Preparation of ethyl cellulose fibers by electrospinning and assessment of antimicrobial activity of the nanofibers containing antibiotic

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Department of Bio New Drug Development
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Advisor: Prof. In Hwa Lee

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Department of Bio New Drug Development
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Thesis submitted in partial fulfillment of the requirement for the Award of the degree of Master of Science

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Graduate School of Chosun University
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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>K$_2$HPO$_4$</td>
<td>Dipotassium Phosphate</td>
</tr>
<tr>
<td>DMAc</td>
<td>N, N dimethylacetamide</td>
</tr>
<tr>
<td>EC</td>
<td>Ethyl cellulose</td>
</tr>
<tr>
<td>HVS</td>
<td>High voltage supply</td>
</tr>
<tr>
<td>NA</td>
<td>Nutrient agar</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Staphylococcus aureus subsp aureus</td>
</tr>
<tr>
<td>SCDB medium</td>
<td>Soybean Casein Digest Broth medium</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>TCD</td>
<td>Tip-collector–distance</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
</tbody>
</table>
요 약
전기방사에 의하여 제조된 항균제를 함유한 에틸셀룰로오스
나노섬유의 항균 활성 평가

Pham Thi Kieu Oanh
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THF (tetrahydrofuran)/DMAc (N, N dimethylacetamide) 혼합용매 시스템에 대하여 두 용매의 성분비를 변화시키며 150g/l의 농도로 용해되어 있는 에틸셀룰로오스 및 항체계 용액으로부터 나노섬유를 제조하여 성분비에 따른 THF와
DMAc 용매가 ethyl cellulose 섭유 표면의 형태에 미치는 영향을 살펴 보았다. 식품부패균의 일종인 S. aureus에 나노섬유의 항균 활동 효과를 증가시키기 위해 암피실린과 페니실린, 스트렙토마이신을 항생제로 선택하여 나노섬유에서 방출되는 항생제의 효과를 물에 녹인 항생제와 한천배지의 디스크확산법 (agar disc
diffusion) 탐도측정 테스트 (optical density test) 방법으로 비교하며 수행하였다. 섭유의 형태는 주사전자현미경 (Scanning electron microscope, SEM)으로 관찰하였다. 다양한 성분의 용매에 DMAc를 첨가하지 나노섬유의 지름이 감소하는 경향을 나타내었다. 항생제를 함유하고 있는 나노섬유의 형태는 항생제를 가지고 있지 않은 섭유에 비해 표면이 매끄러워지고 지름이 감소하였다. 3:2 부피 비의
THF:DMAc 용매일 경우, 나노섬유의 표면에는 작은 결절이 형성되었다. 이 부피 비의 용매에 용해된 에틸셀룰로오스 섭유로부터 항생소질이 나노섬유에 탑재된 항생물질의 항균성 효과 지속시간이 스트렙토마이신과 같은 항생제가 대량으로 박테리아에 투여되었을 때 미치는 항균성 효과의 지속시간보다 더 긴 것으로 나타났다.
반면에, 나노섬유에 함유된 암피실린과 페니실린의 항균성 활동은 용액에 녹여 투여되었을 때의 항균성 활동은 두 경우 모두 크게 나타났다. 그러나 적은 농도로 스트렙토마이신이 나노섬유에 함유되어 있을 경우, *S. aureus*에 미치는 항균성 효과는 나노섬유의 경우가 지속적으로 효과를 보였다.
ABSTRACT

Preparation of ethyl cellulose fibers by electrospinning and assessment of antimicrobial activity of the nanofibers containing antibiotic

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Department of Bio New Drug development,
Graduate School of Chosun University

Electrospinning method was used to produce nanofibers from ethyl cellulose solution having concentration of 150 g/l, using a binary solvent system of THF (tetrahydrofuran): DMAc (N, N dimethylacetamide) of various volume ratios. The influence of the composition of the binary solvent system on the surface morphology of ethyl cellulose nanofiber with or without adhered antibiotics was investigated using SEM.

To assess the effectiveness for releasing drug from the nanofibers and their antibacterial activities on S. aureus; ampicillin, penicillin and streptomycin were selected as antibiotics. Agar disc diffusion and optical density test were applied for the assessment.

The results showed that the addition of DMAc in the binary solvent system led to decrease the fiber diameter. The morphologies of fibers having antibiotic were smoother and of smaller diameter than those of the antibiotic-free fibers. Tubercles were formed on the fiber surface at THF to DMAc volume ratio of 3 to 2. At this solvent concentration, the antibiotic
release from ethyl cellulose fibers was at the best. The antibacterial effective time of the antibiotics loaded in nanofibers was longer than that of the bulk antibiotics against bacteria in the case of streptomycin. On the other hand, the antibacterial activities of ampicillin and penicillin loaded in the fibers were not significant as compared to the effect of bulk antibiotics. However, at low concentrations of ampicillin and penicillin, the antibacterial effect on *S. aureus* was considerably higher than streptomycin loaded in the fibers.
I. Introduction

1. Nanotechnology

*Nanotechnology*, engineering of functional systems at the molecular scale, is the design, characterization, production and application of structures, devices and systems by controlling shape and size at the nano-scale. The term broadly refers to such fields as biology, physics or chemistry, any scientific field, or a combination thereof, which deals with the deliberate and controlled manufacturing of nanostructures.

*Electrospinning* is a process by which a polymer solution is charged to high voltage to produce submicron scaled fibers [1]. At a voltage sufficient to overcome surface tension forces, fine jet is stretched and elongated before it reaches the target. It dries and is collected as an interconnected web of small fibers with typical diameter of several hundreds of nanometers.

![Fig. 1. Schematic of the electrospinning set up.](image)

The advantages of the electrospinning process are its technical simplicity and its easy adaptability. The apparatus used for electrospinning consists of a high voltage electric source with positive and negative polarity, a syringe pump with capillary to carry the solution from
the syringe to the spinneret, and a conducting collector like-aluminum coated foil paper which can be made of any shape like-a flat plate, rotating drum, etc. depending on the requirements. A schematic of the electrospinning process is shown in Fig. 1.

