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Synthesis of Bioapplicable Polymers
Using Atom Transfer Radical Polymerization and Ring Opening Metathesis Polymerization

ATRP 와 ROMP 를 이용한 생체 내 응용 가능한 고분자 합성

2018 년 8 월
서울대학교 대학원
화학부 생화학 전공
김 희 진
Synthesis of Bioapplicable Polymers Using Atom Transfer Radical Polymerization and Ring Opening Metathesis Polymerization

지도교수 이 연

이 논문을 이학박사학위논문으로 제출함
2018 년 8 월

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Abstract

Biomaterials are used to make devices which can be replace a part or function of the body in a safe, reliable and physiologically acceptable manner. A variety of devices and materials are used in the treatment of disease or injury. Biomaterials are classified into metal, ceramic, polymer and composite materials according to type of materials. Whereas metal and ceramic are used mainly for mechanical properties, organic-based materials are applied mainly to biological and pharmacology. The range of application is diverse such as artificial hearts, blood vessels, bones, kidneys and ears etc. Biomaterials are required proper physical properties and excellent biocompatibility because they directly contact with living tissue. They must have tissue compatibility and hemocompatibility that must not cause tissue necrosis and blood coagulation in contact with human body. They have non-allergic or non-immune response as well as non-toxic. Also, they must perform function of replaced body parts and at the same time they are no degradation of the physical properties.

Among them, polymeric materials have a wide variety applications for implantation. They have less resistance to the human body than the metal and ceramic materials. So, they have applied in biomedical fields such as medical device, tissue engineering and drug delivery etc. Especially, synthetic polymers can be used as various biological materials because they can control the
chemical and physical properties of monomers and introduce various functions during synthesis and processing. However, when they are used as biomaterials, nonspecific protein adsorption and blood coagulation can be caused on the surface. These symptoms get worse, they lose the natural functions and cause inflammatory and immune response.

Natural polymeric materials, which have good biocompatibility, such as collagen, cellulose and chitin are used in biomedical field. However, they are difficult to apply for tissues which must bear a lot of the weight such as bone or cartilage due to their weak hardness.

First, I focused to synthesizing biocompatible polymers using atom transfer radical polymerization. I suggested synthesis of biomembrane-mimic polymers. Like 2-methacryloyloxyethyl phosphorylcholine (MPC), monomers were synthesized as custom methacrylate with phospholipid head groups on the side chain for atom transfer radical polymerization (ATRP). I synthesized three different phospholipid-mimic polymers with phosphatidic acid (PA), phosphatidylethanolamine (PE), and phosphatidylserine (PS) head groups. I developed a new synthetic method to prepare monomers and ATRP was used to control the polymerization of the phospholipid-mimic polymers. Considering phospholipid compositions in cell membranes vary between organs, tissues, cells, and even cellular organelles, the biomembrane-mimic polymers based on diverse phospholipid head groups can be a potential platform for the
preparation of cell- or tissue-specific surfaces with both biocompatibility and bioactivity, which are difficult to be obtained by only PC-based polymers for biomedical applications.

Recently, smart materials which have external signal-sensitive degradation of chemical bonds have been developed. These have degradable moieties responding to biologically tolerable signals such as light, enzyme, pH and glutathione. Among these biosignals, pH is commonly applied to selectively trigger degradation because pH in different tissues and cellular compartments exist in human body. Different tissues have individual pH conditions and pH values decrease gradually in the endocytic process. In addition, tumor cells extracellular matrix pH is lower than in healthy cells. Therefore, a degradable bond at a specific pH value can be used for the release of conjugated drugs based on specific conditions and target sites.

So, for biodegradable polymers, I proposed synthesis of pH-responsive polymers having cis-α,β-unsaturated anhydrides. I synthesized polymers with pH-sensitive molecule, maleic acid anhydride-mimic, as a monomer unit using ring opening metathesis polymerization (ROMP). In order to control pH-sensitivity, I synthesized various bicyclic α,β-unsaturated anhydride derivative monomers which were changed the substituents group of the cis-α,β-double bonds. I synthesized monomers which are pH-sensitive moiety and ring strain for ROMP using Diels-Alder reaction. Monomers were tailored to control the
rate of degradability at acidic condition. They were polymerized by ROMP using 2nd generation Grubbs catalyst. In addition, maleic acid amide bond was introduced by amidation between polymer and n-propylamine. And rate of degradability was confirmed at various pH condition by detecting released amine. Furthermore, I studied in vitro bioactivity of alendronate-grafted polymers using breast cancer cell at pH 5.8, 6.5 and 7.4. The ROMP polymers with α,β-unsaturated anhydrides can be controlled pH-sensitivity to environmental signals upon substituents group of the cis-α,β- double bonds and theirs tunable pH-responsiveness is very useful property as the targeting and release of therapeutic agents.

**Keywords:** Biomembrane-mimic polymers, Cytocompatibility, Phospholipid head groups, ROMP, pH-degradability, Bicyclic α,β-unsaturated anhydride, Cis-α,β-unsaturated acid amide, Reversible grafting and release

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PART I. Synthesis of biomembrane-mimic polymers with various phospholipid head groups

1. Abstract

Lipid bilayers in biomembranes consist of diverse phospholipids, including phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) with various compositions according to the cell and tissue types. I synthesized biomembrane-mimic polymers, poly(2-methacryloyloxyethyl phosphoric acid) (PMPA), poly(2-methacryloyloxyethyl phosphorylethanolamine) (PMPE), and poly(2-methacryloyloxyethyl phosphorylserine) (PMPS), with PA, PE, and PS head groups, respectively. PA monomer was synthesized from 2-hydroxyethyl methacrylate (HEMA) and dimethyl chlorophosphate (DCP). PE and PS monomers were synthesized from N-tert-butoxycarbonyl (tBoc) protected ethanolamine and serine through the reaction with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP). Each biomembrane-mimic polymer was successfully synthesized by atom transfer radical polymerization (ATRP) from the monomer. The molecular weight distributions of PMPA, PMPE, and PMPS were analyzed by gel permeation chromatography (GPC) and in vitro cytotoxicity was also examined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and lactate
dehydrogenase (LDH) assay. The new biomembrane-mimic polymers could be used to prepare a polymeric platform that mimic a cell- or tissue-specific membrane for future applications in biomedical fields such as tissue engineering or bioimplants.
2. Introduction

Various functional materials have been developed for biomedical applications, including drug delivery, tissue engineering, and bioimplants. Although different materials based on ceramics, metals, polymers, and their composites have been used in the human body, biocompatibility is still one of the most important issues faced when using biomaterials. Foreign materials with insufficient biocompatibility can cause inflammation, severe immune response or rejection, tissue deformation, or fatal tissue necrosis. Because most reactions to foreign bodies start with the recognition of the surface of the foreign body by the biosystem, the preparation of a sufficiently biocompatible surface is essential for preventing such catastrophic results of foreign body reactions.

Naturally-occurring polymers or polymers derived from biological origins are attractive materials for the preparation of biocompatible surfaces. Polymers based on protein or peptide structures, e.g., collagen or elastin, and polymers based on sugar structures, e.g., hyaluronic acid or dextran, have shown great potential in the preparation of biocompatible hydrogels, drug delivery carriers, and tissue scaffolds because they mimic the extracellular matrix or components in biological fluids. Moreover, polymers based on the
phospholipid structure have also been developed to mimic the cell surface. Starting from poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), which mimics the head group of phosphatidylcholine (PC) in the cell membrane, various PC-based polymers have been used in biomedical applications. PC-based polymers have exceptional hemocompatibility, antiprotein adsorption activity, and antithrombotic activity. Therefore, they are commonly used as coating materials for coronary stents and artificial joints preventing restenosis and periprosthetic osteolysis. In addition, they are also applied for a shell of drug delivery carrier for the enhancement of delivery efficiency and the reduction of cytotoxicity.

Inspired by the successful results obtained with PC-based polymers, I decided to synthesize phospholipid-mimic polymers with head groups other than the phosphocholine moiety. The phospholipids in the cell membrane are a complex mixture of diverse lipids (Figure 1), including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI), and sphingomyelin (SM). Although PC is a major component of phospholipids, other phospholipids also play important roles in the formation of and changes in the membrane structure and in intracellular and intercellular signal transduction. Lipid compositions in cell membranes vary between organs, tissues, cells, and even cellular organelles. The translocation of PS from the inner leaflet to the outer leaflet of the cell
membrane is closely related to apoptosis and the recruitment of phagocytic cells.\textsuperscript{17} Therefore, a polymer library based on diverse phospholipid head groups could provide a platform for the preparation of cell- or tissue-specific surfaces with hemocompatibility and cytocompatibility. Such surfaces are difficult to obtain with only PC-based polymers.

In this study, I synthesized three different phospholipid-mimic polymers poly(2-methacryloyloxyethyl phosphoric acid) (PMPA), poly(2-methacryloyloxyethyl phosphorylethanolamine) (PMPE), and poly(2-methacryloyloxyethyl phosphorylserine) (PMPS) with PA, PE, and PS head groups, respectively (Figure 2). I developed a new synthetic method to prepare PA monomer from 2-hydroxyethyl-methacrylate (HEMA) and dimethyl chlorophosphate (DCP). PE and PS monomers were also synthesized from corresponding simple precursors, ethanolamine and serine, through the reaction with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP). Atom transfer radical polymerization (ATRP) was used to control the polymerization of the phospholipid-mimic polymers. In addition, the cytotoxicity and membrane compatibility of each phospholipid-mimic polymer was checked to assess its suitability for future use in biomedical applications.
3. Experimental Section

3.1 Materials

Ethanolamine, di-tert-butyl dicarbonate, dimethyl chlorophosphate (DCP), 2-hydroxyethyl methacrylate (HEMA), ethyl 2-bromo-2-methylpropionate, methacrylic acid, 18-crown-6, pyridine, triethylamine (TEA), trimethylsilyl bromide (TMSBr), copper (I) bromide (Cu(I)Br), bipyridine (bpy), ethylenediaminetetraacetic acid (EDTA), aluminum oxide, trifluoroacetic acid (TFA), polyethyleneimine 25,000 (PEI 25 kDa) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Aldrich (USA). Polyethylene glycol 10,000 (PEG 10 kDa) was purchased from Creative PEGWorks (USA). 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) and bromotrimethylsilane (TMSBr) were purchased from TCI (Japan). N-Boc-Ser-OtBu was purchased from BACHEM (USA). Column chromatography-grade silica gel (230-400 mesh) was purchased from Merck (Germany). Magnesium sulfate (MgSO4), sodium phosphate monobasic monohydrate (NaH2PO4), sodium chloride (NaCl), (N,N-dimethylformamide (DMF), sodium hydroxide (NaOH), hydrogen chloride (HCl), diethyl ether, 1,4-dioxane, chloroform, tetrahydrofuran (THF), benzene, acetonitrile, methylene chloride (MC), methanol (MeOH), and Triton-X 100 were purchased from Daejung
(Korea). Dulbecco’s modified Eagle’s medium (DMEM), Dulbecco’s phosphate buffered saline (DPBS), and fetal bovine serum (FBS) were purchased from WelGENE (USA). The lactate dehydrogenase (LDH) cytotoxicity detection kit was purchased from Thermo Fisher Scientific, Inc. (USA). (All reagents were used without further purification.

