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Fabrication and Characterization of Electrospun Polyvinyl Alcohol/Poly-ε-caprolactone Antibacterial Agent Composite Nanofiber for Potential Scaffold

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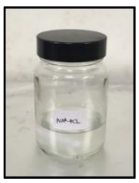
GRAPHICAL ABSTRACT



Clear of PVA-PCL solution

ABSTRACT

Bacterial infection and injury caused by dressing removal are two concerning issues which affect the healing process in treatment of skin damagesd. Attenuated total reflection (ATR) shows that the spectra of polyvinyl alcohol/poly-ε-caprolactone (PVA/PCL) blend is very similar to the spectra of both pure PVA and PCL especially for the absorption of hydroxyl group and carbonyl group which contribute to PVA and PCL groups respectively. In this study, the electrospun of PVA/PCL nanofiber was coated with three different types of antibacterial agents which are poly-L-lysine, chlorhexidine gluconate and chitosan. The effects of three antibacterial agents were examined by Disk Diffusion Test against *S.aureus* and *E. coli*. The zone of inhibition was evaluated by measuring the diameter of the bacterial growth inhibition zone around the membrane. The results show that chlorhexidine gluconate is the best antibacterial agents to supress the growth of the S. aureus and E. coli. The field emission scanning electron microscopy (FESEM) images showed an increase in fiber diameter with the presence of the three antibacterial agents. Besides, corresponding tensile strength values for the electrospun of PVA/PCL/antibacterial agents tested appear to be within the range of tensile strength values for human skin which conclude that the electrospun of PVA/PCL/antibacterial agent nanofiber is suitable for regenerating human skin. Last but not least, the hydrophilicity of the electrospun of PVA/PCL/antibacterial agents nanofiber was confirmed by water contact angle measurement which is 0° and it is theoretically can improve the cell adhesion onto the membrane.

Keywords: Polyvinyl alcohol, poly-ε-caprolactone, antibacterial agent, electrospinning, scaffold

1. INTRODUCTION

Recent studies showed that different methods are available for fabricating a wound healing. Among the various methods, electrospinning demonstrates a great assurance due to its excellent porous structure and a very large surface area-to volume ratio. In addition, the electrospun nanofibers can serve as the substrate for tissue recovery by promoting cell adhesion, spreading, and expansion [1]. Lately there has been an interest in the production of nanofibrous materials. Electrospinning currently is recognized as the most proficient technique for producing continuous polymer fibers with significant in length having nanoscale diameters.

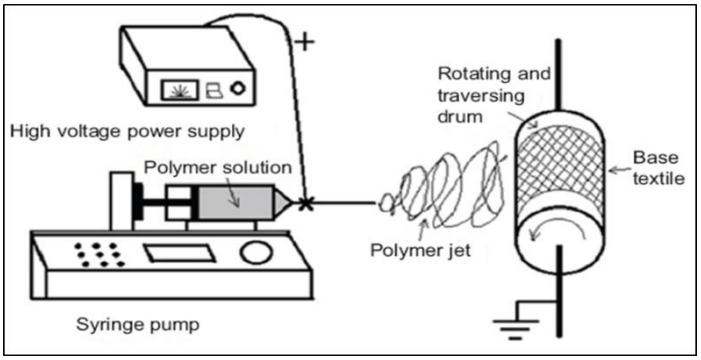
Poly-ε-caprolactone (PCL) is one of the most commonly applied synthetic polymers for medical applications because of its biocompatibility and slow biodegradability. PCL is hydrophobic, so presence of another component which embeds hydrophilicity and cell attachment is necessary in scaffold fabrication [2]. Polyvinyl alcohol (PVA) is a biodegradable polymer, and the presence of hydroxyl groups on the carbon atoms enhanced its degradability through hydrolysis. It is a water-soluble synthetic polymer, with good biocompatibility. Thus, it has been used as a hydrogel substitute material for wound dressing, a drug delivery system, contact lens, and prosthesis because of the affinity for blood, plasma, and living tissue [3,4]. One reason to choose PCL as a hydrophobic additive to electrospun PVA mats is that the electrospun PCL nanofibers in the mats are not easily dissolved in water or cell culture medium owing to their poor solubility at room or body temperature [5].

