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The effect of silver nanoparticle concentration on the antibacterial properties of tri-layer PCL-Ag/PT/PVP wound dressing

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Abstract:

To comprehensively manage wound infection, tri-layer fibre membranes have been designed with two antibacterial agents, including silver nanoparticles (AgNPs) and chitosan oligosaccharide (COS), and their antibacterial properties were evaluated using qualitative and quantitative tests. While the first polycaprolactone (PCL) silver layer and the second plasma-treated PCL layer were fabricated by electrospinning, the third COS layer was constructed via coating and heat-vacuum drying. The formation of the membranes and their fibrous and porous structures were examined using scanning electron microscopy (SEM). The antibacterial activities of the membranes against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* were examined by disk diffusion and percentage reduction assays, which were performed simultaneously as per the certificates from the Ministry of Health Institute of Public Health in Ho Chi Minh city. The tri-layer membranes, named PCLAg50/PT (plasma treatment)/COS and PCLAg500/PT/COS, achieved desirable antibacterial results with extensive inhibition zones and bacterial reduction rates ranging from 99-100%. In addition, while the PCLAg500 membrane exhibited broad inhibition zones and reduction rates ranging from 94.6-97%, the PCLAg50 membrane achieved reduction rates ranging from 67.5-82.1% despite the absence of inhibition zones. As a result, the fabricated tri-layer membranes could reduce local and opportunistic infections. Depending on infectious risks, the concentration of silver utilsed as an infection-prevention agent must be adjusted from 50 to 500 ppm to ensure safety.

Keywords: **antibacterial, chitosan oligosaccharide, electrospinning, plasma treatment, polycaprolactone, polyvinylpyrrolidone, silver nanoparticles.**

Classification number: **3.6**

1. Introduction

Traditional dressings have often favoured cotton gauze to dress all types of wounds [1]. Over centuries, advanced dressings have evolved into numerous forms including adherent dressing, semipermeable films, hydrocolloids, hydrogels, alginates, foam dressings, and antimicrobial dressings, effectively supporting treatment for different types of wounds [2, 3]. Among these, antimicrobial dressings play a crucial role in wound infection management or at least in preventing potential risks from external pathogens [4]. In clinical practice, the use of commercial dressings containing antiseptic substances leads to a significant alleviation in wound severity and treatment-related burdens [5]. Besides antibacterial properties, ideal dressings should enable gaseous exchange, exudate absorption, waterproofing, and painless removal [6]. To meet these requirements, ideal dressings should be designed with three layers: an inner-contact layer, a middle absorbent layer, and an outer waterproof layer with suitable characteristics [7]. The inner layer, in direct contact with the wound site, plays a critical role in treating infection and promoting tissue regeneration, necessitating essential features such as antibacterial capability, absorption, and biocompatibility [8]. Meanwhile, the middle layer primarily absorbs the exudate and maintains moisture in the wound bed, while the outer layer, which covers the wound surface and prevents the invasion of pathogens, needs to be waterproof, mechanically robust, and antibacterial [9]. Consequently, dressing characteristics that promote the healing process have attracted significant interest from scientists for further investigation.

Focusing on antibacterial properties, various antiseptic substances used in wound dressings, such as silver,

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chitosan, chlorhexidine, iodine, etc., have proven effective to treat infectious wounds [10]. Among these, silver in the forms of silver ions or silver nanoparticles is known for its outstanding antibacterial efficiency against a wide range of bacteria, including antibiotic-resistant strains [11, 12]. Historically, silver's mechanism of action involves its positive charges entrapping and penetrating the negatively charged bacterial membrane, triggering the disruption of the respiratory process and resulting in bacterial death [13]. Simultaneously, silver nanoparticles convert into silver ions to prolong the antibacterial effect [14, 15]. However, drawbacks related to overdosing or silver's metal ion form have also been documented [16]. Hoping to achieve low toxicity, COS have been proposed as an additional antibacterial indicator.

Although COS dissolves in water or organic solvents, facilitating its use in wound dressings without causing toxicity, it may be rapidly released and degraded in the exuding wounds, shortening the antibacterial treatment duration due to its low molecular weight. To address these limitations, researchers have studied the combination of polyvinylpyrrolidone (PVP) with COS to increase molecular weight. PVP, a non-ionic and water-soluble polymer, is widely used in pharmaceutical applications and approved by Food and Drug Administration. In wound dressings, its advanced properties include hygroscopicity, attributed to the presence of vinylpyrrolidone, which helps to absorb exudate and maintain moisture in the wound bed, significantly promoting wound healing.

