REVIEW ARTICLE

STUDY OF DIFFERENT PROPERTIES AND APPLICATIONS OF POLY LACTIC-CO-GLYCOLIC ACID (PLGA) NANOTECHNOLOGY: AN OVERVIEW

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ABSTRACT

In past decades poly lactic-co-glycolic acid (PLGA) has been one of the most attractive polymeric candidates used to fabricate devices for diagnostics and other applications of clinical and basic science research, including vaccine, cancer, cardiovascular disease, and tissue engineering. In addition, PLGA and its co-polymers are important in designing nanoparticles with desired characteristics such as biocompatibility, biodegradation, particle size, surface properties, drug release and targetability and exhibit a wide range of erosion times. PLGA has been approved by the US FDA for use in drug delivery. This article represents the more recent successes of applying PLGA-based nanotechnologies and tools in these medicine-related applications, and factors affecting their degradation and drug release. It focuses on the possible mechanisms, diagnosis and treatment effects of PLGA preparations and devices.

Keywords: PLGA, Nanotechnology, Biodegradable polymers, Cancer, Cardiovascular Disease.

INTRODUCTION

Polymeric nanoparticles have in general shown their advantage by their increased stability and the unique ability to create an extended release. Nano materials used for drug delivery must meet several requirements, such as biocompatibility, drug compatibility, suitable biodegradation kinetics and mechanical properties, as well as ease of processing[1, 2]. Polymeric nanoparticles are advantageous in a number of ways over microparticles. They possess high drug-loading capacities, thereby increasing intracellular delivery of the drug and are better suited for intravenous delivery[3]. In the last 25 years, synthetic biodegradable polymers have been used increasingly in a wide variety of approaches to construct molecular imaging agents and therapeutic delivery devices for drugs and genes due to their biocompatibility and biodegradability [4]. Especially excellently biocompatible and biodegradable polyester called poly (D, L-lactide-co-glycolide) (PLGA) is the most frequently used biomaterial and is already commercialized for a variety of drug delivery systems (blends, films, matrices, microspheres, nanoparticles, pellets, etc.)[5]. Furthermore, it has been approved by the US FDA for drug delivery. Polymeric nanoparticles of this polymer are used for the delivery of various drugs (antipsychotics, anesthetics, antibiotics, antiparasites, antitumorals, hormones, proteins, etc.) [6]. This review, therefore, was conducted with the view to summarize the properties of PLGA and more recent successes of applying PLGA-based nanotechnologies and tools in cardiovascular disease, cancers and immunology, vaccines and other diseases and devices. The possible mechanisms, diagnosis and treatment effects of PLGA preparations are also discussed.

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Physicochemical Properties of PLGA

Poly (lactic-co-glycolic acid) is a copolymer synthesized by means of random ring-opening. Co-

polymerization of two different monomers, the cyclic dimers (1, 4-dioxane-2, 5-diones) of glycolic acid and lactic acid. Common catalysts used in the preparation of this copolymer include tin (II) 2-ethylhexanoate, tin (II) alkoxides and aluminum isopropoxide. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding a linear, amorphous aliphatic polyester product[7]. The forms of PLGA are usually identified by the monomer's ratio used. For example, PLGA 50:50, which is most frequently used in nanotechnology, identifies a copolymer whose composition is 50% lactic acid and 50% glycolic acid (Fig.1).

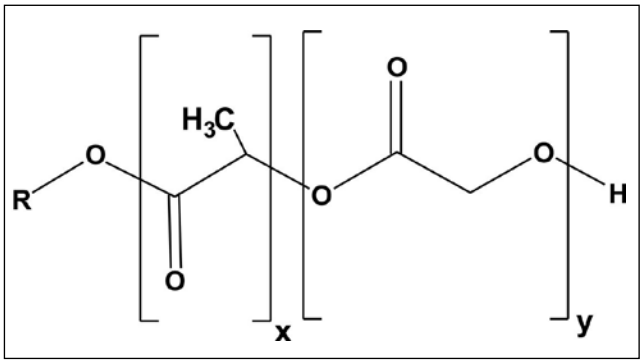

Fig. 1: Structure of poly lactic-co-glycolic acid (x is the number of lactic acid units and y is number of glycolic acid units

Physical properties such as the molecular weight affect the mechanical strength of the polymer and its ability to be formulated as a drug delivery device. Also, these properties may control the polymer biodegradation rate and hydrolysis. The mechanical strength, swelling behavior, capacity to undergo hydrolysis and subsequently, the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The resultant crystallinity of the PLGA co-polymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. PLGA polymers containing a 50:50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing a higher proportion of either of the two monomers. It has a glass transition temperature (Tg) of 45°C and an inherent viscosity of 0.5-0.8 mPa. The Tgs of the PLGA co-polymers are above the physiological temperature of 37°C and hence they are normally glassy in nature. Thus, they have a fairly rigid chain structure, which gives them significant mechanical strength to be formulated as a degradable device. It has been reported that the Tgs of PLGA decrease with the decrease of lactide content in the co-polymer composition with decreasing M.W. [8, 9].

Biodegradation of PLGA [10-15]

The PLGA co-polymer undergoes degradation in an aqueous environment (hydrolytic degradation or biodegradation) through cleavage of its backbone ester linkages. It has been recorded that the PLGA biodegradation occurs through random hydrolytic chain scissions of the swollen polymer. A three-phase mechanism for PLGA biodegradation has been proposed:

1. Random chain scission process. The M.W. of the polymer decreases significantly, but no appreciable weight loss and no soluble monomer products are formed.
2. In the middle phase, a decrease in M.W. accompanied by a rapid loss of mass and soluble oligomeric and monomer products are formed.
3. Soluble monomer products formed from soluble oligomeric fragments. This phase is that of complete polymer solubilization.

