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Research article

Preparation and Characterization of Porous Natural Rubber Loaded with Silver Nanoparticles

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pectin;
carrageenan

Abstract

In this study, porous natural rubber (NR) films loaded with silver nanoparticles (AgNPs) were prepared by the solvent casting method. The porous structure of the NR films was formed with hydrophilic substances such as glycerol, pectin or carrageenan. The film properties including morphology, mechanical test, hydrophilicity evaluation, water absorption, release study, and antibacterial activity were determined. From the results, the morphology of films was a continuous porous structure on the surface of films. The pectin and carrageenan enhanced porosity, hydrophilicity, and water sorption of the porous NR films. The developed porous NR films could sustain the release of Ag^+ ions for 2 days and showed inhibition zones against *Staphylococcus aureus* and *Escherichia coli.* Therefore, these porous NR films have the potential to be used as chronic wound dressing.

1. Introduction

A wound is a destruction in the continuousness of the epithelial layer of the skin caused by physical or thermal damage. Wounds are classified as acute and chronic wounds depending on the healing process. An acute wound is an area of skin damage that occurs immediately from an accident or surgery and heals within 8-12 weeks [1]. However, a prolonged wound healing process (more than 12 weeks) may result in the formation of a chronic wound. Such wounds frequently become infected by pathogens [2, 3]. Consequently, key factors that delay the wound healing process in chronic wounds are also caused by bacterial infection [4, 5]. Bacteria can form biofilms on wound beds. A biofilm is typically a long-term infection that progresses slowly, appears to be incompletely resolved by immune defenses, and responds only temporarily to antimicrobial therapy [6]. Patients with wounds need frequent dressing changes, and this generally involves a number of hospital admissions and physical limitations [4]. Therefore, wound dressing is the first choice for healing a wound.

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Wound dressing is a material used to promote the wound healing process and protect a wound from the environment. It should remove excess wound exudate, maintain high humidity, permit gas transmission, reduce wound surface necrosis, protect the wound from bacteria, and stimulate the growth factors [7]. The type of wound dressing selected depends on the wound type, wound depth, exudate levels, and wound bed characteristics. Nowadays, the most commonly used wound dressings in clinical practice are hydrogels, hydrocolloids, foams, and films [8-11]. Film dressing is the wound-dressing product of first choice because of its extreme flexibility, transparency, and adhesiveness. Film dressing permits the wound to breathe at a suitable level through the process of moisture vapor transpiration. Furthermore, film dressing can be fabricated into types of porous film. The advantages of porous film include its ability to absorb wound exudate and transmit gas or moisture, which are properties that promote the wound healing process [8].

Natural rubber (NR) is a natural polymer obtained from the latex sap of trees (*Hevea brasiliensis*), and is composed of cis-1,4-polyisoprene. NR has been used in biomedical applications such as membranes, urinary or tracheal tubes, and drug delivery [10, 12, 13]. Phaechamud *et al*. [8] fabricated gentamicin sulfate-loaded porous NR films for wound dressing using a solvent casting technique. The films showed sustained release for 7 days and inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa* [8]. Moreover, Phaechamud *et al*. [14] improved the wettability properties of the films by combining the natural rubber with xanthan gum as the hydrophilic substance for wound dressing application. The results showed that adding xanthan gum to NR film exhibited higher wettability than the plain NR film [14]. Therefore, the NR could be used for the treatment of chronic wounds. Also, the hydrophilicity of NR films was improved by the addition hydrophilic substances such as alginate, gelatin, chitosan, carrageenan and pectin.

Carrageenan is a water-soluble polymer consisting of a linear chain of D-galactan that obtained from red algae [15]. It is a type of polysaccharide consisting of repeating galactose units and 3,6-anhydrogalactose joined by alternating α-(1,3) and β-(1,4) glycosidic links [16]. It has potential for use in drug delivery systems and biomedical applications due to its biocompatibility, high viscosity, controlled release properties, and gelling capacity [17]. Moreover, it has been used as a hydrophilic material for the improvement of hydrophobic membranes [18]. Pectin is another hydrophilic polymer. It is found in the major cell walls of plants and is a structural hetero-polysaccharide. The incorporation of pectin caused films to be compostable and wettable. The hydrophilic functional groups of pectin give it the potential for use in biomedical and drug delivery systems [19].

