1. Introduction

The drug delivery system is the effective method in which drugs put into the body and reach the region where they are needed. The purpose of the delivery system is to maximize a drug efficacy and to minimize a side effect through the delayed or sustained dosage. For more adequate delivery, the carriers are designed with degradable synthetic polymers, biochemical macromolecules, pseudo-biopolymers, or their interconnected structure [1–3].

Poly(L-lactic acid) (PLLA) is the biodegradable polymer which is degraded to L-lactic acid, a biological intermediate in carbohydrate catabolism. PLLA have been used for a variety of medical applications [4]; drug carriers, implants for bone fixation, absorbable sutures, surgical dressings, and tissue engineering applications. The copolymers of lactic acid have also been studied to develop an appropriate biomaterial corresponding with their aims such as poly(lactic acid-co-glycolic acid), poly(ethylene oxide-b-lactide), and poly(lactide-b-N-isopropylacrylamide) etc. [5–7].

γ-aminobutyric acid (GABA) is the inhibitory neurotransmitter which is related to blood flow and metabolism in the mammalian central nervous system. GABA is the omnipresent non-protein amino acid which is produced generally by α-decarboxylation of L-glutamic acid catalyzed by the enzyme glutamate decarboxylase. It has been investigated to a role as anxiolytic agent, and is effective in improving symptoms of neurological and psychiatric disorders such as epilepsy, brain ischemia, and mood disorders [8–10]. Recently, many studies focus on new type of anticonvulsant drug including GABA or its derivatives such as gabapentin and vigabatrin [11].

Poly(γ-aminobutyric acid) (PGABA) is the bio-based linear polymer which has repeated unit of GABA in the main chain and is producible from glutamate by bacteria such as Lactobacillus sp. and marine Pseudomonad [12,13]. It can be also synthesized from anionic ring-opening polymerization of 2-pyrrolidone [14]. The man-made PGABA is a sort of polyamide, a so-called polyamide 4, which has excellent thermal and mechanical properties based on high melting point of 260°C [15]. PGABA is some hydrophilic and biodegradable especially by bacteria [16]. Kazuhiko Hashimoto et al. reported the block copolymers with polyoxymethylene glycol for controlled of biodegradability [17].
copolymers have been studied in biomedical membrane for encapsulation of Langerhans islets [18]. Each block of a block copolymer can have a different property according to segmental size or chemical function. The block copolymers are therefore designed to retain several properties simultaneously such as adsorptive ability to drug, biodegradability, plasticity, and targeting. Particularly, amphiphilic block copolymers have been applied extensively in pharmaceutical applications due to their interesting phase transition [19].

In this study, the block copolymer was designed with the segmental block of poly(L-lactic acid) and the segmental block of poly(γ-aminobutyric acid) for a control release of GABA loaded as a drug; poly(L-lactic acid-block-γ-aminobutyric acid). It was synthesized successfully by sequential polymerization of self-initiation. The chemical structure of the block copolymer was analyzed by FT-IR, 1H-NMR and XRD. Surface energy, biodegradability and release behaviors of GABA were investigated on a membrane phase of the block copolymer.

2. Experimental

2.1. Materials
L-lactide acid (Sigma-Aldrich Co., USA) was purified by recrystallization using methanol. 2-pyrrolidone (Sigma-Aldrich Co., USA) was purified by distillation under a reduced pressure. Sodium (Sigma-Aldrich Co., USA) was used after removing oil stabilizer of the surface by adsorbent. Anhydrous tetrahydrofuran, n-butyllithium, stannous octoate were used.

Temperature of the reaction solution was lowered at -78 °C thereafter.

2.2. Syntheses

Polymerization of Poly(L-lactic acid): Poly(L-lactic acid) was prepared by ring-opening polymerization of dimer L-lactide using stannous octoate and acetic acid as co-initiator. The dimer and co-initiator were added in toluene and reacted under dry nitrogen atmosphere at 110°C for 48 hours. Temperature of the reaction solution was lowered at -78°C thereafter.

End-group Substitution of Poly(L-lactic acid): The dehydrated 2-pyrrolidone was obtained by the follow reaction; 2-pyrrolidone was reacted with the same mole ratio of n-butyllithium in anhydrous tetrahydrofuran at -78°C, and the temperature of the reaction was steadily increased until room temperature during 30 min. The dehydrated 2-pyrrolidone solution was added into the polymerization solution of poly(L-lactic acid) at -78°C. The temperature of the reaction was steadily increased until room temperature during 30 min. The polymer was precipitated in diethyl ether and was filtered. The 2-pyrrolidone-terminated poly(L-lactic acid) was obtained.

