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Synthesis of Architecture-Controlled Poly(ethylene oxide)s as Biocompatible Materials

생체적합성 물질인 폴리에틸렌 옥사이드의
구조 제어에 관한 연구

2014년 2월

서울대학교 대학원
재료공학부
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Synthesis of Architecture-Controlled Poly(ethylene oxide)s as Biocompatible Materials

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Abstract

Synthesis of Architecture-Controlled Poly(ethylene oxide)s as Biocompatible Materials

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Poly(ethylene oxide)s are well known biocompatible, non-toxic, anti-fouling materials in biomedical fields and have been used for applications such as drug delivery, gene therapy, imaging modality and surface modification of carriers. Molecular weight, molecular weight distribution and primary architecture of poly(ethylene oxide) can be precisely controlled employing anionic polymerization of ethylene oxide in a high-vacuum system. 4-arm, 6-arm, 8-arm star-shaped poly(ethylene oxide)s were synthesized using pentaerythritol, sorbitol and 4-arm star-shaped poly(ethylene) oxide precursor as initiators. All polymerizations were carried out in DMSO based on 30 mole% activation of hydroxyl

groups in initiators to minimize the side reaction of DMSO activation by DPMK catalyst, known as 'dimsyl anion' formation. Dendritic poly(ethylene oxide)s were prepared in the same condition. Linear, star-shaped, dendritic poly(ethylene oxide)s were synthesized and surface modification at focal point as well as at each peripheral end was successfully controlled. All the polymers were characterized using ^{1}H NMR, GPC and UV spectrometer.

Anionic polymerization of poly(ethylene oxide)s produced the polymers with very narrow molecular weight distribution, which is an important requirement for a biomaterial applicable to drug and cell delivery system *in vivo*. For delivery of anticancer drugs on tumor site, nanostructures were used for efficiency in delivery and minimized side effects. Block copolymers consisting of 5 K poly(ethylene oxide), functionalized middle block and 2 K poly(caprolactone) were synthesized. Cysteine residues with thiol pendent group were introduced as a functionalized block between two polymers for enhanced stability during blood stream and selective degradation at a targeted site. Doxorubicin was used as anticancer drug and drug loaded nanoparticles displayed the size of 221 nm. Doxorubicin-loading amount and efficiency was around 8.7 and

26.0 %, respectively. Release profile of doxorubicin was monitored under two different conditions with the presence as well as absence of DTT and selective drug release at intracellular condition was observed.

8-arm star-shaped and dendritic poly(ethylene oxide)s were obtained without any noticeable side reactions. Molecular weight was 80 K with narrow molecular weight distribution of 1.03 and functionalized end groups were utilized for islet surface modification as well as double layer coating with unfractionated heparin (UFH). Two catechol groups in average were conjugated at the peripheral ends of 8-arm star poly(ethylene oxide)s and reacted with either thiol or amine groups on the cell surface in mild condition. Cell coverage and viability were optically visualized by FITC dyes which were additionally conjugated at the unreacted ends of the polymers. Modification of cell surface with double layers of poly(ethylene oxide)s and ultra fractionated heparin did not significantly affect the viability and biological functions of islets *in vitro* and *in vivo*.

Dendritic poly(ethylene oxide)s were applied for islet modification with the similar method as star-shaped poly(ethylene oxide)s. Dendritic poly(ethylene oxide)s were activated by NHS at the focal point for the conjugation with amine groups on the islet surface. *In vivo*

immunoprotection effects were investigated and dendritic poly(ethylene oxide)-modified islets showed high coverage effect and viability compared to unmodified islets.

Keywords: poly(ethylene oxide)s, anionic polymerization, star-shaped, dendritic, drug delivery system, cell delivery system

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Chapter 1.

Introduction

1.1. Poly(ethylene oxide)s in Biomedical Area

Poly(ethylene oxide)s have been developed as biocompatible polymer in pharmaceutical and biological applications because of their lack of toxicity, low immunogenicity and wide range of solubility both in organic solvents and aqueous system[1]. Since poly(ethylene oxide)s have high mobility and flexible properties in water, poly(ethylene oxide)s modified surfaces have various advantages that increase hydrophilicity as well as reduce protein adsorption, macrophage attack, platelet adhesion and decrease immune response[2]. Poly(ethylene oxide)s are stable polymers which are composed of polyether and are known as polyethylene glycol (PEG) or polyoxyethylene (POE), depending on its molecular weight.

Many researchers have been covalently attached poly(ethylene oxide)s to peptides, protein and antibody to improve their therapeutic effect[3]. This process is called PEGylation that has been developed from Frank F. Davis group in 1970. PEGylation on therapeutic drugs are shown to significant advantages such as increase of drug solubility and stability, extension of circulation time and reduction of drug toxicities[4].

Conjugation of PEG to therapeutic drugs such as protein and peptide has many benefits for formulation and administration with low solubility in

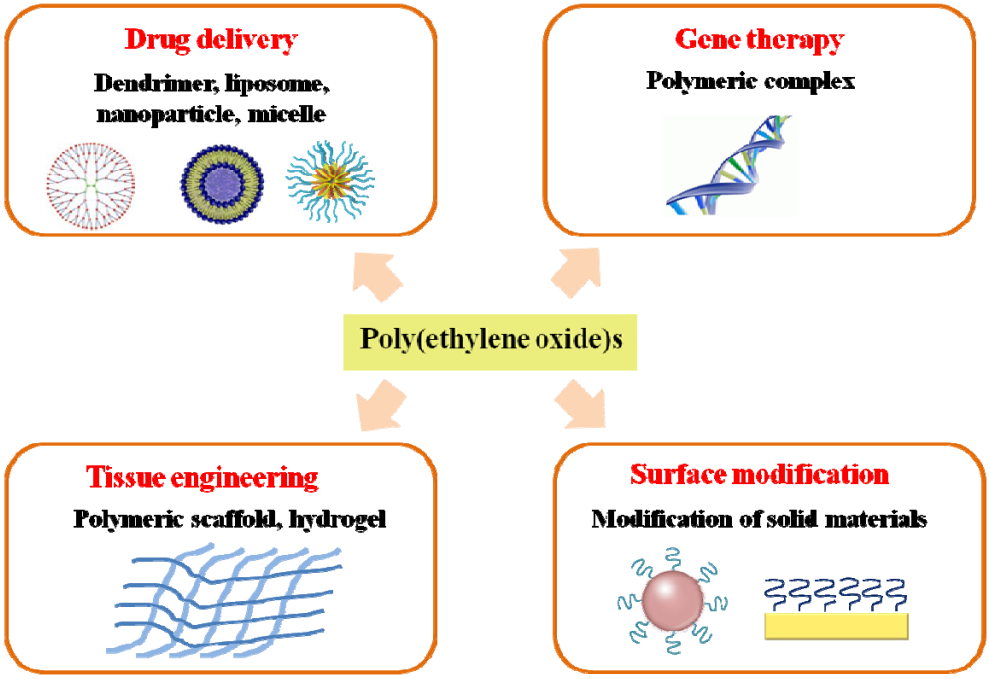

Figure 1-1. Application of poly(ethylene oxide)s in biomedical fields.

physiological condition. It also increases thermal and mechanical stability of therapeutic drugs and reduces drug toxicity since it decreases aggregation of drugs in aqueous condition. PEGylation improves plasma half-life of therapeutic drugs due to reduce cellular clearance by reticuloendothelial system, specific cell-protein binding and degradation by enzymes[3].

Poly(ethylene oxide)s are also used for surface on the nanoparticles for drug delivery systems, gene therapy, tissue engineering and imaging applications[5]. Generally, nanotechnologies for various types of cancer therapy have been developed for many decades. Inorganic metals such as gold nanoparticles[6], iron oxide nanoparticles and titanium oxide nanoparticles are unstable and tend to aggregate in physiological condition because their surface properties are close to hydrophobic even though they have negative charge on surfaces[7]. To stabilize their surface make hydrophilic, poly(ethylene oxide)s are used on surfaces[8]. Poly(ethylene oxide)s block nanoparticles to be removed by immune system and uptake in liver as well as improve their stability. Besides inorganic nanoparticles, organic nanoparticles formed by self-assembly which are liposome, micelles, polymersome and aggregates are used for poly(ethylene oxide)s as hydrophilic regions[9].

Surface modification of poly(ethylene oxide)s on solid substrates which is applied for biosensor, nanoarray or microarray have been also studied for many years[10]. Biocompatible synthetic polymers like poly(ethylene oxide)s and natural polymers like polysaccharides are used for covering surfaces and protect to adsorption of protein. Poly(ethylene oxide)s are commonly exploited for surface modification on account of multiple advantages as mentioned above[11].

There are various applications using poly(ethylene oxide)s in biomedical fields. This is because poly(ethylene oxide)s are possible to modify various kinds of functional groups. Many researcher have been interested in modify the end of poly(ethylene oxide)s and heterobifunctional poly(ethylene oxide)s. M.S. Tomson published review paper about heterobifunctional poly(ethylene oxide)s oligomers in 2008[12]. Terminal hydroxyl groups of poly(ethylene oxide)s are converted to the new functional groups such as amine, carboxylate, maleimide and thiol groups through various synthetic methods. Functionalized poly(ethylene oxide)s are very useful for sophisticated application in pharmaceutical and biomedical fields[13].

1.2. Anionic Polymerization

Anionic polymerization is well-known for addition polymerization of vinyl monomers that are reacted with activated carbanion species[14]. Polymerization takes place in three steps, initiation, propagation and termination. Initiation step is occurred by formation of strong anion to monomer through electron transfer of nucleophilic initiator such as organometallic compounds, hydroxides and alkoxides. Propagation is carried out through complete consumption of monomer. The reaction proceeded in low temperature and short time because the anion is not stable and the reaction rate is so fast. Termination can be occurred through the addition of alcohol or water by quenching of carbanion species because anionic polymerization has no termination step. In 1956 Szwarc and co workers reported living anionic polymerization. The reason of living anionic polymerization is that there is no termination step in absence of impurities. The carbanion reactivity remains continuously and react with another monomers unless chain transfer or deliberate termination[15].

Vinyl monomers with substituent that stabilize a negative charge on the double bond are used for anionic polymerization because they are stabilized the carbanion propagation center through delocalization of

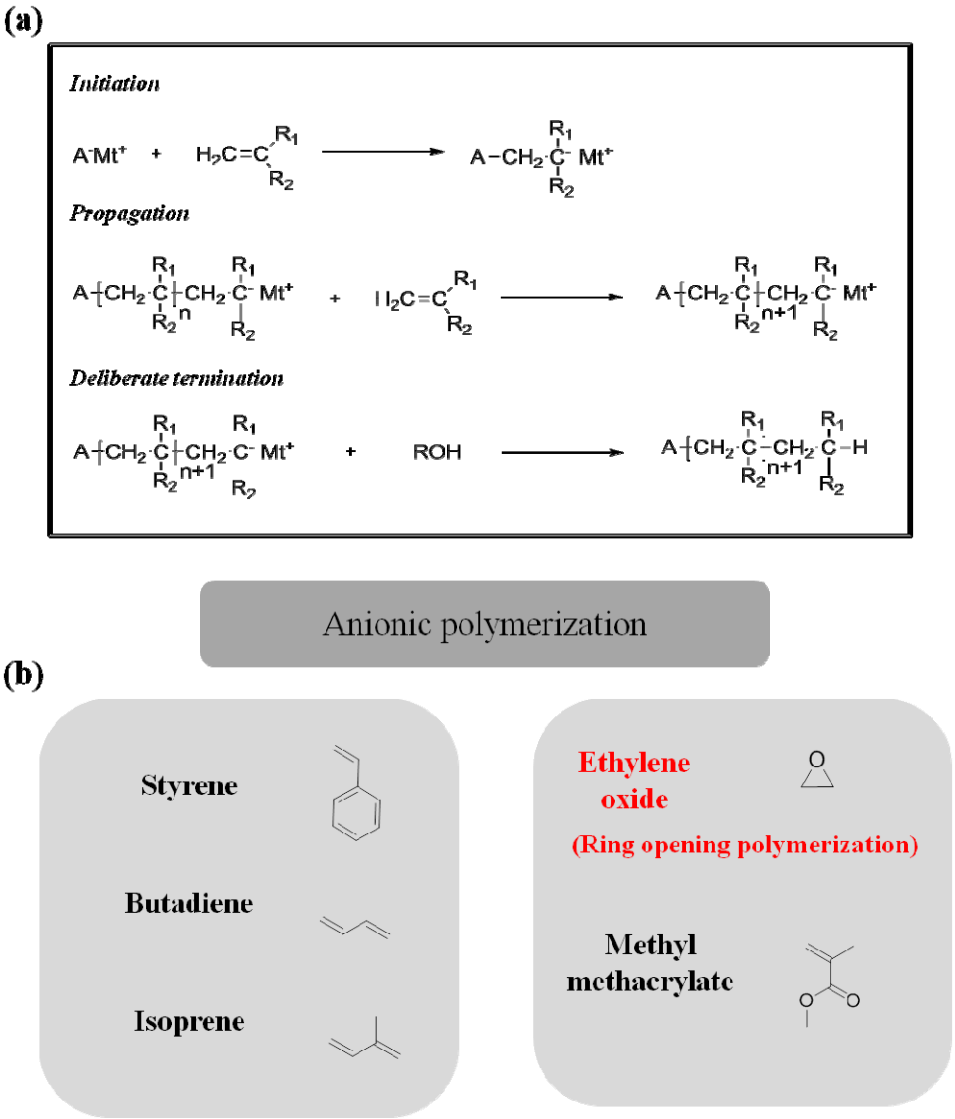

Figure 1-2. (a) The process of anionic polymerization and (b) representative monomers.

negative charge. There monomers are styrene, dienes, methacrylate, etc. Except for vinyl monomers that are representative monomers for anionic polymerization, there are epoxies, cyclic siloxane and lactones which are prepared via anionic ring opening polymerization. Solvents are determined by solubility and reactivity of carbanion of initiator and propagating species[16].

Anionic polymerizations are carried out in a high vacuum apparatus. The equipments are composed of a primary and a secondary manifold. All manifolds are made of Pyrex glass tubing and Teflon stopcocks for high vacuum system without any contaminants like moisture and oxygen. To remain high vacuum, diffusion pump and mechanical pump are connected to vacuum line. The pressure is retained 10^{-3} mTorr in leak free system[17].

Reactors for polymerization and ampoules for initiators, monomers or solvents are composed of Pyrex glassware constructed of glassblowing. All apparatus are cleaned with sulfuric acid, rinsed with distilled water several times and dried in convection oven.

All chemicals including initiators, monomers and catalyst are purified through several steps to remove reactive impurities. The final steps are carried out in vacuum line or in seal evacuated glass ampoules. Before

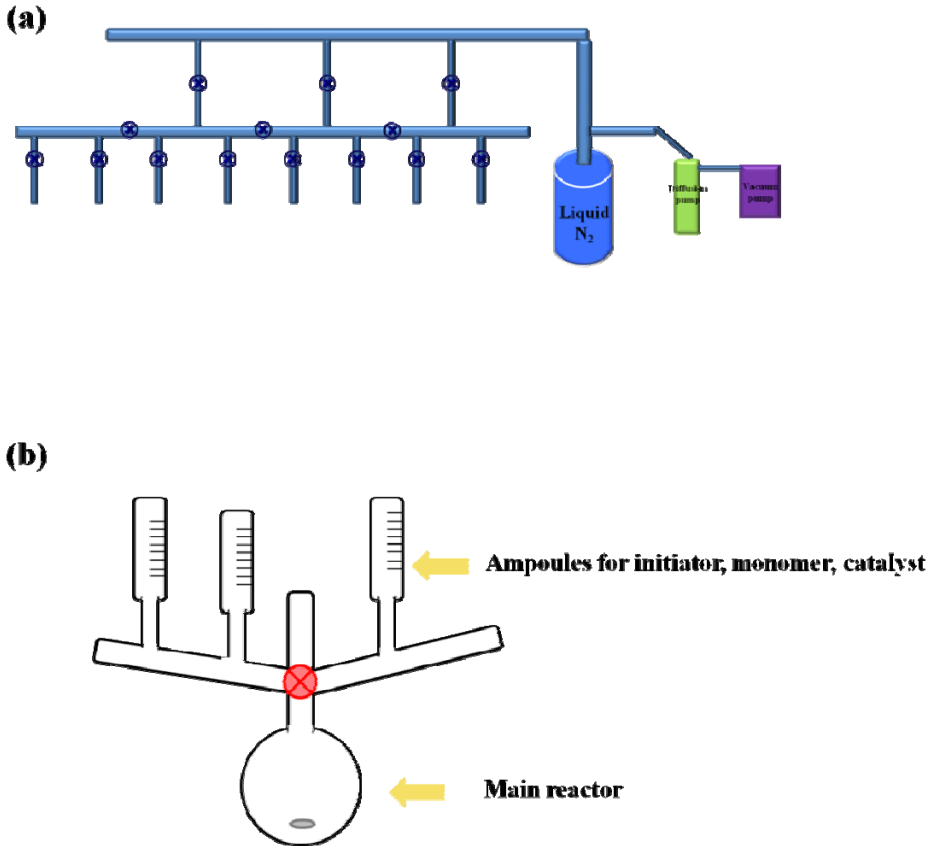

Figure 1-3. (a) Vacuum line system and (b) reactor for anionic polymerization.

starting reaction, all the reactors and apparatus were connected to the vacuum line and confirmed the leak using Tesla coli. All the solvents or monomers for ampoules are moved with dry ice at -78 ^{o}C or liquid nitrogen through vacuum line and finally sealed with flame. When use the liquid under the argon gas, gas tight syringe were employed. It is important that contact with no contact to moisture and air is needed.

Most reactions in anionic polymerization are carried out in low temperature because the reaction is very fast and active. Dry ice in isopropanol (IPA) at -78 ^{o}C or ice bath are usually used during polymerization, especially, when the vinyl monomers are synthesized.

Polymers obtained from anionic polymerization have narrow molecular distribution which is the greatest advantage of anionic polymerization. And it is easy to control of block copolymer composition. Especially, styrene and dienes are well known for composition of synthetic rubber and used for commercial application.

1.3.Drug Delivery System for Cancer Therapy

Cancer treatments have been studied through various methods such as surgery, chemotherapy and radiotherapy depending on the cancer types[18].

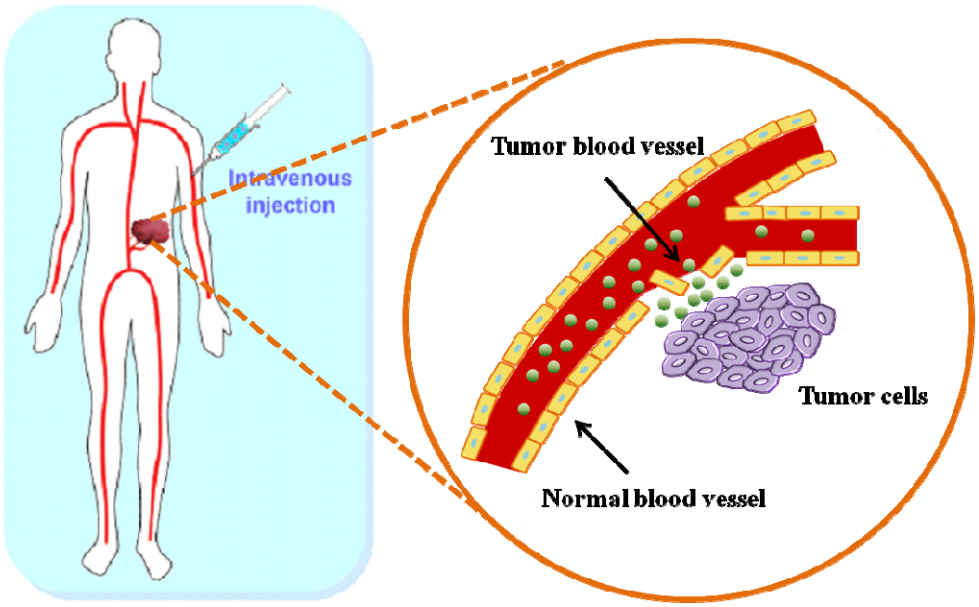

Figure 1-4. Enhanced Permeability and Retention (EPR) effects.

carcinoma which is the most common type of cancer occurring in human body. Local delivery of anticancer drugs can treat specific cancers because the depth of needle of syringe has a limitation[19]. Anticancer drugs that are injected intravenously have lower limitation to treat cancer than local injection[20].

There are diverse severe side effects when anticancer drugs are delivered by intravenous injection in the body since drugs can affect on normal tissue as well as cancer. Most of anticancer drugs have poor solubility in water and removed by immune system in the body that makes drug efficiency very low[21]. To improve drug efficiency and reduce side effects, delivery system using nanoparticles, micelles, and liposome has been researched for many decades.

Nanoparticles encapsulating therapeutic drugs benefit from their size. Between 100 nm and 800 nm size of nanoparticles are accumulated near tumor tissues, the vascular is heterogeneous, leaky, discontinuous, permeable and poor lymphatic system, which is called the enhanced permeability and retention (EPR) effect[22]. Tumor tissues growth rapidly and require to nutrients as well as oxygen, result in angiogenesis[23]. Using this characteristic, passive targeting is available to deliver nanoparticles

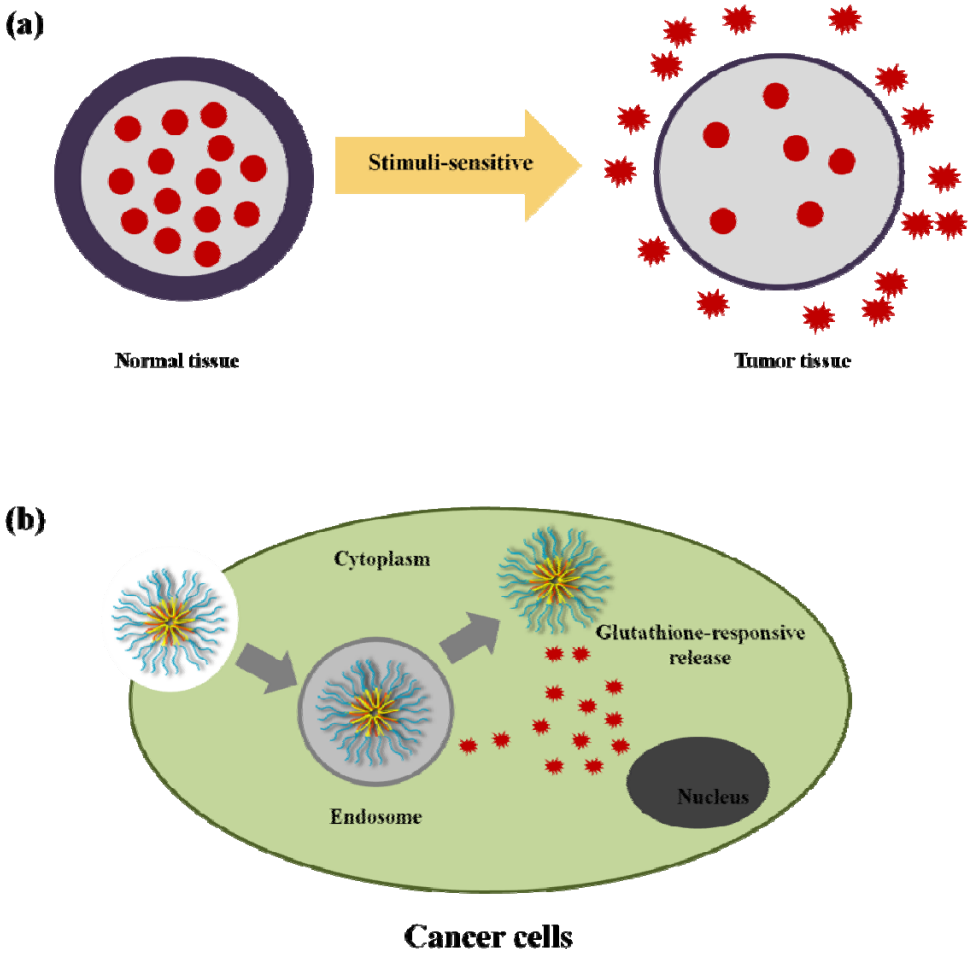

Figure 1-5. (a) Stimuli-sensitive drug delivery and (b) glutathione-sensitive drug delivery in cytoplasm.

depending on nanoparticles size and vascular environment[24]. Active targeting is that ligands or targeting moieties that have high affinity and specificity of binding to cell surface receptors are decorated on surface of nanoparticles. There are a number of receptors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) near tumor tissues[25].

Stability of nanoparticles has also been importance issue for efficient drug delivery. During circulating blood stream, drugs encapsulated in nanoparticles can be defused or nanoparticles can be collapsed. To improve stability, many researchers have been studied for improving particle stability using crosslinkers to bind inner site or outer of nanoparticles[26].

Release of drugs on target site is also important. Nanoparticles are designed to change their formation or degradation to release drugs on specific target by responding on sensitive stimuli such as pH, enzyme. In our group, we used glutathione responsive drug carrier using disulfide bonds[27].

Nanoparticles are removed by macrophage or immune system by recognizing foreign materials. Many kinds of biocompatible polymers are used for outer of nanoparticles to prevent from recognizing by immune

system. Especially, Poly(ethylene oxide)s conjugated nanoparticles are prolonged their circulation and reduced to clearance by reticuloendothelial system (RES) due to flexible and hydrophilic properties of them.