### 3.2. Synthesis of 2-methacryloyloxyethyl phosphoryldimethylester (MPDME) (2)

DCP (5.78 g; 40.0 mmol) and pyridine (3.16 g; 40.0 mmol) were dissolved in chloroform (30 mL). HEMA (1) (1.30 g, 10.0 mmol) dissolved in chloroform (30 mL) was slowly added into the DCP/pyridine solution on ice. The reaction mixture was stirred overnight and extracted with 0.01M HCl (×3). The organic layer was dried with MgSO$_4$ and concentrated to yield MPDME (yield, 1.93 g, 81.1%).

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta = 1.87$ (CH$_3$C=CH$_2$CO$_2$-, s, 3H), $\delta = 3.65–3.71$ (-(CH$_2$)$_2$OPO(OCH$_3$)$_2$-, m, 6H), $\delta = 4.20–4.24$ (-COOCH$_2$CH$_2$OP(OCH$_3$)$_2$-, q, 2H), $\delta = 4.27–4.30$ (-COOCH$_2$CH$_2$OP(OCH$_3$)$_2$-, t, 2H), $\delta = 5.54$ (CH$_3$C=CH$_2$CO$_2$-, s, 1H), $\delta = 6.09$ (CH$_3$C=CH$_2$CO$_2$-, s, 1H)
3.3. Synthesis of 2-methacryloyloxyethyl phosphoryl N-tBoc-ethanolamine (MPE (Boc)) (6)

Ethanolamine (187 mg, 3.05 mmol) was slowly added to the solution of di-tert-butyl dicarbonate (1.00 g, 4.58 mmol) in 1,4-dioxane/water (v/v = 1). After stirring for 5 h at ambient temperature, the solvent was completely removed by evaporation. N-tBoc-ethanolamine (5) was obtained without further purification. For the formation of the phosphorous-oxygen (P-O) bond, 5 and TEA (464 mg, 4.58 mmol) were mixed in 30 mL of benzene and cooled to −30°C. COP (522 mg, 3.67 mmol) in 10 mL of benzene was slowly added to the stirred tBoc-ethanolamine solution. After the addition, the reaction mixture was maintained at ambient temperature with stirring for 4 h. Diethyl ether was poured into the reaction mixture, and precipitated triethylamine hydrochloride was filtered off. The filtrate was concentrated under reduced pressure to evaporate benzene and diethyl ether. The residual liquid was dissolved in acetonitrile (100 mL) and mixed with 2 equivalent of 18-crown-6. The aqueous solution (10 mL) of sodium methacrylate (2 equivalent), which had been prepared previously by titration between methacrylic acid and sodium hydroxide, was added to the reaction mixture. After further stirring at 75°C for 48 h, 18-crown-6 was removed by precipitation in cold acetonitrile. MPE (Boc) (6) was purified by silica gel chromatography with an eluent of MC/MeOH = 2/1. (yield, 0.990 g, 92.1%).
$^1$H-NMR (DMSO, 300 MHz): $\delta = 1.36$ (-C(CH$_3$)$_3$, s, 9H), $\delta = 1.81$ (CH$_3$C=CH$_2$CO$_2$-, s, 3H), $\delta = 3.05$ (-NHCH$_2$CH$_2$PO$_4$-, q, 2H), $\delta = 3.45$ (-O$_4$PCH$_2$CH$_2$O-, q, 2H), $\delta = 3.60$–3.68 (-NHCH$_2$CH$_2$PO$_4$CH$_2$CH$_2$O-, m, 4H), $\delta = 5.39$ (CH$_3$C=CH$_2$CO$_2$-, s, 1H), $\delta = 5.83$ (CH$_3$C=CH$_2$CO$_2$-, s, 1H)

3.4. Synthesis of 2-methacryloyloxyethyl phosphoryl di(tBoc)-serine (MPS (Boc)) (10)

$N$-Boc-Ser-OtBu (9) (500 mg, 1.91 mmol) and TEA (387 mg, 3.82 mmol) were mixed in 30 mL of benzene and cooled to $-30^\circ$C. COP (545 mg, 3.82 mmol) in 10 mL of benzene was slowly added to the stirred $N$-Boc-Ser-OtBu solution. After the addition, the reaction mixture was maintained at ambient temperature with stirring for 4 h. Diethyl ether was poured into the reaction mixture, and precipitated triethylamine hydrochloride was filtered off. The filtrate was concentrated under reduced pressure to evaporate benzene and diethyl ether. The residual liquid was dissolved in acetonitrile (100 mL) and mixed with 3 equivalent of 18-crown-6. The aqueous solution (10 mL) of sodium methacrylate (3 equivalent) was added to the reaction mixture. After further stirring at 75°C for 48 h, 18-crown-6 was removed by precipitation in cold acetonitrile. MPS (Boc) (10) was purified by silica gel chromatography with an eluent of MC/MeOH = 2/1. (yield, 0.881 g, 94.7%).
$^1$H-NMR (DMSO, 300 MHz): $\delta = 1.36$ (-NHCOO(CH$_3$)$_3$, -COO(CH$_3$)$_3$, s, 18H), $\delta = 1.89$ (CH$_3$C=CH$_2$CO$_2$-, s, 3H), $\delta = 3.45$ (-NHCH(COO(CH$_3$)$_3$)CH$_2$PO$_3$-, t, 2H), $\delta = 3.92$–3.98 (-CH$_3$CH$_2$=CCO$_2$CH$_2$CH$_2$PO$_3$-, -NHCH(COO(CH$_3$)$_3$)CH$_2$PO$_3$-, m, 5H), $\delta = 5.69$ (CH$_3$C=CH$_2$CO$_2$-, s, 1H), $\delta = 6.06$ (CH$_3$C=CH$_2$CO$_2$-, s, 1H) $\delta = 7.49$–7.51 (-NHCH(COO(CH$_3$)$_3$)CH$_2$PO$_3$-, d, 1H)

3.5 Polymerization (synthesis of 3, 7 and 11)

Polymerization of MPDME (2), MPE (Boc) (6), and MPS (Boc) (10) was performed by atomic transfer radical polymerization (ATRP). Ethyl 2-bromo-2-methylpropionate, Cu(I)Br, and bpy were used as the initiator, catalyst, and ligand, respectively. The initial composition of the initiator and monomers for each polymer is summarized in Table 1. Cu(I)Br and bpy were used 2 equivalent and 4 equivalent comparing to the initiator, respectively. The initiator and monomers were dissolved in degassed methanol under an argon atmosphere. The catalyst and the ligand were added to the stirred solution, and the reaction mixture was further stirred for 48 h under an argon atmosphere. Small amount of reaction mixture was collected during polymerization to measure the conversion rate. Monomer conversion was calculated by comparing vinyl peak of monomer and methyl/methylene peak of polymer in
1H-NMR spectra (Figure 3). After the confirmation of monomer conversion over 99% by 1H-NMR, the reaction solution passed through an aluminum oxide column to remove the residual ATRP catalyst. After the evaporation of the solvent, poly(methacryloylethyl phosphoryldimethylester) (PMPDME) (3), poly(methacryloylethyl phosphoryl N-tBoc-ethanolamine) (PMPE (Boc)) (7), and poly(methacryloylethyl phosphoryl di(tBoc)-serine) (PMPS (Boc)) (11) were obtained. The yields were over 80%. For comparison, PMPA and PMPC were directly synthesized from 2-methacryloyloxyethyl phosphoric acid (MPA) monomer and 2-methacryloyloxyethyl phosphorylcholine (MPC) monomer using a similar procedure.

