200 nm to > 50 µm. Park et al. has demonstrated microspheres prepared by the gelatin template with drug loading capacity higher than 50% and low initial drug burst release [42].

2.3.4 *Laser-induced forward transfer*

Pulsed laser writing is a relatively new technique with growing focus in microsphere fabrication. Recently, Lin and Huang reported successful production of sodium alginate microspheres using a metallic foil modified laser-induced forward transfer (LIFT) technique (**Figure 5**) [43]. Modified LIFT is a nozzle free laser jetting-based method which incorporates a metallic foil to assist the formation of highly viscous microspheres. A laser pulse is directed perpendicularly through the backside of a quartz disk-based ribbon while the front side is coated with materials to be transferred and a possible sacrificial energy absorption layer. The laser pulse energy is absorbed by the sacrificial layer leading to the formation of a vapor/plasma bubble which releases and ejects the remaining underneath coating as a microsphere toward a receiving substrate underneath. The ability to produce microspheres from viscous materials (e.g., alginate) without the shearing force associated with nozzle-based processes has made the metallic foil modified LIFT uniquely advantageous over other nozzle-based jetting technologies.

2.3.5 *Solvent-free methods*

The fabrication of nano or microspheres with solvent-free methods including the melting ultrasonic dispersion and melting emulsion processes was reported by Li et al. [44]. In the melting ultrasonic dispersion process, a drug was dissolved or dispersed in a polymer at a temperature above the melting point of the polymer. The mixture was then ultrasonicated in

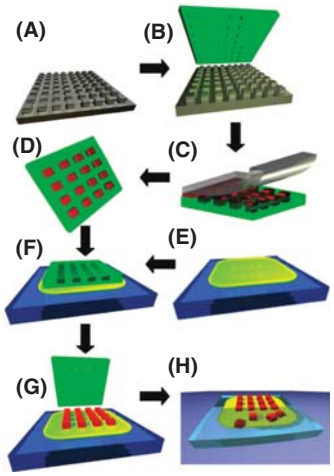

Figure 4. Schematic representation of soft lithography technology PRINT process of protein particles. Silicon master template (A); mold (green) release from master template (B); nanomolding via capillary fill (protein solution red) with countersheet having a higher surface energy than the PFPE mold (C); filled mold lyophilized (D); glass slide (blue) with harvest film (yellow) (E); filled mold rolled onto harvest film (F); mold release from array of isolated features (G); dissolution of the harvesting film to yield free particles (H).
Reprinted with permission from [39]. Copyright 2008. American Chemical Society.

a dispersion medium (i.e., ethanol) to form a colloid dispersion of molten polymer nanoparticles which was subsequently added to an ice bath to obtain solidified drug encapsulated nanoparticles. In the melting emulsion method, a drug and polymer melt was immersed in hot water and extruded through a membrane filter to form a homogenous emulsion with molten polymer microspheres. This was further added into an ice bath to obtain solidified drug-loaded microspheres. These methods eliminated the use of harsh organic solvent which could lead to denaturation of proteins. However, they are not applicable to most of the commonly used conventional polymers such as polylactide and polycaprolactone because of their relatively high viscosity at melting temperature. For this purpose, a family of cholic acid functionalized branched polycaprolactones was developed by Li and coworkers.

2.4 Interfacial polymerization

Polymerization has been a widely used industrial process for manufacturing of microspheres. However, because of the safety concern of the residual monomers in the product and the costly steps for their removal, polymerization has not been established as a pharmaceutical process for microsphere fabrication. In light of the recent vast interest in using microspheres for delivery of biological molecules, polymerization methods have gained increasing attention in pharmaceutical research. Interfacial polymerization involves the reaction of monomers at the interfaces between two immiscible liquid phases forming microcapsules and is commonly carried out in a suspension or an emulsion. In emulsion polymerization [45], the monomer and initiator are in different phases. Emulsion polymerization requires the formation of micelles in an aqueous solution, which poses problems with actual encapsulation of the drug if it is not trapped with the polymer inside the micelle. It is also limited to water-insoluble drugs.

In suspension polymerization, monomer and initiator should be both miscible and in the same phase of a two-phase system. The size of particles formed by suspension polymerization is determined by the droplet size of the polymer-drug mixture which is added to an immiscible dispersion medium. This method also requires mechanical agitation for the formation of microspheres. Qiu et al. [46] reported a facile method for the preparation of microparticles with a highly folded surface via a suspension polymerization process, where a mixture of styrene, divinylbenzene, toluene and water were mixed with 10% HSMA (copolymer: styrene-alt-maleic anhydride hydrolyzed in 3% sodium hydroxide solution) by stirring at 13K rpm for 2 min. Polymerization was conducted by heating the resulting dispersion to 70°C. Seeded dispersion polymerization has also been employed to produce monodispersed microspheres [47]. In this method, a polymer seed mixture is swollen by addition of water or an appropriate solvent and polymerization of monomer takes place in the swollen seeds. Murphy et al. [48] reported their work on preparing hydrogel microspheres via a pH-induced protein aggregation (PIPA) polymerization process. Protein aggregation could be modulated by varying pH around its isoelectric point (pI). These protein aggregations could then serve as nucleation centers for microsphere polymerization. Using protein calmodulin (CaM) as a model compound, the authors have demonstrated formation of PEG-CaM-PEG microspheres with an approximate particle size of 2 μm and narrow particle size distribution at pH (3.5 to 5.2) which is close to the pI of CaM (4.2). Since this approach does not require the use of an organic phase and surfactant, it could be especially useful for the preparation of protein based hydrogel microspheres.

3. Applications

The commercialization of microspheres for parenteral drug delivery has been relatively slow as evidenced by a smaller number of marketed product launches in recent years. However, the advance in biotechnology has led to more well documented research and clinical trials in the application of microspheres to parenteral delivery of biological molecules. Some references on the applications of microspheres for parenteral drug delivery have been provided in the previous discussion on the use of different types of polymers for microsphere fabrication. Additional references will be provided in the sections below.

3.1 Protein delivery

A clinical Phase I–IIa study of gelatin hydrogel microspheres has been reported to deliver basic fibroblast growth factor

