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Current advances in research and clinical applications of PLGA-based nanotechnology

Jian-Ming Lü,
Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA; and, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Xinwen Wang,
Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA; and, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Christian Marin-Muller,
Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA; and, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Hao Wang,
Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA; and, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Peter H Lin,
Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA; and, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Qizhi Yao, and
Molecular Surgeon Research Center, Division of Vascular Surgery and Endovascular Therapy, Michael E DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA; and, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA

Changyi Chen, MD, PhD†
Michael E DeBakey Department of Surgery (R413), Baylor College of Medicine, One Baylor Plaza, Mail Stop: BCM390, Houston, TX 77030, USA Tel.: +1 713 798 4401 Fax: +1 713 798 6633 jchen@bcm.tmc.edu

Abstract

Co-polymer poly(lactic-co-glycolic acid) (PLGA) nanotechnology has been developed for many years and has been approved by the US FDA for the use of drug delivery, diagnostics and other applications of clinical and basic science research, including cardiovascular disease, cancer, vaccine and tissue engineering. This article presents the more recent successes of applying PLGA-based nanotechnologies and tools in these medicine-related applications. It focuses on the possible mechanisms, diagnosis and treatment effects of PLGA preparations and devices. This updated

†Author for correspondence Michael E DeBakey Department of Surgery (R413), Baylor College of Medicine, One Baylor Plaza, Mail Stop: BCM390, Houston, TX 77030, USA Tel.: +1 713 798 4401 Fax: +1 713 798 6633 E-mail: jchen@bcm.tmc.edu.

information will benefit to both new and established research scientists and clinical physicians who are interested in the development and application of PLGA nanotechnology as new therapeutic and diagnostic strategies for many diseases.

Keywords

cancer; cardiovascular disease; diagnosis; drug delivery; imaging; nanoparticle; nanotechnology; polylactic-co-glycolic acid; vaccine

Nanotechnology, or nanoscience, is a highly multidisciplinary field of applied science and technology intended to create, understand and use atomic- and molecular-scale (0.1−100-nm) structures, and to fabricate devices or materials that lie within the nano size range [1]. Nanotechnology applies the principles of engineering, electronics, physical and material science, and manufacturing to the molecular or submicron level [2]. It has already had significant impact in the development of novel products in industry. Many materials of nanostructure, such as nanoparticles (NPs), nanosensors, nanofibers, nanowire, nanosheaf and nanomesh have been developed (Table 1). These materials are generally, with all three dimensions, less than 100 nm. However, many important nanomaterials possess one or more dimensions that are greater than 100 nm, for example, carbon nanotubes are usually 500 nm long, while as little as 2 nm in diameter. Most current nanomaterials could be organized into four types: carbon-based materials, metal-based materials, polymers or dendrimers and composites (Table 2). A variety of other types of nanomaterials are expected to appear in the future [201].

The unique properties arising from the nanoscale dimensions of these nanomaterials give them novel electrical, catalytic, magnetic, mechanical, thermal or imaging features that are highly desirable for applications in commercial, medical, military and environmental sectors. Research and development of these materials have broadened their applications, and many new branches, such as nanomedicine, nanobiology, nanobiotechnology, nanotherapeutics, nanosurgery, nanomechanics and nanotoxicology, have been developed (Table 1). Nanobiotechnology is the branch of nanotechnology with biological and biochemical applications or uses. Nanobiotechnology often studies existing elements of nature in order to fabricate new devices. The application of nanotechnology in biology results in nanobiology. Nanomechanics is a branch of nanoscience studying fundamental mechanical (elastic, thermal and kinetic) properties of physical systems at the nanometer scale. Nanomedicine is the most important application of nanotechnology in the medical field. The application of nanotechnology to disease treatment, diagnosis, monitoring and to the control of biological systems has recently been referred to as nanomedicine by the NIH in the USA. Research into the rational delivery and targeting of pharmaceutical, therapeutic and diagnostic agents is at the forefront of projects in nanomedicine [3,4]. Nanosystems, which can be designed to have different compositions, have unique physical and biological properties that might be used to overcome the limitations of molecular imaging and gene/drug delivery in recent years [1,5].

The medical use of nanomaterials includes advanced drug delivery systems, new therapies and *in vivo* imaging [6–10]. Neuroelectronic interfaces and other nanoelectronics-based sensors are another active goal of research. Significant effort has been devoted to developing nanotechnology for drug delivery since it offers a suitable means of delivering small-molecular-weight NP drugs, as well as macromolecules, such as proteins, peptides or genes by either localized or targeted delivery to the tissue of interest [11]. Several excellent articles have reviewed the application of nanotechnology to the molecular imaging and therapy of the cardiovascular system [6–10], as well as nanomedicine for drug delivery and imaging for cancer therapy and diagnosis using targeted functional NPs [1,12]. Specifically, Patel *et al.*

reviewed the potential application of a variety of NPs and devices in cardiovascular imaging and therapeutics and nanoporous structures for sensing and implant-based drug delivery [13].