Poly-L-lysine (LPL) is widely used as an antibacterial agent because of its broad antimicrobial spectrum [6]. LPL is a homo-poly-amino acid characterized by the peptide bond between the carboxyl and ε-amino groups of L-lysine. LPL shows a wide range of antimicrobial activity and is stable at high temperatures and under both acidic and alkaline conditions. Chitosan is an abundant natural biopolymer obtained from the exoskeletons of crustaceans and arthropods which is a nontoxic, biocompatible and biodegradable copolymer, known to inherent antibacterial activity, which is mainly due to its polycationic nature. Chitosan inhibits the growth of a variety of pathogenic microorganisms – gram-positive and gram-negative bacteria, yeasts, and fungi [7]. Chlorhexidine gluconate (CHG) is the gluconate salt form of chlorhexidine, a biguanide compound used as an antiseptic agent with topical antibacterial activity. CHG is positively charged and reacts with the negatively charged microbial cell surface, thereby destroying the integrity of the cell membrane [8].

This research will emphasize on examining the effects of the three antibacterial agents since infection plays a major role in influencing the rate of wound healing. The three antibacterial agents will be coated on the electrospun of PVA/PCL nanofiber at 5 min, 10 min, 15 min and 20 min.

2. EXPERIMENTAL

The experiment was divided into three main stages. The first stage was focused on the fabrication of PVA/PCL electrospun nanofiber. The electrospinning process used to fabricate the nanofibers membrane was straightforward and could be easily done for fibers formation. Electrospun of fibers is easily generated using semi-automatic machine manufactured by FNM (USA). Scheme 1 shows the schematic diagram of electrospinning process set up. The solution of polymer will be filled up in a syringe and the connection of the circuit is as shown in the figure [9]. The second main step was begun by preparing the three antibacterial agents in a form of solution with different solvent, then the experiment will be proceed by coating the electrospun of PVA/PCL nanofibre with the antibacterial solution. The third stage in this study is the characterization of the electrospun PVA/PCL-antibacterial agent composite fiber. This characterization will be done by attenuated total reflection (ATR) and contact angle. The tensile strength will also be done to determine the maximum load that a material can withstand. Last but not least, the antibacterial acitivity of electrospun PVA/PCL-antibacterial agent composite fiber will be investigated by disk diffusion test (DDT) against two kinds of pathogens, S. aureus and E. coli. Briefly, 100 µl of the bacterial suspension (0.5 MC Farland Standards) was added into and spread out Mueller-Hinton agar (MHA) surface. Then, the samples were placed on the suspension layer. The dishes were incubated at overnight at 37 °C. The zone of inhibition was evaluated by measuring the diameter of the bacterial growth inhibition zone around the membrane.



Scheme 1. Basic setup of the electrospinning apparatus used in this study

3. RESULTS AND DISCUSSION

3.1. Fabrication of electrospun polyvinyl alcohol/poly-ε-caprolactone nanofibrous mat

The combination of hydrophilic polymer and hydrophobic polymer is a good approach to yield a good scaffold. Although hydrophilic polymer is a good candidate for scaffold, they to be unstable when in contact with water. As a result, hydrophobic polymer is combined with hydrophilic polymer in order to solve the problem. In this project, a new nanofibrous membrane containing PVA (hydrophilic polymer) and PCL (hydrophobic polymer) was introduced. 10% (w/v) PVA and 15% (w/v) were prepared separately and then combined with ratio 1:1. The solution is homogenous without using any surfactant or emulsifier. The electrospinning process was conducted at 20kV, 150 mm of working distance and flow rate of 1mL/h.

The fabricated nanofiber of PVA/PCL was cut into small round shape with diameter of 2.5 cm. After that, they were coated by immersion in antibacterial solutions for 5 minutes, 10 minutes, 15 minutes and 20 minutes. The antibacterial solution was successfully coated on the membrane surface throughout the time given. This is due to the good absorption of the antibacterial agents with both PVA and PCL during coating. In addition, the membrane surface also not degraded after the antibacterial coating and it showed that all the antibacterial agents would not change the nanofiber morphology into slightly collapsed nanofiber morphology during the coating. Figure 1 shows the electrospun PVA/PCL nanofiber was coated with (a) LPL, (b) CHG and (c) chitosan.

