Synthesis and Property of Ag(NP)/catechin/Gelatin Nanofiber

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Abstract. Nanomaterial play important role future industry such as for the medical, food, pharmaceutical and cosmetic industry. Ag (NP) and catechin exhibit antibacterial property. Ag(NP) with diameter around 15 nm was synthesis by microwaved method. We have successfully produce Ag(NP)/catechin/gelatin nanofiber composite by electrospinning process. Ag(NP)/catechin/gelatin nanofiber was synthesized by using gelatin from tuna fish, polyethylene oxide (PEO), acetic acid as solvent and silver nanoparticle(NP)/catechin as bioactive component, respectively. Morphology and structure of bioactive catechin-gelatin nanofiber were characterized by scanning electron microscopy (SEM) and fourier transform infrared spectroscopy (FTIR), respectively. SEM analysis showed that morphology of nanofiber composite was smooth and had average diameter 398.97 nm. FTIR analysis results were used to confirm structure of catechin-gelatin nanofiber. It was confirmed by FTIR that specific vibration band peak amide A (N-H) at 3286,209 cm−1, amide B (N-H) 3069,396 cm−1, amide I (C=O) at 1643,813 cm−1, amide II (N-H and CN) at 1538,949 cm−1, amide III (C-N) at 1276,789 cm−1, C-O-C from polyethylene oxide at 1146,418 cm−1, respectively. When examined to S. Aureus bacteria, Ag/catechin/gelatin nanofiber show inhabitation performance around 40.44%. Ag(NP)/catechin/gelatin nanofiber has potential application antibacterial medical application.

1. Introduction
Silver nanoparticle, Ag(NP) is important nanomaterial which shows antibacterial performance and used in in cosmetic, soap and textile application [1]. One versatile process to produce Ag(NP) is produced by using reducing agent with microwave assisted method [2]. Catechin is polyphenolic compound obtained from extract of gambier, leaf green tea, apple, red wine. Catechin is used for food, medical, pharmaceutical and cosmetic industry and showed antioxidant, anti-inflammatory, anti-tumor and anti-bacteria activities properties [3,4]. Gelatin is protein which derived from collagen. Gelatin has wide range application such as for food, biotechnology, medical, pharmaceutical and cosmetic industry [5]. Electrospinning is versatile method to produce nanofiber. Electrospinning process is used direct current (DC) high voltage electric to drive nanofiber formation from polymer solution as main driving force process [6]. Wide spectrum aspects of research and application of gelatin nanofiber are explored and studied by scientist in the world. Laha et al have studied drug release of crosslinked gelatin nanofiber mesh [7]. Lee et al prepared FGF-2 immobilized gelatin nanofibers for tissue engineering [8]. Huang et al studied proanthocyanidin-crosslinked gelatin nanofibers for drug delivering application [9]. Zhuang et al produce chitosan/gelatin nanofibers containing silver nanoparticles [10]. Jalaja et al have studied property and activity of gelatin graphene oxide on S. Aureus and E. Coli bacteria [11]. Biodegradation of PCI/gelatin nanofiber was
studied by Dulnik et al [12]. Sheath/core PVA/gelatin nanofiber was synthesized and studied by Merkle et al [13]. Xu et al. all develop fluoride-containing gelatin nanofiber scaffold [14]. Nasir et al has functionalization of gelatin nanofiber by catechin [15]. In current research, our focus is to synthesized Ag(NP)/bioactive/catechin/gelatin nanofiber by electrospinning and to study its morphology, nanostructure and antibacterial property.

2. Experiment

Materials and equipment. Silver nitrate (Merk), catechin from gambier (LIPI), gelatin from yellow finn skin tuna fish (LIPI), glacial acetic acid (Merck), polyethylene oxide, PEO, with molecular weight 600 kDa (Merck). All materials were used without further purification. Zuheros Nano computerized electrospinning machine (LIPI) was used to fabricate nanofiber.

Preparation of Ag(NP) solution

Sodium citrate (1 % w/v) was added and mixed to 10 mL of silver nitrate (1 mM) solution. Reaction of silver nanoparticle formation was conducted in 300 watts in microwave reactor for 5 minutes.

Electrospinning solution. Electrospinning solution was prepared as follow: a mixed of silver nanoparticle, Ag(NP) solution (58% v/v), catechin solution10% (v/v), gelatin 8% (w/v) and PEO 4 % (w/v) were dissolved in acetic acid 32% (v/v) and gently stirrer at room temperature for 24 hours.

Electrospinning process. Electrospinning process to synthesized nanofiber was carried out by using computerized electrospinning machine. Electrospinning solution which contain Ag(NP), catechin, gelatin and PEO was put into syringe and set up in electrospinning machine. Electrospinning parameters as follow: nozzle to collector distance (18 cm), applied voltage (14 kV), fixed polymer solution concentration, and flow rate (0.008 ml/m) were used to fabricate nanofiber.

