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Controlled Drug Delivery: Transition to Nanosystems

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Over the past few decades, drug delivery systems have advanced significantly to deliver novel drug entities, such as proteins and oligonucleotides, and a number of drug delivery technologies involving smart polymers have been developed. Polymers have been essential in the development of controlled release dosage forms, as they offer a wide range of physicochemical properties, such as aqueous solubility, biodegradability, and molecular weight. Furthermore, emergence of drug delivery techniques such as smart hydrogels, micelles, liposomes, and PEGylation has improved the bioavailability of poorly soluble drugs, protein therapeutics, and other drugs. New approaches in designing better drug delivery systems involve the use of nanotechnology to create polymer particles in the nano range and/or incorporate these nanoparticles into microdevices to allow better control of drug release and more efficient drug targeting.

Key words: Drug delivery, Nanotechnology, Nanoparticle, Micelle, Drug eluting stent (DES)

INTRODUCTION

Controlled drug delivery technologies have advanced during the last five decades. In early days of drug delivery technologies, formulations were largely based on simple mixtures of a drug and a polymer. Controlled drug delivery technologies exist because of drugs which play a central role in medical practice. Drugs are not everything in medicine, but there would be no modern medicine without them.¹⁾ Drugs treat diseases and illnesses, such as infections, hypertension and its arteriosclerotic complications, coronary heart disease/heart attack, cancers, gastric and duodenal ulcers, and pains. Besides serving as diagnostic tools, drug also support other therapeutic techniques, such as surgery, organ transplantation, and psychiatry.¹⁾ Moreover, such treatments can become more efficient by using controlled drug delivery systems.

The main goal of drug delivery systems, also called dosage forms, is to provide the safe and convenient delivery of accurate amount of a drug to the appropriate places. Certain dosage forms are preferred for certain route of administration. Specific dosage forms may also be necessary depending on the physicochemical and other properties of drugs. Conventional dosage forms come in various physical forms including tablets, capsules, caplets, injectables, suspensions, emulsions, ointments, and syrups. The uniqueness of conventional dosage forms is that they are defined by the total amount of a drug contained in a formulation and the duration of action. Con-

trolled-release drug delivery, on the other hand, is characterized by the delivery of a drug to a target site at a predetermined rate and for a predetermined period of time that is controlled by the device (*i.e.*, dosage form) itself. The duration of drug release ranges from days to years. Since the drug release is predominantly controlled by the design of the dosage form itself, the drug release rate is largely independent of external factors.

There are several reasons why drug delivery systems are important. The development of a new drug is estimated to cost approximately \$800 million.²⁾ Drug delivery systems provide an alternative to developing such expensive new drugs. By utilizing suitable drug delivery systems, those chemical entities that lack clinical efficacy can be developed into effective drugs. Furthermore, as more drug delivery technologies were introduced, it was found that the same drug, which had its patent protection expired, could extend its product life if it is formulated into a more advanced drug delivery system leading to a higher efficacy. Procardia[®] (nifedipine) is a calcium channel antagonist used for the treatment of hypertension. When its patent expired, Pfizer introduced the same drug in a novel controlled-release delivery system, called "OROS[®] (Oral Osmotic Tablet)," which provides 24-h release after oral administration. The new product, Procardia XL[®], could extend the product life for several more years. As new types of protein drugs and oligonucleotides were developed, non-conventional drug delivery systems were required. These drugs often require site-specific delivery, and, therefore, demanding the use of advanced delivery systems. Delivery of poorly soluble

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Table 1. Evolution of drug delivery technologies.

1950-60s	Beginning of controlled drug delivery Delayed release and sustained release
1970s	Control of drug release kinetics Various polymers and geometries
1980s	Zero-order release
1990s	Modulated release Smart polymers Protein delivery (PEGylation)
2000s	Nanotechnology Solubility increase Targeting Cellular uptake

drugs has been the subject of intense research for decades. In most cases, drugs are loaded and released in a controlled fashion in the form of nano/microparticles or membranes with polymers.^{3,4)} Advances in the drug delivery technologies over the past several decades are summarized in Table 1.

POLYMERS IN CONTROLLED DRUG DELIVERY

Advances in drug delivery technologies could not have been possible without polymers. As polymers are the engines of drug delivery systems, synthesis of new polymers resulted in improvements in drug delivery, and delivery of new drugs required synthesis of new polymers with desired properties. Numerous books on polymer chemistry, such as theoretical treatment, polymer synthesis, and characterization, exist, but only a handful of books are available describing the history and cultural impacts of the polymers, or plastics.^{5,6)} As described in a 1967 movie, the Graduate, there was a great future in plastics, and the plastics have dominated our lives to the extent that we cannot live without them. While the natural polymers, such as wood (cellulose), skins (proteins), and genetic materials (DNA), have existed since the human history, synthetic polymers were first introduced by the synthesis of Bakelite in 1907, which was formed by the reaction under heat and pressure of phenol and formaldehyde, generally with a wood flour filler. Synthesis of nylon in 1938 marked a milestone in polymer history as it was the first polymer synthesized based on Staudinger's theory of polymeric nature of plastics. Since then, other synthetic polymers, such as poly(hydroxyethyl methacrylate), poly(dimethyl siloxane) and polyurethanes, have been synthesized for various industrial applications. Most of the polymers currently used in controlled drug delivery were initially developed for industrial applications. Only recently poly(α -hydroxy ester)s, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymers (PLGA)s, were specifically developed for drug delivery applications due to their unique advantage of biodegradation and their out-

standing chemical/physical properties.^{7,8)} Those polymers were also made into block copolymers with poly(ethylene glycol) (PEG). One advantage from using block copolymers is that the characteristics of the block polymer can be manipulated by choosing monomers with different properties.⁹⁾