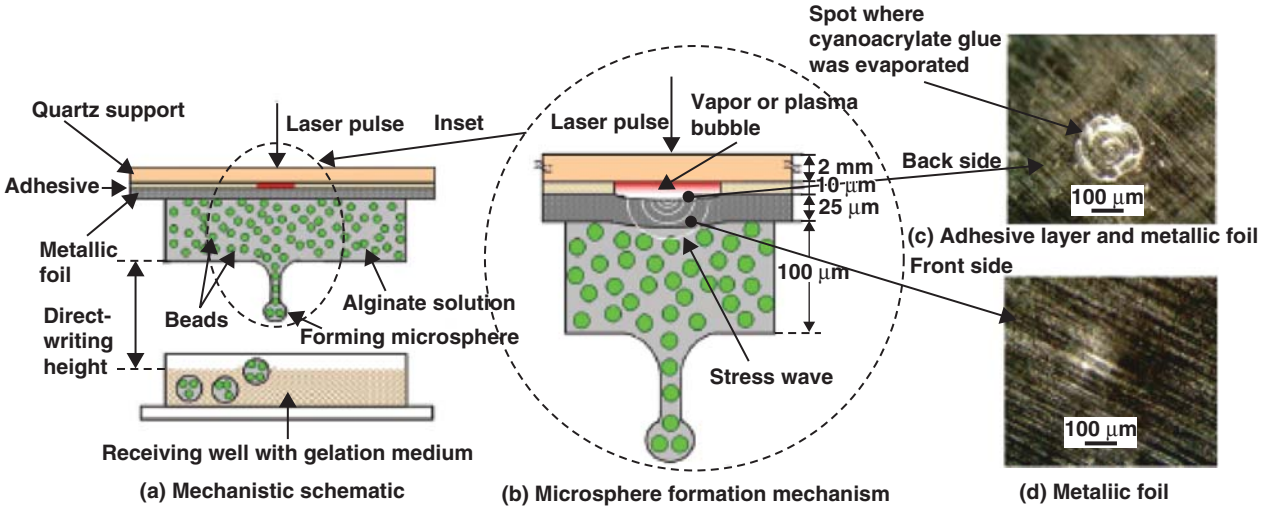

Figure 5. Schematic illustration of metallic foil-assisted LIFT process.
Reprinted with permission from [43]. Copyright 2011. American Institute of Physics.

(bFGF) for the treatment of critical limb ischemia by IM injection of microspheres into the gastrocnemius of the ischemic limb [49]. The same microsphere formulation was also used in a Phase-I trial on patients diagnosed with peripheral arterial disease [50]. Locteron™ is a poly(ether-ester) microsphere formulation of recombinant interferon-alpha2b (IFN-alpha2b), which was evaluated in a Phase-I clinical trial for hepatitis C (HCV) treatment [51]. In another clinical trial in HCV patients, treatment with Locteron™ once every 2 weeks in combination with ribavirin demonstrated rapid antiviral efficacy and 63% less flu-like symptoms over 4 weeks than control group [52].

Biodegradable poly(ether-ester) polymer (Polyactive™) is a block copolymer of poly(ethylene glycol terephthalate) (PEGT) and poly(butylene terephthalate) (PBT) [53]. PEGT/PBT microspheres have been investigated for protein delivery of lysozyme [54]. PLGA microspheres loaded with mono-PEGylated growth hormone-releasing peptide-6 (GHRP-6) showed 1-month sustained release with lower initial burst compared to native GHRP-6 microspheres. The release rate was dependent on the molecular weight of PEG used for pegylation; a faster release was shown with PEG of a low molecular weight [55].

In addition to the biodegradable polymers, other components can be incorporated into the microsphere matrix for stability enhancement and encapsulation efficiency improvement. Glycol chitosan was incorporated in PLGA microspheres as a protein stabilizer for lysozyme delivery [56]. Similarly, sucrose acetate isobutyrate (SAIB) was embedded into PLGA microspheres for protein delivery and resulted in high encapsulation and prolonged release for up to two months [57]. Human serum albumin (HSA) and poly-L-lysine (PK) were used to protect erythropoietin (EPO) and OVA in PLGA microparticles [58]. Protein-HAS/PK complexes were formed and encapsulated into PLGA matrix by w/o/w emulsion method. The preparation resulted in high loading efficiency, improved protein integrity, and sustained release. Ho et al. developed PLGA microspheres containing hydroxyapatite and BSA for controlled release [59]. PLGA microspheres were prepared by a w/o/w emulsion method in the presence of BSA-adsorbed hydroxyapatite. The encapsulation efficiency reached above 90% due to the enhanced absorption capacity. The inclusion of alkaline hydroxyapatite significantly reduced the initial burst effect and prolonged the release of protein from the microspheres for at least 3 weeks. In addition, the alkaline hydroxyapatite was able to neutralize the acidic degradation products generated by PLGA polymer.

As a formulation strategy, surface modification of microspheres by crosslinking or coating has been used to reduce the initial burst effect and achieve controlled release of proteins. Chan et al. prepared novel collagen microspheres for protein delivery, which were modified by photochemical crosslinking [60]. Solorio et al. reported the encapsulation of bone morphogenetic protein 2 (BMP2) in gelatin microspheres for local delivery, which were prepared by the emulsion method followed by crosslinking with genipin [61]. Sustained release of vascular endothelial growth factor (VEGF) from alginate microparticles formed by ionic crosslinking was evaluated [62]. Investigation on the use of silk-coated PLGA and alginate microspheres for protein delivery was also reported [63].

Composite microparticles typically consisting of protein-loaded particles dispersed in another type of microparticles were developed for improved performance. Yu et al. proposed a novel composite alginate/poly(lactic-co-glycolic) acid microparticulate system for stabilization and controlled release of bovine insulin [64]. In this study, alginate hydrogel particles loaded with bovine insulin, prepared by ionic gelation, were

embedded into PLGA microparticles by emulsification and solvent evaporation. The insulin-loaded composite microparticles showed improved encapsulation efficiency and prolonged drug release (4 month) compared to conventional microparticles. Composite microspheres composed of protein-loaded dextran glassy microparticles dispersed in PLGA microspheres were prepared by a solid-in-oil-in-oil-in-water ($s/o_1/o_2/w$) multi-emulsion method [65]. The stability of protein was preserved in the dextran glassy microparticles in the PLGA matrix and complete release of protein in a controlled release manner was achieved [65].

Temperature-responsive microspheres have been designed to release encapsulated proteins at variable rate in response to environmental temperature. Lee et al. fabricated temperature-responsive biodegradable microspheres using poly(epsilon-caprolactone)-Pluronic diblock copolymers [66]. Proteins were loaded into microspheres by a w/o/w emulsion method with a porous structure formed due to presence of hydrophilic Pluronic blocks. An in vitro release study showed that the release rate of proteins was slow at 20°C but faster at 37°C. In addition, the release rate of protein could be modified by changing the length of poly(epsilon-caprolactone) blocks in the copolymer [66].

Dynamic hydrogel microspheres have been investigated for ligand-induced release of encapsulated vascular endothelial growth factor (VEGF) [67]. PEG-CaM-PEG conjugates were synthesized by reacting calmodulin (CaM), a dynamic protein, with poly(ethylene glycol)(PEG) diacrylate. PEG-CaM-PEG microspheres were prepared using w/o/w emulsion polymerization. VEGF was loaded into PEG-CaM-PEG microspheres by incubation. The microspheres underwent significant volume decrease (48.7%) in the presence of a specific ligand, trifluoperazine (TFP), which induced calmodulin's nanometer scale conformation change. The release of VEGF was triggered by the ligand-induced volume decrease of microspheres, which increased the internal fluid pressure and convective fluid flow out of the microparticles. This study suggested protein conformational change could be a useful mechanism to control drug release from microspheres.