Silver nanoparticles (AgNPs) are commonly used as antibacterial agents. Due to their cost effectiveness, safety, lower toxicity, biocompatibility, and powerful antibacterial activities, they are increasingly used in various applications such as health care, medical, food, and industrial purposes. The biological activity of AgNPs relies on various factors such as size, size distribution, shape, and surface chemistry. Gunasekaran *et al*. [20] reported on the high performance of AgNPs on cotton fabrics for wound dressings, which could kill bacteria and speed the wound healing process. Rheima *et al*. [21] reported that the AgNPs obtained by a UV-irradiation method were almost spherical, and had high potential to inhibit *Staphylococcus aureus* and *Escherichia coli*. Nešović *et al*. [22] reported that AgNPs, as potent antimicrobial agents, had drug release behavior that was particularly suited to wound dressing applications, and provided a higher inhibition of bacterial infection.

In this study, the porous NR films loaded with AgNPs were prepared by a solvent casting method. Film properties including morphology, mechanical test, hydrophilicity evaluation, water absorption, release study, and antibacterial activity were determined.

2. Materials and Methods

2.1 Materials

Natural rubber was purchased from Mastex Co, Ltd., Nakornpathom, Thailand. Dichloromethane (DCM) and silver nitrate were purchased from RCL Labscan Limited, Thailand. Glycerol (grade AR, 56-81-5), pectin from citrus peel (galacturonic acid ≥74% dried basis), and I-carrageenan (commercial grade, type II, predominantly iota carrageenan) were bought from Sigma-Aldrich, USA.

2.2 Preparation of porous natural rubber films loaded with silver nanoparticles

The porous NR films were prepared by the solvent casting method. The components of porous NR films loaded with AgNPs are shown in Table 1. First, NR solution was dehydrated in a hot air oven at 80°C until constant weight was achieved. Next, 1.5 g dehydrated NR was cut and then dissolved in 70 g of dichloromethane under magnetic stirrer until a homogeneous solution of NR was obtained. Next, 1 mL of polymer solution (pectin or carrageenan), 0.02 g of silver nitrate, 0.5 g of glycerol, and 0.5 g of Tween20 were added to the NR solution under stirring at room temperature for 1 h. Afterwards, Ag^+ ions in the NR solution were reduced to AgNPs using UV irradiation technique at 254 nm for 1 h [23]. Then, the mixture was poured into a petri dish, and dichloromethane was allowed to evaporate in a laminar hood overnight at room temperature. The mixture was then dried in a vacuum oven at 50°C overnight. Finally, the porous NR films loaded with AgNPs were obtained and stored in a dry condition until use.

Table 1. The component of porous NR films loaded with AgNPs

Code	Pectin (mg)	Carrageenan (mg)	Silver nitrate (mg)	Tween20 (mg)
NR1	-	-	-	-
NR2	-	-	20.0	-
PC1	12.5	-	20.0	500.0
PC2	25.0	-	20.0	500.0
PC3	50.0	-	20.0	500.0
CR1	-	12.5	20.0	500.0
CR2	-	25.0	20.0	500.0
CR3	-	50.0	20.0	500.0

2.3 Morphology study

The surface morphologies of the porous NR films loaded with AgNPs were studied with an optical microscope (OM). Digital optical microscopy was conducted using a Motic BA310 Met instrument with an optical objective of 10X, model LM Plan 10x/0.3 ∞/0 WD17.5. The presence of AgNPs in the NR solution was observed with a transmission electron microscope (TEM, FEI, Model: TECNAI G2 20). One drop of the NR solution containing AgNPs was placed on a grid and dried at room temperature.

2.4 Mechanical test

The determination of mechanical properties was done with a modified method from Phaechamud *et al*. [8]. Tensile strength, Young's modulus, and percentage of elongation at break of the porous NR

films loaded with AgNPs were investigated with an Instron Machine Model 5566 Universal Testing Machine. Before the test, the porous NR films loaded with AgNPs were cut into a rectangular shape with the size of 4×1 cm^2. All samples were pulled at 240 mm/s until breakage.

2.5 Hydrophilicity evaluation

The hydrophilicity of the porous NR films loaded with AgNPs was measured with a goniometer following Phaechamud *et al*. [8] with some modifications. Water was dropped from a syringe at the rate of 2.5757 µL/s. The recording of the contact angle was obtained when the distilled water contacted the films at 3 s.