Regarding the foundation of wound dressings, numerous polymers have been combined to synthesise multi-functional membranes with advanced characteristics such as high tensile strength, waterproofness, wettability, and biocompatibility. Notably, the electrospinning technique has been widely applied to produce fibre membranes with advanced features for wound dressing, thanks to polymers. Many researchers have taken an interest in a synthetic material known as PCL, which is utilised to fabricate electro-spun membranes with exceptional mechanical, physical, elasticity, and hydrophobic properties. Consequently, it can cover and protect the wound from the external forces while preventing unexpected leakage or absorption. Another advantage of PCL-based membranes is their scaffolding, which can entrap and stabilise silver nanoparticles.

One study reported that silver nanoparticles loaded in PCL electrospun membranes, with a silver content of 500 ppm, exhibited excellent growth inhibition against both gram-negative and gram-positive bacterial strains [17]. In contrast to PCL's features, PT is known as a method to improve polymer wettability. In several studies, a time-stable wetting effect on plasma-treated polymers helped manage weak hydrophobic properties. In fact, PCL surface are hydrophobic, which limits their compatibility with human tissue essential for such applications as dressings. In the dressing application of plasma-treated polycaprolactone, its functional group anchors to the PCL backbone, and these groups tend to etch the polymer surface and attract other hydrophilic components [18]. As a result, the plasma-treated membrane is not only wettable but also a scaffold for hydrophilic solution coatings. In addition, this membrane possesses good mechanical strength and biocompatibility, both of which have been widely utilised in biomedical applications. In another investigation, membranes coated with COS/PVP also demonstrated growth inhibition of both gram-negative and gram-positive bacterial strains [19]. However, this research focuses on the antibacterial properties of silver and COS in polymetric electrospun membranes, encompassing not only growth inhibition but also bactericidal performance.

This work aims to evaluate the antibacterial properties of tri-layer membranes containing two antibacterial agents, COS and AgNPs, against *P. aeruginosa*, *E. coli* and *S. aureus* using qualitative and quantitative testing. The tri-layer membranes consist of: (1) an outer layer with a PCL membrane loaded with silver nanoparticle (50 ppm and 500 ppm), serving as the waterproof cover to maintain an aseptic environment; (2) a middle PCL/PT layer primarily for absorption; and (3) an inner layer featuring COS/PVP coatings, intended to treat wound infections, maintain moisture, ensure non-adherence. Additionally, these membranes were re-examined objectively to confirm their high reliability at the Institute of Hygiene and Public Health.

2. Materials and methods

2.1. Materials

PCL (80,000 Da) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co., St. Louis, MO (USA). PVP K30 (40,000 Da), silver nitrate ($AgNO_3$ ≥99%), acetone (AC, CH_3COCH_3, 99.5%), hydrogen peroxide (H_2O_2) 30% (v/v) reagent, EtOH (C_2H_5OH) (100%), and Tween 20 were produced by Xilong Chemical Co., Ltd. (China). Low-viscosity chitosan (CS) (270 kDa) was purchased from Dao Nguyen, Vietnam. Mueller-Hinton broth (MHB)

was supplied by Hi-Media (India). Muller-Hinton agar (MHA) was purchased from Oxoid, UK. CN Agar for *P.* was purchased from Biokar Diagnostics, France. Bacteria *P. aeruginosa* ATCC 9028, *E. coli* ATCC25922, and *S. aureus* ATCC 25913 were supplied from the Marine Laboratory, International University - Vietnam National University, Ho Chi Minh City, Viet Nam. All chemicals were analytical grade and were used directly without further purification.

2.2. Methods

2.2.1. Preparation of PCLAg solutions with silver concentrations of 50 and 500 ppm and PCL solution

$AgNO_3$ solution (100,000 ppm) was prepared by dissolving 1.573 g of $AgNO_3$ in 10 ml DMSO solvent in a dark container, stirring at 200 rpm for 1 hour at room temperature. A PCL solution (12% w/v) was then prepared by dissolving PCL pellets in fresh acetone, stirring at 400 rpm at 70°C for 4 hours. After that, 2.5 ml of freshly prepared liquid $AgNO_3$ was added to 497.5 ml of PCL solution while stirring at 400 rpm for 30 min at room temperature. The solutions of silver nanoparticles stabilised in PCL were successfully prepared by γ-ray (Co-60) irradiation at a dose of 15 kGy and dose rate of 6.5 kGy/h by Gamma GC-5000 (Brit, India), following a previously described procedure [17]. Additionally, the PCLAg50 solution was also obtained by diluting the irradiated PCLAg500 solution ten times with PCL solution (12% w/v).