Glucose-responsive microparticles were developed for self-regulated insulin delivery [68]. The microparticles were fabricated by coupling concanavalin A (Con A) and dextran on the surface of chitosan microparticles. High entrapment efficiency was achieved for insulin (92.2%). Con A has strong affinity for sugar binding including chitosan, dextran, and glucose. Glucose would competitively bind to Con A and disrupt the reversible linkage between Con A and chitosan/dextran leading to the release of insulin. The encapsulated insulin had a high release rate in the presence of glucose at 4 mg/mL whereas the release rate was low in the absence of glucose. When glucose concentration varied stepwise (0 mg/mL to 4 mg/mL to 0 mg/mL), pulsatile insulin release pattern was observed.

3.2 Gene delivery

There have been many clinical trials and extensive preclinical research activities on gene therapies in the past decade. In general, cellular entry of exogenous genetic material requires effective delivery systems which enable DNA or RNA to overcome various physiological barriers (extracellular and intracellular) to reach the final target. Controlled intracellular delivery systems based on microspheres have been widely pursued to meet the challenges of gene therapy.

3.2.1 *Unmodified microspheres*

Microspheres formulated with biodegradable polymers have been reported for local delivery of gene therapeutics for certain diseases. PLGA microparticles were prepared to deliver oxytocin receptor (OTR) antisense oligodeoxynucleotides (ODN) for social recognition inhibition in mice [69]. The sustained release of antisense ODN in mouse brain after intra-amygdala injection allowed for behavioral testing several days after injection without obvious toxicity. PLGA microparticles were also used to deliver plasmid-encoding interleukin-10 (IL-10) to alleviate neuropathic pain [70]. A single intrathecal administration of microparticles in rat model induced phagocytic cell recruitment and relieved neuropathic pain for more than 74 days. De Stefano et al. designed a PLGA microsphere formulation loaded with a decoy ODN against NF-κB for chronic inflammation in rats [71]. Subcutaneous injection of decoy ODN-loaded microspheres inhibited leukocyte infiltration and formation of granulation tissue for 15 days in a rat chronic inflammation model, whereas the effect of naked decoy ODN only lasted 5 days. Chitosan microparticles were prepared to deliver PEDF (pigment epithelium-derived factor) plasmid for treatment of osteosarcoma in mice [72]. The injection of PEDF-loaded chitosan microparticles inhibited primary tumor growth, reduced bone degradation (osteolysis), and decreased lung metastases without gross toxicity.

3.2.2 *Cationized microspheres*

Research has been reported on functionalization of microspheres by inclusion of additional components in the formulations. Microsphere cationization strategy has been widely used to achieve intracellular gene delivery. Various natural or synthetic cationic molecules have been used for fabrication of microspheres, including poly(ethyleneimine) (PEI) [73-75], polyamidoamine (PAMAM) dendrimers [76-78], cationic gelatin [79,80], poly (β-amino ester)s [81], and chitosan [82]. The cytotoxicity of cationic molecules is usually high but can be reduced by incorporation into microspheres. Since genetic materials such as DNA, RNA, or oligonucleotide are negatively charged, the incorporation of cationic molecules in microspheres can result in condensing of genetic materials by electrostatic interaction. Upon cellular internalization, the cationic molecules protect the delivered genes from endolysosomal degradation, presumably via proton sponge mechanism and increase of osmotic pressure [83]. After endolysosomal escape, effective gene therapy also depends on successful nuclear entry of delivered genes.

Davies et al. investigated the use of cationic PLGA microspheres modified by PEI for DNA delivery [73]. PEI-modified microspheres with various size (1.5-4 μm) and capable of

binding DNA to microsphere surfaces were prepared by the oil-in-water (o/w) emulsion-solvent evaporation method. It was found that the surface PEI content and DNA loading capacity increased with higher molecular weight and concentration of PEI used during preparation. An in vitro cell culture study confirmed the microsphere uptake and cellular localization of adsorbed DNA.

Plasmid DNA -loaded PLGA/PEI microspheres were prepared by using three different double emulsion methods: plasmid DNA was entrapped into PLGA/PEI blends, complexed with PEI prior to loading into a PLGA matrix, or adsorbed to PEI-conjugated PLGA microparticles [74]. All microsphere formulations demonstrated strong DNA binding capacity and high cellular uptake in vitro compared to unmodified PLGA microparticles. However, PLGA microspheres prepared by PEI-plasmid DNA (pDNA) complexation prior to entrapment were shown to be the best formulation in terms of pDNA loading and transfection efficiency. The attachment of DNA/PEI molecules on PLGA microparticles can also be achieved by the layer-by-layer (LBL) deposition technique [75]. PLGA microparticles were coated with PEI to form the first layer on the surface, and then DNA as the second layer. The same layering process was continued until formation of a desired number of layers on microparticles. The cellular uptake and transfection activity of the resultant microparticles increased in a dose-dependent manner in J774.1 murine macrophages [75].

Polyamidoamine (PAMAM) dendrimers, acting as transfection agents in gene delivery, are cationic spherical polymers with amino groups on the surface. Intra et al. evaluated PLGA microparticles loaded with PAMAM-pDNA complexes (dendriplexes) as a non-viral gene delivery system [76]. Plasmid DNA was complexed with PAMAM prior to encapsulation into PLGA microparticles by an emulsion method. The use of PAMAM enhanced DNA loading, reduced particle size, and provided positive surface charge compared to PLGA microparticles loaded with pDNA alone. This study showed PAMA-pDNA encapsulated PLGA microparticles significantly increased transfection efficiency and transgene expression in mammalian cells with low toxicity.

A novel approach was reported for the preparation of dendrimer/DNA complexes encapsulated in biodegradable polymer microspheres [77]. PAMAM/DNA complexes were first incorporated into a poly(DL-lactide) film with poly-alpha,beta-[N-(2-hydroxyethyl)-L-aspartamide] (PHEA) added as a stabilizer. Ultrasonication was later applied to the PAMAM/DNA complexes loaded polymer film leading to the formation of microspheres. The presence of PHEA in the microspheres resulted in effective gene expression in HEK293 cells. PLGA microspheres with surface-conjugated PAMAM dendrimers were prepared for gene delivery [78]. Cationic PLGA microparticles were obtained by conjugating various generations of PAMAM dendrimers (G3-G6) to the surface of blank PLGA microparticles. Enhanced gene expression was shown by the surface modified microparticles and increase in the generation level of PAMAN beyond G6 did not improved gene expression.

Cationic gelatin microspheres incorporated with PTEN plasmid DNA were developed to sensitize prostate cancer cells for irradiation [79]. Local administration of metalloproteinase-1 (MMP-1) plasmid DNA encapsulated in cationic gelatin microspheres was reported to prevent the progression of myocardial fibrosis and was shown to improve cardiac function in a rat model [80]. In these studies, gelatin was cationized by adding ethylenediamine and the resultant cationic gelatin microspheres were funtionalized to complex DNA via ionic interaction. Acid-degradable cationic dextran microparticles prepared by spermine-modified acetalated dextran have been reported for delivery of siRNA therapeutics [83]. Tertiary-amine-containing poly(β-amino ester) polymer (PBAE) has been evaluated to enhance transfection in PLGA microspheres [81]. Fucoidan, a polysaccharide of sulfated polyfucose extracted from brown seaweeds was recently used to developed microspheres (fucospheres) for delivery of plasmid DNA encoding human GM-CSF [82]. Fucosphere is prepared through polyion complexation of negatively charged fucoidan and positively charged chitosan.