Nanomaterials 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 [5,6]. In the last 20 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 [14]. Polymers may be linear, branched or globular, and their size can be tightly controlled. Polyamides, poly(amino acids), poly(alkyl-α-cyano acrylates), polyesters, poly orthoesters, polyurethanes and polyacrylamides have been used to prepare various drug-loaded devices [15,16]. Amongst them, the thermoplastic aliphatic polyesters, such as polylactic acid, poly glycolic acid and especially the copolymer poly(lactic-co-glycolic acid) (PLGA), have a long history of use as biomaterials due to their excellent biocompatibility and bio degradability, beginning in the 1970s with biodegradable sutures [10,17]. Furthermore, they have been approved by the US FDA for drug delivery. While different aspects of NPs and microparticles have been reviewed in detail elsewhere, this article presents the 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 and devices are discussed. This updated information will benefit both new and established research scientists and clinical physicians who are interested in the development and application of nanotechnology as new therapeutic strategies for many diseases.

PLGA-based nanotechnology

Poly(lactic-co-glycolic acid) is a copolymer synthesized by means of random ring-opening copolymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid (Figure 1a). Common catalysts used in the preparation of this copolymer include tin (II) 2-ethylhexanoate, tin (II) alkoxides or 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 [18]. The forms of PLGA are usually identified by the monomers' ratio used (e.g., PLGA 50:50), which is most frequently used in the nanotechnology, identifies a copolymer whose composition is 50% lactic acid and 50% glycolic acid.

Different physical characteristics of PLGA NPs (size, size distribution, morphology and ζ potential) can be synthesized by controlling the parameters specific to the synthesis method employed. The preparation methods and properties of PLGA NPs have been reviewed previously [18]. The most commonly used method for PLGA NP formation is the single- or double-emulsion-solvent evaporation. Single-emulsion process involves oil-in-water (o/w) emulsification, while the double-emulsion process is a water-in-oil-in-water (w/o/w) method. The w/o/w method is best suited to encapsulate water-soluble drugs, such as peptides, proteins and vaccines, while the o/w method is ideal for water-insoluble drugs, such as steroids [16]. In some cases, solid/oil/water (s/o/w) techniques have been exploited with PLGA-based microspheres, especially for a higher drug loading of large water-soluble peptides, such as insulin. Briefly, for the o/w method, PLGA is first dissolved in a water-immiscible, volatile organic solvent (e.g., dichloromethane), the drug is then added to the polymer solution to produce a solution or dispersion of the drug particles. This polymer–solvent–drug solution/dispersion is then emulsified with appropriate stirring and temperature conditions in a larger volume of water in the presence of an emulsifier such as poly(vinyl alcohol) (PVA) to yield an o/w emulsion. PVA is used to stabilize the emulsion, since it forms particles of relatively small sizes and uniform size distribution [19]. Followed by solvent removal by either

evaporation or extraction to harden the oil droplets, the solid nanospheres obtained are then washed and collected by filtration, sieving or centrifugation. These are then dried under appropriate conditions or are lyophilized to give the final free-flowing injectable nanosphere product [16,20–23]. We have used this method to produce PLGA NPs approximately 100 nm in size (Figure 1b). PLGA NPs are colloidal systems that typically range from 10 to 1000 nm in diameter, with the therapeutic agent either entrapped into or adsorbed or chemically coupled onto the polymer matrix [15]. A typical procedure for preparation of PLGA NPs and NPs containing plasmid DNA or other drugs is available in the literature [23–25].

The reason why PLGA NPs are biodegradable in the body is 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 byproducts of various metabolic pathways in the body under normal physiological conditions [26]. 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 [16,27]. The degradation products are easily metabolized in the body via the Krebs cycle and are eliminated [28]. Thus, there is very minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications. As reviewed by Athanasiou *et al.*, the *in vivo* and *in vitro* studies testing the toxicity/biocompatibility demonstrated that PLGA nanomaterials have satisfactory bio-compatibility and absence of significant toxicity [26]. The *in vivo* studies involved applications in bone, articular cartilage and the meniscus, and a significant number of other studies performed *in situ* in muscle or other soft tissues. All the results appear to support the *in vivo* use of PLGA biomaterials, although some cases reported inflammatory responses [26].

The biodistribution 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 [29]. This effect is enhanced when the NP is shielding with a poly(ethylene glycol/oxide) surface modification [30,31]. 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 phagocytocis [30].

It is necessary to sterilize all medical implants after fabrication and prior to their surgical placement to reduce the risk of infection and other complications. Athanasiou *et al.* reviewed the advantages and disadvantages of the common terminal sterilization methods of steam, dry heat, ethylene oxide gas and ionizing radiations [26]. Among them, dry heat and steam sterilizations are carried out at high temperatures and can cause severe degradation and hydrolysis of polymeric microparticles; ethylene oxide is not applicable due to its toxic residues. The γ-irradiation causes instability, deterioration and cross-linking breakage of polymer chains but it is currently the more frequently used method for terminal sterilization of PLGA nanodevices. Many studies are still focused on the development of a suitable method for sterilizing PLGA devices [32,33]. For instance, Shearer *et al.* determined the effects of sterilization with ethanol, peracetic acid, ultraviolet irradiation and antibiotic solution on the structure of PLGA hollow-fiber scaffolds and found that none of the sterilization methods are ideal in terms of sterilizing the sample without causing structural changes. However, they

suggested that the antibiotic treatment would provide a convenient, effective method with which to sterilize PLGA hollow fibers for use as a tissue-engineering scaffold [33].