2.2.2. Preparation of membranes: PCLAg50 and PCLAg500

The electrospun membranes were fabricated with an electrospinning machine designed by the School of Biomedical Engineering, International University, Vietnam National University, Ho Chi Minh City. The following parameters were used: feed rate of 2.5 ml/h, voltage of 15 kV, the rotation speed of 150 rpm, collecting membrane area of 600 m^2, 10 ml syringe, and 18G needle. The bilayer membranes were electrospun with three syringes set up in parallel, and the volumes of PCLAg solution and PCL solution were 45 ml and 15 ml, respectively. PCLAg50/PCL and PCLAg500/PCL (10x10 cm) were placed at the center of the plasma chamber (GaLa Instrumente, Germany) for 2 min of air plasma treatment under the following conditions: 30 W RF power and a frequency of 13.56 MHz.

2.2.3. Preparation of solution: COS-PVP

The coating solution containing COS and PVP was formulated in accordance with previous work [19]. Firstly, chitosan (3%w/v) was immersed in H_2O_2 solution (6% v/v) and stirred at 90°C for 4 hours. The COS-formed solution was filtered before the PVP powder (6% w/v) was added to and stirred for 30 min at room temperature.

2.2.4. Preparation of the tri-layer membranes: PCLAg50/PT/COS and PCLAg500/PT/COS

The bilayer membranes were cut into 6x6 cm pieces and placed in a 5x5 cm exposure area mold. Then, 6 ml of COS-PVP solution was coated onto the surface of the PCL-PT layers in three equal portions. After each coating, they were dried in a vacuum oven at 40°C for one day. Finally, the total collection area of the coated membrane was 5x5 cm. All samples were fabricated with the following parameters shown in Table 1 below.

Table 1. The content of components in the PCLAg/PT/COS membrane.

LAYER	PCL	SILVER		COS
PCLAg	12% w/v	50 ppm	500 ppm	-
PCLAg/PT				-
COS	-	-	-	0.18% w/v

2.2.5. Scanning electron microscopy (SEM)

The surface morphologies of all the membranes were determined by SEM (JSM-IT100 and Smart Coater, JEOL, Japan). After that, the fibre diameter and the porosity of the membrane were measured by ImageJ software (NIH, Maryland, USA).

2.2.6. Antibacterial experiments

Quality antibacterial experiment: The agar diffusion standard method was employed as a quick qualitative evaluation of the antibacterial susceptibility of specimens [20]. The prepared bacterial culture of 10^6 CFU/ml was transferred to sterile agar disks of 90 mm diameter containing 15 ml Miller-Hinton agar. Then, 10x10 mm square samples were gently placed on the surface of the inoculated disks. The disks were incubated for 18-24 hours, and the results were demonstrated by measuring the diameter of inhibition zones around the specimens. The experiments were triplicated.

Quantitative antibacterial experiment: The AATCC100 method was employed as a quantitative evaluation of the antibacterial performance of specimens over 24 hours [21]. An antibacterial membrane and two blank membranes (controls) with diameters of 4.8 cm were placed into separate 250 ml flasks and then 1 ml of diluted suspension with 10^5 CFU/ml was added dropwise. Immediately after, 100 ml of sodium chloride 0.9% was added to the control flasks, and

the bacteria was shaken out from the membrane. Then, 100 µl of this solution was spread on the surface of Miller Hinton agar disks (except for the *P. aeruginosa* sample, which used the CN agar disk) and inoculated at 37°C for 18-24 hours. Finally, the number of bacterial colonies was counted as A. The two remaining flasks were simultaneously inoculated at 37°C for 18-24 hours. As in the preceding procedure, the number of bacterial colonies shaken out of the antibacterial membrane and the blank membrane were denoted as B and C, respectively. The experiments were triplicated. The results are expressed by the formula:

$$R\ (\%) = [(A-B)/A]x100$$

where R (%) is the percentage reduction; A is the number of bacteria recovered from the blank membrane immediately after being treated; B is the number of bacteria recovered from the inoculated antibacterial specimen after 24 hours.

2.2.7. Statistical analysis

Statistical analysis and graphs were done using Origin Pro 8.5.1. Differences between samples were analysed by one-way analysis of variance (ANOVA) followed by the Student's T-test. Data were expressed as mean ±SD.

