

# PLGA Nanoparticle Design and Preparation for DDS and Medical Device

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## 1. INTRODUCTION

This paper describes in part the clinical research and development of a nanomedicine drug delivery system (DDS) using the biodegradable poly lactic-co-glycolic acid (PLGA) nanoparticle system, which the authors are working on.

## 2. PLGA NANOPARTICLE DDSs

### 2.1 PLGA Characteristics

Fig. 10.1 shows the chemical structure of PLGA and the hydrolysis of PLGA nanoparticles (average particle size of 200 nm) in a phosphate-buffered saline solution. The *in vivo* degradation of these particles is said to proceed nonenzymatically in the first stage (glass-like) and enzymatically in the second stage (rubber-like) [1]. The breakdown products, lactic acid and glycolic acid, are metabolized in the Krebs cycle.

If a drug can be encapsulated in PLGA nanoparticles, its stability will improve, enabling sustained release by means of hydrolysis [2,3]. Some of the nanosize nonvirus carriers of this type are liposomes, polymer micelles, and nanospheres (nanoparticles), but an especially successful example of microspheres using PLGA as a base is the practical application of microspheres (particle size of about 20–30  $\mu\text{m}$ ), encapsulating luteinizing hormone-releasing hormone (LHRH) derivatives because as a long-term sustained-release system administered once in every 1 or 3 months. If PLGA particles are further reduced to the nanometer scale, they create new functions, which are not found in microparticles, by means of adjusting particle size and surface charge or by surface modification and other means. In other words, because the specific surface area of the particles rapidly increases and their volume becomes minute, interaction with bio-membranes is heightened, the retention, in adhesion to,

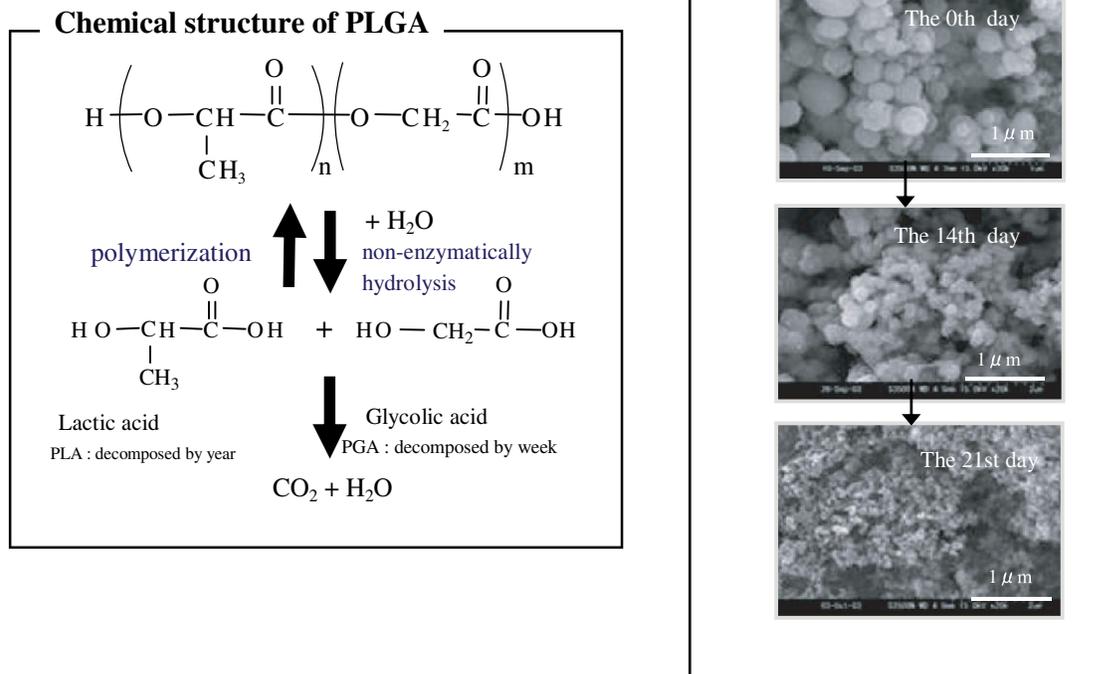
and permeability into mucous membranes are increased, and drug concentration in absorption sites rises. Furthermore, with the additional sustained-release effect, drug absorption improves. Because of endocytosis, such particles can also be used as gene transfer carriers. The nucleic acid of decoy oligonucleotides, siRNA, and anti-sense RNA, which can control the expression of target genes located upstream of disease-onset factors, has strong specificity toward target molecules, and there have been expectations that it will produce pharmaceuticals with strong pharmacological effects and no side effects. Owing to enzymolysis in the blood and high molecular weight, endocytosis is low, and it will surely be used as a DDS in clinical applications.

### 2.2 Basic Technology for Nanoscale PLGA Particles and (Sterile) Mass-Production Technology

The basic technology for nanoscale PLGA particles is the “spherical crystallization technique” [2]. When this method is used in the presence of polymer molecules, not only crystals but also polymer drug carriers with a variety of functions are obtained. PLGA nanoparticles were prepared for the first time using emulsion solvent diffusion (ESD, Fig. 10.2), in which crystallization proceeds via an emulsion. The procedure involves dissolving PLGA and a drug in a water-miscible organic solvent, on which they form nanoparticles due to the formation of tiny emulsion droplets owing to self-emulsification occurring when added to water and also due to PLGA deposition inside emulsion droplets caused by the mutual dispersion of the two solvents, the organic solvent and water.

