CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2025.019

Development of electrospun chitosan/polyvinyl alcohol membranes with antibacterial properties for enhanced open wound healing

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Article info.

Received 10 Apr 2024
Revised 4 May 2024
Accepted 4 Nov 2024

Keywords

Antibacterial ability, chitosan, electrospinning, fiber membrane, open wound healing, polyvinyl alcohol

ABSTRACT

This work focuses on using the electrospinning process to fabricate a microfiber membrane for wound healing by combination of chitosan (CS) and polyvinyl alcohol (PVA). The impacts of several parameters on fiber morphology and antibacterial capabilities, such as PVA concentration, CS/PVA ratio, applied voltage, injection distance, and solution injection speed, were investigated. The ideal parameters for the electrospinning process were identified as follows: PVA concentration of 15%, CS/PVA ratio of 3/7, collecting distance of 10 cm, voltage of 11 kV, and flow rate of 0.085 mL/h. Scanning electron microscopy verified the fiber structure was homogeneous and particle-free, with an average diameter of 457 ± 111 nm. The CS/PVA microfiber membrane demonstrated strong antibacterial action against E. coli and L. monocytogenes strains. After 12 days, in-vivo tests demonstrated that the wound-healing capacity of the CS/PVA microfiber membrane was quicker than that of a self-healed wound sample. These results demonstrate the effective creation of CS/PVA microfibers for wound healing applications.

1. INTRODUCTION

The skin acts as a protective barrier for the body, keeping hazardous things out while also reducing electrolyte and water loss (Giménez-Arnau, 2016). As a result, damaged skin has a significant influence on human health. When a skin wound arises, the body instantly begins the self-healing process, which is divided into four stages: hemostasis, inflammation, proliferation, and regeneration (Singh et al., 2017). During this phase, bacteria can readily infiltrate the wound and cause infection, slow healing, distort the wound tissue, and even jeopardize the patient's life.

Traditional wound dressings, such as cotton, bandages, and gauze, are used to keep pathogens out of the body while also isolating the wound from toxins throughout the healing process (Mirhaj et al., 2022). Furthermore, typical dressings absorb exudate from the wound surface, producing dehydration, and prolonged contact with the lesion may result in adhesions (Pereira et al., 2016). This might create additional harm to the wound, slowing healing. As a result, the creation of wound dressings has an important therapeutic significance, with the objective of promoting skin regeneration and speeding up wound healing. Wound dressings under investigated include membranes, foams, sponges, hydrogels, and fibrous membranes.

Among the different compounds investigated for appropriateness in fiber membrane wound healing applications, chitosan (CS) stands out for its substantial antibacterial characteristics, which are linked to the presence of amine groups with protons (Lim, 2004). Furthermore, the CS has amazing properties, such as non-toxicity, biocompatibility, and biodegradability, prompting substantial research in a variety of domains, including wound healing applications. Incorporating CS into the fiber production process increases the fiber's resistance to bacterial contamination. However, fibers derived purely from the CS have poor mechanical characteristics. As a result, the purpose of this study is to improve the mechanical properties of CS fibers by incorporating polyvinyl alcohol (PVA), a synthetic polymer with high tensile strength and flexibility, which improves the structural integrity of fibers when combined with CS, resulting in increased strength and reduced susceptibility to breakage, particularly in nano-sized fibers (Boudriot et al., 2006; Gaaz et al., 2015). The synergistic combination of these two polymers produces fibers that combine the benefits of both natural and synthetic materials, resulting in a well-balanced set of features.

Electrospinning, a nanofiber creation technology used in this work, may produce fibers with diameters as tiny as a few hundred nanometers (Reneker & Chun, 1996; Shin et al., 2001; Hohman et al., 2001; Luo & Edirisinghe, 2014). The resulting nanofibers have thin surface layers, tiny diameters, flexibility, and high strength, making them ideal for a variety of applications like antibacterial textiles. The electrospinning technique entails injecting the polymer solution through an injector and exposing it to a high voltage at the tip of the needle to produce electrical shear stress (Subbiah et al., 2005). This tension lengthens the solution at the injector output, resulting in an inverted triangle known as the "Taylor Cone". Fiber production occurs largely when the polymer solution has a high viscosity (Geng et al., 2005; Boudriot et al., 2006; Luo & Edirisinghe, 2014).