Polymers used in drug delivery can be classified into water-soluble polymers, water-insoluble polymers, biodegradable polymers, and cross-linked hydrophilic polymers (i.e., hydrogels). Water-soluble polymers and biodegradable polymers are used for designing dissolution-controlled drug delivery systems, while water-insoluble polymers are used for diffusion-controlled delivery systems. Water-soluble polymers with functional groups that can respond to environmental changes, e.g., changes in pH, temperature, or solvent, are known as smart or intelligent polymers. It is these smart polymers that allowed development of advanced drug delivery systems. Biodegradable polymers, polyesters, can be manipulated by incorporating a variety of labile groups in their backbone. Biodegradation can be of enzymatic chemical or microbial origin and these may operate either separately or simultaneously and often are influenced by many other factors.⁹⁾ Depending on their chemical structure polymers that are used for dissolution controlled drug delivery systems have two main degradation modes: bulk erosion and surface erosion. Surface erosion occurs when the rate of erosion exceeds the rate of water permeation into the bulk of the polymer. Bulk erosion occurs when water molecules permeate into the bulk of matrix at a faster rate than erosion.⁹⁾

In the diffusion-controlled drug delivery systems the drug diffuses through a polymer membrane or a matrix into the external environment. Hydrogels, formed from a three-dimensional cross-linked network of hydrophilic polymer chains, enable water absorption and swell without dissolving while maintaining the overall structure. The swelling of the polymer increases the aqueous solvent content within the drug delivery device as well as the polymer mesh size enabling the drug to diffuse through the swollen network into the external environment. PEG-based hydrogels allow control of the hydrogel's mesh size by changing the PEG chain length, which makes it easy to control the extent of swelling and, consequently, the release of drug substances.¹⁰⁾ However, if drug molecules are smaller than the obtained mesh size in the swollen state, additional methods are necessary to control the release from the hydrogels such as the complexation using microparticle systems based on gelatin.¹¹⁾

The pH values around the cancer cells and in the stomach are known to be 6.5-7.2 and less than 3, respectively. This unique property has been exploited for drug delivery to the cancer cells using pH-sensitive polymers. The pH-sensitive polymers, which can bypass drug resistance and deliver sufficient amounts of the anticancer drug, were evaluated both *in vitro* and *in vivo* experiments.¹²⁻¹⁴⁾ The pH-sensitive polymers

containing acidic groups, such as carboxylic and sulfuric acids, or basic amino groups can accept or release protons in response to changes in environmental pH. Recent reports have described two nanoparticle systems that operate according to different mechanisms: (1) triggered release and improved cell interaction by aggregation/shrinking at the pH of cancer cells; and (2) triggered release by destabilization of the drug carrier at the pH of cancer cells. A new pH-sensitive polymer based on sulfonamide derivatives has been used for targeting to a receptor that was over-expressed by acidic extracellular matrix of cancers.¹⁵⁻¹⁷ The solubility transition of the pH-sensitive polymer occurred in a narrow pH range, only 0.2 pH units.¹⁸ Other pH-sensitive polymers, block copolymers of poly(L-histidine), which is a polybase, and PEG, were also synthesized to make polymeric micelles that can respond to the local pH changes in the body.^{19,20} The local pH change around cancer cells has also been used to deliver DNA from nanoparticle complexes formed by electrostatic interactions between DNA, cationic polymer (PEI) and amphiphilic copolymer. The complex formed at physiological pH dissociated to release DNA at pH 6.6.²¹

The potential of using thermally responsive macromolecules as a drug carrier was investigated.^{22,23} Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) and an artificial elastin-like polypeptide were designed to exhibit a lower-critical solution transition (LCST) in response to increase in temperature slightly above 37°C.^{24,25} Below the LCST, poly(NIPAAm) is soluble in water. As the temperature is increased above the LCST, however, the polymer becomes precipitated from the aqueous solution due to hydrophobic associations. Poly(NIPAAm) and its copolymers have been utilized as thermoresponsive drug delivery vehicles,^{22,23,26,27} three dimensional extracellular matrix,^{26,28,29} and detachable substrates for cell culture.^{30,31}

Not only synthetic polymers, but also natural polymers such as polysaccharides,³² proteins,³³ nucleic acids,³⁴ and their hybrids³² have been widely used in drug delivery. One of the advantages of natural polymers is that they cause less irritation when used. One disadvantage, though, is that their release profiles are not easy to control. Liposome is a phospholipid vesicle composed of a bilayered lipid membrane. It can be used to deliver various drugs, vaccines, and even genes to the body. When used in the delivery of cancer drugs, liposomes help to protect healthy cells from the toxicity of drug and lower their concentration in susceptible tissues such as kidney and liver, reducing side effects. Hydrophilic anti-cancer drugs, such as doxorubicin and cytosine, can be trapped inside the aqueous compartment of liposomes. However, water-insoluble drugs, such as paclitaxel, can be incorporated into the bilayer membrane, which plays a role as a solubilization vehicle.³⁵ The clearance of PEG-liposomes from blood circulation was 2 to 5 times slower than conventional liposomes, which enhance the targeting ability of drug-loaded liposomes.

