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초청논문

생체의료용 기능성 고분자 재료의 개발

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Functional Polymeric Materials for Biomedical Application

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초록 : 생체의료용 기능성 고분자재료의 개발에 관한 연구를 고분자의 기능성, 생체적합성 및 생분해성을 토대로 다루었다. 본 연구실에서 합성한 기능성 생분해 고분자들을 비롯하여 생분해성 고분자 생체재료, 온도 감응성 고분자재료, 양이온성 고분자재료, 비축합성 고분자 생체재료, 조직공학을 위한 생체고분자 DNA 매트릭스 및 RNAi 기법을 위한 고분자 등의 기능성 고분자 생체재료에 관련된 연구들을 정리하고, 가까운 장래에 의료용으로 이용될 수 있는 기능성 생체고분자에 대한 연구들을 제안하였다.

Abstract : The development of functional polymeric materials for biomedical application has progressed on the basis of functionality, biocompatibility and biodegradability. In this paper we review the functional polymeric biomaterials-based systems and propose a range of biomedical applications in the near future. These systems include the functional biodegradable polymers synthesized in our research laboratory, biodegradable polymeric materials, thermo-sensitive polymeric materials, cationic polymeric materials, non-condensing polymeric biomaterials, bio-polymeric DNA matrix for tissue engineering, and polymeric biomaterials for RNA interference (RNAi) technology.

Keywords : Functional polymers, polymeric biomaterials, biocompatibility, biodegradability, DNA, RNAi, gene carriers, drug delivery systems, biomedical application.

Content

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Introduction

Functional biodegradable polymers have been recently applied to develop gene and drug delivery systems[1-3] for biomedical applications. The synthesis and characterization of functional biodegradable polymers are elements of this leading frontier of research in polymer science at the present time. The gene and drug delivery for biomedical application can be defined in the most general sense as a method to provide a patient's somatic cells and tissues with the genetic system required to supply the specific therapeutic proteins for correcting or modulating diseases. The purpose of somatic gene therapy is to overcome the limitations associated with the administration of therapeutic proteins, including low bioavailability, systemic toxicity, *in vivo* instability, high hepatic and renal clearance rates, and high cost of manufacturing. Providing a therapeutic gene as a "pre-drug" to a patient may circumvent some of the limitations associated with the use of recombinant therapeutic proteins.[4]

Gene medicine consists of three components: a gene that encodes a specific therapeutic protein, a plasmid-based gene expression system that controls the functioning of a gene within a target cell, and a gene delivery system that controls the delivery of the gene expression plasmids to specific locations within the living body. The gene expression plasmids contain both the therapeutic gene and several other genetic elements, including transcript stabilizers to control transcription and protein stability from the host cells in a body.[5] Gene and drug delivery systems are designed to control the location of a gene within the body by affecting the distribution and access of a gene expression system to the target cell or by enabling recognition by a cell-surface receptor followed by intracellular trafficking and nuclear translocation.[5] Gene and drug delivery systems should both protect a gene expression from premature degradation in the extra-cellular milieu and achieve nonspecific or cell-specific delivery to a target cell. Other elements in a gene delivery system may facilitate the intra-cellular trafficking of a gene expression system.[6]

These include the ability of some viral vectors to integrate with the host genome and permanently alter its genetic structure, the capability of self-replication with a unique possibility of recombination and compliment activation, and their inability to bypass the host's defense mechanism. Over the past decade there have been many attempts to design a non-viral vector that could achieve the level of gene expression and specificity attained by viral vectors, but that could allow greater flexibility in cDNA and bypass the immune response. The design of an optimal synthetic gene carrier is still a limit on effective non-viral gene therapy. Several lipid-, peptide-, and polymer-based biomaterials are currently under investigation for biomedical applications including gene and drug delivery systems.[7-9]

In the present paper, we review the existing functional polymeric biomaterials-based systems, such as functional biodegradable polymers, biodegradable polymeric biomaterials, thermo-sensitive polymeric materials, cationic polymeric materials, non-condensing polymeric biomaterials, bio-polymeric DNA matrix for tissue engineering, and polymeric biomaterials for RNA interference (RNAi) technology, and propose various biomedical applications in the near future.

Functional Polymeric Biomaterials-Based Systems

The functional polymeric biomaterials-based systems have focused on polymeric gene and drug carriers for transferring therapeutic genes into the cell nucleus. There has been some success in reducing both immune response and cytotoxicity, as well as enhancing gene expression. Although cellular internalization followed by intracellular transport to the nucleus is not yet clearly understood, there are several interesting polymers under investigation based on their surface charge distribution, hydrophilicity and hydrophobicity of the structures. Rather rapid degradation of DNA is a fundamental problem for gene therapy, as destruction of incoming genes results in loss during gene expression.[10] In investigating the use of polymers to "carry" the DNA, major attention has focused on cationic polymers, which can both condense large genes into smaller structures and mask the negative charges of pDNA. In addition, non-condensing neutral polymers have been evaluated for DNA protection from extra- and intra-cellular nucleases.[11]