Numerous research projects have been conducted to synthesize CS nanofibers utilizing electrospinning (Sajomsang, 2010). For example, Homayoni et al. (2009) refined the electrospinning technique for CS and evaluated the decay period of CS nanofibers, determining an optimal sample diameter of 140 nm. Kang et al. (2010) effectively created chitosan-coated polyvinyl alcohol nanofibers for skin wound therapy. Li et al. (2013) created fibrous films made of nanofibers containing CS, PVA, chitosan oligosaccharide, and silver nanoparticles, and shown their potential in wound healing using in vitro studies. Lemma et al. (2016) successfully produced chitosan nanofibers mixed with poly(ethylene oxide) using the electrospinning technology. While multiple studies have investigated the production of chitosan nanofibers coupled with other materials, including polyvinyl alcohol, there has been much investigation into their practical utilization as fiber membranes (Wei et al., 2011; Fan et al., 2019). Besides, the studies aimed to combine CS/PVA nanofibers with active materials with the desire to enhance antibacterial properties of fiber membranes (Kharaghani et al., 2018; Iqbal et al., 2020) and improve wound healing efficiency (Chen et al., 2022; Lv et al., 2022) and. In addition, cross-linking agents are introduced into the fibers for the purpose of perfecting their morphology and characterization of PVA/CS mats (Viana et al., 2020). However, only the combination of CS and PVA without the presence of cross-link agents still demonstrated its good morphology, superior antibacterial property and effective wound healing. The CS's microbial resistance, cell growth support and tissue regeneration ability as well as PVA's moisturizing ability and porous architecture contribute to improving wound healing effectiveness. Moreover, due to hydrogen bonding formation (NH_2 groups of CS and OH groups of PVA), the PVA-CS mixture can also form physical crosslinks that can contribute to the unique properties of the material, such as increased tensile strength, toughness, and stability in aqueous environments (Nathan et al., 2023).

This work attempts to contribute the development of CS/PVA nanofiber by demonstrating an application of CS/PVA microfibers as an antibacterial membrane to aid in wound healing without any active agents. Electrospinning was used to create CS/PVA microfiber membranes, with parameters such as PVA concentration, voltage, CS/PVA volume ratio, and solution flow rate were investigated thoroughly, all influencing the fiber creation process. The Kirby-Bauer technique was used to determine the membrane's antibacterial properties. Its capacity to aid in wound healing was evaluated in mice.

2. MATERIALS AND METHOD

2.1. Materials

The chemicals include chitosan (CS, ≥85%, 150 kDa, Viet Nam Food, Viet Nam), polyvinyl alcohol

(PVA, 99.5%, viscosity of 49.6 mPa.s, degree of hydrolysis of 87–89%, Xilong, China), acetic acid (AA, 90%, Jinhuada, China). *Escherichia Coli* (Gram-negative bacteria) and *Listeria Monocytogenes* (Gram-positive bacteria) were utilized. All other materials and reagents used in this study were analytical grade.

2.2. Preparation of CS/PVA fiber membranes

The CS solutions were first prepared by dissolving 0.6 g of CS in 10 mL of 10% AA. The mixtures were then stirred for 24 hours at room temperature to obtain a 6% CS solution (w/v). Afterward, PVA solution was made by dissolving 1.2 and 1.5 g of PVA in 10 mL of deionized water. The solution was agitated for 24 hours at 75°C to get a 12 or 15% PVA solution (w/v). Subsequently, the CS/PVA mixture was created by mixing the CS solution with PVA in different mass ratios of 4:6, 3:7, and 2:8. The mixture was swirled constantly at room temperature for 1 hour. A 1 mL syringe was filled with the prepared solution and then linked to a stainless steel needle. During the electrospining process, the pump speed is set to 0.2, 0.1, and 0.085 mL/h, and voltages of 10, 11, and 12 kV are supplied to the needle tip and collector; the distance between the needle tip and the sample collector is kept constant at 10 cm.

2.3. Analytical methods

For preliminary assessment, the average diameter and morphology of the microfibers in membranes was determined using an optical microscope (Nikon EPIPHOT 200, Japan). To improve their observation, the microfiber membranes were sputter-coated with a platinum layer to observe by a scanning electron microscope (Hitachi S-4800, Japan). The results of SEM images were calculated using ImageJ software (version 1.53e) to determine the fiber average size and distribution.

2.4. Antibacterial evaluation assay

In this work, *Escherichia coli* (*E. coli*) and *Listeria monocytogenes* (*L. monocytogenes*) were used as indicator bacteria to assess the antibacterial properties of the CS/PVA fiber membrane. Bacterial strains were grown in Luria-Bertani (LB) medium at 37°C for 24-48 hours, then incubated overnight (Yamamoto et al., 2021). The bacterial suspension was standardized to a McFarland turbidity of 0.5 with a 0.85% physiological saline solution. CS/PVA fiber membranes were applied to circular portions (1 cm in diameter), and CS/PVA with mass ratios of 4:6, 3:7, and 2:8 were tested. Positive and negative controls were also set up for comparison. The antibacterial ability was assessed using the Kirby-Bauer method (disc diffusion technique) (Hudzicki, 2009; Esser & Elefson, 1970). 100 µL of bacterial suspension (5 x 10^6 CFU/mL) was uniformly distributed on concentrated LB agar plates. Sterilized cloths with CS/PVA fiber membranes (about 1 cm diameter) were put on the agar surface. The experiment was done three times, with the plates incubated at 37°C for 24 hours. The diameter of the sterile ring was measured to assess antibacterial activity.

2.5. Wound healing study

Ethical approval was granted by the Council for Science and Education, Can Tho University (The approval reference number: BQ2023-01/TBK) prior to experimentation. All animal experimentation was conducted in accordance with accepted standards of humane animal care, as outlined in the Ethical Guidelines of the National Health and Medical Research Council.