Morphology of Ag(NP)/catechin/gelatin nanofiber characterization. Morphology of electrospun gelatin nanofiber was characterized by SEM. After that, nanofiber diameter was calculated from SEM image of nanofiber by using image processing software (Adobe Photoshop software).

Structure of Ag(NP)/catechin/gelatin nanofiber characterization. Structure of Ag(NP)/catechin/gelatin nanofiber was characterized by using ATR-FTIR spectrophotometer (Nicolet). For measuring this method, thin film sheet of nanofiber was used.

Antibacterial performance of Ag(NP)/catechin/gelatin nanofiber. Antibacterial property of Ag(NP)/catechin/gelatin nanofiber was test to staphylococcus aureus bacteria by optical density method. The procedure as follow: 6 mL nutrient broth was added to test tube and the add 40 mL staphylococcus aureus and nanofiber sample. Incubation was conducted for 24 hours at 37 °C. Optical density of suspension was measured at 600 nm by using spectrophotometer (Agilent) (15).

3. Result and Discussion

Morphology of silver nanoparticle. Ag(NP) was synthesized by microwave method. Ag(NP) has light yellow color as shown in figure 1. Morphology and sized of nanoparticle was observed by transmittance electron microscopy (TEM). Figure 1b showed morphology of Ag(NP). Ag(NP) diameter is around 15 nm.

Figure 1. Morphology of silver nanoparticle, Ag(NP) analyzed by using TEM

Morphology of Ag(NP)/catechin/gelatin nanofiber. Ag/catechin/gelatin nanofiber was synthesized from mixed solution silver nanoparticle, catechin, gelatin and polyethylene oxide by using electrospinning process. PEO function as spinnability polymer for synthesis of nanofiber. After drying process, nanofiber was analyzed by SEM to obtain its morphology and diameter by using SEM. Morphology of nanofiber was smooth and
homogenous without bead nanofiber string. Range of nanofiber diameter was from 292 nm to 532 nm. The average diameter was 398.97 nm. Size of nanofiber diameter was influence by several factors such as polymer concentration, viscosity, surface tension, applied voltage, distance between nozzle to collector and humidity [16].

3.1. FTIR spectrum of Ag(NP)/catechin/gelatin nanofiber
Figure 3 showed FTIR spectra of Ag(NP)/catechin/gelatin nanofiber and catechin/gelatin nanofiber as control. Figure 3 showed spectra of nanofiber. It was confirmed by FTIR that specific vibration band peak amide A (N-H) at 3286,209 cm$^{-1}$, amide B (N-H) 3069,396 cm$^{-1}$, amide I (C=O) at 1643,813 cm$^{-1}$, amide II (N-H and CN) at 1538,949 cm$^{-1}$, amide III (C-N) at 1276,789 cm$^{-1}$, C-O-C from polyethylene oxide at 1146,418 cm$^{-1}$, respectively. When we compare with our previous result in catechin/gelatin nanofiber [15], presence of Ag(NP) in nanofiber only slightly change of vibration peak of functional group gelatin, catechin and PEO but not change its structure.

3.2. Antibacterial Performance of Ag(NP)/catechin/gelatin nanofiber on bacteria
Ag(NP)/catechin/gelatin nanofiber antibacterial activity was examined to staphylococcus aureus bacteria by optical density/turbidimetry methods. Staphylococcus aureus is well known as dangerous a gram positive of bacteria. S. Aureus bacteria is found on skin, hair, throat and nose.
Figure 4. Appearance of antibacterial suspension samples. a). Control and b). Ag(NP)/catechin/gelatin nanofiber.

Appearance of suspension of test solution results were shown in figure 4. Bioactive catechin-gelatin nanofiber can inhibit S. Aureus bacteria around 40.44%. Gopal et al and Staphleton et al showed green tea of catechin effective in inhibiting bacteria. According to study of Grigor’eva et al, interaction between Ag(NP) with S. Aureus as follow: Ag(NP) adsorbed on cell of bacteria, penetrated and accumulated in cell walls and damage cytoplasm. Cytoplasm of bacteria become amorphous. Ag(NP) was bonded in DNA fiber S. Aureus [16,17,18 and 19].

4. Summary
We have successfully synthesized Ag/catechin-gelatin nanofiber with diameter around 398.97 nm by electrospinning process. Ag(NP)/catechin/gelatin nanofiber can inhibit growth of S. Aureus bacteria around 40.4%. Advanced study interaction of Ag(NP)/catechin with S. Aureus is required to explain the performance of its antibacterial activity. Ag(NP)/catechin-gelatin nanofiber has potential application for medical application.

Acknowledgements
This research is financial support by Ministry of Research and Technology-Higher Education Republic Indonesia (Insinas Project FY 2018-2019)

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