Drug delivery is the method or process of administering a pharma ceutical compound to achieve a therapeutic effect in humans or animals [5]. Medications, such as peptide-, protein-, antibody-, vaccine- and gene-based drugs, may not be delivered using routine delivery methods because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently enough, owing to molecular size and charge issues, to be therapeutically effective. Therefore, many efforts have been focused on the targeted delivery where the drug is only active in the target area of the body, such as tumor tissues, and sustained-release formulations in which the drug is released over a period of time in a controlled manner. A wide variety of natural and synthetic biodegradable polymers have been investigated for drug targeting or prolonged drug release [15,16].

Poly(lactic-co-glycolic acid) is a polymer approved by the FDA for drug delivery owing to its biodegradability, drug biocompatibility, suitable biodegradation kinetics and mechanical properties and ease of processing [16,34,35]. It has been widely used to formulate into different biodegradable devices, such as microparticles, NPs, implants and miscellaneous devices, as well as *in situ*-formed devices. Biodegradable PLGA NPs have been investigated for sustained and targeted/localized delivery of different agents, including drugs, proteins and peptides, and recently plasmid DNA, owing to their ability to protect DNA from degradation in endolysosomes [15,23,36–39].

Owing to their subcellular and sub-micron size, NP delivery systems have distinct advantages for drug delivery. NPs can penetrate deep into tissues through fine capillaries, across the fenestration present in the epithelial lining (e.g., liver) and, generally, are taken up efficiently by the cells [40]. This allows efficient delivery of therapeutic agents to target sites (tissue or organ) in the body. NPs also have the advantage of sustaining the release of the encapsulated therapeutic agent over a period of days to several weeks compared with natural polymers that have a relatively short duration of drug release [15]. PLGA has been a common choice in the production of a variety of biomedical devices, such as grafts, sutures, implants and prosthetic devices [16]. For example, a commercially available drug delivery device using PLGA is Lupron Depot® for the treatment of advanced prostate cancer. Thus, PLGA NPs have great potential in many clinical applications currently under active investigations.

Cardiovascular disease

Cardiovascular disease (CVD) has been the leading cause of death every year since 1900 in the USA and is still the most common cause of death in the USA. More than 50% of these deaths are caused by coronary artery disease, according to the most recent data from the American Heart Association's Heart Disease and Stroke Statistics – 2008 Update [202]. Effective treatments of CVD include preventive lifestyle changes, medications and surgical procedures [17,41,42]. The rapid innovations and expansions of nanotechnology have opened new avenues for the management of CVDs. Several excellent reviews have described and summarized existing literature on the application of general nanotechnology to imaging and therapeutic aspects of CVD [6,8,9]. By contrast, 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 [43]. 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 [44]. 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 [45–48]. This problem has motivated scientists and physicians to explore tissue engineering approaches to replace blood vessels [45–49]. There have been significant advances toward creating functional blood vessel substitute ions. The fabrication of a vascular graft with mechanical, structural and functional properties similar to native vessels has also been investigated. Tissue engineering brings new hope to this field, but still faces many challenges [42]. 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 [50]. Specifically, the scaffold used for cell growth should be biodegradable, strong and elastic, as it has been shown that cyclic mechanical strain during tissue development can improve histological organization and enhance extracellular matrix synthesis [51,52]. A tissue engineered vascular graft appears to be a promising solution that could meet all requirements needed for that purpose.

As described, PLGA is an 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 [42].

A key aspect of tissue-engineering approaches is the development of scaffolds that support, stimulate and direct the growth of specific cells. Sarkar *et al.* developed a technology by incorporating PLGA micro/NPs into molten biopolymer polycaprolac-tone (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 [53]. Since PLGA degrades at a far faster rate than PCL [54], 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 [55].

The incorporation of some contributed genes [56] or proteins, such as basic FGF, delivered by NPs in the scaffolds may improve the mechanical and biologic properties of engineered grafts. Our studies have shown that PLGA NPs formulated with a fluorescence molecule, 6-coumarin, can be effectively delivered to a new tissue-engineered vascular graft derived from a porcine carotid artery with decellularization and heparin immobilization procedures (Figure 2). Vascular tissue concentration and distribution of PLGA NPs can easily be controlled by justifying NP concentration, luminal pressure and delivery time. Furthermore, we have successfully formulated basic FGF-containing PLGA NPs and delivered these NPs into the decellularized and heparinized porcine carotid artery vascular graft. Human coronary artery

endothelial cells seeded on the graft luminal surface showed an enhanced cell proliferation in the graft with basic FGF-PLGA NP delivery compared with control grafts (bovine serum albumin containing NP graft and empty PLGA NP graft) (Figure 3). These data demonstrate PLGA NPs can be used to deliver growth factors to the tissue-engineered vascular graft and enhance the interaction between vascular cells and the graft scaffold, thereby modulating vascular graft healing and remodeling. 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 [57]. 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 [58]. Commonly used cells in tissue-engineered vascular grafts include endothelial cells, smooth muscle cells (SMCs), mesothelial cells, fibroblasts and even bone marrow-derived endothelial/SMC progenitor cells [48,59–62]. These cells isolated from autologous tissues could be modified to counteract the disease condition, then cultured *in vitro* together with the vascular construct for up to several weeks prior to implantation. PLGA NPs could effectively deliver proteins, DNA or other biological reagents to these cells and induce a favorable response of cells for vascular graft healing and remodeling. For example, we have successfully formulated PLGA NPs containing fluorescence molecule 6-coumarin or plasmid DNA with green-fluorescence protein gene and demonstrated their effective delivery of these molecules into human coronary artery endothelial cells (Figure 4). 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 [63]. 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 (imaging)

Effective prevention of cardiovascular morbidity and mortality will require the development of new diagnostic and therapeutic strategies aimed at treating early and subclinical disease stages. Nanotechnology has also 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 [64].