Healthy male mice weighing 40-45 g were chosen and placed in an experimental room for 7 days to relieve stress and become acquainted with the room environment before producing a wound. The mice were sedated with ether before having their backs shaved and a 1 cm skin wound created. The mice were separated into three groups. Group 1 was left untreated (control sample), whereas group 2 received CS/PVA (2/8 mass ratio) membrane and group 3 received CS/PVA (3/7 mass ratio) membrane. To determine the length of a healed wound, it was measured using a ruler during a 12-day period. The wounds were photographed on days 0, 3, 6, 9, and 12.

3. RESULTS AND DISCUSSION

3.1. Results of the factors affecting the electrospinning process

Figure 1 exhibits optical microscope pictures of fiber membranes with PVA concentrations of 12% and 15%, which were used to investigate the effect of PVA concentration on fiber form and size. When a concentration higher, PVA causes higher solution viscosity, which improves fiber elongation and adherence to the collecting surface. However, excessive PVA concentration causes agglomeration, increases fiber diameter, and inhibits microfiber production (Boudriot et al., 2006). On the other hand, decreasing the PVA concentration reduces solution viscosity, reducing microfiber elongation. Furthermore, low PVA concentrations cause

irregular electrospinning of polymer fibers, rendering them impossible to produce fibers (Geng et al., 2005). These findings highlight the need to adjust PVA purity and concentration in order to attain the desired microfiber characteristics. Figure 1a indicates that the sample made with 12% PVA had a discontinuous fiber look due to the low viscosity of the PVA solution. In contrast, Figure 1b depicts the development of fibers with a more complete structure when the PVA concentration was raised to 15% and tested at various voltages, as seen in Figure 2.

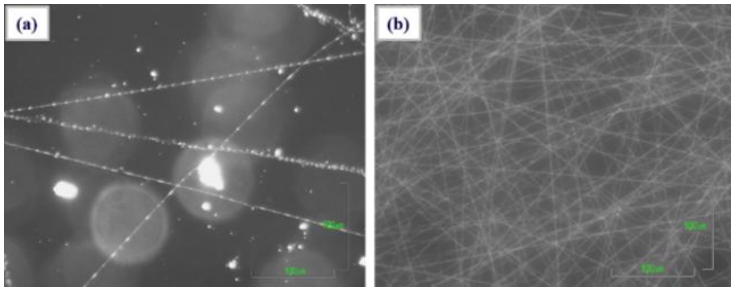

Figure 1. Optical microscope images of CS/PVA fiber membranes at different PVA concentration of (a) 12% and (b) 15%

Additionally, the voltage used during the electrospining process was studied. It is noted that larger voltage results in lower fiber size because it creates excessive heat, which reduces viscosity and moisture content, preventing CS/PVA fiber adherence to the membrane (SalehHudin et al., 2023). In contrast, lower voltage values are more suited for electrospining and aligning the CS/PVA solution into long, uniform microfibers. As a result, voltage is recognized as a significant element influencing fiber production ability, fiber quality, and characteristics. Figure 2 shows detailed information on the parameters impacting fiber development. The samples were tested at voltages of 10 kV, 11 kV, and 12 kV. At 10 kV, the fiber diameter is rather large, and particles are present within the fiber (Figure 2a). When the voltage is increased to 11 kV and 12 kV, image analysis under the optical microscope shows that the sample electrospun with 11 kV (in Figure 2b) has relatively straight fibers, more uniformly distributed, less twisted, and fewer folds than that with 12 kV (in Figure 2c). As a result, the sample with 11 kV voltage was chosen for future studies.

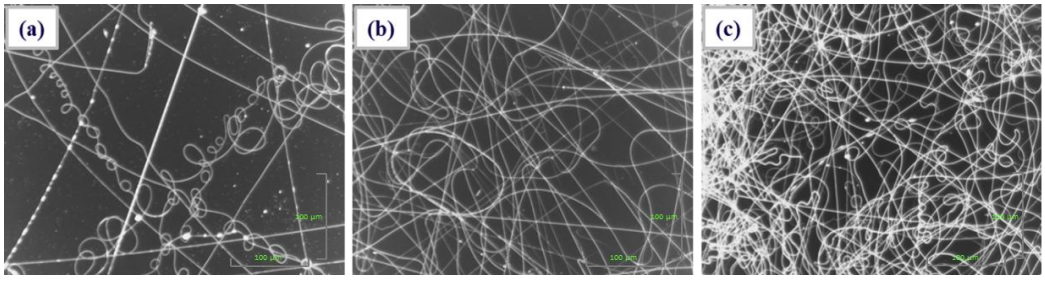

Figure 2. Optical microscope images of CS/PVA fiber membranes at various voltage values: (a) 10 kV, (b) 11 kV, and (c) 12 kV

CS content in CS/PVA fiber membranes was also studied using samples with CS/PVA ratios of 4:6, 3:7, and 2:8 (Figure 3). Higher CS concentrations are thought to have a key role in the production of antibacterial fibers. However, increasing the CS concentration has a negative influence on electrospun fiber production; for example, when the CS:PVA ratio is 4:6, the fibers are structurally unstable, discontinuous, and contain a high number of particles (Figure 3a). When the CS ratio is reduced to 3:7 or 2:8 for CS:PVA, the straight, homogenous fibers are formed (Figure 3b,c). Because the study's goal was to boost antibacterial and hemostatic activity, the subsequent trials employed a CS:PVA ratio of 3:7.