1H-NMR of PMPDME (3) (D$_2$O, 300 MHz): $\delta$ = 1.24–1.40 (-CH$_2$-C(CO)-CH$_3$- in the chain, m, 3H), $\delta$ = 2.21–2.29 (-CH$_2$-C(CO)-CH$_3$- in the chain, m, 2H), $\delta$ = 4.11–4.14 ((CH$_2$)$_2$PO(OCH$_3$)$_2$, d, 6H), $\delta$ = 4.56 (-COOCH$_2$CH$_2$OP(OCH$_3$)$_2$, s, 2H), $\delta$ = 4.62 (-COOCH$_2$CH$_2$OP(OCH$_3$)$_2$, s, 2H)

1H-NMR of PMPE (Boc) (7) (CD$_3$OD, 300 MHz): $\delta$ = 1.14–1.29 (-CH$_2$-C(CO)-CH$_3$- in the chain, m, 3H), $\delta$ = 1.44 (-C(CH$_3$)$_3$, s, 9H), $\delta$ = 2.35–2.38 (- CH$_2$-C(CO)-CH$_3$- in the chain, m, 2H), $\delta$ = 3.15 (-NHCH$_2$CH$_2$PO$_4$, t, 2H), $\delta$ = 3.53 (-O$_4$PCH$_2$CH$_2$O-, q, 2H), $\delta$ = 3.88–3.90 (-NHCH$_2$CH$_2$PO$_4$CH$_2$CH$_2$O-, m, 4H)

1H-NMR of PMPS (Boc) (11) (DMSO, 300 MHz): $\delta$ = 1.05–1.27 (-CH$_2$-
C(CO)-CH$_3$- in the chain, m, 3H), δ = 1.38 (-NHCOOC(CH$_3$)$_3$, -COO(CH$_3$)$_3$, s, 18H), δ = 2.72–2.75 (-CH$_2$-C(CO)-CH$_3$- in the chain, m, 2H), δ = 3.64–3.71 (-NHCH(COO(CH$_3$)$_3$)CH$_2$OPO$_3$-, m, 2H), δ = 3.75–3.80 (-CH$_3$CH$_2$=CCO$_2$CH$_2$CH$_2$OPO$_3$-, -NHCH(COO(CH$_3$)$_3$)CH$_2$OPO$_3$-, m, 5H), δ = 7.73 (-NHCH(COO(CH$_3$)$_3$)CH$_2$OPO$_3$-, s, 1H)

3.6. Deprotection of the polymers (synthesis of 4, 8 and 12)

PMPDME(3) was deprotected using TMSBr. The reaction ratio between TMSBr and the methyl group in PMPDME was 4. TMSBr was added to the PMPDME solution in chloroform. After stirring for 90 min at ambient temperature, chloroform was removed by evaporation. H$_2$O/THF (v/v = 1/7) was added to the reaction mixture, and the mixture was stirred overnight at ambient temperature.

For the deprotection of PMPE (Boc) (7) and PMPS (Boc) (11), each polymer was dissolved in chloroform. Trifluoroacetic acid (TFA) was added to the solution (final concentration (v/v) of TFA = 30%), and the reaction mixture was stirred overnight at ambient temperature. Each reaction mixture was evaporated to remove the solvent, and the polymer was purified by dialysis. The polymers were dialyzed against 0.01 M EDTA aqueous solution (×2) and distilled water (×2) by using a dialysis membrane (MWCO = 1000; Spectrum Laboratories,
Inc., USA) in order to clearly remove low-molecular weight impurities and the residual ATRP catalyst. The deprotected polymers, poly(2-methacryloyloxyethyl phosphoric acid) (PMPA) (4), poly(2-methacryloyloxyethyl phosphorylethanolamine) (PMPE) (8), and poly(2-methacryloyloxyethyl phosphorylserine) (PMPS) (12), were obtained by lyophilization. The PMPA was obtained as a white hydroscopic solid (73.8%). The PMPE and PMPS were obtained as a pale yellow viscous oil (71.3%, 77.1%)

The $^1$H-NMR spectra of 4, 8, and 12 are shown in Figure 3. The molecular weight and polydispersity (PD) of the polymers are summarized in Table 1.

### 3.7. Gel permeation chromatography (GPC)

The synthesized polymers were characterized by gel permeation chromatography (GPC) with a Superdex GPC column. (GE healthcare, UK). 5mM sodium phosphate buffer with 150 mM sodium chloride was used as the mobile phase at a flow rate of 0.7 mL/min. Each sample was detected with a refractive detector (YL9170; Young Lin Instrument Co., Korea). Poly(ethylene glycol) (PEG) standards (Sigma Aldrich, USA) and Poly(methacrylic acid) (PMAA) standards were used for calculating the molecular weights of the polymers.
3.8. MTT Cytotoxicity assay

The cytotoxicity of the synthesized polymers was estimated by the MTT viability assay. HeLa cells (cervical cancer cells) were seeded on a 96-well plate at $5 \times 10^3$ cells per well in 90 μL of DMEM containing 10% FBS. After a 24-h incubation at 37°C, the culture medium was removed, and the cells were washed with DPBS. The culture medium was replaced with 100 μL of fresh DMEM (10% FBS) containing serial dilutions of the polymers (0, 20, 40, 60, 80, and 100 μg/mL). The cells were subsequently incubated at 37°C for 24 h. For the viability assay, the cells were washed with DPBS, and 20 μL of MTT solution (2 mg/mL) was added to each well. After incubating the cells at 37°C for 2 h, the medium was removed carefully. Blue formazan crystals were dissolved in DMSO (150 μL/well). The absorbance was measured in a microplate reader (Molecular Devices Co., Menlo Park, CA) at a wavelength of 570 nm. The relative cell viability (%) was calculated as a percentage of control cells treated with DMEM only.

3.9. LDH assay

Compatibility on the cell membrane was estimated by lactate dehydrogenase (LDH) assay. HeLa cells (cervical cancer cells) were seeded on a 96-well plate at $5 \times 10^3$ cells per well and incubated overnight in 90 μL of DMEM containing
10% FBS. The cell culture medium was changed with 100 μL of fresh DMEM (10% FBS) containing polymers with various concentrations (0, 20, 40, 60, 80, and 100 μg/mL). After incubation at 37°C for 2 h, each sample media was collected and centrifuged at 180 × g for 10 min. Supernatant (50 μL) of was used for the LDH assay. The LDH activity was determined using a commercial kit according to the manufacturer’s protocol. After incubation at room temperature for 30 min, LDH activity was determined by a microplate reader (Molecular Devices Co., Menlo Park, CA) at a wavelength of 490 nm. 0.1% Triton X-100 was used as a positive control (100% release of LDH).
4. Results and discussion

4.1. Preparation of monomers having phospholipid head groups

Among the various phospholipids in biomembranes, PA has the simplest structure, containing a phosphomonoester group with a negative charge at neutral pH (Figure 1). PMPA, the corresponding polymer based on a methacrylate backbone, was synthesized by polymerizing MPA in previous studies. However, the free phosphomonoester group in the MPA monomer could hamper well-coordinated ATRP to produce polymers with wide molecular weight distributions. Therefore, I intended to protect the phosphomonoester group with a phosphoryl dimethyl ester. I synthesized MPDME monomer (2) through the reaction between HEMA (1) and DCP (Figure 2). The monomer was efficiently polymerized by ATRP using an ethyl 2-bromo-2-methylpropionate initiator. After the polymerization, the phosphoryl dimethyl ester-protecting group was easily removed under mild conditions by using TMSBr.

Other phospholipids such as PC, PE, and PS have a phosphodiester linker with a different head groups (Figure 1). Zwitterionic PC and PE are effectively neutral due to the negatively charged phosphodiester and positively charged...
ammonium groups. The trimethylethylammonium head group in PC is larger than the monoethyammonium head group in PE. PS has a phosphodiester, an ammonium, and a carboxylate in the head group, which has a net negative charge.

Regarding PC-based polymers, PMPC has been studied most actively. Its monomer, MPC, was synthesized efficiently from HEMA in previous studies. After the hydroxyl group of HEMA was reacted with COP, the five-membered cyclic phosphotriester was ring-opened by trimethylamine to produce the MPC monomer. However, the synthetic scheme should be changed in the case of MPE or MPS, because ethanolamine and serine have a primary amine group that can attack the methacrylate via the Michael addition. Therefore, the hydroxyl group of \( N-t\text{Boc-ethanolamine} \) (5) and \( N\text{-Boc-Ser-OrBu} \) (9) was reacted with COP to produce cyclic phosphotriesters before nucleophilic ring opening by sodium methacrylate (Figure 2). All types of hydroxyl-containing compounds can produce the corresponding 2-methacryloyloxyethyl phosphodiester derivatives through this synthetic route. In the ring-opening step by sodium methacrylate, 18-crown-6 ether has been used to enhance the nucleophilicity of the attacking carboxylate anion by chelating the sodium ion. Generally, the activation of the nucleophile is an important factor in ring-opening reactions. Cleavage of the dioxaphospholane ring can readily be accomplished by highly reactive anionic reagents rather than neutral
nucleophiles. Monomer (MPE (Boc) (6) or MPS (Boc) (10)) was purified by silica gel chromatography with a high yield of purified material (>90%).

4.2. Synthesis of polymers using ATRP

Three monomers that mimic PA, PE, and PS were polymerized in methanol by ATRP with CuBr and bpy as the catalyst and the ligand, respectively. As a control, MPC was also polymerized under the same conditions. During the polymerization, monomer conversion was monitored by comparing the peaks of the vinyl protons in the monomer and the methyl and methylene protons in the polymer backbone by $^1$H-NMR spectra (Figure 3). The conversion rate of MPDME monomers was faster than MPE (Boc) and MPS (Boc). About 5 hr was required for 50% conversion of MPDME, comparing to about 15 hr for MPE (Boc) and MPS (Boc). All monomers exhibited 99% conversion within 48 hr.

The resulting polymers were deprotected to produce the final products, PMPA (4), PMPE (8), and PMPS (12). The phosphoryl dimethyl ester of PMPDME (3) was removed by TMSBr, as mentioned previously. The ester hydrolysis was highly efficient, and the yield was over 98%. For MPE (Boc) (6) or MPS (Boc) (10), the Boc protection groups were removed by excess trifluoroacetate (TFA). The copper catalyst, which could be toxic in biological applications, was
removed by dialysis against EDTA solution. The $^{1}$H-NMR spectra of PMPA, PMPE, and PMPS are shown in Figure 4.

The molecular distribution of each polymer was analyzed by gel permeation chromatography (GPC). 5mM sodium phosphate buffer with 150 mM sodium chloride was used as an eluent to reduce the nonspecific adsorption of the polymer on the stationary phase. The GPC chromatograms of PMPA, PMPE, and PMPS are shown in Figure 5. The average molecular weights of the polymers were calculated by comparing their retention times with those of poly(ethylene glycol) (PEG) and poly(methacylic acid) (PMAA) calibration standards. The calculated $M_n$ and $M_w$ are summarized in Table 1. The PMAA-based values were slightly higher than PEG-based values. Considering PMAA has a comb-type backbone structure similar with my polymers but PEG has a linear structure, the PMAA-based calculation could be more reliable. For each polymer, the degree of polymerization was similar to the targeted degree of polymerization. All polymers showed narrow unimodal molecular weight distributions with the polydispersity (PD) value lower than 1.3.