The introduction of modern contrast agents is critical in diagnostic ultrasound [65]. Modern ultrasound contrast agents are primarily comprised of microbubble (microspheres with porous or hollow inner structure) formulation that circulates in the intravascular compartment and are designed to enhance acoustic signals reflected from the blood pool. Therefore, optimal density differences are obtained when the contrast agent is a gas because tissues are primarily composed of liquid. The hollow, 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 [66]. 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 [65]. 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 [66,67].

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 [66]. Stent placement is currently the primary intervention for cardiovascular occlusive diseases; however, it may lead to in-stent restenosis [68–70]. 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 [71]. 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 β_3-integrin receptor interactions and associated changes in actin dynamics [72]. 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 [69]. 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 [73].

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 [74–76]. 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 [74]. 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 [77]. 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

[78]. It has also been reported that an antisense oligodeoxynucleotide released from PLGA microparticles inhibited SMC growth *in vitro* [79].

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 didodecyldimethylammonium bromide modification were an effective means of inhibiting proliferative response to vascular injury in a rabbit arterial balloon injury model [80]. 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 [81]. In addition, nanospheres composed of PLGA and containing PDGF-β receptor antisense have been formulated and examined [82]. 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 [83].

Cancers

Cancer is the second leading cause of death, responsible for approximately 23% of deaths in the USA, according to the statistics for 2008 of American Cancer Society [203]. Cancer research is an intensive scientific effort in order to identify the causes and develop specific strategies for prevention, diagnosis, treatments and cures. Many forms of cancer are treatable by present therapies, such as surgery, chemotherapy, radiation therapy, immunotherapy or other methods. However, chemotherapy for cancers are usually limited by the toxicity of drugs to normal tissues (i.e., in the process of killing cancer cells). Chemotherapeutic agents also damage healthy tissues, leading to systemic toxicity and adverse effects that greatly limit the maximal allowable dose of anticancer drugs and, thus, restricts their therapeutic efficacy. 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 [1].

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 macro molecules 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 [1]. 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 [84]. 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 [85]. Forsberg *et al.* used PLGA/camphor/ammonium carbonate particles as a contrast agent to enhance the Doppler signal after PLGA injection [86].

During cancer treatment, a major difficulty is destroying tumor cells without destroying normal tissues. In order to improve the selective toxicity of anticancer therapeutics, cancer-selective delivery systems are highly desired for chemotherapeutic agents for their ability to efficiently deliver the drug load to the tumor site. The most commonly used method is employing molecules that specifically recognize and interact with cancer cells. These molecules include antibodies, growth factors, transferrins, cytokines, protein or other agents. The basic principle that underlies ligand-targeted therapeutics is that the selective delivery of antineoplastic drugs to cancer cells or cancer-associated tissues, such as tumor vasculature, can be enhanced by associating the drugs with molecules that bind to antigens or receptors that are either uniquely expressed or overexpressed on target cells compared with normal tissues. This allows the specific delivery of drugs to cancer cells [87].

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 [87,88]. 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 $MCF7_{TR}$ 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

[31]. 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 [89]. 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 [90]. Mitoxantrone-loaded PLGA microspheres were also demonstrated to deliver therapeutic concentrations of drugs to the tumor and prevent glioma growth without causing side effects [91].

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 [92]. Another study employed the PLGA NP delivery system to specifically deliver monoclonal antibody (mAb) for targeting invasive epithelial breast tumor cells [93]. 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 [94]. 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 ana lysis 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) [95]. 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.

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 [96]. 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 [97], 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) [98,99]. The majority of the existing literature involving PLGA polymers has tended to be focused on PLGA microspheres. In the last 10 years, microspheres have been used extensively for the injectable delivery of vaccine antigens, both for viral and bacterial antigens. As an example of the latter, 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 [100]. 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 [101]. 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 [102].

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 [103,104], while phenotype and functional analysis of DCs *in vitro* revealed no negative effects on their pivotal properties [105].

While PLGA microspheres have been studied more extensively as targeting vehicles, NPs theoretically offer many inherent advantages to targeting and delivery due to their small size: NPs range in size from 10 to 100 nm, whereas microparticles range from 1 to 1000 μm [98, 106,107]. Their smaller size allows the NPs to maneuver through organ-specific physical and physiological barriers [106]. This means that PLGA NPs of different sizes within their range can, in theory, be injected systematically to target specific immune-response modulators, such as DCs and macrophages both *in vitro* and *in vivo*, rather than only being useful in a localized immunization, as larger polymer molecules have demonstrated [98,108].