It was discovered that the solution injection speed is an important component depending on the microfiber diameter, with faster rates resulting in smaller diameters and slower speeds resulting in bigger diameters. It also influences yarn consistency

and surface properties. Excessively, high injection rates cause granulation instead of fiber production, resulting in a loss of product homogeneity. In contrast, extremely low pumping rates result in thicker microfibers with a thin surface layer. As a result, determining the optimal injection rate is critical for managing microfiber characteristics. The injection rates of the solution were tested at 0.2, 0.1, and 0.085 mL/h. Figure 4 shows that at a flow rate of 0.2 mL/h, the sample contains multiple grain-like formations due to the solution's high viscosity. When the gadget pumps at this rate, the needle tip condenses. At low voltage, the deposited particles are not given enough time to electrify and spin. Even after lowering the injection speed to 0.1 mL/h, the needle tip condensate still lacks the time required for electrification and spinning, as seen in Figure 5. Finally, the flow rate is lowered to 0.085 mL/h so that the electrospun can stabilize during the process. When a result, even when the fiber diameter rises, the ideal flow rate for the fiber spraying method remains 0.085 mL/h.

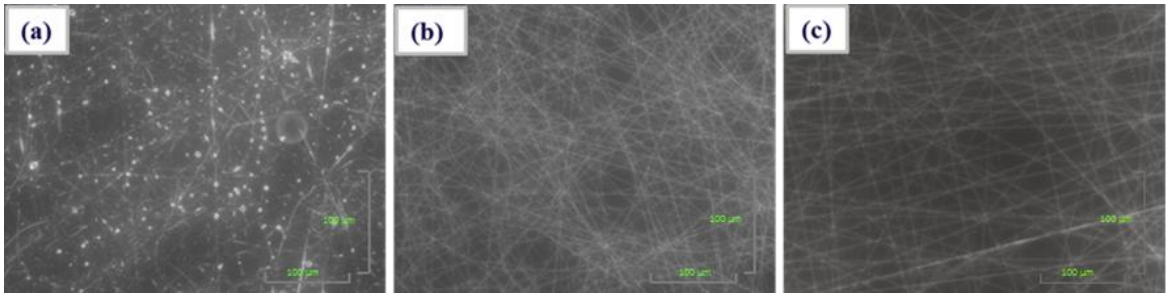

Figure 3. Optical microscope images of CS/PVA fiber membranes at different mass ratio of (a) 4:6, (b) 3:7, and (c) 2:8

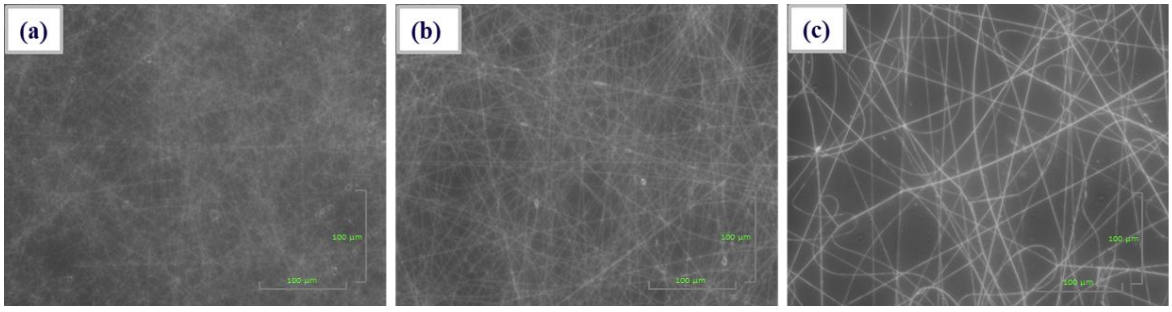

Figure 4. Optical microscope images of CS/PVA fiber membranes at different solution flow rate of (a) 0.2 mL/h, (b) 0.1 mL/h, and (c) 0.085 mL/h

3.2. Morphology of electrospun fibers in the membrane

The surface morphology of CS/PVA fibers was examined using scanning electron microscopy, and the results are depicted in Figure 5. This figure presents the size and morphology of CS/PVA fibers based on the optimal parameters, which include a CS concentration of 6%, a PVA concentration of 15%, a 10% AA concentration, a CS/PVA ratio of 3:7, a flow rate of 0.085 mL/h, a distance of 10 cm between the injector and sample collector, and a voltage of 11 kV. Analysis using Image-J software revealed an average fiber diameter of 457 ± 111 nm, with a diameter distribution ranging from 200 to 800 nm. Although lower voltage settings resulted in larger diameter fibers, the surface morphology did not indicate the presence of particles, suggesting complete dissolution of CS in the solution.