2.6 Water absorption

The porous NR films (1×1 cm^2 strips) were weighed (W_1) and then soaked in phosphate buffer solution (PBS), pH 7.4 for 2 and 24 h. After that, the wet films were removed from PBS. The excess medium around the wet films was wiped off before weighing (W_2). The wet films were washed with distilled water twice, and then dried in a hot air oven at 50°C for 24 h. The percentage of water absorption of each sample was calculated using the equation (1).

$$\%\text{Water absorption} = \frac{(W_2 - W_1)}{W_1} \times 100 \quad (1)$$

2.7 Release study

The release study of the porous NR films loaded with AgNPs was investigated by the total immersion method in PBS (pH 7.4). The porous NR films loaded with AgNPs were performed in a shaker bath (37°C) at different time points (i.e. 10, 20, 40, 60, 90, 120, 180, 300, 480, 600, 1440, and 2880 min). At the specified time point, 3.0 mL of the released medium was kept and fresh PBS was added at equal volume of the sampling solution. Finally, the amount of Ag+ ions released from each sample solution was measured using atomic absorption spectrometry (AAS). The obtained data were used to calculate the cumulative released amount of Ag^+ ions.

2.8 Antibacterial activity

The antibacterial activity of the porous NR films loaded with AgNPs was determined according to the agar diffusion method following the method of Hudzicki with some modifications [24]. *Staphylococcus aureus* and *Escherichia coli* were used to evaluate antibacterial activity. Firstly, the bacteria were inoculated in the test tubes, and the test tubes containing bacteria were shaken in a shaker at 37°C for 24 h. Cultures of these strains containing approximately 10^5 CFU/mL were prepared and used to evaluate antibacterial activity. Inoculum containing 10^5 CFU/mL of each bacterial strain was swabbed on nutrient agar. Subsequently, the porous NR films loaded with AgNPs were placed on inoculum agar plates and incubated at 37°C for 24 h. The diameters of the inhibition zones were measured with a digital Vernier caliper.

2.9 Statistical analysis

The data are shown as means and standard errors of means. In SPSS, one-way analysis of variance and Tukey's post hoc test were used for statistical analysis (IBM SPSS, USA). The statistical significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1 Morphology study

The surface morphology of the porous NR films with 10X magnification is displayed in Figure 1. The pictures show the porous structure of the NR films. The addition of pectin and carrageenan into the NR structure produced roughness and porous surfaces on the surface of the films. Increasing the pectin and carrageenan concentration caused more roughness and porous structure. NR is a hydrophobic substance, while carrageenan and pectin are hydrophilic substances. Thus, the incorporation of hydrophilic substance into the NR phase resulted in the porous structure of the film. A higher content of pectin in the NR phase caused the higher incorporation phases resulting in larger porous structures of the NR film. Carrageenan has a sulfur group in its structure that may crosslink the NR leading to smaller porous structures of the NR films. Figure 2 shows selected TEM micrographs of AgNPs in the NR. The shape of the AgNPs was almost spherical shape. This result confirmed that Ag+ ions were reduced to AgNPs via UV-irradiation technique. In addition, protein in the NR solution could stabilize AgNPs in the NR solution [25].

3.2 Mechanical properties

The mechanical properties of the porous NR films are shown in Figure 3. The NR1 film showed the highest tensile strength and Young's modulus values. The porous NR films containing pectin and carrageenan had a continuous porous structure (see Figure 1) that caused lower deformation resistance, observed as a decrease in tensile strength and Young's modulus values. The PC1 film had the highest tensile strength value when compared with other films (PC2, PC3) since it had a low amount of porous surface structure. Thus, it could resist a higher load, resulting in the highest tensile strength. Moreover, the addition of higher amounts of pectin and carrageenan increased the porosity structure of the surface of the films, resulting in the decrease of the tensile strength value of the films. These mechanical behaviors were also reported for the porous NR films induced by the addition of glycerol and triethyl citrate [14]. However, the addition of polymer increased the elongation at break except the CR3 when compared with the NR1 and NR2 films. The Young's modulus of the porous NR films became low with addition of AgNPs. The addition of pectin affected Young's modulus values except in the case of PC3. The addition of carrageenan increased the Young's modulus values of the porous NR films. This might be due to crosslinking between the carrageenan and NR caused by the sulfur in carrageenan structure.