1.4. Cell Delivery System for Islet transplantation

Cell delivery system is a promising area in pharmaceutical fields that uses cells as active drugs[28]. Unlike chemical drugs for unspecified individuals, cell delivery system is for each person which has different therapeutic effects in accordance with individual condition. It has higher therapeutic effects and stability as well as lower side effects than those of the existing drugs.

Cell delivery system has been studied for diabetes, cancer, dementia and intractable diseases through integrating technologies such as biotechnologies, genetic engineering, pharmacy and materials engineering[29]. It has been expected for invaluable technology that has a wide range of application and therapeutic effects. So far, stem cell delivery has been developed by using cell fabrication, separation of cells, gene recombination, and so on. In recent, the interests of non immunogenic cell transplantation for diabetes have been increased because diabetic patients

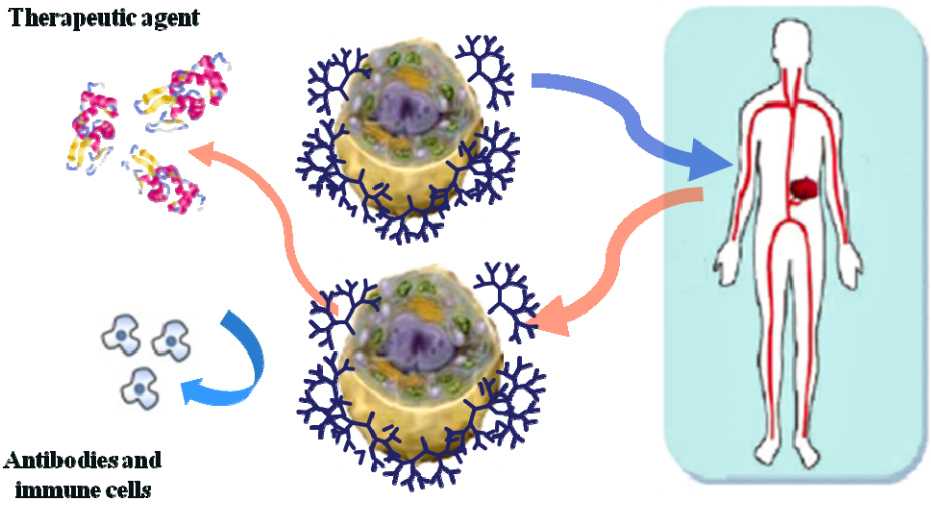

Figure 1-6. Cell surface modification for cell transplantation.

are dramatically raised for many years[30].

Even though transplantation of cells in physiological condition has a lot of advantages, there are tremendous immune reactions that lead to be degraded and faced with cell death. To minimize and suppress immune response after cell transplantation[31], cell surfaces are modified by biocompatible polymers such as poly(ethylene oxide)s, heparin, alginate, amino acid based polymers and so on[32]. Surface density and functionality are very important for regulating polymers on cell surface[33]. Control of size and shape of polymers are also significant since polymers on cell surface function as barriers for protecting approaching proteins and immunity substances[34]. Layer-by-Layer systems using biocompatible polymers via various methods are used for surface modification[35].

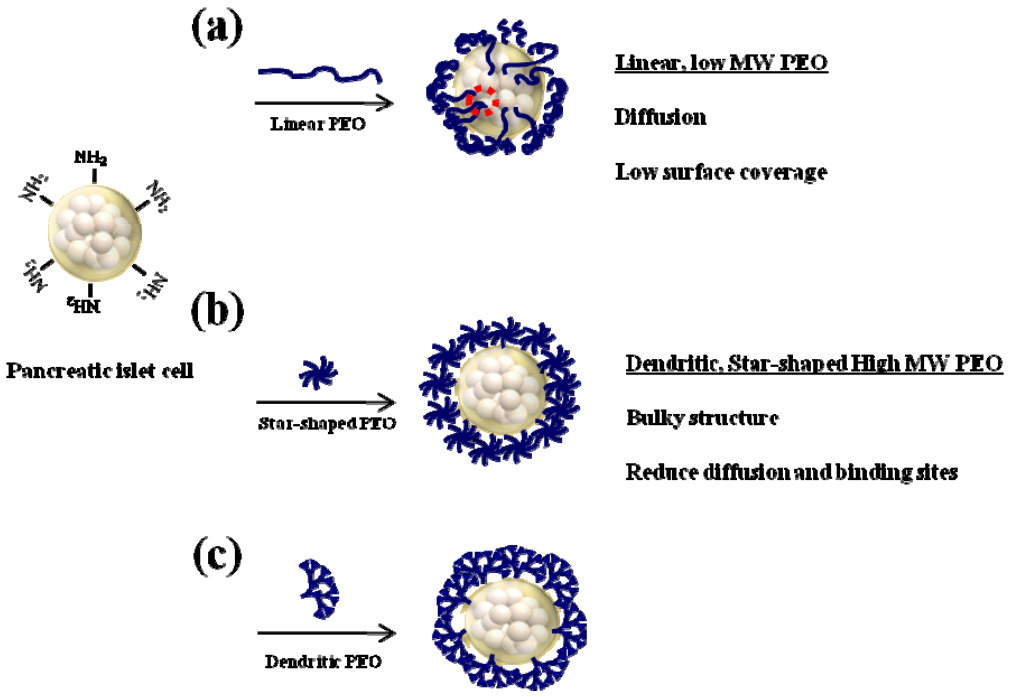

Figure 1-7. Cell surface modification by (a) linear (b) star-shaped (c) dendritic poly(ethylene oxide)s.

1.5. Research Objectives

Poly(ethylene oxide)s have been spotlighted as biocompatible polymers in pharmaceutical and biological application due to their chemical and physical properties. In biomedical field, poly(ethylene oxide)s has been commonly developed for imaging modalities, drug carriers and surface modification to stabilize and offer effective treatment effects.

To synthesize and control the architecture of poly(ethylene oxide)s, anionic polymerization was used. Anionic polymerization is sensitive with moisture, air, and any impurities and is proceed in high vacuum system. All reactors or experiments are carried out with glass blowing and in the vacuum line. For these reasons, well defined polymers with narrow molecular distribution and without any side reaction could be obtained. In chapter 2, the method of synthesizing poly(ethylene oxide)s via anionic polymerization is described. The choice of initiator, catalyst, and solvents has an effect on polymerization. There are different kinds of conditions when the linear, star-shaped and dendritic poly(ethylene oxide)s are synthesized.

In chapter 3, linear poly(ethylene oxide)s were used to making block copolymer with poly(caprolactone) for drug delivery system. Poly(ethylene

oxide)s for hydrophilic part and poly(caprolactone) for hydrophobic part were foamed polymeric micelle in water. To provide stability and response in specific site, cysteine peptides were introduced between the two polymers. The size of polymeric micelles was around 250 nm for EPR effects and the polymeric micelles were shown sensitivity in specific condition by confirming drug release profile.

Poly(ethylene oxide)s were also researched in cell delivery area. Delivery of pancreatic islets has been serious problems due to their inflammation and severe immune rejection responses. To protect these reactions, poly(ethylene oxide)s were used for cover the surface of islet. It needed to control the architecture of poly(ethylene oxide)s for efficient complete modification of cells. Therefore, star-shaped and dendritic poly(ethylene oxide)s were synthesized because star-shaped and dendritic poly(ethylene oxide)s have bulky structure compared to linear poly(ethylene oxide)s and there are many functional groups at the end of polymers can used for further modification or for drugs. In chapter 4, 8-arm star-shaped poly(ethylene oxide)s was applied with heparin layer to reduce the immune responses. In chapter 5, dendritic poly(ethylene oxide)s used for surface modification of islet because dendritic poly(ethylene oxide)s have one

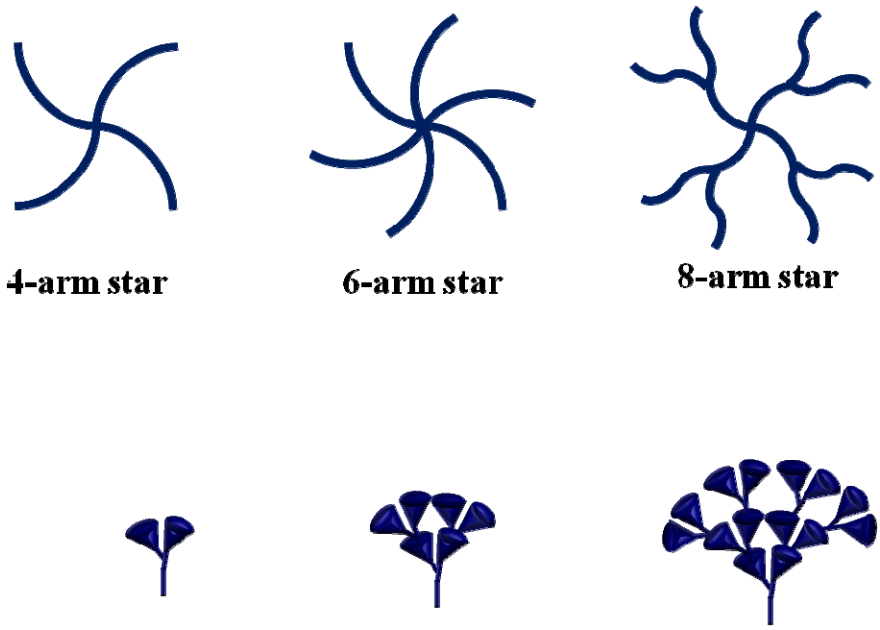

Figure 1-8. Control of the architecture of poly(ethylene oxide)s for cell surface modification.

focal point and multifunctional groups at the end of polymers could make the efficient covering effect for cells. Cell covering effect, viability, function were confirmed by various methods. They showed the possibility of application on cell delivery system without any damage.

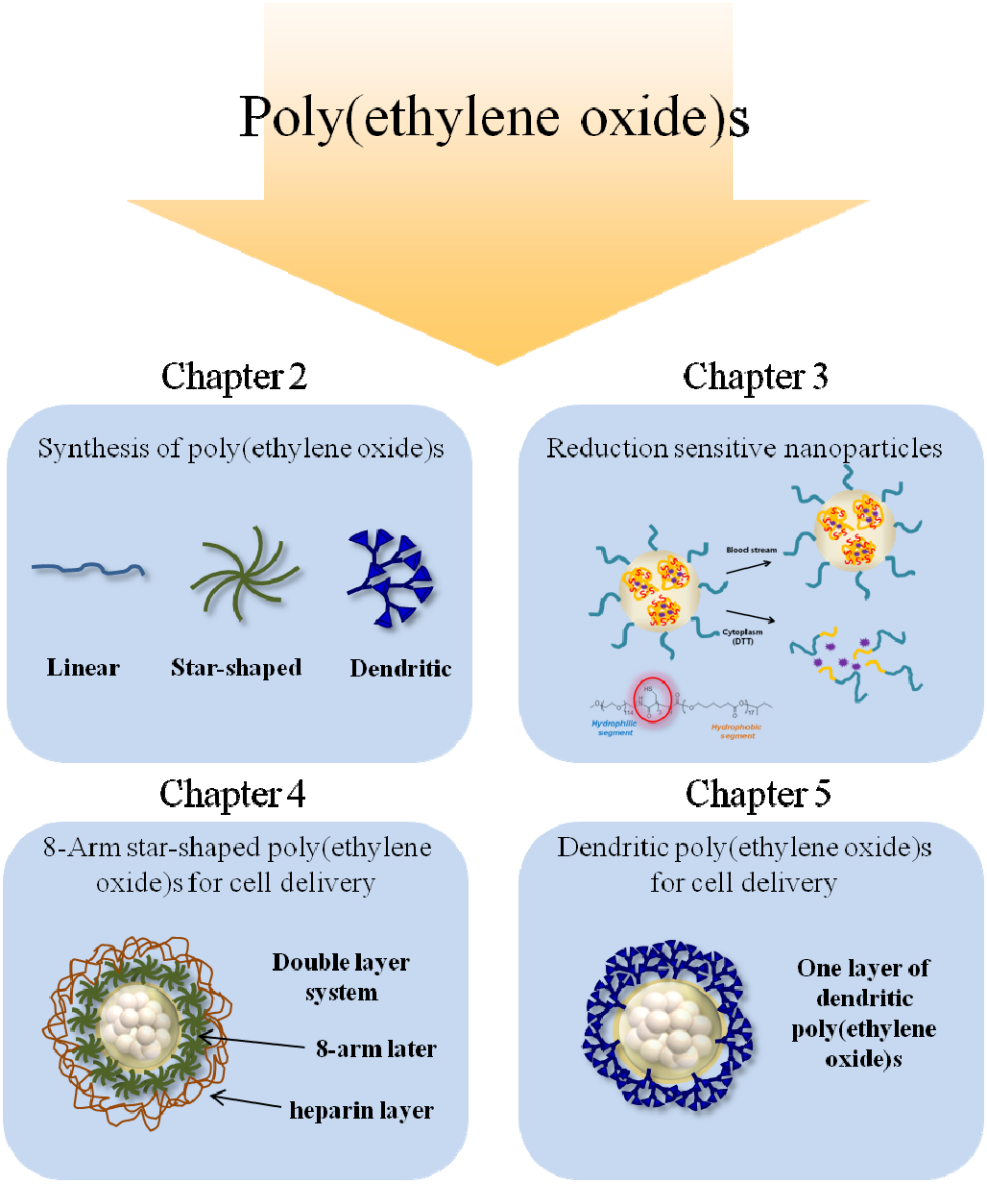

Figure 1-9. Illustration for research of poly(ethylene oxide)s.

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Chapter 2.

Synthesis of Poly(ethylene oxide)s via Anionic Polymerization

2.1. Introduction

Poly(ethylene oxide)s are synthesized by control of initiator, catalyst and solvents. Initiators are important factor to synthesize poly(ethylene oxide)s which have what kinds of shapes: linear, branched, crosslinked, dendritic, etc. Ethylene oxides are polymerized from initiators that are activated with strong base catalyst. Hydroxyl terminated compounds are mainly used for initiators to synthesize poly(ethylene oxide)s[1]. Alkoxides are formed after activating with catalyst such as sodium naphthalide, potassium naphthalide, or diphenyl methyl potassium and their solubility play an important role in polymerization. For this reason, solvents are chosen according to kinds of initiators because solubility of alkoxides effect on initiation activities, kinetics and degree of polymerization. Benzene, toluene, tetrahydrofuran (THF) and DMSO are mainly used for synthesizing poly(ethylene oxide)s. Benzene and toluene are nonpolar solvents and DMSO is the most polar solvent among them[2].

Alkoxides can be associated depending on the metallic counterion which ionic radius has an effect on reactivity of initiators. The higher the ionic metal radius, the reactivity of propagation is increased. The reactivity of metal is increased in following order: lithium, sodium,

potassium, and cesium. To minimize aggregation of alkoxides, polar solvents such as tetrahydrofuran (THF) are preferred to non polar ones like toluene and benzene.

Solvent purification has a significant effect on polymerization. THF is reacted with calcium in one neck round bottom flask for several days on the vacuum line. During vigorous stirring, hydrogen is degassed continuously, and then THF is distilled over sodium mirror with dry ice bath at 78 ^{o}C in the vacuum line. THF is degassed by reacting with sodium for several days until completely be over. Finally, the solvent is distilled over the flask with dry ice at 78 ^{o}C which contains sodium mirror and a few grains of benzophenone. THF is continuously degassed until the color is changed to dark purple.

Unlike THF, DMSO is distilled using constructed glasses because it has high viscosity and boiling temperature. Two ampoules for purified DMSO are connected to one neck flask with glass blowing to minimize the space in the distillation state. The constructed reactor is evacuated on the vacuum line to confirm the leaks with Teslar coil. When it is sure that there are no leaks, DMSO and calcium hydride are stored with vigorous stirring at room temperature for several days. Hydrogen gasses are

removed by opening the Teflon stopcock in the vacuum line. After degassing completely, DMSO in the reactor is heated up to 60 ^{o}C, DMSO is distilled to connected glass ampoules under vacuum. The fresh DMSO ampoules are obtained by sealing with flame between main reactor and connected ampoules.

Benzene is stirred with same volume ratio of sulfuric acid at room temperature. The color of sulfuric acid layer is changed to dark brown after a few days later. At least 7 days later, benzene is extracted from sulfuric acid and washed with double distilled water several times. The solvent from neutral water is stirred with magnesium sulfate to remove remain water. After filtering, benzene is placed in one neck round bottom flask with calcium hydride in the vacuum line. The solvent is degassed at room temperature for several days, distilled to the other flask with dry ice at 78 ^{o}C which is charged with sodium mirror, continued to degassing. This procedure proceeded with two times. Finally, the solvent is distilled to the flask containing butyl lithium and purified styrene monomers. Poly(styrene)s are synthesized in purified benzene solution and the color of solvent turns to orange because of formation of stryl lithium. Poly(stryl lithium)s are used to confirm the complete distillation and additionally

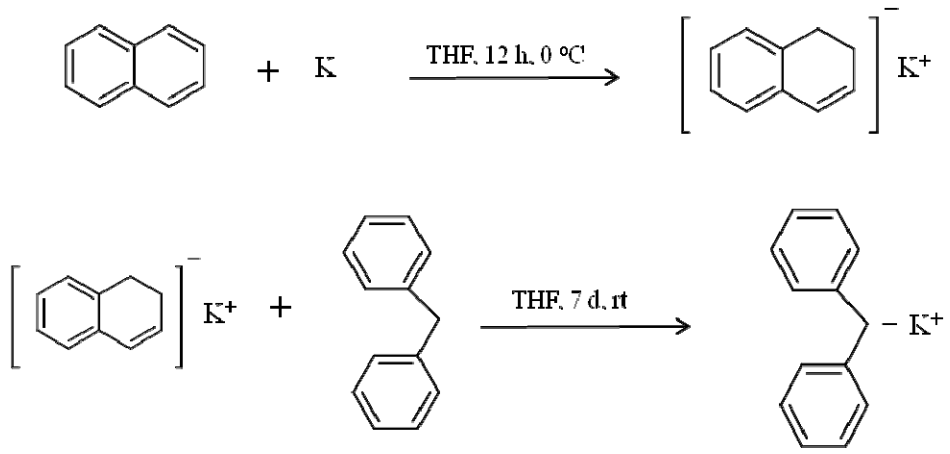
purified by reacting with remain impurities.

Catalyst is decided to sort of counter ion for polymerization and activity of it. In case of poly(ethylene oxide)s, lithium metal is rarely used for initiator because lithium ionic radius is too small and associated strongly, ethylene oxide monomers do not be inserted after one or two monomers are reacted with initiators. Cesium metal has large ionic radius, ethylene oxide monomers are associated with activated initiators easily, and on the other hand, control of polymerization can be difficult because it is too reactive. Prof. Kim in Kyung Hee University developed combination of lithium and potassium as initiators. Butyl lithium (normal, secondary, tertiary) and potassium tertiary butoxide which are commercial available were used for polymerization and molecular weight was controlled by adjusting ratio of them.

Generally, sodium and potassium as counter ion are used for initiator or base catalyst. For many decades, sodium naphthalide and potassium naphthalide have been used for initiator for synthesis of ethylene oxides. Especially, potassium ion is the most popular in synthesis of ethylene oxides because it has a proper reactivity and easy control. Potassium naphthalide has been typically used for initiate ethylene oxide ring opening

polymerization. It has been also used for strong base catalyst when initiators are used as kinds of alcohol or amine. In our group, we usually have been used hydroxyl containing initiators which have heterofunctional groups for application after synthesis. To activate hydroxyl groups, potassium naphthalene was used for strong base catalyst. Potassium naphthalide is reactive to synthesis of polymers via anionic polymerization and has many advantages, but it has to be kept in low temperature at 78 ^{o}C because of its stability. Because of this reason, we were chosen diphenyl methyl potassium (DPMK) as a catalyst to synthesize poly(ethylene oxide)s[3].

The procedure for synthesis and determination of DPMK has been reported. First, diphenyl methane was prepared in glass ampoule and diluted with purified THF in the vacuum line. This ampoule was connected to main reactor which is 500 mL round bottom flask via glass blowing. After confirming leaks of reactor using Teslar coil, pieces of potassium metal (4.1 g, 1.05 x 10^{-1} mol) and naphthalene (6.72 g, 5.25 x 10^{-2} mol) were introduced in the main reactor. Purified THF were distilled because potassium naphthalide was formed in the solution. Diphenyl methane was added to potassium naphthalide solution and stirred more than



Scheme 2-1. Synthetic scheme of diphenylmethyl potassium (DPMK).

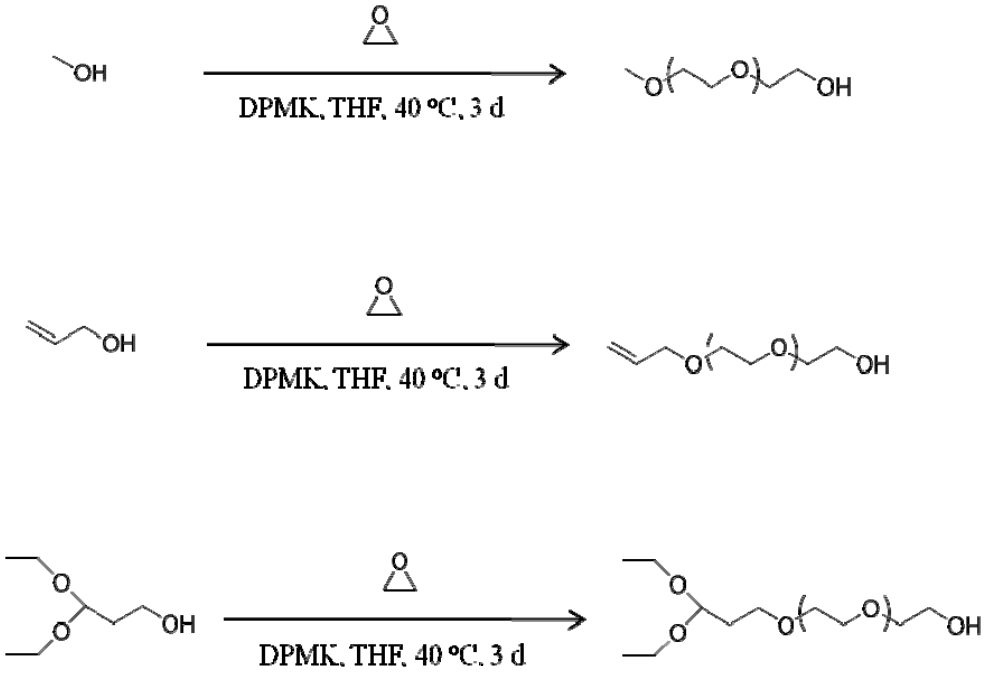
7 days. The color of final solution turned to dark red-orange. Titration with acetanilide was used for determination of concentration of DPMK. DMSO was chosen as a solvent and ampoule with well dried DMSO was constructed to reactor via glass blowing. After checking of leaks in the vacuum line, a few grains of triphenylmethane and DMSO were introduced and then DPMK was added using gas tight syringe under argon atmosphere. Acetanilide (0.2 g, 1.48×10^{-3} mol) was added to the red orange solution, and the solution turned to light yellow color instantly. More DPMK was added to solution and titrated repeatedly. The average concentration of DPMK was used for polymerization[4].

2.2. Experimental Section

Linear poly(ethylene oxide)s are synthesized in various methods by combination of initiator, catalyst and solvents[5]. As mentioned above, counter ions have effect on reactivity with monomer and growing end site of polymers. Lithium ion has the highest association binding with growing anion because it has small ionic radius. Lithium ion can be used with sodium or potassium by controlling the ratio between them. In case of styrene or isoprene, their activities are relatively high, lithium ion is

commonly used for polymerization. Potassium ion has more reactivity than lithium ion, it has difficulty to get well controlled polymer when potassium is used for synthesis of styrene or isoprene.

For synthesizing poly(ethylene oxide)s, potassium ion is mainly used for many decades[6]. Potassium naphthalene has been used not only as an initiator but also as a catalyst. These days, many researchers have been interested in synthesizing of heterofunctional poly(ethylene oxide)s which have potential possibilities for application in various fields. Monofunctional poly(ethylene oxide)s are containing methoxy group or phenyl groups at the one end group are used for block copolymers[7]. Methanol is used as an initiator to synthesize methoxy poly(ethylene oxide)s. In our group, the initiator which has protected functional group in one site and hydroxyl group for growing site was needed to apply on biomedical field. Many kinds of initiators were tried to synthesize linear poly(ethylene oxide)s. Allyl alcohol was a first candidate for synthesis of heterofunctional linear poly(ethylene oxide)s. It contains allyl group in one site and hydroxyl groups for reacting with ethylene oxides.



Scheme 2-2. Synthesis of linear poly(ethylene oxide)s using (a) methanol (b) allyl alcohol (c) 3,3-diethoxyl propanol as an initiator.

3,3-diethoxypropanol was chosen as an initiator because acetal group in this initiator can convert to carboxylic acid after deprotection. This chemical is commercially available. Before use this chemical, reactor for distillation was constructed by glass blowing. 3,3-diethoxypropanol was reacted with calcium hydride for removing water and distilled to small ampoule for use[8]. To synthesis of linear poly(ethylene oxide)s, ethylene oxide was distilled over calcium hydride. Ethylene oxides are in gas state and kept in sealed bottle. They are transferred to round bottom flask with stopcock in dry ice at 78 ^{o}C. Ethylene oxides are reacted with calcium hydride and degassed in the vacuum line for one day. They are transferred into flask which is charged with di-butyl magnesium and stirred for the further purification[9]. For use of ethylene oxides, ampoule is constructed by glass blowing and confirmed leaks in the vacuum line. Purified ethylene oxides are transferred into ampoule and sealed with flame in the vacuum line.

