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Poly Lactic-Co-Glycolic Acid (PLGA) Copolymer and Its Pharmaceutical Application

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Abstract

Poly lactic-co glycolic acid (PLGA) is a biodegradable, biocompatible and FDA approved copolymer chemically synthesized from lactic and glycolic acid as monomers. Depending upon the monomer ratio in the polymeric units, the physicochemical properties and degradation rate differ. By virtue of their properties, these polymers have been widely used for fabrication of controlled drug delivery systems in the form of *in-situ* gels, microspheres, implants, nanoparticles, etc. Although there has been significant progress in both sectors, there is still limited understanding due to the complexities involved in both polymer chemistry and production technologies. This chapter will help in the understanding of the chemistry behind the polymers, their synthetic manufacturing pathway and their pharmaceutical applications.

Keywords: Poly lactic-co glycolic acid, synthetic manufacturing pathway, gene delivery, PLGA-based theranostic tools, bone regeneraton, pulmonary delivery

6.1 Introduction

In the last few decades, much importance has been given to the use of polymer in drug delivery systems. Various synthetic and natural polymers, such as acrylates, chitosan, alginate etc., have been widely investigated in the field of drug delivery. The natural polymers have the advantages of showing better cell adhesion and cell function as compared to their synthetic counterparts; but these natural polymers also have the disadvantages of inducing allergens and having limited purity and mechanical properties. In contrast to natural polymers, synthetic biodegradable polymers such as poly(amides), poly(amino acids), poly(alkyl-a-cyano acrylates), poly(esters), poly(orthoesters), poly(urethanes) and poly(acrylamides) have been widely used as drug delivery carrier. These polymers possess relatively good mechanical property, less interaction with cell

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due to lack of cell recognition signals (which is usually the case with natural polymers), and ease of modification, which makes it easy for carrying out various modification to make the delivery system more specific [1,2]. Among the various synthetic biodegradable polymers, poly(lactic-co-glycolic acid) (PLGA) has been the most widely investigated polymer. PLGA is a copolymer composed of lactic acid and glycolic acid, generally an acronym for poly D, L-lactic-co-glycolic acid, where D- and L-lactic acid forms are in equal ratio. The synthesis of PLGA includes random ring-opening copolymerization of two different monomers, i.e., the cyclic dimers (1,4-dioxane-2, 5-diones) of glycolic acid and lactic acid in the presence of catalyst such as tin(II) 2-ethylhexanoate, tin(II) alkoxides or aluminum isopropoxide. During the copolymerization reaction in the presence of catalysts, consecutive monomeric units (glycolic or lactic acid) get linked together in PLGA by ester linkages and degrade inside the body by their hydrolysis into endogenous monomers, i.e., lactic acid and glycolic acid, which then degrade into water and carbon dioxide, hence presenting negligible or no toxicity [3–5]. By properly choosing molecular weights, the degradation time of PLGA can be modulated from a week to several months, thus enabling drug release in a controlled or sustained manner. The first work on PLGA dates back to the 1960s. A lot of research was done from the 1960s to 1970s with respect to use of PLGA in the biomedical field. After that, researchers kept exploring PLGA for use in several other fields, including surgical dressings, vascular grafts, dental and fracture repairs, drug delivery, gene delivery, cancer therapy, etc. The driving force behind the vast amount of research done on PLGA was its unique properties such as versatile degradation kinetics, nontoxicity, and biocompatibility [6–8]. Apart from its use in the biomedical field and for delivery of drugs, it has also been widely explored for delivery of proteins, peptides, genes, vaccines, antigens, human growth factors, vascular endothelial growth factors, etc. It has been approved by the U.S. Food and Drug Administration (FDA) for use in drug delivery device [7,9–14]. In the case of drug delivery, various types of drug delivery systems (DDS) have been formulated using PLGA, including nanoparticles, microparticles, microspheres, implants and *in-situ* gels. In each case, the release of drug from the DDS depends on the molecular weight of the polymer used. By proper selection of molecular weight according to the system and drug delivery duration, the release of drug from DDS can be modulated from a few weeks to several months in either a sustained or controlled manner. The FDA has approved PLGA microparticle-based DDS which gives controlled release of human growth hormone for a month. Several PLGA-based DDSs have been approved by the FDA, especially for treatment of cancer.

6.2 Physicochemical Properties

As mentioned above, PLGA is a copolymer of lactide and glycolide, which is synthesized by random ring-opening polymerization. The degradation rate of PLGA is dependent on the amounts of glycolic acid and lactic acid. The PLGA containing a higher ratio of lactide is often less hydrophilic than PLGA containing an equal ratio of PLA and PGA or lower ratio of PLA. This is because the presence of methyl side groups in PLA makes PLA more hydrophobic than PGA, and hence an increased ratio of PLA in PLGA copolymer would ultimately be less hydrophilic, absorb less water and hence

will degrade more slowly. This means that by varying the PLA content or by optimizing the hydrophilic content of PLGA, degradation time can be easily modulated [15–17]. The glass transition temperature (Tg) of the PLGA is more than 37°C and hence it is glassy in nature. The good mechanical strength of PLGA can be attributed to the rigid chain structure. The ratio of PLA in PLGA significantly affects the Tg of PLGA. With an increase in the ratio of PLA, Tg increases. Apart from its glassy nature, rigid chain structure and hydrophilic nature, the physical properties of PLGA also depend on a variety of other factors such as the initial molecular weight, the ratio of lactide to glycolide, the size of the DDS, exposure to water (surface shape) and storage temperature; polydispersity and degree of crystallinity affects the biodegradation of PLGA and its mechanical strength [18]. In the case of crystallinity, the ratio of PLA and PGA plays an important role.

6.3 Biodegradation

The biodegradation of PLGA depends on multiple factors such as molecular weight size, shape and morphology; chemical structure, hydrophobicity, crystallinity, and glass transition temperature of the polymer; physicochemical parameters, etc.[10]. The ratio of PLA to PGA significantly affects the biodegradation of PLGA-based DDS. The degradation is at a very slow rate and hence the degradation products have no significant effect on the normal cell function. The degradation products of PLGA have minimal or no systemic toxicity as the degradation products are effectively dealt with by the body. Debate has been raised on the role of enzymes in the degradation of PLGA. Some researcher support the theory that the degradation of PLGA is purely by hydrolysis and enzymes play no role in the biodegradation, while some researchers think that enzymes could play a significant role in the degradation of PLGA apart from simple hydrolysis. This has also been demonstrated by the difference in the *in-vitro* and *in-vivo* degradation rates [17,19]. As mentioned above, the degradation of PLGA depends on a variety of factors. In general, the degradation time will be shorter for low molecular weight, more hydrophilic, more amorphous polymers, and copolymers with higher content of glycolide, whereas the degradation time will be higher as the PLA content increases, providing hydrophilic character to the PLGA copolymers. When L-PLA and PGA are used, the formed PLGA copolymers are crystalline in nature, whereas the PLGA copolymers formed by using D, L-PLA and PGA are amorphous in nature. The degradation time therefore will be shorter for DL-PLA containing PLGA copolymers and higher for L-PLA containing PLGA copolymers [7,8]. In the case of PLGA copolymers obtained from DL-PLA and PGA, the ratio of PLA and PGA will decide the degradation time. A lower ratio of PLA will give PLGA copolymers with shorter degradation time and vice versa. Although DL-PLA and PGA have a shorter degradation time than L-PLA and PGA, if the ratio of PLA is much lower in PLGA copolymers obtained from L-PLA than in D, L-PLA, then it might be possible that the degradation time of L-PLA containing PLGA copolymers will be shorter than DL-PLA containing PLGA copolymers. The most widely used PLGA copolymer composition of 50:50 has the shortest degradation time (d,l-lactide/glycolide), with that polymer degrading in about 50–60 days. The PLGA copolymers with other varying ratios of 65:35, 77:25,

and 88:15 (d,l-lactide/glycolides) have longer *in-vivo* lifetimes, which is highest for 88:15 with degradation time of about 150 days *in vivo* [10]. After the ratio and type of polymers, the second crucial factor affecting degradation time is molecular weight and molecular weight distribution. Both molecular weight and molecular weight distribution may play a role in the degradation behavior *in vivo* and *in vitro*. A PLGA copolymer with a large molecular weight distribution indicates the presence of relatively large numbers of carboxylic end groups, which can facilitate the autocatalytic degradation of the polymer chains; while those with narrow molecular weight distribution would take more time for degradation owing to the absence of a large number of carboxylic acid groups [20]. An interesting work carried out using PLGA copolymers with different molecular weights of 10000 and 20000 demonstrated that the rate of degradation of PLGA copolymers having molecular weight of 10 000 degraded approximately twice as fast as the 20 000 molecular weight PLGA copolymers [21]. Thus, the result suggests that molecular weight significantly affects the degradation rate of PLGA copolymers. Recently, Mohammad and Reineke determined the *in-vivo* degradation kinetics of PLGA nanoparticles of two sizes, distributed in the liver, spleen, and lungs following intravenous administration, by calculating the change in the molecular weight which occurs due to ester bond scission in the polymer backbone [22]. The results demonstrated that the degradation follows first-order kinetics irrespective of nanoparticle size. Although the nanoparticles with particle size of 200 nm degraded at a faster rate as compared to 500 nm nanoparticles in the spleen, in the liver the degradation rate of both types of nanoparticles was similar.