DESIGN OF DRUG DELIVERY SYSTEMS

There is no one protocol that can be followed for the design of a controlled release formulation. Various factors need to be considered simultaneously to develop a delivery system suitable for a drug, as listed in Table 2. Of the factors listed in Table 2, however, the selection of the delivery system is by and large determined by the nature of a drug. A drug can be water-soluble or water-insoluble, low or high molecular weight, and stable or non-stable. These properties are the key for consideration of other factors. Drugs can be divided into different groups depending on their physicochemical properties, such as aqueous solubility and molecular weight. Once the physicochemical properties are understood, it can narrow down the route of administration. For example, delivery of protein drugs (e.g., insulin) by oral administration has been pursued for decades, but it still remains as an elusive dream. Parenteral administration is still the only viable option, even though pulmonary route has been exploited in recent years. Also a good understanding of the biological barriers is of great importance to choose the right administration route. The administration route is also affected by the nature of the delivery vehicle, or vice versa. A broad spectrum of delivery vehicles could be used depending on the properties of the drug and the aim/disease. Some of the used delivery vehicles are: polymeric micelles, polymeric micro/nanoparticles and liposomes. Each of these delivery vehicles has their specific properties that make them suitable for use with certain drugs and certain diseases. The different delivery vehicles have different drug release profiles. In many cases, a sustained drug release is desired. Otherwise, profiles that do not exhibit zero-order release may be preferable in certain circumstances. Pulse release could be divided into programmed and triggered release. The triggered release could be controlled by changes in the physiologic environment or by external stimuli from the environment such as change in pH or temperature.^{36,37}

Table 2. Factors in the design of drug delivery systems.

1. Drug property	Molecular weight, solubility, and stability of a drug
2. Administration route	Oral, ocular, nasal, pulmonary, transdermal, parenteral, or implantable
3. Delivery vehicle	Size and shape, polymer
4. Drug release mechanism	Continuous delivery, modulated delivery, duration of release
5. Targeting	Organ, tissue, cell, cellular component
6. Biocompatibility	Exposure to blood, implantable, oral

Novartis's Ritalin® applied a pulsatile release technology to produce a once-daily dosage form, mimicking the original twice-daily dosage regime. In some cases, delayed-release may also be formulated to protect an acid-labile drug from the low pH in the stomach, or target the lower gastrointestinal tract for local effect, thus minimizing systemic exposure and achieving a target profile. All mentioned factors play a role in the pharmacokinetic properties of the drug.

Conjugation of drugs to polymers is one of the most widely used methods for designing drug delivery vehicle.^{38,39} The drug-conjugated polymers contain cytotoxic drug molecules connected directly or through a suitable linker, and they may contain a cancer recognition moiety. Polymers that have been used for making drug-polymer conjugates include poly(*N*-[2-hydroxypropyl]-methacrylamide) (HPMA),⁴⁰⁻⁴⁴ poly(styrene-co-maleic anhydride),⁴⁵ and PEG.^{46,47} The molecular weight of the drug-polymer conjugates affects the interactions with proteins and circulation time in blood.⁴⁸ For increased blood circulation, grafting drugs to PEG has been the choice.⁴⁰ Macromolecule-ligand conjugation could provide site-specific drug delivery to concentrate the drug in the specific site of the body through manipulation of its biodistribution profile. Target-specific ligands such as carbohydrates must be linked to different types of macromolecular drug carriers to enhance the targeting of drug to specific cells or tissues.

Homogenization in emulsion is relatively more effective in emulsifying viscous solutions. A high-speed homogenizer produced cystatin-loaded PLGA particles ranging from micro to nano depending stirring speed (Figure 1).⁴⁹ Yoncheva *et al.* reported encapsulation of pilocarpin hydrochloride in PLGA by using a combination of a double emulsification and high pressure homogenization procedure.⁵⁰ The particle size was decreased with increasing homogenization pressure and the number of cycles, and was different depending on the type of emulsifier (Table 3). Residual solvent and emulsifiers are a

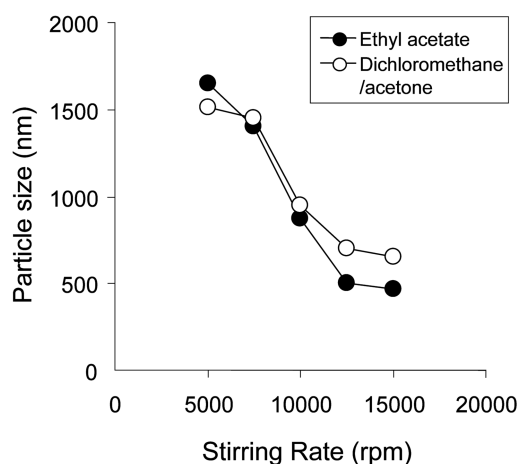


Figure 1. The size of cystatin-loaded PLGA nanoparticles as a function of the stirring rate. From Ref. [49].