Reactor is composed of main reactor and ethylene oxide ampoule. It is constructed by glass blowing. First, initiators are charged via gas tight syringe in the reactor under argon atmosphere, distilled fresh THF in vacuum and stirred for 30 min to dissolve completely. Ander argon

atmosphere DPMK is introduced by gas tight syringe which is 90 % moles of hydroxyl groups in initiator. DPMK is also a good initiator for ethylene oxides in THF, control of the amount is an important factor to get polymers that have and accurate molecular weight and narrow molecular weight distribution. For this reason, the amount of DPMK is used below 100 % moles of initiators. When hydroxyl groups turns to alkoxides by reacting with DPMK, the color of solution is changed to light-yellow. Ethylene oxides are introduced to this solution in cold state after stirring for 30 min. The polymerization is usually carried out at 40 ^{o}C for 72 h and quenched by the addition of a few drops of methanol. The solution was concentrated and precipitated in cold diethyl ether twice. After filtration, the product was dried in vacuum for 24 h to produce linear poly(ethylene oxide)s.

3-mercaptopropionic acid has thiol group and carboxylic group[10]. We were tried to initiate ethylene oxides by thiol group in DMSO because thiol groups after activating by DPMK were insoluble in THF. The procedure of polymerization was same mentioned above. But the polymers were obtained broad molecular weight distribution and hard to use though initiators have a good candidate.

Star-shaped poly(ethylene oxide)s have at least three arms of polymeric chain in the molecules. There are two kinds of methods to synthesize star-shaped poly(ethylene oxide)s, core first and arm first. The core first method is that core molecules have multiple activated centers which have star-shaped polymers after synthesis of monomers. Star-shaped polymers with 3 arms or 4 arms initiated by multifunctional initiators such as trimethylol propane (TMP), trimethylol ethane (TME), glycerol and pentaerythritol are prepared using anionic polymerization of ethylene oxides[11]. In contrast, arm first method is that living linear macromolecules react with low molecular agents having multifunctional groups. Linear poly(ethylene oxide)s are synthesized via anionic polymerization and activated carbanion at the end of polymers are terminated by low molecular agent with multifunctional groups[12].

Both methods core first and arm first of the star-shaped poly(ethylene oxide)s have their advantages and drawbacks. Arm method has some difficulties to remove free linear polymers after reaction[13]. In case of poly(ethylene oxide)s, solubility between linear and star-shaped poly(ethylene oxide)s is not different much, so it is hard to remove free

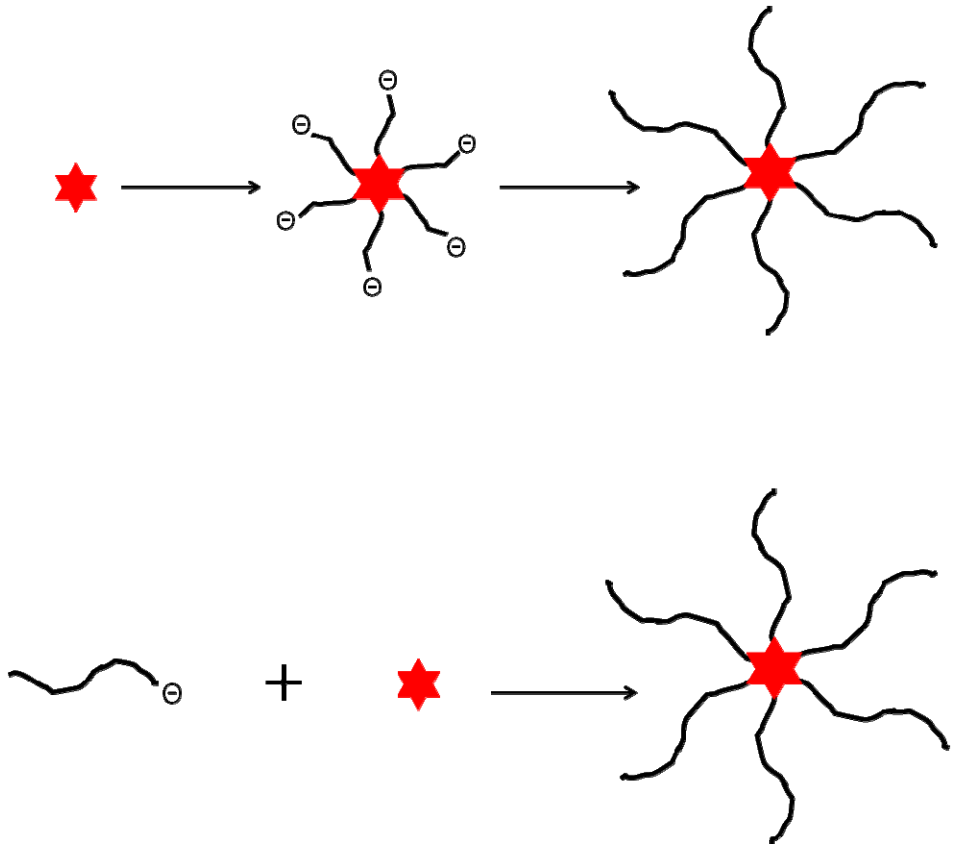
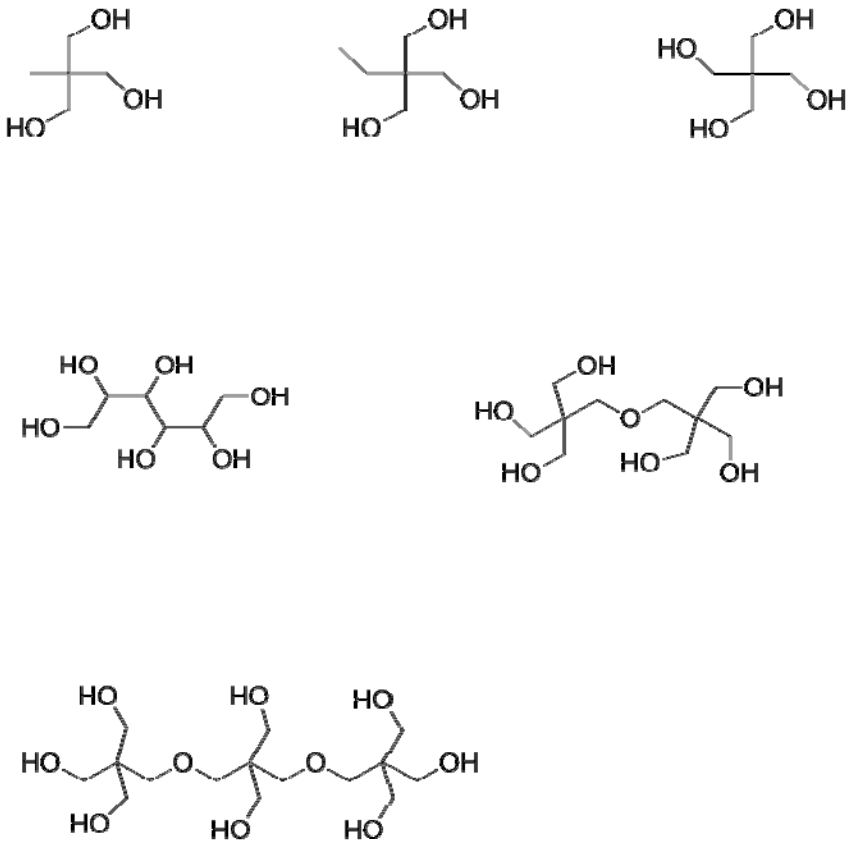


Figure 2-1. Synthetic methods of star-shaped poly(ethylene oxide)s by (a) core first method and (b) arm first method.



Scheme 2-3. Various types of initiators for star-shaped poly(ethylene oxide)s (a) trimethylol propane (b) trimethylol ethane (c) pentaerythritol (d) dipentaerythritol (e) tripentaerythritol.

linear poly(ethylene oxide)s. According to reactivity of low molecular weight, there can be star-shaped poly(ethylene oxide)s which have different arms in their molecules[14].

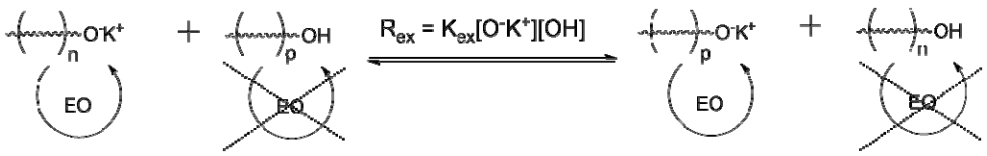
In our research, the application on biomedical fields was the final purpose, the purity of polymers was considered wide range of possibilities. Molecular weight distribution of polymers has an effect on toxicity and limit on application. For this reason, core first method was chosen to synthesize star-shaped poly(ethylene oxide)s.

In the core first method of poly(ethylene oxide)s polymerization, the molecular weights are controlled by adjusting the molar ratio between the initiators and monomers[15]. After polymerization, the hydroxyl groups at the end of polymers are used for initiators of synthesis of ethylene oxides or the other molecules. The reactions are proceed in DMSO system when multiple hydroxyl groups in low molecule are used for initiator for star-shaped poly(ethylene oxide)s, in contrary to use THF as solvents in synthesis of linear poly(ethylene oxide)s[13]. Hydroxyl groups are transformed to alkoxides after reacting with DPMK, lead to associate with each other because of their strong tendency. Thus, polymers with low yield and broad molecular distribution are obtained since all the hydroxyl

groups can not participated in initiate polymers. To solve this problem, DMSO is used as reaction solvent to disassociate and dissolve multiple alkoxides, besides lower amounts of DPMK below 50 % of moles of hydroxyl groups are used for partial activation[16].

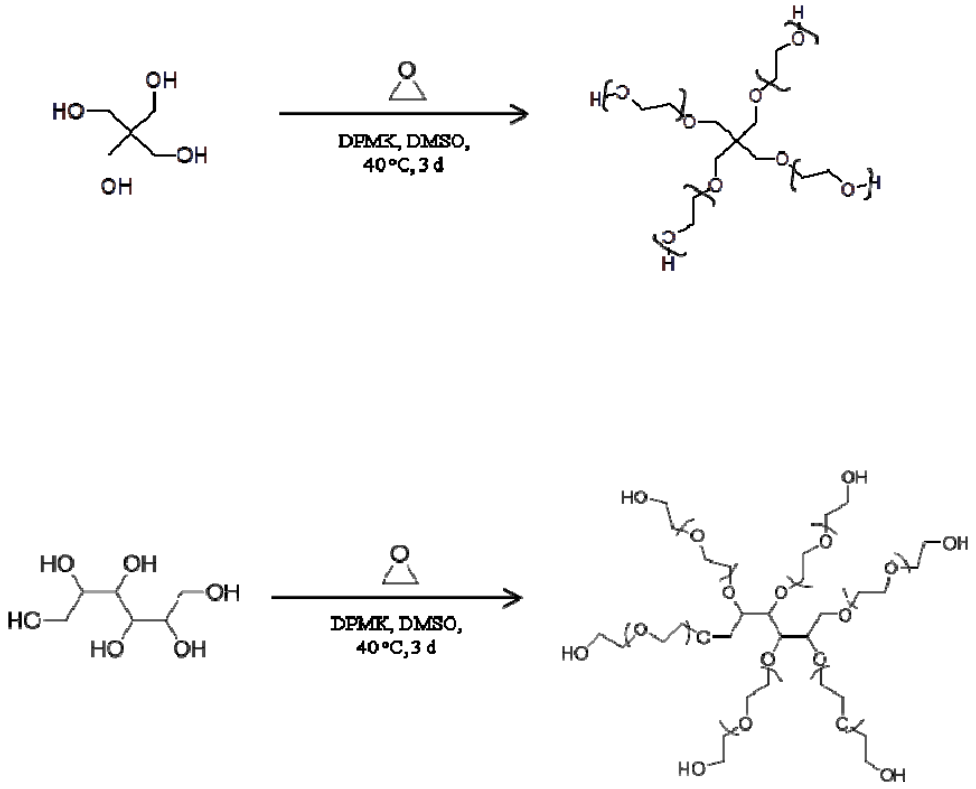
Protons are exchanged between dormant hydroxyl groups and alkoxides groups rapidly, the rate of exchange is much higher than the rate of propagation. This is cause to get well defined polymers that have narrow molecular weight distribution.

4-arm, 6-arm, and 8-arm star-shaped poly(ethylene oxide)s were synthesized with DPMK in DMSO via anionic polymerization. Pentaerythritol was chosen as an initiator of 4-arm poly(ethylene oxide)s, it has 4 hydroxyl groups in its molecules. Pentaerythritol as an initiator was dried in the vacuum oven at 50 ^{o}C for 24 h before use. Sorbitol for 6-arm poly(ethylene oxide)s was recrystallized from ethanol and water (10/1, v/v) to remove impurity[17]. To synthesis well defined poly(ethylene oxide)s, it was particular about control of DPMK. In the DMSO system, DPMK activate DMSO and form dimsyl anion that can initiate ethylene oxides. DPMK was used 20-30 moles % of hydroxyl group of initiators to obtain well defined star-shaped poly(ethylene oxide)s.

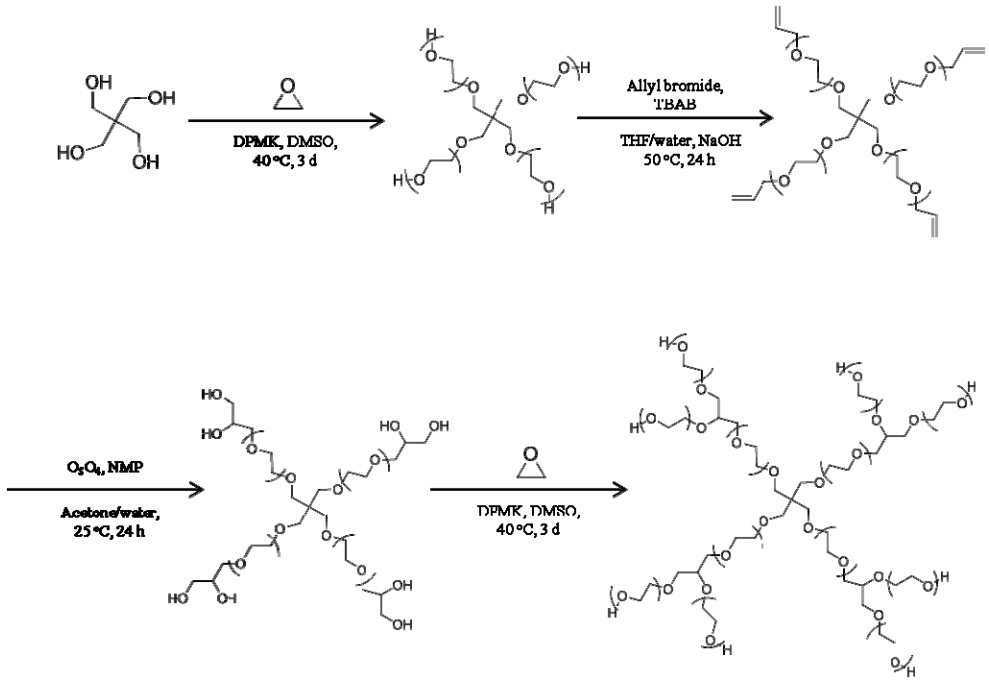


Scheme 2-4. Exchange of protons between dormant and active species during polymerization of EO ($R_{ex} >> R_p$).

Molecular weight and molecular weight distribution were successfully controlled even with 30 mole % activation of hydroxyl groups of initiators based on much faster rate of potassium in exchange reaction between activated and dormant chain ends, compared with the rate of propagation. [15]. Like pentaerythritol for 4-arm and sorbitol for 6-arm star-shaped poly(ethylene oxide)s, 8-arm star-shaped poly(ethylene oxide)s was also tried to synthesize using tripentaerythritol as an initiator. But it was impossible to synthesize 8-arm star-shaped poly(ethylene oxide)s using tripentaerythritol because it has low purity and insoluble in DMSO. 4-arm poly(ethylene oxide)s were used for macroinitiator to synthesis of 8-arm star-shaped poly(ethylene oxide)s. Hydroxyl groups of the end of 4-arm poly(ethylene oxide)s, branch points were introduced to make splitter.



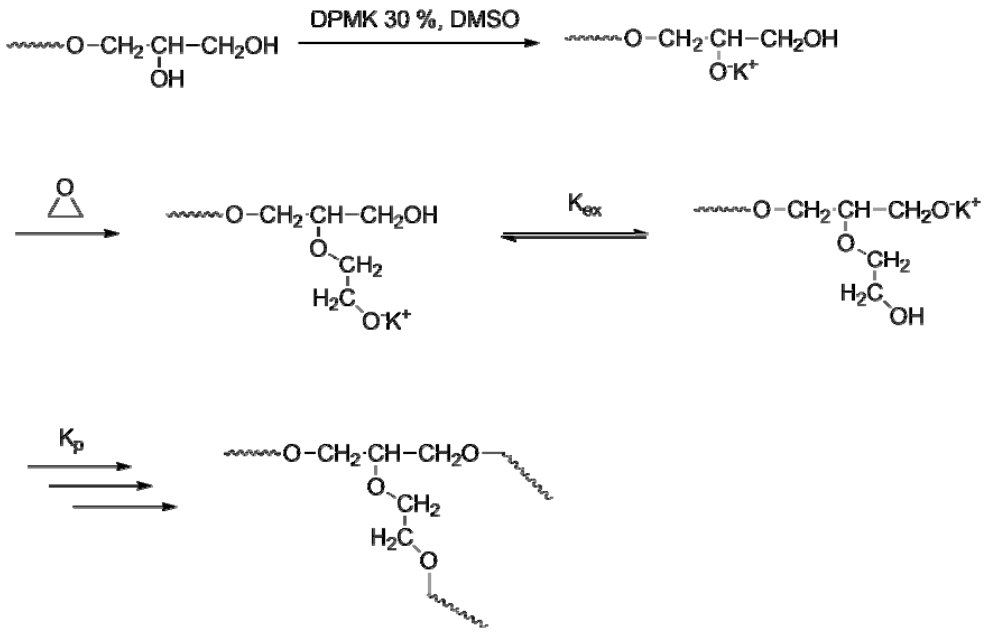
Scheme 2-5. Synthetic scheme of star-shaped poly(ethylene oxide)s (a) 4-arm star-shaped and (b) 6-arm star-shaped poly(ethylene oxide)s using pentaerythritol and sorbitol as an initiator, respectively.



Scheme 2-6. Synthetic scheme of 8-arm star-shaped poly(ethylene oxide)s.

There were many candidates for branch points which are contain ether bond, amide bond and ester bond to make higher generation of macromolecules[18]. The branch points had to be composed of ether bond because they are stable while DPMK activate initiator in anionic polymerization. Even though DPMK is relatively weak base catalyst than potassium naphthalene, ester bonds or amine bonds are possible for activating and degrading by DPMK.

For this reason, allylation and dihydroxylation were carried out for branch points and they consist of ether bonds[19]. Allyl bromide was reacted with hydroxyl groups at the end of polymers to introduce allyl groups, and then dihydroxylation was carried out with osmium tetroxide to obtain 2 hydroxyl groups at one chain end of polymers. As a result, 8 hydroxyl groups from 4-arm star-shaped poly(ethylene oxide)s were obtained and used for macroinitiators for 8-arm star-shaped poly(ethylene oxide)s. Only 30 mole % of hydroxyl groups were deprotonated using DPMK and exchanged of protons between primary and secondary alcohol and the exchange rate of proton is faster than that of propagation. This condition permitted the uniform growth of poly(ethylene oxide)s chains from the two types of hydroxyls carried by the branching points[15] .



Scheme 2-7. Growth of poly(ethylene oxide)s from branch point containing primary and secondary hydroxyl groups.

Dendritic polymers have a focal point, a precise number of branch points and terminal functional groups at the end of polymers. They have similar characteristics with regular dendrimers[4, 20].

Dendrimers have diverse advantages for their unique structures, applied for various fields for many decades. Especially, dendrimers containing poly(ethylene oxide)s have benefits for their chemical and physical properties and are widely used in pharmaceutical and biomedical fields. They have different properties compared to linear polymers, for example, ability of micelle formation, solution and salvation properties.

Y. Gnanou group has been studied for synthesis of star-shaped containing several generations and dendrimers with poly(ethylene oxide)s via anionic polymerization. They developed various kinds of dendrimers such as Janus type of dendrimers using different branch points to each site or dendrimers combination of two different kinds of monomers, poly(ethylene oxide)s and poly(styrene)s. They also synthesized bouquet-type dendrimers with poly(ethylene oxide)s.

In our group, dendritic poly(ethylene oxide)s which contain focal point on it for biomedical application were need, we have followed the method of polymerization that are synthesis of bouquet-type dendrimers

with poly(ethylene oxide)s[5, 21]. The advantages of synthesis for dendritic poly(ethylene oxide)s via anionic polymerization are that control of molecular weight of each branch and the whole of polymers is available, lead to possible to control the architecture of dendritic polymers. Molecular weight and molecular weight distribution of dendritic poly(ethylene oxide)s are obtained as we wanted and expected by control of polymerization condition.

3.3-diethoxyl propanol was chosen as an initiator as we mentioned above about the linear poly(ethylene oxide)s[17]. Branch points were introduced by same method like the method that introduce splitter at the end of 4-arm star-shaped poly(ethylene oxide)s to growing for 8-arm star-shaped poly(ethylene oxide)s[22]. Reaction with allyl bromide and hydroxyl groups is that phase transfer reaction. High concentrated NaOH aqueous solution and THF are separated in two phase and tetrabutylammonium bromide (TBAB) act as a catalyst between polymers and allyl bromide.

The reaction was proceeded with at 50 ^{o}C for 24 h and organic layer was removed by rotary evaporator. The remaining water phase extracted with methylene chloride (MC), the organic layer was dried by stirring with magnesium sulfate. After filtering, the polymers in MC were precipitated

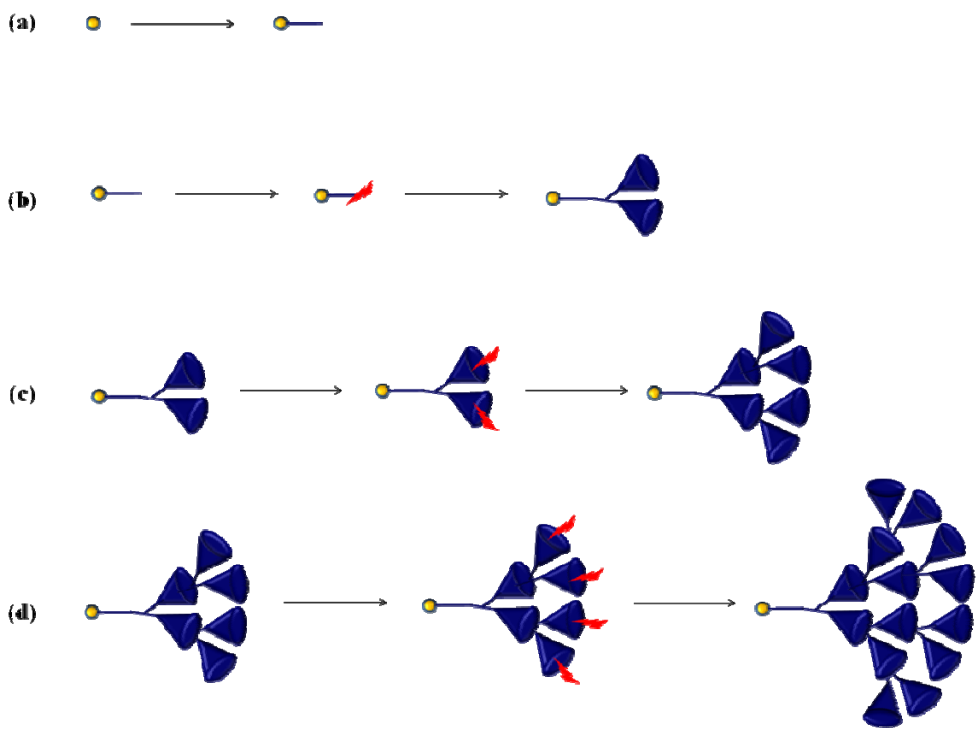
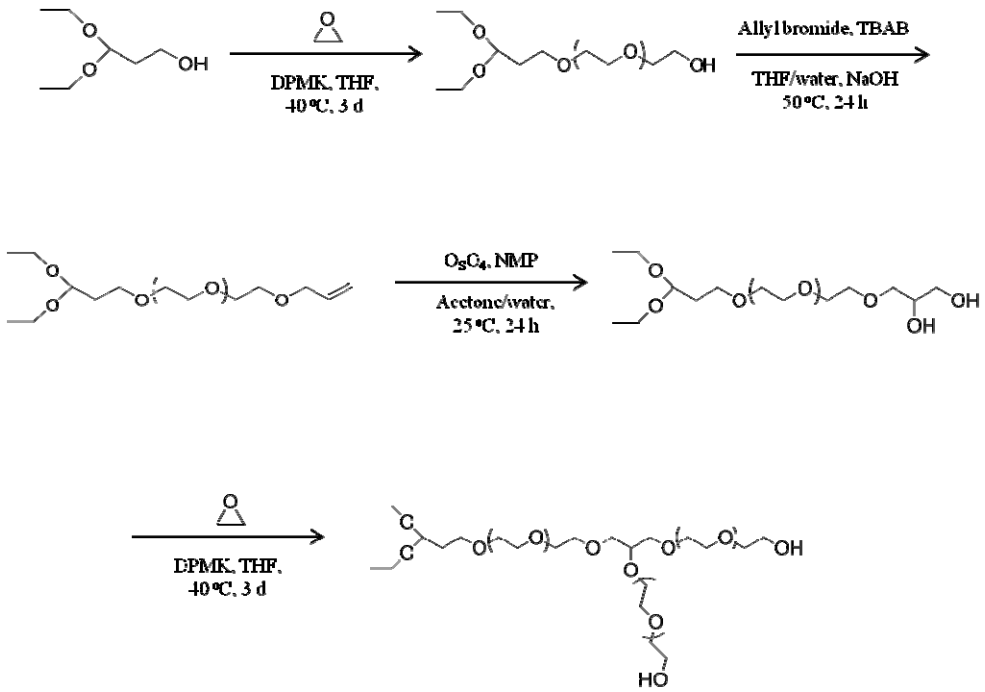


Figure 2-2. Synthesis of (a) G0 (b) G1 (c) G2 (d) G3 dendritic poly(ethylene oxide)s by divergent method.