6.4 Biocompatibiliy, Toxicty and Pharmacokinetics

Biocompatibility and toxicity are the two major issues which must be addressed before selecting polymers for drug delivery application. The biocompatibility of polymers is generally governed by two main factors: the host reactions induced by the material and the degradation of the material in the body [23]. Hence, it is very important to determine the degradation rate of polymer and its clearance from local tissue for predicting the drug and polymer concentration in the tissue. PLGA is well characterized and DDS based on it is commercially available, which confirms the biocompatibility and nontoxic nature of PLGA. A study was performed on understanding the long-term biocompatibility of PLGA and it was found that PLGA-based DDS did not show any unwanted effects after intravascular administration [24]. Semete and coworkers studied the toxicity of PLGA-based nanoparticles in cell lines and mice. *In-vitro* cytotoxicity of the respective particles was tested in two different cell lines. The results demonstrated that the PLGA nanoparticles showed absence of lesions or inflammation, as confirmed by histopathological study. No remarkable change in the pathology of tissues was observed after oral administration of PLGA nanoparticles in mice [25]. Several studies were performed to analyze the pharmacokinetics of PLGA in the body. Studies suggest that the biodistribution and pharmacokinetics of PLGA generally follow a nonlinear and dose-dependent profile, and both blood clearance and uptake by the mononuclear phagocyte system (MPS) may depend on the dose and composition of PLGA carrier systems [26–28]. Whole-body autoradiography and quantitative distribution experiments were

also performed for studying the biodistribution of PLGA-based DDS inside the body. Results indicate that certain formulation of PLGA, mainly nanoparticles, accumulate rapidly in the liver, bone marrow, lymph nodes, spleen and peritoneal macrophages [29]. So, from the reported studies we can get an idea about the PLGA-based DDS, its safety and distribution inside the body.

6.5 Mechanism of Drug Release

There are various factors which affect the release of drug from the PLGA-based DDS (Figure 6.1). The drug release from PLGA-based DDS has been beautifully reviewed by Fredenberg *et al.* [30].

There exist three possible mechanisms of drug release from PLGA-based DDS: (i) transport through water-filled pores, (ii) transport through the polymer, and (iii) transport due to dissolution of the encapsulating polymer. The last mechanism does not involve transport of drug from DDS, as in this case the drug is automatically exposed to the dissolution medium due to erosion of the polymer. As soon as PLGA comes in contact with dissolution medium the medium gets rapidly absorbed, hence creating water pockets which can be considered as pores. In the initial phase this absorption is slow but increases later on [31,32]. The absorption of dissolution media causes hydrolysis of PLGA leading to the start of the scission of ester bonds. Hydrolysis leads to formation of degradation products, which then diffuse out of the polymer matrix. In doing so it leads to the formation of small pores throughout the polymer matrix. The small pores developed throughout the matrix keep on increasing in size and finally coalesce together to form bigger pores [31,33–35]. A schematic representation of various factors involved in the release of drug from polymer matrix is shown in Figure 6.2.

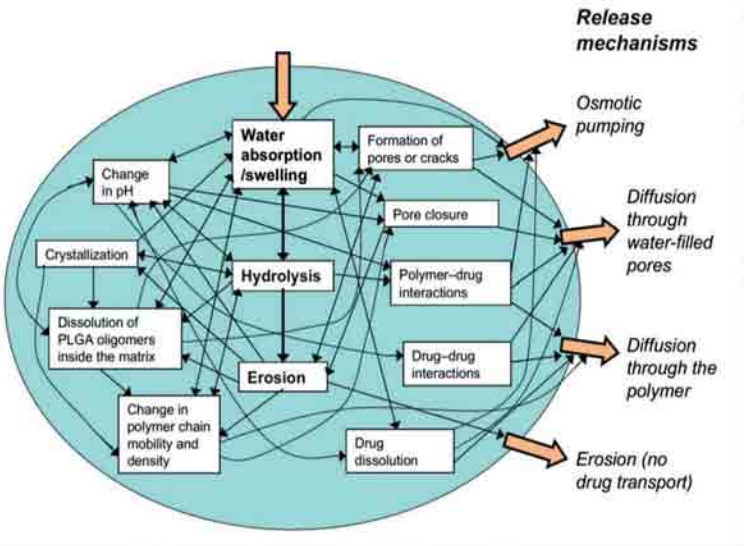

Figure 6.1 Various processes taking part in drug release from PLGA-based DDS (Adapted from Elsevier).

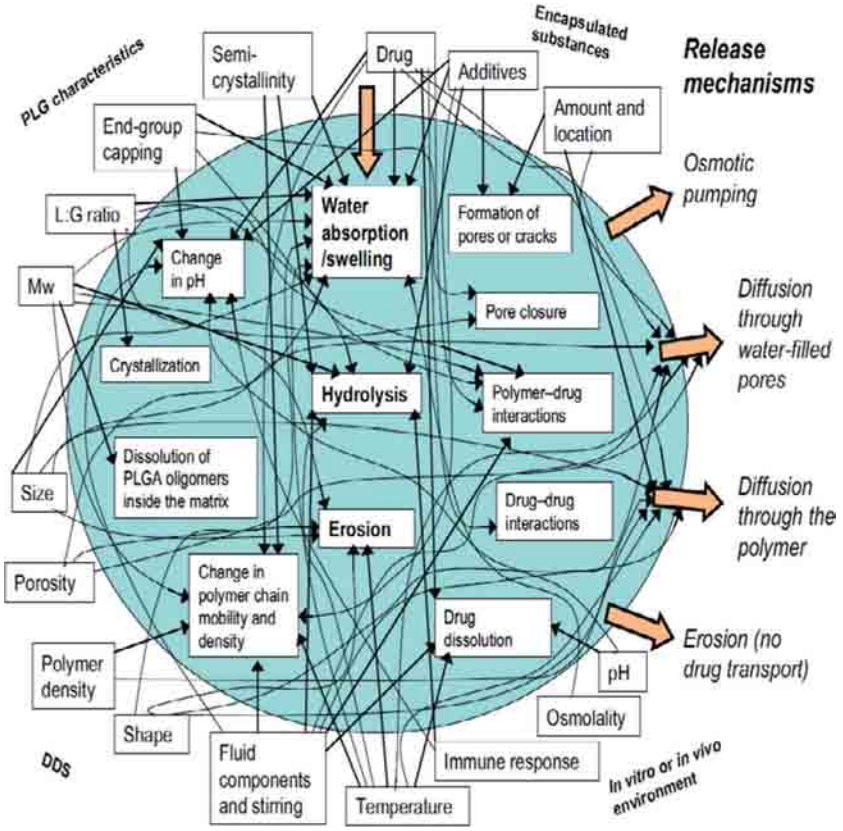

Figure 6.2 Various factors involved in drug release from PLGA matrices (Adapted from Elsevier).

The release profile of drug from PLGA-based DDS is rarely found to be monophasic. Commonly the drug release profile is biphasic or triphasic. A DDS with colloidal nature, i.e., having particles with different sizes, often shows altered release profile from a Fickian diffusion profile and a sigmoidal profile to a zero-order profile [36–40]. In the case of triphasic release profile showing DDS, the first phase is often termed as burst release, which is attributed to the presence of unentrapped drug present as adsorbed layer on the surface of particle, either nanoparticles or microparticles, or drug layer present close to the surface which is easily accessed by hydration of particle. The second phase is slow due to slow diffusion of drug from dense polymer matrix and absence of multiple larger pores. The third phase shows faster drug release as compared to the second phase, which is attributed to the presence of larger pores and degradation of polymer in the second phase [41,42]. The above explanation is not always true for different release profiles. In some cases, the second phase might show faster drug release followed by slower drug release at the end of second phase. The slower drug release in the second phase of triphasic release profile may not always be due to dense polymer matrix or absence of larger pores, but may be due to pore closure, polymer–drug interactions or drug–drug interactions that inhibit the release of the drug as reported earlier. The rapid drug release in the third phase is generally due to the presence of large pores and polymer degradation, but it can also be due to the presence of cracks or

disintegration of particle or collapse of DDS [43–48]. So, we can see that the release as well as the release profile can change due to various factors and is not always constant.

6.6 PLGA-Based DDS

Various DDSs based on PLGA have been developed in recent times. The developed DDS includes microparticles, nanoparticles, injectables, dry powder for inhalation,

Table 6.1 Some of the FDA approved PLGA-based formulations.

Sr. No.	Brand Name	Active Ingredient	Company	Indication	Route	Status
1	Nutropin Depot®	Human growth hormone	Genetech	Growth deficiencies	SC/IM	Marketed
2	Lupron® Depot	Leuprolide acetate	TAP	Prostate cancer		Marketed
3	Suprecur® MP	Buserelin acetate	Aventis	Prostate cancer		Marketed
4	Decapeptyl®	Triptorelin pamoate	Ferring	Prostate cancer	IM	Marketed
5	TrelstarTM Depot	Triptorelin pamoate	Pfizer	Prostate cancer	IM	Marketed
6	Pamorelin®	Leuprolide acetate		Prostate cancer	SC	Marketed
7	Sandostatin® LAR	Octreotide acetate	Novartis	Acromegaly	SC/IM	Marketed
8	Somatuline® LA	Lanreotide	Ipsen	Acromegaly		Marketed
9	Arestin	Minocycline	Orapharma	Periodontal disease	Oral	Marketed
10	Atridox®	Doxycycline hyclate 10%		Chronic adult periodontitis	Topical	Marketed
11	Risperidal® Consta	Risperidone	Johnson & Johnson	Antipsychotic		Marketed
12	Oncogel®	Paclitaxel		Solid tumors	Intratumoral Inj.	Clinical trial
13	Sanvar® SR	Vapreotide		Esophageal bleeding varices (EVB)	SC/IM	Clinical trial

oral administration, etc. Many such PLGA-based DDSs have even gotten approval from the FDA and are being marketed. A few of them are still under clinical trial phase. Some are still in the development stage. A few of the FDA approved PLGA-based formulations are shown in Table 6.1.