Functional Biodegradable Polymers for Medical Applications. The medical applications of biodegradable polymers include absorbable bone plates and other surgical fixation devices, artificial skin substitutes and carrier systems for the controlled release of drugs. Especially, functional biodegradable polymers have been used in the development of polymeric matrices for the controlled and sustained release of low molecular weight therapeutic agents and short-term implants such as sutures and surgical staples for biomedical applications. Such implants should maintain their functionality over a relatively short period of time. However, efforts to develop absorbable implants that will fulfill more demanding functions such as vascular prosthesis and osteoplastic devices (bone screws, plates, pins, etc) have recently been made. Such implants must maintain chemical and mechanical stability *in vivo* over a sufficient period of time for the fulfillment of their primary function of allowing regeneration of the substituted organ or assuring adequate stability of bone fracture during healing. The selected medical applications of absorbable polymers have been addressed in some papers.[12-15] The scarcity of the polymers that meet these demanding requirements has prompted a continuous search for improved biodegradable polymers. One of the advantages of chemically synthesized biodegradable polymers is that their degradation rates can be controlled at will, so as to meet specific requirements by chemical modification of their structures.

There are many factors affecting the biodegradability of polymeric materials. They are related to primary structures (chemical composition, molecular weight, molecular weight distribution, etc.), higher-order structures (melting point, glass transition temperature, crystallinity, crystal structure, etc.), and surface conditions (surface area, hydrophilicity, hydrophobicity, etc.).[16,17] Since these factors are interrelated in a complicated way, it is not easy to clarify the structure-biodegradability relationships for a wide range of polymers. In our laboratory, efforts have been recently directed toward the synthesis of functional biodegradable polymers for bio-modeling and biomedical applications.[18-25] The Krebs cycle acids are good candidates for the development of new polyesters. The tricarboxylic acid cycle, or Krebs cycle, is the process during which acetyl moiety, in acetyl CoA, is oxidized completely to carbon dioxide and water. It is expected that the polyesters prepared using Krebs cycle acid derivatives are biocompatible and biodegradable, and that their degradation residues will be nontoxic. The monomers such as 1,4-butanediol dilactate and 2-acetoxysuccinic acid were synthesized for the development of new biodegradable polymers. 1,4-Butanediol dilactate (BDLA) was synthesized using L-lactic acid and 1,4-butanediol diluted in cyclohexane. The synthesis of 2-acetoxy succinic acid (2-ASA) was also carried out using *L*-malic acid and acetic anhydride. Poly(1,4-butanediol succinate) (PBS) was synthesized from 1,4-butanediol, succinic anhydride, and *p*-toluenesulfonic acid. Poly(1,4-butanediol dilactate succinate) (PBDS) was prepared from BDLA and succinic anhydride. Poly(1,4-butanediol dilactate 2-acetoxy succinate) (PBDAS)

was polymerized using 2-acetoxy succinic acid with 1,4-butanediol as the same method of PBS polymerization. The crosslinked poly(1,4-butanediol *L*-malate) polymers were also synthesized from *L*-malic acid and 1,4-butanediol by varying the functional group ratio.

The hydrolytic behaviors of the synthesized polymers in various pH buffer solutions and their biodegradation by microorganisms were studied. Thus far, the effects of crystallinity and molecular weights on the biodegradation have also been investigated. The swelling ratio of the crosslinked copolyesters were also measured in different pH solutions at 20, 30, and 37 ℃. The swelling degree of crosslinked poly(1,4-butanediol *L*-malate) was increased with decreasing crosslink density at pH 7.4. The hydrolysis of the copolyesters proceeded faster with increasing solution pH. The hydrolytic degradation of poly(1,4-butanediol succinate) was carried out by stirring the dispersion in 0.1 N NaOH alkaline solution at 37 ℃. The weight loss of poly(1,4-butanediol succinate)(Mw: 6300) reached 85% within two days. The degradation of poly(1,4-butanediol succinate) by actinomycete and bacteria is similar to the sequence of events observed with fungi except for the extended existence of the crystalline structure. The degradation of poly(1,4-butanediol succinate)(Mw: 29000) followed a slower trend than that of lower molecular weight of poly(1,4-butanediol succinate) (Mw: 6300). However, the crystalline structure of poly(1,4-butanediol succinate) (Mw: 29000) was maintained for a longer period than that of poly(1,4-butanediol succinate) (Mw: 6300). The biodegradable behaviors of the crosslinked poly(1,4-butanediol *L*-malate) were examined using microorganisms such as fungus and bacterium, which were routinely used in the ASTM procedure for the determination of biodegradation. The degrees of biodegradation after seven weeks were up to 85% in *Aspergillus niger* and up to 75% in *Pseudomonas fluorescens*.[20,24]