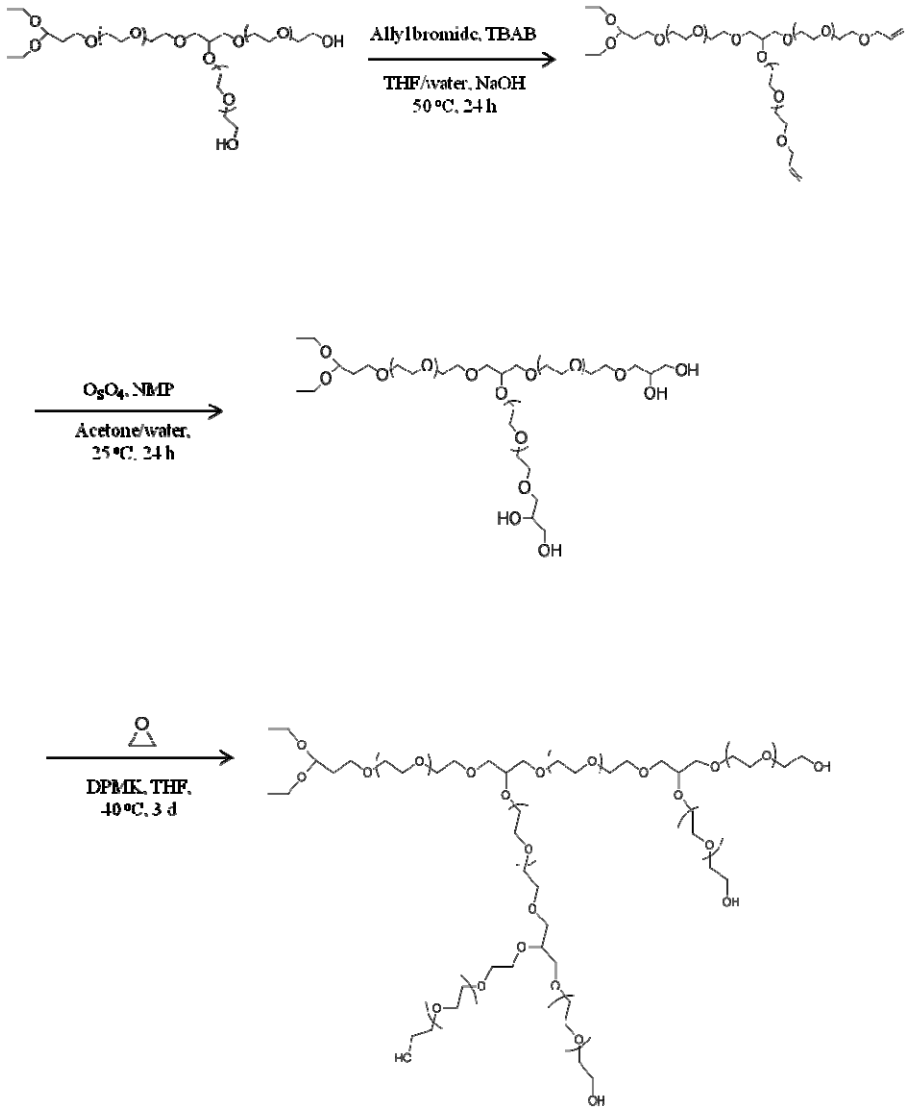
in cold diethyl ether twice, and dried in vacuum oven at 25 ^{o}C for 24 h.

Osmium tetroxide was used to generate 2 hydroxyl groups from 1 allyl group at the end of polymers. Acetone and double distilled water were used for solvent and N-methyl 2-pyrrolidone was used with osmium tetroxide. The reaction was carried out at room temperature for 24 h. After reaction, acetone was removed and the water phase was extracted with MC. The organic layer extracted and stirred with magnesium sulfate to remove remaining water. The polymers in MC were precipitated in cold ether twice, filtered and dried in vacuum oven at 25 ^{o}C for 24 h.

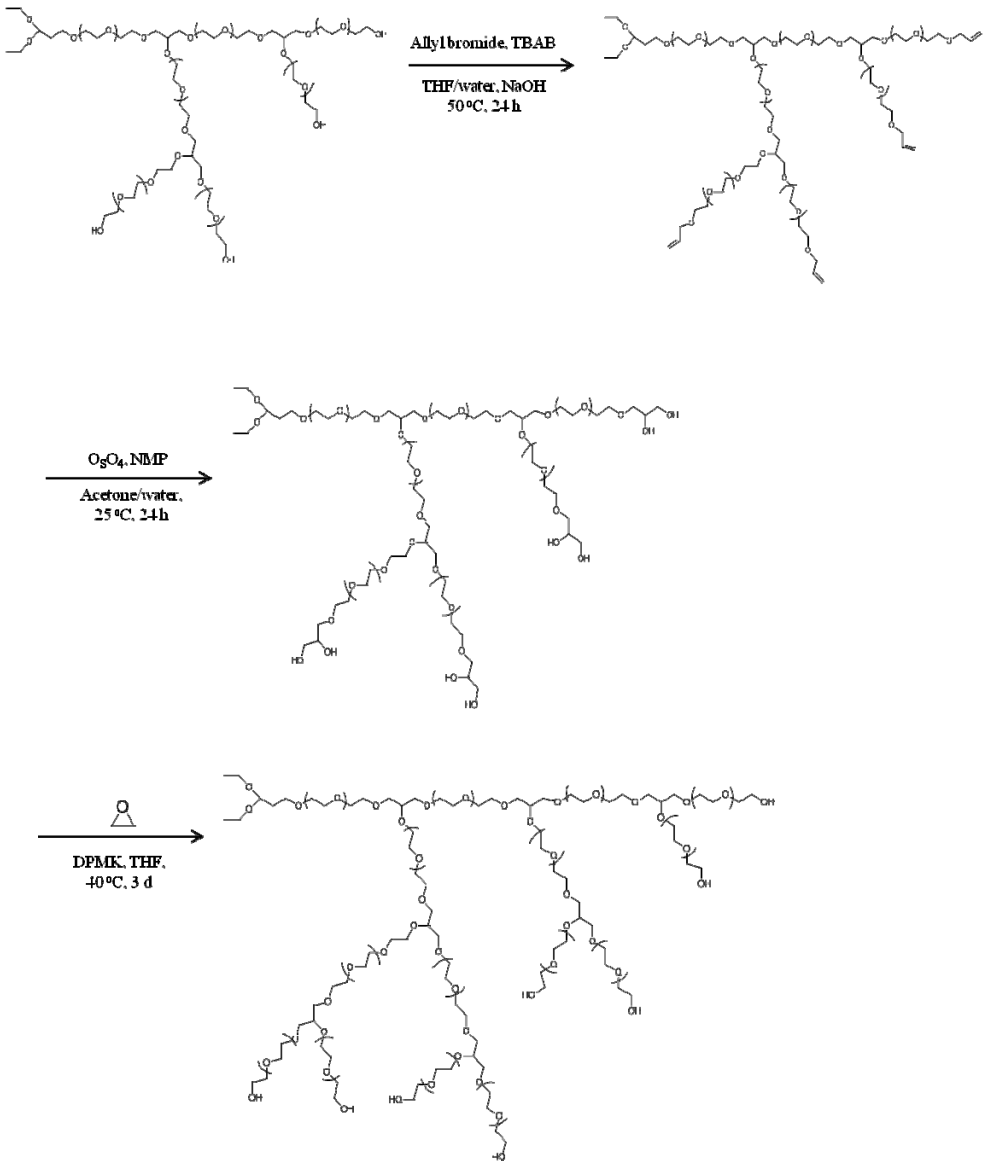
After forming branch points at the end of linear poly(ethylene oxide)s, polymerization method of generation 1 was carried out in 30 % moles of initiators of DPMK and in DMSO, because it is common method when synthesize growing generation of star-shape or dendritic poly(ethylene oxide)s. When multifunctional initiators are converted to alkoxides, they are not so soluble in THF. But we tried to synthesize generation 1 in THF, it worked successfully. Additionally, cosolvent system with THF and DMSO (1/1, v/v) and 50 % of moles of hydroxyl groups in initiator was also showed to obtaining well defined generation 1 poly(ethylene oxide)s. Synthesis of generation 2 and generation 3 were also tried by same method,



Scheme 2-8. Synthetic scheme of G1 poly(ethylene oxide)s.



Scheme 2-9. Synthetic scheme of G2 poly(ethylene oxide)s.



Scheme 2-10. Synthetic scheme of G3 poly(ethylene oxide)s.

and well defined polymers were obtained. It seems that alkoxides in high generation (up to generation 2) were not aggregated together because poly(ethylene oxide)s are well dissolved in solvent and the distance between alkoxides are far from each other.

We synthesized dendritic poly(ethylene oxide)s up to generation 3, and confirmed the polymer peak that are from generation 0 to generation 3 were shifted without any side reaction by GPC.

2.3. Results and Discussion

Star-shaped poly(ethylene oxide)s were synthesized in DMSO using pentaerythritol and DPMK as an initiator and a catalyst. The amount of DPMK was controlled to suppress a possible side reaction of liner PEG formation. The obtained molecular weight of 20 K was in good agreement with the target molecular weight. 4-arm poly(ethylene oxide)s with 8 hydroxyl end groups were prepared via allylation and the consecutive hydroxylation reaction. The presence of allylic double bonds at 5.33-5.11(CH=CH_2) and 6.03-5.79 (C*H*=CH_2) ppm and the disappearance of the peaks in ^{1}H NMR spectra confirmed the successful reaction without any noticeable side reactions. 8-arm PEG was obtained from the macroinitiator

using the same method with the molecular weight of 80 K and M_w/M_n value of 1.03. The shift of peak molecular weights and the narrow distribution in GPC traces supports the successful polymerization.

Polymerization was proceeded in a high vacuum system and 8-arm star-shaped poly(ethylene oxide)s were grown from 4-arm star-shaped poly(ethylene oxide)s macroinitiator was employed for further investigation.

Choice of proper solvent and initiator is an important factor to control the structure and the molecular weight distribution in anionic polymerization. DMSO was the solvent in the polymerization since initiators with several polar hydroxyl groups were not so soluble in THF, the mostly used solvent in anionic polymerization. DPMK was used as a catalyst which deprotonate hydroxyl groups of the initiator. DPMK can also deprotonated the methyl groups in DMSO to produce dimsyl anion, leading to the formation of undesirable linear poly(ethylene oxide)s as a side reaction. The amount of DPMK was carefully controlled less than 30 mol % of hydroxyl groups in the initiator to suppress the side reaction.

3,3-diethoxy propanol was chosen as an initiator of dendritic poly(ethylene oxide)s because it has acetal group that can convert to another functional groups for its application. High molecular weight dendritic

poly(ethylene oxide)s were synthesized by ring opening polymerization of EO under high vacuum system. DMSO was used as solvent during polymerization because multiple alkoxide groups at the end of dendritic poly(ethylene oxide)s were aggregated and not soluble in THF. To activate hydroxyl groups for initiation, DPMK was used as strong base catalyst. In DMSO system, DPMK can transfer to DMSO that leads to make dimsyl carbanion which are able to initiating polymerization. Because of this reason, 30 % DPMK of hydroxyl groups was used during polymerization in DMSO. Each polymerization was proceeded at 40 ^{o}C for 72 h.

Dendritic poly(ethylene oxide)s were synthesized by divergent method and each generation was obtained with a very narrow M_w/M_n values and excellent control of molar mass without any side reactions. We carried out allylation and dihydroxylation at each generation for introduction of branching points and confirmed by ^{1}H NMR. Allylic double bonds were observed at 6.03-5.79 (C*H*=CH_2) and 5.33-5.11(CH=C*H*$_2$) ppm. After dihydroxylation, allylic double bonds were disappeared completely. Polymerization and introduction of branch points were repeated and third generation dendritic poly(ethylene oxide)s were obtained and molecular weight is 78 K and molecular distribution was 1.04. That means

introduction of branching points was carried out successfully and there were no side reaction during polymerization.

2.4. Conclusion

Poly(ethylene oxide)s are attractive materials for their chemical and physical properties. They have been applied in various fields, especially in pharmaceutical and biomedical fields. Linear poly(ethylene oxide)s have been studied and applied in a large number of groups, but the research of star-shaped or dendritic poly(ethylene oxide)s was not so much. Star-shaped or dendritic poly(ethylene oxide)s have a plenty of advantages due to their unique structures. To use these polymers freely, controlling of structure was needed. For this reason, anionic polymerization method was used for synthesis of poly(ethylene oxide)s.

Synthesizing of poly(ethylene oxide)s were attempted in many kinds or methods. They were affected by kinds of initiators, catalyst and solvents. Consequently, linear polymers were synthesized in THF and star-shaped or dendritic poly(ethylene oxide)s were synthesized in DMSO using DPMK as a catalyst. All the polymers we obtained were no side reaction with narrow molecular weight distribution.

2.5. References

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Chapter 3.

Reduction-sensitive Polymeric Nanoparticles for Drug Delivery System

3.1. Introduction

Delivery systems efficiently circulating in the bloodstream require quite different material conditions from those for effectively releasing the encapsulated agents at an aimed place[1–6]. Hydrophilic, neutral, or negatively charged materials with three-dimensional stability are preferred for long-term circulation[7], while selective release via cellular uptake is improved with hydrophobic, positively charged delivery system with structural dissociation at a target place[8,9]. Development of materials sensitive to external stimulus makes it realized to obtain such a delivery system and we define those as stimuli-sensitive, intelligent materials that respond to minute changes in environments by large changes in their physicochemical properties such as solubility, coil-globule transition, three-dimensional stability, and so on[10]. Stimuli-sensitive polymeric micelles and self-aggregates have been explored to encapsulate large amount of bioactive agents as well as to protect them during the circulation, and eventually to selectively release the agents at an aimed place. Most investigated triggers are changes in pH, temperature, enzymatic cleavage, or specific chemicals [8,9,11–15]. Among them, disulfide bonds were reported as a specific stimulus differentiating the environ-mental conditions of delivery

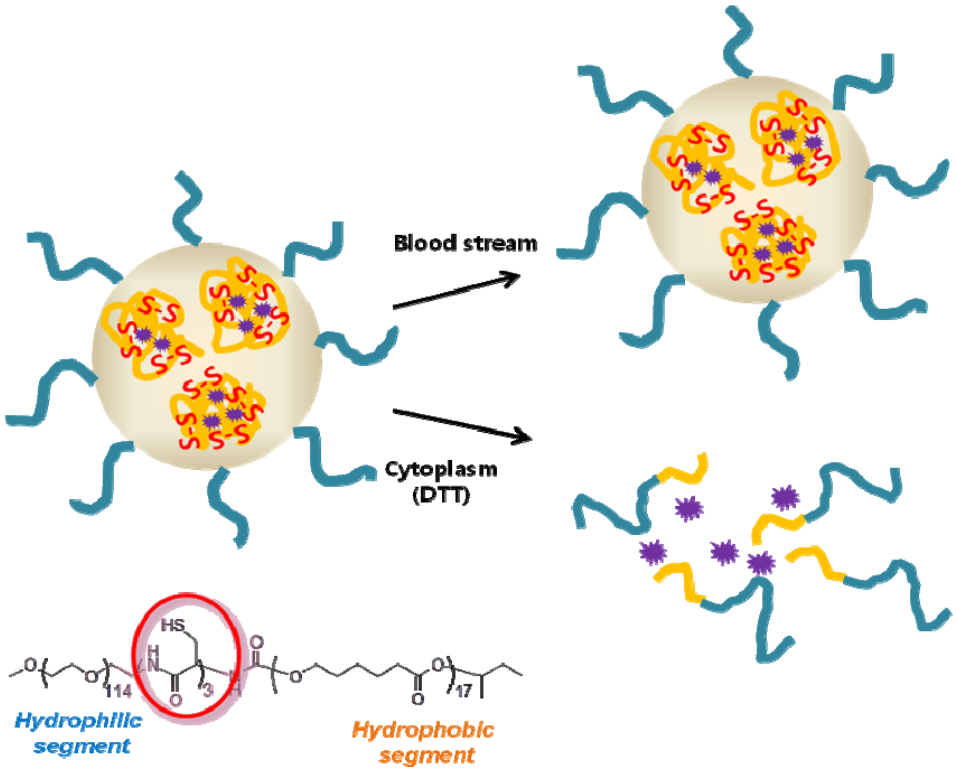


Figure 3-1. Illustration of reductive-sensitive mPEG-Cys-PCL nanoparticles.

system before and after cellular uptakes as a form of endosomes. The tendency of endosomes to lower their pH for eventual fusion with lysosomes and relatively higher concentration of glutathione in the cytoplasm make the environment after cellular uptakes more susceptible to reductive conditions. Most studied reduction-sensitive delivery systems are designed to trigger sudden burst of encapsulated drugs by disintegrating their three-dimensional structures caused from degradation of disulfide bonds when they face a low pH condition in endosomes or increased concentration of thiol-rich compounds[16–19]. In this study, copolymers composed of biocompatible poly(ethylene glycol) (PEG) and poly(e-caprolactone) (PCL) were functionalized by three cysteine amino acid residues incorporated at the junction of the two polymeric blocks.

Synthetic procedure and the formation of self-aggregates were investigated and stimuli-sensitive release profile was evaluated for an application of the developed copolymers as an efficient stimuli-sensitive delivery system.

3.2. Experimental Section

Materials. HCl (2M) in diethyl ether, ε-caprolactone (99 %), 1-

methoxy-2 propanol (99.5 %), trifluoroacetic acid (99.0 %), piperidine (99.0 % succinic anhydride (SA, 99.0 %), 4-dimethylamino pyridine (DMAP, 99.9%), N-hydroxy succinimide (NHS, 98%), and N',N'-dicyclohexyl carbodiimide (DCC, 99.0%) were purchased from Sigma–Aldrich (St. Louis, MO). Methoxy PEG amine (MW: 5 k, 95 % and Fmoc-Cys(trt)-COOH were commercially available from Sunbio (Korea) and Anygene (Korea). D,L-Dithiothreitol (DTT, 99.0%) was obtained from Fluka. Dichloromethane, methanol, and 1,4-dioxane were purchased from Daejung Chemicals and Metals Co. (Korea). Dichloromethane was dried over calcium hydride and 1,4-dioxan was freshly distilled over sodium before use. All other chemical were used as received.

Instruments. Chemical structure was determined by ^{1}H NMR analysis using Bruker Avance 300MHz spectrometer in $CDCl_3$ or DMSO-d_6 at room temperature. Molecular weight and its distribution were determined using gel permeation chromatography equipped with a Shimadzu RID-10A refractometer detector and columns of Styragel HR 3,HR 4, and HR 4E. THF was employed as an eluent with the flow rate of 1mL·min^{-1} and polystyrene or PEG standards were used for calibration.

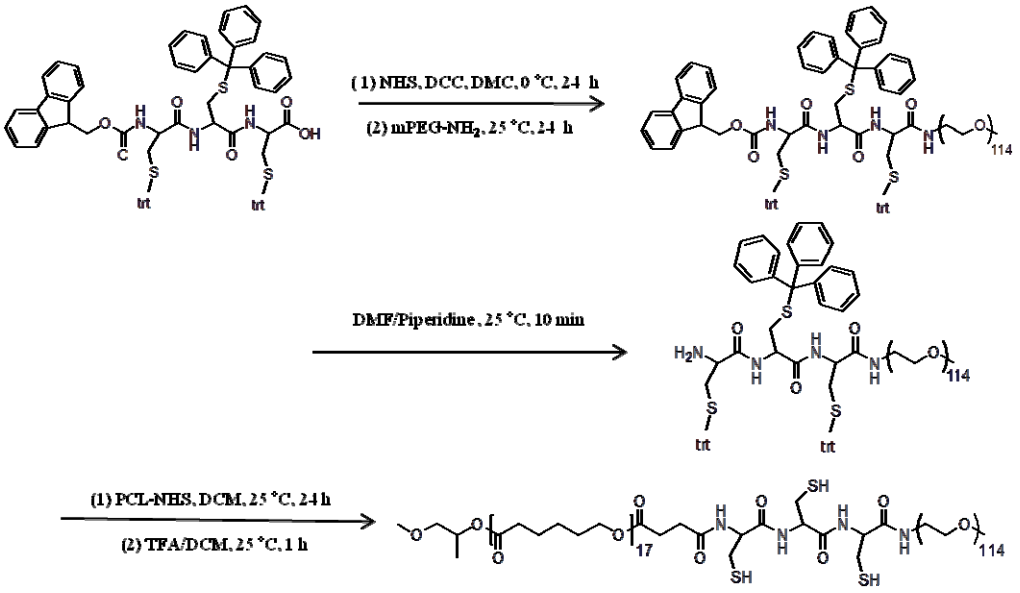
Measurement of particle size based on intensity was carried out using Otsuka ELS-Z size analyzer equipped with He–Ne laser at a wavelength of 630 nm. Critical aggregate concentration (CAC) of mPEG-Cys(trt)-PCL was determined in different concentrations from 1.0 $mg \cdot mL^{-1}$ to 1.0×10^{-3} $mg \cdot mL^{-1}$ as previously reported for the measurement of critical micelle concentration[20] and obtained using Shimadzu UV-1650 PC at the wavelength of 230 nm and Shimadzu RF-500 spectrofluorophotometer. Averaged values of particle size, UV absorption, and fluorescence were calculated with the data from three runs.

Fmoc-Cys(trt)-COOH (0.38 g, 0.30mmol) was dissolved in 10mL anhydrous dichloromethane in a 50 mL round-bottom flask. DCC (0.08 g, 0.36mmol) and NHS (0.04 g, 0.36mmol) were introduced and the reaction mixture was stirred for 12 h under nitrogen atmosphere at 0 ^{o}C. Dicyclohexylurea (DCU) was removed by filtration at least three times, and the mixture was precipitated in 10-fold volume excess cold diethyl ether. The precipitate was filtered to produce Fmoc-Cys(trt)-NHS after drying under vacuum for 24 h (0.35 g, 95%).

Fmoc-Cys(trt)-NHS (0.35 g, 0.25 mmol) was placed in a 100mL two-neck round-bottom flask and dissolved in anhydrous dichloromethane under nitrogen atmosphere. Methoxy PEG amine (1.15 g, 0.23mmol) in dichloromethane was added into the solution and the reaction continued for 12 h. The mixture was precipitated in diethyl ether, filtered, and dried in vacuum at 25 ^{o}C for 24 h to produce mPEG-Cys(trt)-Fmoc (1.30 g, 90%).

Selective deprotection of Fmoc over trityl protecting groups was carried out in 10mL piperidine/DMF (1/1 v/v) solution at 25 ^{o}C for 10min. The reaction mixture was poured into 10-fold excess amount of diethyl ether, filtered, and dried under vacuum for 24 h to produce mPEG-Cys(trt)-NH_2 (0.78 g, 82 %).

Monohydroxy-terminated PCL (PCL-OH) was synthesized by ring-opening polymerization as previously reported[21]. Anhydrous 1,4-dioxane (50 mL) dissolving PCL-OH (5.0 g, 2.5 mmol) and DMAP (0.5 g, 5.0 mmol) were introduced in a 500mL two-neck round-bottom flask equipped with N_2 inlet-out. SA (0.5 g, 5.0mmol) was added to the solution and the reaction was continued for 24 h at 60 ^{o}C. The solution was filtered, condensed, and precipitated in 10-fold volume excess cold methanol. Crude precipitate was dissolved again in dichloromethane and washed with hydrochloride



Scheme 3-1. Synthetic procedure of mPEG-Cys-PCL.

acid (10 % v/v) and with a saturated NaCl solution. The organic phase was dried over magnesium sulfate and condensed solution was precipitated into cold methanol, filtered, and dried under vacuum for 24 h at 25 ^{o}C to produce PCL-COOH (4.1 g, 78 %).