The basic phenomenon of electrospinning is relatively simple to realize in practice. It suffices to apply a high voltage to a capillary filled with polymer fluid by means of an electrode, and then to collect the resulting fibers on a grounded plate. Feed of the polymer fluid to the tip of the capillary results in the formation of a droplet at the capillary tip whose size and shape are dictated by surface tension and gravitational forces. The application of a high voltage to the capillary produces a surface charge on the droplet which offsets the forces of surface tension. This results in elongation of the drop, formation of a Taylor cone and, at sufficiently high voltage, ejection of a continuous stream (“jet”) from the tip of the cone. During the ejection, the jet travels toward the collector, and the solvent evaporates. Both electrostatic and fluid dynamic instabilities can contribute to the basic operation of the process. With low molecular weight liquids, the fluid typically breaks up into small charged droplets. With polymeric fluids, viscoelastic forces stabilize the jet and permit the formation of small diameter, charged filaments.

Electrospinning is the unique process in that it is able to produce polymer fibers with diameters ranging over orders of magnitude, from the micrometer range typical of conventional fibers, down to the nanometer range.

**Nanofibers** are solid state linear nanomaterial characterized by flexibility and an aspect ratio greater than 1000:1. Materials in fiber form are of great practical and fundamental importance. The combination of high specific surface area, flexibility and superior directional strength makes fiber a preferred material form for many applications ranging from clothing to reinforcements for aerospace structures. Fibrous materials in nanometer scale are the fundamental building blocks of living systems.

In recent times, nanofibers have attracted the attention of researchers due to their
pronounced micro and nano structural characteristics that enable the development of advanced materials that have sophisticated applications.

**Application of Nanotechnology**

Polymers with attractive chemical, mechanical, and electrical properties like-high conductivity, high chemical resistance, and high tensile strength have been spun into ultra fine fibers by the electrospinning process, and their application potential in areas like-tissue engineering, catalysis, filtration, nanocomposites, nanofibrous structures, drug delivery systems, protective textiles, and storage cells for hydrogen fuel cells, etc have been examined [2-5]. In the following, some examples for applications of nanofiber are given.

**Medicine**

In a recent report [6], business opportunities for nanostructured materials in biotechnology and medicine were estimated to be of the order of 180 billion US$ in 2015. Particular examples which were highlighted were drug release systems, nanofibers for wound healing obtained by electrospinning as well as nanostructured materials for tissue engineering, where bone tissue engineering was one important aspect. Another aspect is the inclusion of drugs or other functional materials in medicine and pharmacy into polymer nanofibers and polymer nanotubes [7]. The nanoobjects then serve both as drug carrier and drug release systems. It is the particular shape and size of the nanoobjects as well as their internal architecture which control where the nanoobjects will attach in the body and in which manner the release will take place.

**Carbon and graphitic nanofibers**

Carbon nanofibers are finding enormous applications in unconventional energy sources and storage cells due to their enhanced conductivity and high aspect ratio. Their mechanical properties enable them to be used as fillers in composites that fine applications in synthetic and rubber industries [8]. Carbon nanofiber reinforced composites offer increased stiffness, high
strength and low electrical resistivity. Also, aligned nanofiber composites provide enhanced mechanical properties than the randomly aligned nanofiber composite structure [9].

Catalytic nanofibers

Chemical reactions employing enzyme catalysts are important in chemical processes due to their high selectivity and mild reaction conditions [10]. Immobilized enzymes are used largely due to easiness of catalyst separation, enzyme stability, and their availability for continuous operations. Nanomaterials are of recent interest as catalyst substrates because of their large surface area per unit mass and the feasibility for high catalyst loading [10]. Fibrous catalysts offer advantages, such as feasibility of adapting to any geometry and low resistance to the flow of liquids and gases [11]. Carbon nanofiber supports loaded with iron particles have shown high conversion of hydrocarbons in comparison with active carbon and alumina [12]. It has been shown that the intrinsic catalyst effect is more pronounced when loaded in smaller diameter fibers such as nanofibers [12], etc.

Filtration

Polymeric nanofibers have been used in air filtration applications for more than a decade [11]. Due to poor mechanical properties of thin nanowebs, they were laid over a substrate suitable enough to be made into a filtration medium. The small fiber diameters cause slip flows at fiber surfaces, causing an increase in the interception and inertial impaction efficiencies of these composite filter media [12]. The essential properties of protective clothing are high moisture vapor transport, increased fabric breath ability, and enhanced toxic chemical resistance, electrospun nanofiber membranes. The highly porous electrospun membrane surfaces help in moisture vapor transmission. Many researchers have studied the possibility thin nanofiber layers over the conventionally used nonwoven filtration media for protective clothing [13]. Nanofibers also find applications in aerospace and semiconductor industries. Piezoelectric polymers were electrospun and investigated for applicability as a component on the wings of micro-air vehicles [14].
As is evident from the aforementioned brief discussion on the applications of nanomaterials, the potential of nanostructured materials in advanced applications is unlimited.

2. Cellulose

Cellulose is a chief constituent of the cell walls of plants. Chemically, it is a carbohydrate that is a high molecular weight polysaccharide. Raw cotton is composed of 91% pure cellulose; other important natural sources are flax, hemp, jute, straw, and wood. Insoluble in water and other ordinary solvents, it exhibits marked properties of absorption [15].

Cellulose is the most abundant naturally occurring organic substance, being found as the principal component of cell walls in higher plants where it provides the main structural feature. Cellulose is an excellent fiber. Wood, cotton, and hemp rope are all made of fibrous cellulose.