PLGA NPs are biodegradable in the body because they undergo hydrolysis of their ester linkages in the presence of water to produce the original monomers, lactic acid and glycolic acid, which are also byproducts of various metabolic pathways in the body under normal physiological conditions (Fig. 2). The degradation rate of PLGA polymers is related to the monomer ratio used in production; the polymer containing a 50:50 ratio of lactic and glycolic acids is hydrolyzed much faster than those containing higher proportions of either of the two monomers. The degradation products are easily metabolized in the body via the Krebs cycle and are eliminated. Thus, there is very minimal systemic toxicity associated with using PLGA for drug delivery

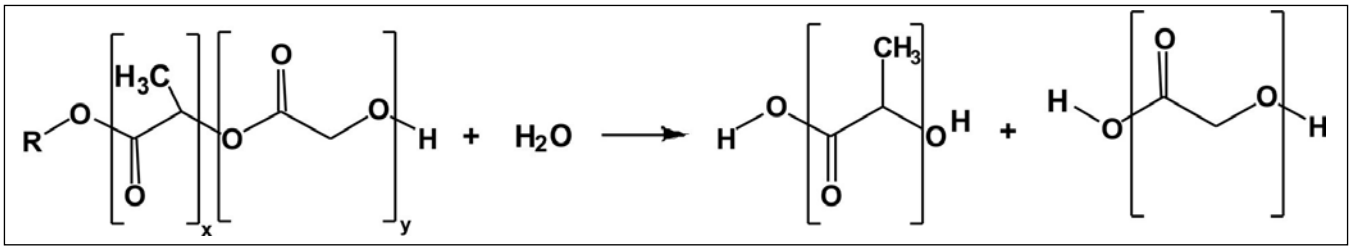

Fig. 2: Hydrolysis of poly lactic-co-glycolic acid

or biomaterial applications. The role of enzymes in any PLGA biodegradation is unclear. Most of the literature indicates that the PLGA biodegradation does not involve any enzymatic activity and is purely through hydrolysis.

Factors affecting Degradation

To enhance the desirable properties of PLGA, it is essential to understand the factors affecting the PLGA degradation and design a drug delivery device accommodating all these factors to make it more efficient and efficacious.

Effect of Composition [16- 19]

Polymer composition is the most important factor to determine the rate of degradation of a delivery matrix, which influence the rate of degradation. A systematic study of polymer composition with its degradation has been shown by many groups. These results show that increase in glycolic acid percentage in the oligomers accelerates the weight loss of polymer. PLGA 50:50 (PLA/PGA) exhibited a faster degradation than PLGA 65:35 due to preferential degradation of glycolic acid proportion assigned by higher hydrophilicity. Subsequently PLGA 65:35 shows faster degradation than PLGA 75:25 and PLGA 75:25 than PLGA 85:15. Thus, absolute value of the degradation rate increases with the glycolic acid proportion.

Effect of Crystallinity [20-24]

Copolymer composition also affects important properties such as glass transition temperature and crystallinity which have indirect effects on degradation rate. At the moment, there are conflicting reports on the effect of crystallinity on the degradation rate. Few groups have proposed that the crystallinity of lactic acid (PLLA) increases the degradation rate because the degradation of semi-crystalline polymer is accelerated due to an increase in hydrophilicity. In contrast, various other studies have shown a decrease of degradation rate with increase in sample crystallinity.

Effect of Average Molecular Weight (MW) [22]

Polymers with higher molecular weight have generally exhibited lower degradation rates. Molecular weight has a direct relation with the polymer chain size. Polymers having higher molecular weight have longer polymer chains, which require more time to degrade than small polymer chains.

Effect of Drug Type and Effect of Drug Load

The mechanism of polymer-drug matrix degradation and the parameters of drug release rate vary as a function of drug type [23]. The presence of drug may change the degradation mechanism from bulk erosion to surface degradation, as well as affect the rate of matrix degradation [27]. It is clear that one must seriously consider the effect of the chemical properties of the drug to explain the drug-release mechanisms of a particular system using biodegradable polymers.

Amount of drug loading in the drug delivery matrix plays a significant role on the rate and duration of drug release. Matrices having higher drug content possess a larger initial burst release than those having lower content because of their smaller polymer to drug ratio. However, this drug content effect is attenuated when

the drug content reaches a certain level depending upon drug type [25].

Effect of Size and Shape of the Matrix

The ratio of surface area to volume has shown to be a significant factor for degradation of large devices. Higher surface area ratio leads to higher degradation of the matrix. It has also been reported that bulk degradation is faster than pure surface degradation for PLGA, which makes the release of the drug faster from the devices with higher surface area to volume [21, 26, 27].

Effect of pH

The in vitro biodegradation/hydrolysis of PLGA showed that both alkaline and strongly acidic media accelerate polymer degradation[28]. However, the difference between the slightly acidic and neutral media is less pronounced due to autocatalysis by the carboxylic end groups [29].

The Bio-distribution of PLGA [30-32]

The bio distribution studies demonstrate that PLGA NP delivery enhances accumulation of diagnostic or therapeutic agents by the enhanced permeability and retention effect. For example, when indocyanine green was delivered through NPs in healthy mice using a fluorometric assay method, the NPs led to higher indocyanine green deposit in organs (two to eight times) as well as in blood (five to ten times) compared with free solution, indicating the enormous potential of PLGA NPs as a delivery system for indocyanine green for its use in tumor diagnosis and photodynamic therapy. This effect is enhanced when the NP is shielded with a poly (ethylene glycol/oxide) surface modification. Coating the nanoparticle surface with a hydrophilic polymer, such as poly(ethylene glycol) (PEG), has been shown to confer long circulation properties to poly(lactic acid), PLGA, polycaprolactone and polyphosphazene nanoparticles. The presence of the hydrophilic polymeric chains on the surface of the nanoparticles is considered to sterically stabilize them against opsonization and subsequent phagocytosis.