PCL-COOH (2.5 g, 1.2 mmol) and DCC (0.5 g, 2.4 mmol) was dissolved in 25 mL anhydrous dichloromethane and introduced into a 50 mL two-neck round-bottom flask equipped with N_2 inlet-out. NHS (0.28 g, 2.40 mmol) was added and the reaction mixture was stirred for 12 h under nitrogen atmosphere at 0 ^{o}C. DCU was removed by filtration at least three times and the solution was precipitated in 10-fold volume excess cold methanol. The precipitate was filtered and dried under vacuum for 24 h to produce PCL-NHS (2.1 g, 80%, M_n =1860 and M_w/M_n = 1.22 by GPC). PCL-NHS (0.19 g, 0.09mmol) dissolved in anhydrous dichloromethane was placed in a 100mL two-neck round-bottom flask equipped with N_2 inlet-out.

mPEG-Cys(trt)-NH_2 (0.50 g, 0.08mmol) in anhydrous dichloromethane was added into the solution and the reaction continued for 12 h. The mixture was precipitated in cold diethyl ether and filtered to produce mPEG-Cys(trt)-PCL. The product was dried in vacuum at 25 ^{o}C for 24 h (0.58 g, 90%, M_n = 6670 and M_w/M_n = 1.25 by GPC).

Deprotection of trityl groups was carried out with TFA in dichloromethane. After 1 h, the reaction mixture was precipitated in diethyl ether and filtered. Obtained mPEG-Cys-PCL was dried under vacuum for 24 h at 25 ^{o}C (0.38 g, 80 %).

mPEG-Cys-PCL (20mg) was dissolved in 10 mL DMF and excess DTT was added as a reducing agent of disulfide. The solution was stirred for 12 h at room temperature and 2 mL aliquot was added to 10 mL double distilled water. After vigorous stirring for 12 h, the solution was dialyzed against double distilled water for 3 d to remove excess DTT and organic solvent. The self-aggregates underwent cross-linking reaction by oxygen present in the air during the dialysis. The solution was passed through a 0.22mm filter to produce disulfide stabilized self-aggregates[22–24].

Accurately weighed doxorubicin and 20 mg copolymer were dissolved in 10 mL DMF and self-aggregates encapsulating drugs were formed using the same procedure as described above. Doxorubicin loading amounts was calculated from the weight percentage of the loaded drug divided by the total weight of the loaded drug and the polymer. One milliliter solution of self-aggregates containing doxorubicin was placed in a pre-swollen cellulose dialysis membrane with the molecular weight cutoff

of 3.5 k and two membranes were incubated in 25 mL distilled water at 37 ^{o}C. After 24 h, a dialysis tube was placed and incubated in 1 mM DTT solution, while the other kept incubated in distilled water as a control. At an appropriate time interval, the media was changed and the amount of doxorubicin was measured by the UV–Vis absorbance at 480 nm.

3.3. Results and Discussion

Block copolymers with three cysteine amino acids at the junction of hydrophilic PEG and hydrophobic PCL was synthesized by conjugating the two polymers to a peptide, Fmoc-Cys(trt)-COOH, where N-terminal of Cys-Cys-Cys was protected with Fmoc group and pendant thiol groups were blocked with trityl groups. Methoxy PEG amine with molecular weight of 5k was conjugated to Fmoc-Cys(trt)-NHS and the conjugation was confirmed by ^{1}H NMR based on the integration ratio between Fmoc aromatic peaks around 7.7 ppm and PEG proton peaks at 3.21–3.86 ppm.

Ring-opening polymerization of cysteine NCA with methoxy PEG amine as a macroinitiator did not work with this system due to the difficulty in controlling the number of oligomerized cysteines down to a small number. Fmoc protecting group was selectively removed to produce PEG-Cys(trt)-

NH_2 under basic condition without affecting acid-sensitive trityl groups[25].

ε-Caprolactone was polymerized with 1-methoxy-3-propanol as an initiator and 2 M HCl in ether as a catalyst to produce PCL with molecular weight of 1960 by ^{1}H NMR[21]. PCL, activated by consecutive reactions with SA and NHS, was conjugated to the N-terminal of PEGylated peptide, followed by deprotection of trityl groups using TFA in dichloromethane.

Figure 3.1 shows a representative ^{1}H NMR spectra of mPEG-Cys(trt)-PCL and mPEG-Cys-PCL, where the disappearance of trityl peak at 7.2–7.4 ppm and the other intact peaks after the reaction support the complete and efficient deprotection. In addition, shift of peak molecular weight after the conjugation and narrow molecular weight distribution in GPC trace without any noticeable shoulder formation in Figure 3.2 confirmed that mPEG-Cys- PCL copolymer was successfully synthesized.

Particle size of nanostructure prepared by amphiphilic copolymers is known to depend in large part on the preparation methods. In case of mPEG-PCL copolymer, dialysis was reported as an inappropriate way to prepare micelles and produced large self-aggregates up to 1 μm;[22] however, in mPEG-Cys-PCL system, dialysis was the most efficient method to endow solvent and the solution was poured into excess amount of water

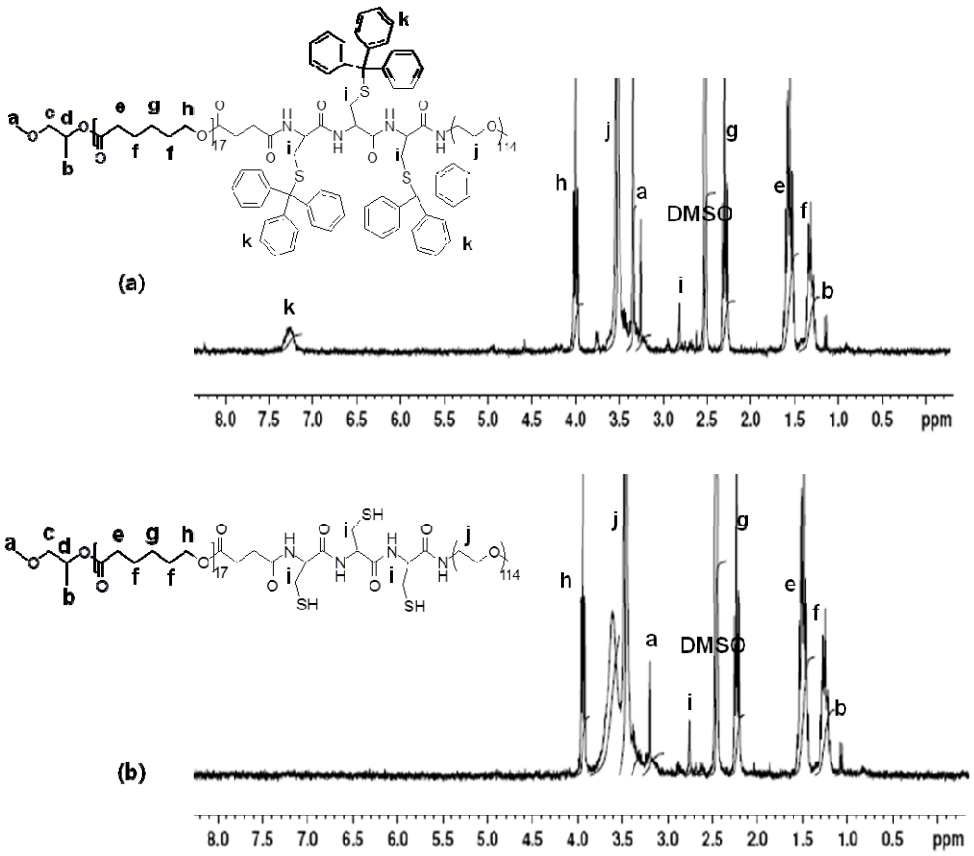


Figure 3-2. ^{1}H NMR spectra of (a) mPEG-Cys(trt)-PCL and (b) mPEG-Cys-PEG in DMSO-d_6.

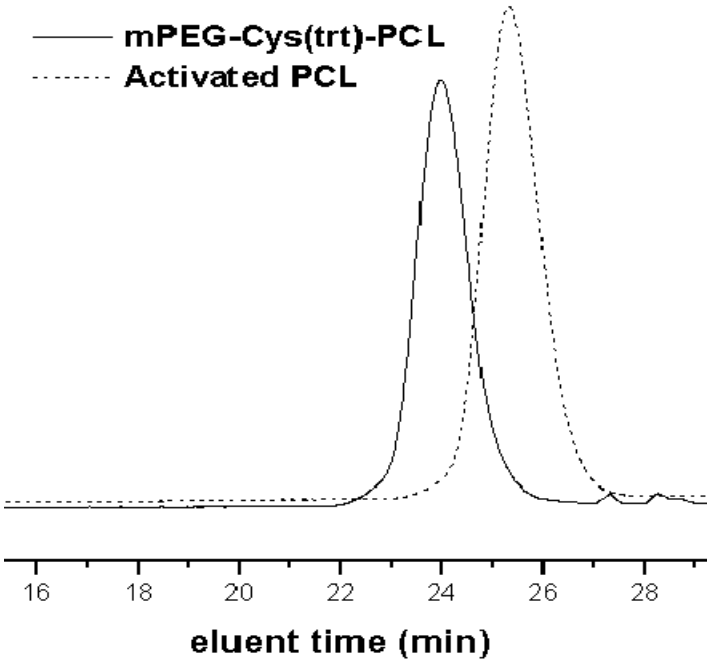


Figure 3-3. GPC traces of activated PCL and mPEG-Cys-PCL using THF.

before the dialysis, was employed to prepare relatively small-sized nanostructures. The nanostructures with intermolecular disulfide bonds even though it could not avoid increasing the size. A modified O/W dialysis system[26], where polymers were dissolved in a small volume of water-miscible organic as an eluent.

mPEG-Cys-PCL copolymer and excess amount of DTT were dissolved in DMF and transferred into distilled water to induce the self-assembling process and dialysis was followed to complete the formation. Average size of the self-assembled structures analyzed by dynamic light scattering was 206 and 184 nm before and after deprotection of trityl protecting groups, respectively, which did not fall in the size range to claim the structures as micelles. Instead, the size was close to be defined as self-aggregates and CAC was determined as 0.07 $mg \cdot mL^{-1}$. CAC was not observed for the stabilized self-aggregates when the solution concentration was diluted down to 0.001 $mg \cdot mL^{-1}$. The size and distributions of self-aggregates in each stage are illustrated in Figure 3.3.

The aggregates were stabilized by the formation of intermolecular disulfide bonds among thiol groups via oxidation during the dialysis against

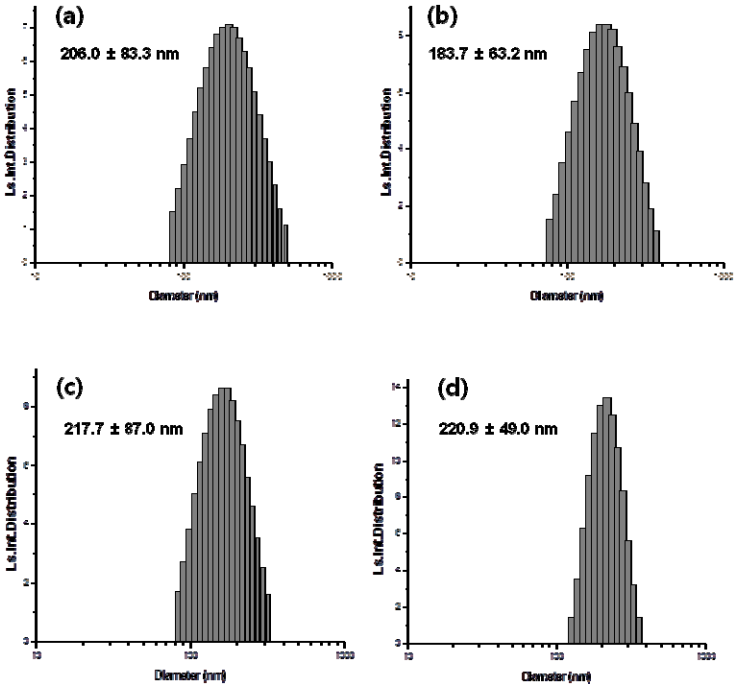


Figure 3-4. Size distribution of self-aggregates (a) before and (b) after deprotection, and size distribution of (c) stabilized and (d) drug-loaded stabilized self-aggregates.

double distilled water to remove DMF and excess DTT. The quantitative analysis to confirm the formation of disulfide bonds between the block copolymers in the aggregates was performed using Ellman's method[27].

The concentration of thiol groups without cross-linking was measured to be 143.5 ± 0.23 μmol, whereas the value decreased down to 12.2 ± 0.19 μmol after the cross-linking. Based on the previous report that less than 10% thiol groups were observed even after the cross-linking in comparison to the initial condition[28], it was concluded that the quantitative formation of disulfide bonds were successfully achieved. Increase in the mean particle size to 218 nm after stabilization was negligible when the size distribution was taken into account, but disulfide-stabilized self-aggregates were free from CAC and remained stable regardless of the concentration due to the intermolecular chemical bonding which prohibited the structural dissociation of the aggregates into individual polymer chains.

Doxorubicin- encapsulated stabilized aggregates were achieved by the same procedure with comparable particle size of 221 nm. The redispersion properties of the technique as a carrier for drug controlled release. After the freeze drying and the redispersion in the aqueous media, the size of self-aggregate without drugs decreased approximately 20 nm

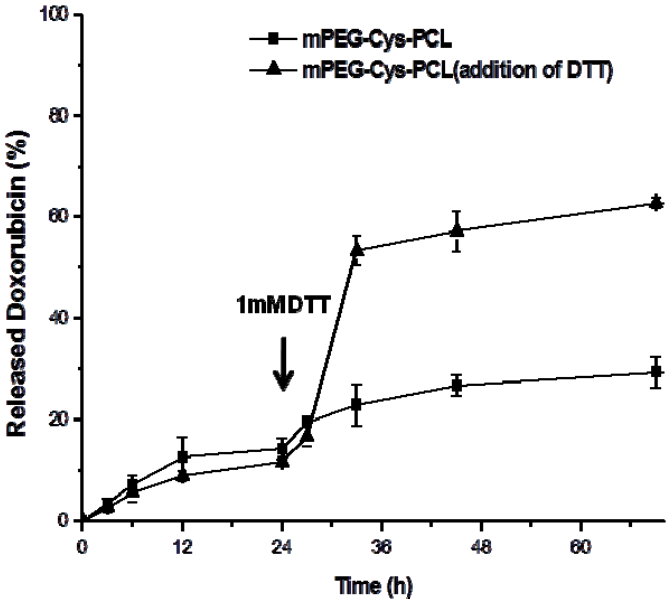


Figure 3-5. In vitro doxorubicin release profile from stabilized self-aggregates below CAC with (▲) and without (■) addition of DTT in aqueous medium at 37 ^{o}C.

down to 190 nm, while that of drug-loaded self-aggregates increased by 5 nm. In both cases, the difference was within the error range and it was concluded that redispersion of the dried nanoparticles did not make a noticeable change.

Doxorubicin-loading amount and efficiency was around 8.7 and 26.0%, respectively, and were not affected by disulfide stabilization. The stabilized-aggregates from mPEG-Cys-PCL copolymer meet the conditions of reduction-sensitive and effective targeting drug delivery system by sudden burst in the cytoplasm, while the delivery system maintains its stability during circulation in the bloodstream.

Release profile of doxorubicin was monitored with two samples, which were prepared and stabilized at the concentration above the CAC, and then incubated in distilled water below CAC at 37 ^{o}C. As shown in Figure 3.4, typical burst effect was not observed for the first 24 h. After 24 h, a dialysis tube was placed and incubated in 1mM DTT solution, where DTT worked as a reducing agent of disulfide bond instead of glutathione to mimic the cytoplasm environment.

The presence of DTT destabilized the self-aggregates via competitive dissociation of preformed disulfide bonds by inherent cysteine

thiol groups and the following disassembly of the aggregates below CAC condition triggered a burst release of doxorubicin, whereas the sustained release profile was maintained from the stabilized self-aggregates encapsulating doxorubicin in water without DTT.

Improved structural stability below CAC and burst release of encapsulated drugs responding to the external stimulus of DTT assess the successful development of a reduction-sensitive and specifically targeted drug delivery system.

3.4. Conclusion

Stabilized self-aggregates via intermolecular disulfide bonds were obtained with mPEG-Cys-PCL copolymer and the release profiles of encapsulated doxorubicin were characterized below CAC at 37 ^{o}C. Intermolecular disulfide bonds stabilized the polymeric aggregates even in the diluted condition below CAC and the stabilized self-aggregates displayed a typical sustained release profile. Destabilized aggregates burst encapsulated doxorubicin in the reductive condition by the addition of DTT. The delivery

System from the investigated copolymers is expected to be stable in

the bloodstream due to the absence of CAC and thiol-sensitive delivery system at a target place after endocytosis.

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Chapter 4.

8-Arm Star-shaped Poly(ethylene oxide)s for Cell Delivery

4.1. Introduction

Star-shaped poly(ethylene oxide)s have been used for surface modification on the solid surfaces[1]. They have lower hydrodynamic volume and good solubility than linear polymers due to their globular structure. Multiple functional groups at the end of polymers have lots of advantages for the further modification or application[2].

Star-shaped poly(ethylene oxide)s are synthesized by ring opening of ethylene oxide via anionic polymerization method. 4-arm, 6-arm, 8-arm star-shaped poly(ethylene oxide)s are synthesized in two kinds of synthesis; core first and arm first[3]. These two methods have some merits and advantages. Core first method was chosen to synthesize star-shaped poly(ethylene oxide)s because there are problems to remove the linear poly(ethylene oxide)s after synthesizing star-shaped poly(ethylene oxide)s via arm first method. 8-arm poly(ethylene oxide)s were selected for application of cell surface modification and was synthesized from 4-arm star-shaped poly(ethylene oxide)s precursor[4].

All polymerizations were carried out in DMSO as a solvent. 4-arm star-shaped poly(ethylene oxide)s and initiator of 4-arm star-shaped poly(ethylene oxide)s have multiple hydroxyl groups, they aggregate

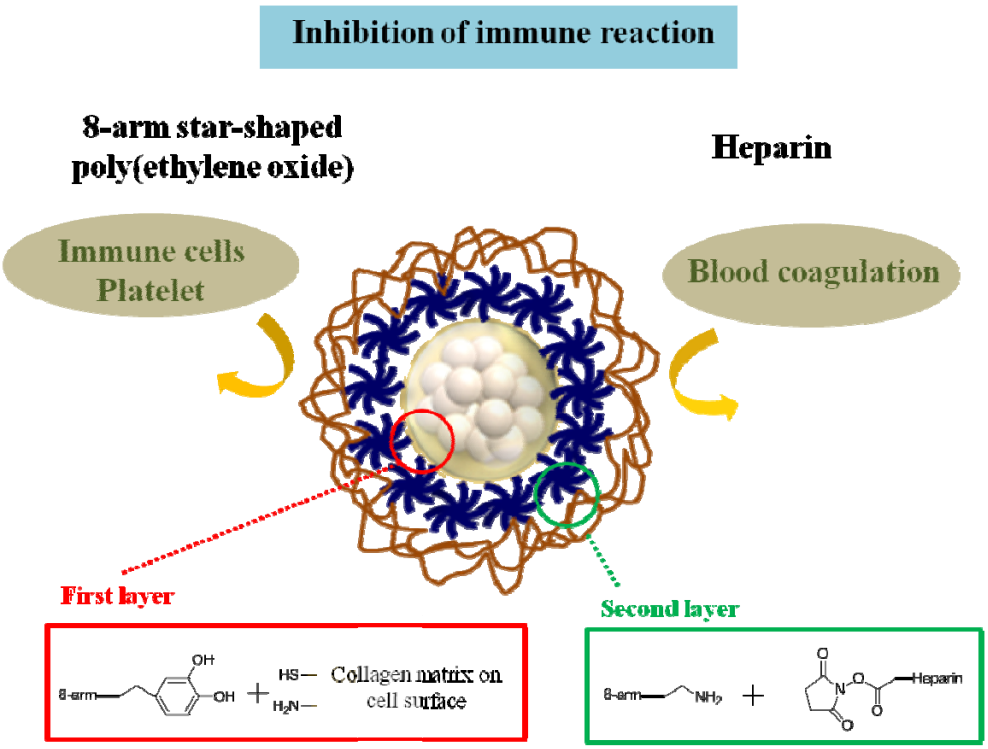


Figure 4-1. Illustration of immunoprotection of 8-arm star-shape PEO and UFH double layer on islet surface.

together due to high tendency in THF. And initiators were activated partially by use of 30 % moles of hydroxyl groups in initiators. Well defined 8-arm star-shaped poly(ethylene oxide)s were applied for modification of islet[5].

Cell transplantation has been studied for diabetes for many years. Pancreatic islets are used for drugs to treat type 1 diabetes with staying for as long as possible after transplantation[6]. But there are serious immune response by contact with surface directly and lead to cell death. We supposed 8-arm star-shaped poly(ethylene oxide)s showed effective shield effects due their bulky structure and were applied for the further modification using their multiple end functional groups[7].

At the end groups of 8-arm poly(ethylene oxide)s catechol groups were conjugated for modification on cell surfaces. Catechol groups react with amines and thiol chemically in pH 8 within 1 h[8]. Two catechol groups were introduced to each 8-arm star-shaped poly(ethylene oxide)s and remaining hydroxyl groups are converted to amine groups for conjugation with unfractionated heparin(UFH)[9]. UFH was used for prevention of blood coagulation and immune reaction against transplanted pancreatic islets through portal vein. The immune suppression effects on pancreatic

islets with this double layer are confirmed by covering effect, cell viability, and survival rate of cells[10].

4.2. Experimental Section

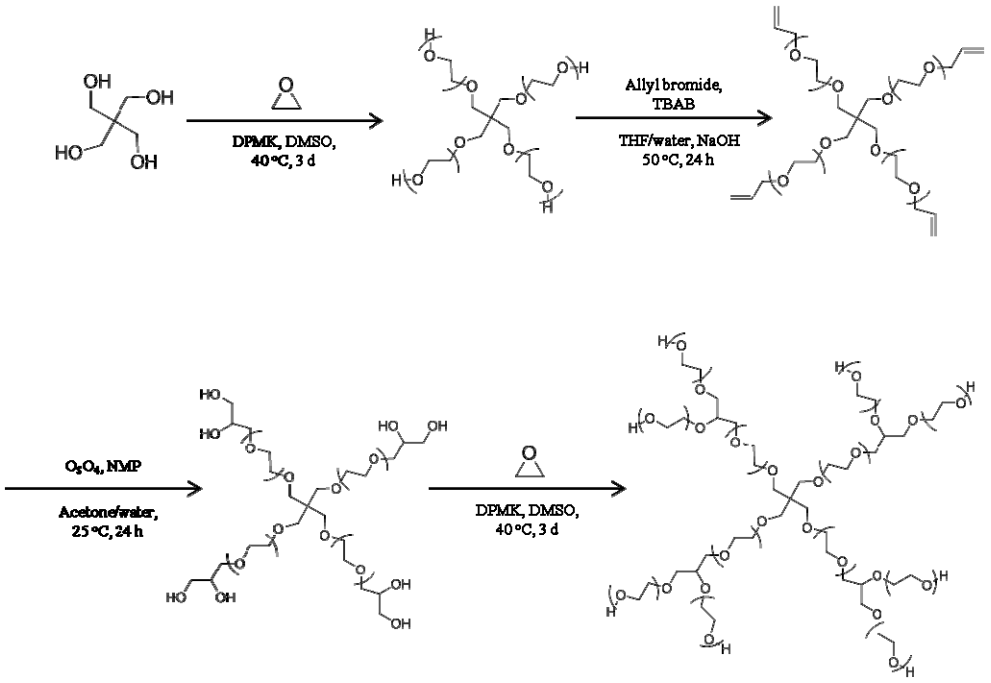
Materials. Ethylene oxide (EO) was distilled over CaH_2. Tetrahydrofuran (THF) and Dimethyl sulfoxide (DMSO) were distilled over CaH_2 prior to use. 1,4-dioxane was distilled over sodium. Diphenylmethyl potassium (DPMK) was prepared and titrated as previously reported. All other chemicals were purchased from Sigma-Aldrich and used without further purification

Instruments. Gel permeation chromatography (GPC) was used to determine molecular weights and molecular weight distributions, M_w/M_n of polymer samples using poly(ethylene glycol) standards (Polymer Laboratories a part of VARIAN). The system configuration was consisted of refractive index detector (Shimadzu RID-10A refract meter) and Styragel HR 3, HR 4 and HR 4E column in series. The flow rate of tetrahydrofuran (THF) as an eluent was 1 mL/min at 40 ^{o}C. ^{1}H NMR spectra of the polymers were obtained on a Bruker Avance-300 spectrometer. Sample

concentrations were about 10 % (w/v) in $CDCl_3$.

4-arm poly(ethylene oxide) was synthesized by living anionic ring opening polymerization of ethylene oxide (EO) in high vacuum system. Diphenylmethyl potassium (DPMK) was introduced at -78 ^oC into a 500 mL flask charged with the pentaerythritol (0.06 g, 0.44 mmol) in 100 mL anhydrous DMSO[11]. The mixture was slowly heated to room temperature and stirred until the color was changed from red-orange to yellow. The flask was chilled down to -78 ^oC and EO (8.82 g, 200 mmol) was added. The polymerization was carried out at 25 ^oC for 72 h and quenched by the addition of a few drops of methanol[5]. The solution was concentrated and precipitated in cold diethyl ether twice. After filtration, the product was dried in vacuum for 24 h to produce 4-arm poly(ethylene oxide). The molecular weight was 20 K and M_w/M_n was 1.02.