The effects of the pendant hydrophobic group and crystallinity were investigated on poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate). Their morphology changes during biodegradation were observed using optical microscopy to compare their biodegradabilities. The quantitative determination of carbon dioxide, generated during the treatment with activated sludge, showed that poly(1,3-propanediol succinate) was biodegraded faster than poly(1,2-propanediol succinate) with the pendant hydrophobic methyl group. The biodegradability of *Aspergillus niger* to degrade the polymers was monitored using gel permeation chromatography. The biodegradation of poly(1,2-propanediol succinate) with the pendant hydrophobic methyl group was slower than that of poly(1,3-propanediol succinate).[19,20] In the crystallinity changes of the polymers during biodegradation, the chain scissions in the crystalline region of poly(1,3-propanediol succinate) proceeded faster than those of poly(1,2-propanediol succinate). Based upon the visual observation of their biodegradation, the crystalline structure of poly(1,2-propanediol succinate) retained its crystallinity longer than the similar structure in poly(1,3-propanediol succinate). These biodegradable polymers were degraded to their lower molecular weight components depending upon both their hydrophobicity of their chain structures.[21-23] The development of new polymeric materials for biomedical applications, including the synthesis and physical properties of biodegradable polyesters, has been carried out in our laboratory.[24-28]

Biodegradable Polymeric Biomaterials-Based Systems. Poly[α-(4-aminobutyl)-*L*-glycolic acid] (PAGA) is a biodegradable analogue of PLL, which can rapidly degrade in aqueous solution to give *L*-oxylysine as a final product. PAGA was synthesized to circumvent the cytotoxicity, non-biodegradability and low transfection efficiency of PLL.[29] This water-soluble polymer efficiently condenses plasmids and demonstrates transfection efficiencies higher than those of PLL-based systems in cultured cells. PAGA is currently being investigated for cytokine gene delivery for the treatment of diabetes[30] and cancer.[31] The expression level of IL-10 in 293 T cells with PAGA/pmIL-10 was significantly higher than that attained with naked DNA. Systemic administration of PAGA /pmIL-10 into NOD mice markedly reduced insulitis compared to the mice injection with naked DNA (15.79% vs. 90.9%).[30] Single injection of PAGA/pmIL-12 complexes to subcutaneous tumor-bearing BALB/c mice significantly enhanced mIL-12 expression and reduced the tumor growth.[31] However, transgene expression following intratumoral injection of PAGA/pLuc or PAGA/pmIL-12 was only 2-fold high than that of naked DNA. PAGA/pLuc or PAGA/pmIL-12 complexes are likely to degrade quickly after entering the cytosol due to autohydrolysis of ester linkage in PAGA, which could explain the relatively low DNA transfection efficiency.

Chemical modifications of PAGA aimed at slowing down the degradation rates are currently being studied. Another biodegradable cationic polymer, poly(4-hydroxy-*L*-proline ester) (PHP ester), has also been reported in the literature. PHP ester can be synthesized from CBZ-4-hydroxy-*L*-proline by melting-condensation polymerization of *N*-cbz-4-hydroxy-*L*-proline followed by deprotection using palladium on activated carbon as a catalyst,[32] or by using dicyclohexylcarbodiimide/(dimethylamino)pyridine (DCC/DMAP)-activated polycondensation of *N*-carboxy-4-hydroxy-*L*-proline.[33] PHP ester can condense DNA, shows low cytotoxicity, and its transfection efficiency is similar to that of PLL. Poly(*D,L*-lactic acid-*co*-glycolic acid) (PLGA) is a commonly used biodegradable and biocompatible polymer. PLGA microspheres have been shown to protect DNA from degradation by nuclease.[34] Using a water-in-oil-in-water (W/O/W) double-emulsion and solvent evaporation method, nanospheres containing plasmid DNA were prepared.[35] The microsphere size, release kinetics and encapsulation efficiency of plasmid DNA were found to be dependent on the emulsification methods, water/oil ratio, primary emulsion and the surfactant concentration. Furthermore, DNA is nicked in the steps of microencapsulation and lyophilization. Therefore, prior to the preparation of microsphere, PLL is used to pre-condense the DNA.[36]

Plasmid DNA was mixed with these nanoparticles and incubated for 1 hr at room temperature. The adsorption of DNA on the surface of the nanoparticle was dependent on the dextran content in the graft copolymer. Maruyama *et al.*[37] conjugated polysaccharide-grafted PLL and mixed this with poly(*D,L*-lactide) (MW 10000 Da) to prepare nanoparticles by either solvent evaporation or diafiltration. The ionic interaction between DNA and PLL moieties on the nanoparticles is thought to be the main driving force for DNA absorption on the

nanoparticles. For the nanoparticles prepared from PLL homopolymer/PLA, the majority of the amino groups in PLL might be interacting with PLA, leading to "train"-form adsorption of PLL on the nanoparticle surfaces. On the other hand, dextran-graft chains partially disturb the ionic interaction between PLA and PLL segments. Therefore, PLL segments on the copolymer nanoparticles adopt the "loop" and "tail" forms. The majority of the amino groups in PLL backbone are free in the surface adsorption form, providing a higher capacity for DNA adsorption. PLL-graft-Dex/PLA nanoparticles with DNA complexes can be produced at a diameter of 250 nm, and which enhance the ability of complex formation by 3 times as compared to PLL/PLA systems.