Fig. 10.3 shows photographs of PLGA nanoparticles taken with an atomic force microscope and the particle-size distribution. This method has broad applicability for various drugs from water-soluble to

## Ex. Degradation of the PLGA nano-particle (200 nm)



**FIGURE 10.1** Chemical structure of PLGA: poly lactic-co-glycolic acid and degradation behavior in the phosphate-buffered saline solution.

hydrophobic types, which is achieved by solvent combinations. They are mucoadhesive and permeating and can be oriented to drug absorption sites (primary targeting). Furthermore, macrophages phagocytize them, and they are shown to have cell permeability (secondary targeting). It has been found that these functions are further amplified by means of surface reformation. The authors have created a method to control the diameters of these PLGA nanoparticles and have achieved industrial mass production. Our procedure controls the sizes of ESD-produced PLGA nanoparticles mainly using the PLGA concentration in solvent and makes the particle size about 150 nm (dynamic light scattering) [3], which makes pressure-filtration sterilization possible. By doing so, we are building good manufacture product (GMP) manufacturing techniques, including guaranteed sterility, that are capable of making, for example, particles needed for injectables.

### 2.3 Nanocomposite Technologies for Practical Use of PLGA Particles

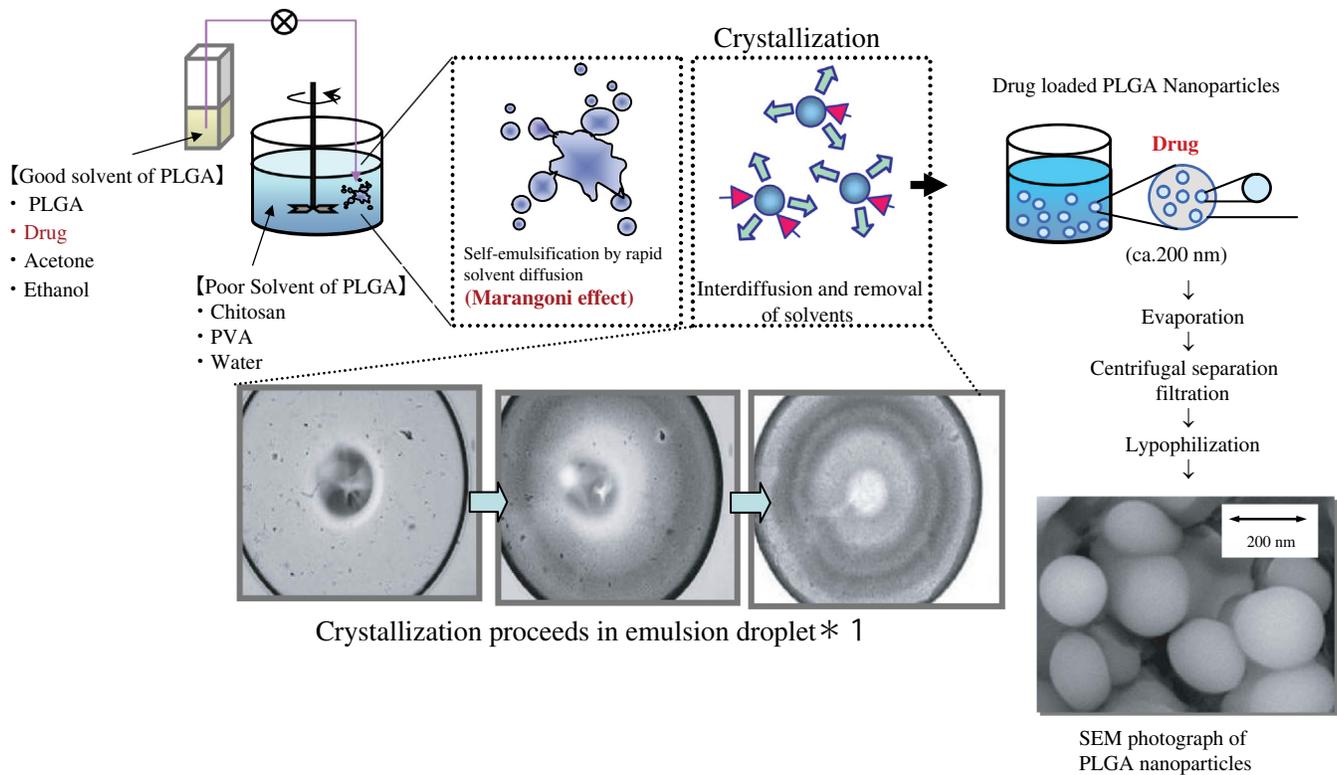
The practical use in DDS products of agglomerating PLGA nanoparticles, which are susceptible to heat and moisture, presents requirements for storage, distribution, and assuring quality stability in secondary processing. For modifying the physical properties of

PLGA nanoparticles, we have developed particle mechanofusion technology, such as compression-shearing particle composing [4], spray-drying fluidized bed granulation [5], and spouted bed binderless granulation [6] technologies. Because PLGA nanoparticles are, in comparison with other DDS ultrafine particle materials, a rigid solid polymer material, particle mechanofusion technology can be used. For example, if one performs secondary processing into microsize PLGA nanoparticles, in which an excipient is the matrix and PLGA nanoparticles are structurally controlled in the dispersed phase, the characteristics are substantially improved storage stability and handling and the ability to produce the final preparation form (tertiary processing, tablets, capsules, granules, and inhalants).