3.2.3 *Other surface modified microspheres*

Besides cationization of microspheres, there are other approaches which can be used to modify microspheres for improved gene delivery efficiency. Microsphere surface modification by attaching ligands, proteins or peptides which have specific affinity to cells, has been reported for gene delivery with increased intracellular uptake [84,85]. Ligand-grafted PLGA microspheres were prepared and shown to achieved enhanced cellular uptake by phagocytosis in pig alveolar macrophages [84]. Bradley et al. developed streptavidin-conjugated polystyrene microspheres to deliver biotinylated DNA into mammalian cells [85]. The high affinity of the biotin–streptavidin interaction allowed the binding of DNA to streptavidin-conjugated microspheres which were shown to attain successful intracellular delivery of DNA. In addition, chemical surface modification of microspheres can be achieved via covalent conjugation, which may improve the property of microspheres. For instance, PEGylated microspheres for siRNA delivery significantly silenced the expression (approximately 90%) of enhanced green fluorescent protein (EGFP) compared to commercial lipofection products [86].

3.3 Vaccine delivery

Microspheres have been widely reported as vaccine delivery systems for a variety of antigens including protein, peptide, DNA, toxin and virus [29,87,88]. A noticeable benefit of microspheres for vaccine delivery is the intrinsic adjuvant effect to induce humoral and cellular immune responses for the enhancement of antigens with poor immunogenicity [89,90]. The adjuvant effect of microspheres has been shown to be superior to conventional adjuvants like aluminum salts [91]. As a delivery system, the controlled release microspheres provide long-lasting immunity and reduce administration frequency—typically multiple times over months. Microspheres also have the capacity to incorporate

and protect multiple antigens or immunostimulatory agents. Furthermore, microparticle-based vaccines are also considered tunable systems in that the physico-chemical properties of microparticles including composition, particle size, charge, and surface characteristics can be modified for optimal immune responses against a particular disease [89,92-94]. Microparticles less than 10 μm have been reported to specifically target antigen presenting cells (APC) upon phagocytosis to improve immunity and reduce side effects with the cellular and molecular mechanisms being investigated [95-98].

PLGA microparticles containing ZYC300, a plasmid DNA of CYP1B1 have been studied in human clinical trials for cancer treatment [99]. CYP1B1 is a universal tumor antigen overexpressed in almost all human tumors with rare expression in normal tissues. In the first human Phase-I clinical trial [100], ZYC300 was administered intramuscularly to treat seventeen patients with advanced stage progressive cancer. Six patients developed T-cell mediated immune response to CYP1B1 and five showed improved response to the next treatment regimen. The treatment was promising and safe, without significant adverse effects such as autoimmunity. Based on the results of this clinical study, co-administration of ZYC300 with cyclophosphamide was proposed to enhance CYP1B1 immune responses. Another study suggested that combination of electroporation (EPT) with ZYC300 PLGA microparticles resulted in high expression of antigen CYP1B1 and enhanced immune response, which was contributed to the increased recruitment of antigen presenting cells to the injection site [101].

In a Phase-II trial, PLGA microparticles encapsulated plasmid DNA vaccine was administered intramuscularly to treat human papilloma virus (HPV) associated high-grade cervical dysplasia [102]. By inclusion of cationic components like cetyltrimethylammonium-bromide (CTAB) [103-106], or polyethyleneimine (PEI) [107-109], cationic PLGA microparticles have been fabricated. Cationic PLGA microspheres with adsorbed DNA were evaluated in a Phase-I clinical trial as a HIV vaccine [110].

Singh et al. reported an improved process for preparing cationic PLGA microparticles with an adsorbed DNA vaccine using modified solvent evaporation techniques with a single lyophilization step [105]. Cationic PLGA microparticles prepared by conjugating PEI on the surface have been attempted to deliver antigen expressing plasmid DNA for B cell lymphoma treatment [109]. Intradermal or intramuscular injection of these microparticles in Balb/c mice provided prophylactic anti-tumor effects and increased long term survival rates. Lin et al. demonstrated a novel ultrasonic atomization approach for preparing malaria DNA vaccine PLGA-PEI cationic microparticles. A cell study showed enhanced cell uptake and expression of antigen of the fabricated microparticles [108]. Cationic poly(orthoester) microspheres which were prepared by blending with PEI were evaluated for DNA vaccines delivery [107]. The pH-triggered degradation of POE led to the formation of electrostatic complexes of DNA and PEI, which were responsible for reported enhanced gene transfection and induced maturation and activation of bone marrow-derived dendritic cells in vitro.

Anionic microparticles have been used to deliver non-DNA vaccines such as proteins. The interaction between adsorbed protein and PLGA microparticles is thought to be both electrostatic and hydrophobic in nature as demonstrated by acidic or basic proteins [111]. Chesko et al. reported the preparation of anionic PLGA microparticles in the presence of dioctyl sodium sulfosuccinate (DSS)—an anionic surfactant used to adsorb ovalbumin, lysozyme, a recombinant HIV envelope glycoprotein and a Neisseria meningitidis B protein [112]. Caputo et al. assessed anionic microspheres which were made from poly(methylmethacrylate) (PMMA) and Eudragit L100-55 for HIV-1 Tat protein vaccine [113]. The protein was adsorbed onto microspheres mainly via electrostatic interactions with a loading efficiency of 20%. The results showed that the microsphere/Tat vaccines induced enhanced long-lasting cellular and humoral responses in mice after systemic or mucosal immunization [113].

The nature of the polymer plays a critical role in the antigen-specific adjuvanticity of encapsulated CpG (cytosine-phosphate-guanine) motifs [93]. Co-delivery of OVA (ovalbumin) and CpG oligonucleotide in PLGA 502 and PLGA 756 microparticles failed to show the typical adjuvant effects of CpG motif when closely associated with an antigen. Compared to OVA-loaded PLGA 502, co-delivery of CpG with OVA in PLGA 502 particles significantly improved the antibody response in mice after intradermal immunization. However, the cellular immune response of co-encapsulated CpG was negative. In fact, CpG co-delivery decreased the level of strong humoral immune response induced by OVA-loaded PLGA 756 microparticles.

4. Expert opinion

Organic solvents have been widely used in the fabrication of microspheres; currently marketed microsphere products are primarily manufactured by well established industrial solvent based processes with a long history of regulatory approval. In spite of their negative environmental impact, organic solvents offer unique advantages in producing microspheres simply because of the high solubility of biodegradable polymers in selected organic solvents, allowing the formation of a polymer-organic solvent phase immiscible with the aqueous solution containing a water soluble drug. The single or double emulsion subsequently produced via emulsification of the two- or three-phase system yields hardened microspheres with entrapped drug upon solvent evaporation. Formulation variables influencing the properties of microspheres fabricated by emulsion methods have been extensively investigated as discussed in Part 1 of this review. In addition to the use of organic solvents, one key drawback of an emulsion method is the relatively low encapsulation efficiency mainly attributed to the loss of drug in the aqueous external phase. The recent

advances in emulsion technologies as a result of equipment innovation and novel process design have significantly expanded the capability of emulsion methods in producing microspheres with improved properties such as uniform size-distribution and more complex morphologies with enhanced product performance.