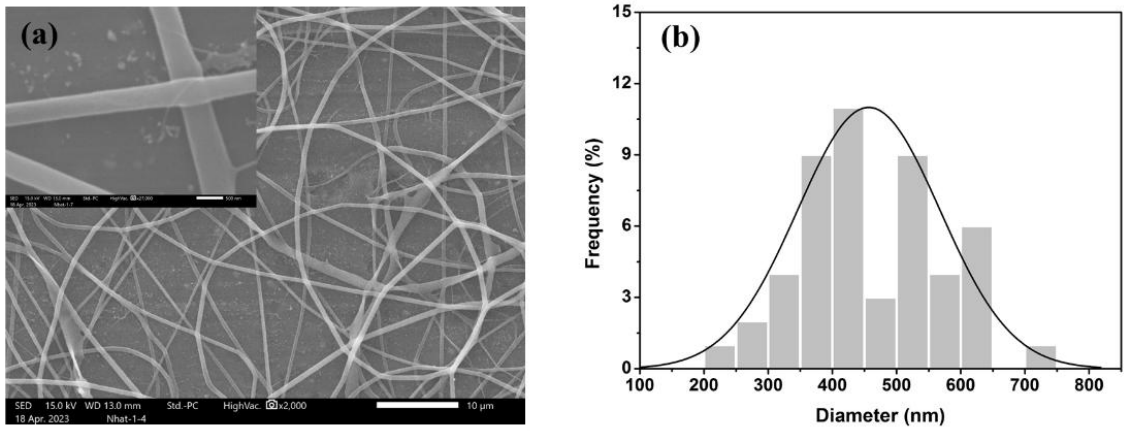

Figure 5. CS/PVA fiber membrane: (a) SEM images in different scales (x1,200 and x27,000) and (b) fiber diameter distribution histograms of under optimal conditions

3.3. Results of antibacterial testing

The antibacterial properties of the produced CS/PVA microfiber membrane as the CS mass ratio increased were evaluated against *E. Coli* and *L. Monocytogenes* using disc diffusion. The CS:PVA = 2:8 sample had an inhibitory zone of 13.833 ± 1.041 mm (Figure 6a1) and 14.867 ± 0.231 mm (Figure 6a2) for *E. Coli* and *L. Monocytogenes*. Increasing the CS mass ratio (e.g., CS:PVA = 3:7) resulted in a growth inhibition zone diameter of 14.500 ± 0.500 mm for *E. Coli* and 14.900 ± 0.100 mm for *L. Monocytogenes* (Figure 6b1, 6b2). Further increasing the CS mass ratio (e.g. CS:PVA = 4:6) resulted in a growth inhibition zone diameter of 15.667 ± 0.764 mm (Figure 6c1) and 15.033 ± 0.252 mm (Figure 6c2) for *E. Coli* and *L. Monocytogenes*. As a result, raising the CS mass ratio improves the antibacterial properties of the CS/PVA fiber membrane, which is consistent with the theory of antibacterial properties of CS (El-Tahlawy et al., 2005; Shekarforoush et al., 2015). This finding validated CS's antibacterial activity and promised to help decrease the healing period of open wounds.

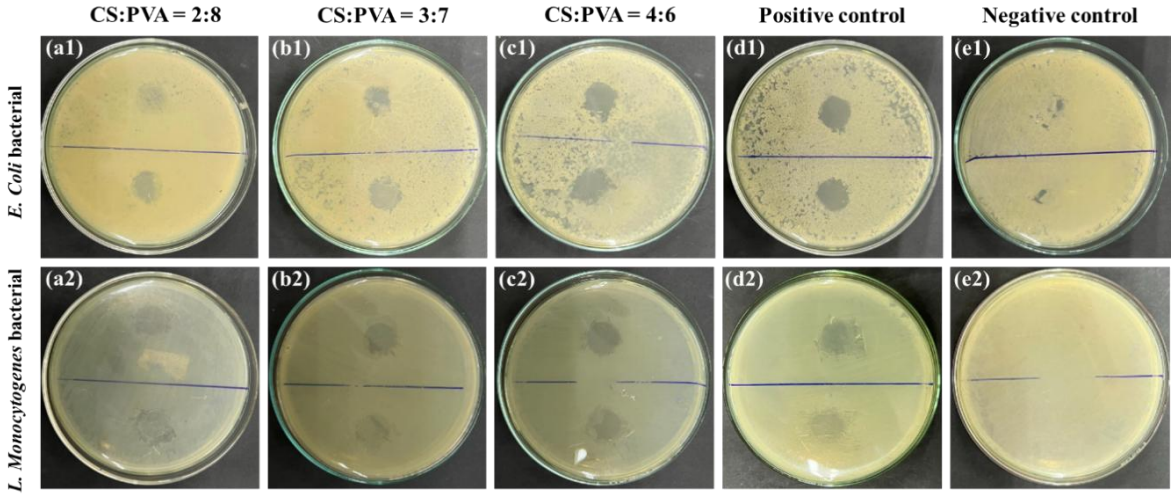

Figure 6. Antibacterial efficacy of fiber membranes in different mass ratio of (a1, a2) CS:PVA = 2:8, (b1, b2) CS:PVA = 3:7, (c1, c2) CS:PVA = 4:6, (d1, d2) positive, and (e1, e2) negative control sample against *E. Coli* and *L. Monocytogenes* bacterial, respectively