Drug Release Behavior: Biphasic Release [33- 37]

PLGA copolymer undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkages into oligomers and, finally monomers. This has been demonstrated in both *in vivo* and *in vitro* for various drug types and proteins with different polymer ratios. The degradation process for these polymers is mainly through uniform bulk degradation of the matrix where the water penetration into the matrix is higher than the rate of polymer degradation. Furthermore, the increase of carboxylic end groups as a result of biodegradation autocatalyses the process. The degradation of PLGA copolymer is the collective process of bulk diffusion, surface diffusion, bulk erosion and surface erosion. Since there are many variables that influence the degradation process, the release rate pattern is often unpredictable. The release of drug from the homogeneously degrading matrix is more complicated. A biphasic curve for drug release because of PLGA biodegradation has been shown to display following pattern (Fig. 3):

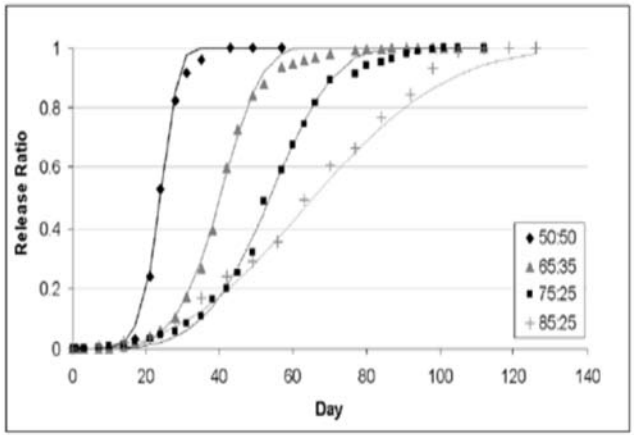

Fig. 3: *in vivo* release profiles for 50:50, 65:35, 75:25 and 85:15 poly lactic-co-glycolic acid. Notation 65:35 PLGA means 65% of the copolymer is lactic acid and 35% is glycolic acid. A biphasic release profile with an initial zero release period followed by a rapid drug release has been observed

1. Initial burst of drug release is related to drug type, drug concentration and polymer hydrophobicity. Drug on the surface, in contact with the medium, is released as a function of solubility as well as

penetration of water into polymer matrix. Random scission of PLGA decreases molecular weight of polymer significantly, but no appreciable weight loss and no soluble monomer products are formed in this phase.

2. In the second phase, drug is released progressively through the thicker drug depleted layer. The water inside the matrix hydrolyzes the polymer into soluble oligomeric and monomeric products. This creates a passage for drug to be released by diffusion and erosion until complete polymer solubilization. Drug type also plays an important role here in attracting the aqueous phase into the matrix.

The role of enzymes in any PLGA biodegradation is unclear. Most literature indicates that the PLGA biodegradation does not involve any enzymatic activity and is purely through hydrolysis. However, some investigators have suggested an enzymatic role in PLGA breakdown based upon the difference in the in vitro and in vivo degradation rates. The PLGA polymer biodegrades into lactic and glycolic acids. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water. Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and is eventually eliminated as carbon dioxide and water. Ideally, PLGA polymer systems should have considerable mechanical strength, since the drug delivery devices formulated using them are subjected to significant physical stress, which can also influence mechanical breakdown of implants and alter surface area and hydration/hydrolysis.

PLGA-Based Nanotechnology and its Medicine-Related Application

Cancers

To reduce the toxicity and increase the therapeutic efficacy of anticancer agents, the applications of cancer nanotechnology have attracted great attention in recent years. PLGA-based nanotechnology is currently under intense development for applications in cancer imaging and targeted therapy. PLGA NPs or microspheres, linked with biotargeting ligands, such as cytokines, hormones, vaccines and chemotherapeutic agents, are used to target malignant tumors with high affinity and specificity. PLGA NPs also have large surface areas and functional groups for conjugating to multiple diagnostic (e.g., optical, radioisotopic or magnetic) agents. These PLGA particles were implanted in cancer patients for early cancer detection and screening. PLGA-based nanotechnology has been used widely in the diagnosis and treatment of cancer [38] (Fig. 4).

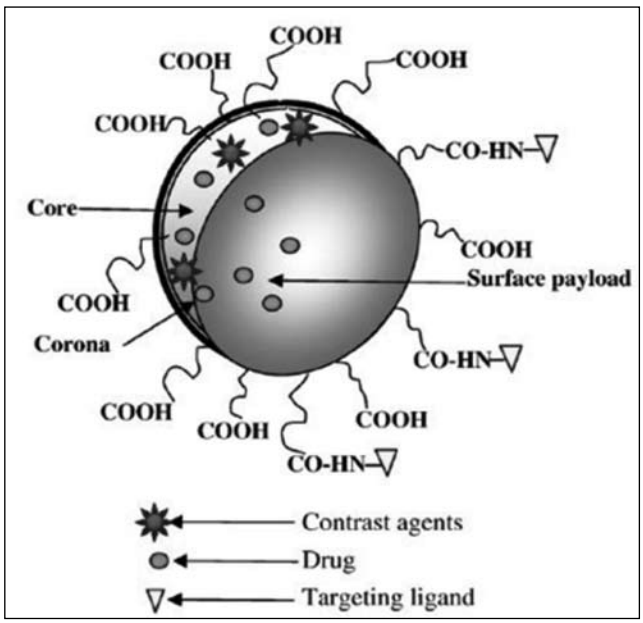

Fig. 4: Multifunctional PLGA nanoparticles used as diagnostics and imaging agents on one hand and as specific drug delivery agents on other hand as both drug and contrast agents can be encapsulated in the core of nanoparticles helping them serve the dual purpose

Nanoparticles as drug carriers or imaging tools have been developed as new modalities for cancer therapy and diagnosis. The NP delivery systems are attractive because they target tumors and enhance the tumor accumulation of anticancer agents in tumor cells more than in healthy tissues. The general mechanism is based on the unique pathophysiology of the tumor vasculature. In contrast to normal tissue, tumors contain a high density of abnormal blood vessels that are dilated and poorly differentiated,

with a chaotic architecture and aberrant branching. Many functions of the tumor vasculature are impaired, which leads to the higher concentration of plasma proteins detected in tumor tissues than in normal tissues. This is due to an enhanced permeability and retention effect, which result from the combination of an increased permeability of tumor blood vessels and a decreased rate of clearance caused by the lack of functional lymphatic vessels in the tumor, and results in an increased accumulation of macromolecules or NPs in tumors. These findings support the use of NPs in tumor diagnosis and therapy as carriers because they passively accumulate in solid tumors after their intravenous administration [39].