In case of PMPA, I also synthesized from a MPA monomers without phosphoryl dimethyl ester protecting group for a comparison with my new synthetic method. The synthetic scheme and molecular weight distribution was summarized in Figure 6 and Table 2. PMPA based on the MPA monomer
(PMPA (MPA) showed wider molecular distribution than PMPA based on the MPDME monomer (PMPA (MPDME)). Especially, PMPA (MPA; DP = 51) exhibited a PD value of 1.43, significantly higher than PMPA (MPDME; DP = 48) (PD = 1.27). Therefore, ATRP from the protected MPA monomers were preferred to produce a more well-defined polymer. Considering that PD values of approximately 1.3 were obtained in the ATRP of PC-based homopolymers in previous studies,\textsuperscript{26} and the PD values of my biomembrane-mimic polymers were acceptable.

4.3. \textit{In vitro} cytotoxicity of polymers

The in vitro cytotoxicity of PMPA, PMPE, and PMPS was examined using the MTT assay to assess their suitability for future biomedical applications. The relative viability of HeLa cells treated with PMPA (DP = 48), PMPE (DP = 49), or PMPS (DP = 49) was compared (\textbf{Figure 7 (a)}) with PMPC (DP = 51)-treated cells. In addition, the viability of other cell types (HepG2 and HEK293) was also compared in \textbf{Figure 7 (b) and (c)}. PEG (10 kDa) was used as a negative control, and branched polyethylenimine (b-PEI) (25 kDa), a well-known polymer with high amine density for gene delivery,\textsuperscript{27} was used as a positive control. Because both non-cytotoxicity of PEG as well as the cytotoxicity of b-PEI is well-known, I could compare the cytotoxicity of my
polymers with pre-existing polymers. None of the biomembrane-mimic polymers showed significant cytotoxicity at a concentration of 100 μg/mL, whereas PEI (25 kDa) showed less than 30% viability at a concentration of 20 μg/mL. Similarly, biomembrane-mimic polymers with low degrees of polymerization (PMPA (DP = 26), PMPE (DP = 28), and PMPS (DP = 28)) also exhibited almost no cytotoxicity (Figure 7).

Furthermore, potential damages on cell membrane were also examined using the LDH assay. When the plasma membrane is damaged, LDH, a cytosolic enzyme is released into cell exterior through the damaged membrane.\textsuperscript{28} Figure 8 showed the LDH release from polymer-treated HeLa cells. PMPC (DP = 51) and \textit{b}-PEI (25 kDa) were used as controls. Comparing to cells damaged by Triton-X 100 detergent, \textit{b}-PEI has approximately 40% LDH enzyme activity. The amine-based surface of \textit{b}-PEI significantly destabilized the cell membrane. On the other hand, PMPA, PMPE and PMPS exhibited almost no LDH activity. Therefore, my biomembrane-mimic polymers did not destabilize the cell membrane, even though PMPE and PMPS have many primary amine residues. Similarly, polymers with low degrees of polymerization (PMPA (DP = 26), PMPE (DP = 28), and PMPS (DP = 28)) also exhibited almost no LDH enzyme activity (Figure S8).
Based on the cytotoxicity and membrane compatibility data, biomembrane-mimic polymers based on phospholipid head groups, similar to PMPC, the prototypical PC-based mimic, can be useful as biomedical materials.
5. Conclusions

I successfully developed a new synthetic method to prepare biomembrane-mimic polymers with various phospholipid head groups including PA, PE, and PS from simple precursors. PMPA, PMPE, and PMPS with narrow molecular weight distributions were synthesized by ATRP. Highly biocompatible biomembrane-mimic polymers have great potential to ensure biocompatibility for the surface of biomedical implants or devices. Furthermore, given that the phospholipid compositions of biomembranes vary, PMPA, PMPE, PMPS, and PMPC can be used to form a polymeric library for the preparation of a cell- or tissue-specific surface with biocompatibility and biofunctionality.
6. References


Figure 1. Biomembrane-mimic polymers with various phospholipid head groups
Figure 2. Synthetic scheme of biomembrane-mimic polymers
Figure 3. Monomer conversion of MPDME (2) (♦), MPE (Boc) (7) (▲), and MPS (Boc) (■) in the synthesis of PMPA (DP = 48), PMPE (DP = 49), and PMPS (DP = 49).
Figure 4. $^1$H-NMR spectra of biomembrane-mimic polymers (D$_2$O, 400MHz)
Figure 5. GPC profiles of biomembrane-mimic polymers
Figure 6. GPC profile of the PMPA polymers which were synthesized from MPA monomers without phosphoryl dimethyl ester protecting group
(a) HeLa

![Graph showing cell viability (%) against concentration of polymer (μg/mL) for different polymers including PEI 25K, PEG 10K, PMPA (DP = 26), PMPC (DP = 29), PMPE (DP = 28), and PMPS (DP = 28).](image)

![Graph showing cell viability (%) against concentration of polymer (μg/mL) for different polymers including PEI 25K, PEG 10K, PMPA (DP = 48), PMPC (DP = 51), PMPE (DP = 49), and PMPS (DP = 49).](image)
(b) HepG2

![Graphs showing cell viability for different concentrations of polymer](image)

- PEI 25K
- PEG 10K
- PMPA (DP = 26)
- PMPC (DP = 29)
- PMPE (DP = 28)
- PMPS (DP = 28)

![Graphs showing cell viability for different concentrations of polymer](image)

- PEI 25K
- PEG 10K
- PMPA (DP = 48)
- PMPC (DP = 51)
- PMPE (DP = 49)
- PMPS (DP = 49)
Figure 7. MTT assay of biomembrane-mimic polymers on (a) HeLa, (b) HepG2, and (c) HEK293 cells. Error bar means standard deviation (n = 3).
**Figure 8.** LDH assay of biomembrane-mimic polymers. Error bar means standard deviation (n = 3)
**Table 1. Synthesized biomembrane-mimic polymers**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Target</th>
<th>Synthesized&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>$M_n$</td>
<td>$DP_n$</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>PMPA</td>
<td>5.25 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>25</td>
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<tr>
<td>(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.05 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>50</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>PMPE</td>
<td>6.30 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>25</td>
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<tr>
<td>(8)</td>
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<td>1.26 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>50</td>
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<td></td>
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<tr>
<td>PMPS</td>
<td>7.40 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>25</td>
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<tr>
<td>(12)</td>
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<td></td>
<td>1.48 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>50</td>
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<sup>a</sup>Molecular weights were determined by GPC using PMMA standards. Molecular weights calibrated with PEG standards are shown in parentheses.
Table 2. The molecular weight and polydispersity (PD) of the PMPA polymers which were synthesized from MPA monomers without phosphoryl dimethyl ester protecting group

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Target $M_n$</th>
<th>$DP_n$</th>
<th>Synthesized $^a$ $M_n$</th>
<th>$M_w$</th>
<th>PD</th>
<th>DP$_n$</th>
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<tbody>
<tr>
<td>PMPA</td>
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<td>25</td>
<td>5.89 x $10^3$</td>
<td>7.83 x $10^3$</td>
<td>1.33</td>
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<td>(5.13 x $10^3$)</td>
<td>(6.62 x $10^3$)</td>
<td>(1.29)</td>
<td>(24)</td>
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<td>$1.05 \times 10^4$</td>
<td>50</td>
<td>1.07 x $10^4$</td>
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<td>1.45</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.01 x $10^4$)</td>
<td>(1.44 x $10^4$)</td>
<td>(1.43)</td>
<td>(48)</td>
</tr>
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</table>

$^a$Molecular weights were determined by GPC using PMMA standards.
Molecular weights calibrated with PEG standards are shown in parentheses.
PART II. Ring Opening Metathesis Polymerization of Bicyclic \( \alpha,\beta \)-Unsaturated Anhydrides for Ready-to-be-grafted Polymers Having Tailored pH-Responsive Degradability

1. Abstract

Polymers having various \( cis-\alpha,\beta \)-unsaturated anhydride moieties as repeating units were synthesized via ring opening metathesis polymerization (ROMP). The \( cis-\alpha,\beta \)-unsaturated anhydride moieties were ‘ready-to-be-grafted’ with amine-containing molecules in high density to form acid-labile \( cis-\alpha,\beta \)-unsaturated acid amide linkages in side chain. The pH-responsive degradability of the grafted side chains can be controlled by changing the accessibility between acid and amide carbonyl groups in the structure. The ROMP polymers having \( \alpha,\beta \)-unsaturated anhydrides are expected to be applied as pH-responsive platform materials in biomedical applications, which is supported by the \textit{in vitro} result that alendronate-grafted ROMP polymers showed distinct pH-dependent cytotoxicity on a human breast cancer cell line according to the anhydride structures even in biologically tolerable pH conditions.
2. Introduction

pH-responsive polymer is one of the key components in smart drug delivery systems because many important target sites of drug delivery exhibit unique pH environments, which are different from neutrality in most physiological fluids.\(^1\) Selective and controllable degradation of polymers is required for the spatiotemporally specific release of cargo drugs in the gastrointestinal tract,\(^2\) on skin,\(^3\) in cancerous or inflammatory tissues,\(^4\) or in intracellular organelles such as endosome or lysosome.\(^5\) Many pH-responsive degradable bonds, including imine,\(^6\) hydrazone,\(^7\) acetal,\(^8\) orthoester,\(^9\) are introduced to polymeric backbones or side chains to obtain suitable pH-responsiveness for each application. However, pH-responsive degradability of such bonds is generally restricted to a narrow range and difficult to be tailored intentionally and delicately.\(^10\) Moreover, drug conjugation to polymers often requires complex reactions, including protection and deprotection, due to vulnerability of degradable bonds in many conjugation conditions.\(^11\) In addition, it is preferable to avoid the conjugation in aqueous conditions for minimizing hydrolysis of the bonds.\(^12\)