Thermosensitive Polymeric Materials-Based Systems. Thermosensitive polymers can control the release of encapsulated DNA in response to extreme temperature changes that lead to swelling or deswelling of the polymer. Poly(*N*-isopropylacrylamide) (pNIPAAm) is an uncharged thermosensitive polymer that is water soluble at low temperature but precipitates when the temperature is raised above 31-32 ℃. This transition temperature is referred to as the lower critical solution temperature (LCST).[38] The LCST of copolymers containing NIPAAm can be raised to higher temperatures by changing compositions of copolymers of NIPAAm and other co-monomers such as butyl methacrylate (BMA), *N',N*-dimethylacrylamide (DMA) or DMAEMA. DMAEMA acts as a hydrophilic comonomer, increasing the LCST of the copolymer with increased DMAEMA content. This observation suggests that the co-polymerization of DMAEMA and pNIPAAm of various monomer ratios and molecular weights by free radical polymerization, and that these polymers should be evaluated as carrier systems for gene delivery.[39]

The size of poly(DMAEMA-*co*-NIPAAm)/pDNA complexes was about 200 nm, which increased in proportion to the NIPAAm content of poly(DMAEMA-*co*-NIPAAm). However, the complexes using high molecular weight poly(DMAEMA-*co*-NIPAAm) or lower ratios of NIPAAm with plasmid DNA were relatively stable at 37 ℃, when compared to other fractions of poly(DMAEMA-*co*-NIPAAm). The zeta potential of the complexes was independent of the molecular weight of the copolymer but dependent on the copolymer/pDNA ratios and NIPAAm content of the copolymer. The cytotoxicity of poly (DMAEMA-*co*-NIPAAm)/pDNA decreased with increasing NIPAAm concentration. The transfection efficiency was a function of the copolymer/pDNA ratio and showed a bell-shaped curve. At the optimal conditions, the transfection efficiency of poly(DMAEMA-*co*-NIPAAm)/pDNA increased with decreasing NIPAAm content. The formation of stable copolymer/pDNA complexes with a size of around 200 nm is a prerequisite for efficient transfection. The transfection efficiency of poly (DMAEMA-*co*-NIPAAm)/pDNA was lower than that of pDMAEMA/pDNA, suggesting that poly(DMAEMA-*co*-NIPAAm) has more potential for gene delivery due to its lower toxicity than pDMAEMA.

Another thermosensitive terpolymer, pNIPAAm-*co*-DMAEMA-*co*-butylmethacrylate (BMA) was synthesized and its transfection efficiency was evaluated at different incubation temperatures.[40] In the terpolymer, BMA is the hydrophobic component, and thus the solubility of terpolymer/pDNA complexes is probably regulated by both ionic and hydrophobic interactions. A terpolymer containing 8 mol% of DMAEMA and 11 mol% of BMA had an LCST at 21 ℃. Therefore, the terpolymer was insoluble above 21 ℃ and soluble below 21 ℃. The terpolymer/pDNA complexes showed partial dissociation at 20 ℃, but no dissociation at 37 ℃, suggesting that the formation and dissociation of the complexes were also modulated by temperature. The transfection efficiency of pDMAEMA/pDNA incubated at 37 ℃ for 48 hrs was higher than if the complex was incubated at either 20 ℃ for 3 hrs or at 37 ℃ for 45 hrs. However, the transfection efficiencies of terpolymer/pDNA complexes incubated at 20 ℃ for 3 hrs and 37 ℃ for 45 hrs were much higher than for those incubated at 37 ℃ for 48 hrs. Poloxamers, which are non-ionic copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), offer the unique property of reversible thermal gelation.[41] Poloxamers have been shown to significantly increase gene expression in skeletal muscle,[42] possibly by protecting plasmid DNA from enzymatic degradation and by enhancing distribution of plasmid DNA across the tissue. Because poloxamers are non-biodegradable and likely to be toxic, Jeong *et al.*[43] synthesized a series of triblock and diblock, biodegradable, thermosensitive polymers consisting of PEG or PEO and polyesters, such as poly(*D,L*-lactide) (PLL) or poly(*D,L*-lactide-*co*-glycolide) (PLGA). The aqueous solution of these triblock or diblock copolymers exhibits temperature-dependent, solution-to-gel transitions in the range of 30～35 ℃ and gel-to-solution transition in the range of 40～70 ℃.

