Study of parameter affecting morphology of electrospun poly (lactic acid) (PLA) fibers loaded with Ag/CaCO₃ filler

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Silver nanoparticles (AgNPs) are widely used as antimicrobial agent in commercial products like textiles, cosmetics and drugs. AgNPs deposited on the surface of calcium carbonate (CaCO₃) particles, serve as sustained release of antimicrobial activity. The silver nanoparticles embedded calcium carbonate particles (Ag/CaCO₃) are prepared by a precipitation method. In this study, precipitated (Ag/CaCO₃) filler will be used for preparation of Ag/CaCO₃-poly(L-lactic acid) (Ag/CaCO₃-PLA) nanofibers by electrospinning. Polymer concentration and functional filler amount in binary solvent system will be studied. The condition for fabricating electrospun PLA and Ag/CaCO₃-PLA was a 15 cm of collection distance and 15 kV of working voltage. Morphology of Ag/CaCO₃ loaded PLA electrospun fibers will be investigated by the field emission scanning electron microscope (FE-SEM). The electrospun fibers will be further applied as the antimicrobial material.

Keywords PLA, Ag/CaCO₃ filler, Electrospinning, Antimicrobial Applications

1. Introduction
Silver nanoparticles (AgNPs) are widely used as antimicrobial agent in health care, food and commercial products like textiles. AgNPs have now been established as an effective biocidal agent and act on a wide range of both gram-negative and gram-positive bacteria [1]. AgNPs incorporated into polymer matrix are currently a popular type of nanocomposite that can provide excellent antimicrobial properties. Antimicrobial nano polymer composite could be applied as packaging films [2], wound dressing materials [3] and other antimicrobial applications [4,5] according to their outstanding antimicrobial activity. To reduce agglomeration and maintain the nano size of Ag particles, calcium carbonate is one of a good choice as AgNPs carrier for incorporation into polymer matrix [6]. Polylactic acid (PLA) has been widely used in various applications including biomedical applications and packaging because of its biodegradability, biocompatibility and ability to be dissolved in common solvents for processing [7].

Electrospinning is simple and efficient for the fabrication of nano to microscale fibers. The morphology and diameter of the electrospun fibers depend on solution properties, processing and ambient factors [8]. In this research work, the electrospinning was employed to prepare mat of PLA and PLA loading with Ag/CaCO₃. Then, effects of PLA solution concentration, applied voltage, solution flow rate and Ag/CaCO₃ filler content on morphology of electrospun fibers and distribution of fiber diameter were studied. This research work is preliminary work for further study on application of Ag/CaCO₃ for food packaging.
2. Experimental
The commercial PLA grade (4043D) was purchased from NatureWorks. Chloroform RPE was purchased form Carlo Erba Reagents and acetone was purchased from Thermo Fisher Scientific. Silver nanoparticles loaded calcium carbonate particles (Ag/CaCO₃) were prepared by a coprecipitation method mentioned by S. Zapotoczny (2012) [6]. The particle size range of the Ag/CaCO₃ is around 2 to 5 µm with D(v,0.9) of 4.66 µm. Various concentrations of PLA were prepared with a binary solvent system of chloroform and acetone (chloroform: acetone = 2:1 in volume). The PLA solution with concentrations of 7.5%, 10%, 12.5% and 15% w/v were prepared by stirring at room temperature for 24 hrs using a magnetic stirrer. For preparing Ag/CaCO₃ filled PLA nanofibers, only 10% w/v PLA solution with the binary solvent system of chloroform and acetone was employed. Firstly, 3.00 g of PLA was dissolved in 20 mL of chloroform for 22 h and different Ag/CaCO₃ contents which were dispersed in 10 mL of acetone were added to the dissolved PLA solution. Then, the mixtures were magnetically stirred at room temperature for 2 h.