Among pH-responsive bonds, β-carboxylic acid amide having a \(cis-\alpha,\beta\)-double bond has a unique degradation property. Unlike other amide bonds that can only be hydrolyzed at pH conditions below 1 or above 13, β-carboxylic
acid amide having a cis-α,β-double bond, i.e., maleic acid amide derivative, can be hydrolyzed at mild acidic conditions around pH 3-7. Furthermore, because the intramolecular attack of β-carboxylic acid to amide carbonyl group is the main factor for prompting the hydrolysis of amide bond at mild acidic conditions, the kinetics and responding pH condition of the degradation can be tuned by controlling substituents on the cis-α,β-double bond.\textsuperscript{13} Additionally, maleic acid amide derivatives can be easily synthesized via a one-step reaction between corresponding anhydrides and amines even in aqueous conditions. Due to such characteristics, maleic acid amide derivatives have been used as one of the key functionalities in smart biomaterials, while their degradation can be induced by mild pH change.\textsuperscript{14}

Despite their attractive characteristics, the strategy for the introduction of maleic acid amide derivative functionalities into polymers is quite limited. In all previous reports, maleic acid amide derivatives and their corresponding anhydrides have been introduced to pre-formed polymeric backbones.\textsuperscript{15} There are few reports on polymerization of monomers containing maleic acid amide/anhydride moieties, probably due to reactivity of the double bond or the anhydride of possible monomers in conditions of traditional radical, cationic, anionic, or other step-growth polymerization.\textsuperscript{16}

In this study, I intended to polymerize monomers possessing maleic acid anhydride-mimic moieties having both cis-double bond and anhydride groups
As a polymerization method which is free from reactivity issues that are mentioned above, I selected ring-opening metathesis polymerization (ROMP) using Grubbs’ catalysts. The Grubbs’ catalyst can polymerize electron-rich olefin monomers but not react with electron-deficient α,β-olefins or anhydrides. Moreover, the possible ROMP monomers having the structure of maleic acid anhydride derivative can be readily synthesized via the Diels-Alder reaction. The expected polymers may possess the amine-reactive maleic acid anhydride derivatives in every repeating unit and be well ‘ready-to-be-grafted’ with amine-possessing molecules in high density. The densely grafted polymers may release the grafted molecules in pH-dependent manners. Because the pH-responsive degradability is determined by the accessibility of the β-carboxylate to the amide carbonyl group in maleic acid amide derivative, I can tune the pH-responsive release kinetics of the grafted molecules by controlling the angle between cis-substituents. After confirmation of my main concept for the synthesis of the ready-to-be-grafted ROMP polymers having tailored pH-responsive degradability, I checked the potential of the polymers for pH-responsive delivery of a model drug, alendronate, in vitro.
3. Experimental Section

3.1 Materials and characterization

Dicyclopentadiene, furan, propylamine, ferric chloride, acetic anhydride, triethylamine (TEA), the second-generation Grubbs’ catalyst, methanol, chloroform, anhydrous tetrahydrofuran (THF), fluorescamine and alendronate sodium were purchased from Sigma-Aldrich (USA). Acetylene dicarboxylic acid was obtained from TCI (Japan). Magnesium sulfate (MgSO₄), sodium hydroxide (NaOH), acetone and acetic acid were purchased from Daejung (Korea). Dulbecco’s modified Eagle’s medium (DMEM), Dulbecco’s phosphate buffered saline (DPBS), and fetal bovine serum (FBS) were purchased from WelGENE (USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (USA). The solvents were handled in a glovebox under argon gas condition. All reagents were used without further purification.

Thin-layer chromatography (TLC) was carried out on MERCK TLC silica gel plates. Dialysis membrane (MWCO = 1000) was purchased from Spectrum Laboratories, Inc. (USA). ¹H-NMR was recorded by a Bruker (300 MHz) spectrometer. Spectrofluorometer (Jasco, FP-8300 (Japan)) was used to quantify the amount of amines released from polymers.
3.2. Synthesis of bicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic anhydride (1)

1,3-Cyclopentadiene was obtained through distillation of dicyclopentadiene at 180°C. 1,3-Cyclopentadiene (1.50 mL, 17.8 mmol) was reacted with acetylene dicarboxylic acid (1.0 g, 8.8 mmol) in 20 mL of THF under vigorous stirring at room temperature overnight. The solvent was removed by evaporation to produce an oily residue. The residue was dissolved in acetic anhydride (8.3 mL, 88 mmol) and the solution was heated at 60°C for 2 h. After evaporation of volatile substances, the remaining mixture was dissolved in chloroform and washed with distilled water (×5). The organic layer was dried with MgSO₄ and concentrated to yield product 1 as white power (Yield: 87%).

¹H-NMR (CDCl₃, 300MHz) δ (ppm): 6.95 (m, 2H), 4.04 (m, 2H), 2.35 (m, 1H), 2.19 (m, 1H), ¹³C-NMR (CDCl₃, 75MHz) δ (ppm): 168.74, 142.31, 77.99, 56.51, LC-MS calculated for 162.03, observed 162.40

3.3. Synthesis of 7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic acid anhydride (2)

Furan (2.00 mL, 27.2 mmol) was reacted with acetylene dicarboxylic acid
(1.0 g, 8.8 mmol) in 20 mL of diethyl ether under vigorous stirring at room temperature for 5 days. Crystallized white solid was filtrated and washed using diethyl ether. The solid was dissolved in THF and excess acetic anhydride was added to the solution. After overnight stirring at 60°C, volatile substances were removed by evaporation. After crystallization in ethyl acetate, the crystal was heated at 150°C for 3 min. The product 2 was obtained as pale yellow oil (Yield: 62%).

$^1$H-NMR (THF-$d$, 300 MHz) δ (ppm): 6.70 (s, 2H), 5.15 (s, 2H), $^{13}$C-NMR (THF-$d$, 75MHz) δ (ppm): 168.13, 142.85, 138.20, 87.94, LC-MS calculated for 164.01, observed 164.32

### 3.4. General procedure for polymerization (3 and 4)

ROMP was performed using the second-generation Grubbs’ catalyst. The initial composition of the initiator and monomers for each polymer is summarized in Table 1. The monomer was dissolved in anhydrous THF under argon atmosphere. Subsequently, the catalyst was added to the stirred solution. After confirmation of complete consumption of the monomer by TLC, the reaction was quenched by adding excess ethyl vinyl ether. The reaction mixture was concentrated by evaporation. Each polymer was precipitated by adding the concentrated solution into methanol or diethyl ether. The precipitated polymer
(3 or 4) was obtained as a gray solid after drying under vacuum.

\[ ^1 \text{H-NMR (THF-}d, 300 \text{ MHz)} \delta (\text{ppm}) \]

- 3 (DP=42): 7.19-7.35 (m, 5H), 6.91 (m, 1H), 6.37-6.43 (m, 28H), 5.30-6.17 (br, 56H), 5.14-5.25 (m, 2H), 4.99-5.06 (m, 2H), 3.90-4.23 (br, 33H), 2.94-3.58 (br, 51H), 1.86-2.07 (m, 41H), 1.53-1.84 (m, 42H)

- 4 (DP=48): 7.31-7.41 (m, 5H), 6.95 (m, 1H), 6.39-6.52 (m, 40H), 5.34-6.23 (br, 90H), 4.79-5.24 (m, 62H), 4.63-4.77 (m, 1H), 4.32-4.58 (m, 2H)

3.5. Cleavage of the cyclic ether (5)

Polymer 4 (30 mg) was reacted with ferric chloride (5 mg) in acetic anhydride (2 mL) at room temperature for 3 h. The reaction mixture was dialyzed against MeOH (×3) and distilled water (×2) using a dialysis membrane. The product 5 was obtained as a white solid after freeze-drying.

\[ ^1 \text{H-NMR (THF-}d, 300 \text{ MHz)} \delta (\text{ppm}): 7.17-7.41 (m, 5H), 6.94 (m, 1H), 6.18-6.72 (br, 64H), 5.61-6.14 (br, 72H), 5.25-5.59 (br, 26H), 5.15-5.23 (m, 2H), 4.97-5.12 (m, 2H), 1.88-2.19 (m, 188H) \]
3.6. Gel permeation chromatography (GPC)

The synthesized polymers were characterized using gel permeation chromatography (GPC) with a Shodex GPC column (KF-803, Japan). THF was used as the mobile phase at a flow rate of 0.7 mL/min. Each sample was detected with a refractive detector (YL9170; Young Lin Instrument Co., Korea). Polystyrene (PS) standards (Agilent Technologies, USA) were used for calculating the molecular weights of the polymers.

3.7. General procedure of polymer grafting

Each polymer was dissolved in a THF/methanol solvent and added with excess amine compounds. After stirring at room temperature under argon atmosphere overnight, the reaction mixture was evaporated. The grafted polymer was purified by dialysis against MeOH (×2), sodium bicarbonate solution (×2) and distilled water (×3). The grafted polymer was obtained as a white solid after freeze-drying.

$^1$H-NMR (THF-$_d$, 300 MHz) δ (ppm)

- 6: 8.52-8.59 (m, 20H), 7.24 -7.38 (m, 5H), 6.97 (m, 1H), 6.23-6.77 (m, 28H), 5.21-6.19 (br, 56H), 5.16-5.23 (m, 2H), 4.93-5.17 (m, 2H), 3.91-4.25 (br, 33H), 3.36-3.57 (br, 57H), 3.14-3.38 (br, 66H), 1.81-2.19 (m, 41H), 1.60-1.82 (m,
42H), 1.33-1.42 (m, 66H), 0.58-0.76 (m, 99H)

- 7: 8.55-8.59 (m, 20H), 7.21-7.58 (m, 5H), 6.98 (m, 1H), 6.38-6.80 (m, 40H), 5.39-6.21 (br, 90H), 4.80-5.25 (m, 62H), 4.69-4.80 (m, 1H), 4.42-4.65 (m, 2H), 3.22-3.49 (m, 76H), 1.19-1.42(m, 74H), 0.00-0.43 (m, 112H)

- 8: 7.19-7.49 (m, 5H), 6.92 (m, 1H), 6.15-6.75 (br, 65H), 5.51-6.17 (br, 72H), 5.22-5.52 (br, 26H), 5.13-5.203 (m, 2H), 4.91-5.09 (m, 2H), 3.18-3.52 (m, 86H), 1.83-2.21(m, 187H), 1.16-1.49 (m, 85H), 0.00-0.39 (m, 127H)

3.8. Density functional theory (DFT) calculation

The density functional theory (DFT) calculations at unrestricted B3LYP/6-31G(d,p) level was carried out using the quantum chemical package Gaussian 09W. The geometry of the monomeric units of polymers was fully optimized in the gas phase and the empirical-dispersion correction was used during optimization.

