WALAILAK JOURNAL
http://wjst.wu.ac.th Engineering and Physical Sciences

Ammonia-Acetylene PECVD Coating on Wound Dressing to Control Delivery of Silver Sulfadiazine

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Received: 1 May 2016, Revised: 27 December 2016, Accepted: 23 January 2017

Abstract

A novel wound dressing surface modification was developed by applying plasma-enhanced chemical vapor deposition (PECVD). The cotton wound gauze dressings were treated by the mixed composition of 10 %NH_3+He and 10 %NH_3+C_2H_2 plasmas followed by immersion in silver sulfadiazine (AgSD) solution. A high functionality and a high surface area can thus be obtained by plasma deposition of C_2H_2 + NH_3. We reported the effect of a strong interaction between AgSD and cotton gauze yarn by observing the reactive amorphous carbon layer. It had an ability to slow down the release rate of AgSD compounds into the solution, reported as the UV-visible absorbance of the solution released from the plasma-treated gauze. The results showed that the release rate of AgSD molecules was lower, compared to the untreated gauze. Scanning electron microscopy (SEM) micrographs showed the interaction between AgSD and the cotton fibers of the plasma-treated gauze. In the untreated gauze, no such interaction was found. According to the energy dispersive X-ray spectroscopy (EDS) data, %weight of the Ag compound on the treated gauze was higher than the untreated gauze. A test on the antibacterial effect, using Fluid Thioglycollate Medium (FTM) as the culture media, clearly demonstrated that the plasma treated gauze which was immersed with AgSD, had ability to inhibit bacterial growth.

Keywords: Plasma polymerization, surface functionality, covalent grafting, silver sulfadiazine

Introduction

Open wounds and chronic ulcers need to be protected from environmental factors by covering the wounded area with wound dressings such as cotton gauze. The primary wound healing process has several conditions, including protecting the wound by preventing entry of harmful bacteria into the wound; maintaining the wound dryness by allowing evaporation of wound exudate and ensuring wound healing progresses. Traditionally, a tropical antimicrobial agent, silver sulfadiazine (AgSD) cream, has been used as an antibiotic for burning injuries in humans [1,2]. AgSD is directed to the wound area and covered by the dry gauze dressing. Some gauze dressing can provide certain bacterial protection, however, it can be lost when the outer surface of the dressing becomes moistened, either by wound exudates or external fluids [3]. The limitations of the traditional wound healing process are the direct delivery of AgSD agent to the wound site and the frequent dressing changes required. These can cause the accumulation of silver or silver sulfide (Ag_2S) particles in the human organs such as hair, skin, kidneys, liver and may delay wound healing [1]. A novel wound dressing was developed to target many aspects of

the complex wound healing process to ensure the impact of reducing the silver dosage and shortening the healing time, especially for chronic wounds and other difficult to heal wounds. The basic silver-based dressings can be broadly conceptualized in terms of the characteristics and silver delivery of the carrier dressing to the wound. In solution, AgSD is dissociated, and silver is released, as well as sulfadiazine ions. Campos *et al*. [4] reported that the chemical interaction between the silver ions and chitosan occurs as chitosan contains amino groups which are able to bind to silver and control silver release. Therefore, surface modifications are required on dressings to create a high functionality and a high surface area to optimize the interaction with the AgSD compounds. Plasma-enhanced chemical vapor deposition (PECVD) of acetylene mixed with ammonia (C_2H_2+NH_3) was used to create a carbon film. Amorphous carbon is currently attractive in biomedical applications due to its typical biocompatibility and chemical stability under non oxidizing conditions [5].

In this study, a novel wound dressing was developed by using PECVD of (1) an ammonia and acetylene mixture and (2) helium and ammonia mixture to modify the gauze surface. The aim is use plasmas to produce active sites on the dressing surface and introduce covalent bonds to AgSD. The novel wound dressing was developed in order to control the delivery rate of silver, avoid bacterial infection and keep the wound dry while covered and protecting the wound.

Materials and methods

Cotton gauze of 24 × 20 mesh and 3″ × 3″, 8-ply was provided by United Medical Instrument Co., Ltd. (Bangkok, Thailand). Silver sulfadiazine (AgSD), 98 % of $C_{10}H_9AgN_4O_2S$, was provided by Sigma-Aldrich Co., Ltd. (USA).

Cotton gauze samples were cleaned by ethanol, and then air-dried overnight in laminar flow cabinets before the PECVD treatment. Plasma depositions were produced by using an in-house-developed 13.56 MHz capacitively coupled plasma reactor [6]. The radiofrequency (RF) power at 13.56 MHz was coupled to the chamber via a manual impedance matching network, using 30 cm in diameter parallel stainless steel electrodes that were separated by a distance of 10 cm. **Figure 1** shows the onsite PECVD treatment of the sample. The PECVD parameters applied are shown in **Table 1**.