Cellulose fiber is useful in widely applications, such as filtration, biomedicine, clothing and textile and the film prepared from cellulose has quite good permeability, it has been widely used industrial air filter. There are few researches reported on the fiber, the properties of good thermostability and electricity is discovered. Ethyl cellulose was used in this study because it was selected as a model encapsulation material. It is a water insoluble polymer, and widely used in pharmaceutics as a wall material for sustained-release microcapsule [16]. This is due to its high safety, good stability, easy fabrication and cheapness.

Fig. 2. Molecular structure of ethyl cellulose.
3. Antibiotics

Antibiotics are low molecular weight metabolites that inhibit the growth of other microorganisms at low concentrations [17]. Antibiotics are drugs derived wholly or partially from certain microorganisms and are used to treat bacterial or fungal infections. They are ineffective against viruses. Antibiotics either kill microorganisms or stop them from reproducing, allowing the body's natural defenses to eliminate them [18].

In this study, antimicrobial activity of antibiotics as streptomycin, ampicillin and penicillin were tested and the activities of the antibiotics loaded in nanofibers made by electrospinning were also studied on *S. aureus*.

Ampicillin and penicillin are beta-lactam antibiotics. They are bactericidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial walls.

![Chemical structures of ampicillin (a) and penicillin (b).](image-url)

Fig. 3. Chemical structures of ampicillin (a) and penicillin (b).
Streptomycin belongs to aminoglycosides group; it stops bacterial growth by damaging cell membranes and inhibiting protein synthesis.

![Chemical structure of streptomycin.](image)

Fig. 4. Chemical structure of streptomycin.

The study was designed to demonstrate the incorporation and controlled–release of the antibiotic drugs from electrospun fibrous scaffolds, as well as its ability to inhibit bacterial growth. To control the drug release profile, ethyl cellulose (EC) in tetrahydrofuran (THF) and N,N-dimethylacetamide (DMAc) solvent system was used to encapsulate the hydrophilic drugs. EC was selected as a model encapsulation material. EC is a water insoluble polymer and widely used in pharmaceutics as a wall material for sustained release microcapsules. This is due to its high safety, good stability and easy fabrication. In this study, the nanofibers were formed by EC dissolving in the solvent of THF/DMAc with their various ratios. The viability and bioactivity of the released drugs was examined using in vitro *S. aureus* inhibition tests. Disc diffusion method was also employed as assessment method in special properties of the antibiotics.

In the thesis, the conditions of the solution properties and electrospinning technique preparing ethyl cellulose nanofibers in which optimal antibiotic release time was obtained as well.

This is an initial study to apply electrospinning technique for production of the new antimicrobial medicines and drug delivery.
II. Materials and Methods

1. Electrospinning method

a. Materials

Antibiotic agents as ampicillin (Sigma Aldrich Co., Germany), penicillin G sodium salt (Sigma Aldrich Co., Germany) and streptomycin sulfate salt (Sigma Aldrich Co., Germany).

Tetrahydrofuran (THF) (99% purity, Sigma Aldrich Co., Germany) and N, N dimethylacetamide (DMAc) (99% purity, Sigma Aldrich Inc., Germany) were chemically pure reagents.

Ethyl Cellulose (EC) powder ($M_N = 30,000$) (Sigma Aldrich Inc., Germany).

b. Preparing EC solution and antibiotic mixing EC solutions

Nanofibers adhering antibiotics were prepared in the binary solvent system (THF, DMAc). Nanofibers were prepared formed from EC dissolved in the binary component solvent system (THF, DMAc) with the volume ratio of THF to DMAc of 1 to 4, 3 to 2 and 4 to 1 (v/v). EC solution concentration was 150 g/l.

<table>
<thead>
<tr>
<th>THF:DMAc (v/v)</th>
<th>1:4</th>
<th>3:2</th>
<th>4:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC solution viscosity</td>
<td>2160 cP/19.8°C</td>
<td>2832 cP/19.8°C</td>
<td>3528 cP/19.8°C</td>
</tr>
</tbody>
</table>

Nanofibers, containing streptomycin 7 wt%, were formed from EC dissolved in various THF:DMAc solvent system volume ratio of 1:4, 3:2 and 4:1.
Table 2. Viscosity of EC solution with various volume ratio of the binary solvent system containing streptomycin 7 wt% 

<table>
<thead>
<tr>
<th>THF:DMAc (v/v)</th>
<th>1: 4</th>
<th>3: 2</th>
<th>4: 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC solution viscosity</td>
<td>2724 cP/20.7°C</td>
<td>2336 cP/20.5°C</td>
<td>2088 cP/21.6°C</td>
</tr>
</tbody>
</table>

Nanofibers were formed from EC dissolved in THF:DMAc solvent system (3:2 v/v), having ampicillin, penicillin and streptomycin at various concentrations. The concentrations of 0.1 wt%, 0.3 wt%, 0.5 wt%, 0.7 wt% and 1.0 wt% were used to test of the antibacterial activities by agar disc diffusion assay; the concentrations of 5.0 wt%, 7.0 wt% and 10.0 wt% were used to observe the fiber morphologies and test of the antibacterial activities by optical density method.

Table 3. Viscosity of EC solution containing antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration (wt %)</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>5.0</td>
<td>1230 cP/20.6°C</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>2472 cP/20.6°C</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>2608 cP/20.0°C</td>
</tr>
<tr>
<td>Penicillin</td>
<td>5.0</td>
<td>2460 cP/20.7°C</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>2616 cP/20.7°C</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>2760 cP/20.7°C</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>5.0</td>
<td>2160 cP/18.3°C</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>2832 cP/18.8°C</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>3528 cP/18.6°C</td>
</tr>
</tbody>
</table>
c. Electrospinning

A schematic diagram for electrospinning is depicted in Fig. 1. The electrospinning setup used in this study consisted of a syringe pump (i.d. 10 mm) (200 series, KD Scientific Inc., USA) and needle (i.d. 0.8 mm), a ground electrode (stainless steel sheet) and a high-voltage supply (Series ER/DM, Glassman high voltage, Inc.). The needle was connected to the high-voltage supply, which can generate positive direct current voltage up to 50 kV.