3. Results and discussion

3.1. Evaluation of surface morphology

The successful fabrication of the tri-layer membranes (including the outer PCLAg layer with two silver concentrations of 50 and 500 ppm, the middle PCLAg/PT layer, and the inner COS-coated layer) and the PCL membrane is demonstrated in Fig. 1. Fig. 1A provides the SEM micrographs, Fig. 1B shows the distributions of the fibre diameters, and Fig. 1C shows the distributions of the pore diameters. All membranes were produced with non-beaded fibres stacked on top of each other to create a bulging and porous structure. There is no significant difference in fibre diameters between groups containing silver concentrations of 50 and 500 ppm. The outer layers of PCLAg50 and PCLAg500 have mean fibre diameters of 1.07 and 1.05 µm, respectively. Compared to those in the outer layers, the fibres in the middle layers, including PCLAg50/PT and PCLAg500/PT, increased slightly to approximately 1.18 and 1.17 µm, respectively. Changes in the surface of all layers were also observed in the mean diameter of porosity. The outer PCLAg layers had mean porosity sizes around 11.51 and 12.89 µm. However, the coated COS layers had significantly reduced mean porosity diameters of 7.76 and 9.45 µm, with only 10% of porosity visible on the surface. The coating method allowed for covering over 90% of the membrane surface. Both diameter and porosity contributed to creating an ideal membrane for wound dressing applications.

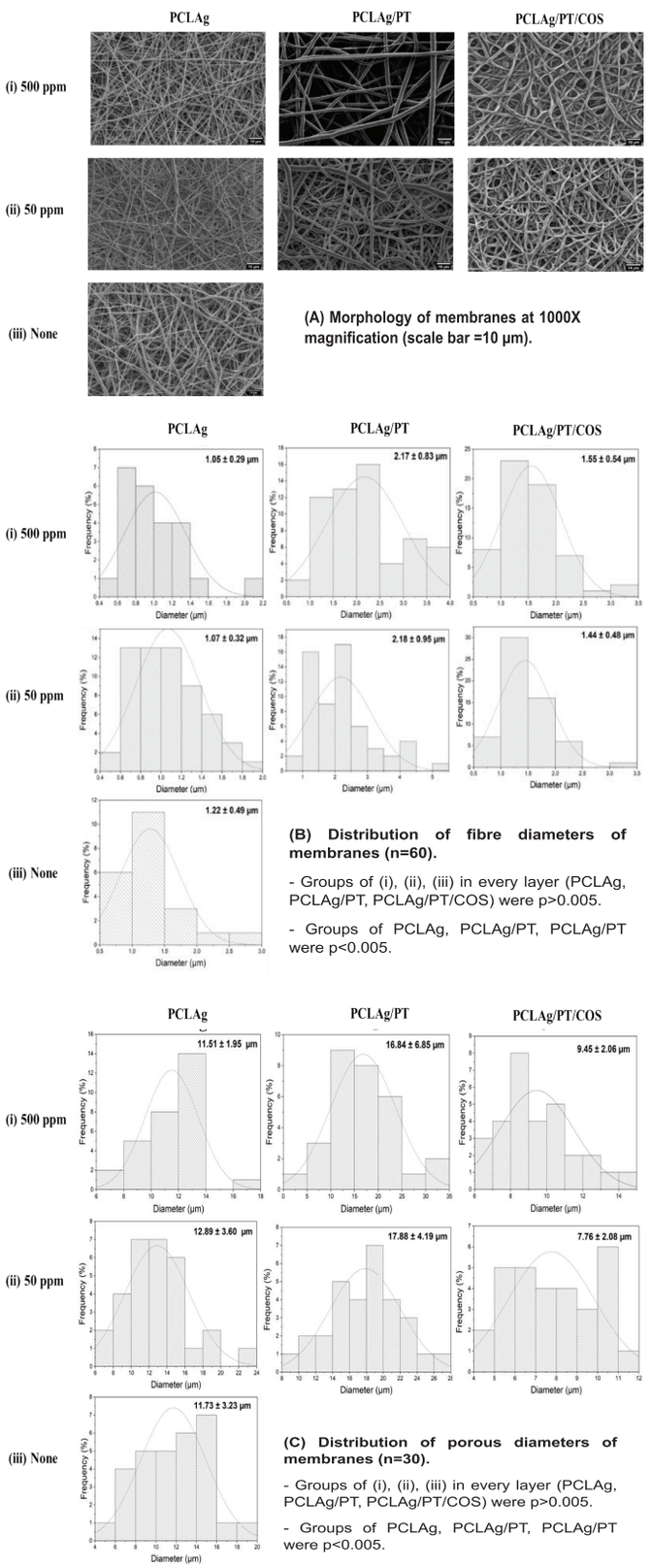

Fig. 1. SEM results of tri-layer membranes including the PCLAg layers, the PCLAg/PT layers, and the COS layers at two concentrations of silver (i) 500 ppm and (ii) 50 ppm.