Each PLA solution, unfilled and filled, was loaded into a 10 mL plastic syringe (NIPRO syringe), and the needle (inner diameter 0.9 mm) was used for electrospinning. Two flow rates (1 mL/h and 1.5 mL/h) were varied and high voltage of up to 15 kV were applied. Electrospun fibers were collected on a grounded aluminum collector at a distance of 15 cm from the needle tip. The spinning time for pure PLA solutions and Ag/CaCO₃ loaded PLA solutions was 30 min and 15 min, respectively. All experiments were performed at about 30°C and relative humidity around 60%. Morphology of electrospun fibers made of pure PLA and Ag/CaCO₃ loaded PLA were investigated by the field emission scanning electron microscope (Carl Zeiss, Auriga) using an accelerating voltage of 3.0 kV. Before observation, each sample was coated with a gold sputter coater for 2 min (Neo Coater).

3. Results and Discussion
SEM micrographs in Figure 1 show morphology of the nanofibers electrospun with different PLA concentrations, flow rates and applied voltages. The SEM micrographs show characteristic of porous structure of PLA fibers electrospun from PLA solution with the binary solvent system of chloroform:acetone. The concentration of PLA solution does affect PLA nanofiber morphology and diameter. The micrographs that smaller fibers with beads were collected from 7.5% w/v PLA solution (Figure 1A-D). The formation of beads indicates insufficient chain entanglements [9]. On increasing PLA concentration to 10%w/v, continuous fibers with various diameter fibers were produced (Figure 1E-H) and uniform fibers were collected at 12.5%w/v PLA solution (Figure 1I-L). At the highest PLA concentration of 15%w/v, electrospun fibers were folded back and forth not randomly oriented as observed from electrospun fiber with lower concentration of PLA solution (Figure 1M-P). Casasola et al. [9] have mentioned that increasing polymer concentration results in more chain entanglements, an increase in the solution viscosity. As a result, hence an increase in the viscoelastic force will be increased and will counterbalance the Coulombic stretching force. Therefore, it results in continuous fibers with fewer beads.

Uniform electrospun nanofibers in size could be prepared via a critical flow rate of a polymeric solution. At higher flow rate of 1.5 mL/h, fibers with larger diameter were produced comparing to the prepared fiber with lower flow rate of 1 mL/h. Increasing flow rate beyond a critical value not only leads to increase in the pore size and fiber diameter but also bead formation. This might be due to incomplete drying of the nanofiber jet during the flight between the needle tip and metallic collector [10]. Upon concerning applied voltage, smaller diameter of fibers was produced at higher applied voltage of 18kV compared to produced fibers at 15kV, as shown in Figure 1. The formation of smaller diameter nanofibers with an increase in the applied voltage is attributed to the stretching of the polymer solution in correlation with the charge repulsion within the polymer jet [10].

SEM micrographs in Figure 2 show electrospun fibers obtained from PLA solutions filled with 1 and 3% w/v Ag/CaCO₃ using 1 mL/h flow rate, and 18 and 20 kV applied voltages. At 1%w/v Ag/CaCO₃ loading, the electrospun filled PLA fibers contained some particles blooming along the fiber, as shown in Figure 2(A) and 2(B). However, with 3%w/v Ag/CaCO₃ loading, the electrospun fiber comparatively contained more particle blooming along the fiber due to higher degree of the local particle agglomeration.
during electrospinning process, as illustrated in Figure 2(C) and 2(D). The local particle agglomeration might be caused by heterogeneous distribution of the particles in PLA solutions during electrospinning.

Figure 1. SEM micrographs of electrospun PLA fiber prepared from various concentrations, flow rates of 1 and 1.5 mL/h, and applied voltages of 15 and 18 kV.
4. Conclusions
Highly porous fibers were prepared by electrospinning from pure PLA solution. PLA solutions of 10 and 15% w/v concentrations would give rise to electrospun continuous fibers without beads. Larger diameter electrospun fibers were obtained at higher flow rate of 1.5 mL/h and applying higher voltage of 18 kV resulted in smaller diameter fibers than applying lower voltage. PLA electrospun fibers filled with 1% w/v Ag/CaCO$_3$ contained some particles blooming along the fiber however, more particle blooming and nonuniform shape were observed from the electrospun fibers filled with 3% w/v Ag/CaCO$_3$. The electrospun fibers obtained from 1% w/v Ag/CaCO$_3$ loading with applied voltage of 20 kV were more uniform in shape.

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