Research is still going on regarding the development of PLGA-based formulations for delivering therapeutics. Given below are brief descriptions of PLGA-based systems which have been developed so far and on which research is still ongoing.

6.7 Bone Regeneration

Bone healing and remodeling is a very complex process which takes place by a coordinated effort of cells, bioactive factors and extracellular matrix, which together stimulate the proliferation, differentiation and migration of osteoprogenitor cells, leading to bone regeneration and healing [49,50]. PLGA has been widely investigated for its use in bone and tissue regeneration. Various types of DDS, including nanoparticles, scaffolds, microspheres and hydrogels, have been developed for bone regeneration therapy. Several PLGA-based scaffolds have been developed in the past for bone regeneration using different techniques, including gas forming and particulate leaching technique, porogen leaching and melt-molding technique, thermally induced phase separation technique, solid freeform fabrication (SFF) technology and selective laser sintering [51–55]. Kim *et al.* fabricated a polymeric/nano-hyaluronic acid (HA) composite scaffold by using the technique of gas forming and particulate leaching (GFPL) [51]. This technique had the advantage of not involving organic solvents. The scaffold prepared by GFPL technique helped in exposing the HA nanoparticles at the scaffold surface significantly more than what was observed in scaffolds prepared by techniques other than GFPL such as the conventional solvent casting/particulate leaching technique. The GFPL scaffolds were highly porous with improved mechanical properties and demonstrated higher cell growth *in vitro* compared to scaffolds fabricated by other techniques. Among all the techniques used, thermally induced phase separation technique has gained lot of attention and has been employed for fabrication of microporous membranes or microcellular foams from medicine to the chemical industry, scaffolds for tissue engineering, and as a drug carrier for controlled release DDS [56,57]. Although PLGA has been used widely in the area of drug delivery, there has been much less work reported on PLGA-based scaffolds. This is attributed to the surface chemistry of PLGA which does not promote cell adhesion efficiently, which is important for bone growth and proliferation owing to its hydrophobic nature [58]. But this limitation has been overcome by researchers by including filler along with PLGA. PLGA microspheres entrapping bone morphogenetic protein-2 (BMP-2) were incorporated in calcium phosphate cement (CPC) for fabrication of a degradable bone substitute with osteogenesis materials [59]. Nie and coworkers studied different types of scaffolds loaded with BMP-2 plasmid by loading it into fibrous matrices [60]. The result demonstrated release of plasmid from all types of scaffolds. In another work, endothelial progenitor cells were loaded onto porous PLGA scaffold [61]. From the study it was found that pre-seeding endothelial progenitor cells onto highly porous,

biocompatible PLGA scaffolds creates a favorable microenvironment for the regeneration of osteochondral regions by promoting growth of chondrocyte and hyaline cartilage, thus providing complete osteochondral integration and a vascularized bone matrix. The results demonstrated that PLGA grafts enhance the formation of hyaline cartilage and the neo-vascularization in the subchondral bone of osteochondral defects. Autologous adipose-derived stromal cells were delivered for treatment of tibial defect by seeding the cells in three-dimensional poly(lactic)-glycolic acid (PLGA) scaffolds and culturing them in osteogenic medium [62]. The osteogenesis was calculated using von Kossa staining in three-dimensional cultures, which showed the presence of multiple calcified extracellular matrix nodules. Plain radiographs and micro-CT findings confirmed the complete healing of tibial defect, making adipose-derived stromal cells-seeded PLGA scaffold a promising candidate for bone regeneration. Similar to adipose-derived stromal cells, bone morphogenetic protein-4 (BMP4) was spatially immobilized in a collagen-PLGA hybrid scaffold with a fusion BMP4 composed of an additional collagen-binding domain derived from fibronectin [63]. The osteogenic differentiation of MSCs was analyzed by real-time RT-PCR for osteogenic gene expression. The *in-vivo* implantation study demonstrated that the immobilized CBD-BMP4 maintained its osteoinductive activity, being capable of up-regulating osteogenic gene expression and biomineralization. From the study it was concluded that the BMP4-immobilized collagen-PLGA hybrid scaffold showed osteogenic induction activity to human mesenchymal stem cells with prolonged stimulation effects even after 4 weeks of implantation. Along similar lines, bone-forming peptide 1 (BFP1) derived from the immature region of bone morphogenetic protein 7 (BMP7) was immobilized on electrospun PLGA nanofibers [64]. The BPF1 was immobilized with the aim of supporting the distribution of collagen I and the spreading of human mesenchymal stem cells. The fabricated scaffold was implanted onto mouse calvarial defects. The results showed that there was significant improvement in bone defect after 2 month as observed from semi-quantification of bone growth from representative X-ray images. A comparison was done between poly(lactic-co-glycolic acid)/tricalcium phosphate scaffolds incorporated or coated with osteogenic growth factors for enhancement of bone regeneration [65]. The result indicated that poly(lactic-co-glycolic acid)/tricalcium phosphate coated with BMP2 showed better bone regeneration ability as compared to the uncoated one. PLGA has also been used in combination with hydroxyappatite for fabrication of scaffold for bone regeneration [66,67]. Curran and coworkers reported the development of an injectable two-phase injectable PLGA system, utilizing plasma techniques, for the repair of bone defects [68]. Quantitative assays for cell viability and histological analysis for key markers of differentiation demonstrated that the developed injectable PLGA microspheres had the ability to induce MSC osteogenic and chondrogenic differentiation. Apart from cells and growth factors, peptides have also been immobilized on PLGA-based systems. Lin *et al.* and Pan *et al.* incorporated BMP-2-related peptide P24 and amphiphilic peptide Ac-RADA RADA RADA RADA S[PO4] KIPKASSVPTELSAISTLYLDDD-CONH2 (RADA16-P24) on PLGA respectively. Both exhibited strong osteogenic capability and effective ectopic bone formation [69,70]. Many such works have been reported, which proves that PLGA is a promising candidate in fabrication of scaffold/DDS for bone regeneration.

6.8 Pulmonary Delivery

It is a well-known fact that certain features of the formulation such as aerodynamic size, flow and aerosolization properties, particle size and shape does affect the deposition pattern of inhaled particles in lungs [71–73]. Particulate systems, including nanoparticles, microspheres, liposomes, etc., containing therapeutic agents have been extensively investigated to overcome the problems for effective treatment of various lung diseases such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF), which require an effective local delivery of therapeutic agents instead of systemic delivery. Since PLGA is biodegradable it has been widely explored in the form of microparticles and nanoparticles in pulmonary drug delivery for treatment of various lung diseases. The PLGA nanoparticles and microparticles have been used for encapsulating a variety of therapeutic moiety, including drug, protein, peptide, gene, etc. The PLGA-based microparticles that have been developed used various techniques, but solvent extraction/evaporation technique is the most widely used technique for microencapsulation of therapeutic molecule [74]. Rifampicin-loaded PLGA microparticles were developed to target alveolar macrophages in order to reduce systemic toxicity [75]. Results demonstrated that rifampicin-PLGA particles administered by nebulization and insufflation methods showed a 10-fold reduction in lung bacterial burden as compared to rifampicin administered alone. PLGA-PEG copolymer was used for delivering low molecular weight heparin for pulmonary delivery [76]. Fluorescent tagged PEG–PLGA particles were used for studying particle uptake in alveolar cells. The developed particle showed no cytotoxic effect on bronchial epithelial cells on incubation with PEG–PLGA-based formulations. Ungaro *et al.* developed antibiotic-loaded PLGA nanoparticles embedded in lactose microparticles to form nano-embedded microparticles (NEM) using the spray drying technique [77]. Silicon microfluidic flow focusing device (SMFFD) was used for fabrication of PLGA microparticles [78]. *In-vivo* biodistribution studies demonstrated that PLGA nanoparticles were found both in the deep part of the lung as well as the upper part, depending on the type of surface coating. The SMFFD is advantageous for giving precise geometry and dimensions of the flow focusing channel, which helps in easy optimization of microparticle by varying polymer, flow rate, continuous phase and disperse phase. The results indicated that SMFFD can be employed for obtaining microparticles with optimized size and surface morphology. Supercritical fluid pressure-quench technology was also employed for development of large porous microparticles of celecoxib [79]. The formed porous microparticles exhibited sustained drug delivery and anti-tumor efficacy, without causing any significant toxicity. A dry powder inhaler for delivery of insulin via pulmonary route was developed by Hamishehkar *et al.* [80]. Microcapsule/carrier powder mixtures were prepared using insulin-loaded PLGA microcapsules along with sorbitol or mannitol as the carriers, with various particle surface morphologies prepared by spray-drying and freeze-drying techniques. Insulin was also delivered using PLGA by formulating large porous particles using a double emulsion method with the aid of hydroxypropyl-β-cyclodextrin [81]. The obtained results indicated the viability of a dry powder formulation based on biodegradable