3.2. Evaluation of antibacterial activities

The antibacterial activities of the PCLAg membranes and the tri-layer PCLAg/PT/COS membranes were examined by qualitative and quantitative tests against *P. aeruginosa*, *E. coli*, and *S. aureus* to comprehensively understand their characteristics over a wide range of applications.

In terms of the antibacterial activity, Fig. 2 and Table 2 demonstrate the results of the PCLAg membranes consisting of silver nanoparticles at 50 and 500 ppm, which show that the inhibition zones of the PCLAg500 membrane against *P. aeruginosa*, *E. coli*, and *S. aureus* were 15.3, 11.4, and 13.6 mm, respectively, whereas those of PCL served as the control. For the quantitative results, the reduction percentages of the membrane with a silver content of 500 ppm against *P. aeruginosa*, *E. coli*, and *S. aureus* achieved expected numbers (over 90%). Regardless of the absence of inhibition zones, the PCLAg50 membrane obtained valuable reduction percentage of over 60%. With this reduction in concentration, there is great potential for using low concentrations of silver nanoparticles in dressings to reduce the accumulation of silver in the skin and blood while still possessing a bactericidal potential of more than 50%.

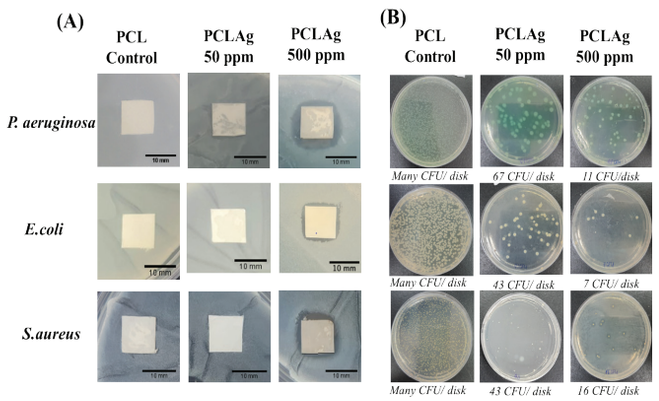

Fig. 2. Images of the antibacterial results of PCLAg50, PCLAg500, and PCL (control) membranes against *P. aeruginosa*, *E. coli*, and *S. aureus*. (A) Inhibition zone with a 10-mm scale bar; **(B)** bacterial colonies 24 hours after injection (n=3).

Table 2. The inhibition diameter and reduction percentage of PCLAg50, PCLAg500, and PCL (control) membranes against *P. aeruginosa*, *E. coli*, and *S. aureus*.

Sample	Inhibition zone diameter (mm)			Reduction percentage (%)		
	P. aeruginosa (-)	*E. coli* (-)	*S. aureus* (+)	*P. aeruginosa* (-)	*E. coli* (-)	*S. aureus* (+)
PCLAg500	15.5±0.05	11.4±0.06	13.6±0.06	94.6	96.8	97
PCLAg50	-	-	-	67.5	86.5	82.1
PCL (Control)	-	-	-	-	-	-

Table 3. The inhibition zone diameter and reduction percentage of PCLAg50/PT/COS, PCLAg500/PT/COS, and PCL (control) membranes against *P. aeruginosa*, *E. coli*, and *S. aureus*.

Sample	Inhibition zone diameter (mm)			Reduction percentage (%)		
	P. aeruginosa (-)	*E. coli* (-)	*S. aureus* (+)	*P. aeruginosa* (-)	*E. coli* (-)	*S. aureus* (+)
PCLAg500/PT/COS	13.7±0.03	13.2±0.01	22.4±0.06	100	99	99.4
PCLAg50/PT/COS	11.9±0.03	12.3±0.02	15.2±0.05	100	99	99.3
PCL (Control)	-	-	-	-	-	-

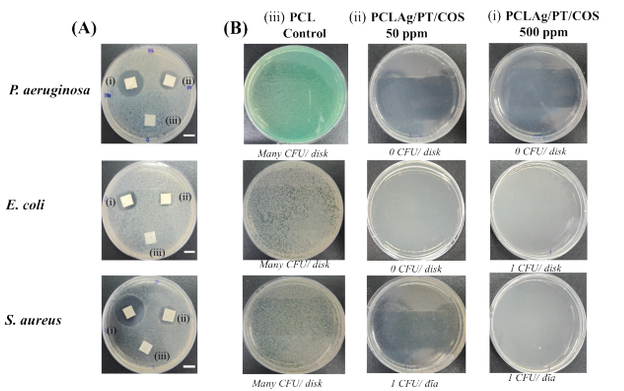

Fig. 3. Images of the antibacterial results of PCLAg50/PT/COS, PCLAg500, and PCL (control) membranes against *P. aeruginosa*, *E. coli*, and *S. aureus*. (A) Inhibition zone with a 10 mm scale bar and **(B)** Bacterial colonies 24 hours after injection (n=3).