(a) (b) (c)

Figure 1. Electrospun PVA/PCL nanofiber coated with (a) LPL, (b) CHX, and (c) chitosan

3.2. ATR Analysis

Attenuated total reflectance (ATR) was used to characterize the PVA, PCL, and PVA-PCL. Figure 2 shows the ATR spectra of (a) pure PVA, (b) pure PCL, and (c) PVA/PCL blend nanofibers; the distinguished feature for PVA is the hydroxyl stretching absorption around 3285.15cm-1 and the distinguished feature for PCL is the carbonyl stretching absorption around 1724.13cm-1, and the spectra of PVA and PCL blend very similar to the spectra of both pure PVA and PCL especially for the absorption of hydroxyl group which contribute to PVA groups as described in FTIR spectra of PVA. In the area of 1720.06cm-1, there is a deeper absorbance in PVA/PCL which is related to the carbonyl groups of PCL. So, this area is identically related to interaction between PVA and PCL.

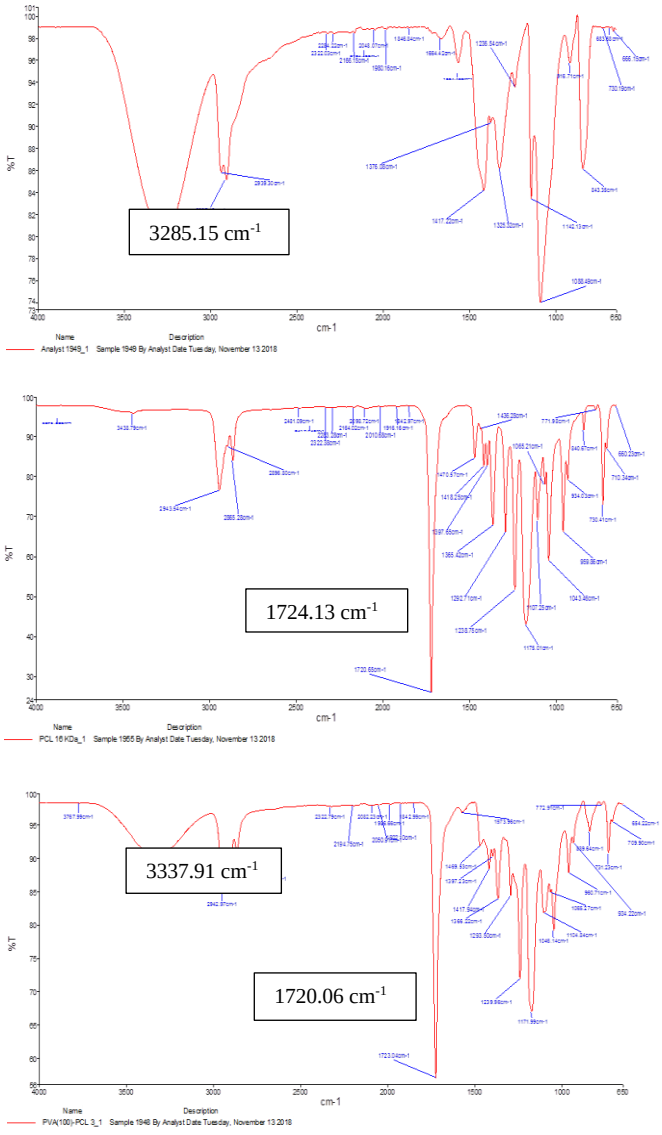


Figure 2. ATR spectra of (a) pure PVA, (b) pure PCL, and (c) PVA-PCL

3.3. Antibacterial Activity

A plausible mechanism proposed is to explain the inhibitory effect of antibacterial agents on microbial growth based on the their morphological appearance on the *E.coli* and *S. aureus* that were studied by disk diffusion test (DDT), which can be concluded that the electrostatic adsorption of antibacterial agents to the cell surface, followed by a stripping of the outer membrane. This ultimately leads to the abnormal distribution of the cytoplasm causing physiological damage to the bacterial cells. The results obtained show that all the samples can inhibit the growth of both gram-positive and gram-negative bacteria as the antibacterial agents were adsorbed on the scaffold successfully with different rate; LPL at 20 min, CHG at 5 min and chitosan at 20 min. The table below shows that CHG is the best antibacterial agents since the diameter of the bacterial growth inhibition zone is the highest though it needs only 5 minutes to supress the growth of both *S. aures* and *E. coli*.