porous particles for the controlled release of insulin to the lungs. *In-vivo* data demonstrated that PLGA/HPβCD/insulin porous particles were able to reach alveoli and release insulin, which was then absorbed in its bioactive form. Similar work was done by development of inhalable dry powder for delivery of siRNA for treatment of severe lung disease [82]. Rifampicin is the most commonly used model drug for targeting the lung using PLGA nanoparticles. Apart from drugs, several other proteins and growth factors have been loaded on PLGA-based DDS. The DOTAP-modified PLGA was used for development of nanoparticles and mannitol was used for formulation of nanoparticle embedded microparticle by spray-drying technique [83]. This study demonstrated that spray drying is an excellent technique for engineering dry powder formulations of siRNA nanoparticles, which might enable the local delivery of biologically active siRNA directly to the lung tissue. DNA encoding *Mycobacterium tuberculosis* latency antigen Rv1733c was entrapped in co-formulation of PLGA-PEI (polyethylenimine) nanoparticles to evaluate the immunogenicity of DNA vaccine on host immunity [84]. The result demonstrated that the PLGA-PE nanoparticles were able to stimulate dendritic cells to secrete IL-12 and TNF-α comparable to levels observed after lipopolysaccharide (LPS) stimulation. Rv1733c DNA adsorbed on PLGA–PEI nanoparticles increased T cell proliferation and IFN-α production in lungs more potently. Working along similar lines of loading DNA encoding Rv1733c, Osman *et al.* loaded DNaseI on PLGA nanoparticle and assessed its cytotoxicity on lung epithelial cells [85]. It was found that a high respirable fraction (71.3%) of PLGA nanoparticles reached lungs after nebulization of nanoparticle suspension. The loaded DNaseI retained 76% biological activity, revealing the suitability of PLGA for delivery of DNaseI. Prostaglandin E1 (PGE1), a potent pulmonary vasodilator, was loaded on PLGA (85:15) using double emulsion/solvent evaporation method [86]. Similar porous particles of PLGA were also formulated keeping PEI as the core. The effect of PGE1-loaded PLGA was evaluated by measuring the mean pulmonary arterial pressure, right ventricular hypertrophy, degree of muscularization, platelet aggregation, matrix metalloproteinase-2 (MMP-2), and proliferating cell nuclear antigen. Microscopic and immunohistochemical results revealed that porous particles of PGE1 also reduced the degree of muscularization, von Willebrand factor (vWF), and PCNA expression in the lungs of rats. The overall study showed that PGE1-loaded inhalable PLGA formulation is suitable for inhibiting the progression of disease. In a recent published literature, Kim and coworkers used PLGA-based microparticles for dual drug delivery [87]. They formulated inhalable porous PLGA microparticles with incorporated doxorubicin and attached TRAIL on the surface of doxorubicin-loaded PLGA microparticles (TRAIL/Dox PLGA MP) using the double emulsification method with ammonium bicarbonate as a gas-foaming agent for the treatment of lung cancer. The loading efficiency of both doxorubicin and TRAIL was more than 85%. The obtained results from animal studies revealed that the doxorubicin and TRAIL exhibited a synergistic effect on tumor and so the inhibition was effective. Recently, an excellent review on engineered PLGA-based micro- and nanoparticles for pulmonary administration was published by Ungaro *et al.,* and the authors recommend referring to it for detailed information about PLGA-based DDS for pulmonary administration [88].

6.9 Gene Therapy

Non-viral gene carriers are preferred over viral gene carriers due to the safety of the former, although the transfection efficiency may be lower than the latter. Polyethylenimine has been considered as the gold standard for non-viral gene carrier and has been most widely investigated as gene carrier. PLGA has also been explored by various researchers as non-viral gene carrier. PLGA-based gene carriers have the ability to provide sustained release of gene. DNA is protected from nucleases when it is encapsulated into PLGA microspheres [89]. The DNA-loaded PLGA microsphere after intracellular uptake undergoes endo-lysosomal escape, releasing the encapsulated DNA at a sustained rate resulting in sustained gene expression. PLGA-based emulsion containing alkaline phosphatase was used as marker gene for coating gut suture [90]. The gene expression was observed afer weeks of implantation, which confirms the sustained release of gene from PLGA-based carriers. Similarly, marker gene was used for the study of sustained release of gene in cell culture in the presence of serum in a rat bone osteotomy model [91]. The effect of particle size on gene delivery was also studied by formulating PLGA nanoparticles using a double emulsion-solvent evaporation technique, and then nanoparticle fractions were separated by membrane filtration (100 nm size cutoff) and the transfection levels of the different fractions were evaluated in cell culture. The result demonstrated that lower size nanoparticle fraction produced a 27-fold higher transfection in COS-7 cells and 4-fold higher transfection in HEK 293 cells for the same dose of nanoparticles [92]. The toxicity studies for PLGA-based gene carrier did not reveal any toxicity to *in-vitro* cell culture and nanoparticles administered intravascularly in arterial tissue did not show untoward effects in animal models, demonstrating biocompatibility of nanoparticles [93]. Apart from nanoparticle and microsphere, many other systems have been explored for gene delivery using PLGA. PLGA films were explored as reservoirs for gene complex [94]. They demonstrated no cytotoxicity effects *in vitro*. Table 6.2 summarizes the different PLGA-based systems explored for gene delivery.

6.10 Tumor Trageting

PLGA-based materials have been used for tumor targeting, but amongst them, PLGA-based nanoparticles have been most widely explored. Various PLGA-based nanocarriers have been reported with effective tumor targeting and tumor inhibition ability. Many small anti-cancer drugs have been encapsulated in PLGA-based nanoparticles and have been evaluated *in vitro* and *in vivo* to treat various cancers. Apart from drugs, proteins and genes have also been entrapped in PLGA nanoparticles for cancer therapy. Entrapment of paclitaxel in PLGA nanoparticles exhibited enhanced antitumor efficacy as compared with free paclitaxel [108]. Similarly, intravenous administration of PLGA-based polymeric nanoparticles incorporating paclitaxel and C6-ceramide exhibited a longer retention time in blood and enhanced accumulation inside the tumor as compared to free paclitaxel [109]. Along similar lines, docetaxel was also incorporated for enhancing its antitumor activity. Research demonstrated that the antitumor activity

Table 6.2 Various PLGA-based systems explored for gene delivery.

Formulation	System	Cargo	Ref.
Nanoparticles	Poloxamer and PLGA	FITC-labeled plasmid DNA	[95]
Microsphere	PLGA and PEI	Phosphodiester oligothymidilate pdT16 oligonucleotide	[96,97]
Disk	PLG and PEI	Nuclear targeted β-galactosidase	[98]
Nanoparticles	Calcium phosphate and PLGA	Plasmid DNA	[99]
Nanoparticles	PLGA	SOX9 genes and anti-Cbfa-1 siRNA	[100]
Micelles	PLGA and PEI	Plasmid DNA	[101]
Micelles	PLGA and PEI	siRNA	[102]
Scaffold	PLGA and PLA-PEG	Plasmid DNA	[103]
Nanosphere	PLGA, DOTAP and Chitosan	pDNA (pCMV-Luciferase)	[104]
Sponge	PLGA and PEI	pNGVL plasmid encoding for nucleus-targeting galactosidase	[105]
Electrospun composite fiber	PLGA and Chitosan	PT7T3D-PacI encoding BMP-2 plasmid	[106]
Gel construct	PLGA and Fibrin	lipofectamine/pDNA-TGF-β1 complexes and mesenchymal stem cells (MSCs)	[107]

of PLGA entrapped docetaxel was higher as compared to free drug [110]. PEGylated PLGA nanoparticles were used for entrapment of cisplatin, which demonstrated higher residence time in prostate cancer [111]. Based on the above results, it can be concluded that the antitumor activity of anticancer drugs is enhanced after being entrapped in PLGA-based nanoparticles and so can be considered as promising nanocarrier for delivery of anticancer drugs. PLGA-based nanoparticles targeting the tumor cells or tumor endothelium using cyclic peptide, Cyclo-(1,12)-pen ITDGEATDSGC (cLABL), demonstrated enhanced inhibition of LFA-1/ ICAM-1 along with high cellular internalization [112]. Various proteins and genes have been studied after entrapment in PLGA-based nanoparticles for their antitumor activity. The anti-angiogenic peptide, Endostar, when loaded into PLGA-based polymeric nanoparticles, exhibited a longer halflife and decrease in dosing frequency as compared to those without Endostar. Study on a murine tumor model demonstrated higher tumor inhibition rate owing to its anti-angiogenic effect after being loaded into PLGA nanoparticles. Recombinant human granulocyte colony-stimulating factor and toxin protein PE38KDL exhibited sustained

release when administered via PLGA nanoparticles for more than 1 week and also showed enhanced *in-vitro* cytotoxicity. PLGA nanoparticle encapsulating plasmid coding for AnxA2 shRNA, expression of AnxA2, shRNA, CLDN3, cDNA demonstrated significant reduction in the tumor growth along with an increase in average survival rate among animals [113–118]. When entrapped in PLGA nanoparticles, plasmid coding for siRNA sequence targeting Methyl-CpG binding domain protein 1 showed enhanced uptake by BxPC3 tumor cells, leading to efficient cell growth inhibition and tumor cell apoptosis [119]. Similarly, BCL-w siRNA was loaded onto PLGA nanoparticles in combination with PEI or cet-PEI. The addition of PEI enhanced the cellular uptake of nanoparticles and more than 60% silencing of BCL-w mRNA was observed in BCL-w siRNA loaded with PLGA-PEI nanoparticles [120]. A lot of similar research involving genes, peptides and drugs has been carried out using PLGA-based nanoparticles, and results have supported the fact that this delivery system holds the potential for delivery of therapeutic moieties. With the FDA approving various PLGA-based DDSs which also include various anticancer formulations, the future seems to embrace the PLGA-based DDS for various other cancers, which probably could minimize the toxicity to normal cells and enhance the efficacy of cancer therapy.