Furthermore, NP carriers possess a higher stability in biological fluids and avoid enzymatic metabolism than other colloidal carriers, such as the liposomes or lipidic vesicles. Poly(lactic-co-glycolic acid) microparticles have also been used for diagnosing in the area of cancer. Tumor imaging plays a key role in clinical oncology. It requires sufficient intensity of a corresponding signal from targeted area in order to differentiate this area from the surrounding tissues. Conventional tumor imaging approaches, such as MRI, PET, ultrasound and optical imaging techniques, are useful only when relatively large tissue areas are involved in the pathological process. PLGA microbubbles have been developed for use as ultrasound contrast agents to improve ultrasound imaging in the diagnosis of CVD, as mentioned previously. This development of NPs as imaging-contrast agents may also offer enhanced sensitivity and specificity for *in vivo* tumor imaging. For example, an *in vivo* study demonstrated that PLGA microparticles induced a significant enhancement of 47% intratumoral vessels detected after injection [40]. Goldberg *et al.* developed PLGA perfluorocarbon-filled microparticles and injected them in rabbits with VX2 tumors. Gray-scale and color Doppler were performed to better detect and estimate the lesion dimensions treated by radiofrequency [41]. Forsberg *et al.* used PLGA/camphor/ammonium carbonate particles as a contrast agent to enhance the Doppler signal after PLGA injection [42]. Many anticancer drugs have been clinically applied to treat various cancers, but they cannot be used effectively due to poor cell penetration. For example, paclitaxel is a mitotic inhibitor used in cancer chemotherapy. The success of its clinical application is mainly limited by its low therapeutic index and low solubility in water, as well as in many other pharmaceutical solvents acceptable for intravascular administration. Incorporation of paclitaxel in the PLGA NPs strongly enhances its antitumoral efficacy compared with the free drug (Taxol®), and this effect being more relevant for prolonged incubation times with cells. Based on these results, it can be concluded that the formulations developed in this work may be considered promising systems for *in vivo* paclitaxel delivery [43, 44]. In an animal-mode study, paclitaxel and the apoptotic signaling molecule, C6-ceramide, were encapsulated in a PLGA/poly(β-amino ester)-blended polymer. When particle formulations were administered intravenously to MCF7 and MCF7TR tumor-bearing mice, higher concentrations of paclitaxel were found in the blood due to longer retention time and an enhanced tumor accumulation relative to administration of the free drug. In addition, the PLGA/poly (β-amino ester)-blend nanoparticles were effective in enhancing the residence time of both drugs at the tumor site by reducing systemic clearance[32]. Hypericin, a natural photosensitizer extracted from *Hypericum perforatum*, is a potential tool for the treatment and detection of ovarian cancer and other cancers. Due to its hydrophobicity, systemic administration of hypericin is problematic. Hypericin-loaded PLGA NPs regress ovarian tumor growth effectively[45]. Cisplatin, another chemotherapy drug used to treat various types of cancers, was loaded to PLGA-mPEG NPs for targeting prostate cancer in mice, resulted in prolonged cisplatin residence in systemic circulation[46]. Mitoxantroneloaded PLGA microspheres were also demonstrated to deliver therapeutic concentrations of drugs to the tumor and prevent glioma growth without causing side effects[47]. Encapsulation of PLGA NP has been investigated for the delivery of other anticancer agents, including plasmid DNA, antibodies and proteins. One study demonstrated a novel approach in delivery of plasmid DNA with cationic

microparticles synthesized by conjugating branched polyethyleneimine to the surface of presynthesized PLGA microparticles. These formulations were sufficiently cationic to adsorb plasmid DNA with no toxicity at increasing doses and finally enhanced gene transfer *in vitro,* suggesting their ability to transfect antigen-presenting cells (APCs). Preclinical evaluation of surface-functionalized cationic microparticles demonstrated their high efficacy as an efficient carrier of plasmid DNA vaccines in a murine model of B-cell lymphoma[48]. Another study employed the PLGA NP delivery system to specifically deliver monoclonal antibody (mAb) for targeting invasive epithelial breast tumor cells [49]. The mAb was attached to the NP surface either covalently or noncovalently. The mAb-coated NPs localized solely to MCF-10A neoT cells, whereas noncoated NPs were distributed randomly, indicating specific targeting of the immuno-NPs. These mAb-coated NPs were more likely to be bound to the targeted cells than noncoated NPs. For protein delivery, modified PLGA microspheres were developed to release endogenous antiangiogenic proteins for tumor inhibition *in vivo* and *in vitro* in a human glioma mouse model[50]. The modified PLGA particles are loaded with the endogenous inhibitor hemopexin or platelet factor four fragment (PF-4/CTF). The study shows the successful loading of hemopexin and PF-4/CTF in PLGA particles without affecting their biological activity.

Immunohistochemical analysis of the treated tumors showed a marked decrease in tumor vessel density compared with untreated tumors. IL-18 protein was encapsulated into PLGA microspheres by two procedures (i.e., w/o/w and s/o/w)[51]. These microparticles could be implanted stereotactically within the CNS and could release active IL-18 in a controlled manner in accordance with the specifications for *in vivo* immunotherapeutic applications against gliomas. The *in vitro* studies on the releasing rate and biological activity of IL-18 suggested that recombinant IL-18-releasing microspheres may represent a useful device for the treatment of brain cancers.

Cardiovascular disease

Effective treatments of CVD include preventive lifestyle changes, medications and surgical procedures[52,53,54]. The rapid innovations and expansions of nanotechnology have opened new avenues for the management of CVDs. We focus on the current advances in the application of PLGA-based nanotechnology to CVD, with specific emphasis on vascular tissue engineering, diagnosis and treatment.