For antibiotic loading nanofibers producing, the voltage of 15 kV was applied. The distance between the needle tip and the ground electrode tip collector distance (TCD) was 10 cm. The ground electrode was connected to collectors coating aluminum foil. The polymer solutions were delivered via the syringe pump to control the mass flow rate. Mass flow rate of solutions was 100 µl/min.

Electrospinning process was carried out at room temperature and humidity.

Fig.5. Photo of electrospinning apparatus.
2. Drug release test

To monitor the drug releasing rate from nanofiber, UV-visible spectrophotometer was used. The energy of ultraviolet and visible lights corresponded to the difference of electron condition energy in atoms and molecules. The spectrum obtained through the detector showed intensity of absorbed light as well as the information of sample molecules. Using this principle, the antibiotic release concentration of the nanofiber samples in testing liquid was determined. And at wavelength of 203 nm, antibiotic absorption capacity was the best. 0.02 g nanofiber adhering antibiotic as streptomycin concentration of 7 wt% was soaked in 100 ml distilled water.

3. Antimicrobial activity test

a. Bacterial strain

A *Staphylococcus aureus* subsp. *aureus* (*S. aureus*) strain (KCCM 12214) was used in this study from the Korean Culture center of Microorganism.

b. Growth conditions

*S. aureus* were grown on Soybean Casein Digest Broth medium (Becton Dickinson, USA) at 37°C.
Table 4. Composition of Soybean Casein Digest Broth medium

<table>
<thead>
<tr>
<th>Components</th>
<th>Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>17.0 g</td>
</tr>
<tr>
<td>Pancreatic Digest of Soybean Meal</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Dipotassium Phosphate (K$_2$HPO$_4$)</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Trypticase soy agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.0 L</td>
</tr>
</tbody>
</table>

+ Adjust pH of the media at 7.3 ± 0.2.

All subcultures were sterilized by autoclave at 121°C for 15 minutes and kept at 4°C then warmed at room temperature before using in the experiments.

Agar media were poured into Petri dishes immediately after autoclaved. The dishes solidified were kept at 4°C before using in the experiments.

Subcultures were carried out by 300 ml flasks which contained 100 ml of LB medium. The culture was incubated at 36.5°C for 24 hours before using as inoculum.

c. Antimicrobial activity assays

_The disc-diffusion assay_ was used to determine the growth inhibition caused by antibiotic agents against the bacterial strains as _S. aureus_ strain.

According to the disc-diffusion assay, the antimicrobial activity of antibiotic agents was tested individually against the above microorganisms. The 100 µl indicator microorganism cells were inoculated into a 250 ml flask containing 100 ml of Soybean Casein Digest Broth medium for _S. aureus_ the media with each tested microorganism in a shaking incubator.
overnight at 36.5°C. The microorganism was used to test antimicrobial activity. Antimicrobial activity test was followed standard disc diffusion method [65]. A plate of nutrient agar (NA) was inoculated with 0.1 ml of bacterial suspension. The suspension was streaked over the entire surface of the medium to ensure an even distribution of the suspension. Then, sterile filter paper discs (i.d. 8 mm) or 0.01 g nanofibers discs (i.d. 6 mm) including antibiotic agents were placed individually with forceps. Diffusion of 100 µl the antibiotics in the discs began immediately. Then, the discs or the nanofibers were incubated at 37.2°C for 24 hrs. After the incubation, the diameter of the zones of complete inhibition was measured in mm.

The antibacterial activities of the fibers loading the various antibiotic concentrations of 0.1 wt% to 1.0 wt % were investigated.

*Optical density test for assessment of antimicrobial activity on bacteria*

This method was applied for an approximate evaluation of the susceptibility of *S. aureus* strain to the bulk antibiotics and the antibiotics which were added into fibers with various antibiotic concentrations. The effect of antibiotics having concentration loaded in the fibers of 5 wt%, 7 wt%, and 10 wt% was test. The weight of the fibers was 0.4 g. The bacterial inoculums and the fibers in bacterial cultured medium were prepared in small sterile tubes. Bacteria inhibition activity was checked every 4 hours. In this study, *S. aureus* strain was evaluated on the basis of the optical absorbance of the culture. Turbidity changes effects by the bacteria–antibiotics reaction were followed by optical density measurements at wavelength of 640 nm for *S. aureus*.

4. Measurement and characterization

The viscosity, temperature of cellulose solutions in THF/DMAc reagent mixed antibiotic agents was determined with a Viscometer (Brookfield, model DV-II).

The morphology of the electrospun fibers was observed on a scanning electron microscope (FE-SEM, S-4800, Hitachi, Japan).

The inhibitory concentrations of the antibiotics were determined by UV spectrophotometer (UV- VIS recording spectrophotometer, UV–2401PC, Shimadzu, Japan).
III. Results

1. Preparation of nanofibers from ethyl cellulose solution

Nanofibers were prepared from ethyl cellulose solution (150 g/l) in the binary solvent system THF and DMAc with the volume ratio of 3 to 2 by electrospinning method, at a high voltage of 15 kV, the TCD of 10 cm, with an EC solution flow rate of 100 µl/min. The produced nanofibers were shown in Fig. 6.

Fig. 6. Morphology of fiber observed by SEM at high magnification of x 1,000.

2. Morphologies of nanofibers prepared by using ethyl cellulose solution loading antibiotics

The binary component THF:DMAc solvent was used to dissolve the ethyl cellulose. The jet from the needle tip was formed and sustained because the power supply overcame the viscosity and surface tension of the EC solution.