6.11 Miscellaneous Drug Delivery Applications

Apart from the above-mentioned delivery systems, PLGA-based carriers have also been employed for delivering drug for various other applications. In an attempt to enhance bioavailability, flurbiprofen was loaded onto PLGA nanoparticles. The nanoparticle showed controlled release of drug in addition to a twofold increase in bioavailability. The studies demonstrated the absence of ocular toxicity or damage to eye tissues after administration of flurbiprofen-loaded PLGA nanoparticles [121]. Similarly, Araújo *et al.* loaded fkurbiprofen on PLGA nanoparticle to minimize inflammation induced by surgical trauma [122]. The results revealed long-term stability for ophthalmic use, and HETCAM assay showed absence of ocular irritancy. In another work, Gupta *et al.* loaded sparfloxacin onto PLGA nanoparticles for ocular delivery [123]. Precorneal residence time of prepared sparfloxacin PLGA nanoparticles was studied in albino rabbits by gamma scintigraphy after radiolabeling of sparfloxacin by Tc-99 m. Results demonstrated the absence of radioactivity in systemic circulation even after 6 hr of administration, suggesting an increase in residence time of PLGA nanoparticles on corneal surface. Olanzapine-loaded PLGA nanoparticles were fabricated for nose-to-brain delivery [124]. The results of *in-vivo* pharmacokinetic studies demonstrated 6.35 and 10.86 times higher uptake of intranasally delivered PLGA nanoparticle than pain drug solution delivered through intravenous and intranasal route, respectively, which proves the superiority of PLGA-based nanoparticle for delivery of centrally acting drugs. For enhancing the corneal permeation of drug, chitosan-PLGA nanoplexes were formulated using fluorescent dye rhodamine [125]. Confocal microscopy of the corneas revealed paracellular and transcellular uptake of the nanoplexes, but no alteration was microscopically observed after ocular surface exposure to nanoplexes. Recently, daunorubicin was loaded into oxidized porous silicon microparticles, and microparticles were then encapsulated with a layer of PLGA to investigate their synergistic effects

in control of daunorubicin release from microparticles [126]. The PLGA–silica microparticle composite displayed a more constant rate of daunorubicin release than the porous silica control formulation. It was concluded from the study that this system is encouraging and may be of value in managing unwanted ocular proliferation through a single intravitreal injection.

PLGA-based systems were also developed for vaginal drug delivery for treatment of HIV infections. Topical PLGA nanoparticles entrapping camptothecin were formulated for the prevention of intravaginal tumor [127]. The effectiveness of the developed formulation was tested on a vaginal tumor model in mice. The results of the study demonstrated that campothecin-loaded PLGA nanoparticles completely prevented growth of tumors and hence can be used for effective treatment of intravaginal tumors. Further surface modification of PLGA using poly(ethylene glycol) was done to increase the penetration of PLGA nanoparticles inside the mucus layer of vaginal epithelium [128]. Delivery of siRNA using PLGA nanoparticle had deep vaginal penetration of nanoparticle and exhibited prolonged gene silencing activity. Various other studies involving delivery of drugs using PLGA nanoparticles have been done for exploring the use of PLGA-based carriers in the field of drug delivery such as cerebral delivery, cardiovascular disease, arthritis and regenerative medicine. Research on PLGA-based carriers for drug delivery is still going on, with PLGA-based nanoparticles holding the most promising potential.

6.12 Conclusion

The above discussion leads us to conclude that PLGA-based carriers continue to be widely explored in the field of drug delivery owing to their biocompatibility and nontoxic nature. They can protect drugs from degradation and enhance their stability. Use of surface functionalizing agent can further allow a specific delivery of drugs, proteins, peptides or nucleic acids to their target tissue. The approval of PLGA by the FDA opens up new avenues for PLGA-based DDS ongoing clinical trials. Further research on PLGA-based systems, especially nanoparticles, which hold the most promising position in recent times for being brought to market in the near future, can change conventional drug delivery system by increasing efficacy. The future may even see the development of various PLGA-based imaging tools or, more specifically, PLGA-based theranostic tools, which would help in the delivery of therapeutic moiety while simultaneously allowing imaging of its release.

References

1. I. Armentano, M. Dttori, E. Fortunati, *et al*. Biodegradable polymer matrix nanocomposites for tissue engineering: A review, *Polym Degrad Stab* 95, 2126–46, 2010.
2. X. Wang, L. Yang, Z.G. Chen, D.M. Shin. Application of nanotechnology in cancer therapy and imaging. *CA Cancer J Clin* 58, 97–110, 2008.
3. C.E. Astete, C.M. Sabliov. Synthesis and characterization of PLGA nanoparticles. *J Biom Sci, Polymer Ed* 17, 247–89, 2006.

4. J.M. Lü, X. Wang, C. Marin-Muller, *et al*. Current advances in research and clinical applications of PLGA-based nanotechnology. *Expert Rev Mol Diagn* 9, 325–41, 2009.
5. S.K. Sahoo, V. Labhasetwar, in: M. Amiji (ed.), *Biodegradable PLGA/PLA Nanoparticles for Anticancer Therapy*, CRC Press, pp. 243–250, 2007.
6. X. Wu. Synthesis and properties of biodegradable lactic/ glycolic acid polymers. In: D.L. Wise, D.J. Trantolo, D.E. Altobelli, M.J. Yaszemski, J.D. Gresser, E.R. Schwartz (eds.), *Encyclopedic Handbook of Biomaterials and Bioengineering. Part A. Materials*, vol 2. Marcel Dekker, New York, pp 1015–1054, 1995.
7. R.A. Jain. The manufacturing techniques of various drug loaded biodegradable poly (D,L-lactide-coglycolide) (PLGA) devices. *Biomaterials* 21(23), 2475–90, 2000.
8. K.M. Huh, Y.W. Cho, K. Park. PLGA-PEG block copolymers for drug formulations. *Drug Deliv Technol* 3(5), 2003.
9. E. Alarçin, O. Sipahigil, M. Türkoğlu, *et al*. Cell proliferation and cytotoxicity evaluation of vascular endothelial growth factor loaded poly(lactic-coglycolic acid) microspheres, *15th International Pharmaceutical Technology Symposium, Proceeding Abstracts*, p: 121–2, Antalya (2010).
10. I. Bala, S. Hariharan, M.N. Kumar. PLGA nanoparticles in drug delivery: The state of the art. *Crit Rev Ther Drug Carrier Syst* 21(5), 387–422, 2004.
11. M.N. Ravi Kumar, U. Bakowsky, C.M. Lehr. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. *Biomaterials* 25(10), 1771–7, 2004.
12. O. Sipahigil, E. Alarçin, M. Türkoğlu, *et al*. Characterization, cell proliferation and cytotoxicity evaluation of vascular endothelial growth factor loaded poly(lactic-co-glycolic acid) microspheres. *Nobel Med* 8(1), 77–82, 2012.
13. Y. Tabata, M. Miyao, M. Ozeki, Y. Ikada. Controlled release of vascular endothelial growth factor by use of collagen hydrogels. *Biomater Sci Polym Ed* 11(9), 915–930, 2000.
14. R.C. Mundargi, V.R. Babu, V. Rangaswamy, P. Patel, T.M. Aminabhavi. Nano/micro technologies for delivering macromolecular therapeutics using poly (D,L-lactide-co-glycolide) and its derivatives. *J Contr Release* 125(3), 193–209, 2008.
15. E. Nieddu, L. Mazzucco, P. Gentile, *et al*. Preparation and biodegradation of clay composites of PLA. *Reactive Functional Polymers* 69(6), 371–9, 2009.
16. B.D. Ralner, S. Hoffman, F.J. Schoen, *et al*. *Biomaterials Science: An Introduction to Materials in Medicine*, 2nd ed., Elsevier Academic Press, China, 2004.
17. X.S. Wu. Synthesis and properties of biodegradable lactic/glycolic acid polymers. In: Wise, *et al*. (eds.), *Encyclopedic Handbook of Biomaterials and Bioengineering*, p. 1151–200, Marcel and Dekker, New York, 1995.
18. M.L. Houchin, E.M. Topp, Physical properties of PLGA films during polymer degradation. *J Appl Polym Sci* 114, 2848–2854, 2009.
19. D.H. Lewis. Controlled release of bioactive agents from lactide/glycolide polymers. In: M. Chasin, R. Langer (eds.), *Biodegradable Polymers as Drug Delivery Systems*, p. 1–41, Marcel Dekker, New York, 1990.
20. M.S. Shive, J.M. Anderson. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev* 28(1), 5–24, 1997
21. S. Kamei, Y. Inoue, H. Okada, M. Yamada, Y. Ogawa, H. Toguchi. New method for analysis of biodegradable polyesters by high performance liquid chromarography after alkali hydrolysis. *Biomaterials* 33(13), 953–8, 1992.
22. A.K. Mohammad and J.J. Reineke, Quantitative detection of PLGA nanoparticle degradation in tissues following intravenous administration. *Mol Pharm* 10, 2183–2189, 2013.
23. V.R. Sinha, K. Bansal, R. Kaushik, R. Kumria, A. Trehan. Poly-(epsilon-caprolactone) microspheres and nanospheres: An overview. *Int J Pharm* 278, 1–23, 2004.