## 3. APPLIED TECHNOLOGY WITH PLGA NANOPARTICLES AS BASE CARRIER

### 3.1 Examples of Research on PLGA Nanoparticle Preparations

Table 10.1 presents some examples of DDS research on PLGA nanoparticles. There are reports that, for example, PLGA nanoparticles 200 nm in size permeate digestive tract mucous membranes and are retained in



**FIGURE 10.2** Preparation process of PLGA nanoparticles by the spherical crystallization technique (emulsion solvent diffusion method). PLGA, poly lactic-co-glycolic acid; PVA, polyvinyl alcohol; SEM, scanning electron microscope. \*1: Photos provided by Prof. Kawashima, Aichi Gakuin University.

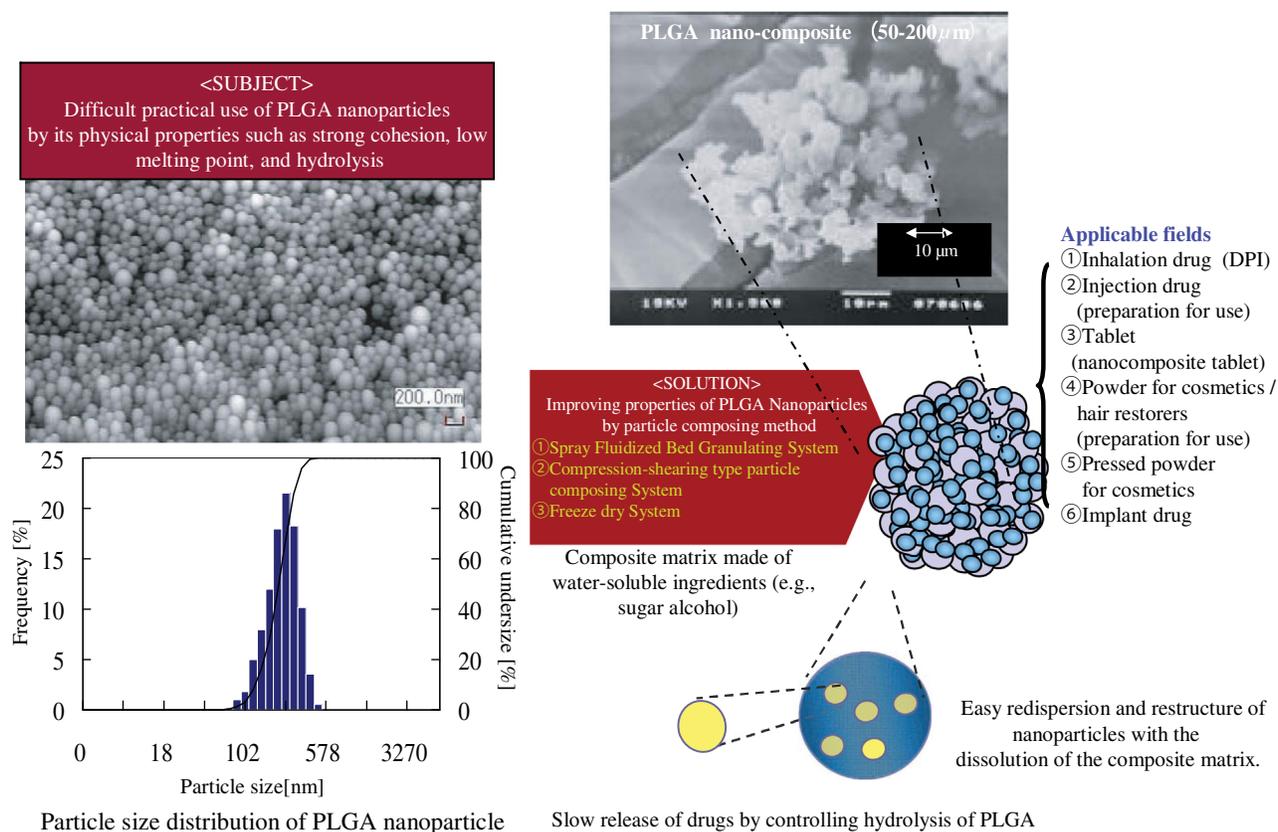
(are adsorbed into) [7] mucosal layers and the epidermis surface, and that in the rat colitis model and the vascular endothelial inflammation model, the particles target the inflammation site [8] and, several hours after oral administration, pass through the intestinal epithelium [9].