Table 3. The effect of particle size for pilocarpin hydrochloride encapsulation in PLGA (From Ref. [50])

Emulsifier	Pressure, gauge (bar)	Cycles	Particle diameter (nm)
Poly(vinyl alcohol)	0		332
	100	1	283
	100	3	232
	500	1	231
	500	3	204
Carbopol	0		1125
	100	1	631
	100	3	366
	500	1	467
	500	3	309
Poloxamer	0		572
	100	1	692
	100	3	424
	500	1	467
	500	3	304

matter of concern with respect to the toxicological risk, especially for injectable formulations. The most common emulsifier is poly(vinyl alcohol) (PVA) for PLGA-based particle formation. The interfacial PVA influences particle size, zeta potential, polydispersity index, surface hydrophobicity, and drug loading.⁵¹ Both residual solvent and emulsifier can be reduced by cross-flow microfiltration,⁵² evaporation under reduced pressure, or ultracentrifugation. Recently, several researchers have utilized albumin protein stabilizer because of its complete compatibility with even the injectable formulation (Figure 2).⁵¹

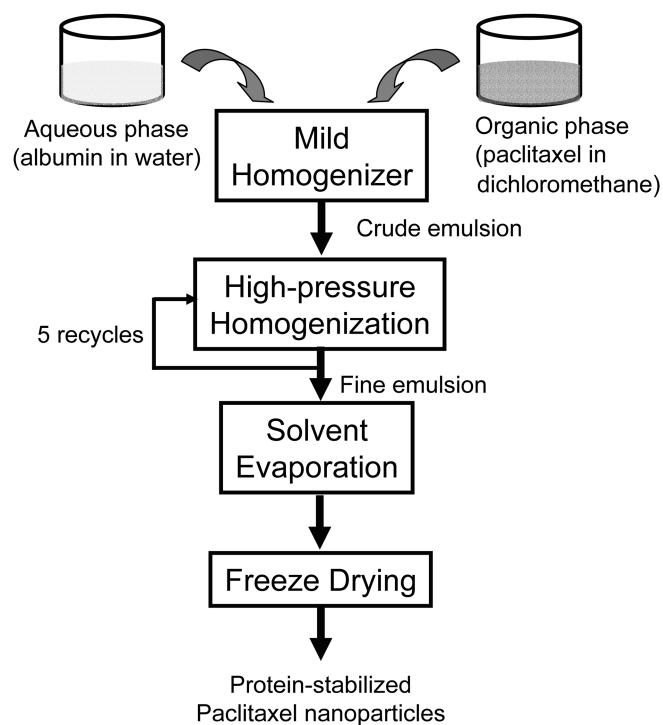


Figure 2. Schematic description of the formation of protein-stabilized drug nanoparticles. From Ref. [51].

A more systematic design and manufacture of particulate systems including micro- or nano-particles has been done by application of traditional as well as novel technologies, such as supercritical fluid (SCF) technology. A fluid such as carbon dioxide (CO₂) is supercritical when it is compressed beyond its critical pressure and heated beyond its critical temperature. Non-porous or highly porous PLGA microparticles encapsulating proteins (e.g., basic fibroblast growth factor and deslorelin) were produced by modified SCF technology.^{53,54}

Drug targeting with nanotechnology continues to be the main focus of the drug delivery system, as the ultimate target of a drug can be a specific site inside a cell, which is quite often hard to reach. For effective targeting at the cellular level, the drug delivery vehicle needs to be in micro- down to nano-dimension for cellular absorption as well as interaction with the target molecules. Different drug targeting methods are used with the different vehicles, passive targeting and physical targeting. Physical targeting could be based on abnormal pH or temperature in the delivery zone or use of a vector molecules possessing high specific affinity toward the affected zone.^{37,55} The PLGA nanoparticles can be easily designed to have targeting properties to specific tissues by conjugating ligands as a targeting moiety in the formulation, and they can also be modified to include PEG pendant chains for increased circulation time.^{7,56} PLGA nanoparticles (~200 nm in diameter) loaded with plasmid DNA demonstrated a sustained anti-proliferative effect whose magnitude increased with incubation time in a breast cancer cell, indicating that a therapeutic protein concentration was maintained in the target tissue.⁵⁷ In practice, it has already been demonstrated that organ or tissue accumulation could be achieved by the passive targeting via the enhanced permeability and retention (EPR) effect or by antibody-mediated active targeting, which requires carrier prolonged circulation, while the subsequent intracellular delivery specifically into target cells could be mediated by site-recognizable ligands (folate, transferrin) or by cell-penetrating peptides (CPP, such as TAT or polyArg). However, such targeting systems, which first accumulate in the target tissue and after penetrate inside target cells, still represent a challenge.