24. L. Guzman, V. Labhasetwar, C. Song, Y. Jang, A. Lincoff, R. Levy, E. Topol. Local intraluminal infusion of biodegradable polymeric nanoparticles. A novel approach for prolonged drug delivery after balloon angioplasty. *Circulation* 94,1441–1448, 1996.
25. B. Semete, L. Booysen, Y. Lemmer, L. Kalombo, L. Katata, J. Verschoor, H.S. Swai. In vivo evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems. *Nanomedicine: Nanotechnology, Biology, and Medicine* 6, 662–671, 2010.
26. Y.Y. Yang, T.S. Chung, N.P. Ng. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. *Biomaterials* 22, 231–241, 2001.
27. Z. Panagi, A. Beletsi, G. Evangelatos, E. Livaniou, D.S. Ithakissios, K. Avgoustakis. Effect of dose on the biodistribution and pharmacokinetics of PLGA and PLGA-mPEG nanoparticles. *Int J Pharm* 221, 143–152, 2001.
28. H.K. Makadia, S.J. Siegel. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier, *Polymers* 3, 1377–1397, 2011.
29. D.V. Bazile, C. Ropert, P. Huve, T. Verrecchia, M. Marlard, A. Frydman, M. Veillard, G. Spenlehauer. Body distribution of fully biodegradable [14C]-poly(lactic acid) nanoparticles coated with albumin after parenteral administration to rats. *Biomaterials* 13, 1093–1102, 1992.
30. S. Fredenberga, M. Wahlgren, M. Reslow, A. Axelsson, The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems – A review. *Int J Pharm* 415, 34–52, 2011.
31. R.P. Batycky, J. Hanes, R. Langer, D.A. Edwards. A theoretical model of erosion and macromolecular drug release from biodegrading microspheres. *J Pharm Sci* 86, 1464–1477, 1997.
32. P. Blasi, S.S. D'Souza, F. Selmin, P.P. DeLuca. Plasticizing effect of water on poly(lactide-co-glycolide). *J Control Release* 108, 1–9, 2005.
33. A. Mochizuki, T. Niikawa, I. Omura, S. Yamashita. Controlled release of argatroban from PLA film – Effect of hydroxylesters as additives on enhancement of drug release. *J Appl Polym Sci* 108, 3353–3360, 2008.
34. A. Shenderova, T.G. Burke, S.P. Schwendeman. The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharm Res* 16, 241–248, 1999.
35. X. Chen, C.P. Ooi. Effect of ganciclovir on the hydrolytic degradation of poly(lactide-co-glycolide) microspheres. *J Biomater Appl* 20, 287–302, 2006.
36. N.S. Berchane, K.H. Carson, A.C. Rice-Ficht, M.J. Andrews. Effect of mean diameter and polydispersity of PLG microspheres on drug release: Experiment and theory. *Int J Pharm* 337, 118–126, 2007.
37. C. Berkland, K.K. Kim, D.W. Pack, PLG microsphere size control drug release rate through several competing factors. *Pharm Res* 20, 1055–1062, 2003.
38. S. Fredenberg. PLG films in controlled release pharmaceuticals – Release mechanisms. Doctoral Dissertation. Department of Chemical Engineering, Lund University, Sweden, 2011.
39. S. Fredenberg, M. Wahlgren, M. Reslow, A. Axelsson. Pore formation and pore closure in poly(d,l-lactide-co-glycolide) films. *J Control Release* 150, 142–149, 2011.
40. P. Sansdrap, A.J. Moës. In vitro evaluation of the hydrolytic degradation of dispersed and aggregated poly(d,l-lactide-co-glycolide) microspheres. *J Control Release* 43, 47–58, 1997.
41. C. Berkland, M. King, A. Cox, K.K. Kim, D.W. Pack. Precise control of PLG microsphere size provides enhanced control of drug release rate. *J Control Release* 82, 137–147, 2002.
42. J. Wang, B.M. Wang, S.P. Schwendeman. Characterization of the initial burst release of a model peptide from poly(d,l-lactide-co-glycolide) microspheres. *J Control Release* 82, 289–307, 2002.

43. X. Huang, C.S. Brazel. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *J Control Release* 73, 121–136, 2001.
44. S.E. Bae, J.S. Son, K. Park, D.K. Han. Fabrication of covered porous PLGA microspheres using hydrogen peroxide for controlled drug delivery and regenerative medicine. *J Control Release* 133, 37–43, 2009.
45. B. Han, S.-Z. Gao, X.-H. Zhang, H.-B. Tian, H.-T. Wang. Preparation of aclarubicin PLGA nanospheres and related in vitro/in vivo studies. *J Appl Polym Sci* 117, 2754–2761, 2010.
46. M.D. Blanco, M.J. Alonso. Development and characterization of proteinloaded poly(lactide-co-glycolide) nanospheres. *Eur J Pharm Biopharm* 43, 287–294, 1997.
47. J. Kang, O. Lambert, M. Ausborn, S.P. Schwendeman. Stability of proteins encapsulated in injectable and biodegradable poly(lactide-co-glycolide)-glucose millicylinders. *Int J Pharm* 357, 235–243, 2008.
48. J. Kang, S.P. Schwendeman. Pore closing and opening in biodegradable polymers and their effect on the controlled release of proteins. *Mol Pharm* 4,104–118, 2007.
49. G.L. Barnes, P.J. Kostenuik, L.C. Gerstenfeld, T.A. Einhorn. Growth factor regulation of fracture repair. *J Bone Miner Res* 14, 1999.
50. A. Schindeler, M.M. McDonald, P. Bokko, D.G. Little, Bone remodeling during fracture repair: The cellular picture. *Semin Cell Dev Biol* 19, 459–466, 2008.
51. S.S. Kim, M.S. Park, O. Jeon, C.Y. Choi, B.S. Kim. Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. *Biomaterials* 27, 1399–1409, 2006.
52. Y. Cui, Y. Liu, Y. Cui, X.B. Jing, P.B.A. Zhang, X.S. Chen. The nanocomposite scaffold of poly(lactide-co-glycolide) and hydroxyapatite surface-grafted with L-lactic acid oligomer for bone repair. *Acta Biomater* 5, 2680–2692, 2009.
53. M. Okamoto, B. John. Synthetic biopolymer nanocomposites for tissue engineering scaffolds. *Prog Polym Sci* 38, 1487–1503, 2013.
54. C. Forgacs, W. Sun. *Biofabrication: Micro- and Nano-Fabrication, Printing, Patterning, and Assemblies*, William Andrew Publishing: Norwich, NY, USA, pp. 1–265, 2013.
55. J.H. Shim, T.S. Moon, M.J. Yun, Y.C. Jeon, C.M. Jeong, D.W. Cho, J.B. Huh. Stimulation of healing within a rabbit calvarial defect by a PCL/PLGA scaffold blended with TCP using solid freeform fabrication technology. *J Mater Sci Mater Med* 23, 2993–3002, 2012.
56. J.K. Park, J.H. Shim, K.S. Kang, J. Yeom, H.S. Jung, J.Y. Kim, K.H. Lee, T.H. Kim, S.Y. Kim, D.W. Cho, *et al*. Solid free-form fabrication of tissue-engineering scaffolds with a poly(lactic-co-glycolic acid) grafted hyaluronic acid conjugate encapsulating an intact bone morphogenetic protein-2/poly(ethylene glycol) complex. *Adv Funct Mater* 21, 2906–2912, 2011.
57. Y. Xia, P. Zhou, X. Cheng, Y. Xie, C. Liang, C. Li, S. Xu. Selective laser sintering fabrication of nano-hydroxyapatite/poly-epsilon-caprolactone scaffolds for bone tissue engineering applications. *Int J Nanomed* 8, 4197–4213, 2013.
58. S.J. Lee, K. Gilson, Y.M. Lee, H.B. Lee. Interaction of human chondrocytes and NIH/3T3 fibroblasts on chloric acid-treated biodegradable polymer surfaces. *J Biomater Sci Polym Ed* 13, 197–212, 2002.
59. Z.Q. Fei, Y. Hu, D. Wu, H. Wu, R. Lu, J. Bai, H. Song. Preparation and property of a novel bone graft composite consisting of rhBMP-2 loaded PLGA microspheres and calcium phosphate cement. *J Mater Sci: Mater Med* 19, 1109–1116, 2008.
60. H. Nie, M.-L. Ho, C.-K. Wang, C.-H. Wang, Y.-C. Fu. BMP-2 plasmid loaded PLGA/HAp composite scaffolds for treatment of bone defects in nude mice. *Biomaterials* 30, 892–901, 2009.
61. N.-J. Chang, C.-F. Lam, C.-C. Lin, W.-L. Chen, C.-F. Li, Y.-T. Lin, M.-L. Yeh. Transplantation of autologous endothelial progenitor cells in porous PLGA scaffolds create a microenvironment for the regeneration of hyaline cartilage in rabbits. *Osteoarthritis and Cartilage* 21, 1613–1622, 2013.