3.3 Hydrophilicity evaluation

The low contact angle values displayed the high hydrophilic properties of the NR film. The wettability of the NR2 film was lower than that of the NR1 film due to the incorporated AgNPs. The AgNPs filled the free spaces of the NR structure. Thus, the water could not permeate into the NR structure, leading to lower the wettability of the NR2 film. However, the wettability of the PC and CR films was higher than that of the NR films (Figure 4). The addition of hydrophilic substances such as pectin and carrageenan into the NR films resulted in the high wettability of the NR films. This result was similar to the result of a previous report in which the NR films were modified by adding xanthan gum and triethyl citrate [8]. The higher wettability of films showed the better water absorption and release characteristics. The higher wettability films could enhance the release of drug from the films. As reported previously, the addition of hydrophilic substances increased the release of nicotine from the NR transdermal patches [12].

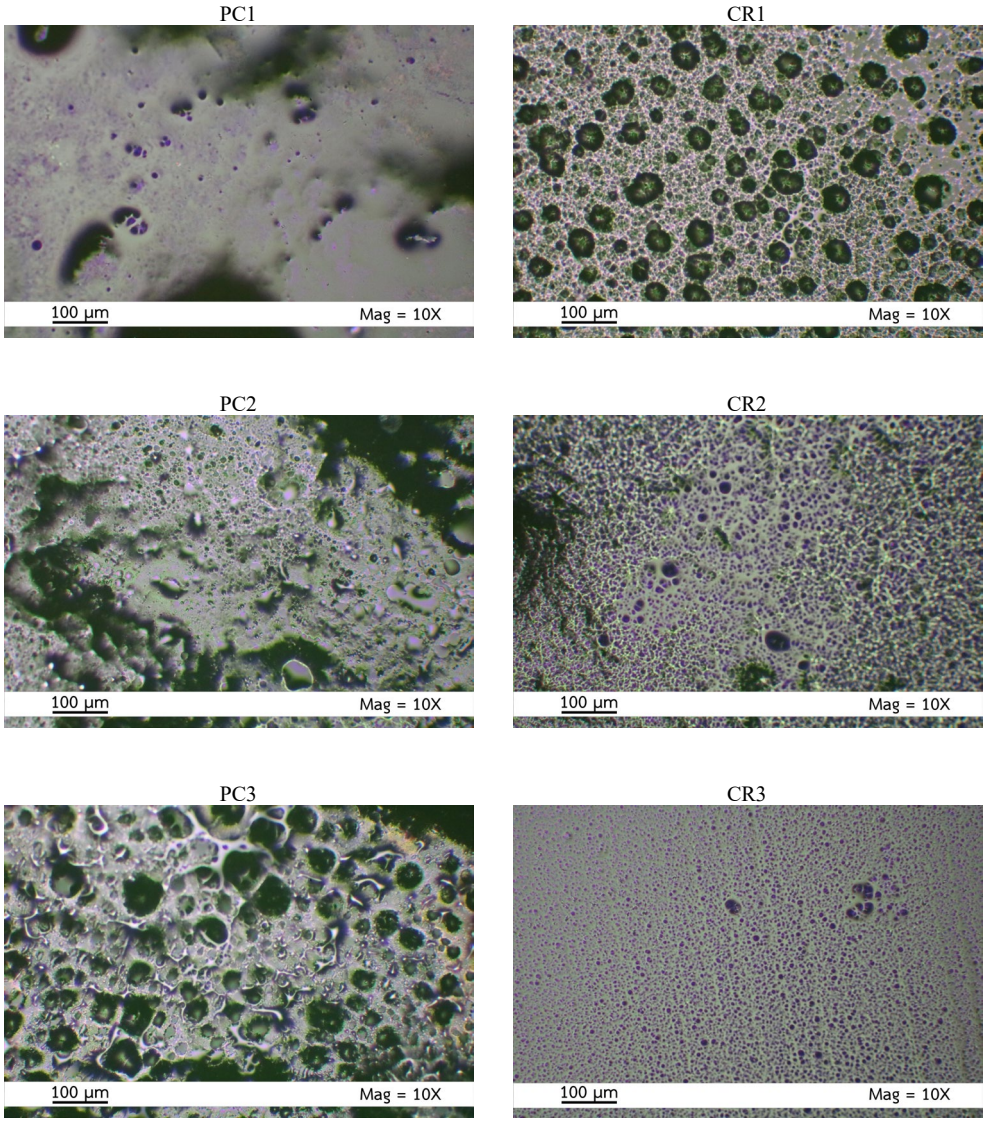

Figure 1. Optical microscope images of the porous NR films

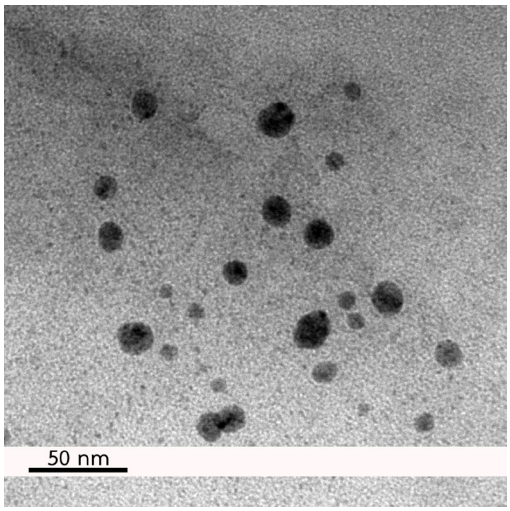

Figure 2. TEM image of AgNPs in NR solution after UV exposure

3.4 Water absorption

The percentages of water absorption are illustrated in Figure 5. The PC and CR films showed higher water absorption than the NR films, due to higher hydrophilicity of the PC and CR films. Pectin and carrageenan could absorb water into their structures leading to higher water absorption [8]. The hydrophilicity had an effect on water absorption in the first 2 h. However, there were no apparent differences in water absorption of the NR films after 24 h.

3.5 Release profile