DELIVERY OF POORLY SOLUBLE DRUGS

Bioavailability of a poorly soluble drug is low. Those drugs with high cellular permeability but with low solubility would benefit significantly with the delivery system. The intrinsic solubility, or equilibrium solubility, of a poorly soluble drug does not change by the delivery system. The right delivery system, however, can release the drug at a much faster rate than simple dissolution from drug particles, and this is how the bioavailability is increased, as shown in Figure 3. Even if the dissolved drug is absorbed, more drug can be released from the delivery system fast enough to increase the bioavailability

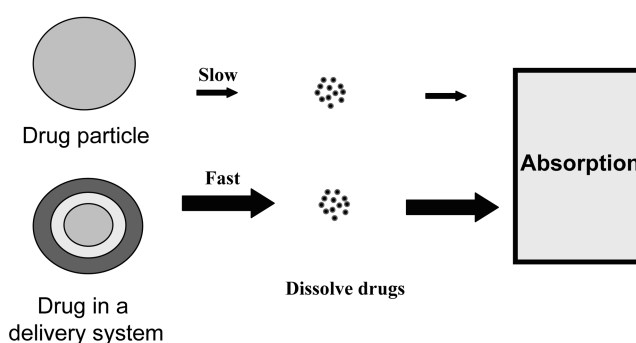


Figure 3. Improved bioavailability of a poorly soluble drug by increasing the dissolution kinetics.

by orders of magnitude. For example, paclitaxel is extremely hydrophobic and sparingly water-soluble due to its cyclic components, and this poor solubility has limited its clinical applications.⁵⁸ A number of formulations with paclitaxel have utilized polymeric micelles,⁵⁹⁻⁶¹ micro- or nano-particles,^{8,62} solid dispersion,⁶³ complexation with cyclodextrin,⁶⁴ and cosolvents⁶⁵ to overcome its poor water-solubility.

The cosolvent approach is the simplest one to dissolve paclitaxel, but the use of organic solvent is not encouraged.^{8,64} One of the drug delivery systems to increase the bioavailability for poorly water-soluble drugs is to make nanocrystals or nanoparticles of a drug, extremely increasing the surface area. The nanosize approach has a limitation in that the increase in bioavailability is only several folds at best after oral administration. There have been attempts to deliver drug nanocrystals directly to the target cells by modifying the crystal surface with a targeting moiety, such as antibody.⁶⁶ Fifty times smaller than conventional microparticles, nanocrystal particles, which have an approximately 80-400 nanometer in diameter, are produced by a proprietary milling technique and stabilized against agglomeration to create a suspension that behaves like a solution. A number of pharmaceutical products that incorporate nanocrystal technology have been successfully commercialized. The new tablet developed with nanocrystal technology gives patients a far more convenient means to administer the drug. Wyeth's first solid-dose formulation of the immunosuppressant Rapamune[®] (sirolimus) received marketing approval from the FDA in 2000. Emend[®] (Merck), which is a capsule containing aprepitant, was approved by the FDA in 2003. This technology is also useful for moderately soluble drugs when a high concentration of drug in a low volume of fluid is desired.

In alternative approaches, self-assembled polymeric colloidal nanoparticles (micelle) composed of hydrophilic shell and hydrophobic core were commonly used where the sizes of colloidal particles range from several tens to hundred nanometers.^{19,67} Micelles are safe for parenteral administration as compared with other solubilizing agents, permitting an increase in the dose of potent yet toxic and poorly water-sol-

Table 4. Advantages of polymeric micelles as drug carriers (From Ref. [47]).

1. Very small diameter (10-100 nm)
2. High structural stability
3. High water solubility
4. Low toxicity
5. Separated functionality

uble compounds.⁶⁰ Hydrophobic drugs can be solubilized into the hydrophobic core structures of polymer micelles to concentrations much higher than their intrinsic water-solubility. Table 4 summarizes these advantages.⁴⁷ Otherwise, one of disadvantages of polymeric micelles is their physical instability during circulation due to their amorphous state. Nicotinamide-derivatized hydrotropic polymers were found to increase the water-solubility of paclitaxel by several orders of magnitude.⁶⁸ To improve the bioavailability of the paclitaxel in formulation, hydrotropic polymer-based micelles, consisting of a hydrophilic PEG shell and a hydrophobic core that contains a significant amount of hydrotropic moieties (poly(2-[4-vinylbenzyloxy]-*N,N*-diethylnicotinamide) (PDENA)) were developed (Figure 4).^{61,69} With the unique micellar characteristics and hydrotropic activity, the hydrotropic-based micelles exhibit a high drug loading capacity and enhanced long-term stability in aqueous medium.⁶¹

Hydrotropic hydrogels were examined to improve the aqueous solubility of paclitaxel.⁶⁸ The loading of paclitaxel into the hydrogels was carried out by solubilizing paclitaxel in aqueous solutions of 2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide (2-VBOPNA) and 6-(4-vinylbenzyloxy)-*N*-picolylnicotinamide (6-VBOPNA), followed by the *in situ* crosslinking reaction to form hydrogels. Paclitaxel solubility in hydrogels increased as the concentration of 2-VBOPNA or 6-VBOPNA used in hydrogel synthesis increased (Figure 5). Poloxamers or Pluronics are commercially-available triblock copolymers composed of a central block of polypropylene (PPO) surrounded by two poly(ethylene oxide) (PEO). They have been used as solubilizers for insoluble drugs as well as nanocontainers for site-spe-