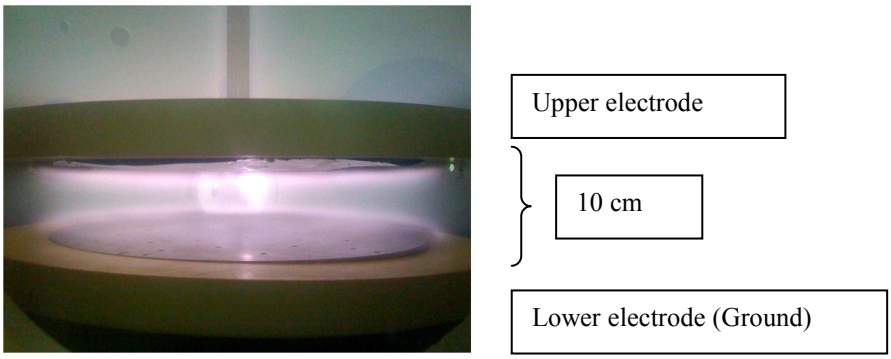


Figure 1 A photograph of the onsite PECVD reactor.

A typical base pressure of 8.0×10^{-3} Torr was achieved by using a rotary pump (18 $m^{-3}h^{-1}$). The carbon film was deposited on the gauze surface with a gas mixture of 10 % NH_3 and C_2H_2 and the nitrogen-containing functional groups were induced with gas mixture of 10 % NH_3 and He.

Table 1 PECVD treatment conditions.

Parameters	Conditions
Base pressure	About 2.0×10^{-2} Torr
RF, 13.56 MHz power	100 W
Temperature	Room temperature
Distance between top electrode and substrate	10 cm
Mixed gas (10 % NH_3 + C_2H_2)	1.0×10^{-1} Torr
Mixed gas (10 % NH_3 + He)	1.0×10^{-1} Torr
Treatment time	10 min

The manufacture method of immobilizing AgSD molecules is based on the formation of covalent bonds. The immobilizing methods can be divided into 2 main processes: (1) creating a reactive amorphous carbon (a-C) film and functionalization by addition of a reactive gas (NH_3 gas) to produce an activated group or active sites and (2) immobilization by specific adsorption which is mainly based on physical and chemical adsorption to create a stable bond between the silver compound and the activated cotton gauze surface, as shown in **Figure 2** [7]. The total pressure of the gas mixture was kept constant at 1.0×10^{-1} Torr. The RF power was kept at 100 watt and the deposition time was 10 min. After plasma treatment, the gauze was immersed in a 1.0 % w/v AgSD solution in deionized water for 5 min and air-dried overnight. The gauze was finally immersed in distilled water to wash out residual un-grafted molecules and then dried in air. The gauze dressing samples were kept in a desiccator for subsequent measurements, characterizations and tests.

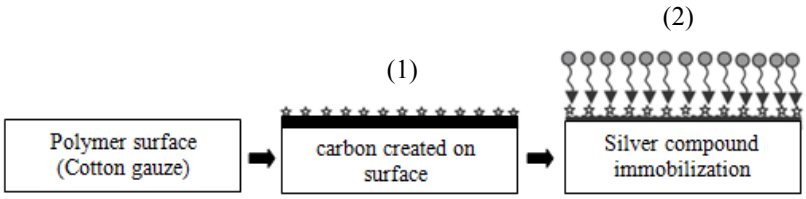


Figure 2 Concept of silver compounds immobilization onto modified surface using 2 main processes: (1) creating a reactive amorphous carbon films by using the mixed gas of NH_3+C_2H_2 and (2) immobilization of silver compounds.

Optical emission spectroscopy (OES) measurements were carried out to investigate the chemical species in the mixture of NH_3/He and NH_3/C_2H_2 plasmas. An optical fiber probe was settled at a position that contacts with the glass in the glow discharge zone of chamber. The fiber transferred the 200 - 800 nm light collected from the plasma, to a photomultiplier through a S2000 Miniature Fiber Optic Spectrometer (Ocean Optic, Inc.) and a monochromator.

Gauze samples with and without PECVD treatment were prepared and used to detect the release rate, using UV spectrophotometry. Each gauze sample was kept into the test tube and immersed in distilled water for 24 h. The 3 ml released-silver solution was collected and transferred into a 1 cm pathlength quartz cuvette for scanning at the range of 200 - 500 nm.

The water resistance of the gauze surface was measured by the sessile drop technique, using 20 µl de-ionized water droplet. The image of the water droplet was captured and exported for analysis with software to determine the contact angle. The angle size was related to the hydrophobicity of the surface. The greater angle indicated higher water resistance.

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to examine the surface morphology, as well as the surface chemical composition, of the gauze dressing. The SEM instrument used in this work was a JSM (Jeol, Japan).