In our experience with administering PLGA nanoparticles encapsulating insulin for diabetes sufferers to beagles under spontaneous respiration via pulmonary systemic circulation delivery, the insulin was absorbed throughout the body from the alveolar epithelium. The effect of lowering blood sugar was about 3.5 times greater than in the subcutaneous administration group perhaps because the insulin released from the PLGA nanoparticles that had been efficiently delivered to the alveoli, which are the fast absorption site, is slowly released from the particles with little breakdown by enzymes [10,11]. This kind of PLGA nanocomposite dry powder inhalation aerosol, including nucleic acid drug transpulmonary DDS preparations (lung cancer, pulmonary fibrosis, and pulmonary hypertension), can be made by the three aforementioned particle-composing methods as shown in Fig. 10.4. The granules with which these are made fill capsules well. Furthermore, they are delivered efficiently to the lungs via the respiratory tract

because in a self-inhalation respirator the spouting effect of exhalation instantaneously disperses the granules in the air as fine particles of several  $\mu\text{m}$  in size. In the lungs, the preparation breaks down when the lactose and sugar alcohol absorb moisture and dissolve, on which the nanosize PLGA particles are rebuilt and their functions manifested.

To histopathologically assess the *in vivo* dynamics of PLGA nanoparticles when inhaled into the lungs, the authors made rats inhale the suspension of fluorescein isothiocyanate (FITC) encapsulated in PLGA nanoparticles sprayed with compressed air. After 5 min, FITC was taken into the epithelial cells of type-1 alveoli, entered the blood via the alveolar membrane, and then 15 min later was detected in the liver and kidneys [12]. In other words, after administration the FITC was quickly absorbed from the type-1 alveoli epithelial cells and was more effective than when just administering FITC solution. This effect is pronounced when PLGA nanoparticles are about 80 nm in size, while particles that are several hundred nm or larger are phagocytized preferentially by alveolar macrophages [13].

Such PLGA nanocomposite particles have excellent formability, and they can also be used to make enteric nanocomposite tablets [14] by mixing with an



**FIGURE 10.3** Atomic force microscopic image of poly lactic-co-glycolic acid (PLGA) nanoparticles and structure of PLGA nanocomposite.

enteric base. Using this technology, researchers are exploring the creation of practical oral administration that could be seen, from the perspective of the patient's quality of life, as the ultimate nucleic acid DDS (oral nucleic acid drugs), and in advance of that, we have, as the world's first research, confirmed the effectiveness of NF- $\kappa$ B decoy oligodeoxynucleotides (NDONs) when they are encapsulated in PLGA nanoparticles and orally administered to colitis model lab animals [15].

#### 4. PLGA NANOPARTICLE SYSTEM PLATFORMS AND IMPLEMENTATION IN NANOMEDICAL SYSTEMS

PLGA nanoparticles are efficiently taken in by human umbilical vein endothelial cells and human skeletal muscle cells [16] and are used to facilitate endothelial cell angiogenesis. Genes and molecularly targeted drugs [17] that control smooth muscle proliferation and migration, which are the main causes of ischemic diseases, are encapsulated in PLGA nanoparticles, which

find application in inhalants [18] and intramuscular injection drugs [19], as well as in medical appliances such as stents [20] and balloon catheters [21].

#### 4.1 Injectables Using PLGA Nanoparticles for Treating Ischemic Diseases

In a case in which pitavastatin encapsulated in PLGA nanoparticles was administered locally [19] in a mouse limb ischemia model, reendothelialization by the angiogenesis effect was confirmed. Usually in lab animal experiments, subcutaneous injections and oral administration require general and daily administration of high-dose statins at 1–5 mg/kg/day. This carries a significant risk of rhabdomyolysis and other side effects, and clinical application has therefore been deemed difficult. But intramuscular localized injections (0.4 mg/kg) of these PLGA nanoparticles in four limb sites induced angiogenesis in mouse limbs, with a significant improvement in blood circulation compared with the control group. It has also been determined that the transpulmonary administration of these PLGA nanoparticles is effective against pulmonary hypertension [22].