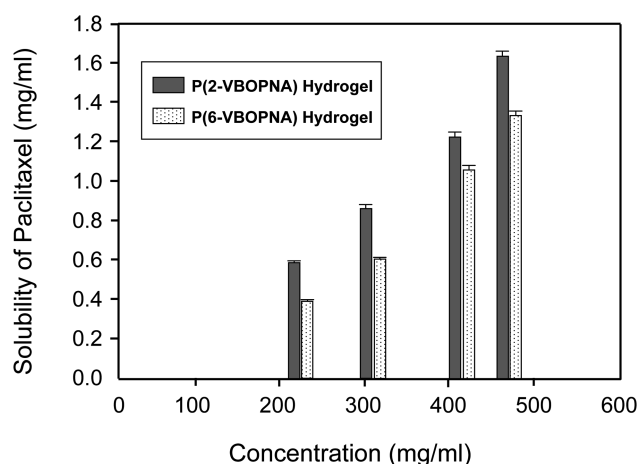


Figure 5. Paclitaxel solubility in *N*-picolylnicotinamide hydrogels as a function of the concentrations of 2-VBOPNA and 6-VBOPNA. From Ref. [68].

cific drug delivery in body.⁷⁰

Dendritic polymer architectures (dendrimer), which provide multi-valent surfaces, are another type of nanoparticles, that facilitate immobilization of drugs.⁷¹⁻⁷⁸ Dendrimers consisting of an apolar core and polar shell have been referred to as unimolecular micelles. Unlike conventional micelles, however, the dendritic structure is independent of dendrimer concentration.⁷⁹⁻⁸² Due to their unique structure and properties, dendrimers with amphiphilic moieties are known to have micelle-like behavior and have guest-host properties in solution.⁸³ The inherent stability of dendritic micelles and their capability to encapsulate drug molecules make them good candidates for novel drug delivery systems. The highly branched poly(amidoamine) (PAMAM) dendrimer conjugated with fluorescein isothiocyanate (FITC) was utilized as multi-functional cancer therapeutic nano-carriers.^{74,77} A nano-structural PAMAM dendrimer model as a carrier (< 5 nm in diameter) and method of attaching drugs to the dendrimer were demonstrated.⁸⁴ However, dendrimers have often been used for increasing the solubility, but each dendrimer molecule is not large enough to dissolve a drug inside the dendrimer

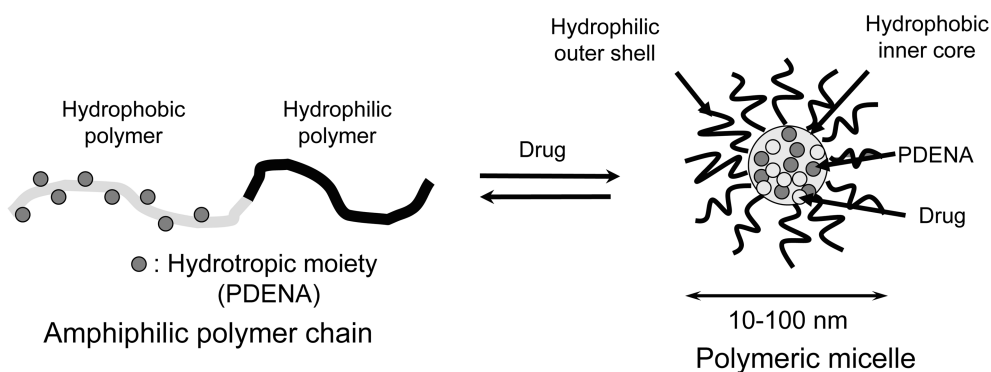


Figure 4. Self-assembly of amphiphilic polymers with hydrotropic moieties into polymeric micelles.

structure. Rather, the aggregates of dendrimers wrap around a drug, and this results in unstable formulations. Furthermore, the safety of dendrimers has not been fully established. In the end, it is the safety and efficacy that will distinguish the clinical useful delivery systems from others. A poorly soluble drug can also be complexed with protein nanoparticles, such as paclitaxel complexed with albumin microparticles (Abraxis Bioscience).

DELIVERY OF PROTEIN DRUGS

The formulation and delivery of therapeutic proteins for systemic activity continues to challenge development scientists. Protein drugs and oligonucleotides are very large in size in comparison with traditional low molecular weight drugs. Protein drugs require maintenance of their tertiary structures to be bioactive, and this brings difficulty in protein formulations. Protein drugs are vulnerable to gastrointestinal (GI) proteolytic enzymes (peptidases) and extremes of pH that are present in the GI tract and once absorbed are often removed to a high degree by hepatic metabolism.⁸⁵ Thus, protein drugs are delivered by injections, and because of short half-lives of most protein drugs, daily injections, sometimes multiple daily injections are required,⁸⁶ and also most delivery systems for protein drugs are designed to deliver them for weeks or months. Since it is practically unrealistic to remove the delivery systems after their lifetime, biodegradable polymers have been used for long-term delivery of protein drugs.