Fourier transform infrared spectroscopy (FTIR) was used to extract the chemical bonding on the surface of the samples. The IR spectra were obtained by using a Thermo Scientific Nicolet 6700 FTIR spectrophotometer (Bruker, Germany), operated in the attenuated total reflectance (ATR crystal) mode. The spectra were collected and averaged from 64 scans at a resolution of 4 cm^{-1} from 400 - 4000 cm^{-1}.

Staphylococcus aureus ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used to evaluate the potential inhibition of the treated gauze in vitro. Furthermore, Difco™ Fluid Thioglycollate Medium (FTM) from Becton Dickinson (BD) was used for culturing the tested bacterial strains. It was used as a standard test medium recommended by the United States Pharmacopeia, National Formulary, National Institutes of Health, and Food and Drug Administration for the detection of anaerobic bacteria and aerobic bacteria [8-10]. For the aseptic technique, 25 µl of each bacterial strain (10^4 - 10^5 CFU/ml) were dropped on nutrient agar plate before placing gauze samples of plasma-treated dressing + AgSD and gauze dressing standard. The plates were left at room temperature (25 °C) for 4 h. Then the bacteria were transferred to FTM for observation of antimicrobial effectiveness of gauze dressing. For positive control, 25 µl of each bacterial strain were dropped into FTM and incubated at 35 - 37 °C for 5 days. The FTM without gauze sample and bacteria was used as a negative control. After incubation, the presence of turbidity in the medium was observed to detect the growth of the microorganisms.

Results and discussion

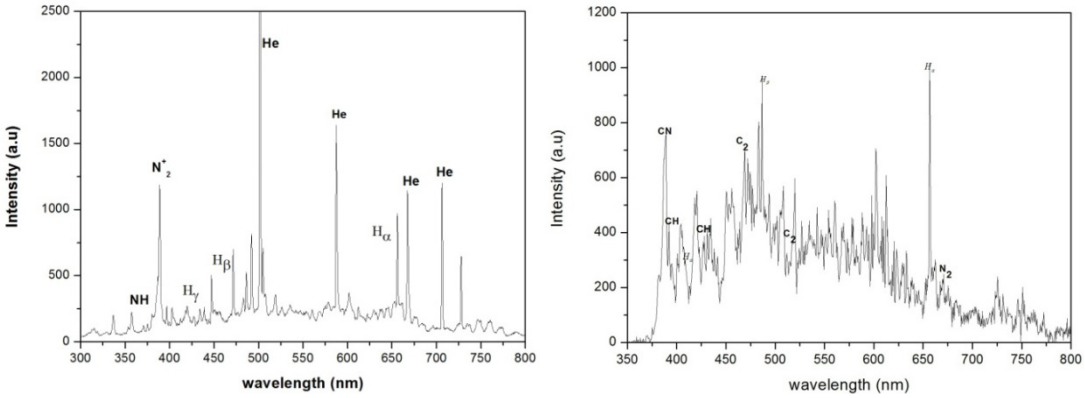
Optical emission spectroscopy (OES) of plasma species

OES measurements were carried out to investigate the chemical species in the gas mixtures of 10 % NH_3+He and 10 % NH_3+C_2H_2 plasmas. **Figure 3(a)** shows the chemical species, identified from the optical emission. The OES spectra of NH_3/He plasma arise from atomic and molecular species. It mainly includes NH at 336 nm, N_2^+ at 357.8 nm, H_γ at 434 nm, H_β at 486 nm, H_α at 656 nm and metastable He at 501, 587, 667 and 706 nm [11], respectively. In **Figure 3(b)**, the OES spectrum of NH_3/C_2H_2 plasma shows that the species are composed mainly of CN at 388.8 nm, CH at 390.5, 431 nm, H_α at 410, 656.5 nm, H_β at 486.2 nm, C_2 at 468.8, 516.5 nm, and N_2 at 672.5 nm [12,13]. It could be suggested that hydrogen radicals and carbon source radicals, such as C_2 and CH might be generated from the NH_3+C_2H_2 plasma.

Surface characterization

The results from the water contact angle test are shown in **Table 2**. The NH_3+C_2H_2 plasma-treated gauze surface shows high hydrophobicity. Therefore, the 20 µl water droplet with a contact angle of more than 110 degrees could stay on the upper gauze surface until it evaporated (as shown in **Figure 4**). The standard gauze dressing and the NH_3+He treated-gauze showed complete wetting as the liquid spreads on the surface and it was absorbed by the textile immediately. The water contact angle before and after immersing with AgSD decreased in the case of NH_3+C_2H_2 plasma treatment, while it increased in the case of NH_3+He plasma treatment due to the different plasma process. In the case of NH_3+He, the AgSD directly interacted with the hydrophilic NH_3+He treated gauze surface, while in case of NH_3+C_2H_2, the AgSD interacted with the gauze surface via the hydrophobic carbon film surface. This point also needs to be studied in detail and the other additional factors. The results indicated that the NH_3+C_2H_2 plasma-treated gauze had ability to keep the wound dry such that the wound exudates or external fluids evaporate too.