To introduce branch point at the periphery of 4-arm poly(ethylene oxide)s, tetrabutylammonium bromide (0.10 g, 0.02 mmol,) and NaOH (0.18 g, 2.5 mmol) in 1.7 mL water were introduced in a 50 mL 2-neck round bottom flask equipped with nitrogen inlet-out. 4-arm poly(ethylene

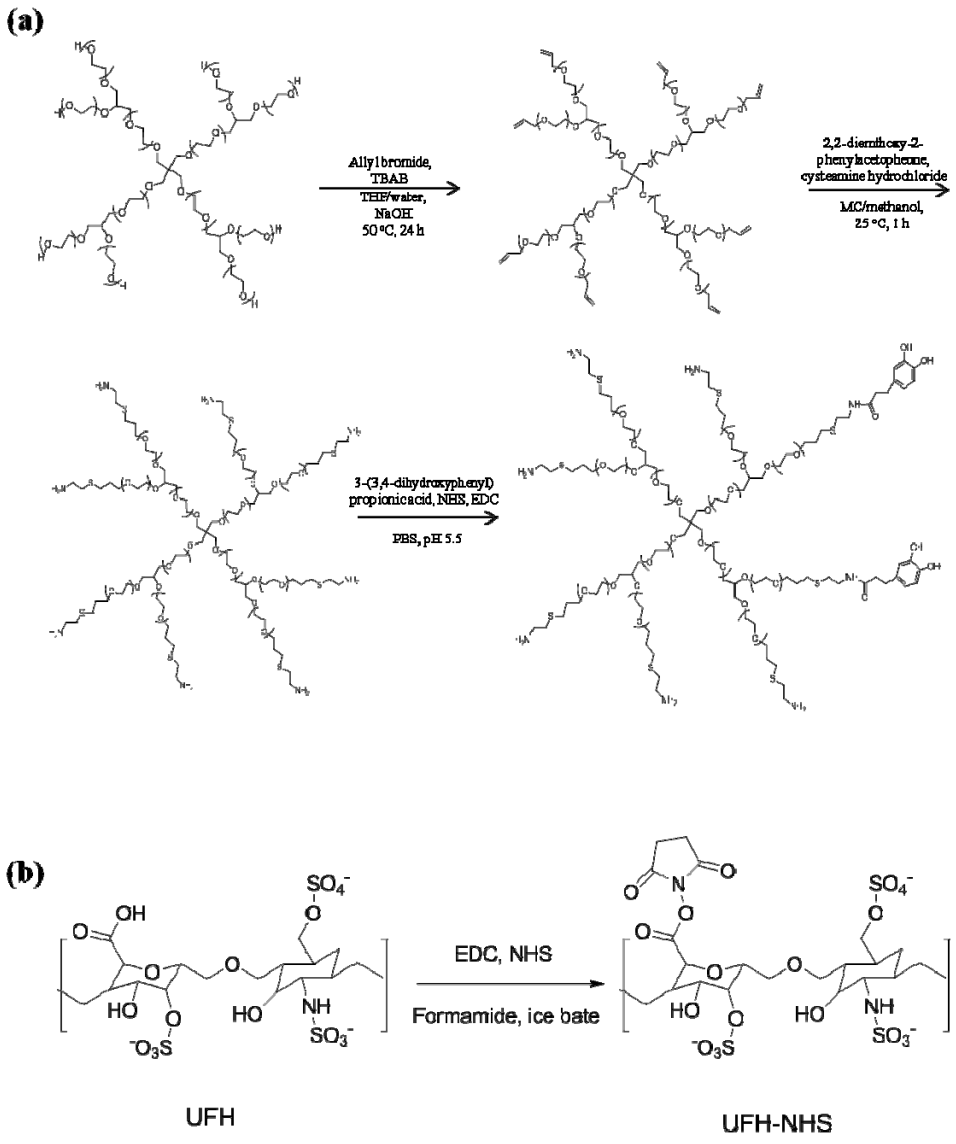


Scheme 4-1. Synthetic scheme of 8-arm star-shaped poly(ethylene oxide)s.

oxide)s (5.00 g, 0.25 mmol) in 4 mL THF was added and the mixture was stirred at 50 ^{o}C for 30 min followed by the addition of allyl bromide (0.21 mL, 7.5 mmol). The reaction was continued at 50 ^{o}C for 24 h. The solution was concentrated and the residues were extracted with dichloromethane. The organic layer was dried over anhydrous magnesium sulfate and concentrated. The solution was precipitated into excess amount of cold diethyl ether to obtain allylated 4-arm poly(ethylene oxide)s. (Yield=80 %)

Allyl terminated 4-arm poly(ethylene oxide)s (2.00 g, 0.10 mmol) and N-methylmorpholine-N-oxide (0.14 g, 1.20 mmol) in 4.5 mL acetone and 4.5 mL distilled water were introduced in a 50 mL 2-neck round bottom flask equipped with nitrogen inlet-out. 0.5 mL of 2.5 % OsO_4 solution in butanol was added and the reaction was continued at 25 ^{o}C for 24 h. The organic solvent was concentrated and the residues were extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate and condensed solution was precipitated into excess amount of cold diethyl ether to obtain 4-arm poly(ethylene oxide)s containing 8 hydroxyl group at the end of polymer. (Yield = 70 %)

Under high vacuum system, 4-arm poly(ethylene oxide)s precursor



Scheme 4-2. Synthetic scheme of (a) end functionalization of 8-arm star-shaped poly(ethylene oxide)s and (b) UFH-NHS.

was freeze dried from 1,4-dioxane. Synthesis of 8-arm poly(ethylene oxide)s was followed same procedure of 4-arm poly(ethylene oxide)s through living anionic polymerization. Molecular weight of 8-arm poly(ethylene oxide)s was 80 K and M_w/M_n was 1.03. Method of allylation of 8-arm poly(ethylene oxide)s was same as 4-arm allylation[12].

Allyl terminated 8-arm poly(ethylene oxide)s (2.000 g, 0.024 mmol) and cysteamine hydrochloride (0.065 g, 0.576 mmol) were reacted using 2,2-dimethoxy-2-phenylacetophenone (0.002 g, 0.008 mmol) as photo-initiator in methanol and dichloromethane (1/1, v/v) in a 50 mL 2-neck round bottom flask equipped with nitrogen inlet-out. The reaction mixture was stirred for 30 min and exposed to the UV light (λ_{exc} = 365 nm) for 1 h. The organic layer was condensed and the aqueous layer was extracted with dichloromethane. The organic layer was dried over anhydrous magnesium sulfate and condensed solution was precipitated excess amount of cold diethyl ether to produce amine terminated 8-arm poly(ethylene oxide)s. (Yield = 80 %)

After 8-arm poly(ethylene oxide)s amine was dissolved in 10 mL dichloromethane, 3,4 dihydroxyhydrocinnaminc acid (0.003 g, 0.036 mmol), N-hydroxybenzotriazole (HOBt) (0.006 g, 0.048 mmol), O-benzotriazole-

N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (0.018 g, 0.048 mmol) were added to solution in a 50 mL 2-neck round bottom flask equipped with nitrogen inlet-out. After 1 h, the solution was filtered and precipitated in cold diethyl ether[13]. The product was dried under vacuum for 24 h and resuspended in pH 3.5-4.0 aqueous solution and dialyzed against acidic condition for 72 h. The aqueous phase was frozen and lyophilized to obtain the catechol conjugated 8-arm poly(ethylene oxide)s. (Yield = 80 %).

UFH-NHS was synthesized as follows. The UFH sodium salt was dissolved in distilled water and passed through a column to exchange sodium ion with hydrogen in order to make desalted UFH. Desalted UFH (200 mg) was dissolved in 5 ml formamide at 50 oC for 1 h and the reaction mixture was cooled down under an ice bath. 63.9 mg EDC was added and the solution was stirred for 10 min before the addition of 38.36 mg of NHS. The reaction was continued overnight in ice bath. Unreacted materials were removed by precipitation with ethanol, followed by lyophilizing. The final product, UFH-NHS, was obtained after the lyophilized product was dissolved in distilled water again and re-lyophilized.

4.3. Results and Discussion

Living anionic polymerization is the most powerful synthetic method to produce well defined and controlled molecular structure of poly(ethylene oxide)s, which is a popular anti-fouling material in bio-related application fields[14]. Polymerization of EO was performed in a high vacuum line maintaining extremely anhydrous reaction conditions. 4-arm star-shaped poly(ethylene oxide)s were synthesized in DMSO using pentaerythritol and DPMK as an initiator and a catalyst. The amount of DPMK was controlled to suppress a possible side reaction of liner PEG formation. The obtained molecular weight of 20 K was in good agreement with the target molecular weight. 4-arm poly(ethylene oxide)s with 8 hydroxyl end groups were prepared via allylation and the consecutive hydroxylation reaction. The presence of allylic double bonds at 5.33-5.11(CH=CH_2) and 6.03-5.79 (CH=CH_2) ppm and the disappearance of the peaks in ^{1}H NMR spectra confirmed the successful reaction without any noticeable side reactions. 8-arm PEG was obtained from the macroinitiator using the same method with the molecular weight of 80 K and M_w/M_n value of 1.03. The shift of peak molecular weights and the narrow distribution in GPC traces supports the successful polymerization.

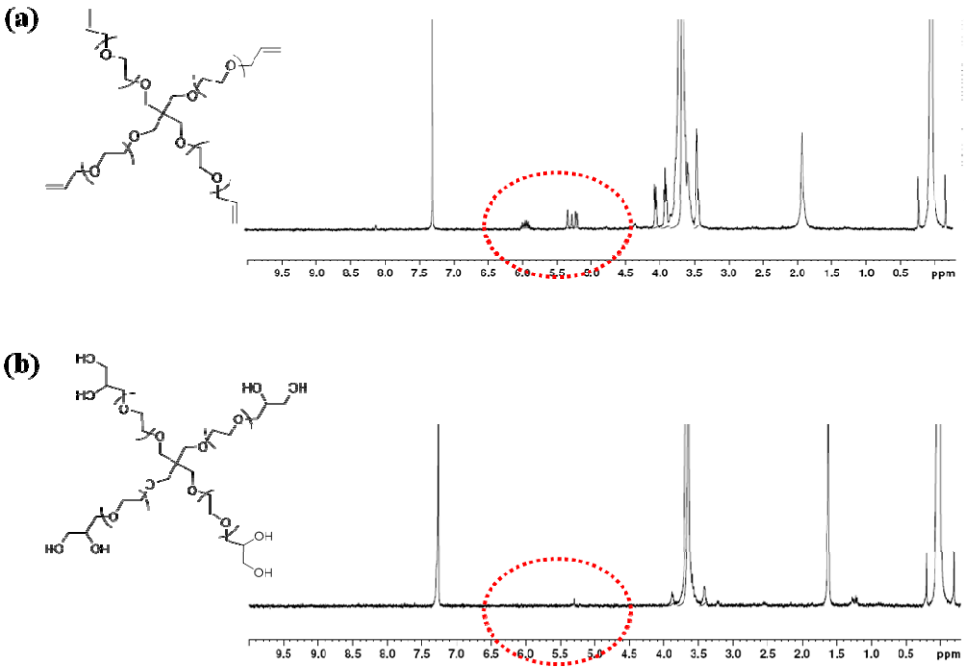


Figure 4-2. ^{1}H NMR spectra of (a) allylation and (b) dihydroxylation of 4-arm poly(ethylene oxide)s.

Polymerization was proceeded in a high vacuum system and 8-arm star-shaped poly(ethylene oxide)s were grown from 4-arm star-shaped poly(ethylene oxide)s macroinitiator was employed for further investigation. 8-arm star-shaped poly(ethylene oxide)s is expected to display better immune protective properties of the encapsulated cells than linear poly(ethylene oxide)s, due to the higher hydrodynamic volume caused by the architecture as well as the larger number of end groups utilized for the conjugation of various functional groups.

Choice of proper solvent and initiator is an important factor to control the structure and the molecular weight distribution in anionic polymerization. DMSO was the solvent in the polymerization since initiators with several polar hydroxyl groups were not so soluble in THF, the

mostly used solvent in anionic polymerization. DPMK was used as a catalyst which deprotonate hydroxyl groups of the initiator. DPMK can also deprotonated the methyl groups in DMSO to produce dimsyl anion, leading to the formation of undesirable linear poly(ethylene oxide)s as a side reaction. The amount of DPMK was carefully controlled less than 30 mol % of hydroxyl groups in the initiator to suppress the side reaction[15].

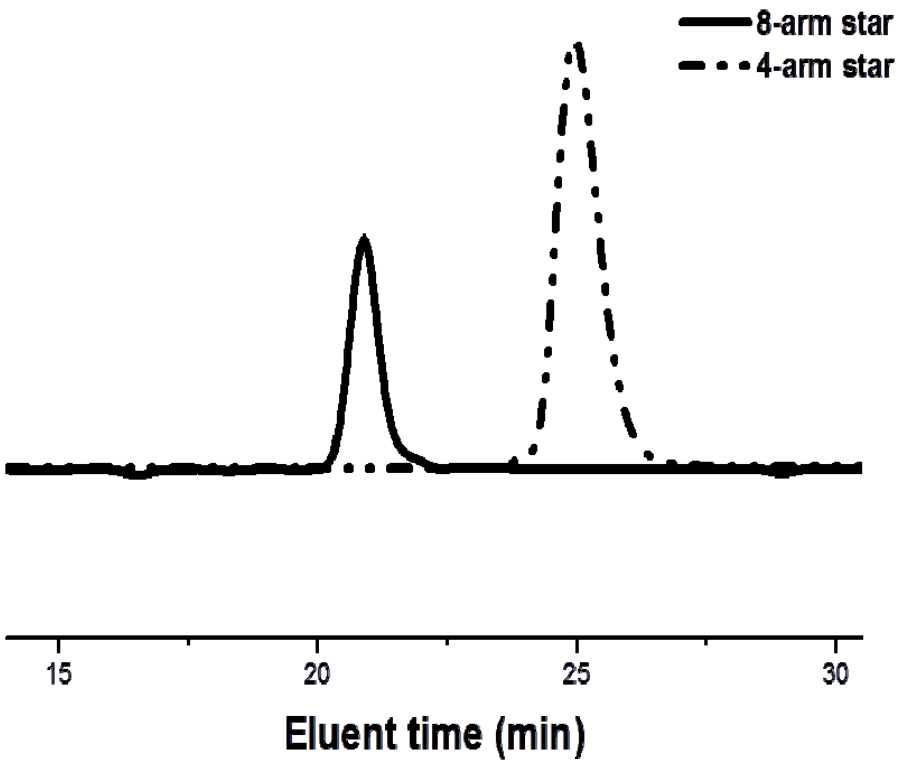


Figure 4-3. GPC traces of the 4-arm and 8-arm star-shaped poly(ethylene oxide)s.

Amine terminated 8-arm star-shaped poly(ethylene oxide)s was prepared by allylation and amination using cysteamine hydrochloride as a chain transfer agent to the allyl groups via a photo-initiated reaction. Peaks at 2.80-2.85 (CH_2-CH_2-S) and 2.65-2.70 (S-CH_2-CH_2-NH_2) in ^{1}H NMR spectrum confirmed the stoichiometric introduction of amino groups, which were further functionalized to the conjugation of bioadhesive catechol groups and visualizable FITC dyes.

Catechol groups are actively investigated as a bioadhesive material in wet conditions. Two catechol groups were conjugated to the 8-arm star-shaped poly(ethylene oxide)s and expected to form covalent bonds with amine or thiol groups presented on the surface of the cell membrane[16].

The rest of the amino groups in the periphery of 8-arm star-shaped poly(ethylene oxide)s were utilized for the reaction with NHS-activated heparins to endow the encapsulated cells with anti-coagulant properties in the blood stream. Unfractionated heparin (UFH-NHS) was synthesized for double layer with 8-arm star-shaped poly(ethylene oxide)s. This polymer was from prof. Byun's lab. To conjugate with amine groups at the end of 8-arm star-shaped poly(ethylene oxide)s NHS groups were modified on UFH using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and NHS in

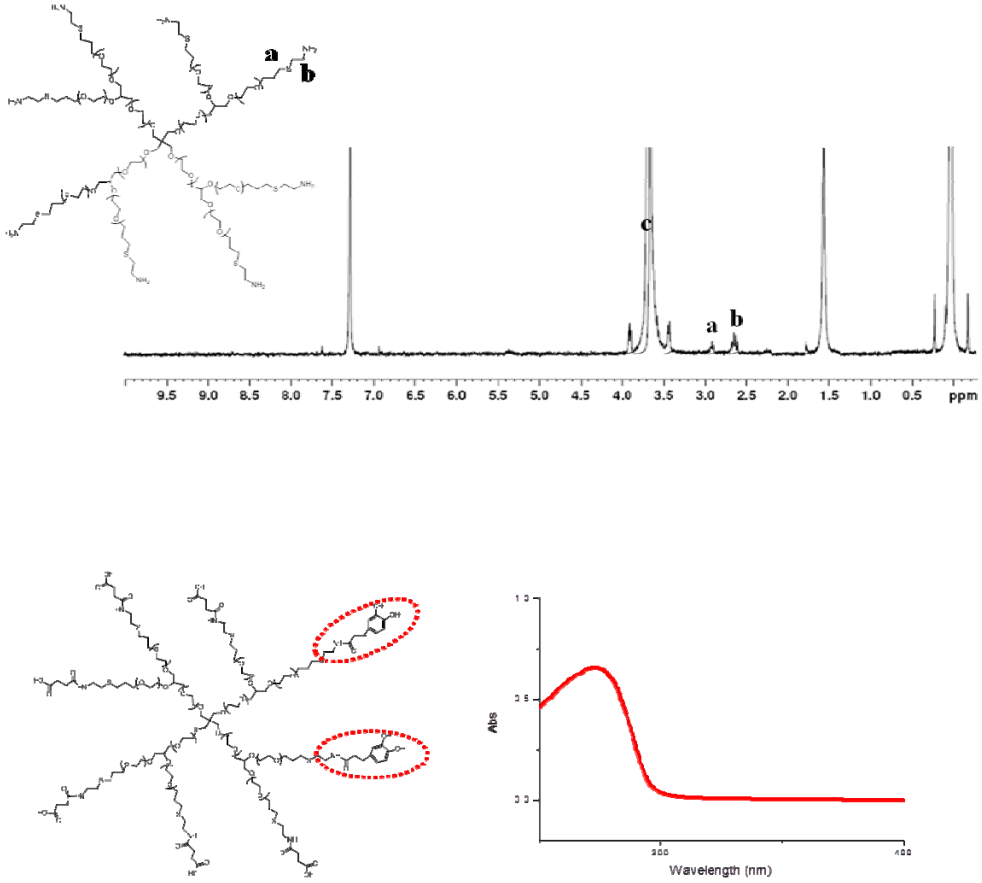


Figure 4-4. (a) ^{1}H NMR spectra of the amine terminated and (b) UV spectroscopy of the catechol conjugated 8-arm star-shaped poly(ethylene oxide)s.

formamide. UFH was used for cell modification after precipitation and lyophilizing[17].

Coverage effect of 8-arm star-shaped poly(ethylene oxide)s and UFH-NHS were observed by conjugation of FITC dye on their polymers. The viability tests were also carried out using CCK-8 assay, the Live/Dead viability and cytotoxicity kit. The viabilities of islets modified using 1% and 2% 8-arm star-shaped poly(ethylene oxide)s solutions were 94.2 ± 8.1% and 72.2 ± 3.3%, respectively, compared to that of the unmodified islets when examined using CCK-8 assay. According to the viability results, 1 % solution of 8-arm star-shaped poly(ethylene oxide)s for 1 h were optimized for islet surface modification[6]. UFH-NHS was used for second layer on this optimized condition. The islets with below 1 % of solutions of UFH-NHS was not enough to cover the islet surfaces compared to 2 % and 5 % of UFH-NHS. The viabilities of double-layer shielded islets using 2 % and 5 % solution of UFH-NHS after first-layer immobilization[18] using 8-arm star-shaped poly(ethylene oxide)s were 110.8 ± 4.8% and 75.0 ± 16.1%, respectively, compared to that of the unmodified islets. 1 % of 8-arm star-shaped poly(ethylene oxide)s and 2 % of UFH-NHS were chosen for islet surface modification because they showed the highest viability[19].

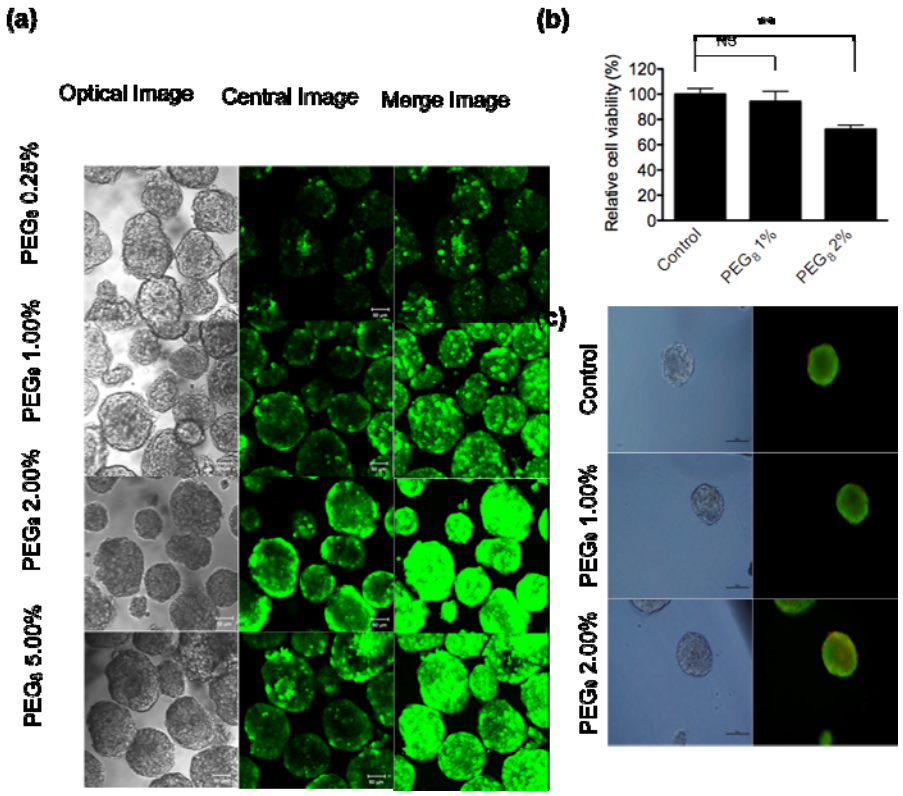


Figure 4-5. (a) Confocal laser scatter microscopy image, (b) viability (c) Live/dead cell image of cells.

Functionality was measured by the GSIS test which is observed to secret insulin in response to exogenous glucose solution. There was no significant difference in the insulin secretion between the control islets and the double-layer shielded islets. The stimulation index (SI) values of the control islets and double-layer shielded islets were 7.7 ± 2.0 and 8.7 ± 1.0, respectively. This result is shown that the insulin secretion function of cells is not affected by 8-arm star-shaped poly(ethylene oxide)s and UFH-NHS[20].

Anti-coagulant activity of double layer was measured by anti-FXa assay and anti-FIIa assay. The activities of anti-FXa and anti-FIIa were 0.7 IU and 0.4 IU per 100 IEQs 8-arm star-shaped poly(ethylene oxide)s/UFH double-layer shielded islets, respectively. The specific activity of UFH is around 150 ~ 190 IU/mg. APTT is also an indicator to measure endogenic coagulation and primarily dependent of the factors in common pathway. The aPTT value measured from the solution containing 100 IEQ 8-arm star-shaped poly(ethylene oxide)s /UFH double-layer shielded islets was 0.3 IU anticoagulant activity which is similar with anti-FIIa activity. From this result, UFH was effective for preventing blood coagulation.

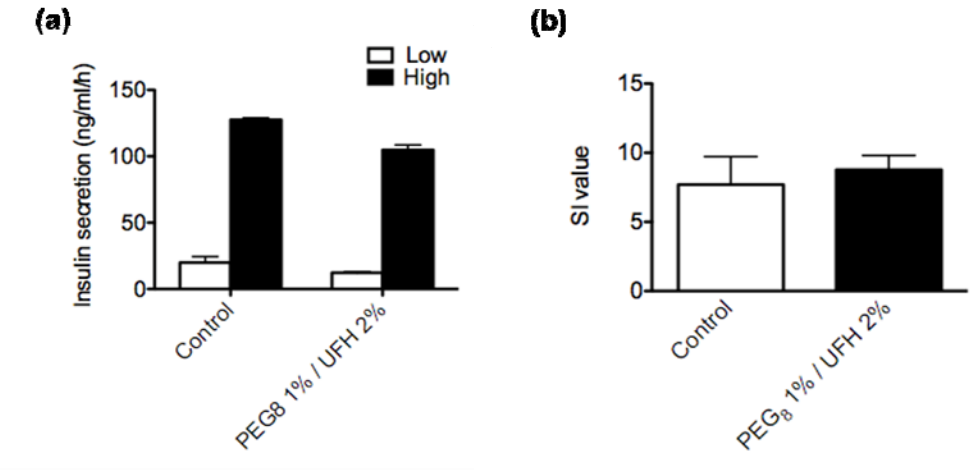


Figure 4-5. (a) The glucose-stimulated-insulin secretion (GSIS) and (b) stimulation index (SI) of islets.

Immunoprotection effect of double layer shielding using 8-arm star-shaped poly(ethylene oxide)s and UFH was evaluated by measuring non-fasting blood glucose levels of recipients after transplantation of surface modified islets. The mean survival times (MST) of unmodified islet and PEG8/UFH double-layer shielded islets were 3.6 ± 1.1 days and 6.8 ± 1.6 days (mean ± SEM, $p < 0.05$), respectively.

FK506 was used for the synergistic effect of poly(ethylene oxide)s and UFH double layer. 0.5 mg/kg of FK506 was daily administrated after double-layer shielded islets or unmodified islets were transplanted.