Electrospinning was performed for the volume ratios of THF:DMAc (1:4, 3:2 and 4:1) (v/v) at a 100 µl/min of flow rate for ethyl cellulose solution under 15 kV of electricity; 10 cm, TCD. Morphology of the fibers was evaluated by using SEM image with various magnifications.

The morphologies of fiber prepared from THF:DMAc solvent with various volume ratios of 1:4, 3:2 and 4:1 were investigated. The solutions having different binary component
THF and DMAc solvent showed various viscosities. The solvent volume ratio of 1:4, 3:2 and 4:1 had the viscosity of 2160 cP, 2832 cP and 3528 cP, respectively. The Fig. 7A shows that diameter of the fiber which was prepared from the binary solvent system THF:DMAc of 1:4 were smaller and the fiber morphologies were more uniform than that of the others.

And the addition of DMAc in the system led to the decrease of the fiber diameter and so the average diameter of the fiber decreased with increasing volume of DMAc. The morphologies of the fiber were shown more evidently in the Fig 7B. The fiber from the EC solution having THF:DMAc ratio of 1:4 (v/v) was smoother than that of THF:DMAc solvent volume ratio of 3:2 which appeared tubercles. Specially, the fiber spinned from the EC solution having the THF:DMAc solvent system ratio of 4:1 (v/v) was porous along the fibers.

Fig. 7. Morphologies of fiber prepared from EC solution concentration of 150 g/l in THF–DMAc solvent with various ratios.
A, low magnification (x 300 - SEM); B, higher magnification (x 25,000 - SEM); (a) THF:DMAc=1:4 (v/v); (b) THF:DMAc=3:2 (v/v); (c) THF:DMAc=4:1 (v/v).
In this study, morphology of fiber which loaded drug as antibiotic was studied. Fig. 8 shows a selected SEM image of the antibiotics loading nanofibers. Clearly, nanofibers with smooth surface were obtained.

At the same concentration, different antibiotics loading in nanofibers led various fiber morphologies. The evidence is presented in Fig. 8.

![Fig. 8](image)

Fig. 8. Morphologies of fiber prepared from EC solution whose concentration was 150 g/l in THF to DMAc solvent having the volume ratio of 3 to 2, unloading and loading Streptomycin (magnification of x 25,000 – SEM).

(a) Fiber without antibiotic;

(b) Fiber with streptomycin; (c) Fiber with ampicillin; (d) Fiber with penicillin.
Fig. 9 shows the SEM images of the nanofibers having streptomycin of various concentrations. On increasing streptomycin concentration in the nanofiber solution, the EC concentration decreased. At low viscosity, beads along with the fibers deposited on the collection plate, were found throughout the fibers. The shape of the beads varied with varying the viscosity. The amount of the fibers containing beads reduced with the increasing streptomycin concentration. It is clearly evident in Fig. 9A.

Fig. 9. Morphologies of fiber prepared from EC solution having THF–DMAc solvent ratio of 3 to 2 and loading Streptomycin with various concentrations.

A, low magnification (x 300 - SEM); B, higher magnification (x 10,000 - SEM);

(a) Streptomycin 5 wt%; (b) Streptomycin 7 wt%; (c) Streptomycin 10 wt%.
3. Drug release test

Figure 10 shows that the released antibiotic concentration is dependent on the composition of the solvent mixture in the EC solution having concentration of 150 g/l.

For THF:DMAc volume ratios of 1:4, 3:2, and 4:1; the maximum absorbance of the release antibiotic concentrations measured at wavelength of 203 nm were 3.98, 9.27 and 1.34, respectively.

The release of antibiotics adhered to the nanofibers prepared from the solvent ratio of 3 to 2 (v/v) was faster than that of the others. However, the antibiotic continuously released for 8 hrs.

The antibiotic adhered to different nanofibers dispersed gradually and steadily after 6, 8 and 8 hrs for THF:DMAc ratio of 1:4, 3:2 and 4:1 (v/v), respectively.

The above-mentioned results show that the nanofibers took long time to release the drug, while the concentration of the drug was high.

The solvent ratio of 3 to 2 may be a suitable solution for the diffusion of streptomycin of 7 wt%. In this solvent, the antibiotic was diffused better than other solutions.

This result leads us to select the binary solvent system of THF:DMAc volume ratio of 3:2 for further study.
Fig. 10. Assessment of optimal release of antibiotic content.

where:  
● the volume ratio of binary component THF-DMAc solvent of 1 to 4  
○ the volume ratio of binary component THF-DMAc solvent of 3 to 2  
▼ the volume ratio of binary component THF-DMAc solvent of 4 to 1.

The EC solution for preparing nanofibers was 150 g/l and streptomycin concentration loaded in nanofibers was 7 wt%.
4. Assessment of antimicrobial activity of bulk antibiotics and antibiotic loaded in nanofibers on bacteria

a. Agar disc diffusion assay

In agar disc diffusion assay, the bulk antibiotics and the antibiotics loading in the fibers showed considerable activity against *S. aureus* except streptomycin concentration of 0.1 wt% loading in the fibers. The bacterial growth inhibition zones expressed antibacterial activities of the antibiotics at various concentrations on *S. aureus*. The higher the antibiotic concentrations, the larger the inhibition zones, and the higher antibacterial activities of antibiotics on *S. aureus* (Table 5 and Figures 11-16).

It was also found that the fibers containing antibiotic inhibited bacteria growth in a larger area that the fiber size due to the diffusion of the antibiotics.