PLGA has been most widely used, but traditional double emulsion methods of making microparticles by solvent extraction/evaporation, which has to be used organic solvents and generated a low pH during degradation (acidic), result in denaturation of protein drugs.^{87,88} For this reason, new microencapsulation methods, such as addition of a poorly sol-

uble basic additive,⁸⁹ combination of PLA/PEG,⁹⁰ and solvent exchange method, have been developed. Enzymatically biodegradable hydrogels such as dextran and amphiphilic poly(ether ester) multiblock copolymers were utilized for the protein delivery.⁹¹ Currently, various nanotechnologies are utilized for preparing protein-containing nano/microparticles, and the issue at hand is to scale up the processing steps.

PEG conjugation to a biomacromolecule is a largely exploited strategy to improve the properties of many drugs. It was in fact often found to improve physical and chemical stability, to increase aqueous solubility, to provide protection against enzymatic degradation, to prolong *in vivo* half-life, and to decrease plasmatic clearance of conjugated drugs when compared to the unmodified parent molecules. Reduced antigenicity, immunogenicity and toxicity are other positive properties often observed with PEGylated molecules. Several PEGylated proteins have been successfully tested in clinical trials, and a few cytokines have recently reached the market.⁹²

DRUG-BIOMEDICAL DEVICE COMBINATION PRODUCTS

Despite the decades of advances in drug delivery technologies, there are relatively low number of biomedical devices combined with drug delivery systems. Urinary catheters that can deliver antibiotics can be easily developed, but there are not readily available. Restenosis is the maladaptive response of the coronary artery to injury. Coronary stents, which made from stainless steel 316L, plastically open the channel of arterial segments that are constricted by fatty deposits or calcium accumulations through the inflation of a balloon, and reduce restenosis rates after balloon angioplasty.⁹³⁻⁹⁵ Recently, drug-eluting stents (DES) for interventional cardiology have been developed, and their clinical successes have brought the

Table 5. Restenosis and in-stent restenosis, 1977-2006 (From Ref. [96]).

Year and procedure	Indications	Restenosis rate	Problem
1977-87: percutaneous transluminal coronary angioplasty	Simple lesion, chronic syndromes	30-50%	Restenosis, subacute closure
1988-92: Newer devices - rotablator, excimer laser coronary angioplasty, coronary atherectomy	Complex, calcific, tortuous, bifurcations, left main, multiple lesions	50%	Restenosis 50-70%
Stent	Acute coronary syndromes	20-30%	Restenosis, subacute thrombosis in-stent restenosis
1993-2002: newer stents (biodegradable, covered, radioactive, intravascular Atherectomy)	Carotides, iliacs, renals, saphenous vein grafts, abdominal aortic aneurysms direct, rupture	10-20%	In-stent restenosis, re-restenosis
1995-2002: brachytherapy-Photodynamic therapy, Ultrasonography, cryotherapy, Cutting balloon	in-stent restenosis	< 10%	Restenosis, re-restenosis 10% late thrombosis, edge failure, geographical miss
2002-: Drug eluting stents	Simple lesions, chronic syndromes, some complex lesions	0-5%	Delayed restenosis

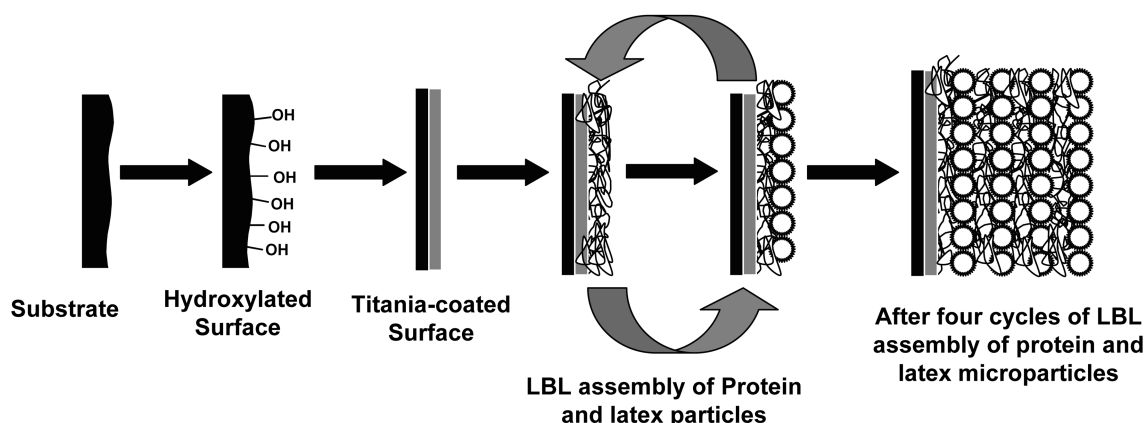


Figure 6. Schematic diagram depicting the steps involved in the assembly of polymer and latex particles on substrate surface.

potential benefits of combination products into our attention. DES significantly reduced restenosis and in-stent restenosis compared to bare stent (Table 5).⁹⁶ Drug coating on cardiovascular stents is not as simple as it may seem. Currently, spray coating is used for coating drug-releasing layer, but new approaches, such as self-assembly and layer-by-layer (LbL), have been applied to develop more efficient methods of drug-containing layer with more control on drug release kinetics. The sequence of deposition of different materials defines the multilayer architecture and its properties. The fabrication of multicomposite films by the LbL assembly procedure involves the nanoscopic assembly of different materials onto solvent accessible surfaces of almost any kind and shape. Coatings comprised of pure polyelectrolyte multilayer and negatively charged latex particles and protein layers have been fabricated, and the resulting coated layers have been assembled into ordered arrays by physical adsorption (Figure 6).