Similar to microspheres, PLGA NPs have been shown to effectively enhance immune responses. Hamdy *et al.* recently demonstrated that particulate delivery of ovalbumin and 7-acyl lipid A to DCs leads to an increase in antigen-specific $CD8^+$ and CD4+ T-cell immune responses, as evidenced by 3000-fold increase in $CD8^+$ T-cell proliferative responses *in vitro* and a 13-fold increase clonally expanded $CD4^+$ T cells *in vivo* [109]. The focus of studies has been on understanding how DC delivery and priming of naive T cells, with the goal of enhancing antigen vaccine delivery to induce the maturation of DCs [110,111]. Elamanchili *et al.* assessed the extent of maturation of DCs after treatment with monophosphoryl lipid A encapsulated in NPs [110]. 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 [112]. 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 [113]. Other studies have sought to improve on the ability of M-cell targeting by grafting arginine-glycine-aspartic (RGD) peptides onto PEGylated PLGA NPs [114]. Interactions between the RGD ligand and the β^1-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 [115]. Another issue that has prevented widespread use of NPs as the vaccine system of choice is the new push for producing multivalent vaccines that encapsulate not only the antigen or antigens of choice but also include adjuvants to help the efficacy of the immune response. Studies using a multivalent approach have demonstrated that coencapsulation of several antigens may intrinsically improve entrapment of the antigen during the process of creating the polymers [116], as well as produce an enhanced immune response by broadening the response against a single infectious agent [117–119]. For example, Heit *et al.* determined that full maturation of and cytokine secretion by APCs as well as crosspresentation of antigen were enhanced when a coencapsulation of antigen with Toll-like receptor 7 and 9 ligands was used [120].

It is in this respect that the size of the NPs has a negative impact, as it becomes increasingly more difficult to maintain the stability of the NPs when attempting to encapsulate multiple-conjugated vaccines. Several studies have documented different methods to improve the encapsulation efficiency of NPs and to effectively quantify the levels of materials that actually become encapsulated during the emulsion process [121]. For example, a study by Ribeiro *et al.* using NPs encapsulating the dendriplexes, complexes of dendrons and condensed plasmids containing the gene for protective antigen of *Bacillus anthracis*, revealed that it is possible to design NPs in a way that allowed for well-defined and prolonged release of encapsulated DNA [122].

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 [114].

Other applications

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 [123,124]. 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 [125]. 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 liposolubility of insulin [126].

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 [124]. 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 suggest the feasibility of using cationic PLGA/E100 NPs for *IL-10* gene delivery for the prevention of autoimmune diabetes [127].

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 [128–130]. In one of the recent animal studies, PLGA derivatized with the peptide g7 (g7-NPs) was loaded with loperamide and with a fluorescent dye, rhoda-mine-123 [128]. 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 adsorbption-mediated endocytosis by the brain capillary endothelial cells, due to their amphypathic character and helical conformation [128]. 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 [131]. 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 [131].

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 [132,133]. For the treatment study of inflammatory bowel disease, anti-inflammatory drug incorporated into PLGA NPs was orally administered by experimental colitis rats, the NP groups continued to show reduced inflammation levels after the administration stopped. 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 [134].

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 [135–137]. 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 [16].

Expert commentary

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.

Five-year view

Drug delivery using PLGA or PLGA-based polymers is an attractive area with innumerable opportunities for biomedical research. 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.

Key issues

- Poly(lactic-co-glycolic acid) (PLGA) is a polymer approved by the US FDA for drug delivery owing to its biodegradability, drug biocompatibility, suitable biodegradation kinetics, mechanical properties and ease of processing.
- Biodegradable PLGA nanoparticles (NPs) have been investigated for sustained and targeted/localized delivery of different agents, including drugs, proteins and peptides and, recently, plasmid DNA, owing to their ability to protect DNA from degradation in endolysosomes.
- PLGA NPs can be used to deliver growth factors to the tissue-engineered vascular graft and enhance the interaction between vascular cells and the graft scaffold, thereby modulating vascular graft healing and remodeling. PLGA NPs drug delivery to the graft extended the period of drug release, thus reducing initial burst release. It can also protect the encapsulated agent from enzymatic degradation.
- 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. PLGA NP-coated stents can effectively deliver genes or drugs to vessel walls. It improves DNA controlled release and this composite enhanced plasmid.
- PLGA NPs 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 widely used in diagnosis and treatment of cancer.
- PLGA microbubbles have been developed for use as ultrasound contrast agents to improve ultrasound imaging in the diagnosis of cardiovascular disease and tumors. This development of NPs as imaging contrast agents may also offer enhanced sensitivity and specificity for *in vivo* tumor imaging.
- Incorporation of several anticancer drugs, such as paclitaxel, cisplatin and hypericin, in the PLGA NPs strongly enhances their antitumoral efficacy compared with the free drugs. PLGA NP encapsulation for delivery of other

anticancer agents, including plasmid DNA, monoclonial antibodies and proteins, has been investigated for tumor inhibition in *in vivo* and *in vitro* animal models.