The total immersion method was performed to study the release of Ag^+ ions from the porous NR films loaded with AgNPs. The samples were immersed in the PBS medium at 37°C for 2880 min. In Figure 6, the cumulative released amounts of Ag+ ions revealed that all samples were highly released within the first immersion time, which increased further after 600 min, and reached a plateau value at 1440 min. The highly released amounts of Ag+ ions from the samples were due to AgNPs on surface of the films. Then, as the films swelled with increasing submersion time, there was a gradual release of Ag+ ions from the films. The maximum cumulative released amount of Ag^+ ions from the NR2 films was 3.39±0.62%. The maximum cumulative released amounts of Ag^+ ions from the CR1, CR2, CR3 films were 3.89±0.23%, 3.69±0.30%, and 2.09±0.10%, respectively. The maximum cumulative released amounts of Ag^+ ions from the PC1, PC2, PC3 films were 1.63±0.17%, 3.10±0.43%, and 3.33±0.66%, respectively.

3.6 Antibacterial activity

The agar diffusion method is a qualitative method used to measure the ability of materials to inhibit bacterial growth. Bacteria are the cause of the inflammation and infection in the wound site [26], thus the ability of wound dressing to inhibit the bacterial growth was a significant objective of this study. Sterilized paper discs containing amoxicillin and the NR1 films represented positive and negative controls, respectively. From Figure 7, the porous NR films loaded with AgNPs showed inhibition zones against *S. aureus* and *E. coli.* The addition of pectin and carrageenan increased the

inhibition zones of both *S. aureus* and *E. coli* since the hydrophilicity and water absorption properties promote the release of the Ag^{+}. The inhibition zone against *E. coli* was higher than that against *S. aureus* due to the different cell wall structures of bacteria. *S. aureus* are gram-positive bacteria and *E. coli* are gram-negative bacteria. Gram-positive bacteria have thicker cell wall structure. This was probably the reason why the inhibition zone of the NR films loaded with AgNPs against *E. coli* might be higher.