3.4. Evaluation of the wound healing ability

The wound healing ability of CS/PVA membranes with different CS/PVA ratio (e.g. 2:8 and 3:7) was investigated through wounding in mice, observing the wound healing process, and comparing with the self-healed wound (as a control sample). The changes of wound in mice were observed on various time intervals of 0, 3, 6, 9 and 12 days after the wound formation (Figure 7). As a result, the wounds applied CS/PVA membranes improved wound closure ability compared with self-healing wounds. On day three, the wounds on the mice in all three tests improved dramatically, with no bleeding or exudate, but there were no significant differences between the three treated wound samples. On day six, all wounds developed satisfactorily, with the most important being the lesion treated with a 3:7 ratio of CS/PVA fiber membrane. This might be attributed to the hemostatic, anti-inflammatory, and antibacterial properties of CS. Only the wound treated with CS/PVA fiber membrane at a 3:7 ratio was nearly healed after 9 days and entirely healed after 12 days. This finding is consistent with those of Dhurai et al. (2013) and Thuy et al. (2013). The CS/PVA film with a 3:7 ratio shows higher wound healing capabilities than the control sample. In other words, the CS and PVA combination membrane offers enormous potential for wound healing.

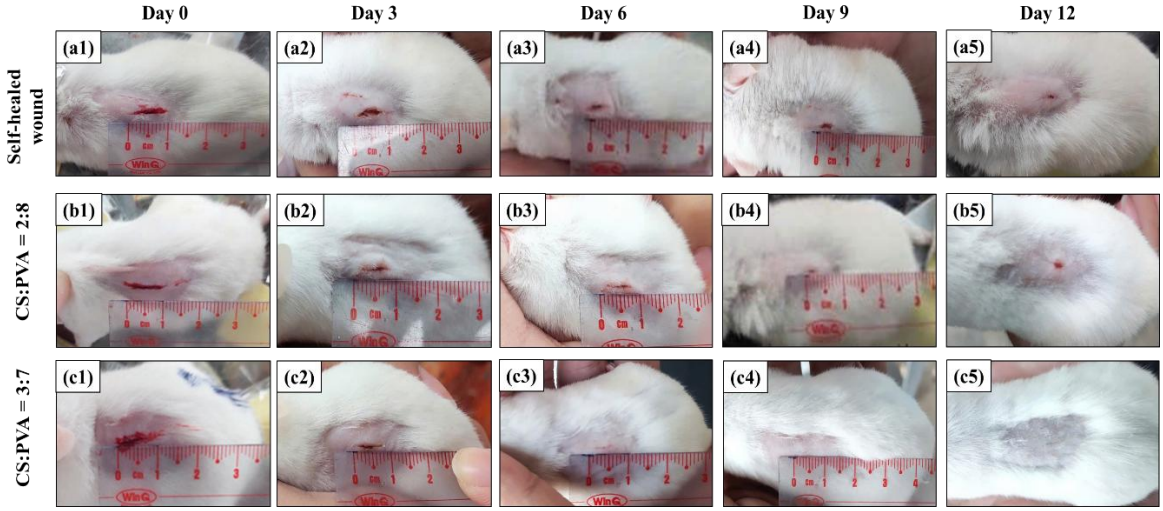

Figure 7. Pictorial representation of wound d surface healing for (a) control samples (the self-healed wound) and after treatment with fiber membranes in different mass ratio of (b) CS:PVA = 2:8 and (c) CS:PVA = 3:7 at different time points

4. CONCLUSIONS

This study successfully investigated a CS/PVA fiber membrane with antibacterial properties utilizing the electrospinning process. The results showed that the requisite fiber characteristics and antibacterial activity could be achieved. The best conditions for fiber synthesis were 6% CS dissolved in 10% AA, 15% PVA, a CS/PVA ratio of 3:7, a distance of 10 cm between the sample collecting and injector, an 11 kV voltage, and a flow rate of 0.085 mL/h. These conditions produced homogeneous CS microfibers with high adhesion. Scanning electron microscopy investigation showed the lack of particles in the fiber structure, despite the average fiber diameter being around 432 ± 111 nm, which is bigger than normal CS microfibers. Maintaining the antibacterial activity of the CS/PVA fiber membrane is critical for rapid wound healing. The antimicrobial test showed that the CS/PVA fibrous fabric was efficient against two indicator bacterial strains, *E. coli* and *L. monocytogenes*. A higher chitosan percentage correlated with better antibacterial properties. *In-vivo* testing after 12 days revealed that the CS/PVA microfiber membrane had a faster wound-healing capability than a self-healed wound sample. Overall, our findings demonstrate the effective creation of CS microfiber-based antimicrobial materials that aid in the healing of open wounds.

ACKNOWLEDGMENT

This work is supported by funding from Can Tho University, Viet Nam (Number code: T2023-98) and Ministry of Education and Training, Viet Nam (Number code: B2024-TCT-07).