Block Copolymerization of Poly(γ-aminobutyric acid): The 2-pyrrolidone was activated by sodium ion. The activated 2-pyrrolidone was used as monomer of ring-opening copolymerization for poly(γ-aminobutyric acid) segmental block.

2.3. Fabrication

The membrane of poly(L-lactic acid-block-γ-aminobutyric acid) were prepared as the follow. The block copolymer was dissolved in 2,2,2-trifluoroethanol. The solution cast on a poly(ethylene terephthalate) (PET) film and was solidified by the evaporation of solvent at room temperature for 24 hours.

2.4. Characterization

Instrumental Analyses: 1H-NMR spectra were recorded in DMSO-d6 at 500 MHz by a JOEL JNM-AL FT-System spectrometer and TMS as a reference standard for chemical shifts. Fourier transform infrared (FT-IR) spectra were measured by Fourier transformation infrared spectrometer (IRAffinity-1, Shimadzu Corp., Japan) in a wavelength range of 400 cm⁻¹ to 4000 cm⁻¹ by KBr pellet method. X-ray diffraction analyses (XRD) were measured by vertical diffractometer (DMAX 2000V, Rigaku Corp., Japan) with reflection method using monochromatic CuKα radiation at 40 kV, 30 mA, and a scan speed 10°/min. The surface contact angles of the block copolymers were measured by contact angle meter (AM2001 G-1, Mirero system, Korea) at 23°C by sessile drop method using deionized water. The surface energy was calculated from the contact angle.

Biodegradation Measurement: In vitro biodegradation of poly(L-lactic acid-block-γ-aminobutyric acid) was determined using lysozyme. The membraneous block copolymer were immersed in 0.1 M PBS solution of pH 6.6 containing 4 mg/ml of lysozyme at 37°C during 14 days. Biodegradation ratio was calculated by the follow equation.

\[ R(\%) = \frac{W_1 - W_2}{W_0} \times 100 \]

\( R \): biodegradation ratio, \( W_0 \): mass of the block copolymer before degradation, and \( W_1 \): mass of the block copolymer.
after degradation.

**Drug Release Test:** In order to investigate a sustaining ability of poly(L-lactic acid-block-γ-aminobutyric acid) as GABA carrier, the release behavior was measured using the membrane of the block copolymer loaded GABA as a model drug. The block copolymers were dissolved in 2,2,2-trifluoroethanol. The GABA was mixed evenly in the solution. The mixed solution was cast on a PET film and was solidified by the evaporation of the solvent. The in vitro release of GABA from the membrane of the block copolymer was performed in 0.1 M PBS solution (pH 7.4) at 37°C for 1, 3, 6, 9 and 12 hours. The GABA concentration of PBS solution was determined at 202 nm by UV spectrophotometry.

### 3. Results and Discussion

#### 3.1. Copolymerization of Poly(L-lactic acid-block-γ-aminobutyric acid)

The novel block copolymer was designed for delivery of GABA which is a neurotransmitter and an anticonvulsant drug. The structure of the copolymer is linear di-block copolymer composed of hydrophobic poly(L-lactic acid) block and relatively hydrophilic poly(γ-aminobutyric acid) block. The aim of this design is an effective delivery of GABA loaded as a drug. The PLLA block is for controlling a microstructure of the block copolymer and for sustaining the release of GABA by the biodegradation of PLLA, and the PGABA block is for retaining the loaded GABA.

Poly(L-lactic acid) was prepared by ring opening polymerization of lactic acid with stannous octoate and acetic acid as co-initiators. The mechanism of the polymerization was shown in Scheme 1. The stannous octoate anion and acetic acid reacted to carbonyl carbon and oxide of L-lactide respectively resulting in an opening cation of L-lactide. Subsequently PLLA block was polymerized by a segment addition reaction of L-lactide in this cation. Next, in order to link PGABA block to the propagating terminal of the PLLA, we designed a self-initiative polymerization as shown in Scheme 2. For making self-initiative motif at PLLA block terminal, firstly, the dehydrated 2-pyrrolidone was prepared by removing hydrogen from amino group of 2-pyrrolidone as the strong electron negativity of butyl ion drew proton from amine of 2-pyrrolidone (Scheme 2(a)). The 2-pyrrolidone-terminated PLLA was synthesized by reacting the dehydrated 2-pyrrolidone to cation terminal of PLLA (Scheme 2(b)). Secondly, for linking γ-aminobutyric acid to 2-pyrrolidone-terminated PLLA, 2-pyrrolidone was activated using sodium ions (Scheme 2(c)). The activated 2-pyrrolidone can be polymerized in 2-pyrrolidone-terminal of PLLA by self-catalyst ring-opening polymerization as shown in Scheme 3. As the result, The poly(L-lactic acid-block-γ-aminobutyric acid) block copolymer was synthesized.