Tissue engineering

Tissue engineering is the technology combining genetic engineering of cells with chemical engineering to create artificial organs and tissues, such as skin, bones and blood vessels. Diseases of the blood vessels, principally of the small-caliber arteries (<6 mm), account for the majority of deaths in the USA annually[54]. Examples include cardiac infarction with coronary artery occlusion, claudication with peripheral arterial disease or stroke with occlusion of carotid or cerebral arteries. Arterial replacement is now a common treatment for arterial occlusive diseases with over 1.4 million arterial bypass operations performed each year in the USA alone. As described, PLGA is FDA-approved elastomeric copolymers for drug delivery owing to its biodegradability, biocompatibility, mechanical properties and ease of processing. It has been used in the production of a variety of vascular tissue–engineering devices, such as grafts and prosthetic devices. In the past several years, many groups have investigated different approaches with PLGA to achieve the biological and biomechanical properties that mimic the native vascular tissue[55]. For cases lacking suitable autologous vessels for bypass procedures, surgeons have turned to synthetic grafts, such as expanded polytetrafluoroethylene (ePTFE), which have had only limited success in small-caliber vessels. This problem has motivated scientists and physicians to explore tissue engineering approaches to replace blood vessels[56-60]. One of the major challenges has been the development of biomaterials that would recreate mechanical and biochemical characteristics in order to promote long-term graft survival[61]. A tissue

engineered vascular graft appears to be a promising solution that could meet all requirements needed for that purpose.

Sarkar *et al.* developed a technology by incorporating PLGA micro/NPs into molten biopolymer polycaprolactone (PCL) to fabricate porous PCL scaffolds. The significantly higher melting temperature of PLGA enabled the PCL to be in a molten state, while leaving the PLGA micro/nanospheres intact. These composite blocks were then used in the mechanical heat press, resulting in thin micropatterned PCL scaffolds with embedded PLGA particles. PLGA-incorporated thin-film scaffolds were immersed in a solution of sodium hydroxide to rapidly degrade the micro/nanospheres[62]. Since PLGA degrades at a far faster rate than PCL[63], the PCL microstructure is not affected by the PLGA micro/nanosphere leaching process; thus, a 2D biodegradable vascular PCL graft scaffold with micron-scale feature was formed. The technology may enable the fabrication of patterned cell sheets that can be layered to create vessel walls with specific and systematically adjustable cellular organizations.

Hwang *et al.* developed a method to produce PLGA microfibers within a polydimethylsiloxane-based microfluidic chip for the generation of 3D tissue-engineering scaffolds. The PLGA fibers were comprised of a dense outer surface and a highly porous interior. Cell culture tests suggest that these PLGA microfibers may be useful for 3D cell culture tissue-engineering applications[64].

Another application of PLGA-based drug delivery was for improving clinical performance of ePTGE graft. Although paclitaxel-coated ePTFE grafts could prevent neointimal hyperplasia in the arteriovenous hemodialysis graft, large quantities of initial burst release of paclitaxel have remained a problem[65]. To achieve controlled drug release, paclitaxel was formulated into PLGA NPs, which were then transferred to the luminal surface and inner part of ePTFE vascular grafts. Thus, PLGA NPs in the graft extended the period of paclitaxel release while reduced its initial burst release[66].

Davda *et al.* reported that a rapid uptake of BSP-PLGA NPs by endothelial cells was observed, and the particles were localized mainly in the cytoplasm in the cells [67]. PLGA NPs localized in the endothelium could provide prolonged drug effects because of their sustained-release characteristics and also could protect the encapsulated agent from enzymatic degradation. Thus, PLGA NPs could be used for localizing therapeutic agents or gene delivery into endothelial cells or other types of cells for improving the design of tissue-engineered grafts.

Diagnosis

Nanotechnology has significantly impacted diagnostic intervention in cardiology. The imaging capability of NPs has been involved in the construction of particles with use of imaging-contrast agents, for targeted biomedical imaging of vulnerable plaques, for detection of specific pathologic targets signaling the onset of atherosclerosis and for tracking inflammatory events[68].

The biodegradable PLGA microcapsules were developed for use as ultrasound contrast agents to improve ultrasound imaging since it may slowly degrade *in vivo* into lactic and glycolic acid, neither of which produce *in vivo* toxic effects and further degrade into carbon dioxide and water via the tricarboxylic acid cycle[69]. Cui *et al.* fabricated a kind of absorbable PLGA microbubble-based contrast agent (PLGA microspheres with porous or hollow inner structure) by an improved double emulsion-solvent evaporation method. *In vitro* acoustic measurements demonstrated the good scatter ability of these polymer based agents. *In vivo* imaging experiments showed PLGA microbubbles could remain stable under high Mechanical Index, which is the ratio of the peak negative pressure and the square root of the frequency. The resistance of PLGA microbubbles to ultrasound destruction allows for their potential applications in left ventricle opacification and myocardium imaging, and demonstrates the ability of PLGA micro-bubbles to detect myocardial perfusion defects[70]. Wheatley *et al.* also developed the PLGA microbubbles used as ultrasound contrast agents. PLGA microbubbles

were prepared by an adapted double emulsion w/o/w solvent evaporation process. Significant acoustic enhancements (up to 24 dB) were reported both *in vitro* and *in vivo*. Moreover, the rabbits used in the *in vivo* study did not show any adverse side effects from multiple injections of the agent[69,71].