**TABLE 10.1** DDS Research of Poly Lactic-co-Glycolic Acid (PLGA) Nanoparticles

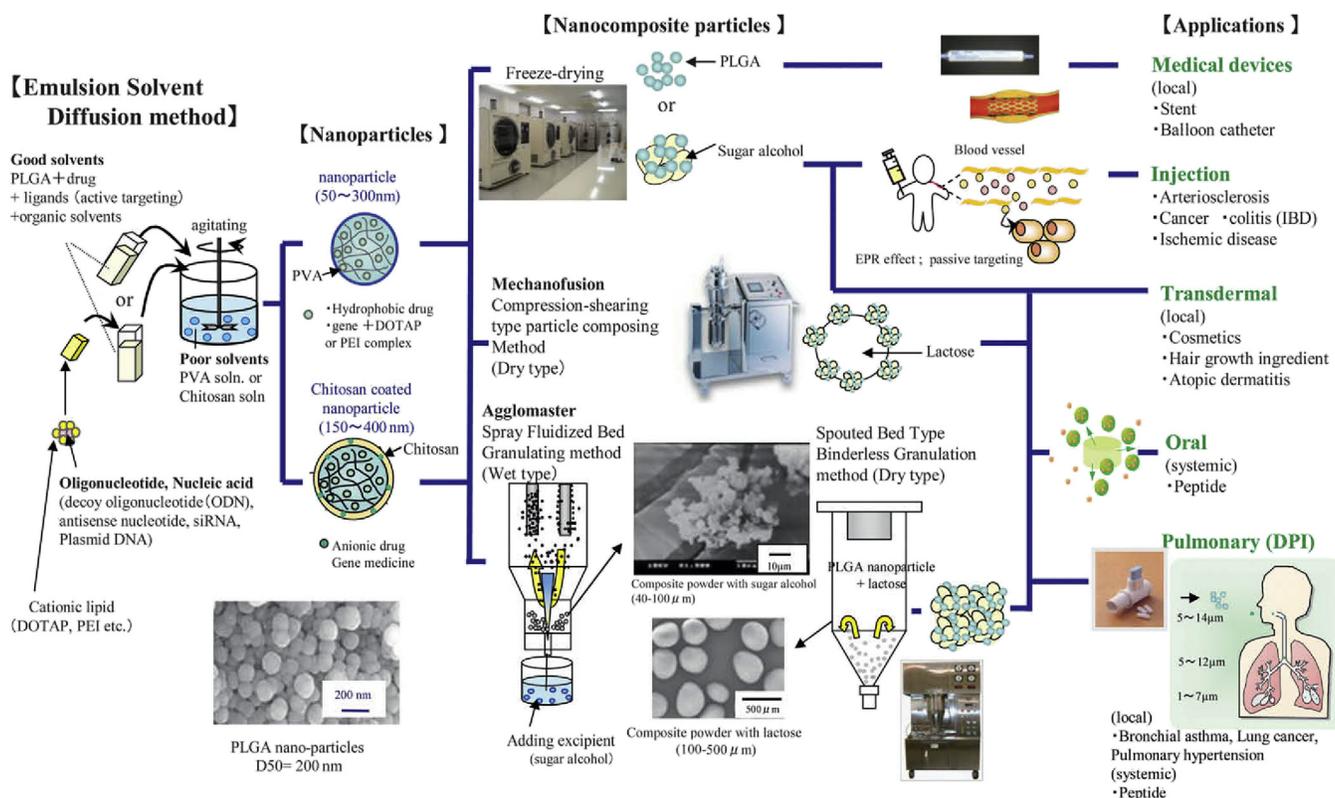
Administration	Target region	Action	Particle size	Model drug	Method	Result (Dynamic state of PLGA nanoparticles)	Application	Quote
Eye	Conjunctival endothelial cell	Local	101 nm	(Fluorescence: Coumarin)	In vitro	Uptake by rat's conjunctival endothelial cell	Conjunctival disease	Pharm. Res. (2004) 21 (4) 641–648 (M.G. Qaddoumi, et al.)
Nose	Mucosa	Local	<200 nm	(green fluorescent protein plasmid)	In vivo (mouse)	Gene expression (nasal mucosa)	Nasal mucosa disease (allergenic, etc.)	J. Nanosci. Nanotechnol. (2004) 4 (8) 990–904 (M.N. Kumar, et al.)
Mouth	Enteric mucosa	Systemic	400 nm	Calcitonin	In vivo (rat)	Adhesion and uptake into the mucosa	Alternate product of injection (peptide, etc.)	Pharm. Develop. Technol. (2000) 5 77–85 (Prof. Kawashima, Aichi Gakuin Univ.)
					In vivo	Permeability across intestinal epithelium within a matter of hours after administration		C. Damge, et al., J. Control. Release (1990) 13 233–239
Mouth	Colon (inflammatory cells)	Local	200 nm	Rolipram	In vivo (rat)	Effective delivery to the inflammatory cells and continuation of pharmacological effect	Inflammatory bowel disease	J. Pharmacol. Exp. Ther. (2001) 299 775–781 (Prof. Kawashima, Aichi Gakuin Univ.)
Lung	Alveolar	Systemic	650 nm	Elcatonin	In vivo (cavy)	Decrease in the level of blood elcatonin	Alternate product of injection (peptide, etc.)	J. Control. Release. (2005) 102 373–381 (Prof. Kawashima, Aichi Gakuin Univ.)
			200 nm	Insulin	In vivo (beagle)	Decrease in the level of blood sugar		J. Soc. Powder Technol. (2005) 42 (11) 765–772 (HPTRI) 23rd DDS conference, (2007) 22 (3) 356 (M.D. Tsutsumi, Saiseikai Chuwa Hospital.)
Glenoid cavity	Glenoid cavity	Local	265 nm	(Fluorescence: Fluorescein amine)	In vivo (rat)	Uptake by macrophages	Chronic rheumatism, degenerative joint disease	Pharm. Res. (2002) 19 (2) 132–139 (E. Horisawa, et al.)
Blood vessel (ischemia lesion)	Vascular endothelial cells	Local (stent)	200 nm	(Fluorescence: FITC) (GFP plasmid)	In vitro In vivo (pig)	Uptake by human coronary artery smooth muscle cells and gene expression	Arteriosclerosis (prevention of restenosis)	Jpn. J. Interv. Cardiol. (2007) 22 (3) 201–210 (Prof. Egashira, Kyushu Univ., HMC)