Theoretical degree of polymerization (DP) and mass ratios of PGABA/PLLA for the block copolymers were calculated by yielding mass and 1H-NMR as shown in Table 2. With decreasing the mole of initiator for the block copolymer, DP for PLLA block increased slightly while DP for PGABA block increased largely. The yielding mass ratio of PGABA/PLLA block for sample GL25 was 0.29, which drew near the block ratio calculated by 1H - NMR. The mole of PGABA homopolymer decreased with decreasing the mole of the block copolymer.

#### 3.2. Structural Analyses of the Block Copolymer

The molecular structure of the block copolymers were

Scheme 1. Mechanism of the polymerization of L-lactic acid.

Scheme 2. Reaction mechanisms of (a) the dehydration of 2-pyrrolidone, (b) the substitution of the end group of poly(L-lactic acid), and (c) the activation of 2-pyrrolidone.

Scheme 3. Mechanism of the copolymerization of L-lactic acid and γ-aminobutyric acid.
analyzed by FT-IR, ¹H-NMR, and XRD. The FT-IR spectra are shown in Figure 1. The N-H (amide I), N-H (amide II), and C=O vibration bands of PGABA appeared at 3292 cm⁻¹, 1538 cm⁻¹, and 1633 cm⁻¹, respectively. The bands from asymmetric and symmetric stretching vibration of CH₂ of aliphatic chain appeared at 2954 cm⁻¹~2872 cm⁻¹ (Figure 1(a)). As shown in Figure 1(b), the C=O and C-CH₃ vibration bands of PLLA appeared at 1751 cm⁻¹ and 1454 cm⁻¹. The bands from asymmetric and symmetric bending vibration of C-O-C of PLLA appeared at 1095 cm⁻¹ and 1192 cm⁻¹. The bands from asymmetric and symmetric bending vibration of CH₃ in PLLA appeared at 2965 cm⁻¹~2937 cm⁻¹. The amide band from cyclic vicinity of 2-pyrrolidone-terminal appeared at 1653 cm⁻¹ (* mark in Figure 1). As shown in Figure 1(c) for the block copolymer, the bands of C=O and C-CH₃ vibration bands of PLLA block appeared at 1747 cm⁻¹ and 1455 cm⁻¹, respectively. The bands from asymmetric and symmetric bending vibration of C-O-C of PLLA appeared at 1092 cm⁻¹ and 1184 cm⁻¹. The bands from asymmetric and symmetric bending vibration of CH₃ in PLLA block appeared at 2958 cm⁻¹~2913 cm⁻¹. The N-H(amide I), N-H(amide II), and C=O vibration bands of PGABA block also appeared at 3294 cm⁻¹, 1535 cm⁻¹, and 1634 cm⁻¹, respectively. The bands from asymmetric and symmetric bending vibration of CH₃ of aliphatic chain in PGABA appeared at 2945 cm⁻¹~2876 cm⁻¹.

The ¹H-NMR spectrum of poly(L-lactic acid-block-γ-aminobutyric acid) was showed in Figure 2. The resonance peak at 5.19 ppm and 1.46 ppm were assigned to the methine and methyl proton resonances respectively for the stereo sequence of PLLA block (Figure 2(a), (b)) [20]. The resonance peak at 3.4 ppm, 1.94 ppm, and 2.45 ppm were assigned to methylene proton resonances for the stereo sequence of PGABA block (Figure 2(c), (d), (e)) [21]. Therefore the molecular structure of poly(L-lactic acid-block-γ-aminobutyric acid) was confirmed by FT-IR spectra and ¹H-NMR spectrum.