Treatment of FK506 for double-layer shielded islets could increase the survival time of islets more than that of unmodified islets in allotransplantation. It meant that of poly(ethylene oxide)s and UFH double prevented the immune cell activation and inflammation reaction, thereby effectively improving the survival time.

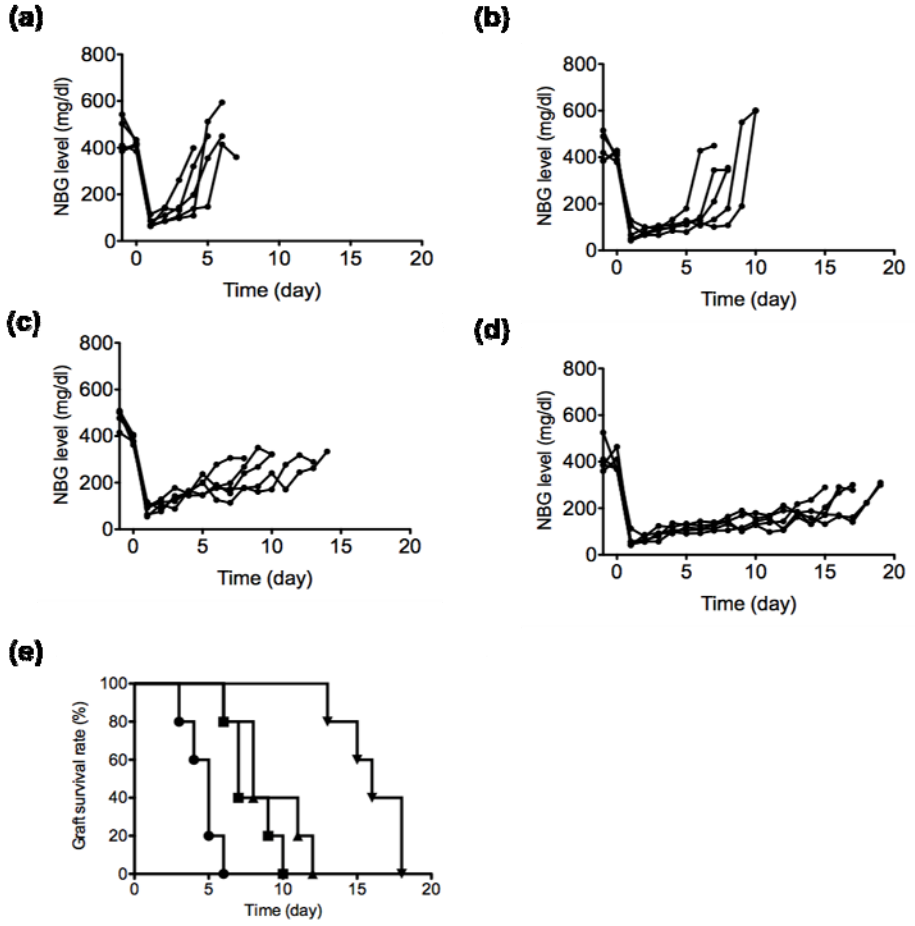


Figure 4-6. Non-fasting blood glucose level after islet transplantation into diabetic mice. (a) Unmodified islet recipients, (b) double-layer shielded islet recipients, (c) Unmodified islet recipients with FK506 treatment, (d) double-layer shielded islet recipients with FK506 treatment, (e) Graft survival rate of each group. (●) unmodified islet recipients, (■) double-layer shielded islets recipients, (▲) unmodified islets recipients with FK506 treatment and (▼) double layer shielded islet recipients with FK506 treatment.

4.4. Conclusion

8-arm star-shaped poly(ethylene oxide)s and UFH double layer are conjugated by amine and NHS chemistry on islet surfaces. Both of the polymers had no effect on cell viability and cell function. In addition, they showed low immune reaction at low dose of immunosuppressive drug when islet transplantation. This protocol serves as an extremely potent treatment to reduce graft loss of intraportally transplanted islets.

4.5. References

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Chapter 5.

Dendritic Poly(ethylene oxide)s for Cell Delivery

5.1. Introduction

Hyperbranched or dendritic macromolecules have been used for surface modification due to their bulky structure. Compared to linear molecules, hyperbranced or dendritic molecules have low viscosity and high solubility which may provide additional advantages by reason of multiple functional groups[1]. With these properties, they are used for wide range of application including surface modification, catalyst, and biological molecular detection. In case of surface modification, dendritic macromolecules are effective for coating on the surface with bulk structure[2].

Poly(ethylene oxide)s are well known for water soluble materials and they are composed of hydrocarbon and ether linkage in every repeating unit[3]. Poly(ethylene oxide)s which are swollen, flexible and increasing mobility in aqueous system make them available for use in biomedicine and pharmaceutical areas[4]. Because of their “stealth” properties, poly(ethylene oxide)s are prevented from approaching protein and immune cell adhesion that are used for drug conjugation, delivery system, and transplantation of biomaterials, etc[5].

Poly(ethylene oxide)s are synthesized by anionic polymerization

which proceeded by ring opening of ethylene oxide using carbanionic initiator in organic solvent[6]. Anionic polymerization is the most powerful method to obtain well-defined poly(ethylene oxide)s[7]. In high vacuum system, it can be controlled to minimize side reaction through blocking off water, air and impurities.

Poly(ethylene oxide)s are commonly synthesized in non polar solvent such as benzene or tetrahydrofuran (THF). In case of hyperbranced or dendritic poly(ethylene oxide)s, they are polymerized in strong polar solvent like dimethyl sulfoxide (DMSO) since multiple alkoxides have strong tendency for association in non polar solvent. DMSO is decisive factor for anionic polymerization of ethylene oxide without multiple initiators aggregation though DMSO can be deprotonated and formed "dimsyl anion" by strong catalyst.

Xiaoshuang Feng et al. developed a novel approach that prepared dendritic poly(ethylene oxide)s by way of partial deprotonation of hydroxyl groups in DMSO system[8]. To introduce branch points for next generation in hyperbranched or dendritic poly(ethylene oxide)s, two step procedures were progressed by allylation and dihydroxylation[9].

In this study, we designed well-defined dendritic poly(ethylene

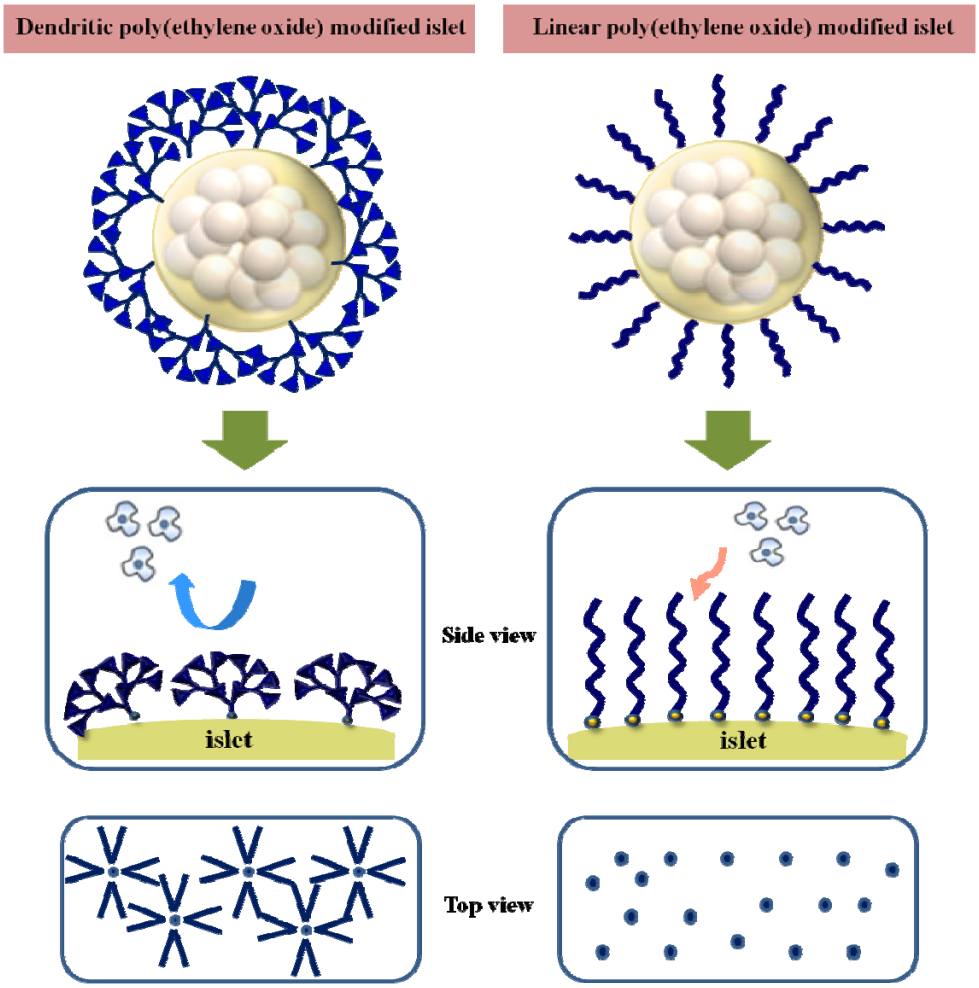


Figure 5-1. Illustration of effective coverage of dendritic poly(ethylene oxide)s compared to linear poly(ethylene oxide)s modified on islet surface.

oxide)s for islet transplantation. Surface modification on the cell surface is important factor that can lead to prolong cell viability and cell lives[10]. To maximize poly(ethylene oxide)s effects, hyperbranched and dendritic poly(ethylene oxide)s have bulky and immense structure, are good candidates for protecting immune substances and protein to adhere on surface. Especially, dendritic poly(ethylene oxide)s have one focal point and a number of end groups can be modified on living cell surfaces. They can minimize the contact points on living cell surface and maximize covering effects.

For this reason, 3,3-diethoxy propanol was chosen for an initiator of dendritic poly(ethylene oxide)s that have acetal group can convert to another functional group for application. On the basis of AFM images and protein adsorption were evaluated for surface effect on solid materials, cell viability and functionality were carried out after living cell surface modification

5.2. Experimental Section

Materials. Ethylene oxide (EO) was distilled over CaH_2. Tetrahydrofuran (THF) and Dimethyl sulfoxide (DMSO) were distilled over

CaH_2 prior to use. 1,4-dioxane was distilled over sodium. Diphenylmethyl potassium (DPMK) was prepared and titrated as previously reported. EO was donated from Lotte Chemical. THF and DMSO were purchased from Daejung chem. All other chemicals were purchased from Sigma-Aldrich and used without further purification

Instruments. Gel permeation chromatography (GPC) was used to determine molecular weights and molecular weight distributions, M_w/M_n of polymer samples using poly(ethylene glycol) standards (Polymer Laboratories a part of VARIAN). The system configuration was consisted of refractive index detector (Shimadzu RID-10A refract meter) and Styragel HR 3, HR 4 and HR 4E column in series. The flow rate of tetrahydrofuran (THF) as an eluent was 1 mL/min at 40 ^oC. ^1H NMR spectra of the polymers were obtained on a Bruker Avance-300 spectrometer. Sample concentrations were about 10 % (w/v) in $CDCl_3$. Surface morphology was analyzed by using atomic force microscope (AFM) with a contact mode (SPA-400).

Anionic ring-opening polymerization of ethylene oxide (EO) was

carried out using a vacuum line. All polymerization reactors and ampoules are constructed with glass blowing. EO was prepared into ampoule with anhydrous THF solution. 3,3-diethoxy propanol (0.27 mL, 1.76 mmol) was introduced into a 500 mL flask under argon atmosphere. 200 mL anhydrous THF was transferred into flask in vacuum line. The solution was stirred at room temperature for 30 min. Diphenylmethyl potassium (DPMK) (1.76 mmol) was added into flask through gas tight syringe at argon atmosphere.

The color of mixture was changed from red-orange to yellow immediately. The flask was chilled down to -78 ^{o}C and then EO (8.82 g, 200 mmol) was added. The polymerization was carried out at 40 ^{o}C for 72 h and quenched by the addition of a few drops of methanol. The solution was concentrated and precipitated in cold diethyl ether twice. After filtration, the product was dried in vacuum for 24 h to produce Generation 0 (G0).

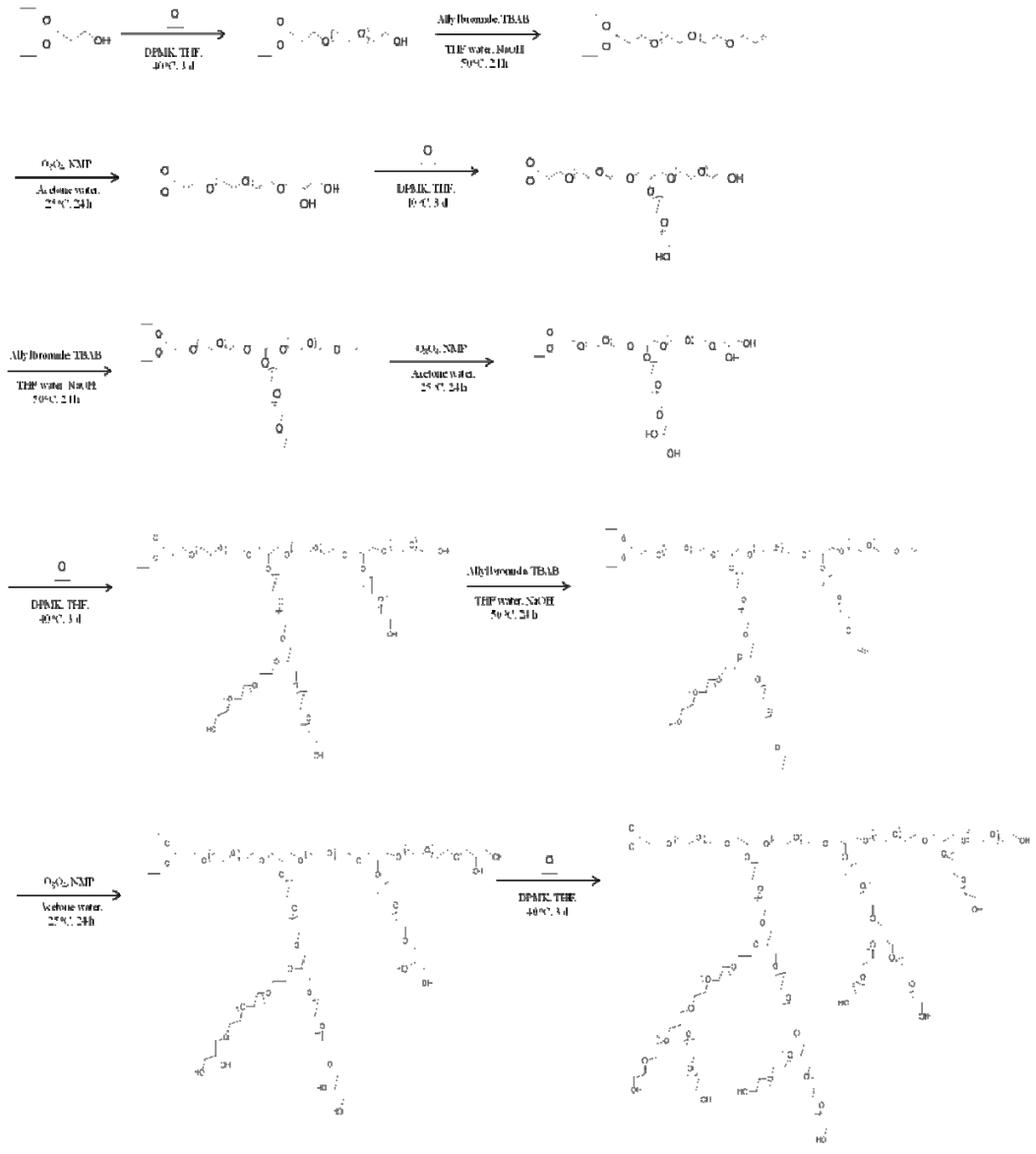
To introduce branch point at the end of G0 polymer, tetrabutylammonium bromide (0.03 g, 0.09 mmol) and NaOH (0.36 g, 9.00 mmol) were dissolved in 2 mL water and then G0 (4.5 g, 0.90 mmol) in 3 ml THF were added in a 50 mL 2-neck round bottom flask equipped with nitrogen inlet-out. Allyl bromides were introduced after 30 min at 50 ^{o}C. The reaction was continued for 24 h. The solution was concentrated and the

residues were extracted with dichloromethane. The organic layer was dried over anhydrous magnesium sulfate and concentrated. The solution was precipitated into excess amount of cold diethyl ether to obtain allylated G0.

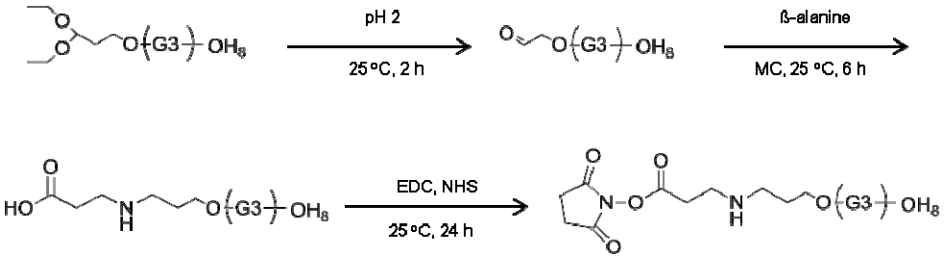
Allylated G0 (4.00 g, 0.80 mmol) and N-methylmorpholine-N-oxide (0.56 g, 4.80 mmol) in 4.5 mL acetone and 4.5 mL distilled water were introduced in a 50 mL 2-neck round bottom flask equipped with nitrogen inlet-out. 0.2 mL of 2.5 % OsO_4 solution in butanol was added and the reaction was continued at room temperature for 24 h. The organic solvent was concentrated and the residues were extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate and condensed solution was precipitated into excess amount of cold diethyl ether to obtain two hydroxyl groups at the end of polymer.

G0 precursor was freeze dried from 1,4-dioxane under high vacuum system for next generation. Synthesis of G1 was followed same procedure of G0 through living anionic polymerization. Introduction of branch points in each generation was followed by allylation and dihydroxylation as mentioned above.

Synthesis of G2 and G3 were carried out in dimethyl sulfoxide (DMSO) with DPMK which concentration was 30 % of hydroxyl moles.



Scheme 5-1. Synthetic scheme of G3 dendritic poly(ethylene oxide)s.



Scheme 5-2. Synthetic scheme of the functionalization of dendritic poly(ethylene oxide)s.

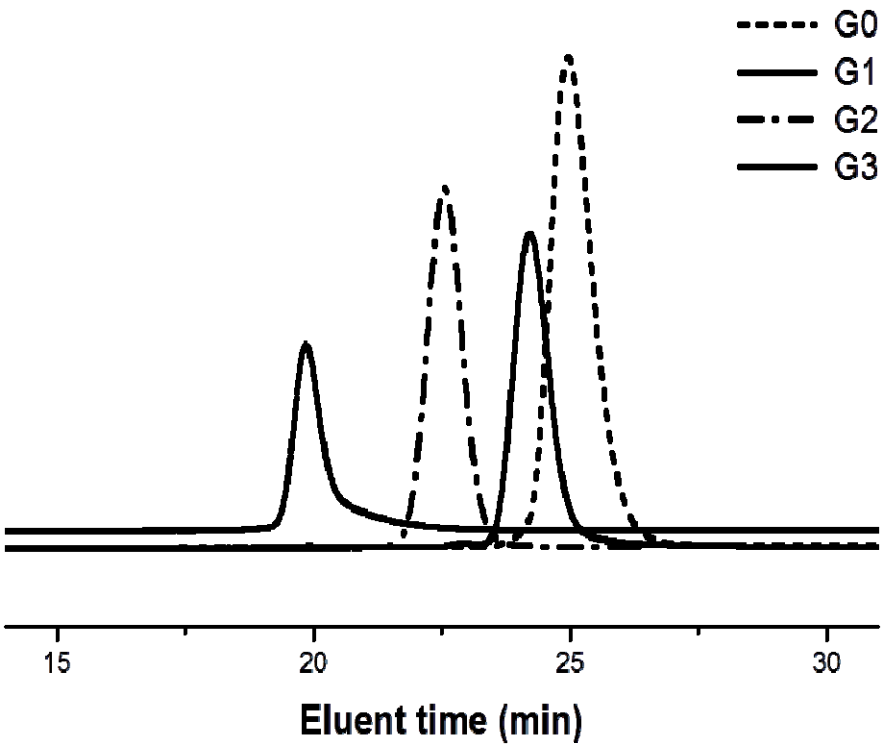


Figure 5-2. GPC traces of dendritic poly(ethylene oxide)s.

The reaction was proceed at 40 ^{o}C for 72 h and quenched by the addition of a few drops of methanol. The solution was concentrated and precipitated in cold diethyl ether twice. After filtration, the product was dried in vacuum for 24 h to produce G1 and G2 respectively.

Acetal group in 3,3-diethoxy propanol was deprotected in acidic condition. Dendritic poly(ethylene oxide)s were stirred in pH 2 aqueous solutions at room temperature for 2 h and adjusted to pH 7 by dropping 1 N NaOH solution. Dendritic poly(ethylene oxide)s were extracted with dichloromethane and dried over anhydrous magnesium sulfate. After filtering, condensed solution was precipitated into cold diethyl ether, filtered and dried under vacuum for 24 h at 25 ^{o}C to produce aldehyde functionalized at the core of dendritic poly(ethylene oxide)s.

ß-alanine was chosen for core functionalization of dendritic poly(ethylene oxide)s because it has amine group for reaction with aldehyde and carboxylic acid group for conjugation to cell surface. Aldehyde functionalized dendritic poly(ethylene oxide)s were dissolved in anhydrous MC with large excess of ß-alanine and $NaBH_4$ as reducing agent. The reaction was carried out at room temperature for 6 h. The polymers were precipitated into cold diethyl ether, filtered and dried under vacuum for 24 h

at 25 ^{o}C to produce carboxylic acid functionalized at the core of dendritic poly(ethylene oxide)s.

Carboxylic acid functionalized dendritic poly(ethylene oxide)s and EDC were dissolved in distilled water at room temperature for 30 min and NHS was added. After stirring for 12 h, the polymers were extracted with dichloromethane and dried over anhydrous magnesium sulfate. After filtering, condensed solution was precipitated into cold diethyl ether, filtered and dried under vacuum for 24 h at 25 ^{o}C. Finally, NHS activated dendritic poly(ethylene oxide)s were obtained.

Silicon wafers and slide glasses (1cm x 1cm) were treated with acetone and distilled water and dried with nitrogen gas. Piranha solution (conc. H_2SO_4, H_2O_2 30 % w/w) was used for 30 min in order to clean the surfaces. After rinsing with water and drying with nitrogen gas, silicon wafers and slide glasses are treated with 1:6 mixture of HF (48 %) and NH_4F (40 %) for 30 min, 1:1:6 mixture of NH_4OH (28 %), H_2O_2 (30 %) and distilled water for 30 min, 1:1:6 mixture of HCl (37 %), H_2O_2 (30 %) and distilled water for 30 min, respectively.

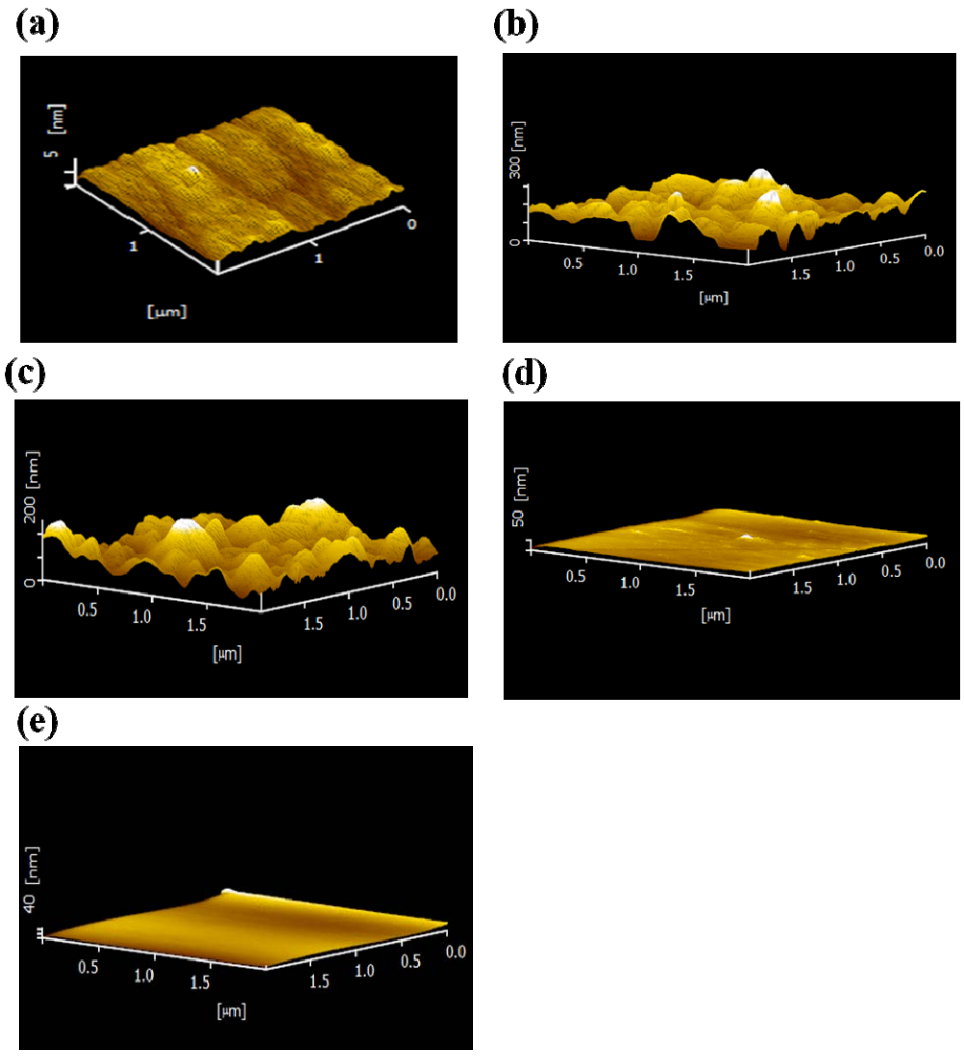


Figure 5-3. AFM image of (a) control (b) 0.25 wt% (c) 1 wt% (d) 2 wt % (e) 4 wt % of dendritic poly(ethylene oxide)s.