Spray drying of a solution or dispersion of a drug dissolved or dispersed in an organic solvent has also been successfully developed for producing microspheres in a laboratory and industrial scale. Although high encapsulation efficiency of drug in microspheres can be achieved by spray drying, the loss of product in the drying chamber may make it unsuitable to be used with limited supply of drugs in the early development phase. Furthermore, the size of microspheres produced by spray drying is limited by the size of the drying chamber; it is difficult to produce microspheres with a particle size over 10 μm using a laboratory spray dryer. Therefore, the drug release profile obtained with the smaller sized microspheres will not reflect the desirable profile to be achieved during the development phase. New design of spray nozzles and drying chambers are the key features of some newly developed spray drying processes, which have demonstrated the capability of producing microspheres with larger particle size and more uniformity in size distribution as well as reduced drug loss in the drying chamber. However, the combined deleterious effects of organic solvent and heat associated with spray drying remain the main concern for the use of spray drying for the fabrication of microspheres containing biological molecules, even though new stabilization approaches have been reported to mitigate these negative effects.

New microsphere fabrication processes have also been developed utilizing technologies from other industries. In comparison with the conventional solvent based processes, these novel processes are designed with the key features of precise particle-size control, non-spherical shape formation, complex structure generation, organic solvent free, and/or room temperature operation conditions. However, the majority of these new methods are still in the early development phase and available information was derived mainly from studies carried out at the laboratory scale. More in-depth investigations are necessary in order to compare and contrast their suitability for scale up as well as their process compatibility with different types of biological molecules. The success in utilizing these novel processes for commercial manufacturing will also necessitate major investments in developmental effort, cost, and time because of the ever increasing demands for compliance with cGMP regulatory requirements for equipment qualification and process validation.

With the solvent free and ambient processing temperature features, some of these new fabrication methods have found applicability in producing microspheres composed of newly functionalized biodegradable polymers for delivery of bioactive macromolecules (genes and vaccines). This has become an area drawing increasing research interest with promising treatment potential being demonstrated in ongoing preclinical and early clinical studies. Although the particle size of microspheres prepared by these novel methods can be approaching the nanoscale, microspheres by definition are not submicron particles; they are not suitable for IV administration and less effective for intracellular delivery as compared to nanoparticles. In spite of this size related limitation, the utility of microspheres as the parenteral delivery system for biological molecules has significantly broadened the therapeutic applications of microspheres and has also enriched the drug delivery research of microspheres.

Declaration of interest

LC Li, C Guo and Y Shi are employees of Abbott.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. Int J Pharm 2004;282(1-2):1-18
2. Berkland C, Pollauf E, Raman C, et al. Macromolecule release from monodisperse PLG microspheres: control of release rates and investigation of release mechanism. J Pharm Sci 2007;96(5):1176-91
3. Choy YB, Choi H, Kim KK. Uniform biodegradable hydrogel microspheres fabricated by a surfactant-free electric-field-assisted method. Macromol Biosci 2007;7(4):423-8
4. Kim K, Pack D. Microspheres for drug delivery. In: Ferrari M, editor-in-chief. Biomems and biomedical nanotechnology Volume I: Biological and Biomedical Nanotechnology; Lee AP, Lee LJ, editors, Springer, 233 Spring Street, New York, NY 10013, USA; 2006:19-50

•• **An excellent review on the precsion particle fabrication technology.**

5. Berkland C, Kim K, Pack DW. Fabrication of PLG microspheres with precisely controlled and monodisperse size distributions. J Control Release 2001;73(1):59-74
6. Pollauf EJ, Berkland C, Kim KK, et al. In vitro degradation of polyanhydride/polyester core-shell double-wall microspheres. Int J Pharm 2005;301(1-2):294-303
7. Berkland C, Pollauf E, Varde N, et al. Monodisperse liquid-filled biodegradable microcapsules. Pharm Res 2007;24(5):1007-13
8. Pollauf EJ, Pack DW. Use of thermodynamic parameters for design of double-walled microsphere fabrication methods. Biomaterials 2006;27(14):2898-906

• **More detailed description of the fabricaton of double-walled microspheres.**

9. Cooley P, Wallace D, Antohe B. Application of ink-jet printing technology to BioMEMS and microfluidic systems. JALA 2002;7(5):33-9
10. Radulescu D, Schwade N, Wawro D. Uniform paclitaxel-loaded biodegradable microspheres manufactured by ink-jet technology. Proceedings of the Winter Symposium and 11th International Symposium on Recent Advances in Drug Delivery Systems; Salt Lake City, UT, USA; 2003
11. Multilayer microspheres. MicroFab Technologies, Inc. Plano, Texas. Available from: http://www.microfab.com/index.php?option=com_content&view=article&id=71&Itemid=134 [Last accessed 16 July 2012]
12. Veldhuis G, Girones M, Bingham D. Monodisperse microspheres for parenteral drug delivery. Drug Deliv Technol 2009;9(1):24-31

• **A good review on the preparation of monodisperse microspheres.**

13. Duque LF, Lathuile A, Limousin MFP, et al. Biodegradable monodisperse microspheres with sustained release of goserelin acetate for the treatment of prostate and breast cancer. Poster# 136. The 39th Annual Meeting & Exposition of the Controlled Release Society; Quebec City, Canada; 2012
14. Hiemstra C, Lathuile A, Zuidema J, et al. IGF-1 loaded monospheres for treating ischemic heart disease. Poster# 166. The 39th Annual Meeting & Exposition of the Controlled Release Society; Quebec City, Canada; 2012
15. Kang L, Chung BG, Langer R, et al. Microfluidics for drug discovery and development: from target selection to product lifecycle management. Drug Discov Today 2008;13(1-2):1-13
16. Xu Q, Hashimoto M, Dang TT, et al. Preparation of monodisperse biodegradable polymer microparticles using a microfluidic flow-focusing device for controlled drug delivery. Small 2009;5(13):1575-81