REFERENCES

Boudriot, U., Dersch, R., Greiner, A., & Wendorff, J. H. (2006). Electrospinning approaches toward scaffold engineering—A brief overview. *Artificial Organs*, *30*, 785-792. https://doi.org/10.1111/j.1525-1594.2006.00301.x

Chen, J., Han, S., Huang, M., Li, J., Zhou, M., & He, J. (2022). Green crosslinked nanofibers membrane based on CS/PVA combined with polybasic organic acid for tympanic membrane repair. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *71*(4), 291-301. https://doi.org/10.1080/00914037.2020.1825084

Dhurai, B., Saraswathy, N., Maheswaran, R., Sethupathi, P., Vanitha, P., Vigneshwaran, S., & Rameshbabu, V. (2013). Electrospinning of curcumin loaded chitosan/poly (lactic acid) nanofilm and evaluation of its medicinal characteristics. *Frontiers of Materials Science*, *7*, 350-361. https://doi.org/10.1007/s11706-013-0222-8

El-Tahlawy, K. F., El-Bendary, M. A., Elhendawy, A. G., & Hudson, S. M. (2005). The antimicrobial activity of cotton fabrics treated with different crosslinking agents and chitosan. *Carbohydrate polymers*, *60*, 421-430. https://doi.org/10.1016/j.carbpol.2005.02.019

Esser, V. M. & Elefson, D. E. (1970). Experiences with the Kirby-Bauer method of antibiotic susceptibility testing. *American Journal of Clinical Pathology*, *54*(2), 193-198. https://doi.org/10.1093/ajcp/54.2.193

Fan, J. P., Luo, J. J., Zhang, X. H., Zhen, B., Dong, C. Y., Li, Y. C., ... & Chen, H. P. (2019). A novel electrospun β-CD/CS/PVA nanofiber membrane for simultaneous and rapid removal of organic micropollutants and heavy metal ions from water. *Chemical Engineering Journal*, *378*, 122232. https://doi.org/10.1016/j.cej.2019.122232

Gaaz, T. S., Sulong, A. B., Akhtar, M. N., Kadhum, A. A. H., Mohamad, A. B., & Al-Amiery, A. A. (2015). Properties and applications of polyvinyl alcohol, halloysite nanotubes and their nanocomposites. *Molecules*, *20*, 22833-22847. https://doi.org/10.3390/molecules201219884

Geng, X., Kwon, O.-H., & Jang, J. (2005). Electrospinning of chitosan dissolved in concentrated acetic acid solution. *Biomaterials*, *26*, 5427-5432. https://doi.org/10.1016/j.biomaterials.2005.01.066

Giménez-Arnau, A. (2016). Standards for the protection of skin barrier function. *Skin Barrier Function*, *49*, 123-134. https://doi.org/10.1159/000441588

Hohman, M. M., Shin, M., Rutledge, G., & Brenner, M. P. (2001). Electrospinning and electrically forced jets. I. Stability theory. *Physics of Fluids*, *13*, 2201-2220. https://doi.org/10.1063/1.1383791

Homayoni, H., Ravandi, S. A. H., & Valizadeh, M. (2009). Electrospinning of chitosan nanofibers: Processing optimization. *Carbohydrate Polymers*, *77*, 656-661. https://doi.org/10.1016/j.carbpol.2009.02.008

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*, *15*, 55-63.

Iqbal, H., Khan, B. A., Khan, Z. U., Razzaq, A., Khan, N. U., Menaa, B., & Menaa, F. (2020). Fabrication, physical characterizations and in vitro antibacterial activity of cefadroxil-loaded chitosan/poly (vinyl alcohol) nanofibers against Staphylococcus aureus clinical isolates. *International Journal of Biological Macromolecules*, *144*, 921-931. https://doi.org/10.1016/j.ijbiomac.2019.09.169

Kang, Y. O., Yoon, I. S., Lee, S. Y., Kim, D. D., Lee, S. J., Park, W. H., & Hudson, S. M. (2010). Chitosan-coated poly (vinyl alcohol) nanofibers for wound dressings. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *92*, 568-576. https://doi.org/10.1002/jbm.b.31554

Kharaghani, D., Khan, M. Q., Tamada, Y., Ogasawara, H., Inoue, Y., Saito, Y., ... & Kim, I. S. (2018). Fabrication of electrospun antibacterial PVA/Cs nanofibers loaded with CuNPs and AgNPs by an in-situ method. *Polymer Testing*, *72*, 315-321. https://doi.org/10.1016/j.polymertesting.2018.10.029

Lemma, S. M., Bossard, F., & Rinaudo, M. (2016). Preparation of pure and stable chitosan nanofibers by electrospinning in the presence of poly (ethylene oxide). *International Journal of Molecular Sciences*, *17*(11), 1790. https://doi.org/10.3390/ijms17111790

Li, C., Fu, R., Yu, C., Li, Z., Guan, H., Hu, D., Zhao, D., & Lu, L. (2013). Silver nanoparticle/chitosan oligosaccharide/poly (vinyl alcohol) nanofibers as wound dressings: a preclinical study. *International Journal of Nanomedicine*, *8*, 4131-4145. https://doi.org/10.2147/IJN.S51679

Lim, S. H. (2003). *Synthesis of a fiber-reactive chitosan derivative and its application to cotton fabric as an antimicrobial finish and a dyeing-improving agent* (Dissertation). North Carolina State University.