The crystallinity of a block copolymer can contribute as a recognition factor for estimating its biodegradation and

### Table 2. Compositions of the synthesized block copolymers

<table>
<thead>
<tr>
<th>Code</th>
<th>Code</th>
<th>Block copolymer* (mol)</th>
<th>DP of the block**</th>
<th>Mass ratio of PGABA/PLLA block</th>
<th>GABA unit in homo-PGABA (mol)</th>
<th>Yielding rate of block copolymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL04</td>
<td>1.4×10⁻⁴</td>
<td>393</td>
<td>18.57</td>
<td>0.05</td>
<td>6.5×10⁻⁴</td>
<td>0.82</td>
</tr>
<tr>
<td>GL14</td>
<td>1.3×10⁻⁴</td>
<td>423</td>
<td>61.53</td>
<td>0.17</td>
<td>6.3×10⁻⁴</td>
<td>0.61</td>
</tr>
<tr>
<td>GL25</td>
<td>1.2×10⁻⁴</td>
<td>458</td>
<td>1166</td>
<td>0.29</td>
<td>5.8×10⁻⁴</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* The mole of block copolymer was assumed to be equal to the mole of initiator and ** DP: degree of polymerization, calculated by yielding mass.
drug-release, or whether the properties of individual blocks are preserved. Wide angle X-ray diffractograms of the block copolymers were shown in Figure 3. The 2-pyrrolidone-terminated PLLA had only diffractions of crystalline phase for PLLA; a strong reflection at 5.32 Å d-spacing arising from (200)/(100) planes and a weak reflection at 4.6 Å d-spacing from (203) plane of PLLA crystalline phase[22]. On the other hand, poly(L-lactic acid-block-γ-aminobutyric acid) showed these reflections for PLLA as well as the reflections at 4.36 Å d-spacing from (200) plane and 3.73 Å d-spacing from (020) plane of crystalline phase for PGABA simultaneously [23]. It was confirmed that each block of poly(L-lactic acid-block-γ-aminobutyric acid) has individually inherent crystalline phase. This means that each block of the copolymer matrix has individually inherent property.

3.3. Surface Energy
The surface energy of drug carrier is an important factor in sustaining and releasing of drugs. The surface energies of the block copolymers were obtained by contact angle measurements as shown in Table 3. The surface energy of poly(L-lactic acid-block-γ-aminobutyric acid) increased with increasing the ratio of PGABA block. It may be due to the hydrophilicity of poly(γ-aminobutyric acid) segments. The surface hydrophilicity of the block copolymer will be able to be controlled by regulating the amount of the PGABA block.

3.4. Biodegradability
The degradation of poly(L-lactic acid-block-γ-aminobutyric acid) was estimated using lysozyme. Figure 4 showed in in vitro biodegradation behavior of the block copolymers. Degradation of the block copolymer began slowly after 2 days. Particularly, the GL04 was more rapidly degraded, which was degraded approximately 30% at 14 days. With increasing PGABA block, the block copolymer was degraded slowly. The PLLA block was mostly influenced by lysozyme, because deesterification of PLLA block occurs more easily than a splitting of amide group of PGABA block.

3.5. Drug Release Behavior
The drug release behavior of poly(L-lactic acid-block-γ-aminobutyric acid) was estimated for drug carrier. Anticonvulsant GABA was used as model drug, which is water-soluble neurotransmitter. The GABA was loaded into the matrix of poly(L-lactic acid-block-γ-aminobutyric acid) by mixing with solution of the block copolymer. The solution cast to membrane form. For release measurement, the membrane was immersed in PBS solution. Figure 5 showed the release profiles of GABA. In all samples, GABA was released quickly up to 3 hours, and was released gradually since. With increasing the proportion of PGABA block, the release amount of GABA decreased. It seems that GABA was entrapped in the domain agglutinated by PGABA blocks.

4. Conclusion
The novel block copolymer was designed for a specific drug delivery. The two block copolymer was prepared by ring opening polymerization of L-lactide and 2-pyrrolidione; Poly(L-lactic acid-block-γ-aminobutyric acid) was synthesized by self-initiative polymerization of the activated 2-pyrrolidione at 2-pyrrolidione terminal of poly(L-lactic acid). The molecular structure of poly(L-lactic acid-block-γ-aminobutyric acid) was confirmed by FT-IR, 1H-NMR, and XRD. The block copolymer had respective crystalline phase in both PLLA block and PGABA block. The surface energy increased with increasing the ratio of PGABA block in the block copolymer. The lysozyme degradation of the block copolymer was influenced in a relative size of PLLA block. The release rate of the loaded GABA decreased with increasing the PGABA block in the block copolymer.
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References