Continued

TABLE 10.1 DDS Research of Poly Lactic-co-Glycolic Acid (PLGA) Nanoparticles—cont'd

Administration	Target region	Action	Particle size	Model drug	Method	Result (Dynamic state of PLGA nanoparticles)	Application	Quote
Injection	Vascular endothelial cells	Local		Dexamethasone	In vivo (rat)	Prevention of neointima formation	Arteriosclerosis	Circulation (1996) 94 (6) 1441–1448 (L.A. Guzman, et al.)
Skin	Skin	Local	200 nm	Provitamin C	In vitro	Permeability into epidermis and dermis (human skin)	Transdermal therapeutic system (allergic disease, analgesic, etc.)	J. Soc. Powder Technol. (2004) 41 (12) 867–875 (Prof. Miwa, Prefectural University of Hiroshima/Miwa, HMC)
Skin	Skin	Local	200 nm	NF-κB decoy oligodeoxynucleotides	In vivo (mouse)	Inhibition of delayed-type allergic reaction	Transdermal therapeutic system (atopic dermatitis)	The First Asian Symposium on Pharmaceutical Sciences and Technology, 2007, 86–89, July 28–30 (Prof. Morishita, Osaka Univ. HMC)

*FITC*, fluorescein isothiocyanate; *HMC*, Hosokawa Micron Corporation.



**FIGURE 10.4** Poly lactic-co-glycolic acid (PLGA) nanoparticles' designs and its processing technologies for novel drug delivery system preparations. EPR, enhanced permeability and retention; PVA, polyvinyl alcohol.

## 4.2 DDS Development Using NF- $\kappa$ B Decoy Oligodeoxynucleotides Encapsulated in PLGA Particles

NDONs [23] (molecular weight of 14,000) are decoy nucleic acid drugs that suppress the activation of the transcription factor NF- $\kappa$ B, which is in cytoplasm and nuclei and controls the gene expression of various inflammatory cytokines.

Chitosan (CS)-modified cation PLGA nanoparticles have been developed for the DDSs of NDONs. Fig. 10.5 illustrates an example of a PLGA nanoparticle DDS. Research of new drug-eluting medical devices, which have PLGA nanoparticles coated onto the surfaces of stents and of balloon catheters, is progressing.

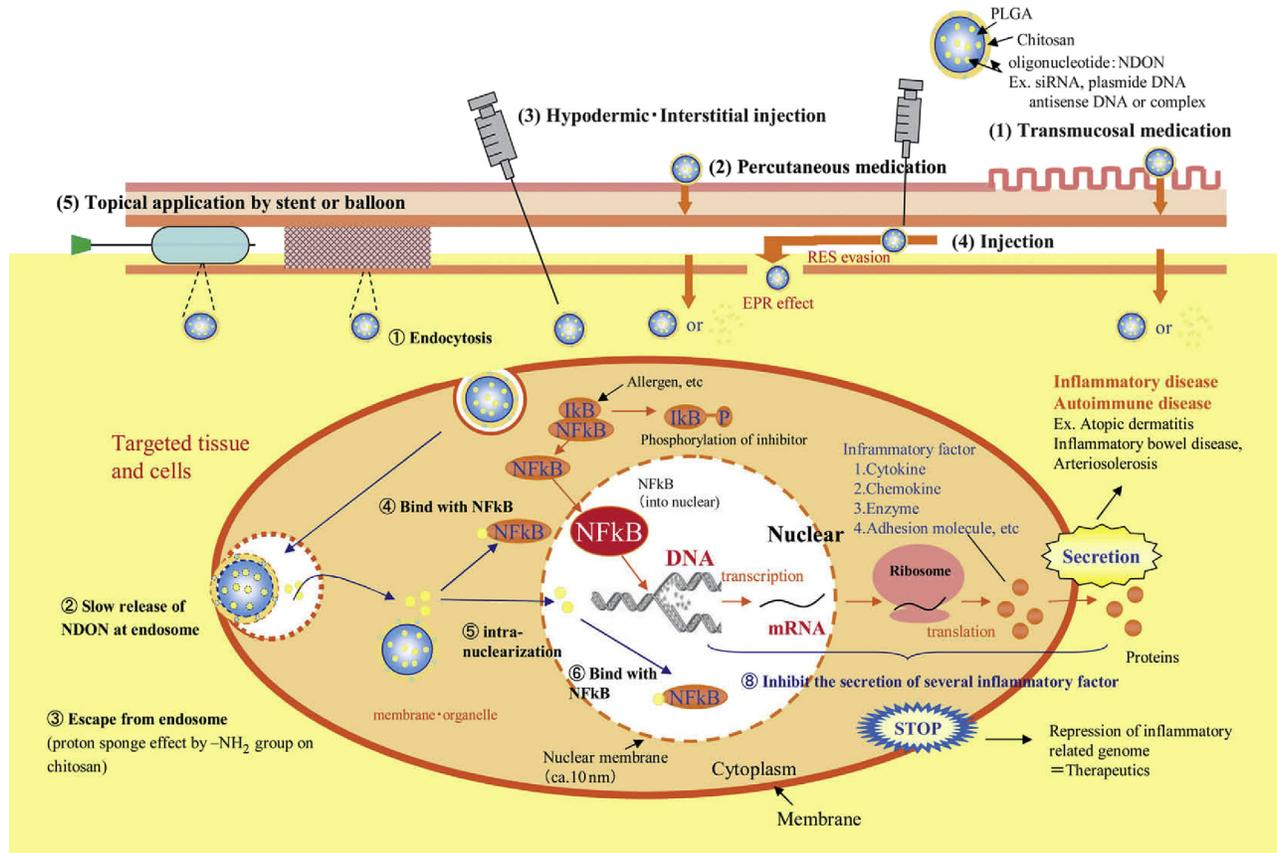
CS-modified PLGA nanoparticles encapsulating NDONs have excellent affinity for and adsorption to the surfaces of anionic cells derived from phosphate groups, and they are introduced into cells through endocytosis. The transfer of NDONs from endosomes to cytoplasm may be accomplished by the amino group-derived buffer effect of the CS on the PLGA nanoparticle surfaces and perhaps also when the cationic chain arising in conjunction with the breakdown of PLGA nanoparticles in the low-pH environment inside endosomes directly interacts with endosome membranes