Lim, S.-H. & Hudson, S. M. (2004). Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. *Carbohydrate research*, *339*, 313-319. https://doi.org/10.1021/ma5016616

Luo, C. & Edirisinghe, M. (2014). Core-liquid-induced transition from coaxial electrospray to electrospinning of low-viscosity poly (lactide-co-glycolide) sheath solution. *Macromolecules*, *47*,

7930-7938.
https://doi.org/10.1021/ma5016616

Lv, H., Zhao, M., Li, Y., Li, K., Chen, S., Zhao, W., ... & Han, Y. (2022). Electrospun chitosan–polyvinyl alcohol nanofiber dressings loaded with bioactive ursolic acid promoting diabetic wound healing. *Nanomaterials*, *12*(17), 2933. https://doi.org/10.3390/nano12172933

Mirhaj, M., Labbaf, S., Tavakoli, M., & Seifalian, A. M. (2022). Emerging treatment strategies in wound care. *International Wound Journal*, *19*(7), 1934-1954. https://doi.org/10.1111/iwj.13786

Nathan, K. G., Genasan, K., & Kamarul, T. (2023). Polyvinyl alcohol-chitosan scaffold for tissue engineering and regenerative medicine application: a review. *Marine Drugs*, *21*(5), 304. https://doi.org/10.3390/md21050304.

Pereira, R. F., & Bartolo, P. J. (2016). Traditional therapies for skin wound healing. *Advances in Wound Care*, *5*(5), 208-229. https://doi.org/10.1089%2Fwound.2013.0506

Reneker, D. H. & Chun, I. (1996). Nanometre diameter fibres of polymer, produced by electrospinning. *Nanotechnology*, *7*, 216. https://doi.org/10.1088/0957-4484/7/3/009

Sajomsang, W. (2010). Synthetic methods and applications of chitosan containing pyridylmethyl moiety and its quaternized derivatives: A review. *Carbohydrate Polymers*, *80*(3), 631-647. https://doi.org/10.1016/j.carbpol.2009.12.037

SalehHudin, H. S., Mohamad, E. N., Afifi, A. M., Mahadi, W. N. L. W. (2023). Simulation and experimental study of parameters in multiple-nozzle electrospinning: Effects of voltage and nozzle configuration on the electric field and electrospun jet attributes. *Journal of Manufacturing Processes*, *85*, 544-555. https://doi.org/10.1016/j.jmapro.2022.11.051

Singh, S., Young, A., & McNaught, C. E. (2017). The physiology of wound healing. *Surgery (Oxford)*, *35*(9), 473-477. https://doi.org/10.1016/j.mpsur.2017.06.004

Shekarforoush, S. S., Basiri, S., Ebrahimnejad, H., & Hosseinzadeh, S. (2015). Effect of chitosan on spoilage bacteria, Escherichia coli and Listeria monocytogenes in cured chicken meat. *International Journal of Biological Macromolecules*, *76*, 303-309. https://doi.org/10.1016/j.ijbiomac.2015.02.033

Shin, Y., Hohman, M., Brenner, M. P., & Rutledge, G. (2001). Electrospinning: A whipping fluid jet generates submicron polymer fibers. *Applied Physics Letters*, *78*, 1149-1151. https://doi.org/10.1063/1.1345798

Subbiah, T., Bhat, G. S., Tock, R. W., Parameswaran, S., & Ramkumar, S. S. (2005). Electrospinning of nanofibers. *Journal of Applied Polymer Science*, *96*, 557-569. https://doi.org/10.1002/app.21481

Thuy, N. T. T., Ghosh, C., Hwang, S.-G., Lam, D. T., Park, J. S. (2013). Characteristics of curcumin-loaded poly (lactic acid) nanofibers for wound healing. *Journal of Material Science*, *48*, 7125-7133. https://doi.org/10.1007/s10853-013-7527-y

Viana, V. R., Ferreira, W. H., Azero, E. G., Dias, M. L., & Andrade, C. T. (2020). Optimization of the electrospinning conditions by box-behnken design to prepare poly (vinyl alcohol)/chitosan crosslinked nanofibers. *Journal of Materials Science and Chemical Engineering*, 8(4), 13-31. https://doi.org/10.4236/msce.2020.84002

Wei, Y., Zhang, X., Song, Y., Han, B., Hu, X., Wang, X., ... & Deng, X. (2011). Magnetic biodegradable Fe_3O_4/CS/PVA nanofibrous membranes for bone regeneration. *Biomedical Materials*, *6*(5), 055008. https://doi.org/10.1088/17486041/6/5/055008

Yamamoto, K., Toya, S., Sabidi, S., Hoshiko, Y., & Maeda, T. (2021). Diluted Luria-Bertani medium vs. sewage sludge as growth media: comparison of community structure and diversity in the culturable bacteria. *Applied Microbiology and Biotechnology*, *105*, 3787-3798. https://doi.org/10.1007/s00253-021-11248-4