• **A good reference on the design and operation of the microfluidic device.**

17. Palmer D, Pattison S, Cheung I, et al. Master the microsphere. PFQ;December/January 2012
18. Palmer D, Zhao X, Seaman P. Encapsulation of biotherapies: new technology for synthesis of monodisperse microspheres. PFQ; August/September 2010
19. Bae SE, Son JS, Park K, et al. Fabrication of covered porous PLGA microspheres using hydrogen peroxide for controlled drug delivery and regenerative medicine. J Control Release 2009;133(1):37-43
20. Mok H, Park TG. Water-free microencapsulation of proteins within PLGA microparticles by spray drying using PEG-assisted protein solubilization technique in organic solvent. Eur J Pharm Biopharm 2008;70(1):137-44
21. Monodisperse spray drying technology. Nanomi B.V., the Netherlands. Available from: http://www.nanomi.com/monodisperse-spray-drying-nozzle.html [Last accessed 16 July, 2012]
22. Van der Gucht F, De Ridder I; Particle engineering with a lab scale spray-dryer. ProCepT nv, Belgium. Available from: http://www.pro-c-ept.com/files/Articles/Poster%20AAPS%202007%20Particle%20Engineering%20with%20Spray%20Drying.pdf [last accessed 16 July, 2012]
23. Semeels G, Van der Gucht F, Van den Mooter G; Optimization of process conditions for spray drying drug-loaded poly lactic-co-glycolic acid (PLGA) microspheres. ProCepT nv, Belgium. Available from: http://www.pro-c-ept.com/files/Articles/Poster%20AAPS%202009%20Spray%20Drying%20PLGA.pdf [Last accessed 16 July, 2012]
24. Wan F, Maltesen MJ, Bjerregaard S, et al. Design and characterization of biodegradable polymer-coated protein micropartices by spray drying method with a three-fluid nozzle. Poster# 154. The 39th Annual Meeting & Exposition of the Controlled Release Society; Quebec City, Canada; 2012
25. Bock N, Woodruff MA, Hutmacher DW, et al. Electrospraying, a reproducible method for production of polymeric microspheres for biomedical applications. Polymers 2011;3(1):131-49
26. Almeria B, Deng W, Fahmy TM, et al. Controlling the morphology of electrospray-generated PLGA microparticles for drug delivery. J Colloid Interface Sci 2010;343(1):125-33
27. Xie J, Ng WJ, Lee LY, et al. Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray. J Colloid Interface Sci 2008;317(2):469-76
28. Davies OR, Lewis AL, Whitaker MJ, et al. Applications of supercritical CO2 in the fabrication of polymer systems for drug delivery and tissue

engineering. Adv Drug Deliv Rev 2008;60(3):373-87

29. Baxendale AJ, van Hooff P, Durrant LG, et al. Single shot tetanus vaccine manufactured by a supercritical fluid encapsulation technology. Int J Pharm 2011;413(1-2):147-54

30. Whitaker MJ, Hao J, Davies OR, et al. The production of protein-loaded microparticles by supercritical fluid enhanced mixing and spraying. J Control Release 2005;101(1-3):85-92

31. Jordan F, Naylor A, Kelly CA, et al. Sustained release hGH microsphere formulation produced by a novel supercritical fluid technology: in vivo studies. J Control Release 2010;141(2):153-60

32. Bahrami M, Ranjbarian S. Production of micro- and nano-composite particles by supercritical carbon dioxide. J Supercrit Fluids 2007;40(2):263-83

33. Reverchon E, Adami R, Caputo G, et al. Spherical microparticles production by supercritical antisolvent precipitation: interpretation of results. J Supercrit Fluids 2008;47(1):70-84

34. Mishima K. Biodegradable particle formation for drug and gene delivery using supercritical fluid and dense gas. Adv Drug Deliv Rev 2008;60(3):411-32

35. Lee LY, Smith KA, Wang C-H. Fabrication of controlled release devices using supercritical antisolvent method. Available from: http://hdl.handle.net/1721.1/7479 [Last accessed 16 July 2012]

36. Lee LY, Smith KA, Wang C-H. Fabrication of micro and nanoparticles of paclitaxel-loaded poly L lactide for controlled release using supercritical antisolvent method: effects of thermodynamics and hydrodynamics. Available from: http://hdl.handle.net/1721.1/30387 [Last accessed 16 July 2012]

• **A good reference on the application of supercritical CO_2 for preparation of microparticles.**

37. Guan J, Ferrell N, James Lee L, et al. Fabrication of polymeric microparticles for drug delivery by soft lithography. Biomaterials 2006;27(21):4034-41

38. Guan J, Chakrapani A, Hansford DJ. Polymer microparticles fabricated by soft lithography. Chem Mater 2005;17(25):6227-9

39. Kelly JY, DeSimone JM. Shape-Specific, Monodisperse Nano-Molding of Protein Particles. JACS 2008;130(16):5438-9

40. Rolland JP, Maynor BW, Euliss LE, et al. Direct Fabrication and Harvesting of Monodisperse, Shape-Specific Nanobiomaterials. JACS 2005;127(28):10096-100

41. Canelas DA, Herlihy KP, DeSimone JM. Top-down particle fabrication: control of size and shape for diagnostic imaging and drug delivery. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2009;1(4):391-404

• **A review article providing more details on the PRINT technology.**

42. Acharya G, Shin CS, McDermott M, et al. The hydrogel template method for fabrication of homogeneous nano/microparticles. J Control Release 2010;141(3):314-19

43. Lin Y, Huang Y. Laser-assisted fabrication of highly viscous alginate microsphere. J Appl Phys 2011;109(8):083107-8

• **An article which described the laser-assisted fabrication of microspheres in more details.**

44. Zhang H, Tong S-Y, Zhang X-Z, et al. Novel solvent-free methods for fabrication of nano- and microsphere drug delivery systems from functional biodegradable polymers. J Phys Chem C 2007;111(34):12681-5

45. Crespy D, Landfester K. Miniemulsion polymerization as a versatile tool for the synthesis of functionalized polymers. Beilstein J Org Chem 2010;6:1132-48

46. Zhao T, Qiu D. One-pot synthesis of highly folded microparticles by suspension polymerization. Langmuir 2011;27(21):12771-4

47. Nguyen D, Duguet E, Bourgeat-Lami E, et al. An easy way to control the morphology of colloidal polymer-oxide supraparticles through seeded dispersion polymerization. Langmuir 2010;26(9):6086-90

48. King WJ, Toepke MW, Murphy WL. A general route for the synthesis of functional, protein-based hydrogel microspheres using tailored protein charge. Chem Commun (Camb) 2011;47(1):526-8

49. Marui A, Tabata Y, Kojima S, et al. A novel approach to therapeutic angiogenesis for patients with critical limb ischemia by sustained release of basic fibroblast growth factor using biodegradable gelatin hydrogel: an initial report of the phase I-IIa study. Circ J 2007;71(8):1181-6

•• **A clinical study which used gelatin hydrogel micropsheres to delivery basic fibroblast growth factor.**

50. Hashimoto T, Koyama H, Miyata T, et al. Selective and sustained delivery of basic fibroblast growth factor (bFGF) for treatment of peripheral arterial disease: results of a phase I trial. Eur J Vasc Endovasc Surg 2009;38(1):71-5

51. De Leede LG, Humphries JE, Bechet AC, et al. Novel controlled-release Lemna-derived IFN-alpha2b (Locteron): pharmacokinetics, pharmacodynamics, and tolerability in a phase I clinical trial. J Interferon Cytokine Res 2008;28(2):113-22

•• **A phase 1 clinical trial which assessed the use of polymeric microspheres to treat hepatitis C.**

52. Krastev Z, Kotzev I, Tchernev K, et al. Randomized, open-Label 12-Week comparison of controlled-release interfffron alpha2b+ribavrin vs. pegylated-interferon alpha2b+ribavrin in treatment-naive genotype1 hepatitis C: 4 week results from 480study (panel a). J Hepatol 2010;52:S27-S