or otherwise breaks them. Because the pharmacological effect of NDONs is heightened by using these PLGA nanoparticles, it is clear that PLGA nanoparticles suppress the breakdown of NDONs and accelerate their movement into cytoplasm. Additionally, analyses have partially revealed the mechanism of the intracellular DDS of NDONs and other encapsulated drugs [24].

## 4.3 Applications in Medical Devices

### 4.3.1 Drug-Eluting Stents

A stent is a device that is expanded inside a constricted coronary artery and left inside the blood vessel to maintain blood flow. Stent treatment accounts for 85% of transdermal coronary angioplasty operations. Annually, there are 150,000 cases in Japan and at least 1.5 million worldwide. Progress is being made by Egashira et al. in clinical application research [25] on drug-loaded PLGA nanoparticle-eluting stents. While use of bare-metal stents (BMSs) with no drug coating is common, in 20%–30% of cases stenosis of blood vessels recurs, causing recurrences of angina pectoris and acute myocardial infarctions. Subsequently, the advent of drug-eluting stents (DESs) with (1) immunosuppressants or (2) anticancer drugs applied to their surfaces



**FIGURE 10.5** Drug delivery system mechanism of NF- $\kappa$ B decoy oligonucleotide (NDON)-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles.

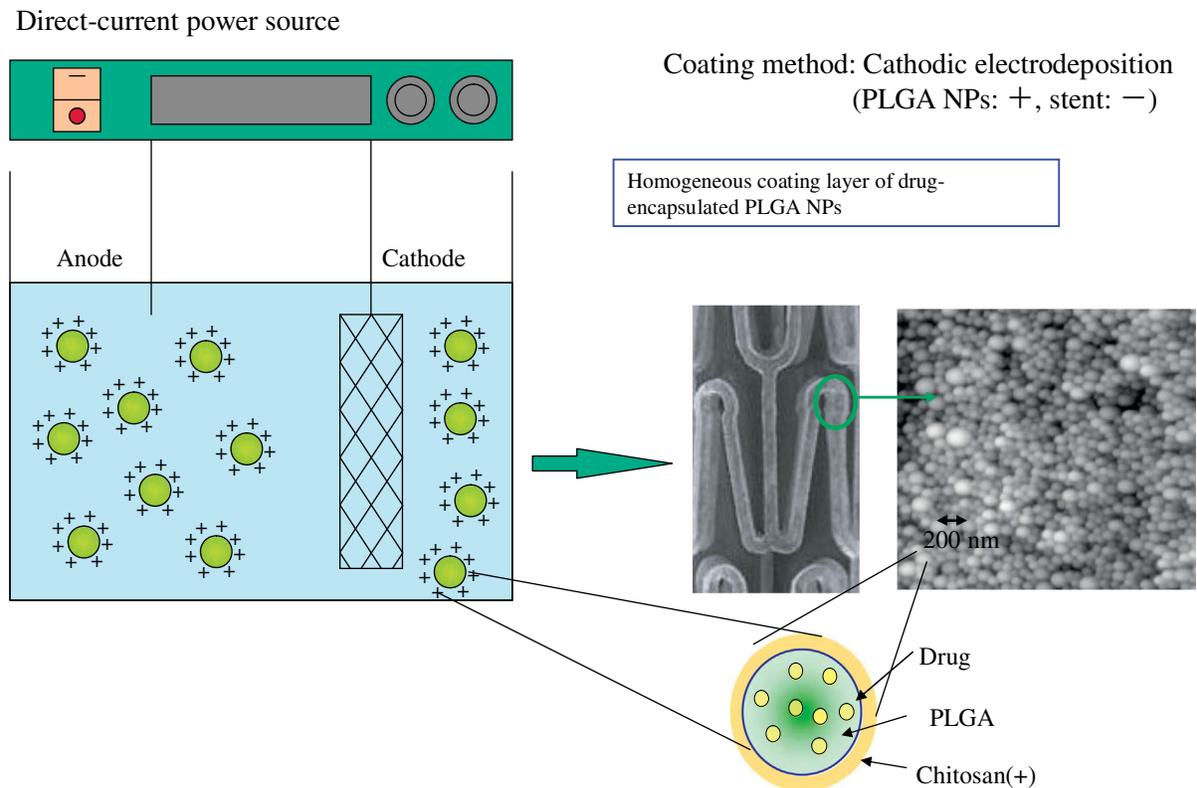
dramatically reduced restenosis, and DESs became widely used worldwide. However, recently, it has become evident that although DESs suppress hyperplasia, they also have a nonspecific antiproliferation effect on the endothelial cells where a stent is inserted, thereby causing blood vessel (endothelial) regeneration failure and having a higher frequency than with BMSs of very late stent thrombosis, which can lead to acute myocardial infarctions and sudden death.