The solution-to-gel and gel-to-solution transition temperatures depend on the polymer composition, concentration and PEG molecular weight. The critical gel concentration, above which the gel phase exists, is about 16 wt%. This triblock copolymer can be loaded with plasmid DNA in aqueous phase at 4～20 ℃. The solution-to-gel transition temperature and its biodegradability could make this triblock copolymer ideal for an injectable system that can be formulated at room temperature and forms gel at body temperature, and thus may be useful for local injection of plasmid DNA for sustained and controlled delivery. The effect of polyelectrolyte on the lower critical solution temperature of pNIPAAm in the copolymer of p(NIPAAm-*co*-AAc) was investigated.[44] The interaction between poly(*L*-lysine)/poly(allylamine) and p(NIPAAm-*co*-AAc) in aqueous solution has also been studied.[45-47] The synthesis and characterization of the temperature/pH-sensitive polymers for drug delivery systems have been carried out in our laboratory.[48-53] Drug delivery through surface modified, stimuli-responsive, polymeric membranes was evaluated in our group.[54]

Cationic Polymeric Biomaterials-Based Systems. DNA can be condensed into mononuclear or polynuclear particles with an excess of polycations in aqueous solutions.[55] The cationic polymer spontaneously forms complexes with DNA because of electrostatic interactions between positively charged amine groups of the polycations and negatively charged phosphate groups of the DNA. The interaction between cationic polymer/DNA complexes and negatively charged cell membranes can enhance DNA uptake by the cells and thus increases the

transfection efficiency. The cationic polymers for the biomaterials-based systems are mostly prepared from the following compounds.

Poly(*L*-lysine) (PLL) : PLL is a well-known polycation, which has been used to condense plasmid DNA under various salt conditions.[56] PLL has a sufficient number of primary amines with positive charges to interact with the negatively charged phosphate groups of DNA. PLL/ DNA complexes are prone to aggregation under physiological conditions. To increase the solubility of PLL/DNA complexes in aqueous media, PLL has been modified with hydrophilic dextran by a reductive amination reaction between ε-amino groups of PLL and the reductive ends of dextran.[57] The dextran chains of poly(*L*-lysine)-*graft*-dextran(PLL-*g*-Dex) do not considerably hinder the electrostatic interaction between DNA and PLL. However, they efficiently increase the solubility of the complex as well as the thermal stability of triplex helices of poly(dA) · 2poly(dT) by more than 50 ℃.[58] In addition to the undesirable effect of aggregation of PLL/DNA complexes, PLL is toxic to the cells. To decrease cytotoxicity, PLL was glycosylated using methylglycolate.[59] The glycosylated PLL is less toxic, but also less biocompatible, than PLL. Thus, in order to decrease both cytotoxicity and aggregation, PLL was covalently linked to polyethylene glycol (PEG).[60] PEG-*g*-PLL was found to be relatively non-toxic to cultured cells, compared to PLL. In addition, the transfection efficiency of poly (ethylene glycol)-*graft*-poly(*L*-lysine)/pDNA is about 30-fold higher than that of PLL/pDNA complexes.

Chitosan Derivatives : Chitosan is a non-toxic biodegradable polysaccharide composed of two subunits, *D*-glucosamine and *N*-acetyl-*D*-glucosamine, linked together by a (1,4) glycosidic bond. Cationic-charged chitosan interacts with the negatively charged phosphate groups of DNA. The colloidal and surface properties of chitosan/plasmid complexes have been shown to depend on the molecular weight of chitosan, the ratio of plasmid to chitosan and the preparation medium. Several cell lines, such as COS-1 cells, can be transfected with plasmid/chitosan complex.[61] High molecular weight chitosan (over than 100 kDa) is dissolved in water after addition of acetic acid, and the resulting low molecular weight chitosan (22 kDa) is well soluble in physiological conditions and efficiently condenses DNA.[62] Although the transfection efficiency of low molecular weight chitosan/DNA complexes is slightly higher than that of PLL/DNA complexes, it is also less toxic to the cells. Lee *et al.*[63] synthesized hydrophobically modified chitosan with deoxycholic acid and demonstrated efficient transfection of chitosan/DNA complexes into COS-7 cell lines. Erbacher *et al.*[64] partially substituted with various amounts of lactosyl residues by reductive amination in the presence of sodium cyanoborohydride α-lactose in order to target cells expressing a galactose-binding membrane lectin. Lactosylated chitosans, substituted with 3, 10, 20 % lactose residues, did not enhance the gene transfection into BNL CL2 or Hep G2 cells. In comparison, *in vitro* transfection efficiency was enhanced 1000-fold (RLU/mg of protein up to 10^8) with lactosylated PEI. Moreover, the reductive amination with lactosylation may significantly reduce the affinity of chitosan for DNA. However, lactosylated chitosan/DNA complexes can efficiently transfect HeLa cells, in the presence of 10% serum, although the transfection efficiency was not improved further enhanced when an endosomolytic agent such as chloroquine was added. It is also of interest that gene expression gradually increased from 24 to 96 hrs in cells transfected with the lactosylated chitosan, whereas under the same conditions the efficiency of PEI-mediated transfection dropped by two orders of magnitude. The transgene expression in the intestinal epithelium following oral administration of chitosan/pCMV-Arah2 complexes was reported.[65] Compared with non-immunized mice or mice treated with 'naked DNA', mice immunized with chitosan/pDNA complexes showed a substantial reduction in allergen-induced anaphylaxis associated with reduced levels of IgE, plasma histamine and vascular lineage. These results suggest that oral allergen-gene immunization with chitosan/DNA nanoparticles may be an effective approach to modulate murine anaphylactic responses. Chitosan is a mucoadhesive polymer, and thus chitosan/DNA complexes are an attractive candidate for transfecting gastrointestinal epithelia and/or immune cells in the gut-associated lymphoid tissue after being carried across the mucosal boundaries. Chitin-based wound dressing containing silver sulfurdiazine was also investigated.[66] The synthesis and characterization of chitosan derivatives were carried out in our research laboratory.[67-69]