53. van Dijkhuizen-Radersma R, Metairie S, Roosma JR, et al. Controlled release of proteins from degradable poly(ether-ester) multiblock copolymers. J Control Release 2005;101(1-3):175-86

54. van Dijkhuizen-Radersma R, Wright SJ, Taylor LM, et al. In vitro/in vivo correlation for 14C-methylated lysozyme release from poly(ether-ester) microspheres. Pharm Res 2004;21(3):484-91

55. Park EJ, Na DH, Lee KC. In vitro release study of mono-PEGylated growth hormone-releasing peptide-6 from PLGA microspheres. Int J Pharm 2007;343(1-2):281-3

56. Lee ES, Park KH, Park IS, et al. Glycol chitosan as a stabilizer for protein encapsulated into poly(lactide-co-glycolide) microparticle. Int J Pharm 2007;338(1-2):310-16

57. Lee ES, Kwon MJ, Lee H, et al. In vitro study of lysozyme in poly(lactide-co-glycolide) microspheres with sucrose acetate isobutyrate. Eur J Pharm Sci 2006;29(5):435-41

58. Yeh MK, Chen JL, Chiang CH, et al. The preparation of sustained release erythropoietin microparticle. J Microencapsul 2007;24(1):82-93

59. Ho ML, Fu YC, Wang GJ, et al. Controlled release carrier of BSA made by W/O/W emulsion method containing PLGA and hydroxyapatite. J Control Release 2008;128(2):142-8

60. Chan OC, So KF, Chan BP. Fabrication of nano-fibrous collagen microspheres for protein delivery and effects of photochemical crosslinking on release kinetics. J Control Release 2008;129(2):135-43

61. Solorio L, Zwolinski C, Lund AW, et al. Gelatin microspheres crosslinked with genipin for local delivery of growth factors. J Tissue Eng Regen Med 2010;4(7):514-23

62. Jay SM, Saltzman WM. Controlled delivery of VEGF via modulation of alginate microparticle ionic crosslinking. J Control Release 2009;134(1):26-34

63. Wang X, Wenk E, Hu X, et al. Silk coatings on PLGA and alginate microspheres for protein delivery. Biomaterials 2007;28(28):4161-9

64. Yu CY, Yin BC, Zhang W, et al. Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improved pH-sensitive drug release property. Colloids Surf B Biointerfaces 2009;68(2):245-9

• **A representative paper which reported the utilization of composite microparticles for protien delivery.**

65. Yuan W, Wu F, Guo M, et al. Development of protein delivery microsphere system by a novel S/O/O/W multi-emulsion. Eur J Pharm Sci 2009;36(2-3):212-18

66. Lee JI, Yoo HS. Biodegradable microspheres containing poly(epsilon-caprolactone)-Pluronic block copolymers for temperature-responsive release of proteins. Colloids Surf B Biointerfaces 2008;61(1):81-7

67. King WJ, Pytel NJ, Ng K, et al. Triggered drug release from dynamic microspheres via a protein conformational change. Macromol Biosci 2010;10(6):580-4

68. Yin R, Han J, Zhang J, et al. Glucose-responsive composite microparticles based on chitosan, concanavalin A and dextran for insulin delivery. Colloids Surf B Biointerfaces 2010;76(2):483-8

• **A good paper which evaluated glucose-responsive microparticles for protein delivery.**

69. Choleris E, Little SR, Mong JA, et al. Microparticle-based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. Proc Natl Acad Sci USA 2007;104(11):4670-5

70. Soderquist RG, Sloane EM, Loram LC, et al. Release of plasmid DNA-encoding IL-10 from PLGA microparticles facilitates long-term reversal of neuropathic pain following a single intrathecal administration. Pharm Res 2010;27(5):841-54

71. De Stefano D, De Rosa G, Maiuri MC, et al. Oligonucleotide decoy to NF-kappaB slowly released from PLGA microspheres reduces chronic inflammation in rat. Pharmacol Res 2009;60(1):33-40

72. Dass CR, Contreras KG, Dunstan DE, et al. Chitosan microparticles encapsulating PEDF plasmid demonstrate efficacy in an orthotopic metastatic model of osteosarcoma. Biomaterials 2007;28(19):3026-33

73. Davies OR, Head L, Armitage D, et al. Surface modification of microspheres with steric stabilizing and cationic polymers for gene delivery. Langmuir 2008;24(14):7138-46

74. Zhang XQ, Intra J, Salem AK. Comparative study of poly (lactic-co-glycolic acid)-poly ethyleneimine-plasmid DNA microparticles prepared using double emulsion methods. J Microencapsul 2008;25(1):1-12

75. Kakade S, Manickam DS, Handa H, et al. Transfection activity of layer-by-layer plasmid DNA/poly (ethylenimine) films deposited on PLGA microparticles. Int J Pharm 2009;365(1-2):44-52

76. Intra J, Salem AK. Fabrication, characterization and in vitro evaluation of poly(D,L-lactide-co-glycolide) microparticles loaded with polyamidoamine-plasmid DNA dendriplexes for applications in nonviral gene delivery. J Pharm Sci 2010;99(1):368-84

• **A representative paper describing the use of dendrimer and polymeric microparticles for DNA delivery.**

77. Fu HL, Li YQ, Shao L, et al. Gene expression mediated by dendrimer/DNA complexes encapsulated in biodegradable polymer microspheres. J Microencapsul 2010;27(4):345-54

78. Zhang XQ, Intra J, Salem AK. Conjugation of polyamidoamine dendrimers on biodegradable microparticles for nonviral gene delivery. Bioconjug Chem 2007;18(6):2068-76

79. Tomioka A, Tanaka M, De Velasco MA, et al. Delivery of PTEN via a novel gene microcapsule sensitizes prostate cancer cells to irradiation. Mol Cancer Ther 2008;7(7):1864-70

80. Lin X, Jo H, Ishii TM, et al. Controlled release of matrix metalloproteinase-1 plasmid DNA prevents left ventricular remodeling in chronic myocardial infarction of rats. Circ J 2009;73(12):2315-21

81. Parsa S, Wang Y, Fuller J, et al. A comparison between polymeric microsphere and bacterial vectors for macrophage P388D1 gene delivery. Pharm Res 2008;25(5):1202-8

82. Sezer AD, Akbuga J. Comparison on in vitro characterization of fucospheres and chitosan microspheres encapsulated plasmid DNA (pGM-CSF): formulation design and release characteristics. AAPS PharmSciTech 2009;10(4):1193-9

83. Frechet JMJ, Cohen JL, Schubert S, et al. Acid-degradable cationic dextran particles for the delivery of siRNA therapeutics. Bioconjug Chem 2011;22(6):1056-65

84. Brandhonneur N, Chevanne F, Vie V, et al. Specific and non-specific phagocytosis of ligand-grafted PLGA microspheres by macrophages. Eur J Pharm Sci 2009;36(4-5):474-85

85. Bradley M, Alexander L, Sanchez-Martin RM. Cellular uptake of fluorescent labelled biotin-streptavidin microspheres. J Fluoresc 2008;18(3-4):733-9