All results from the tests on the tri-layer PCLAg/PT/COS membranes containing silver and COS are shown in Fig. 3 and Table 3. From the qualitative results, Fig. 3A and Table 3 show the inhibition zones of the PCLAg500/PT/COS membranes against three strains of bacteria were considerably higher than those of the PCLAg50/PT/COS membrane and the COS-containing surface in direct contact with bacteria. In detail, while the inhibition diameters of the PCLAg500/PT/COS membranes against *P. aeruginosa*, *E. coli*, and *S. aureus* were 13.7, 13.2, and 22.4 mm, those of the PCLAg50/PT/COS membranes were slightly lower at 11.9, 12.3 mm, and 15.2 mm, respectively. In the quantitative results, Fig. 3B and Table 3 show that the aforementioned membranes have comparable reduction percentages against *P. aeruginosa* (100%) and *E. coli* (99%) with the exception of *S. aureus*, which was about 99.4% for PCLAg500/PT/COS and 99.3% for PCLAg50/PT/COS (the difference was not statistically significant). Therefore, the antibacterial efficiency of the tri-layer membrane at the wound site increased when combined with COS, and the silver nanoparticles seem to be a subsequent antibacterial factor to maintain an aseptic environment around to wound, supporting the healing process. In conclusion, the synergistic effect of COS and silver nanoparticles in the PCLAg/PT/COS membranes not only reduces the wound

infection but also disinfects the surrounding environment to promote healing.

3.3. Certification of the antibacterial activities of the tri-layer membranes

PCLAg50/PT/COS and PCLAg500/PT/COS at the Ministry of Health Institute of Public Health in Ho Chi Minh city. To objectively guarantee high accuracy of the antibacterial properties of the tri-layer PCLAg/PT/COS membranes, all membranes were examined through a qualitative test (the AATCC147 Standard) and a quantitative test (the AATCC100 Standard) by the Ministry of Health Institute of Public Health in Ho Chi Minh city. According to the AATCC145 results, both PCLAg500/PT/COS and PCLAg50/PT/COS samples had relatively similar inhibition zones against *P. aeruginosa* (7.6 and 6.7 mm), *E. coli* (3.0 and 3.1 mm), and *S. aureus* (11.1 and 11.6 mm), respectively (Fig. 4 and Table 4). According to the AATCC100 results, both membranes attained reduction percentages of 100% (Table 4). In conclusion, the investigations conducted by the Ministry of Health Institute of Public Health in Ho Chi Minh city demonstrated that PCLAg50/PT/COS and PCLAg500/PT/COS had excellent antibacterial efficacy, agreeing with the aforementioned results.

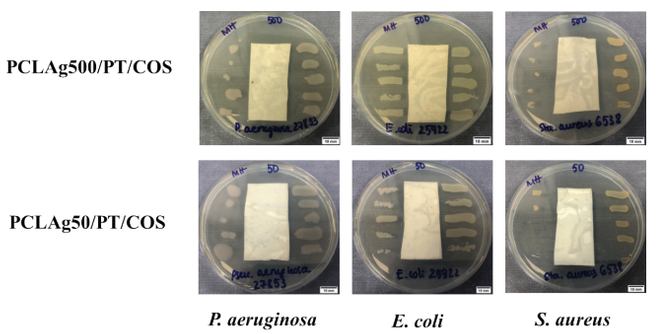

Fig. 4. The inhibition zone images of PCLAg500/PT/COS and PCLAg50/PT/COS membranes against *P. aeruginosa*, *E. coli*, and *S. aureus* implemented by the Ministry of Health Institute of Public Health in Ho Chi Minh city.

Table 4. The inhibition diameters and reduction percentages of the PCLAg50/PT/COS and PCLAg500/PT/COS membranes examined at the Ministry of Health Institute of Public Health in Ho Chi Minh city against *P. aeruginosa*, *E. coli*, and *S. aureus*.