Treatment

Therapeutic applications of nanomaterials in cardiovascular medicine include cardiovascular devices for delivery of drugs and bioactive molecules, or novel technologies for reducing cholesterol accumulation and for dissolving clots[69]. Stent placement is currently the primary intervention for cardiovascular occlusive diseases; however, it may lead to in-stent restenosis[72-74]. Successes in early clinical trials with drug-eluting stents using the anti-proliferative agents have been promising. PLGA NP-coated stents can effectively deliver genes or drugs to vessel walls. Controlled release of DNA from vascular stents was investigated in a series of studies using green-fluorescence protein plasmid DNA incorporated into a PLGA emulsion coating on stainless-steel stents[75]. The study demonstrated that green-fluorescence protein was efficiently expressed in cultured vascular SMCs, as well as in pig coronary arteries, with 1% of neointimal arterial SMCs transfected. Subsequently, a study from the same group investigated a denatured-collagen–PLGA composite vascular stent coating to improve DNA controlled release, and found that this composite enhanced plasmid DNA transfection to 10.4% through mechanisms involving β3-integrin receptor interactions and associated changes in actin dynamics[76]. Another study showed stent-based controlled release of intra vascular angiostatin limits plaque progression and in-stent restenosis, in which controlled-release biodegradable microspheres delivering angiostatin (PLGA-polyethylene glycol) were loaded in channeled stents, anchored and deployed in the aorta of rabbits[73]. Banai *et al.* reported that stent-based delivery of tyrphostin AGL-2043 from a PLGA coating reduces in-stent neointimal hyper-plasia in porcine coronary arteries by 50% after 28 days and preserves lumen area[77]. Vascular SMC proliferation plays an important role in atherosclerosis, restenosis and venous bypass graft disease. A large number of drugs have been shown to produce antiproliferative effects on SMCs. Since stenosis is a focal lesion that usually occurs at the graft anastomosis, local delivery of drugs to the target site is a better strategy to achieve adequate therapeutic concentrations while minimizing the systemic adverse effects. In addition to coated stents, several local drug delivery systems, including different perfusion balloon catheters and hydrogel-coated balloon catheters, have been developed to treat cardiovascular disease and succeeded in animal models. However, no optimal therapy for restenosis and bypass graft disease has been achieved in a clinical setting. The major disadvantages of those methods are low uptake of delivered drugs by the tissue and a rapid washout of the drugs by blood flow[78-80]. To avoid these disadvantages, local drug delivery systems made of NPs have been studied, with promising results. For instance, Yang *et al.* reported that heparin released from PLGA microspheres effectively reduced human SMC proliferation[78]. Suh *et al.* formulated a paclitaxel, an antimicrotubule agent, into a biodegradable poly (ethylene oxide)-poly (lactide/ glycolide) nanosphere as a sustained drug delivery system to study its effects on vascular SMCs in culture[81]. They found that the paclitaxel-loaded nanospheres, prepared by an emulsion solvent evaporation method, showed a sustained-release profile over 4 weeks. Zhu *et al.* developed a combination of PLGA microspheres with ReGel®, an injectable copolymer, as a sustained-release system for perivascular delivery of an antiproliferative drug, dipyridamole. Incorporation of dipyridamole into PLGA microspheres decreased the initial burst and prolonged the release, with the release kinetics dependent on the molecular weight of PLGA[82]. It has also been reported that an antisense oligodeoxynucleotide released from PLGA microparticles inhibited SMC growth *in vitro*[83].

The sustained-release drug or gene profile and cellular internalization from *in vitro* study suggest that PLGA microspheres could be used to encapsulate antiproliferative agents and may provide a new

approach for local drug delivery after PTCA and, in turn, prevent restenosis or bypass graft disease. It has been reported that paclitaxel-loaded PLGA NPs with didodecyldimethyl ammonium bromide modification were an effective means of inhibiting proliferative response to vascular injury in a rabbit arterial balloon injury model[84]. Another study of NPs for local delivery of paclitaxel for restenosis treatment also showed the antirestenotic effect of paclitaxel-loaded NPs *in vivo*. The paclitaxel-loaded NPs, consisting of poly(vinyl alcohol)-graft-PLGA (PVA-g-PLGA) with varying PLGA chain length, as well as PLGA, were administered locally to the wall of balloon-injured rabbit iliac arteries using a porous balloon catheter[85]. In addition, nanospheres composed of PLGA and containing PDGF-β receptor antisense have been formulated and examined[86]. The study showed that PLGA nanospheres containing PDGF-β receptor antisense significantly inhibited the restenosis in a balloon-injured rat model. Kaul *et al.* explored the effect of a PLGA-based periadventitial delivery of a nitric oxide-releasing diazeniumdio-late, spermine/nitric oxide, on balloon injury induced neointimal hyperplasia in rat ileofemoral arteries. They found that the treatment produces a marked localized inhibition of neointimal proliferation in balloon-injured arteries. This phenomenon is associated with suppression of NF-κ-light-chain-enhancer of activated B cells (NF-κB) activation and elevation of the vascular cyclic GMP (cGMP) at the site of injury[87].

Immunology & Vaccines

The characteristics of biodegradable synthetic polymers, including their safety record and biocompatibility, make them attractive candidates for long-term vaccination treatments. Several studies have been aimed at achieving their usage in a clinically relevant manner, particularly for the delivery of subunit vaccines using nano/microparticles prepared from biodegradable and bio-compatible polymers to induce both humoral and cellular immune responses[88]. PLGA is one of the most widely studied polymers of interest in the vaccine field. Since PLGA polymers can offer long-term release of their contents in a recurring, pulsatile manner, the primary focus of past studies has been in using them to replace the multiple immune boosting administrations typically required to induce protective immunity. As a controlled delivery system, PLGA polymers can potentially deliver antigens or adjuvants to a desired location at predetermined rates and durations[89], effectively regulating the immune response over a period of time. As a vehicle for targeted drug delivery, PLGA polymers have been reported to effectively aid in directing antigens to APCs by efficiently trafficking through local lymphoid tissue for uptake by dendritic cells (DCs)[90-91]. In the last 10 years, microspheres have been used extensively for the injectable delivery of vaccine antigens, both for viral and bacterial antigens.

Peyre *et al.* tested PLGA microspheres as a divalent vaccine against tetanus and diptheria in guinea pigs and found that, after 6 weeks, the microspheres encapsulating tetanus or diptheria toxins produced protective immunity that was comparable to or better than that induced by the licensed divalent vaccine[92]. Yet another example of the wide-reaching usage of microspheres as vaccines is their use against dental caries. Smith *et al.* tested induction of salivary IgA and serum IgG antibody responses using *Streptococcus sobrinus* glucosyltransferase encapsulated in microspheres and found that intranasal delivery of glucosyltransferase-containing bioadhesive microparticles induced the highest and longest-lasting salivary immune response of any mucosal or systemic route or vehicle tested[93].