Polyethyleneimine (PEI) : PEI is a cationic polymer composed of 25% primary amines, 50% secondary amines and 25% tertiary amines. It has been shown to effectively condense plasmids into colloidal particles that effectively transfect pDNA into a variety of cells both *in vitro* and *in vivo*.[70] These condensed particles are of spherical shape and have a narrow particle size distribution, which presumably allows high cellular uptake of the plasmids leading to high transfection efficiency.[71] PEI has a high charge density, due to every third atom on the PEI backbone being a nitrogen atom. In linear PEI, all of these nitrogen atoms are protonable, whereas in branched PEI, only two-thirds of them can be charged.[72] The overall protonation level of PEI increases from 20 to 40% at pH 7 and 5, respectively.[73]

In the presence of PEI in endosome, the accumulation of protons brought in by the endosomal ATPase is coupled to an influx of chloride anion. This causes a large increase in the proton concentration within the endosome, resulting in swelling of the polymer by internal charge repulsion, with consequent osmotic swelling of the endosome. Therefore, PEI may enhance intracellular trafficking by buffering the endosomal compartments, thus protecting the DNA from lysosomal degradation by endosomal DNA release via lysosomal disruption. The homogeneous nature and small size of the PEI/DNA complex has been shown to produce a high level of gene expression in mature mouse brain. Transient gene expression in the brain was reported to be due to cell dysfunction and death. Over-expression of an anti-apoptotic gene could block the activation of caspases during the post-transfection phase. Therefore, co-delivery of anti-apoptotic gene bcl-X_L with luciferase significantly increased expression of both of these genes at one-week post-injection.[74] To enhance the water solubility and reduce the aggregation of PEI/pDNA complexes, these complexes were PEGylated. There was little change seen in the mean particle size of PEGylated PEI/pDNA complexes, and PEGylation reduced the surface charge,[75]

The covalent coupling of PEG to PEI was carried out via the primary amino groups in the PEI molecules by reduction with the succinimidyl derivative of propionic acid poly(ethylene glycol) M-PEG-SPA, which has a molecular weight of 5000 Da. PEO-*g*-PEI/pDNA complexes have a small particle size (～100 nm). The solubility of PEI/pDNA complex in water can also be improved by modifying PEI with polyethylene oxide.[76] The phase-separated multiblock copolymers with PEO have been synthesized and characterized by reacting functionalized telechelic oligomers of each block to repeating linkages between blocks.[77-80]

Poly(2-dimethylamino)ethyl methacrylate (pDMAEMA) : pDMAEMA is a water-soluble cationic polymer that can efficiently bind and condense DNA. It can mediate transfection into a variety of cell types. The effects of tertiary amine groups (pKa～7.5) versus quaternary amine groups were evaluated by comparing pDMAEMA with its quaternary amine analogue poly(2-(trimethylamino)ethyl methacrylate) (pTMAEMA).[81, 82] Based on the results obtained from cellular interaction and transfection, they concluded that pTMAEMA does not have an intrinsic endosomal escape property, in contrast to the 'proton sponge' effect seen with pDMAEMA or PEI. The transfection efficiency of pDMAEMA is significantly higher than that of pTMEAMA.[83] Fluorescence and CD spectroscopy, as well as gel electrophoresis, all demonstrate that pDMAEMA and PLL have similar DNA association/dissociation characteristics, whereas pTMAEMA tightly condenses DNA, and thus is unable to fully dissociate plasmid DNA inside the cells, which might explain the lower transfection efficiency of pTMAEMA compared with pDMAEMA. The optimal transfection efficiency was found at a pDMAEMA/DNA ratio of 3:1 (w/w), a ratio at which homogeneous complexes of ～150 nm in diameter could be formed. In addition, the stability of pDMAEMA/DNA has been tested in 20 mM Hepes (pH 7.4) buffer containing 10% sucrose at 4, 20 and 40 ℃.[84] pDMAEMA/DNA complexes were found to be stable over 10 months at 4 ℃ and 20 ℃, but lost a substantial fraction of their transfection potential when stored at higher temperatures. Some of tertiary amine-containing polymers, such as PEI, have been shown to have endosomolytic properties. The chemical structure of *N,N'*-diethylaminoethyl methacrylate (DEAEMA) is similar to that of DMAEMA. Tertiary amine groups of 4-vinylpyridine (4VP) and DEAEMA have pKa values of 5.45 and 9.5, respectively. Therefore, the copolymerization of 4VP with DEAEMA will produce a pH-sensitive polymer, which could enhance membrane fusion.[85]