Table 1. Antibacterial activity on gram positive bacteria and gram-negative bacteria

Type of Antibacterial Agent	Type of Bacteria	Optimum Time to Inhibit Bacterial Growth (min)	Diameter of the bacterial growth inhibition zone (mm)
LPL	*S. aureus*	20	9.5
LPL	*E. coli*	20	9.6
CHG	*S. aureus*	5	18.5
CHG	*E. coli*	5	15.0
Chitosan	*S. aureus*	20	9.0
Chitosan	*E. coli*	20	8.5

3.4. Tensile Strength

The experiments presented were intended to determine whether electrospun of PVA/PCL/antibacterial agent shows sufficiently similar behaviour to human skin. The tensile testing provides an indication of the strength and elasticity of the fiber membrane, which can be reflected by tensile strength and elongation at break. It is suggested that fiber membrane suitable for wound dressing should preferably strong but flexible.

Electrospun of PVA/PCL/antibacterial agent nanofiber aim to replace or restore damaged organs. The mechanical properties of such materials should emulate the human tissues they want to replace; to provide the required anatomic shape, the materials must have the capacity to endure the mechanical forces they will experience when embedded at the defect site. The presented tensile tests on electrospun PVA/PCL/antibacterial agent nanofiber and human skin are in most respects similar. Ultimate nominal tensile strength value for humans was reported as 3–14 MPa for males and 4–13 MPa for females [10]. Corresponding values for the electrospun of PVA/PCL/LPL and electrospun of PVA/PCL/CHG appear to be within the range of tensile strength values for both female and male but corresponding values for the electrospun of PVA/PCL/chitosan only acceptable for males. Based on a comparison with tensile test data from human skin, it was concluded that electrospun of PVA/PCL/antibacterial agent nanofiber is an acceptable simulant for human skin with respect to tensile strength. The table below shows the corresponding value for the electrospun of PVA/PCL nanofiber with three antibacterial agents.

Table 2. Tensile Strength of PVA/PCL/antibacterial agents electrospun nanofiber composite

Type of antibacterial agent	Tensile Strength Value (MPa)
LPL	4.5268
CHX	4.2639
Chitosan	3.1476

3.5. Contact Angle

The hydrophilicity of PVA/PCL/antibacterial agent electrospun was determined by the contact angle measurement. A sessile drop method was applied to measure the contact angle of the electrospun surface at ambient temperature. The droplets of distilled water were placed on the electrospun mats until equilibrium and contact angles were measured by a contact angle system OCA. The water contact angle was measured by the video contact analyzer (Dataphysics, USA Corp). To confirm the uniform distribution of water on the PVA/PCL/antibacterial agent electrospun mats, the contact angle was measured 10 times from different positions on each mat and an average value was calculated by statistical method.

The results of contact angle measurement of electropun of PVA/PCL nanofiber with the three antibacterial agents showed the contact angle of 0°, indicating that all of the electrospun of PVA/PCL/antibacterial agent nanofiber are hydrophilic. It is well known that initial cell adhesion could be affected by the surface hydrophilicity of scaffold, and the hydrophilic surface would lead to higher cell adhesion than the hydrophobic surface [11].

Since all the electrospun of PVA/PCL/antibacterial agnets nanofibre show same hydrophilicity properties, the comparison between them could be made by the time taken for water to complete absorbed on the membrane surface. The table below shows that CHG takes the shortest time for water to penetrate onto the membrane, which may because the higher porosity of randomly oriented fiber membrane.

Table 3. Contact angle of PVA/PCL/antibacterial agents electrospun nanofiber composite

Type of antibacterial agent	Time taken for water completely absorbed on the membrane surface (s)
LPL	42.0
CHG	6.0
Chitosan	180.0

4. CONCLUSION

In this study, we have successfully using a biodegradable polymer such as composite of PVA/PCL to produce electrospun scaffold. The three different antibacterial agents; LPL, CHX and chitosan, which are known for their beneficial effects on wound healing were coated on the scaffold to provide antibacterial activity for the scaffold. The ATR spectra show that, there is a deeper absorbance in PVA/PCL which is related to the hydroxyl group of PVA and carbonyl groups of PCL. So, this area is identically related to interaction between PVA and PCL. Besides, corresponding values for the electrospun of PVA/PCL/antibacterial agents tested appear to be within the range of tensile strength values for human skin which conclude that the electrospun of PVA/PCL/antibacterial agent nanofiber is suitable for regenerating human skin. Last but not least, the contact angle indicates that all of the electrospun of PVA/PCL/antibacterial agent nanofiber are hydrophilic which can improve the cell adhesion onto the membrane. All these results show that this product can be act a scaffold to promote wound healing and improve cell adhesion proliferation.

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