After washing to remove un-grafted AgSD particles, SEM micrographs of the control gauze, the AgSD untreated-gauze, the AgSD NH_3+He treated-gauze and the NH_3+C_2H_2 treated-gauze are shown in **Figures 5(a) - 5(d)**, respectively. The grafting/immobilizing of the AgSD particles can still be observed in the washed NH_3+C_2H_2 treated-gauze. **Figure 5(d)** showed that the AgSD particles were deposited locally on the knot of the carbon treated-gauze yarn, as indicated by the black arrows. In contrast, the yarn of the washed AgSD untreated-gauze and NH_3+He treated-gauze showed a smooth surface with no deposition of AgSD particles. It is evident that the binding of durable AgSD particles on the gauze yarn can be achieved via the carbon deposition.



(a) (b)

Figure 3 The OES spectrum of the mixture of (a) 10 % NH_3+He and (b) 10 % NH_3+C_2H_2 plasmas applied.

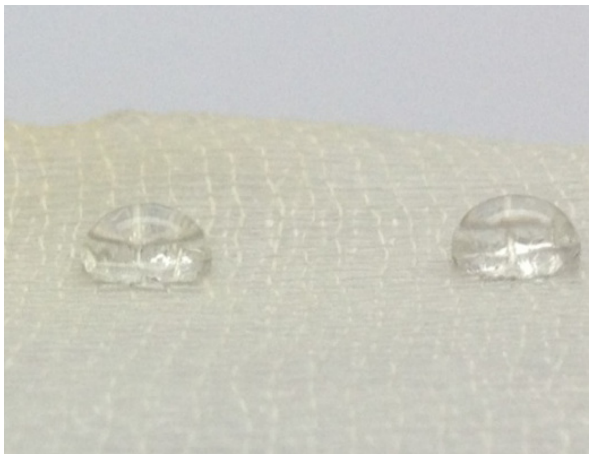
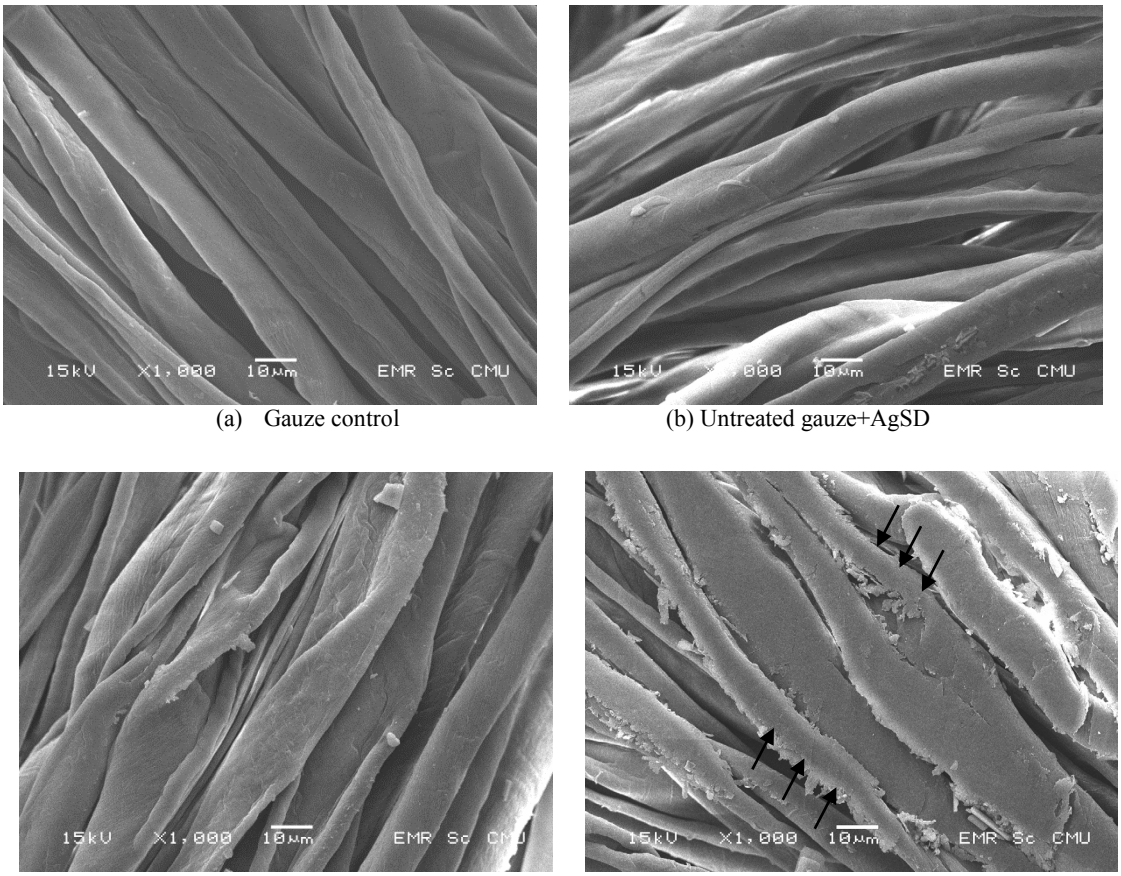