Dendrimers : Dendrimers condense plasmids via electrostatic interactions of their terminal primary amines with the DNA phosphate groups. The particle size, surface charge and gene transfer efficiency of dendrimer/plasmid complexes prepared with the 5th generation of dendrimers has been shown to be influenced by the dendrimer concentration in the complexes. Starburst™ polyamidoamine (PAMAM) dendrimers are a class of highly branched spherical polymers. The surface charge and diameter of any given PAMAM dendrimer is determined by the number of synthetic steps taken to produce it,[86] e.g., five polymerization cycles produce the fifth generation dendrimers. The major structural differences in PAMAM dendrimers relate to the core molecules, either ammonia or ethylenediamine, with which the stepwise polymerization process begins and which dictates the final overall shape, density and surface charge of the molecule. Dendrimers can be either "intact" or "fractured." Intact dendrimers have two arms extending from every branch point, whereas a fractured dendrimer can have zero, one, or two arms extending from each branch point.[87] The transfection efficiency of dendrimer-based formulations into cells is, in turn, dependent on the size, shape, and number of primary amine groups present on the surface of the polymer. Starburst dendrimers have been used to mediate efficient, but non-specific *in vitro* gene transfer to eukaryotic cells.[88] In a search for efficient gene delivery systems, Epstein-Barr virus (EBV)-based plasmid vector coupled with PAMAM dendrimer (EBV/polyplex) was investigated for its potential to deliver therapeutic genes to cancer cells.[89]

Non-condensing Polymeric Biomaterials-Based Systems. Non-condensing polymers, such as polyvinyl alcohol (PVA) or polyvinyl pyrrolidone (PVP), and poly(2-hydroxyethylmethacrylate) (PHEMA) are amphiphilic molecules, having both a hydrophilic and a hydrophobic group.[90-94] The hydrophilic portion of these polymers may interact with plasmid DNA by hydrogen bond (via hydrogen bond acceptor or donor groups), van der Waals interactions, and/or by ionic interactions. PVP is a hydrogen bond acceptor, whereas PVA is a hydrogen bond donor. The interaction between the hydrophobic monomer units *via* van der Waals interaction can form a hydrophobic coating of vinyl backbone around the plasmid DNA. PVP-based formulations are hyperosmotic and result in an improved dispersion of plasmids through the extracellular matrix of solid tissues. The intramuscular injection of PVP-based-plasmid formulation in rats increases significantly the number and distribution of expressing cells, as compared to unformulated plasmid.[95] Up to a 10-fold enhancement of gene expression over unformulated plasmid has been observed in mouse and rat muscle. The interaction between polyvinyl pyridinium and DNA can protect DNA from nuclease degradation and facilitate its cellular uptake via hydrophobic interactions with cell membranes.[96] The increased stability of the PVP-plasmid complex in aqueous solution reduces the precipitation of the complexes and facilitates cellular uptake. This is enhanced by the addition of hydrophilic poly(ethylene oxide) (PEO) segments.

Polyethylene oxide-*block*-poly(*N*-methyl-4-vinylpyridinium sulfate) (PEO-*b*-PVP) was synthesized by a sequential anionic polymerization process followed by quaternization with dimethylsulfate.[97] PEO-*b*-PVP recognizes the tertiary structure of the DNA and selectively binds with the cDNA when both circular and linearized plasmid DNA is present.[97] To enhance the transfection efficiency, poly(*N*-ethyl-4-vinylpyridium bromide) (PEVP)/pDNA complexes with poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) copolymer (Pluronic P85) were prepared. The transfection efficiency of PEVP/pDNA with 0.1% Pluronic P85 was significantly higher than that seen with PEVP/pDNA without copolymer solution.[98] The albumin release from the multiblock copolymer consisting of poly(benzyl *L*-glutamate) (PBLG) and PEO was studied.[99] The preparation and characterization of meth-

oxy poly(ethylene glycol)/caprolactone amphiphilic block copolymeric micelle containing indomethacin were carried out.[100] The micelle formation and drug release behaviours were also investigated.[101] The block copolymers consisting of PBLG as the hydrophobic block and PEO as the hydrophilic block were synthesized and characterized.[102] Clonazepam release from the core-shell type nanoparticles *in vitro* has been also studied.[102]

DNA-based tissue engineering is being investigated as a way to grow new tissues and organs. Ideally, the local delivery of plasmid DNA will allow appropriate levels of transgene expression for a prolonged period. For example, sustained release of plasmid DNA from biodegradable poly(*D,L*-lactide-*co*-glycolide) (PLGA) matrices can lead to the transfection of a large number of cells at a localized site, leading to the production of therapeutic proteins needed for tissue regeneration. PLGA matrices containing plasmid DNA can be targeted physically, and can express protein for a prolonged period of time. Shea *et al.*[103] incorporated plasmid DNA encoding platelet-derived growth factor (PDGF) directly into PLGA three-dimensional matrices.