To deal with this problem, Egashira et al. encapsulated within CS-modified PLGA nanoparticles a drug that suppresses only neointimal proliferation but not reendothelialization or fibrotic scars, and they coated these particles to stent surfaces using the cathodic electrodeposition method [26] illustrated in Fig. 10.6. This method uses the stent as the cathode and causes electrocoagulation of the cationic PLGA nanoparticles. As such, the thickness of the PLGA nanoparticle coating (the drug amount) can be controlled by the current and by adjusting the surface charge of the PLGA nanoparticles, while the drug-release timing can be controlled by changing the molecular weight of the PLGA itself and the ratio of components. When a stent is expanded, the coated particles move to the surfaces

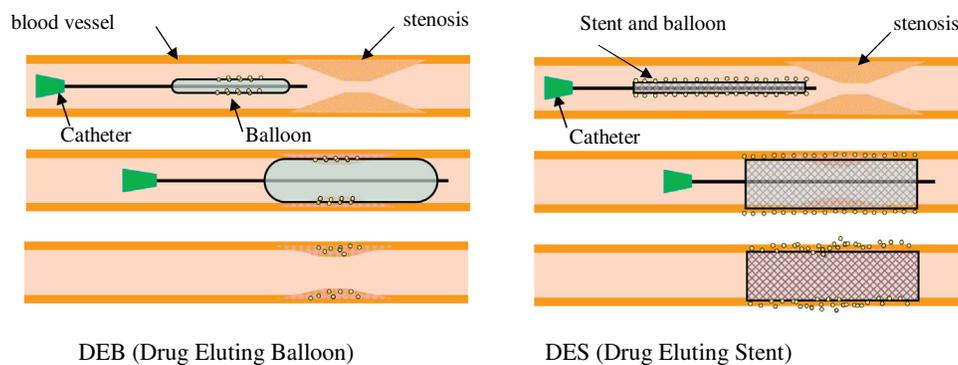
of the inflamed cells and are taken into the cells through endocytosis. It is reported that in an experiment in which this DES was implanted in a pig coronary artery, many PLGA nanoparticles moved to the vascular intima and vascular media [25,27].

#### 4.3.2 Decoy-Eluting Balloon Catheters [21]

A balloon catheter is a medical device comprising a long, thin tube and a balloon on its tip, which enlarges blood vessels. Such catheters are used for percutaneous transluminal angioplasty (PTA) in the peripheral vascular system. A tube called a sheath is inserted in a blood vessel in the foot or hand, then the catheter is pushed through the sheath to the blood vessel's lesion, and the balloon is inflated. This is used in treatments which return constricted or obstructed sites in a blood vessel to near-normal diameter. In particular, they are often used to treat stenosis in vascular shunts that form in the arms of people with obstructive arteriosclerosis or dialysis patients and have already established themselves as an effective treatment method. Nevertheless, in about 30% of instances of PTA balloon catheter treatment, restenosis occurs.



**FIGURE 10.6** Cathodic electrodeposition method for coating poly lactic-co-glycolic acid (PLGA) nanoparticles (NPs) onto the metal stent.



**FIGURE 10.7** Drug-eluting balloon and drug-eluting stent coated with drug-loaded poly lactic-co-glycolic acid nanoparticles as drug delivery system type of medical device.

To reduce these occurrences of restenosis, Morishita et al. are developing decoy-eluting balloon catheters. The procedure involves coating the balloon's outside surface with PLGA nanoparticles, which encapsulate NDONs that are effective in suppressing the acute-phase inflammatory response, which arises when dilating a blood vessel. When the balloon is inflated, the particles come off and are directly transferred to and taken into the inflamed tissue and cells. As such, this is a DDS medical device that makes possible the delivery of NDONs into cells. The conceptual diagram in Fig. 10.7 compares drug-eluting balloons with DESs. This type of balloon catheter has yet to be used

commercially anywhere in the world for not only peripheral vascular but also cardiovascular procedures, and there are expectations that it will contribute greatly to patients' quality of life and also reduce the cost of medical treatment.

## 5. CONCLUSION

This paper has presented examples of pharmaceuticals and medical devices being developed in DDS programs. Clinical trials are being prepared for some of

them. We intend to make our contribution to clinical application by providing manufacturing technologies and other means for PLGA nanoparticle preparations that respond to diverse pharmaceutical needs.

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