As shown in Table 5, at the lowest antibiotic concentration of 0.1 wt% loading in the fibers, the bacterial growth inhibition zone was the smallest for all antibiotics, and at the highest concentration of 1.0 wt%, the zone was the largest. Further, in the assays, *S. aureus* was sensitive to both ampicillin and penicillin. Hence, the inhibition zone diameters were quite large although the activities of the antibiotics loading in the fibers were markedly reduced on comparing with that of bulk antibiotics.
Table 5. Antibacterial activity of the antibiotics on *S. aureus*

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<th>Antibiotics</th>
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<th>Bacterial growth inhibition zones (mm)</th>
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<tr>
<td></td>
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<td>Bulk antibiotic</td>
<td>Antibiotic loaded in nanofibers</td>
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<tr>
<td><strong>Ampicillin</strong></td>
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<td>0.3</td>
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(i.d. of paper disc, 8 mm; i.d. of fiber disc, 6 mm)
Fig. 11. Pictures of *S. aureus* growth inhibition zones of various concentrations of bulk penicillin.

Fig. 12. Pictures of *S. aureus* growth inhibition zones of various concentrations of penicillin loading in the fibers.
Fig. 13. Pictures of *S. aureus* growth inhibition zones of various concentrations of bulk ampicillin.

Fig. 14. Pictures of *S. aureus* growth inhibition zones of various concentrations of ampicillin loading in the fibers.
Fig. 15. Pictures of *S. aureus* growth inhibition zones of various concentrations of bulk streptomycin.

Fig. 16. Pictures of *S. aureus* growth inhibition zones of various concentrations of streptomycin loading in the fibers.
b. The optical density test

The effect of bulk streptomycin on *S. aureus* is shown in Fig. 17 and Fig. 18. The bulk streptomycin completely inhibited the bacterial growth within 12 hrs, 20 hrs at low and higher concentration ratios, respectively. The antibacterial effect of streptomycin against *S. aureus* decreased gradually on increasing the time. After the above mentioned hours, the antibacterial effect of streptomycin varied depending on its concentration.

![Graph showing optical density vs time for different streptomycin concentrations and bacterial growth curves for *S. aureus*.](image)

Fig. 17. The effectiveness of various concentrations of bulk streptomycin on *S. aureus* growth.

where:  
- (●) growth curve of *S. aureus*
- (○) growth curve of *S. aureus* when bulk streptomycin concentration of 0.1 g/l inhibited
- (▼) growth curve of *S. aureus* when bulk streptomycin concentration of 0.2 g/l inhibited
- (▼) growth curve of *S. aureus* when bulk streptomycin concentration of 0.4 g/l inhibited.
Fig. 18. The effectiveness of various concentrations of bulk streptomycin on *S. aureus* growth.

where:  
- (●) growth curve of *S. aureus*  
- (○) growth curve of *S. aureus* when streptomycin concentration of 1 g/l inhibited  
- (▼) growth curve of *S. aureus* when streptomycin concentration of 2 g/l inhibited  
- (▼) growth curve of *S. aureus* when streptomycin concentration of 4 g/l inhibited.

After 20 hrs of exposure, the streptomycin was completely inactive if its concentration was less than 1 g/l (Fig. 17).

For the concentration of 1 g/l to 4 g/l, the streptomycin was almost effective for further 20 hrs. It shows almost similar effect for the 1-2 g/l concentration, while significant effect was shown for 4 g/l (24 hrs). However, it was completely inactive after 36 hrs of investigation since the starting time (Fig. 18).

The antimicrobial activities of streptomycin were studied when the antibiotic was loaded in nanofibers. The results were shown in Fig. 19 and 20.
Fig. 19. The effectiveness of various concentrations of streptomycin loaded in the nanofibers with the fiber weight of 0.40 g on *S. aureus* growth.

where:  

- (●) *S. aureus* growth curve as adding antibiotic-free nanofibers into the culture medium
- (○) growth curve of *S. aureus* when streptomycin concentration of 0.20 g/l contained in nanofibers were added in the culture medium. Streptomycin concentration was 5 wt% in the EC solution. The EC solution concentration was 150 g/l
- (▼) growth curve of *S. aureus* when streptomycin concentration of 0.28 g/l contained in nanofibers were added in the culture medium. Streptomycin concentration was 7 wt% in the EC solution. The EC solution concentration was 150 g/l
- (▽) growth curve of *S. aureus* when streptomycin concentration of 0.40 g/l contained in nanofibers were added in the culture medium. Streptomycin concentration was 10 wt% in the EC solution. The EC solution concentration was 150 g/l.
Fig. 20. The effectiveness of various concentrations streptomycin loaded in nanofibers with the fiber weight of 0.80 g on *S. aureus* growth.

where:  
● *S. aureus* growth curve as adding antibiotic-free nanofibers into the culture medium

○ growth curve of *S. aureus* when streptomycin concentration of 0.40 g/l contained in nanofibers were added in the culture medium. Streptomycin concentration was 5 wt% in the EC solution. The EC solution concentration was 150 g/l

∇ growth curve of *S. aureus* when streptomycin concentration of 0.56 g/l contained in nanofibers were added in the culture medium. Streptomycin concentration was 7 wt% in the EC solution. The EC solution concentration was 150 g/l

▼ growth curve of *S. aureus* when streptomycin concentration of 0.80 g/l contained in nanofibers were added in the culture medium. Streptomycin concentration was 10 wt% in the EC solution. The EC solution concentration was 150 g/l.
At investigating concentrations, the antimicrobial activities of streptomycin adhered on nanofibers on *S. aureus* were the best within the first 10 hrs for streptomycin concentration of 0.20 g/l and with twice of the fiber’s weight, the effective time was 16 hrs. The antibacterial activity was stable at the 24\textsuperscript{th} hour, during studied further 40 hours and the effect was almost nil at or after 56 hrs of exposure. The effective time increased with the increased streptomycin concentration.

On comparing antimicrobial activity of bulk antibiotic and antibiotic adhered to nanofibers, it was found that at the concentration of 0.20 g/l, the most effective time for bulk streptomycin and streptomycin loaded in nanofibers on *S. aureus* was 12 hrs and 20 hrs, respectively. While at 0.40 g/l, it was 20 hrs and 24 hrs, respectively.