- PLGA NPs of different sizes within their range can, in theory, be injected systematically to target specific immune-response modulators, such as dendritic cells and macrophages, both *in vitro* and *in vivo*, rather than only being useful in a localized immunization.
- PLGA NPs have been shown to effectively enhance immune responses. 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.

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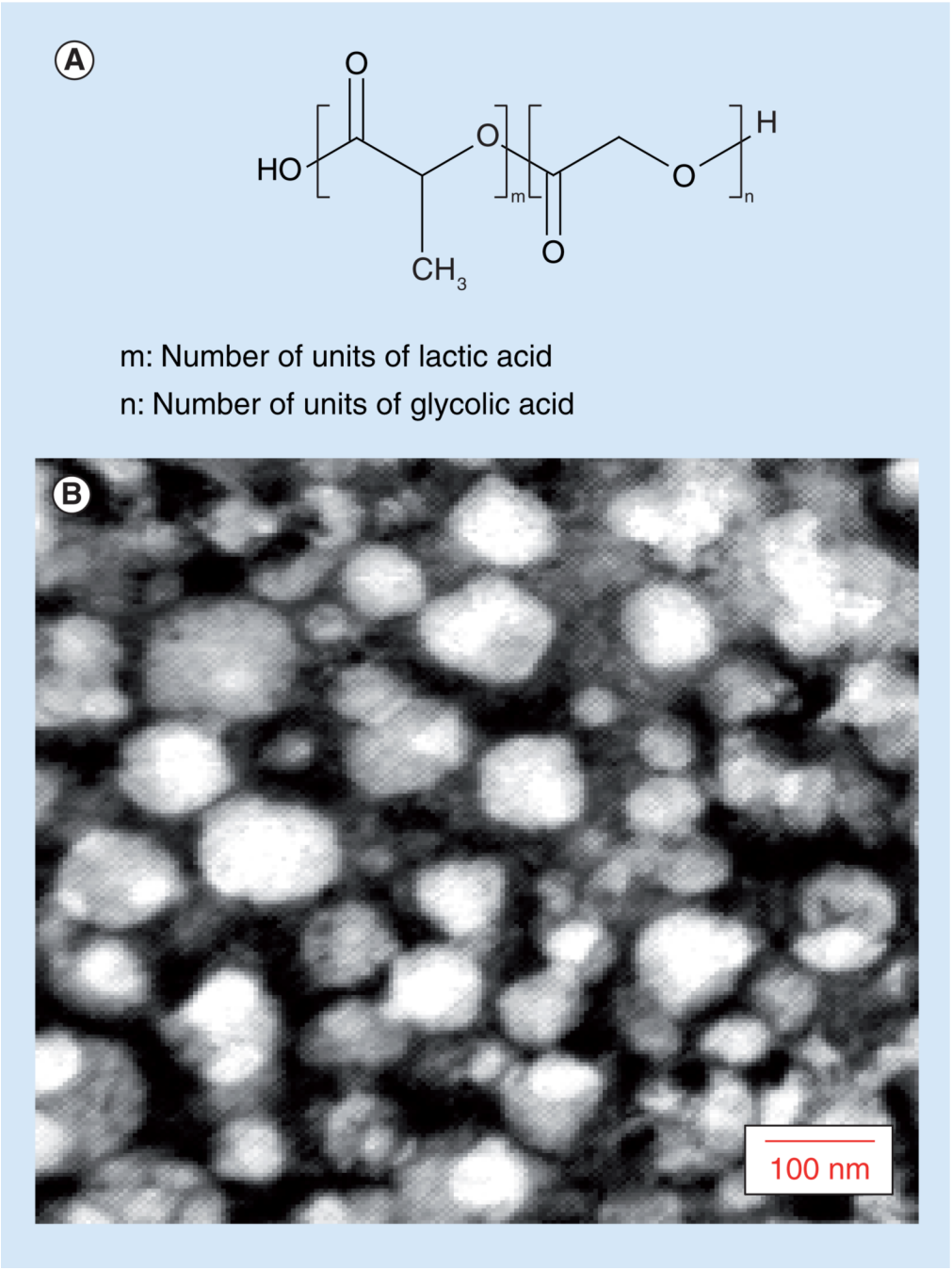

Figure 1. Poly(lactic-co-glycolic acid) chemical structure and nanoparticles

(A) Chemical structure of poly(lactic-coglycolic acid) (PLGA). **(B)** PLGA nanoparticles (NPs). PLGA NPs were produced by single emulsion process involving oil-in-water emulsification at 50:50 of lactic acid and glycolic acid. PLGA NPs were directly visualized under transmission electron microscope with negative staining.