Figure 4 Photograph showing water droplets staying on the plasma-treated gauze surface after the washing process.

Table 2 Water contact angle of gauze control and treated gauze dressing after washing.

Gauze	Contact angle (degree) ±SD
1) Gauze control	0°
2) Untreated gauze+AgSD	0°
3) NH_3+C_2H_2 treated-gauze	110° ± 0.5
4) NH_3+C_2H_2 treated gauze+AgSD	103° ± 1.2
5) NH_3+He treated-gauze	0°
5) NH_3+He treated-gauze+AgSD	15°± 1.5



(a) Gauze control (b) Untreated gauze+AgSD

(c) NH_3+He treated+AgSD (d) NH_3+C_2H_2 treated+AgSD

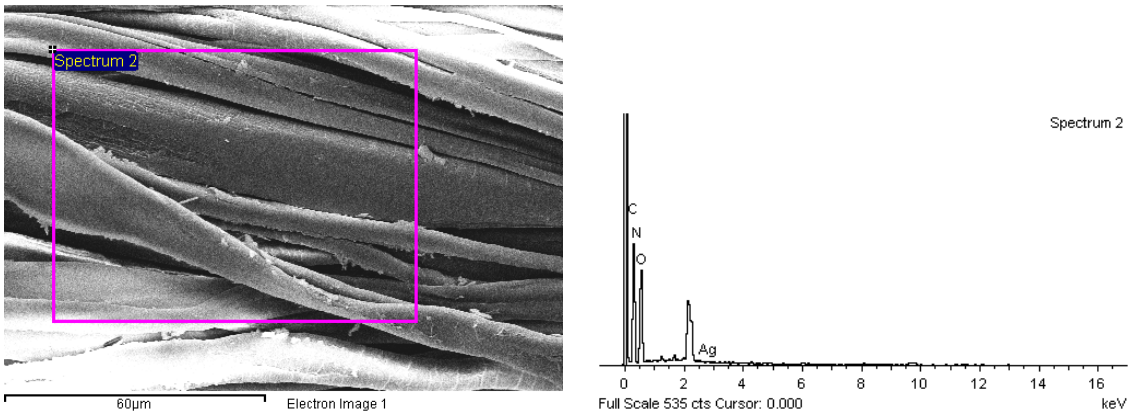
Figure 5 SEM images of gauze control (a), untreated gauze+AgSD (b), NH_3+He treated+AgSD (c) and NH_3+C_2H_2 treated+AgSD (d) after the washing process.

Energy-dispersive X-ray spectroscopy (EDS) is used to confirm the elemental analysis of a sample. The EDS spectrum, as shown in **Figure 6(d)**, affirms that the cluster observed in the SEM micrograph is likely to be AgSD particles. **Table 3** shows the result of quantitative analysis of the elemental contents in various gauze samples by means of EDS. The AgSD content in the treated gauze was found to increase by about 1 times for Ag and about 15 times for N, compared with those in the untreated gauze. These higher levels of N contents in the treated gauze implied the nitrogen-containing functional groups, such as amino group (negatively charge), which are able to bind with silver ion [4]. The results clearly indicated that the NH_3+C_2H_2 plasma treatment is necessary, in order to achieve durable interactions between the AgSD particles and the gauze yarn. The plasma deposition used in this work allowed AgSD to bind with the gauze yarn via covalent bonds provided by the deposited carbon film.