Current DES delivers only one drug, and it may be necessary to deliver two drugs or more in the future, since the physiological response after stent implantation is time-dependent. The initial thrombus deposition and inflammation is followed by smooth muscle cell proliferation and more long-term remodeling processes. Furthermore, it is most desirable to encourage the growth of endothelial cells on the stent surfaces, and unfortunately most drugs used for preventing restenosis also inhibit endothelial cells. Thus, developing delivery technologies for different drugs at different release kinetics will be required for development of the second generation DES.

NANOTECHNOLOGIES IN DRUG DELIVERY

Recent advances in nanotechnology have certainly enhanced the ability to design better drug delivery systems. Nanotechnologies, however, have not produced new drug delivery technologies.⁹⁷ For drug delivery, most of the sites are accessible through either microcirculation by blood capillaries or pores present at various surfaces and membranes. The cross-

sectional diameter of the narrowest capillaries is about 2000 nm, and then for efficient transport the nanoparticle should be smaller than 300 nm.⁹⁸ The rapid clearance of circulating particles from the bloodstream coupled with their high uptake by liver and spleen can be overcome by reducing the particle size, and by making the particle surface hydrophilic.⁴⁵ Gaur et al. observed that 100 nm particles of polyvinylpyrrolidone had a negligible uptake by the macrophages in liver and spleen, and 5-10% nanoparticles remain in circulation even eight hours after intravenous injection.⁹⁹ The uptake of 100 nm PLGA particles by the intestinal tissue was 15-250 fold higher compared to the larger-size microparticles.¹⁰⁰ Cellular uptake is greater for nanoparticles compared to microparticles.¹⁰¹ With 20 nm particles, the uptake by the 1 cm² cell monolayer was as high as ~20%. Intracellular uptake of nanoparticles is one of the most attractive approaches for cancer therapy in drug delivery. In 2005, the U.S. Food and Drug Administration approved intravenously administered 130-nm albumin nanoparticles loaded with paclitaxel (AbraxaneTM) for cancer therapy, which offers elimination of toxicity and improved efficacy with increased drug dosage.⁹⁸

The smart drug delivery systems, such as pH-dependent drug delivery, drug targeting, and molecular imaging of drug-containing nanoparticles, were already available. Nanoparticles with targeting ability can be obtained by conjugation of site-recognition moieties to the surface of the nanoparticles. Use of the targeting nanoparticles affords several potential advantages over conventional antibody-guided therapy. These include delivery of much higher therapeutic payloads per target biorecognition event, the ability to carry multiple, potentially different targeting agents for enhanced selectivity, the ability to integrate means to bypass biological barriers, and co-localized delivery of multiple agents, resulting in targeted combination therapy. Nanotechnologies have brought the ability to manipulate the drug delivery devices in nanoscale better than before. This has been most prominent in the transdermal drug delivery area. Various transdermal patches with microneedles

have been prepared for more efficient delivery of drugs, including macromolecular drugs. Because the microneedles can penetrate the epidermis, it is possible to deliver protein drugs, such as insulin, by transdermal route. Furthermore, the ability to deposit drug particles at the tip of microneedles allows more efficient delivery of a drug.¹⁰²⁾ The ability to nanofabricate various polymers and hydrogels in a microdevice, e.g., microvalve, allows better control of drug release.¹⁰³⁾ Since drug delivery systems will most likely be prepared by bottom-up approach,¹⁰⁴⁾ development of new polymers and supramolecular systems that can self-assemble into suitable delivery systems would be most desirable.

Nanoparticulate drug delivery systems, such as micelles and liposomes, have been applied for treating cancers, but more improvements are necessary for the nanoparticulate formulations to be useful in clinical applications. First, the stability and biological activity of the drug should not be adversely affected during the nanoencapsulation processes or in the final nanoparticle formulations. Second, the yield of the nanoparticles having the required size range and the drug encapsulation efficiency should be high. Third, the nanoparticle quality and the drug release profiles should be reproducible within specified limits. Finally, the nanoparticles should be produced as a free flowing powder in the dry state and should not aggregate or adhere each other. Intravenously injected PEG-grafted liposomes (PEGylated liposomes) exhibit prolonged circulation times in the blood. As a result of enhanced permeability retention phenomenon these liposomes can efficiently accumulate at well-perfused and low-pressure regions of solid tumor.

While there is no doubt that nanotechnology has brought a new dimension in drug delivery technologies, caution has been exercised not to over-engineer the delivery systems. The ultimate goal of drug delivery technologies is to cure the diseases, and this requires delivery of a certain amount of a drug. Application of micro- and nano-devices to drug delivery is certainly the future,¹⁰⁵⁾ but one has to remember that such devices can deliver only a limited amount of a drug.

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