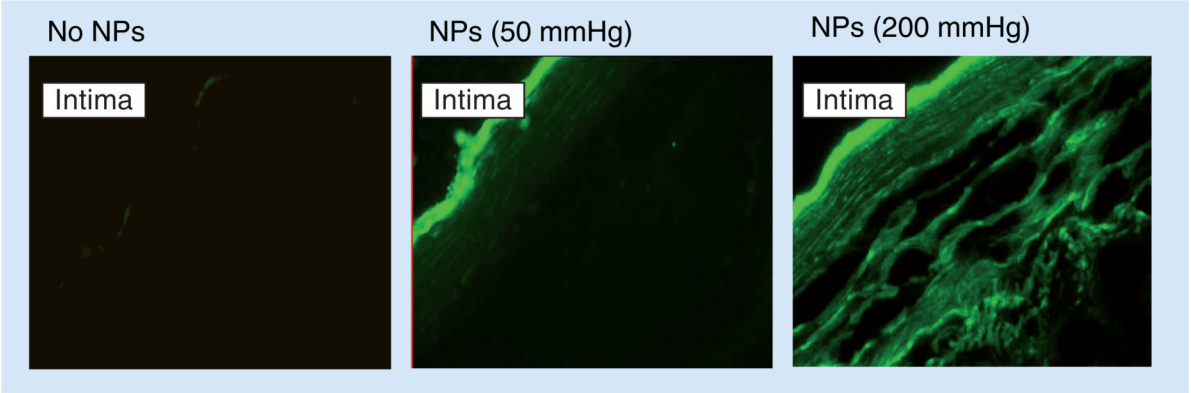

Figure 2. Delivery of poly(lactic-co-glycolic acid) nanoparticles to a tissue engineered vascular graft (decellularized and heparin covalently linked porcine carotid artery graft)

Fluorescence molecule 6-coumarin was formulated into poly(lactic-co-glycolic acid) (PLGA) NPs, which were delivered into the graft through the lumen at pressure 50 and 200 mmHg for 10 min. The graft was sectioned in 7-μm tissue slides and the fluorescence signal from PLGA NPs was directly observed under fluorescence microscope.

NP: Nanoparticle.

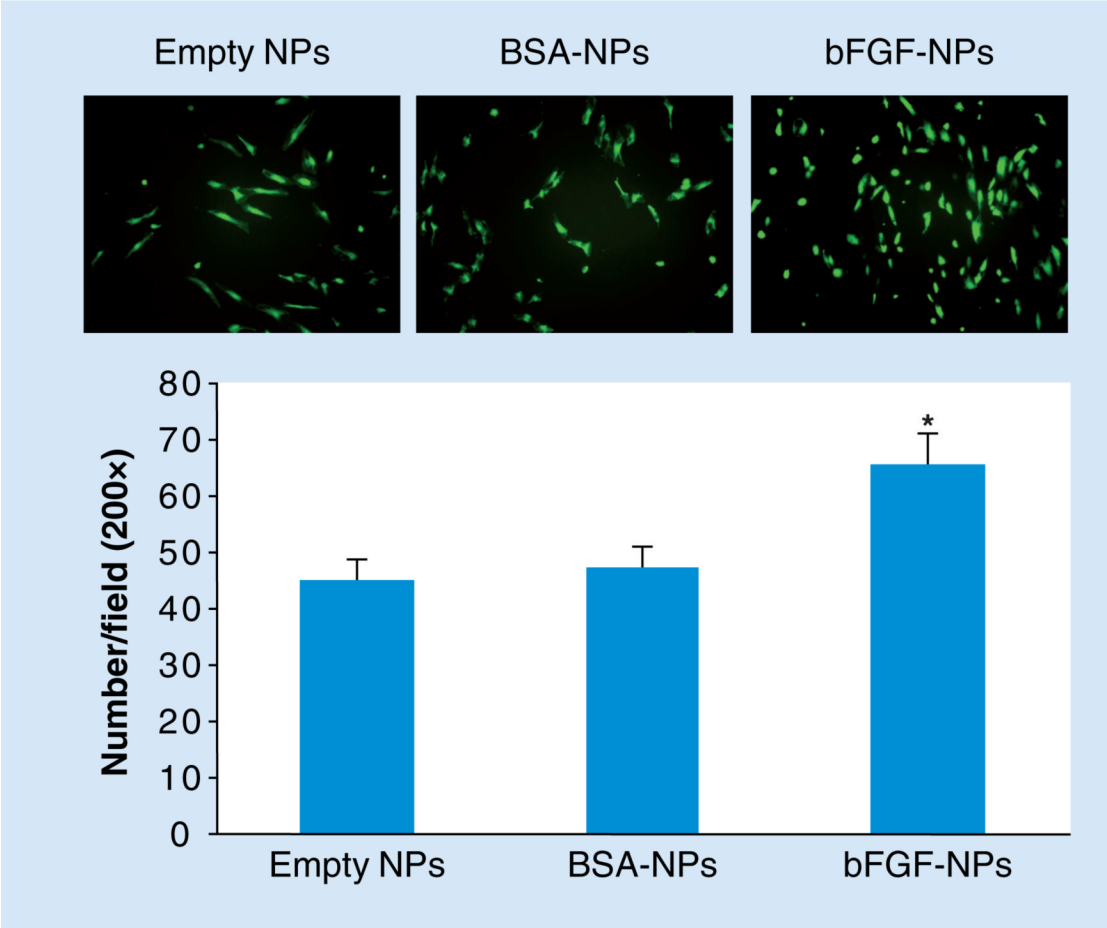

Figure 3. Poly(lactic-co-glycolic acid) nanoparticles deliver bFGF into the tissue engineered vascular graft and increase endothelial cell proliferation on the graft surface

Human recombinant bFGF or BSA was formulated in the poly(lactic-coglycolic acid) (PLGA) NPs, which were delivered to the decellularized and heparinized porcine carotid artery vascular graft. Empty PLGA NPs were served as a negative control. Human coronary artery endothelial cells were seeded on the graft luminal surface and cultured for 48 h. Living cells were stained with a florescence dye calcein-AM. The cells were observed and counted under fluorescence microscope. Magnification: ×200.

bFGF: Basic FGF; BSA: Bovine serum albumin; NP: Nanoparticle.