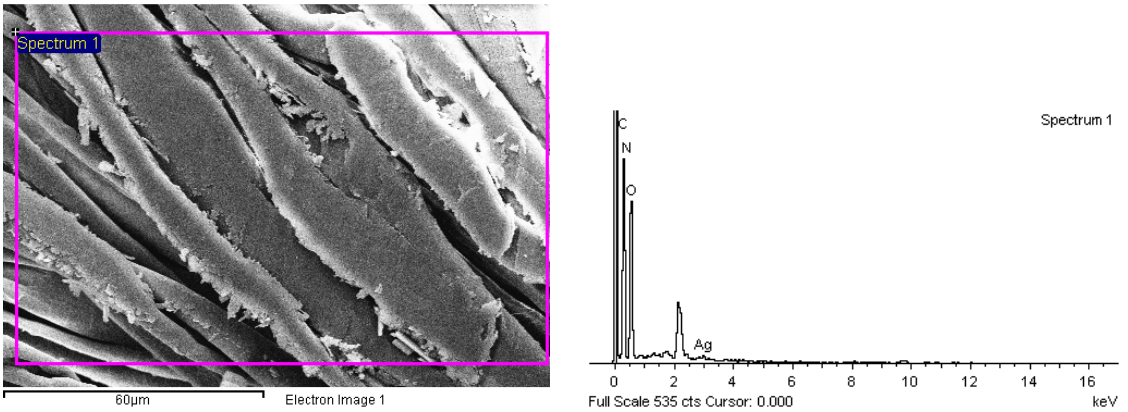
FTIR spectra of the gauze control and NH_3+C_2H_2 treated gauze are shown in **Figure 7(a)**. Infrared C-H vibrations from 2850 - 3100 cm^{-1} and 1380 - 1460 cm^{-1} were observed. These indicate that the cotton surface consists mainly of non-polar groups, such as methyl (CH_3) and methylene (CH_2). The mixed NH_3+C_2H_2 gas makes the carbon films to contain methyl and methylene and the surface becomes hydrophobic. When NH_3 was mixed with C_2H_2 in the feed, the plasma was identified to contain active nitrogen-containing species and hydrocarbon species, necessary for forming C-N bonds on the carbon films and producing oxygen- and nitrogen-containing groups on films surface. The plasma treated-gauze shows new peaks at around 1100 cm^{-1}, 1270 cm^{-1} and 1630-1680 cm^{-1} corresponding to the C-O bond and amide group (H_2N-C=O), respectively [14]. Therefore, it affirms the coordination between the active plasma species and the cotton structure.

Table 3 Element contents of various gauze dressings after washing process by means of Energy-dispersive X-ray spectroscopy (EDS).

Gauze dressing	Element (Weight %)			
	C	O	N	Ag
1. Gauze control	44.88	55.12	-	-
2. Untreated gauze+AgSD	44.71	55.24	-	0.05
3. NH_3+He treated-gauze+AgSD	34.24	52.15	13.28	0.33
3. NH_3+C_2H_2 treated-gauze+AgSD	36.49	49.91	12.64	0.96



(a) NH_3+He treated gauze+AgSD



(b) NH_3+C_2H_2 treated gauze+AgSD

Figure 6 EDS spectrum of (a) NH_3+He treated gauze+AgSD and (b) NH_3+C_2H_2 treated gauze+AgSD after washing.

Figure 7(b) shows the peak at 1515 - 1650 cm^{-1} which represents the $–NH_2$ (amide II) and **Figure 7(c)** shows the peaks at about 600 cm^{-1} and at 1200 - 1250 cm^{-1} corresponding to the S-N stretch and S=O stretch [15], respectively. In addition, a new peak at around 800 - 900 cm^{-1} represents the silver complex, which is consistent with the EDS result, as shown in **Table 3**. This confirms the coordination and conjugation of embedded Ag-SD particles with the carbon structure of the cotton gauze which are not present in the pure gauze.