3.9. Measurement of pH-dependent degradation

The pH-dependent degradation of each polymer was quantified by measuring fluorescence of a fluorescamine derivative produced from the released amine and alendronate. Each polymer was dissolved in a phosphate buffer (100 mM;
pH 3.0, 5.5, 6.5 and 7.4) at a concentration corresponding to 25 μM of the conjugated amine. The solution was placed in a dialysis bag that was immersed in the beaker containing a buffer solution (150 mL; pH 3.0, 5.5, 6.5 or 7.4). The dialysis was performed at room temperature. Aliquots (20 μL) of the buffer outside the dialysis membrane were collected for the fluorescence measurement at different time points. The aliquot was mixed with 20 μL of a fluorescamine solution in acetone (200 μM). After 5 min, the fluorescence of the mixture was measured by a spectrofluorometer at the excitation wavelength of 390 nm and the emission wavelength of 475 nm.

3.10. In vitro bioactivity of alendronate-grafted polymers

Alendronate was grafted to each polymer via the general grafting procedure as mentioned above. The bioactivity of the alendronate-grafted polymer was estimated by the measurement of relative viability of MDA-MB-231 (human breast cancer) cells. The cells were plated in a 96-well plate at $1 \times 10^4$ cells per well in 90 μL of DMEM containing 10% FBS and incubated at 37°C for 24 h. The culture medium was removed and the cells were washed using DPBS. The culture medium was replaced with 100 μL of DMEM (10% FBS) containing each polymer sample, while the pH was adjusted by adding HCl. The cells were subsequently incubated at 37°C. In the experiment in Figure 27, the cells
were incubated with polymer samples for 24 h, but in the experiment in Figure 29, I measured the viability at various time points. For the viability assay, the cells were washed using DPBS, and a 10% CCK solution in DMEM was added to each well. After incubation at 37°C for 1 h, the medium was transferred to a new plate, and the absorbance was measured by a microplate reader (Molecular Devices Co., Menlo Park, CA) at a wavelength of 450 nm. The relative cell viability (%) was calculated as a percentage of absorbance compared with that of the control cells treated by DMEM only at each adjusted pH without polymer samples.
4. Results and discussion

4.1. Design of bicyclic monomers with $\alpha,\beta$-unsaturated anhydride

ROMP uses the ring strain enthalpy of cyclic olefins to compensate entropic penalties during polymerization. The most common ROMP monomers are based on the structures of four- or five-membered ring and norbornene-like bicycles having considerable strain enthalpy (> 5 kcal/mol). In order to produce a maleic anhydride-like moiety having a $\alpha,\beta$-cis-double bond after the ring opening metathesis, there is a need to prepare a cyclic monomer possessing two double bonds. One of them is for the metathesis polymerization, while the other is for the $\alpha,\beta$-cis-double bond. I selected a norbordiene-based structure having a 2,3-dicarboxylic acid anhydride (monomer 1) because it can be readily synthesized from corresponding cyclodiene and alkyne diacid by Diels-Alder reaction (Figure 2a). Nevertheless, it is rather difficult to synthesize possible cyclobutadiene or cyclopentadiene-based structures due to instability or asymmetry. After the ring opening of 1, a polymer having cyclopentene 1,2-dicarboxylic anhydride moieties (3) can be produced (Figure 2b). The anhydride in the polymer 3 can react with amine-possessing molecules, and the resulted grafted polymer 6 can release the grafted amine.
molecules at acidic pH due to the acid-labile β-carboxylic acid amide moieties (Figure 1).

The pH-dependent degradability of maleic acid amide derivatives is highly dependent on the substituents on the $\alpha,\beta$-cis double bond. The substituents determine the accessibility between the acid and amide groups and the effective concentration of each group in the intramolecular cyclization reaction. The pH-responsive degradability and kinetics are also varied by the change of the accessibility between two groups. It was reported that cyclopentene 1,2-acid amide was not readily degraded at mild acidic conditions. On the other hand, a ring-free maleic acid amide having two alkyl substituents such as dimethylmaleic acid amide showed fast degradation at mild acidic conditions, because the vicinal dialkyl groups can accelerate the cyclization reaction between the acid and amide (Thorpe-Ingold effect) without limitation of freedom in the cyclopentene ring structure. If five-membered ring-free polymers can be obtained by the ring cleavage, the resulted acid amide would be much more labile at mildly acidic pH conditions.

Starting from furan instead of cyclopentadiene, the same Diels-Alder reaction procedure can produce monomer 2 with an oxonorbordiene structure (Figure 2a). The dihydrofuran ring in the resulting ROMP polymer 4 could be cleaved by a Lewis acid, such as ferric chloride, because the cyclic ether is a de facto allyl ether (Figure 2c). The ring-cleaved polymer 5 has a repeating unit
similar to dialkyl maleic anhydride. I expect that the amine-grafted polymer 8 can release the side chain at milder pH conditions compared with polymer 6 and 7 that have restricted five-membered ring structures.

4.2. Synthesis of monomers and polymers

Distilled 1,3-cyclopentadiene was reacted with acetylene dicarboxylic acid to form bicyclo[2.2.1]hepta-2,5-dieine-2,3-dicarboxylic acid (Figure 2a). The diacid compound can be easily converted into the anhydride form (1) by anhydride exchange reaction with acetic anhydride (Figure 7). Although bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride as the corresponding mono-ene compound is frequently used in ROMP,22 the diene compound (1) is rarely tried as a ROMP monomer as far as we know. Monomer 1 was polymerized with the second-generation Grubbs’ catalyst at monomer/initiator ratios ([M]/[I]) of 15, 25 and 50 (Figure 2b). After complete consumption of 1 which was checked with TLC, the polymerization was quenched by adding excess ethyl vinyl ether. The $^1$H-NMR spectra of polymer 3 are shown in Figure 8. For the peak characterization, I also synthesized the ring-opened one-mer by treatment of excess catalyst (Figure 3). On the basis of the NMR spectra of the one-mer (12) (Figure 4a), I confirmed that polymer 3 was successfully synthesized. The degrees of polymerization ($DP$s) of two shorter
polymer ($DP = 16$, and 24) were similar to the targeted values ($DP = 15$, and 25), but that of the long polymer ($DP = 42$) was slightly lower than the targeted value ($DP = 50$) (Table 1). Relatively poor solubility of polymer 3 in the reaction solvent might be one reason causing the low DP. The E/Z ratio of polymer 3 was around 0.5, which is similar to those of ROMP polymers from mono-ene compounds in a previous report.23 The Ru-based metathesis catalysts generally showed such Z-selectivity in a wide variety of cross-metathesis reactions,24 although the mechanism has not been fully understood. GPC analysis showed unimodal molecular weight distributions of polymer 3 samples (Figure 10). The $M_n$ values estimated by both NMR and GPC are similar to each other (Table 1). The polydispersity ($PD$) values of the polymer samples with $DP$s of 16, 24, and 42 were 1.34, 1.39 and 1.27, respectively.

For the synthesis of the oxonorborbodiene monomer (2), furan was reacted with acetylene dicarboxylic acid (Figure 2a). However, unlike the case of 1,3-cyclopentadiene, two furan molecules reacted with acetylene dicarboxylic acid molecule to produce the difuran adduct. After anhydride formation, additional furan was removed by flash vacuum pyrolysis.25 Fragmentation through retro Diels-Alder reaction occurred at high temperature to produce 7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic acid anhydride (2) (Figure 11). The monomer 2 was polymerized with the second-generation Grubbs’ catalyst at [$M$]/[I] of 15, 25 and 50 (Figure 2b). The NMR spectra of polymer
4 (Figure 12) and the corresponding one-mer (13) (Figure 5) were compared to support the successful polymerization. The estimated $DP$ values are similar to the targeted values even in the case of the long polymer, probably owing to the better solubility of polymer 4 than that of polymer 3 in the reaction solvent. GPC analysis also showed unimodal molecular weight distribution of polymer 4 having slightly lower PD values than those of polymer 3 (Figure 14 and Table 1).

I intended to cleave the cyclic ether bond in the polymer 4 for delicate control of pH-responsiveness. Sodium borohydride, palladium-based reagents, and Lewis acids are generally used for allyl ether cleavage. I treated the one-mer (13) with FeCl$_3$ in acetic anhydride as one of the mildest reagents for checking the cleavage activity (Figure 3).$^{21}$ As shown in Figure 6, the ether bond of 13 was successfully cleaved to produce 14, a ring-cleaved product having two acetyl esters. Polymer 4 was then exposed to the same reagent (Figure 2). 65% of the allyl ether bonds in polymer 4 were cleaved after 3-h reaction at room temperature. No more cleavage was observed even after further incubation (Figure 15). The GPC analysis showed that the molecular weight distributions of ring-cleaved polymer 5 samples were similar to those of polymer 4 samples having slightly molecular weight. The result supported that the ROMP polymeric backbone was not degraded in the condition of the allyl ether cleavage (Figure 17 and Table 1).
4.3. Polymer grafting and pH-responsive release of grafted molecules

Polymer 3, 4, and 5 contain α,β-unsaturated anhydride moieties that are able to react with primary or secondary amine molecules in basic conditions. Each polymer was stirred at room temperature in the presence of \( n \)-propylamine as a model compound to produce the grafted polymer (Figure 2d). The formation of β-carboxylic acid amide could be confirmed by the \(^1\text{H}-\text{NMR}\) spectra of 6, 7, and 8 which show characteristic amide and acid peaks at 8.6 and 9.8 ppm, respectively (Figure 18). 79\% (6), 78\% (7) and 88\% (8) of anhydride moieties were reacted to form the acid amide linkages by only simple mixing in basic conditions. The polymeric backbone was maintained during the \( n \)-propylamine grafting as shown in the GPC chromatograms (Figure 19 and Table 2)

The pH-responsive degradation of the β-carboxylic acid amide linkages were quantified using fluorescamine, which shows strong fluorescence only after the reaction with a free primary amine.\(^{26}\) The release kinetics of \( n \)-propylamine from polymer 6, 7, and 8 at different pH conditions are shown in Figure 20a, 20b, and 20c respectively. All three polymers showed negligible degradation at pH 7.4, indicating the stability of the acid amide linkages. On the other hand, incubation at pH 3.0 clearly accelerated the hydrolysis of the linkages in all polymers. At pH 3.0, polymer 6 and 7 showed approximately 60\% degradation after 8 h, whereas polymer 8 reached the point of 60\% degradation within 4 h.
The differences were more dramatic at pH 5.5 and pH 6.5. Both polymer 6 and 7 degraded only slowly and hardly reached the point of 10-20% degradation even after 72 h at the mildly acidic conditions. Polymer 8, however, degraded much faster to show more than 30-40% within 8 h. The degradation rate of polymers were not so much changed in the pH range of 5.5-6.5.