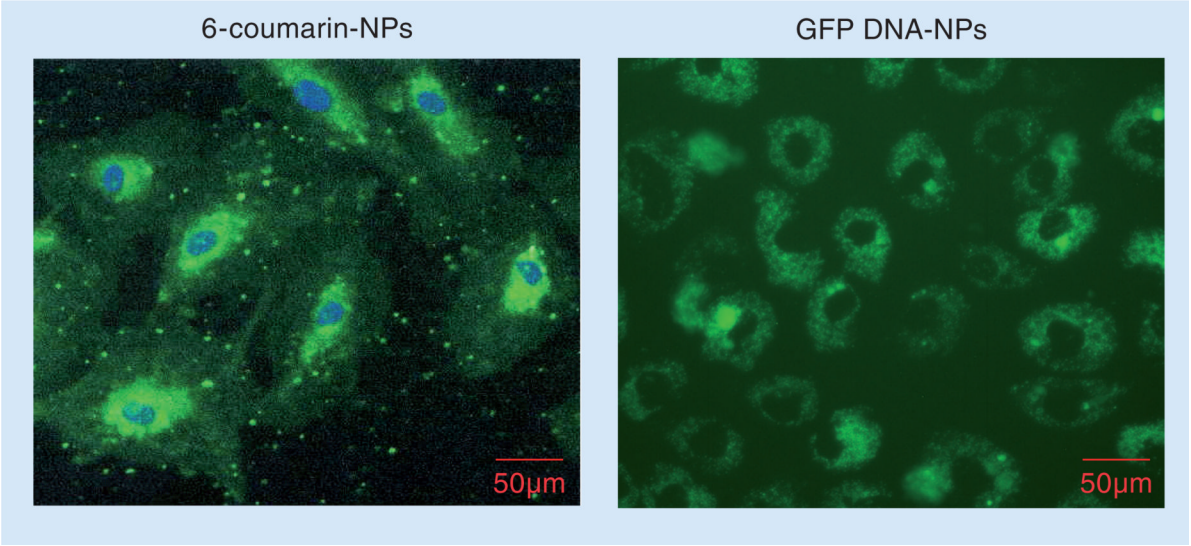

Figure 4. Poly(lactic-co-glycolic acid) NPs deliver fluorescent molecule 6-coumarin and *GFP* gene (plasmid DNA) into the human coronary artery endothelial cells

6-coumarin or GFP containing plasmid DNA was formulated into the poly(lactic-co-glycolic acid) (PLGA) NPs, which were delivered to human coronary artery endothelial cells for 24 h. Florescence signal from PLGA NPs was directly observed under fluorescence microscope. *GFP* gene was successfully delivered into the cells and the gene expression was occurred. Magnification: ×400.

GFP: Green-fluorescence protein; NP: Nanoparticle.

Table 1

Examples of nanotechnology.

Item	Description
Nanomaterials:	
3D <100 nm	Nanoparticles, quantum dots, hollow nanospheres and nanomats
2D <100 nm	Nanofibers, nanotubes, nanowires nanoplatelets, nanofilaments and nanosheafs
1D <100 nm	Nanofilms, nanomeshs, nanocoatings and nanomultilayers
Nanodevices	Nanosensor, nanobiosensor and nanoprobe
Nanomedicine	Application of nanotechnology in the medical field
Nanostructure	An object of intermediate size between molecular and micrometer-sized structures
Nanobiology	Application of nanotechnology in biology
Nanobiotechnology	The nanotechnology with biological and biochemical applications or uses
Nanotherapeutics	Therapeutic approaches of nanomedicine
Nanomechanics	A branch of nanoscience studying fundamental mechanical (elastic, thermal and kinetic) properties of physical systems at the nanometer scale
Nanoscopy	Nanoscale resolution in light microscopy
Nanosurgery	Selectively deletion or knocking out nanometer-sized regions within the nucleus of living cells
Nanosystems	Systems with atomic and molecular precision that make atomically specified structures and devices under programmatic control
Nanotoxicology	The study of the toxicity of nanomaterials

Table 2

Classification of nanomaterials and applications.

Type	Characteristics	Applications
Carbon based	Hollow spheres or ellipsoids: fullerenes; tubes: nanotubes	Films (nanofilms) and coatings (nanocoating), stronger and lighter materials and applications in electronics
Metal based	Quantum dots, nanogold, nanosilver and metal oxides, such as titanium dioxide	Semiconductor crystal of hundreds or thousands of atoms in nanometer scale
Dendrimers or polymers	Nanosized polymers built from branched units with numerous chain ends	Used for catalysis, drug delivery
Composites	Composites combine nanoparticles with other nanoparticles or with larger, bulk-type materials	Added to products ranging from auto parts to packaging materials, to enhance mechanical, thermal, barrier and flame-retardant properties