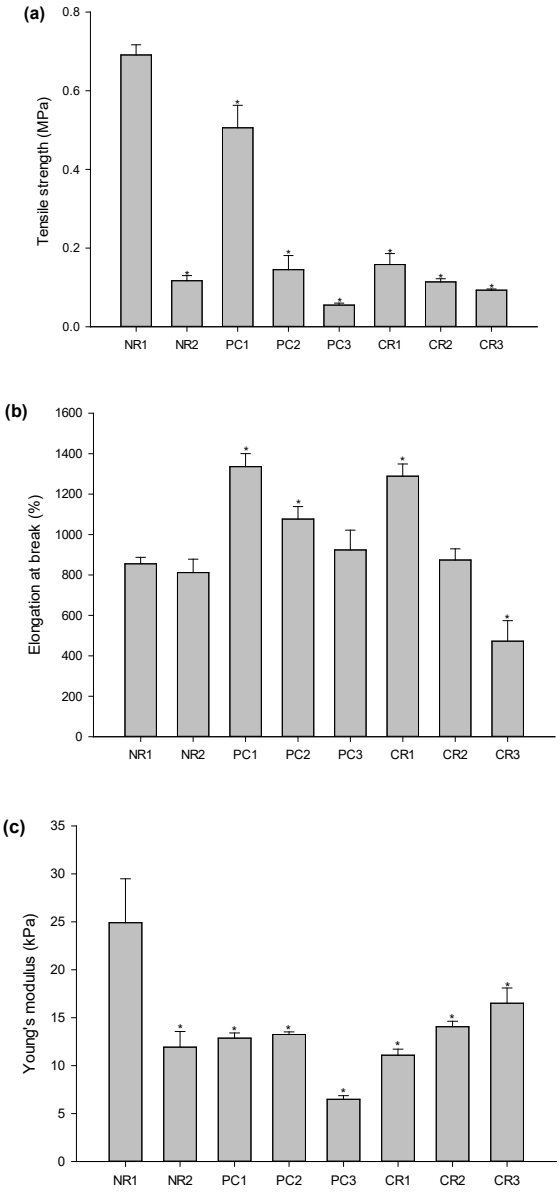

Figure 3. (a) Tensile strength (b) elongation at break and (c) Young's modulus of the porous NR films (n=3). *p < 0.05 compared with NR1 film

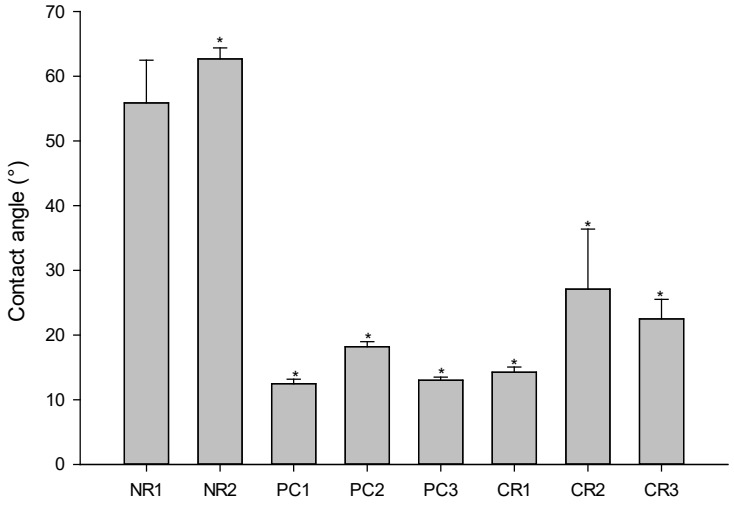

Figure 4. Wettability of the porous NR films (n=3). *p < 0.05 compared with NR1 film

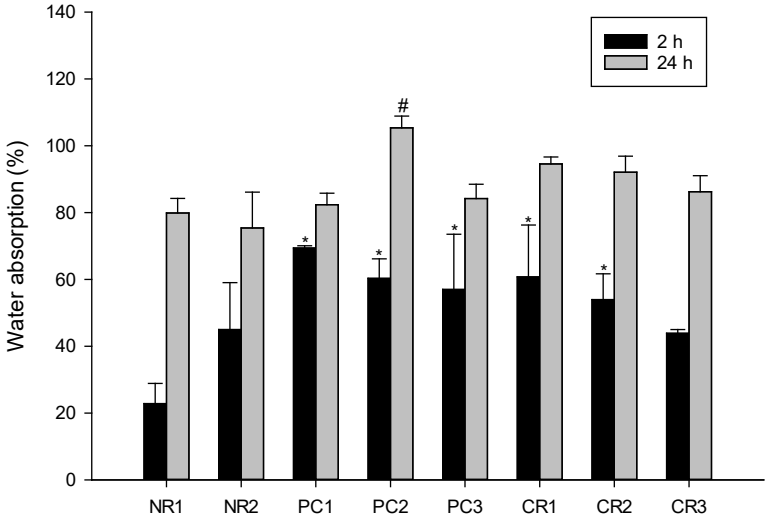

Figure 5. Water absorption of the porous NR films (n=3). *p < 0.05 compared with NR1 film at 2 h and #p < 0.05 compared with NR1 film at 24 h