62. B.H. Park, L. Zhou, K.Y. Jang, H.S. Park, J.M. Lim, S.J. Yoon, S.Y. Lee, J.R. Kim. Enhancement of tibial regeneration in a rat model by adipose-derived stromal cells in a PLGA scaffold. *Bone* 51, 313–323, 2012.
63. H. Lu, N. Kawazoe, T. Kitajima, Y. Myoken, M. Tomita, A. Umezawa, G. Chen, Y. Ito. Spatial immobilization of bone morphogenetic protein-4 in a collagen-PLGA hybrid scaffold for enhanced osteoinductivity. *Biomaterials* 33, 6140–6146, 2012.
64. Y.J. Lee, J.H. Lee, H.J. Cho, H.K. Kim, T.R. Yoon, H. Shin. Electrospun fibers immobilized with bone forming peptide-1 derived from BMP7 for guided bone regeneration. *Biomaterials* 34, 5059–5069, 2013.
65. S. Chen, L. Zheng, X. Xie, X. Wang, Y. Lai, S. Chen, M. Zhang, Y. Wang, J.F. Griffith, L. Qin. Comparative study of poly (lactic-co-glycolic acid)/tricalcium phosphate scaffolds incorporated or coated with osteogenic growth factors for enhancement of bone regeneration. *Journal of Orthopaedic Translation* 2, 91–104, 2014.
66. S.-W. Choi, Y. Zhang, S. Thomopoulos, Y. Xia. In vitro mineralization by preosteoblasts in poly(DL-lactide-co-glycolide) inverse opal scaffolds reinforced with hydroxyapatite nanoparticles. *Langmuir* 26(14), 12126–12131, 2010.
67. M. Li, W. Liu, J. Sun, Y. Xianyu, J. Wang, W. Zhang, W. Zheng, D. Huang, S. Di, Y.-Z. Long, and X. Jiang. Culturing primary human osteoblasts on electrospun poly(lactic-coglycolic acid) and poly(lactic-co-glycolic acid)/nanohydroxyapatite scaffolds for bone tissue engineering. *ACS Appl Mater Interfaces* 5, 5921–5926, 2013.
68. J.M. Curran, S. Fawcett, L. Hamilton, N.P. Rhodes, C.V. Rahman, M. Alexander, K. Shakesheff, J.A. Hunt. The osteogenic response of mesenchymal stem cells to an injectable PLGA bone regeneration system. *Biomaterials* 34, 9352–9364, 2013.
69. Z.-Y. Lin, Z.-X. Duan, X.-D. Guo, J.-F. Li, H.-W. Lu, Q.-X. Zheng, D.-P. Quan, S.-H. Yang. Bone induction by biomimetic PLGA-(PEG-ASP)n copolymer loaded with a novel synthetic BMP-2-related peptide in vitro and in vivo. *J Control Release* 144, 190–195, 2010.
70. H. Pan, S. Hao, Q. Zheng, J. Li, J. Zheng, Z. Hu, S. Yang, X. Guo, Q. Yang. Bone induction by biomimetic PLGA copolymer loaded with a novel synthetic RADA16-P24 peptide in vivo. *Mater Sci Eng C* 33 (2013) 3336–3345.
71. S. Newman, *et al*. *Respiratory Drug Delivery: Essential Theory & Practice*. Richmond, VA: RDD Online, 2009.
72. S. Onoue, *et al*. New treatments for chronic obstructive pulmonary disease and viable formulation/device options for inhalation therapy. *Expert Opin Drug Deliv* 6, 793–811, 2009.
73. B.M. Ibrahim, *et al*. Challenges and advances in the development of inhalable drug formulations for cystic fibrosis lung disease. *Expert Opin Drug Deliv* 8, 451–466, 2011.
74. R.C. Mundargi, *et al*. Nano/micro technologies for delivering macromolecular therapeutics using poly (D,L-lactide-co-glycolide) and its derivatives. *J Control Release* 125, 193–209, 2008.
75. S. Suarez, P. O'Hara, M. Kazantseva, C.E. Newcomer, R. Hopfer, D.N. McMurray, A.J. Hickey. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: Screening in an infectious disease model. *Pharm Res* 18, 1315–1319, 2001.
76. B. Patel, V. Gupta, F. Ahsan. PEG–PLGA based large porous particles for pulmonary delivery of a highly soluble drug, low molecular weight heparin. *J Control Release* 162, 310–320, 2012.
77. F. Ungaro, I. d'Angelo, C. Coletta, R. d'Emmanuele di Villa Bianca, R. Sorrentino, B. Perfetto, M.A. Tufano, A. Miro, M.I. La Rotonda, F. Quaglia. Dry powders based on PLGA nanoparticles for pulmonary delivery of antibiotics: Modulation of encapsulation efficiency, release rate and lung deposition pattern by hydrophilic polymers. *J Control Release* 157, 149–159, 2012.

78. K. Keohane, D. Brennan, P. Galvin, B.T. Griffin. Silicon microfluidic flow focusing devices for the production of size controlled PLGA based drug loaded microparticles. *Int J Pharm* 467, 60–69, 2014.
79. D.S. Dhanda, P. Tyagi, S.S. Mirvish, U.B. Kompella. Supercritical fluid technology based large porous celecoxib–PLGA microparticles do not induce pulmonary fibrosis and sustain drug delivery and efficacy for several weeks following a single dose. *J Control Release* 168, 239–250, 2013.
80. H. Hamishehkar, J. Emami, A. Rouholamini Najafabadi, K. Gilani, M. Minaiyan, H. Mahdavi, A. Nokhodchi. Effect of carrier morphology and surface characteristics on the development of respirable PLGA microcapsules for sustained-release pulmonary delivery of insulin. *Int J Pharm* 389, 74–85, 2010.
81. F. Ungaro, R. d'Emmanuele di Villa Bianca, C. Giovino, A. Miro, R. Sorrentino, F. Quaglia, M.I. La Rotonda. Insulin-loaded PLGA/cyclodextrin large porous particles with improved aerosolization properties: In vivo deposition and hypoglycaemic activity after delivery to rat lungs. *J Control Release* 135, 25–34, 2009.
82. D.K. Jensen, L.B. Jensen, S. Koocheki, L. Bengtson, D. Cun, H.M. Nielsen, C. Foged. Design of an inhalable dry powder formulation of DOTAP-modified PLGA nanoparticles loaded with siRNA. *J Control Release* 157, 141–148, 2012.
83. D.K. Jensen, L.B. Jensen, S. Koocheki, L. Bengtson, D. Cun, H.M. Nielsen, C. Foged. Design of an inhalable dry powder formulation of DOTAP-modified PLGA nanoparticles loaded with siRNA. *J Control Release* 157, 141–148, 2012.
84. M. Bivas-Benita, M.Y. Lin, S.M. Bal, K.E. van Meijgaarden, K.L.M.C. Franken, A.H. Friggen, H.E. Junginger, G. Borchard, M.R. Klein, T.H.M. Ottenhoff. Pulmonary delivery of DNA encoding *Mycobacterium tuberculosis* latency antigen Rv1733c associated to PLGA–PEI nanoparticles enhances T cell responses in a DNA prime/protein boost vaccination regimen in mice. *Vaccine* 27, 4010–4017, 2009.
85. R. Osman, P.L. Kan, G. Awad, N. Mortada, A.E. El-Shamy, O. Alpar. Enhanced properties of discrete pulmonary deoxyribonuclease I (DNaseI) loaded PLGA nanoparticles during encapsulation and activity determination. *Int J Pharm* 408, 257–265, 2011.
86. V. Gupta, N. Gupta, I.H. Shaik, R. Mehvar, E. Nozik-Grayck, I.F. McMurtry, M. Oka, M. Komatsu, and F. Ahsan. Inhaled PLGA particles of prostaglandin E1 ameliorate symptoms and progression of pulmonary hypertension at a reduced dosing frequency. *Mol Pharm* 10, 1655–1667, 2013.
87. I. Kim, H.J. Byeon, T.H. Kim, E.S. Lee, K.T. Oh, B.S. Shin, K.C. Lee, Y.S. Youn. Doxorubicin-loaded porous PLGA microparticles with surface attached TRAIL for the inhalation treatment of metastatic lung cancer. *Biomaterials* 34, 6444–6453, 2013.
88. F. Ungaro, I. d'Angelo, A. Miro, M.I. La Rotonda, F. Quaglia. Engineered PLGA nano- and micro-carriers for pulmonary delivery: Challenges and promises. *J Pharm Pharmacol* 64, 1217–1235, 2012.
89. V. Labhasetwar, J. Bonadio, S.A. Goldstein, R.J. Levy. Gene transfection using biodegradable nanospheres: Results in tissue culture and a rat osteotomy model. *Colloids Surfaces B: Biointerfaces* 16, 281–290, 1999.
90. V. Labhasetwar, J. Bonadio, S. Goldstein, W. Chen, R.J. Levy. A DNA controlled release coating for gene transfer: Transfection in skeletal and cardiac muscle. *J Pharm Sci* 87, 1347–1350, 1998.
91. H. Cohen, R.J. Levy, J. Gao, I. Fishbein, V. Kousaev, S. Sosnoski, S. Slomkowski. Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. *Gene Ther* 7, 1896–1905, 2000.
92. S. Prabha, W.Z. Zhou, J. Panyam, V. Labhasetwar. Size-dependency of nanoparticle mediated gene transfection: Studies with fractionated nanoparticles. *Int J Pharm* 244, 105–115, 2002.