86. Alexander LM, Sanchez-Martin RM, Bradley M. Knocking (anti)-sense into cells: the microsphere approach to gene silencing. Bioconjug Chem 2009;20(3):422-6

87. Wang D, Molavi O, Lutsiak ME, et al. Poly(D,L-lactic-co-glycolic acid) microsphere delivery of adenovirus for vaccination. J Pharm Pharm Sci 2007;10(2):217-30

88. Ahire VJ, Sawant KK, Doshi JB, et al. Chitosan microparticles as oral delivery system for tetanus toxoid. Drug Dev Ind Pharm 2007;33(10):1112-24

89. Torres MP, Wilson-Welder JH, Lopac SK, et al. Polyanhydride microparticles enhance dendritic cell antigen presentation and activation. Acta Biomater 2011;7(7):2857-64

90. Gunbeyaz M, Faraji A, Ozkul A, et al. Chitosan based delivery systems for mucosal immunization against bovine herpesvirus 1 (BHV-1). Eur J Pharm Sci 2010;41(3-4):531-45

91. Mata E, Igartua M, Hernández RM, et al. Comparison of the adjuvanticity of two different delivery systems on the induction of humoral and cellular responses to synthetic peptides. Drug Deliv 2010;17(7):490-9

92. Thomas C, Gupta V, Ahsan F. Particle size influences the immune response produced by hepatitis B vaccine formulated in inhalable particles. Pharm Res 2010;27(5):905-19

93. Román BS, Irache JM, Gómez S, et al. Co-encapsulation of an antigen and CpG oligonucleotides into PLGA microparticles by TROMS technology. Eur J Pharm Biopharm 2008;70(1):98-108

94. Foged C, Brodin B, Frokjaer S, et al. Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. Int J Pharm 2005;298(2):315-22

95. Yoshida M, Babensee JE. Differential effects of agarose and poly(lactic-co-glycolic acid) on dendritic cell maturation. J Biomed Mater Res A 2006;79A(2):393-408

96. Schliehe C, Schliehe C, Thiry M, et al. Microencapsulation of inorganic nanocrystals into PLGA microsphere vaccines enables their intracellular localization in dendritic cells by electron and fluorescence microscopy. J Control Release 2011;151(3):278-85

97. Yoshida M, Babensee JE. Molecular aspects of microparticle phagocytosis by dendritic cells. J Biomater Sci Polym Ed 2006;17(8):893-907

98. Waeckerle-Men Y, Groettrup M. PLGA microspheres for improved antigen delivery to dendritic cells as cellular vaccines. Adv Drug Deliv Rev 2005;57(3):475-82

99. Luby TM. Targeting cytochrome P450 CYP1B1 with a therapeutic cancer vaccine. Expert Rev Vaccines 2008;7(7):995-1003

100. Gribben JG, Ryan DP, Boyajian R, et al. Unexpected association between induction of immunity to the universal tumor antigen CYP1B1 and response to next therapy. Clin Cancer Res 2005;11(12):4430-6

•• **A phase I clinical study which reported the use of PLGA microparticles to deliver plasmid DNA of CYP1B1 for immunotherapy.**

101. Barbon CM, Baker L, Lajoie C, et al. In vivo electroporation enhances the potency of poly-lactide co-glycolide (PLG) plasmid DNA immunization. Vaccine 2010;28(50):7852-64

102. Matijevic M, Hedley ML, Urban RG, et al. Immunization with a poly (lactide co-glycolide) encapsulated plasmid DNA expressing antigenic regions of HPV 16 and 18 results in an increase in the precursor frequency of T cells that respond to epitopes from HPV 16, 18, 6 and 11. Cell Immunol 2011;270(1):62-9

•• **A phase II trial that used PLGA microparticles encapsulated plasmid DNA vaccine to treat human papilloma virus.**

103. Pan CH, Nair N, Adams RJ, et al. Dose-dependent protection against or exacerbation of disease by a polylactide glycolide microparticle-adsorbed, alphavirus-based measles virus DNA vaccine in rhesus macaques. Clin Vaccine Immunol 2008;15(4):697-706

104. Liman M, Peiser L, Zimmer G, et al. A genetically engineered prime-boost vaccination strategy for oculonasal delivery with poly(d,l-lactic-co-glycolic acid) microparticles against infection of turkeys with avian Metapneumovirus. Vaccine 2007;25(46):7914-26

105. Singh M, Fang J-H, Kazzaz J, et al. A modified process for preparing cationic polylactide-co-glycolide microparticles with adsorbed DNA. Int J Pharm 2006;327(1-2):1-5

106. Wischke C, Borchert HH, Zimmermann J, et al. Stable cationic microparticles for enhanced model antigen delivery to dendritic cells. J Control Release 2006;114(3):359-68

107. Nguyen DN, Raghavan SS, Tashima LM, et al. Enhancement of poly (orthoester) microspheres for DNA vaccine delivery by blending with poly(ethylenimine). Biomaterials 2008;29(18):2783-93

108. Liu S, Danquah MK, Forde GM, et al. Microparticle-mediated gene delivery for the enhanced expression of a 19-kDa fragment of merozoite surface protein 1 of Plasmodium falciparum. Biotechnol Prog 2010;26(1):257-62

109. Pai Kasturi S, Qin H, Thomson KS, et al. Prophylactic anti-tumor effects in a B cell lymphoma model with DNA vaccines delivered on polyethylenimine (PEI) functionalized PLGA microparticles. J Control Release 2006;113(3):261-70

110. Spearman P, Lally MA, Elizaga M, et al. A trimeric, V2-deleted HIV-1 envelope glycoprotein vaccine elicits potent neutralizing antibodies but limited breadth of neutralization in human volunteers. J Infect Dis 2011;203(8):1165-73

•• **A phae I clinical trial that used cationic PLGA micropsheres to deliver DNA vaccine to treat HIV.**

111. Singh M, Kazzaz J, Chesko J, et al. Anionic microparticles are a potent delivery system for recombinant antigens from Neisseria meningitidis serotype B. J Pharm Sci 2004;93(2):273-82

112. Chesko J, Kazzaz J, Ugozzoli M, et al. Characterization of antigens adsorbed to anionic PLG microparticles by XPS and TOF-SIMS. J Pharm Sci 2008;97(4):1443-53

113. Caputo A, Castaldello A, Brocca-Cofano E, et al. Induction of humoral and enhanced cellular immune responses by novel core-shell nanosphere- and microsphere-based vaccine formulations following systemic and mucosal administration. Vaccine 2009;27(27):3605-15

Affiliation

Shirui Mao[1], Chunqiang Guo[2], Yi Shi[3] & Luk Chiu Li[†2]
[†]Author for correspondence
[1]Shenyang Pharmaceutical University, School of Pharmacy, China
[2]Animal Health, Abbott Laboratories, J48 200 Abbott Road, Abbott Park, IL 60064, USA
Tel: +1 847938 0391; Fax: +1 847 937 9010; E-mail: lukchiu.li@abbott.com
[3]Global Pharmaceutical Research and Development, Abbott Laboratories, Illinois, USA