A more recent study, using PLGA microspheres encapsulating rotavirus strain SA11 and serum albumin as a stabilizer during the emulsification process indicated that a single-dose oral immunization with 20 µg of antigen elicited improved IgA and IgG antibody titer in comparison to soluble antigen[94].

As with any vaccine treatment, a main concern is whether positive functional changes in the behavior of immune response cells can be induced while limiting any negative phenotypic effects. This is the case for PLGA polymers. Encapsulation of proteins in PLGA polymers enhances and prolongs antigen

presentation by DCs, while phenotype and functional analysis of DCs *in vitro* revealed no negative effects on their pivotal properties[95-97].

Elamanchili *et al.* assessed the extent of maturation of DCs after treatment with monophosphoryl lipid encapsulated in NPs[98]. The treatment of DCs upregulated the expression of surface maturation markers and demonstrated an enhanced allostimulatory capacity that released high amounts of proinflammatory and Th1 polarizing cytokines, over-riding self-tolerance mechanisms. This study was in contrast with another study, where a correlation between the size of the particles and the type of T-cell response induced. Microparticles elicited a potent type 1 T-cell response and potent antibody response, whereas NPs favored the induction of Th2 cells. This may reflect that a high percentage of protein on the surface of the NPs will increase the amount of soluble protein available for presentation by APCs[99]. This characteristic may limit the use of NPs against viruses but may enhance responses against extracellular pathogens. While another benefit of NPs is that they are usually administered intravenously, the versatility for delivery of PLGA NPs has led to their study as potential vaccine candidates for oral administration as well. NPs can target effectively to M-cells in the follicle-associated epithelium of the Peyer's patches, where the transcytotic capability of M-cells allows for uptake of the NPs in the intestine and delivery of the NPs to APCs. These NPs have been observed to act to stimulate the immune response, as measured by an increase in IL-2 and IFN-γ in spleen homogenates [100]. Other studies have sought to improve on the ability of M-cell targeting by grafting arginine-glycine-aspartic (RGD) peptides onto PEGylated PLGA NPs[101]. Interactions between the RGD ligand and the β1-integrins detected at the apical surface of cocultures enhance the concentration of NPs at M-cells.

Aside from these advantages, however, the evidence indicates that there is little benefit in enhancement of the immune response between microspheres and NPs when compared side by side through the same immunization routes. One recent study tested the efficacy of these delivery systems with two protein antigens, including a recombinant antigen from *Neisseria meningitides* type B administered intramuscularly or intraperitonealy, and an antigen from HIV-1 env glycoprotein (gp) 140 administered intranasally. This study determined that there were no differences between the NP and microparticle formulations and the immune responses they produced in mice[102].

As our understanding of PLGA NPs results in preparation and treatment improvements, these polymers are increasingly becoming feasible candidates for vaccine immunotherapy, in addition to their uses as drug delivery systems and anticancer agents, particularly given the current state of the immunotherapeutic vaccine field, where efficacy continues to remain a problem. As with all vaccine development, the major obstacle is providing delivery vehicles with the adequate surface molecules for recognition by the immune system and for more effective targeting. It is likely, therefore, that future studies of PLGA NPs as vaccine candidates will focus on improving these features, as Garinot *et al.* recently tested by grafting RGD peptides covalently onto PEG moieties on the surface of PLGA NPs[100].

Other applications[103-105]

Poly(lactic-co-glycolic acid) has been investigated for drug delivery for pharmaceutical and biomedical applications for a variety of other diseases, such as diabetes, pain, arthritis, bowel disease and brain imaging, owing to its biodegradability and biodistribution, as described previously. Many studies focused on the use of PLGA NPs for drug delivery to the targeted diseases in the animal models by oral or injection administration. Such a strategy of local drug delivery would be a distinct improvement compared with existing delivery devices for these diseases.

Diabetes develops due to a diminished production of insulin. Thus, insulin is used to treat some forms of diabetes mellitus. Studies investigated the preparation of PLGA NPs and PLGA-Hp55 NPs as potential drug carriers for oral insulin delivery by a modified

emulsion–solvent diffusion method in water. Their physicochemical characteristics, drug release *in vitro* and hypoglycemic effects in diabetic rats were evaluated. Insulin stability during microencapsulation and subsequent release is essential for retaining its biological activity. A novel s/o/w anhydrous encapsulation method was developed with a combination of stabilizers for maintaining the integrity of insulin during formulation and delivery. Liposolubility of insulin is another consideration when preparing insulin-loaded PLGA NPs.

Cui *et al.* prepared insulin–phospholipid complex-loaded NPs by a novel reverse micelle-solvent evaporation method, where soybean phosphatidylcholine was employed to improve the lipid solubility of insulin[106].

Several studies demonstrated that modified PLGA, by blending with other polymers, showed improved or enhanced characteristics and physiological properties. For example, a simple and versatile delivery platform for peptide and protein based on physically crosslinked PVA hydrogels containing insulin-loaded PLGA NPs was successfully fabricated. When insulin loaded PLGA NPs were administered intraperitoneally as a single dose (20 U/kg) to streptozotocin-induced diabetic mice, blood glucose levels of these mice decreased and sustained over 24 h. *In vitro* study suggested that PLGA NPs entrapped into the PVA hydrogels showed more suitable controlled-release kinetics for protein delivery and caused a reduction in both the release rate and the total amount of insulin released[104]. Basarkar *et al.* prepared cationic NPs PLGA/E100 by blending PLGA and methacrylate copolymer (Eudragit® E100) to deliver a therapeutic gene, encoding mouse IL-10, leading to prevention of autoimmune diabetes. PLGA/E100 NPs increased the expression of IL-10 *in vitro* and *in vivo*; it led to increased expression of IL-10, which resulted in effective protection against insulitis compared with PLGA NPs or methacrylate copolymer alone. This study suggests that feasibility of using cationic PLGA/E100 NPs for *IL-10* gene delivery for the prevention of autoimmune diabetes[107].