Sample	Inhibition zone diameter (mm)			Percentage reduction (%)		
	P. aeruginosa (-)	*E. coli* (-)	*S. aureus* (+)	*P. aeruginosa* (-)	*E. coli* (-)	*S. aureus* (+)
PCLAg500/PT/COS	7.6	3.0	11.1	100	100	100
PCLAg50/PT/COS	6.7	3.1	11.6	100	100	100

3.4. Discussion

The aim of this study is to gain a comprehensive understanding of the antibacterial properties of tri-layer membranes to apply the concept of antibacterial dressings for the prevention and treatment of infections. The monolayer PCLAg membranes and the tri-layer PCLAg/PT/COS membranes, consisting of two antibacterial agents of AgNPs in the outer layers and COS in the inner layer, were evaluated for their antibacterial effects using two qualitative and quantitative tests. Simultaneously, the completed tri-layer membranes were objectively reassessed for reliability and transparency at a second prestigious institute prior to conducting pilot-scale production process experiments.

With this aim, all fabrication parameters of the PCLAg/PT/COS membranes incorporating the two antibacterial agents - AgNPs and COS - were determined by consulting and selecting appropriate recent research results [19]. Regarding AgNPs, a silver concentration of 500 ppm in PCL (12% w/v) solution was gamma-irradiated at 15 kGy and analysed by UV-vis spectrometry, displaying an absorption peak of 416 nm indicating the formation of AgNPs [17]. Subsequently, the PCL electrospun membrane was produced to encapsulate AgNPs while preserving their structure from rapid silver ion release. PCL, known for possessing advanced qualities such as biocompatibility, high mechanical and physical strength, and hydrophobicity, was used to fabricate the electrospun membrane as the waterproof covering layer. The shape and size ranged from 15-20 nm, with a distribution of around 0.06%, and notably exhibited a high biological effect (*in vitro*), including bacterial inhibition and biocompatibility. Their antibacterial mechanisms have been reported in numerous studies. However, concerns regarding toxicity due to overdosing and metal ions remain. For this reason, a thorough understanding of their antibacterial characteristics in relation to silver concentration is necessary to minimise potential hazards. In this research, silver nanoparticle concentration in the PCL electro-spun membranes were examined at 500 and 50 ppm to broaden applications. Three bacterial strains - *P. aeruginosa* (gram-negative), *E. coli* (gram-negative), and *S. aureus* (gram-positive) - were selected due to their potential to exacerbate wound infections and the persistent treatment burden posed by their antibiotic resistance. In Fig. 1, SEM results showed the formation of electrospun PCL 12 %w/v,

PCLAg500, and PCLAg50 membranes, which had fibre and porous diameters ranging from 1.05-1.22 µm and 11.51-12.89 µm, respectively. These fibre and porous structures allowed these membranes to effectively distribute and capture AgNPs, enhancing their antibacterial capabilities. In Fig. 2 and Table 2, the PCLAg500 effectively demonstrated its antibacterial properties against *P. aeruginosa, E. coli,* and *S. aureus,* with inhibition diameters of 15.5, 11.4, and 13.6 mm, respectively, and reduction percentages of 94.6, 96.8, and 97%, respectively. In contrast, the PCLAg50 membrane, with a silver concentration ten times lower at 50 ppm compared to PCLAg500, showed reduction rates of 67.5, 86.5, and 82.1% against *P. aeruginosa*, *E. coli*, and *S. aureus*, respectively, despite the absence of inhibition zones. Owing to their superior characteristics, the PCLAg layer should be utilised as a covering layer to prevent pathogen spread and offensive odours.

For the construction of the absorption layer as the middle, the PCLAg membrane was treated with air plasma to transform it into a hydrophilic agent [19, 21]. The effectiveness of this air plasma treatment and its mechanism have been investigated in numerous previous studies [22]. In this research, Figs. 1B and 1C demonstrate that the fibre (approximately 2.17 and 2.18 µm) and porous (around 16.84 and 17.68 µm) structures of the PCLAg/PT membrane treated with air plasma were slightly higher than those of PCLAg. In fact, air plasma interacts with the surface of the polymer membrane, changing its surface structures [18]. Simultaneously, it has also been reported that air plasma triggers the formation of new polar groups such as hydroxyl (-OH) and carbonyl (-C=O), attracting water molecules [23]. Therefore, the air plasma treatment of the PCLAg layers produced the middle layers, which underwent structural changes accompanied by the modification of hydrophilic properties. These characteristics facilitate coating of hydrophilic solutions to form the inner layer.