93. V. Labhasetwar, C. Song, R.J. Levy. Nanoparticle drug delivery for restenosis. *Adv Drug Del Rev* 24, 63–85, 1997.
94. A.U. Bielinska, A. Yen, H.L. Wu, K.M. Zahos, R. Sun, N.D. Weiner, *et al*. Application of membrane-based dendrimer/DNA complexes for solid phase transfection in vitro and in vivo. *Biomaterials* 21, 877–87, 2000.
95. N. Csaba, A. Sánchez, M.J. Alonso. PLGA: Poloxamer and PLGA: Poloxamine blend nanostructures as carriers for nasal gene delivery. *J Control Release* 113, 164–172, 2006.
96. G. De Rosa, F. Quaglia, A. Bochot, F. Ungaro, E. Fattal. Long-term release and improved intracellular penetration of oligonucleotide; Polyethylenimine complexes entrapped in biodegradable microspheres. *Biomacromolecules* 4, 529–36, 2003.
97. G. De Rosa, F. Quaglia, M.I. La Rotonda, M. Besnard, E. Fattal. Biodegradable microparticles for the controlled delivery of oligonucleotides. *Int J Pharm* 242, 225–8, 2002.
98. Z. Bengali, A.K. Pannier, T. Segura, B.C. Anderson, J.-H. Jang, T.A. Mustoe, *et al*. Gene delivery through cell culture substrate adsorbed DNA complexes. *Biotechnol Bioeng* 90, 290–302, 2005.
99. J. Tang, *et al*. Calcium phosphate embedded PLGA nanoparticles: A promising gene delivery vector with high gene loading and transfection efficiency. *Int J Pharm* 431, 210–221, 2012.
100. S.Y. Jeon, J.S. Park, H.N. Yang, D.G. Woo, K.-H. Park. Co-delivery of SOX9 genes and anti-Cbfa-1 siRNA coated onto PLGA nanoparticles for chondrogenesis of human MSCs. *Biomaterials* 33, 4413–4423, 2012.
101. D. Mishra, H.C. Kang, Y.H. Bae. Reconstitutable charged polymeric (PLGA)2-b-PEI micelles for gene therapeutics delivery. *Biomaterials* 32, 3845–3854, 2011.
102. S.H. Lee, H. Mok, Y. Lee, T.G. Park. Self-assembled siRNA–PLGA conjugate micelles for gene silencing. *J Control Release* 152, 152–158, 2011.
103. Y.K. Luu, K. Kim, B.S. Hsiao, B. Chu, M. Hadjiargyrou, Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA–PEG block copolymers. *J Control Release* 89, 341–353, 2003
104. K. Tahara, T. Sakai, H. Yamamoto, H. Takeuchi, Y. Kawashima. Establishing chitosan coated PLGA nanosphere platform loaded with wide variety of nucleic acid by complexation with cationic compound for gene delivery. *Int J Pharm* 354, 210–216, 2008.
105. Y.-C. Huang, K. Riddle, K.G. Rice, D.J. Mooney. Long-term in vivo gene expression via delivery of PEI DNA condensates from porous polymer scaffolds. *Hum Gene Ther* 16, 609, 2005.
106. H. Nie, C.-H. Wang. Fabrication and characterization of PLGA/Hap composite scaffolds for delivery of BMP-2 plasmid DNA. *J Control Release* 120, 111–21, 2007.
107. B. Li, F. Li, L. Ma, J. Yang, C. Wang, D. Wang, and C. Gao. Poly(lactide-co-glycolide)/fibrin gel construct as a 3D model to evaluate gene therapy of cartilage in vivo. *Mol Pharm*, in press.
108. C. Fonseca, S. Simoes, R. Gaspar. Paclitaxel-loaded PLGA nanoparticles: Preparation, physicochemical characterization and in vitro anti-tumoral activity. *J Control Release* 83(2), 273–286, 2002.
109. L.E. Van Vlerken, Z. Duan, S.R. Little, M.V. Seiden, M.M. Amiji. Biodistribution and pharmacokinetic analysis of paclitaxel and ceramide administered in multifunctional polymer-blend nanoparticles in drug resistant breast cancer model. *Mol Pharm* 5(4), 516–526, 2008.
110. F. Esmaeili, R. Dinarvand, M.H. Ghahremani, S.N. Ostad, H. Esmaily, F. Atyabi. Cellular cytotoxicity and in-vivo biodistribution of docetaxel poly (lactide-co-glycolide) nanoparticles. *Anticancer Drugs* 21(1), 43–52, 2010.
111. E.C. Gryparis, M. Hatziapostolou, E. Papadimitriou, K. Avgoustakis. Anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on LNCaP prostate cancer cells. *Eur J Pharm Biopharm* 67(1), 1–8, 2007.

112. C. Chittasupho, S.X. Xie, A. Baoum, T. Yakovleva, T.J. Siahaan, C.J. Berkland. ICAM-1 targeting of doxorubicin-loaded PLGA nanoparticles to lung epithelial cells. *Eur J Pharm Sci* 37, 141–150, 2009.
113. S.H. Choi, T.G. Park, G-CSF loaded biodegradable PLGA nanoparticles prepared by a single oil-in-water emulsion method. *Int J Pharm* 311, 223–228, 2006.
114. H. Chen, J. Gao, Y. Lu, G. Kou, H. Zhang, L. Fan, Z. Sun, Y. Guo, Y. Zhong. Preparation and characterization of PE38KDEL-loaded anti-HER2 nanoparticles for targeted cancer therapy. *J Control Release* 128, 209–216, 2008.
115. A.R. Braden, M.T. Kafka, L. Cunningham, H. Jones, J.K. Vishwanatha. Polymeric nanoparticles for sustained down-regulation of annexin A2 inhibit prostate tumor growth. *J Nanosci Nanotechnol* 9, 2856–2865, 2009.
116. L.B. Rangel, R. Agarwal, T. D'Souza, E.S. Pizer, P.L. Alo, W.D. Lancaster, L. Gregoire, D.R. Schwartz, K.R. Cho, P.J. Morin. Tight junction proteins claudin-3 and claudin-4 are frequently overexpressed in ovarian cancer but not in ovarian cystadenomas. *Clin Cancer Res* 9, 2567–2575, 2003.
117. B. Sharma, W. Ma, I. Adjei, J. Panyam, S. Dimitrijevic, V. Labhasetwar. Nanoparticle-mediated p53 gene therapy for tumor inhibition. *Drug Deliv Transl Res* 1, 43–52, 2011.
118. C. Sun, T. Yi, X. Song, S. Li, X. Qi, X. Chen, H. Lin, X. He, Z. Li, Y. Wei, X. Zhao. Efficient inhibition of ovarian cancer by short hairpin RNA targeting claudin-3. *Oncol Rep* 26, 193–200, 2011.
119. G. Luo, C. Jin, J. Long, D. Fu, F. Yang, J. Xu, X. Yu, W. Chen, Q. Ni. RNA interference of MBD1 in BxPC-3 human pancreatic cancer cells delivered by PLGA-poloxamer nanoparticles. *Cancer Biol Ther* 8, 594–598, 2009.
120. M.O. Andersen, A. Lichawska, A. Arpanaei, S.M. Rask Jensen, H. Kaur, D. Oupicky, F. Besenbacher, P. Kingshott, J. Kjems, K.A. Howard. Surface functionalisation of PLGA nanoparticles for gene silencing. *Biomaterials* 31, 5671–5677, 2010.
121. E. Vega, F. Gamisans, M.L. García, A. Chauvet, F. Lacoulonche, M.A. Egea. PLGA nanospheres for the ocular delivery of flurbiprofen: Drug release and interactions. *J Pharm Sci* 97, 5306–5317, 2008.
122. J. Araújo, E. Vega, C. Lopes, M.A. Egea, M.L. Garcia, E.B. Souto. Effect of polymer viscosity on physicochemical properties and ocular tolerance of FB-loaded PLGA nanospheres. *Colloids and Surfaces B: Biointerfaces* 72, 48–56, 2009.
123. H. Gupta, M. Aqil, R.K. Khar, A. Ali, A. Bhatnagar, G. Mittal. Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. *Nanomedicine: Nanotechnology, Biology, and Medicine* 6, 324–333, 2010.
124. U. Seju, A. Kumar, K.K. Sawant. Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: In vitro and in vivo studies. *Acta Biomaterialia* 7, 4169–4176, 2011.
125. G.K. Jain, S.A. Pathan, S. Akhter, N. Jayabalan, S. Talegaonkar, R.K. Khar, F.J. Ahmad. Microscopic and spectroscopic evaluation of novel PLGA–chitosan Nanoplexes as an ocular delivery system. *Colloids and Surfaces B: Biointerfaces* 82, 397–403, 2011.
126. K. Nan, *et al.* Porous silicon oxide–PLGA composite microspheres for sustained ocular delivery of daunorubicin. *Acta Biomater*, 2014, http://dx.doi.org/10.1016/j.actbio.2014.04.024.
127. J. Blum, C. Weller, C. Booth, I. Babar, X. Liang, F. Slack, W. Saltzman. Prevention of K-Ras- and Pten-mediated intravaginal tumors by treatment with camptothecin-loaded PLGA nanoparticles. *Drug Deliv Transl Res* 1, 383, 2011.
128. Y. Cu, C.J. Booth, W.M. Saltzman. In vivo distribution of surface modified PLGA nanoparticles following intravaginal delivery. *J Control Release* 156, 258–264, 2011.