Poly (lactic-co-glycolic acid) NPs are one of the most innovative noninvasive approaches for the drug delivery to the CNS. Modified PLGA NPs can cross the blood–brain barrier and deliver the drugs to exert their pharmacological activity in the central nervous district[108-110]. In one of the recent animal studies, PLGA derivatized with the peptide g7 (g7-NPs) was loaded with loperamide and with a fluorescent dye, rhodamine-123[108]. It concluded that g7-NPs are able to cross the blood–brain barrier, ensuring a sustained release of the embedded drug, and that these NPs are able to reach all the brain areas examined. The ability to enter the CNS appears to be linked to the sequence of the peptidic moiety present on their surface. As the mechanism of action is not clear, it was hypothesized that the parent opioid peptides crosses the blood–brain barrier by adsorption-mediated endocytosis by the brain capillary endothelial cells, due to their amphypathic character and helical conformation[108]. In another study, rhodamine-123 alone was encapsulated into PLGA NPs to examine the biodistribution of delivered drugs in the targeted sites cochlea, liver and kidney of pigs[111]. Drug delivery to the cochlea is difficult due to the limited blood flow to the cochlea and the blood–labyrinth barrier, which limits the transportation of molecules from blood to cochlear tissues. Rhodamine NPs by intravenous injection were identified in the cochlea after systemic or local application, suggesting that PLGA NPs have a potential use in drug delivery to the cochlea. The transfer of PLGA NPs through the round-window membrane to the perilymph was also demonstrated, indicating the efficacy of encapsulating drugs in PLGA NPs as a strategy for sustained and targeted drug delivery to the cochlea[111].

Poly (lactic-co-glycolic acid) NPs are also used as drug carriers for the treatment of inflammation diseases, such as arthritis and bowel disease, in the animal models. Arthritis is a group of conditions involving damage to the joints of the body and is the leading cause of disability in older people. Medications are based on the types of arthritis. Glucocorticoids are highly effective in treating joint inflammation, but their systemic application is limited because of a

high incidence of serious adverse effects, especially related to long-term treatment. However, gluco corticoid or other agents encapsulated in PLGA NPs have shown slow release and are targeted to inflamed joints after intravenous administration in experimental arthritis models. PLGA encapsulation can also strongly promote the therapeutic efficacy of drugs as an intravenous treatment for inflammatory diseases[112,113,114]. For the treatment study of inflammatory bowel disease, experimental colitis was induced by trinitrobenzenesulfonic acid in male Wister rats, Rolipram, an anti-inflammatory drug model, was incorporated within PLGA NP's and administered orally once a day for 5 days. The NP groups continued to show reduced inflammation levels after the administration stopped, orally administered anti-inflammatory drug incorporated into PLGA NPs. This new delivery system enabled the drug to accumulate in the inflamed tissue with higher efficiency than the vehicle control group. The NP deposition in the inflamed tissue should be given particular consideration in the design of new carrier systems for the treatment of inflammatory bowel disease[114].

Several other studies also described using PLGA NPs to deliver the antioxidant vitamin E or antioxidant enzymes, such as super-oxide dismutase and coenzyme Q10, to the desired sites[115-116]. PLGA has been also a common choice in the production of a variety of other biomedical devices, such as sutures, implants, prosthetic devices and *in situ*-formed devices[117].

Owing to their subcellular and submicron size, PLGA NP delivery systems have distinct advantages for drug delivery, such as reducing dosage, ensuring the pharmaceutical effects, minimizing side effects, protecting drugs from degradation and enhancing drug stability. PLGA NPs can penetrate deep into tissues through fine capillaries, cross the fenestration present in the epithelial lining or blood–brain barrier and are generally taken up efficiently by the cells.

This allows efficient delivery and accumulation of therapeutic agents, such as conventional medicines, vaccine antigens, proteins and genes, to target sites (tissues or organs) in the body. PLGA NPs also have the advantage of sustained and controlled release of the encapsulated therapeutic agent over a period of days to several weeks compared with natural polymers, which have a relatively short duration of drug release. Poly (lactic-co-glycolic acid) encapsulation can strongly promote the therapeutic efficacy of drugs for treatment of diseases, such as CVD and cancer, and enhance the immunologic effects of vaccines. PLGA NPs have also been used in the production of a variety of vascular tissue engineering devices, such as grafts, stents and other prosthetic devices that mimic the native vascular tissue, and in the development of ultrasound contrast agents to improve ultrasound imaging for CVD and cancer diagnosis.

The promise of these technologies and approaches using PLGA NPs represents a new avenue to the management of CVD, cancer and other diseases and medical conditions. However, thorough evaluation for pharmacokinetics, biodistribution and toxicity is still required before widespread use of PLGA NPs in clinical trials, and the expected result of which is solid proof of efficacy.

CONCLUSION

PLGA polymers have been shown to be excellent delivery carriers for controlled administration of drugs, peptides and proteins due to their biocompatibility and biodegradability. In general, the PLGA degradation and the drug release rate can be accelerated by greater hydrophilicity, increase in chemical interactions among the hydrolytic groups, less crystallinity and larger volume to surface ratio of the device. All of these factors should be taken into consideration in order to tune the degradation and drug release mechanism for desired application. During the last few years, research on the PLGA NP drug delivery has resulted in hundreds of publications. These studies of PLGA NPs result in a significant improvement in PLGA NP preparations and treatment strategies. These polymers are increasingly becoming feasible candidates for drug delivery systems, anticancer agents and vaccine immunotherapy. Along with better

understanding of diseases, new methods will be designed to improve the treatment and diagnosis. The PLGA NP materials need to be further developed and to be accepted by the market. However, in the next 5 years, more attention will be focused on the thorough *in vivo* evaluation for pharmacokinetics, biodistribution and toxicity before the use of PLGA NPs in more clinical trials. Further solid proof of efficacy is expected to be achieved from clinical trials, particularly from patients with CVD and cancer. The studies of PLGA NPs as vaccine candidates will focus on improving such features as providing delivery vehicles with the adequate surface molecules for recognition by the immune system and for more-effective targeting. We also believe that PLGA NPs will be developed to the treatment and diagnosis of a variety of other diseases. Furthermore, PLGA technology should play more important roles in tissue engineering and stem cell research.

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