Cleaned silicon wafers and glasses were reacted with 2 wt % of 3-(aminopropyl) trimethoxysilane (TMS) in toluene at 80 ^{o}C for 12 h, rinsed with toluene, methanol and distilled water, dried using N_2 gas and baked at 100 ^{o}C oven for 12 h.

NHS activated dendritic poly(ethylene oxide)s of 0.25, 1, 2, 4 wt % in pH 8 aqueous solutions were prepared for surface modification. TMS modified silicon wafers and glasses were incubated in each solution at 37 ^{o}C for 1 h, rinsed with distilled water several times, blew using N_2 gas.

Bovine serum albumin (BSA) and fibrinogen solutions were prepared with the concentration of 100 ug/ml of PBS solution (Phosphate-buffered saline, pH 7.4, ion strength 0.15). Silicon wafers and glasses modified with dendritic poly(ethylene oxide)s at various concentrations were hydrated in PBS solution for 1h, refilled 3 ml of protein solution and incubated at 37 ^{o}C for 2 h. After reaction, each sample was rinsed and agitated carefully using PBS solution to remove weakly adsorbed protein.

Adsorbed protein on the surface was obtained by sonicating in 1 wt % SDS solution for 1 h. The amount of albumin was detected by UV spectroscopy (λexc = 562 nm) calibrated using BSA. Fibrinogen adsorption was developed by enzyme-linked immunosorbent assay (ELISA).

5.3. Results and Discussion

In this study, we developed dendritic poly(ethylene oxide)s for surface modification of islet to reduce immune responses and increase cell viability. poly(ethylene oxide)s are a very well known material as biocompatible, non-toxic, and effective for immune system. poly(ethylene oxide)s modified surfaces increase hydrophilicity as well as reduce protein adsorption, macrophage attack, platelet adhesion and decrease immune response. Linear poly(ethylene oxide)s are mostly used for delivery system such as organic and inorganic nanoparticles, polymeric micelles, and liposome in biotechnology and biomedical field.

Dendritic poly(ethylene oxide)s have different shapes and properties compared to linear poly(ethylene oxide)s. They have a focal point and lots of end groups that can be controlled according to the generation. Because of their spherical shape and multiple functional groups, dendritic poly(ethylene oxide)s show additional advantages compared to linear poly(ethylene oxide)s. We designed third generation dendritic PEOs for islet delivery.

In cell delivery system, coverage effect and cell viability are very important for protecting cell. Dendritic poly(ethylene oxide)s have advantages of attaching on cell surface with lower contact points and higher

coverage effect because of their architecture. To make bulky structure, high molecular weight dendritic PEOs were synthesized.

Dendritic poly(ethylene oxide)s which have the functional group on the focal point are useful for conjugation to protein or living cell surfaces. 3,3-diethoxy propanol was chosen as an initiator of dendritic poly(ethylene oxide)s because it has acetal group that can convert to another functional groups for its application. High molecular weight dendritic poly(ethylene oxide)s were synthesized by ring opening polymerization of EO under high vacuum system. DMSO was used as solvent during polymerization because multiple alkoxide groups at the end of dendritic poly(ethylene oxide)s were aggregated and not soluble in THF. To activate hydroxyl groups for initiation, DPMK was used as strong base catalyst. In DMSO system, DPMK can transfer to DMSO that leads to make dimsyl carbanion which are able to initiating polymerization. Because of this reason, 30 % DPMK of hydroxyl groups was used during polymerization in DMSO. Each polymerization was proceeded at 40 ^{o}C for 72 h.

Dendritic poly(ethylene oxide)s were synthesized by divergent method and each generation was obtained with a very narrow M_w/M_n values and excellent control of molar mass without any side reactions. We carried

out allylation and dihydroxylation at each generation for introduction of branching points and confirmed by ^{1}H NMR. Allylic double bonds were observed at 6.03-5.79 (C*H*=CH_2) and 5.33-5.11(CH=C*H*$_2$) ppm. After dihydroxylation, allylic double bonds were disappeared completely. Polymerization and introduction of branch points were repeated and third generation dendritic poly(ethylene oxide)s were obtained and molecular weight is 78 K and molecular distribution was 1.03. That means introduction of branching points was carried out successfully and there were no side reaction during polymerization[11].

To attach on living cell surface, dendritic poly(ethylene oxide)s were functionalized on the focal points. 3,3-diethoxy propanol was chosen to convert carboxylic acid via formation aldehyde group in acidic condition. Carboxylic acid groups were activated by NHS to react with amine groups on solid substrates and cell surfaces[10]. After introduction of NHS groups, measuring ratio of NHS groups on dendritic poly(ethylene oxide)s by ^{1}H NMR was difficult because total molecular weight was too high.

Conjugation ratio of NHS groups on the dendritic poly(ethylene oxide)s was determined by UV spectroscopy after coupling with FITC-NH_2 at absorbance peak appears at 495 nm, about 0.9 NHS group were

conjugated to each dendritic poly(ethylene oxide)s.

NHS-activated dendritic poly(ethylene oxide)s were used for cell surface modification to conjugate with amine groups on collagen matrix of islet surface. NHS chemistry is well known to coupling primary amine and carboxylic groups in biological condition[12].

NHS activated dendritic poly(ethylene oxide)s were modified on TMS-silanized substrates with various concentration, 0.25, 1, 2 and 4 wt %. After 2 h incubation, unreacted dendritic poly(ethylene oxide)s were removed by washing substrates several time[13]. Coverage effects of chemically modified dendritic poly(ethylene oxide)s on solid substrate were measured by AFM images[14]. TMS-silanized substrates showed a very flat and thickness was 5 nm. Dendritic poly(ethylene oxide)s modified on surfaces with 0.25 and 1 wt % displayed rough and depth of the polymer surface were about 165 nm and 153 nm, respectively.

On the other hand, depth of 2 and 4 wt % dendritic poly(ethylene oxide)s on surfaces showed 25 nm and 18 nm respectively and morphology became smooth. As polymer concentration on the solid surface increased, depth of the polymer surface and surface roughness decreased[11, 13].

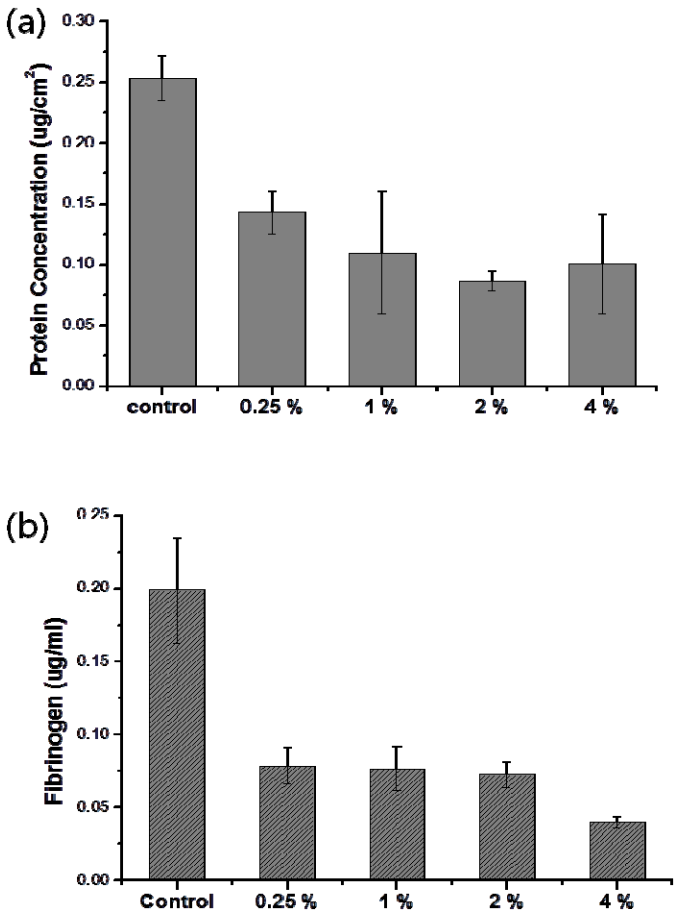


Figure 5-4. (a) Albumin and (b) fibrinogen adsorption of dendritic poly(ethylene oxide)s modified solid surface.

Protein adsorption on substrate modifying dendritic poly(ethylene oxide)s were carried out with albumin and fibrinogen[15]. Dendritic poly(ethylene oxide)s modified substrates that were coated with various concentrations were incubated in protein solution and incubated at 37 ^{o}C for 2 h. After washing several times, adsorbed proteins on substrates were measured by UV spectroscopy and ELISA kit. The amount of protein on TMS-silanized substrate was higher than that of dendritic poly(ethylene oxide)s modified substrate[16].

AFM images and protein adsorption were carried out onto solid substrate such as silicon and glasses. Before using these solid substrates, 3-(aminopropyl) trimethoxysilane (TMS) was treated in order to introduce amine groups on the surfaces.

AFM images show coverage effects on solid substrates according to concentration of dendritic poly(ethylene oxide)s. As concentration of dendritic poly(ethylene oxide)s increased, surface roughness changed smooth and flat. We assumed that morphology was changed because dendritic poly(ethylene oxide)s were densely filled onto substrate as increasing concentration. In contrary, there were no enormous changes of concentration effect in protein adsorption even though there were great

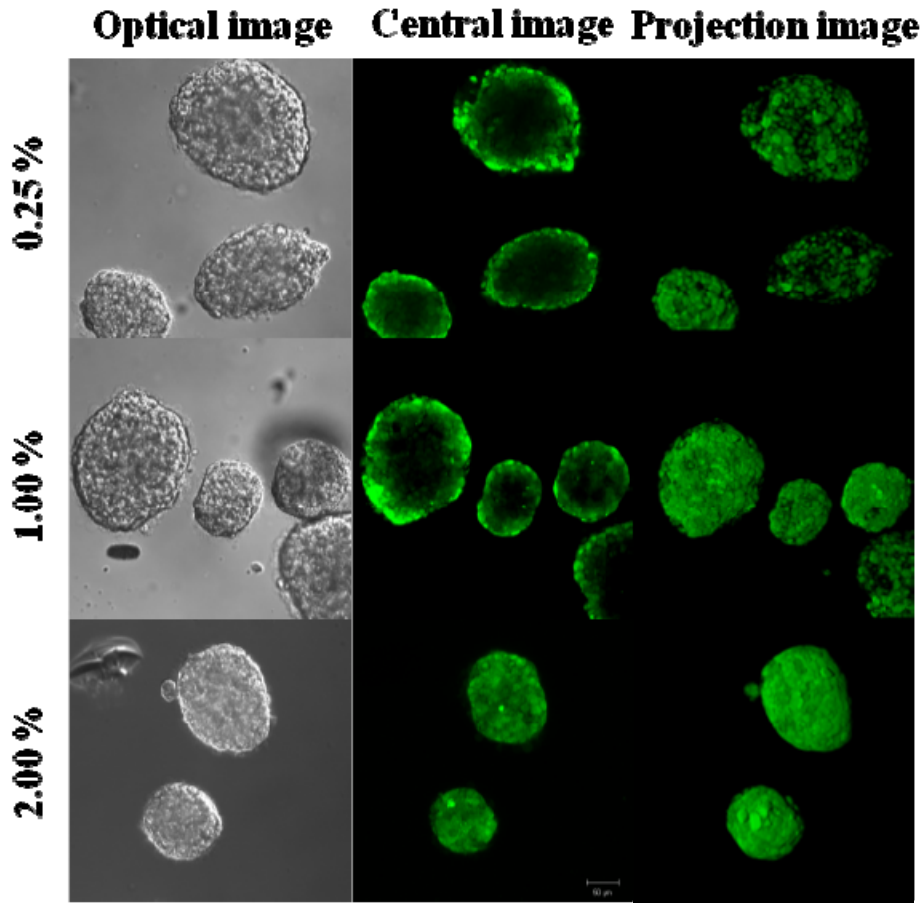


Figure 5-5. Confocal laser scatter microcopy images of dendritic poly(ethylene oxide)s modified islets.

changes in comparison with control. To blocking adsorbing protein on the surface, flexibility of poly(ethylene oxide)s is important to repel approaching protein. These results can be explained by structure of dendritic poly(ethylene oxide)s which are more rigid but less flexible than linear poly(ethylene oxide)s.

Islet coverage effect after coating with FITC conjugated dendritic poly(ethylene oxide)s were measured by confocal laser scanning microscopy (CLSM). All the polymer concentration of islet surfaces were shown that the fluorescence intensity was strong. That means islets were modified by dendritic poly(ethylene oxide)s completely. Viability was observed by Live/Dead Viability and Cytotoxicity Kit. Each polymer concentration and islets were incubated and measured the viability[17]. CCK-8 assay was used to quantify the cell viability of 0.25 wt %, 1 wt % and 2 wt % of dendritic poly(ethylene oxide)s and they showed 113.5 ± 18.0%, 130.7 ± 5.5%, and 116.4 ± 13.7%, respectively. According to this, islet viability was not affected by covering of dendritic poly(ethylene oxide)s. The glucose-stimulated insulin secretion (GSIS) was performed to evaluate the ability of islets the insulin release in response to glucose solution. The secretion rates of insulin at low glucose solution from unmodified islets and

dendritic poly(ethylene oxide)s modified islets (0.25%, 1.00%, and 2.00%) were 0.024 ± 0.008, and 0.035 ± 0.005, 0.048 ± 0.004, and 0.048 ± 0.008, respectively. In addition, the secretion rates of insulin at high glucose solution from unmodified islets and PEG-dendron nano-shielded islets (0.25%, 1.00%, and 2.00%) were 0.076 ± 0.058, and 0.108 ± 0.016, 0.075 ± 0.008, and 0.087 ± 0.021, respectively. There was no effects on functionality of islets by modifying dendritic poly(ethylene oxide)s on islet surfaces[12b].

The immunoprotective effect of dendritic poly(ethylene oxide)s modified on islet surfaces was observed by incubating with splenocytes. When unmodified and dendritic poly(ethylene oxide)s modified islets were incubated with splenocytes, unmodified islets were damaged after 7 days and many dead cells (red fluorescence emitting cells) were detected when analyzed by Live/Dead cell assay kit while dendritic poly(ethylene oxide)s were maintained viability. This result was shown that dendritic poly(ethylene oxide)s had an effect on inhibition of the splenocytes infiltration into islets because of their bulky structures. In addition, CFS labeled splenocytes were incubated with dendritic poly(ethylene oxide)s modified and unmodified islets to analyze the splenocytes proliferation.

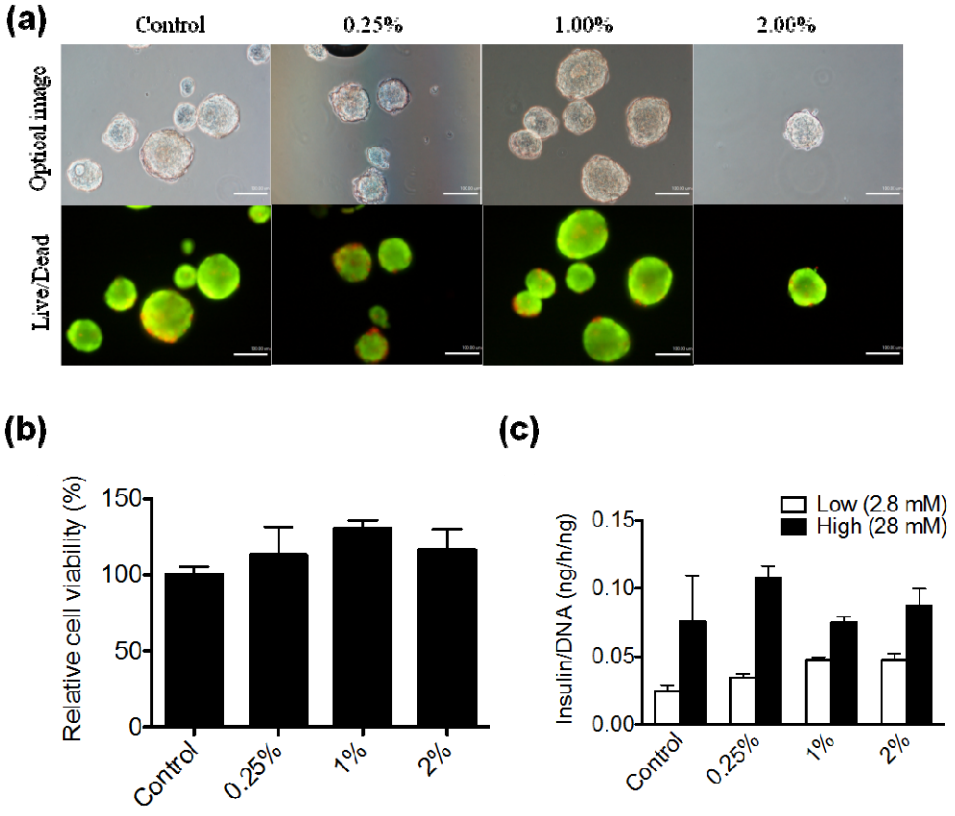


Figure 5-6. (a) Live/dead cell images (b) the relative viability (c) insulin secretion test of unmodified cells and dendritic poly(ethylene oxide)s modified cells.

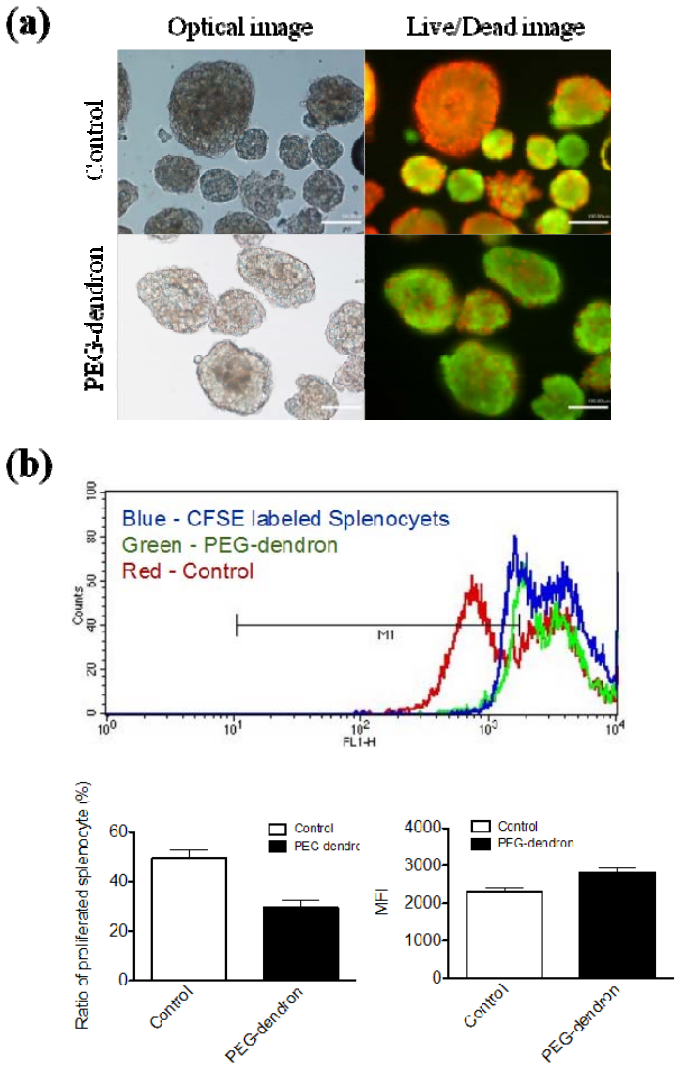


Figure 5-7. (a) Live/dead assay and (b) coculture with splenocytes of unmodified islets and dendritic poly(ethylene oxide)s modified islets.

CFSE-labeled splenocytes incubated with unmodified islets had increased proliferation (49.6 ± 5.8%) when compared to splenocytes incubated with dendritic poly(ethylene oxide)s modified islets (29.72 ± 4.6%) at day 7. Mean fluorescence intensity (MFI) of unmodified islet group and dendritic poly(ethylene oxide)s were measured. MFI of control islets group (2308 ± 195) was statistically lower than PEG-dendron nano-shielded islets group (2822 ± 198). From these data, dendritic poly(ethylene oxide)s modified islets were very effective to inhibit the immune cell recognition as antigen and immune cell activation.

5.4. Conclusion

We developed well defined dendritic poly(ethylene oxide)s for surface modification on islets. Dendritic poly(ethylene oxide)s were activated by NHS on focal points and conjugated by chemical reaction. Dendritic poly(ethylene oxide)s modified islets were shown low toxicity and high viability. Islet functions were not different from unmodified islets. In addition, immunoprotective effect of dendritic poly(ethylene oxide)s were observed by incubating with splenocytes. We could suggest dendritic poly(ethylene oxide)s had an effect on inhibition of the infiltration and

protection of immunity substance into islets because of their bulky structures.

5.5. References

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국문 요약

폴리에틸렌 옥사이드는 구조적인 특성으로 유기용매와 수용액 상에서의 용해도가 우수하고, 추가적인 화학반응 조건에서의 안정성이 확보된 생체 적합성 물질로 알려져 있다. 생체 내로 도입되는 전달체의 필수 구성 요소로 인식되어 약물 전달, 유전자 전달, 조직 공학, 표면 개질 등 바이오의료 분야에서 많은 응용이 보고되고 있다.

폴리에틸렌 옥사이드는 고진공 조건에서 음이온 중합법으로 합성되고, 분자량 및 고분자 구조의 조절이 가능하다. 중합에 사용되는 개시제의 구조, 촉매, 반응용매, 온도 등을 조절하여 반응 조건에 따른 중합도 및 합성 결과를 확인하고 중합 조건을 최적화하여 부반응이 억제된 고순도의 폴리 에틸렌 옥사이드를 선형, 스타형, 덴드리머의 구조로 합성하였다. 중합된 폴리에틸레 옥사이드를 이용하여 약물 전달체와 면역 반응이 제어된 세포 전달체 표면 개질제로의 응용에 관한 연구를 수행하였다.

세포 내 선택적 약물 전달을 극대화하는 항암제 전달체의 개발을

위하여 수용성 폴리에틸렌 옥사이드와 소수성 폴리카프로락톤을 다이설파이트 결합이 가능한 아미노산인 시스틴으로 연결한 블락 공중합체 기반 나노 약물 전달체를 제조하였다. 다이설파이드 결합으로 안정화된 나노 전달체는 혈액 내 조건에서 안정성을 가지고, 상대적으로 사이올 농도가 높은 세포 내에서 선택적으로 불안정화됨을 확인하였고, 나노 입자 의 안정화/불안정화에 따른 선택적인 약물 방출 거동을 확인하였다.

동종 혹은 이종 간 세포 이식 시 발생하는 면역 거부 반응을 최소화하는 방안으로 세포 표면의 고분자 개질이 제안되어 왔다. 생체적합성이 우수한 폴리에틸렌 옥사이드가 사용되어 왔고, 기존 보고된 선형 폴리에틸렌 옥사이드의 개질과는 차별성을 가지는 스타형 또는 덴드리머 형태의 폴리에틸렌 옥사이드를 합성하고, 최적화된 세포 표면 개질 조건을 확립하여 세포 전달 시스템에 응용하였다. 세포 표면으로의 도입 및 추가적인 코팅을 위하여 스타형 및 덴드리머형 폴리에틸렌 옥사이드의 말단과 코어 기능기의 선택적 개질 합성법을 확립하였고, 도입된 기능기는 세포 표면으로의 고분자 도입과 추가 코팅 재료와의 화학적

결합에 사용되었다. 형광 물질이 도입된 고분자로 세포 개질 효과를 확인하였고, 세포 개질 정도와 세포 생존률과의 최적화된 조건을 확립하였다. 폴리에틸렌 옥사이드의 단독 코팅으로는 효율적인 면역 반응 억제에 한계점을 보였으며, 헤파린 등 추가적인 코팅 층과 면역 억제재의 도입으로 높은 세포 생존률과 효율적인 면역 억제 능력을 보이는 세포 전달체의 제작이 가능하였다.

구조와 분자량이 제어된 폴리에틸렌 옥사이드의 음이온 중합법을 확립하고, 약물과 세포 전달체로서의 응용 및 효율성을 확인하여 새로운 구조의 전달체 개발 연구를 수행하였다.

주요어: 폴리에틸렌 옥사이드, 음이온 중합, 스타형, 덴드리머형, 약물 전달 시스템, 세포 전달 시스템

학번: 2007-20694