Plasmid DNA was subsequently released from the matrices over a period ranging from days to month *in vitro*, and led to enhancement of matrix deposition and blood vessel formation in developing tissues *in vivo*. The inefficient delivery of growth factors locally in a transient but sustained manner is a major barrier to effective tissue regeneration. However, systems for localized and sustained delivery of plasmids should be suited for growth factor therapeutics. Bonadio *et al.*[104] investigated the possible use of polymer matrices containing pMat-1 plasmid DNA, which encodes a secreted peptide fragment of human parathyroid hormone, for bone regeneration in a beagle tibia critical defect model. The implantation of polymer matrices with plasmid DNA at the site of bone injury was associated with retention and gene expression of the plasmid DNA for at least 6 weeks. Using biocompatible materials for local and sustained-controlled release of plasmid DNA carrying a HST-1/FGF-4 cDNA, the plasmid DNA delivery systems were developed for tissue engineering,[105,106]

Polymeric Biomaterials for RNA Interference Technology. RNA interference (RNAi) represents a powerful, naturally occurring, biological strategy for inhibition of gene expression. It is mediated through small interfering RNAs (siRNAs), which trigger specific mRNA degradation. In mammalian systems, however, the application of siRNAs is severely limited by the instability and poor delivery of unmodified siRNA molecules into the cells *in vivo*. It is clear that the efficient delivery of intact siRNAs will play a rate-limiting role in any mammalian gene therapy application and has so far widely limited *in vivo* applications of RNAi. Recently, Urban-Klein *et al.*[107] have shown that the noncovalent complexation of synthetic siRNAs with low molecular weight PEI efficiently stabilizes siRNAs and delivers siRNAs into cells where they display full bioactivity at completely nontoxic concentrations. More importantly, in a subcutaneous mouse tumor model, the systemic administration of complexed siRNA, but not of naked siRNAs, leads to the delivery of the intact siRNAs into the tumors. The intraperitoneal injection of PEI-complexed, but not of naked siRNAs targeting the c-erbB2/neu (HER-2) receptor, resulted in a marked reduction of tumor growth through siRNA-mediated HER-2 downregulation. A similar strategy has been developed for the construction of a target-specific delivery system of green fluorescent protein (GFP) siRNA plasmid DNA by utilizing folate-modified cationic PEI. Application of this system to folate receptor positive cells (KB cells) resulted in a marked reduction of GFP expression.[108]

The extent of GFP gene inhibition and cellular uptake behaviors appeared to be more effective with pSUPER-siGFP/PEI-PEG-folate complexes than with pSUPER-siGFP/PEI complexes with no folate moieties, while such inhibition was largely dependent on the presence of folate receptors in the cells. These observations indicate the use of folate receptor-mediated endocytosis as a major pathway in the process of cellular uptake of siRNA-plasmid, suggesting that targeted delivery of siRNA vector could be achieved by cell-specific manners. On the other hand, self-assembling nanoparticles with siRNA have been constructed with PEI that is PEGylated with an Arg-Gly-Asp (RGD) peptide ligand attached at the distal end of the polyethylene glycol (PEG), as a means to target tumor neovasculature expressing integrins, and have been used to deliver siRNA inhibiting vascular endothelial growth factor receptor-2 (VEGF R2) expression and thereby tumor angiogenesis.[109] Intravenous administration into tumor-bearing mice gave selective tumor uptake, siRNA sequence-specific inhibition of protein expression within the tumor and inhibition of both tumor angiogenesis and growth rate.

These observations imply the potential for biomedical applications by targeting gene and drug delivery at two levels: tumor tissue selective delivery via the nanoparticle ligand and gene pathway selectivity via the siRNA oligonucleotide. When the organic-inorganic hybrid nanoparticles entrapping oligodeoxynucleotide or siRNA were prepared through the self-associating phenomenon of the block copolymer, poly (ethylene glycol)-*block*-poly(aspartic acid) (PEG-*b*-PAA), with calcium phosphate, the calcium phosphate core dissociates in the intracellular environment with appreciably lowered calcium ion concentration compared to the exterior, facilitating the release of the incorporated oligodeoxynucleotide and siRNA in a controlled manner.[110] The utilization of polymeric biomaterials in the development of better methods for highly efficient delivery of siRNA in a controlled manner will develop the RNAi technique as a useful biomedical application in the near future.

Summary

The synthesis and characterization of functional polymers for the biomedical application were reviewed. The development of functional polymeric biomaterials was treated on the basis of functionality, biocompatibility and biodegradability. The following functional polymeric biomaterials-based systems were proposed for biomedical applications in the near future: the functional biodegradable polymers synthesized in our research laboratory, biodegradable polymeric materials, thermo-sensitive polymeric materials, cationic polymeric materials,

non-condensing polymeric biomaterials, bio-polymeric DNA matrix for tissue engineering, and polymeric biomaterials for RNA interference (RNAi) technology.

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