The antibacterial activities were investigated with other antibiotics, for example; ampicillin and penicillin at the similar concentration of streptomycin. The results are far more different than that of streptomycin. After 100 hrs of investigation, *S. aureus* growth population was lower than $0.4 \times 10^7$ cells per ml, while the growth of *S. aureus* uninhibited by the antibiotics was more than $15 \times 10^7$ cells per ml (1 OD = $3 \times 10^7$ cells per ml)[63]. Figures 21-24 show the same results.

It was found that the most effective time was long. In the case of ampicillin, it lasted 100 hrs, 52 hrs for bulk ampicillin, ampicillin loading in the fibers, respectively, at antibiotic concentration loading in the fibers of 5 wt\% (0.20 g/l in culture medium). At 10 wt\% (0.40 g/l in culture medium), the effective time was 124 hrs and 68 hrs. The effective time of varous ampicillin concentrations was not significantly different. However, antibacterial activity of bulk ampicillin at concentration of 0.04 g/l was the highest and quite different from other concentrations left. The result was the same to ampicillin loaded in the fibers.

The effective time of penicillin was shorter than that of ampicillin. It was 44 hrs and 36 hrs for bulk antibiotic and the antibiotic loaded in the fiber, respectively at penicillin concentration of 0.20 g/l in culture medium. At 0.40 g/l, the effective time was 54 hrs and 36 hrs, respectively.

Within the mentioned periods, the antibacterial activities increased significantly on increasing the time. After that time, the activities reduced gradually.
Fig. 21. The effectiveness of various concentration of bulk ampicillin on *S. aureus* growth.

where:  
- (■) *S. aureus* growth curve - Control curve
- (●) growth curve of *S. aureus* when bulk ampicillin concentration of 0.20 g/l was added in the culture medium
- (○) growth curve of *S. aureus* when bulk ampicillin concentration of 0.28 g/l was added in the culture medium
- (▼) growth curve of *S. aureus* when bulk ampicillin concentration of 0.40 g/l was added in the culture medium.
Fig. 22. The effectiveness of ampicillin loading in the fibers on *S. aureus*.

where:  

■ the curve of *S. aureus* growth as adding antibiotic – free nanofibers into the culture medium – Control curve

● growth curve of *S. aureus* when ampicillin concentration of 0.20 g/l contained in nanofibers was added in the culture medium. Ampicillin concentration in the EC solution was 5 wt%. EC solution concentration was 150 g/l

○ growth curve of *S. aureus* when ampicillin concentration of 0.28 g/l contained in nanofibers was added in the culture medium. Ampicillin concentration in the EC solution was 7 wt%. EC solution concentration was 150 g/l

▼ growth curve of *S. aureus* when ampicillin concentration of 0.40 g/l contained in nanofibers was added in the culture medium. Ampicillin concentration in the EC solution was 10 wt%. EC solution concentration was 150 g/l.
Fig. 23. The effectiveness of bulk penicillin on *S. aureus* growth.

where:  
- (■) *S. aureus* growth curve – Control curve
- (●) growth curve of *S. aureus* when bulk penicillin concentration of 0.20 g/l was added in the culture medium
- (○) growth curve of *S. aureus* when bulk penicillin concentration of 0.28 g/l was added in the culture medium
- (▼) growth curve of *S. aureus* when bulk penicillin concentration of 0.40 g/l was added in the culture medium.
Fig. 24. The effectiveness of various concentrations of penicillin loading in the fibers on *S. aureus* growth.

where:  
- (■) the curve of *S. aureus* growth as adding antibiotic-free nanofibers into the culture medium – Control curve
- (●) growth curve of *S. aureus* when penicillin concentration of 0.20 g/l contained in Nan fibers was added in the culture medium. Penicillin concentration in the EC solution was 5 wt%. EC solution concentration was 150 g/l.
- (○) growth curve of *S. aureus* when penicillin concentration of 0.28 g/l contained in nanofibers was added in the culture medium. Penicillin concentration in the EC solution was 7 wt%. EC solution concentration was 150 g/l.
- (▼) growth curve of *S. aureus* when penicillin concentration of 0.40 g/l contained in nanofibers was added in the culture medium. Penicillin concentration in the EC solution was 10 wt%. EC solution concentration was 150 g/l.
IV. Discussion

At the high voltage of 15 kV, so the electric field strength was high and the acceleration of the jet to the collector was also high. With the tip to collector distance of 10 cm, the jet had a short distance to travel before it reaches the collector plate. The distance was very short, excess solvent caused the fibers to merge when they contact to form junctions resulting in inter- and intra- layer bonding. Moreover, the flow rate applied in this the study was 100 µl/min. It was high, the bead and fiber diameters were long [19]. The higher volume of the EC solution drawn from the needle tip, the jet took a longer time to dry. As a result, the solvents in the deposited fibers did not have enough time to evaporate given the same flight time.

In the EC solution having the volume ratio of THF to DMAc of 4 to 1, the formation of holes on the fiber surface is the result of the difference in volatility of the two components, THF and DMAc, in the binary solvent system. The boiling points of THF and DMAc are 65°C and 165°C, respectively [62]. Because of the lower boiling point of the THF, THF shows higher vapor pressure. Therefore, EC fiber holes were formed on the surface of the fiber. The morphologies varied as the solvent ratios changed.

The fiber morphology depends on the concentration DMAc, with decrease DMAc concentration, the fiber diameter increased; the viscosity and DMAc concentration affected the morphologies of the fibers. Sheynoy et al. also observed the same results [34].

These results indicate that the antibiotic/polymer ratios and polymer concentration in the spinning solution can have an impact on the average fiber diameter and morphology. The results for streptomycin/EC indicate that a lower antibiotic/polymer ratio leads to smaller fiber diameter. The effect could also be related to the concentration of the polymer in the spinning EC solution. On increasing the antibiotic concentration in the EC solution, the EC solution increased. Therefore, viscosity of the solution increased, hence, and the fiber diameter increased.