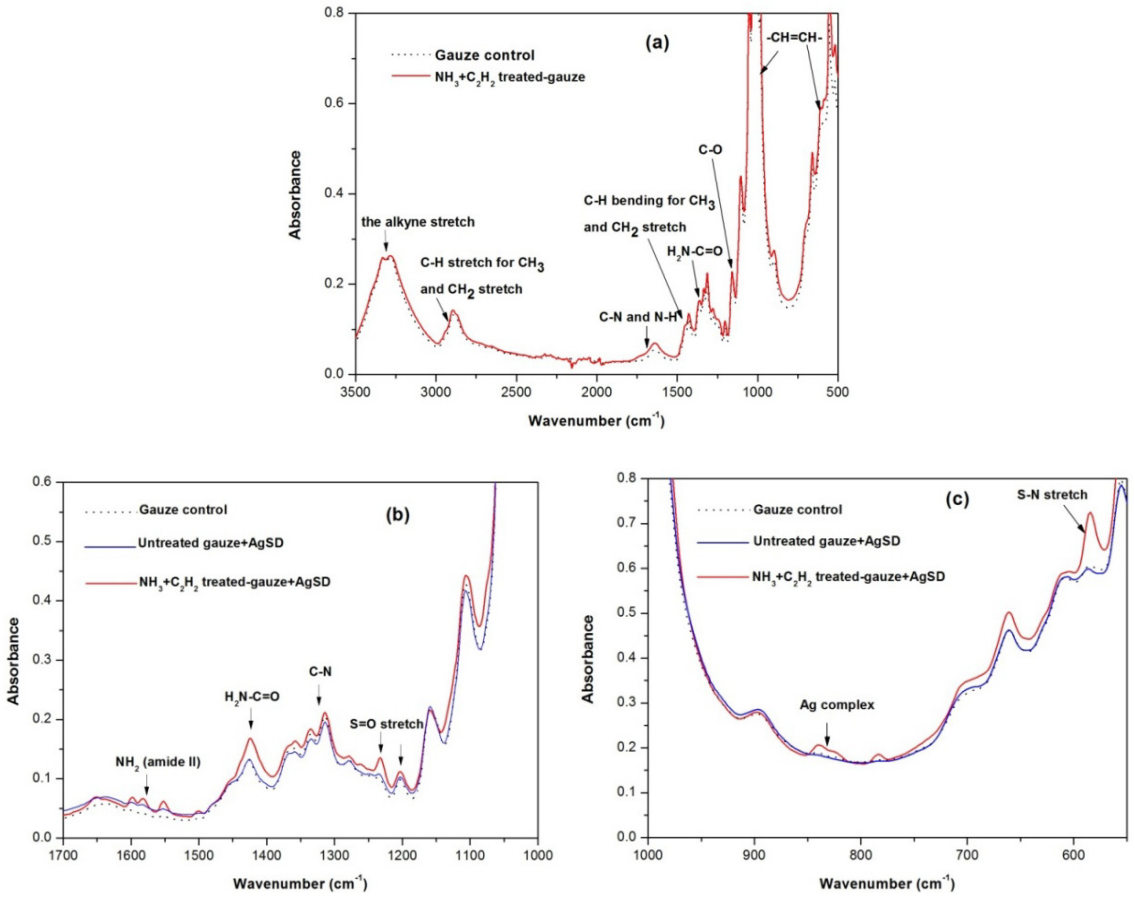


Figure 7 Comparison in FTIR spectra from (a) gauze control and NH_3+C_2H_2 treated gauze, (b) untreated gauze+AgSD and treated gauze+AgSD in the wavenumber range of 1000 - 1700 cm^{-1} and (c) 3500 - 500 cm^{-1}.

The release rate detection of Ag-SD molecules in solution

UV-vis absorbance of the AgSD solution released from untreated and plasma-treated gauzes were scanned with the scanning range at 200 - 500 nm as shown in **Figure 8**. Both spectra showed a broad absorbance peak at around 405 - 410 nm without a clear maximum in the studied wavelength region. A similar maximum absorbance peak appears at 400 nm for the silver nanoparticles solution [16]. From the result, it was evident that the Ag absorbance due to the treatment was about 10 % lower than that without treatment. The lower Ag absorbance was detected from the solution resulted in more Ag was retained in the treated gauze.

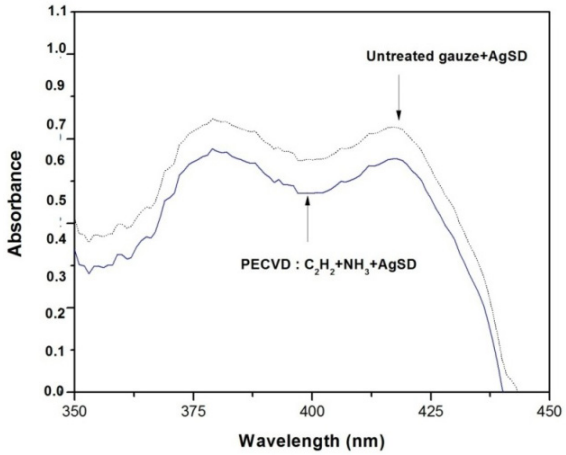


Figure 8 UV-vis absorption spectrum of the released AgSD into solution.

Test on effectiveness of gauze as an antimicrobial dressing

The antimicrobial activity of the dressing tests the effectiveness of the cotton wound dressing after exposure to *E. coli*, *P. aeruginosa*, and *S. aureus*. Novel wound dressings (NH_3+C_2H_2 plasma treated-gauze dressing + AgSD) demonstrate a clear appearance of FTM (no bacterial growth) when compared with the turbid appearance of the standard gauze dressing and the control (bacterial growth). This result proves that the plasma treated-gauze dressings perform well and result in growth inhibition on tested bacterial strains that are commonly found on wounds [17]. In the presence of body fluids and other exudates, the silver complex present in the dressing, are ionized to release the biologically active Ag^+ ions. These ions can bind to negatively charged proteins, RNA, and DNA in most gram-negative and gram-positive bacteria, fungal cells and viruses [17,18]. Therefore, it can imply that the novel gauze can act as an antimicrobial dressing due to the durable bonding with AgSD.