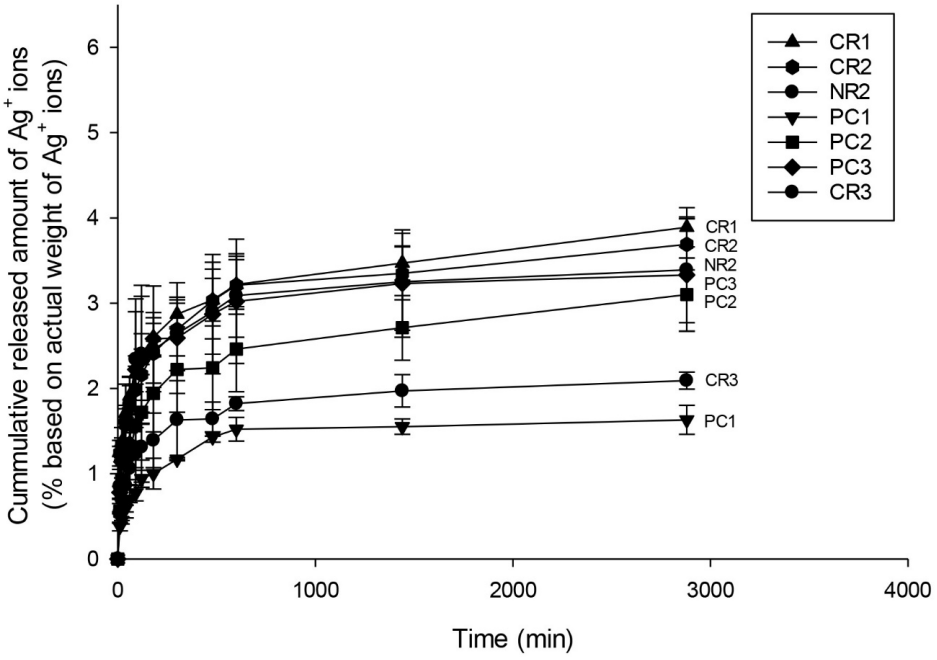

Figure 6. Cumulative released amount of Ag^+ ions from the porous NR films for 2880 min

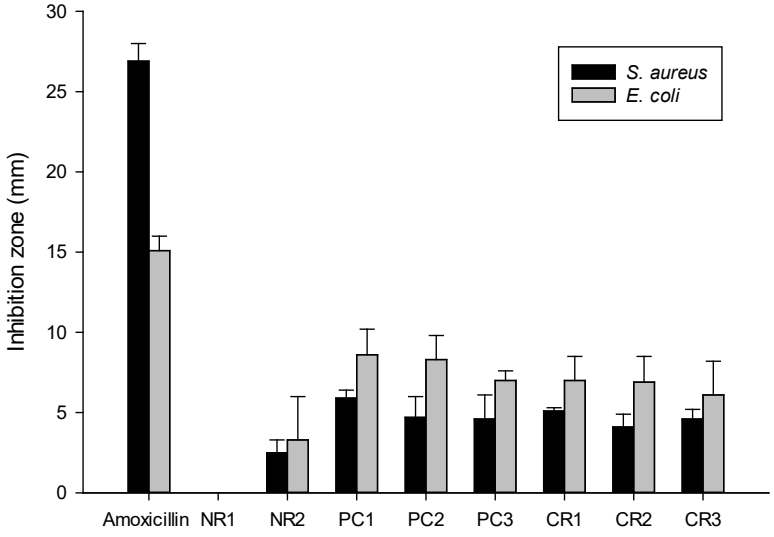

Figure 7. Antibacterial activity of the porous NR films

4. Conclusions

The porous NR films were prepared by solvent casting method. The addition of pectin and carrageenan promoted the porous structure of the NR film loaded with AgNPs. These films with added pectin and carrageenan demonstrated good mechanical properties with good elasticity. The pectin and carrageenan increased the wettability and water absorption of the porous NR films. In addition, the porous NR films loaded with AgNPs showed inhibition zonse against *S. aureus* and *E. coli* with sustained release of Ag^{+} ions. Therefore, these porous NR films can potentially be used as wound dressing materials.

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