At low viscosity, beads along with the fibers deposited on the collection plate, were
found along the fibers. The shape of the beads varied with varying the viscosity. This is probably due to the resistance of the solution to be stretched by the charged on the jet. Beads on fiber reduced with the increase streptomycin concentration. Beads on fibers were less likely to be formed for the more viscous solutions. The higher streptomycin concentration mixing in the EC solution, the lower was the viscosity. The diameter of the beads increases as the viscosity increases. One of factors affecting to form fiber morphology is viscosity of the polymer solution [19].

The concentration of antibiotic loaded in nanofibers influences the spinning of fibers and controls the fiber structure and morphology. Each antibiotic has specific physical properties. They would have different physical state in the polymer. Hence, their morphologies varied.

It is concluded that the production of nanofibers by the electrospinning process is influenced by both the electrostatic forces and the viscoelastic behavior of the polymer. Process parameters such as solution feed rate, applied voltage, tip collector distance, and material properties such as solution concentration, viscosity, substance ingredient ratio, solvents ratio and solvent vapor pressure influence the structure and morphology of electrospun nanofibers.

EC solution for electrospinning, which has low viscosity, produces small fibers, and vice versa. The smaller fiber diameter, the shorter is drug release time.

In the present study, the antimicrobial activities of antibiotic loading in nanofibers were compared to that of bulk antibiotics.

Firstly, in agar disc diffusion assay, on comparing the antimicrobial activity of bulk antibiotics and antibiotics loaded in the nanofibers, the bacterial growth inhibition zones of investigated antibiotics were found to be smaller than that of the bulk antibiotics, tested once in 24 hrs. The bulk antibiotics were released wholly, while the antibiotics loaded in the fiber were dispersed partially. The antibacterial activities varied with various bulk antibiotics, the effect of the antibiotic loading in the fiber also varies.
Secondly, each antibiotic has specific effectiveness on *S. aureus* growth. The effect increases with the increased antibiotic concentration, that’s true to bulk antibiotics and antibiotic loading in the nanofibers. And the antibacterial activities of the fibers are different; in other words, the content and time of different antibiotics exposing from the fibers is different.

In the case of streptomycin, in order to show similar active time like the antibiotic loaded in the nanofibers, the bulk antibiotic concentrations should be as high as 5 times of that.

*S. aureus* is very sensitive to ampicillin and penicillin. At the same concentration and the fiber weight investigated of 0.4 g, the effectiveness of ampicillin and penicillin is quite different from that of streptomycin. Their effective time is much longer and their activities against *S. aureus* are much higher. However, the effective content releasing of ampicillin and penicillin from the fiber was not much, for this reason, bacterial growth inhibition time is much shorter than that of bulk antibiotics.

The antibiotics adhered to nanofibers’ mechanism bases on adsorption of EC with antibiotics. Antibiotics are adsorbed on EC because on the surface of EC has -OH function group which has strong affinities. Therefore, they keep the group of antibiotics by coordination bonds such as N (III) function group of beta-lactam group in ampicillin, penicillin and amino group in streptomycin. In this study, the dispersion of different antibiotics varies because of the force of absorption of the nanofibers to the antibiotics. The force is weak so that the antibiotic dispersion is fast, and vice versa.

The beta lactam antibiotics are inhibitors of bacterial cell wall synthesis. They inhibit the growth of sensitive bacteria by inactivating enzymes located in the bacterial cell membrane, which are involved in the third stage of cell wall synthesis. It is during this stage that linear strands of peptidoglycan are cross-linked into a fishnet-like polymer that surrounds the bacterial cell and confers osmotic stability in the hypertonic milieu [17].

Besides, streptomycin is an aminoglycoside which acts by binding to a specific S12
protein in the 30S ribosomal subunit and causes the ribosome to misread the genetic code [63]. The aminoglycoside-type drugs can combine with other binding sites on 30S ribosomes, and they kill bacteria by inducing the formation of aberrant, nonfunctional complexes as well as by causing misreading. The antibiotic is just a bacteriostatic. It elucidates the above results.

V. Conclusion

1. The addition of DMAc in the binary component THF/DMAc solvent system leads to the decrease of the viscosity of the solution and the decrease in the fiber diameter.

2. Because of the difference in volatilization of the binary solvent system, tiny tubercles on the surface of the fiber are formed when the binary solvent system is used in electrospinning.

3. The load of the antibiotics in the EC solution affects the morphological changes. More tubercles on the surface of the fiber are formed and the concentration of antibiotics influences the spinning of fibers and controls the fiber structure and morphology.

4. The released antibiotic content and the releasing time are dependent on the composition of the binary component THF:DMAc solvent mixture. The drug release time is the longest for the binary solvent system ratio is 3 to 2 (v/v) on comparing the other ratios of 1 to 4 and 4 to 1 (v/v).

5. The adsorption of ethyl cellulose with antibiotics leads the antibiotic content releasing from the fibers less than bulk antibiotics in bacterial culture medium, hence, their antibacterial effect is less than that bulk antibiotics.

6. The antibacterial active time of streptomycin loaded nanofibers is as high as 5 times that of bulk streptomycin at the same concentration.

7. At very low concentration of ampicillin and penicillin, the antibacterial effect on *S. aureus* is much higher than streptomycin when they were loaded in the fibers. However, the release time is less effective than with streptomycin.
Reference:


University, Philadelphia, P. 19104, U.S.A


[46] Taepaiboon P, Rungsardthong U, Supaphol P. Drug-loaded electrospun mats of poly (vinyl alcohol) fibres and their release characteristics of four model drugs. Nanotechnology. 17: 2317-
2329, 2006.


저작물 이용 허락서

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본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 - 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다음 -
1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의변경을허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물이 영리적 목적을 위한 복제, 저장, 전송 등을 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사를가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여발생하는타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한저작물의 전송·출력을 허락함.

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