Discussion

Mainly, our study is based on evaluating the grafting of AgSD molecules onto the gauze surface by covalent bonding. The chemical functional groups of surface and the interaction mechanism are discussed. From the AgSD molecular formula, a positively charged silver ion is attached weakly with a negatively charged sulfadiazine. Gaseous mixtures, such nitrogen and ammonia, can also be added to the acetylene to make carbon thin films incorporating constituent elements in the film structure to increase the nitrogen-containing functional groups, such as C-N, N-H and H_2N-C=O. The presence of nitrogen containing functionalities in the film provides a high surface energy and contributes active functional groups to increase the active sites to react with active particles. However, the NH_3+C_2H_2 mixture produces higher concentrations of C-H in this study. This makes the films contain high amounts of methyl and methylene and yields a hydrophobic surface, as shown in **Figure 4**. From FTIR results, the amorphous carbon films contains negatively charged functional groups, such as amino (-NH_2), amide (-N-C=O), and carbonyl (-C=O) groups (as shown in **Figure 7**) which are capable of binding the silver ion which is positively charged. Hence, the release of the silver compound also depends on the chemical interaction between the silver ions and gauze yarn via carbon films. The movement of charge of the reactively functional groups and chemical interaction of silver particles is shown in **Figure 9**. The highly reactive functional groups, such as –C=O, -NH-C=O in the upper surface of the gauze are probably polarized, followed by the movement of H atom. In this case, the AgSD particle probably binds to the gauze surface in the same way as explained by Sawant *et al*. [14]. The weak bond of the AgSD particles to the reactive groups of the treated gauze surface is confirmed by FTIR spectroscopy.

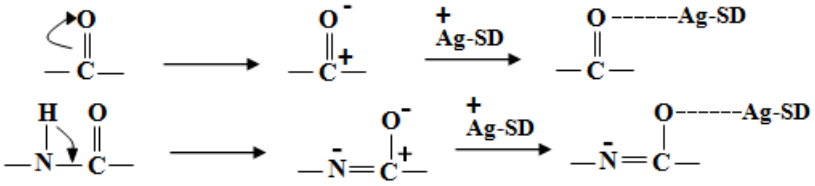


Figure 9 Chemical interaction of AgSD particle with active species on treated surface.

From the FTIR results, the new peaks of treated-gauze dressing are due to the formation of coordination bonds between the AgSD particles (positively charged) and the electron rich groups such as oxygen/nitrogen (negatively charged) present in the treated-gauze. Moreover, the treated-gauze shows a higher absorbance peak than the untreated gauze, especially in the range of 500 - 1600 cm^{-1} as a higher amount of AgSD particles were embedded into the treated-gauze surface. Comparing the results, one can expect that with additional layer of carbon, AgSD can be retained in the gauze.

According to the release rate detection results (**Figure 8**), it indicates that the gauze dressing with carbon films and AgSD immersion shows lower peaks of AgSD than the untreated gauze dressing. AgSD is poorly water-soluble. After dissociation in DI water, the released solution contains both released-silver and sulfadiazine ions. Thus, the release of AgSD can be divided into 2 steps; sulfadiazine releases and silver releases. The sulfadiazine release depends on the solubility of silver sulfadiazine in the dissolution medium. Therefore, less Ag is released and also leads to less sulfadiazine release.

The NH_3+C_2H_2 treated-cotton gauze surface contains abundant functional groups which are capable of binding silver. Hence, AgSD release also depends on the chemical interaction between the silver ions and active species carbon film on the gauze surface. The prominent feature of the carbon film such as a high specific-area induces porous carbon to become a more active binding material and more resistant to structural change caused by hydrolytic effects in aqueous environments [5]. This initial release is very important for fast control of bacterial infection and can slow down the direct delivery of the AgSD agent to the wound area to avoid accumulation of silver sulfide particles in the body [1]. Additionally, a novel wound dressing is able to keep the wound dry while covering and protecting the wound. Stability and security of the sample will be the subject of future studies.

Conclusions

This novel wound dressing with AgSD grafting has been developed by PECVD of NH_3+C_2H_2 gas mixture. It has been shown that the carbon film is necessary to achieve durable interactions between the AgSD particles and gauze yarn. The modified gauze dressing can provide the wound dryness and control the release of AgSD directly to the wound. Our observations suggest that plasma treated gauze dressings with AgSD will have important uses in a wide range on wound management, including preventing wound injection and promoting healing of burns.

Acknowledgements

We thank Thailand Center of Excellence in Physics (ThEP), the National Research University (NRU) Project under Thailand's Office of the Higher Education Commission (OHEC) for the supporting finance. We sincerely thank Assoc. Prof. Dr. Yu Liangdeng, Faculty of Science, Chiang Mai University for critical reading of the paper and Prof. Kom Sukontason, M.D., Associate Dean of Faculty of Medicine, Chiang Mai University for offering to test the effectiveness of the gauze as an antimicrobial dressing.

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