As I predicted previously, the five-membered ring cleavage can clearly facilitate the degradation of \textit{cis}-\textit{\(\alpha,\beta\)}-unsaturated acid amide linkages between the grafted side chain and the backbone of the ROMP polymer. The bond angles in C=C-C(ONH) and (HOO)C-C=C were estimated to be around 132-134° and 131-134° for the five-membered ring monomeric unit in polymer 6 and 7, which were 119° and 123° for the ring-cleaved monomeric unit in polymer 8 by density functional theory (DFT) calculation (\textbf{Figure 21}). The significant decrease of both angles between the amide and the carboxylic acid may cause the elevation of effective concentrations of two reactive groups for the intramolecular cyclization for the pH-responsive degradation of the acid amide linkage. The intramolecular cyclization-based degradation of the acid amide linkage was further confirmed by the regenerated structure of polymer 3, 4, and 5 after release of \textit{n}-propylamine from polymer 6, 7, and 8 (\textbf{Figure 22}). The cyclization-induced degradation was probably one reason for the result that the degradation kinetics was not so much dependent upon \textit{DPs} of the polymers, as shown in \textbf{Figure 23}. I can tune the pH-responsive degradability of grafted
side chains by controlling the angles in the monomeric units in ROMP polymers.

4.4. Bioactivity of alendronate-grafted polymers

In order to show the possible applicability of the ready-to-be-grafted ROMP polymers having pH-responsive degradability, I checked cytotoxicity of alendronate released from the polymers in pH-dependent manners. Alendronate is a bisphosphonate drug possessing a primary amine group (Figure 2d). It is well-known for the inhibitory effect on osteoclasts. Alendronate also exerts direct effects on cancer cells due to induction of apoptosis, inhibition of angiogenesis and adhesion, stimulation of lymphocytes, and inhibition of matrix metalloproteinase. Because both osteoporotic and cancerous tissues are characterized as acidic environments compared with other tissues, I thought that pH-responsive release of alendronate in the target tissues might be beneficial to reduce possible serious side effects of alendronate in other tissues (e.g. abdominal pain, arthralgia, and osteonecrosis of the jaw).

Following the same procedure for the n-propylamine grafting, simple mixing of alendronate and ROMP polymers (6, 7, and 8) in basic conditions produced the alendronate-grafted polymers (9, 10, and 11). From the $^1$H-NMR spectra of 9, 10, and 11 (Figure 24), I calculated the grafting degrees of alendronate, i.e.,
81% (9), 83% (10) and 83% (11), respectively. The polymeric backbone was also maintained during the alendronate grafting, as shown in the GPC chromatograms (Figure 25).

The pH-dependent bioactivities of the alendronate-grafted polymers were compared by measurement of cytotoxicity on MDA-MB-231 cells (human breast cancer cells). After incubation for 24 h, free alendronate exhibits similar half maximal inhibitory concentration (IC$_{50}$) values around 15 μM irrespective of pH conditions. However, the cytotoxicity of the alendronate-grafted polymers was highly dependent upon the incubation conditions. At pH 7.4, all three polymers exhibited negligible toxicity up to 50 μM on the cancer cells (Figure 27c). However, at pH 6.5, polymer 11 showed only 40% viability at 25 μM, whereas polymer 9 and 10 still showed almost no toxicity in the experimental concentration range (Figure 27b). On the other hand, at pH 5.8, all three polymers showed evident toxicity. Among them, polymer 11 showed the highest toxicity similar to free alendronate (Figure 27a). The comparison of IC$_{50}$ values clearly showed the induction of toxicity at acidic pH on the cells treated with the alendronate-grafted polymers (Figure 27d). Polymer 11 has faster degradability at mildly acidic pH conditions than polymer 9 and 10 (Figure 26), while non-grafted polymer 3, 4, and 5 showed no toxicity on the cells in the concentration range of the treatment (Figure 28a). In addition, polymer 11 gave the toxic effect on the cells rather slowly than free alendronate
Therefore, I inferred that the cytotoxicity of the alendronate-grafted polymers was originated from alendronate released from the polymers in pH-dependent manners. Although further investigation, including pharmacokinetics, biodistribution, and *in vivo* efficacy, should be required for biomedical applications, it was clearly shown that pH-responsive release and efficacy of amine-containing drugs can be delicately controlled by the structure of the *cis*-α,β-unsaturated acid amides in the ROMP polymers.
5. Conclusions

In summary, ROMP polymers having α,β-unsaturated anhydrides were prepared for both efficient grafting with amine-containing side chain molecules and pH-responsive release of the side chains. The cis-α,β-unsaturated anhydride moieties were ready-to-be-grafted with amine-containing molecules to form acid-labile β-carboxylic acid amide linkages in high density. The ring structure in the ROMP polymer can be cleaved for modulation of pH-responsive degradation of the acid amide linkages. The grafted ROMP polymers with different accessibility between acid and amide groups showed clearly distinct kinetics of pH-responsive degradation, especially in mildly acidic conditions at pH 5-6. As shown in a proof-of-concept experiment using alendronate-grafted ROMP polymers, the ROMP polymers having α,β-unsaturated anhydrides can provide a useful platform for smart functional materials in the biomedical field where tunable pH-responsiveness is needed in biologically tolerable conditions.
6. References


[22]  a) C. M. Dettmer, M. K. Gray, J. M. Torkelson, S. T. Nguyen,


[29] a) P. Fournier, S. Boissier, S. Filleur, J. Guglielmi, F. Cabon, M.


**Figure 1.** Schematic diagram of synthesis, grafting, and pH-responsive release of ROMP polymers having α,β-unsaturated anhydride.
Figure 2. Synthesis of monomers and polymers with α,β-unsaturated anhydrides with different ring structures and grafting of the polymers.
Figure 3. Synthetic scheme of the one-mers (12, 13, and 14)
Figure 4a. $^1$H-NMR spectra of 12

Figure 4b. $^{13}$C-NMR spectra of 12.
Figure 4c. Mass spectra of 12.
Figure 5a. $^1$H-NMR spectra of 13.

Figure 5b. $^{13}$C-NMR spectra of 13.
Figure 5c. Mass spectra of 13.
Figure 6a. $^1$H-NMR spectra of 14.

Figure 6b. $^{13}$C-NMR spectra of 14.
Figure 6c. Mass spectra of 14.
Figure 7a. $^1$H-NMR spectra of bicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic anhydride (1)

Figure 7b. $^{13}$C-NMR spectra of bicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic anhydride (1)
Figure 8a. $^1$H-NMR spectra of polymer 3 (DP =16)

Figure 8b. $^1$H-NMR spectra of polymer 3 (DP =24)
Figure 8c. $^1$H-NMR spectra of polymer 3 (DP = 42)
Figure 9. $^{13}$C-NMR spectra of polymer 3 (DP=42)
Figure 10. GPC profiles of polymer 3
Figure 11a. $^1$H-NMR spectra of 7-oxabicyclo[2.2.1]hepta-2,5 diene-2,3-dicarboxylic acid anhydride (2)

Figure 11b. $^{13}$C-NMR spectra of 7-oxabicyclo[2.2.1]hepta-2,5 diene-2,3-dicarboxylic acid anhydride (2)
Figure 12a. $^1$H-NMR spectra of polymer 4 (DP =16)

Figure 12b. $^1$H-NMR spectra of polymer 4 (DP =26)
Figure 12c. $^1$H-NMR spectra of polymer 4 (DP =48)
Figure 13. $^{13}$C-NMR spectra of polymer 4 (DP = 48)
Figure 14. GPC profiles of polymer 4
Figure 15a. $^1$H-NMR spectra of polymer 5 (DP=16)

Figure 15b. $^1$H-NMR spectra of polymer 5 (DP=26)
Figure 15c. $^1$H-NMR spectra of polymer 5 (DP=48)
Figure 16. $^{13}$C-NMR spectra of polymer 5 (DP=48)
Figure 17. GPC profiles of polymer 5
Figure 18a. $^1$H-NMR spectra of polymer 6 (DP=42)

Figure 18b. $^1$H-NMR spectra of polymer 7 (DP=48)
Figure 18c. $^1$H-NMR spectra of polymer 8 (DP=48)
a) Polymer 6

![Graph of Polymer 6]

b) Polymer 7

![Graph of Polymer 7]

c) Polymer 8

![Graph of Polymer 8]

Figure 19. GPC profiles of \(n\)-propylamine-grafted polymers
Figure 20. Degradation kinetics of the acid amide side chain in the n-propylamine-grafted ROMP polymers at pH 3.0 (●), 5.5 (▲), 6.5 (■) and 7.4 (♦). Error bar means the standard deviation (n=3).
Figure 21a. The predicted structure of n-propylamine-grafted one-mers 12. (by the DFT calculation)