For the fabrication of the third layer, bioactive substances are often incorporated and directly applied to the wound site to promote healing. In the case of an infected wound, several inevitable issues, such as pathogens, excessive exudate, and physical soft tissue injury, impede the healing process. Hence, a membrane coated with COS-PVP solution has proven biological advantages to promote wound healing owing to its excellent antibacterial activity, hemostatic action, and biocompatibility. In detail, COS, a naturally derived bioproduct from shrimp, is known for its antibacterial properties and, especially, its outstanding biocompatibility in tissue engineering applications [24]. Although the exact mechanisms remain unclear, several theories suggest that protonated amine groups in COS interact with the bacterial membrane, leading to leakage of intracellular constituents or disrupted nutrient transportation. However, COS exhibits weak stability due to the low molecular weight, making it difficult to achieve maximum antibacterial performance.

To enhance the benefits of COS, PVP is combined with COS in the coated layer as a delivery agent [19], which supports exudate absorption, moisture retention, and COS delivery to the wound bed. This means that the dressing could absorb exudates and release the maximum amount of COS with the assistance of PVP, increasing its antibacterial performance and healing progress. Additionally, the coating the COS/PVP layer onto the other two layers during use facilitates painless removal. In previous studies, the electrospun membrane coated with 6 ml COS (3% w/v) and PVP (6% w/v) solution was demonstrated to inhibit bacterial growth (*in vitro*) and promote wound healing (*in vivo*). In this work, the formation of a coated layer was evidenced by a significant decrease in porous diameters to 9.45 (PCLAg500/PT/COS) and 7.76 µm (PCLAg50/PT/COS) (Fig. 1). In Fig. 3 and Table 3, although the inhibition zones of PCLAg500/PT/COS membrane against *P. aeruginosa*, *E. coli*, and *S. aureus* were 13.7, 13.2, and 22.4, wider than those of the PCLAg50/PT/COS at 11.9, 12.3, and 15.2 mm, the bacterial reduction results of both membranes were comparable around 100, 99, and 99.4%, respectively.

Finally, the antibacterial tests reassessed at the Ministry of Health Institute of Public Health in Ho Chi Minh city showed that the PCLAg500/PT/COS and PCLAg50/PT/COS membranes achieved broad inhibition zones of approximately 7.6, 6.7, 3.0 and 3.1 11.1, and 11.6 mm, respectively, against *P. aeruginosa*, *E. coli*, and *S. aureus*, respectively, will all exhibit reduction percentages of 100% (Fig. 4 and Table 4). In fact, both tri-layer membranes obtained optimal antibacterial performance despite the silver concentration being 50 ppm or 500 ppm, comparable to the results above. While a silver concentration of 500 ppm captures attention for its high antibacterial performance in sterilising dressing, a silver concentration of 50 ppm still

achieves an acceptable value for infection prevention. In this experiment, silver played a key role in preventing infection by maintaining a sterile environment around the wound by preventing pathogens from entering the wound and helping disinfect the wound fluid. Therefore, a silver concentration of 50 or 500 ppm should be selected by considering the degree of safety and infection.

4. Conclusions

This study reported the antibacterial capabilities of tri-layer membranes containing two antibacterial agents, AgNPs and COS, designated as PCLAg50/PT/COS and PCLAg500/PT/COS, to exploit their synergistic impact for the development of ideal antibacterial wound dressings. The main difference between these two membranes is the concentration of silver nanoparticles in the PCLAg layers of which 50 and 500 ppm were compared to investigate its toxicity due to overdose. The fibre and porous structures of all three layers of the membranes were examined by SEM. The antibacterial performance of both tri-layer membranes reached almost 100%. Meanwhile, the reduction percentages of PCLAg500 were high, approximately 94.6% (*P. aeruginosa*), 96.8% (*E. coli*), and 97% (*S. aureus*). The PCLAg50 membrane values were slightly lower at 67.5, 80.6, and 82.1%, respectively. Although the silver concentration of the outside layer was reduced 10 times to 50 ppm, its reduction percentage was only reduced by 15%. Therefore, applying silver concentrations of 50 or 500 ppm to the outer layer to maintain a sterile wound environment should consider safety and the degree of infection. Finally, applying two antibacterial agents to wound dressings could provide comprehensive antibacterial efficacy to facilitate wound healing.

CRediT author statement

Thi Thanh Ngoc Nguyen: Investigation, Sample analysis, Data processing, Methodology, Writing the manuscript; Thi Thanh Tam Phan: Writing the editing, Methodology. Thi Hiep Nguyen: Supervise and comment on editing the manuscript for completeness.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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