Figure 21b. The predicted structure of n-propylamine-grafted one-mers 13. (by the DFT calculation)
Figure 21c. The predicted structure of n-propylamine-grafted one-mers 14. (by the DFT calculation)
Figure 22. $^1$H-NMR of the polymer after pH-dependent degradation
Figure 23. pH-dependent release study of short (left) and long (right) $n$-propylamine-grafted polymers. Error bar means the standard deviation ($n=3$).
Figure 24a. $^1$H-NMR spectra of polymers 9 (DP=42)

Figure 24b. $^1$H-NMR spectra of polymers 10 (DP=48)
Figure 24c. $^1$H-NMR spectra of polymers 11 (DP=48)
Figure 25. GPC profiles of alendronate-grafted polymers
Figure 26. pH-dependent release study of alendronate-grafted polymer 9, (DP = 42), 10 (DP = 48), and 11 (DP = 48). Error bar means the standard deviation (n=3).
Figure 27. Relative viability of *MDA-MB-231* cells treated by the alendronate-grafted ROMP polymers (polymer 9; ● (dot and dashed), polymer 10; ■ (short dashed), polymer 11; ▲ (long dashed)) and free alendronate (▼; line) at pH 5.8 (a), 6.5 (b) and 7.4 (c). Error bar means the standard deviation (*n*=5). (d) $IC_{50}$ values [μM] of the alendronate-grafted ROMP polymers and free alendronate on *MDA-MB-231* cells at each pH condition. The polymer concentration indicated corresponding alendronate grafted on the polymer.
Figure 28. *In vitro* cytotoxicity of polymers on *MDA-MB-231* cells. Error bar means the standard deviation (n=5). (a) non-grafted polymers, (b) polymer 11 and free alendronate.
Table 1. Molecular weight distributions of the ROMP polymers having α,β-unsaturated anhydrides

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[a] The $M_n$ (GPC) and $M_w$ (g/mol) were calculated using polystyrene standards.

[b] The DP was calculated from NMR data. DPs of 3, 4 and 5 were calculated from the comparison of the terminal phenyl peak with the -CH2- peak of the cyclopentene ring (3) or the -CH-peaks of the dihydrofuran ring and olefin (4 and 5).
### Table 2. Molecular weight distribution of grafted polymers

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<th>M&lt;sub&gt;w&lt;/sub&gt; (GPC)</th>
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<th>DP&lt;sub&gt;n&lt;/sub&gt; of non-grafted polymers</th>
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List of Publications

1. Geun-woo Jin, Heebeom Koo, Kihoon Nam, Heejin Kim, Seonju Lee, Jong-Sang Park*, and Yan Lee* Polymer 2011, 52, 339-346 “PAMAM dendrimer with a 1,2-diaminoethane surface facilitates endosomal escape for enhanced pDNA delivery”

2. Heejin Kim, Seonju Lee, Minwoo Noh, So Hyun Lee, Yeongbong Mok, Geun-woo Jin, Ji-Hun Seo, and Yan Lee* Polymer 2011, 52, 1367-1374 “Thermosensitivity control of polyethylenimine by simple acylation” (co-first author)

3. Minwoo Noh, Yeongbong Mok, Seonju Lee, Heejin Kim, So Hyun Lee, Geun-woo Jin, Ji-Hun Seo, Heebeom Koo, Tae Ha Park, and Yan Lee* Chemical Communications 2012, 48, 3845-3847 “Novel lower critical solution temperature phase transition materials effectively control osmosis by mild temperature change”

4. Geun-woo Jin, Heejin Kim, Ji-Hun Seo, Jiyeon Ham, Jong-Sang Park*, and Yan Lee* Macromolecular Research 2012, 20, 1249-1256 “Formation of polyion complex micelles with tunable isoelectric points based on zwitterionic block copolymers”
5. Sangmok Jang, Seonju Lee, Heejin Kim, Jiyeon Ham, Ji-Hun Seo, Yeongbong Mok, Minwoo Noh, and Yan Lee* *Polymer* 2012, 53, 4678-4685 “Preparation of pH-sensitive CaP nanoparticles with a phosphate-based block copolymer for efficient gene delivery”

6. Heejin Kim, Wonmin Choi, Seonju Lee, Sooyeol Kim, Jiyeon Ham, Ji-Hun Seo, Sangmok Jang, and Yan Lee* *Polymer* 2014, 55, 517-524 “Synthesis of Biomembrane-Mimic Polymers with Various Phospholipid Head Groups”


8. Yeongbong Mok, Minwoo Noh, Gyu Chan Kim, Youngjun Song, Heejin Kim, Seulah Kim, Sihyeong Yi, Ji-Hun Seo, Yan Lee* *Polymer* 2016, 107, 37-43 “Light-tunable thermoresponsive behavior of branched polyethyleneimine derivatives in water”

생체재료들은 질병이나 사고에 의해서 손상된 조직의 기능을 치환, 대체하기 위하여 체내에서 일시적 또는 지속적으로 주위 생체 조직과 직접 접촉하여 사용되고 있다. 생체재료를 이루는 구성물질에 따라 금속, 세라믹, 고분자, 복합재료로 분류된다. 금속과 세라믹은 기계적 성질을 주로 필요로 하는 분야에 사용되는 반면, 유기물을 기반으로 한 재료들은 생물학, 약리학적 응용에 많이 사용된다. 생체재료는 인공 심장, 혈관, 뼈, 신장, 귀 등 다양한 분야에 응용되고 있다. 생체재료들은 직접적으로 생체 내의 조직과 접촉하므로 물리적 성질뿐만 아니라 뛰어난 생체적합성이 요구된다. 조직 적합성과 혈액 적합성을 가진 신체 내에 이식되었을 때, 주변 조직 파사 및 혈액 응고 등과 같은 반응이 일어나서는 안 된다. 독성을 나타내지 않아야 하며, 어떠한 알레르기 및 면역 반응이 일어나서는 안 된다. 또한 대체된 신체 일부의 본래 기능을 제대로 수행하여야 하며, 시간이 지남에 따라서 물리적 성질 저하가 일어나서는 안 된다.

이러한 생체재료 중, 고분자 재료가 다양한 응용분야에 많이 사용되고 있다. 고분자 재료는 금속과 세라믹 재료들과는 달리 인체 거부감이 덜하여 의료재료, 조직공학, 약물 전달 등 생의료분야에 적용되고 있다. 특히, 합성 고분자는 합성 및 가공 과정에서 단량체의 화
학, 물리적 성질을 조절할 수 있기 때문에 다양한 재료로 이용되고 있다. 그러나 합성 고분자 재료를 생체재료로 사용하였을 때, 표면에 단백질 흡착 및 혈액 응고 등의 부작용이 나타나며, 이러한 증상이 심해지면 본래의 기능을 제대로 수행하지 못한 뿐더러 염증 및 면역 반응을 일으키기도 한다.

그래서 생체적합성이 뛰어난 콜라겐, 셀룰로오스, 키틴과 같은 천연 고분자를 사용하기도 한다. 하지만, 이들은 강도가 약하기 때문에 높은 하중을 견뎌야 하는 조직에는 적용하기가 힘들다는 단점이 있다.

따라서 본인은 첫 번째 연구로, 생체막 모방 고분자 합성을 제안하여, atom transfer radical polymerization (ATRP) 을 이용한 생체적합성 고분자 합성을 하였다. 기존에 잘 알려진 2-methacryloyloxyethyl phosphorylcholine (MPC) 처럼, 인지질 head group을 갖는 단량체를 합성하였고, ATRP를 통하여 고분자 중합을 하였다. 본인은 포스파티디산, 포스파디딜 에탄올아미, 포스파디딜 세린 기를 갖는 세 가지 고분자를 합성하였다. 각각의 단량체 합성을 개발하였고, ATRP를 이용하여 인지질 모방 고분자의 중합 정도를 조절하여 중합하였다. 생체내의 기관, 조직, 세포, 세포내 기관의 세포막에 각기 다르게 존재하는 인지질 구성물질을 고려할 때, 다양한 인지질 구조를 갖는 생체막 모방 고분자는 생체재료의 표면을 세포 및 조직 특이적으로 개질하는데 중요한 재료로 사용될 수 있을 것이라고 여겨진다.
최근 빛, 온도, pH, 효소 등과 같은 외부 신호에 따른 화학 분해 결합을 갖는 스마트 물질에 대한 연구가 활발하게 진행되고 있다. 다양한 외부 신호 중, 생체 내의 조직에 따라 다양한 pH 환경이 존재하기 때문에 pH 변화에 따른 선택적 분해 연구가 많이 주목 받고 있다. 암세포주변 조직에서는 급격하게 자라나는 암세포들에 의해 glucose uptake가 활발하게 진행되면서 주변에 lactic acid가 축적됨에 따라 정상세포 조직보다 낮은 pH를 나타내고, 세포내의 endolysosome은 endocytosis에 의해 cytoplasm보다 pH가 낮다. 그러므로 특정 pH에서 분해가 일어나는 화학 결합을 이용하여 원하는 장소에 약물을 방출 할 수 있도록 할 수 있다.

이를 가지고, 다양한 pH 조건에서 방출되는 아민 물질을 확인 및 정량하여 각기 다른 분해 속도를 확인하였다. 유방암 치료제로 사용되고 있는 알렌드로네이트와 고분자의 아민화반응을 통해 얻은 중합체를 이용하여 pH 5.8, 6.5, 7.4 환경에서의 유방암 세포의 활성도를 측정하여 pH에 따른 약물 방출 속도를 확인하였다. 알파-베탈 불포화 무수물을 갖는 ROMP고분자의 pH 응답성을 시스-알파-베탈 이중 결합의 치환체를 달리 함으로써 조절 할 수 있음을 확인하였다. 이러한 pH 응답성 조절은 치료제를 원하는 장소에서 방출하는 데에 매우 유용하여 특정 위치로의 약물 전달 시스템에 많이 이용될 수 있을 것이다.

주요어 : ATRP, 생체막 모방 고분자, 세포적합성, 인지질 그룹, ROMP, pH 분해능, pH 응답성 조절, 시스-알파-베탈-불포화 말레산 아미드, 아민화 그래프팅 및 방출

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