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Research Article

Preparation and Characterization of Bilateral Composite (Metal-Polymer) for Antibacterial Activity Against Wound Pathogen

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ABSTRACT

The adherence of any tissue gets injured called as wounds. During this time skin needs to be covered with a dressing immediately after it is damage. In this paper silver nitrate incorporated with chitosan sustained antimicrobial capability has been developed by an antimicrobial activity well diffusion method. The chitosan consists of a dense skin and porous material, which can fit the requirements for this to be used as a wound dressing. Silver nitrate is traditionally used antibacterial effects for the prevention of wound infection; however, it has raised concern of potential silver toxicity. The chitosan material acts as a rate- controlling wound dressing to incorporate silver nitrate and release silver ions from the porous layer of chitosan. The bacteria culture *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterococcus* sp, *Klebsilla Pneumoneae*, *Planomicrobium* sp and *Staphylococcus aureus* showed a great result of antimicrobial activity and decreased potential silver toxicity. Further, the morphological shape and crystalinity of silver-chitosan composites characterization was confirmed by SEM and EDAX analysis. The responsible functional groups in the chitosan coated silver nitrate composites were identified by FTIR. These studies indicate that the new type of silver-chitosan wound dressing may be effective in the treatment of infected wounds.

Keywords Chitosan, FTIR, SEM, Silver nitrate, wound dressings.

INTRODUCTION

Generally skin act as an anatomical barrier[1]. Skin plays an important role in protecting the body against pathogens and internal and external environment damages. Wound is the colonization of the microorganisms[2]. Commonly used in clinically based wound dressing have three divisions i) biological dressings ii) synthetic dressings iii) biologic – synthetic dressings. The biological dressings have some disadvantages such as limited supplies, high antigenicity and poor adhesiveness eg. Alloskin. Synthetic dressings have long shelf life and carry almost no risk of pathogen transmission e.g. silicone meshes. Nowadays the researchers have focused on biologic – synthetic dressings[3]. This type of dressing producing a new, improved and modifying the biocompatible materials for wound dressings[4]. The requirements for this new wound dressings are control the infection of wounds under dressings. In more recent a variety of silver based products used for wound dressings. In metal regions silver compounds have high enslaves for their medicinal properties for centuries. In early days of the 19th and 20th century silver used before the advent antibiotics for colds and gonorrhoea and popular remedy for tetanus and rheumatism[5]. Several products have incorporated to silver for use as a antimicrobial agent such as silver nitrate, silver ion, actisorb silver and etc. The source of silver based dressings commercially available released from creams, solutions and ointments (or) nanocrystalline. Silver nitrate cream has been traditionally used for open wound infections. Many polymers have been involved for the purpose of everyday life. Synthetic and natural polymers are play as an essential and ubiquitous role in pharmaceutical fields. These polymer materials such as alginate, chitosan, gelatin, collagen and etc are natural polymers. Synthetic polymers like polyethylene, nylon, polyimide, polyvinyl alcohol and etc. Chitosan is a well known natural biopolymer that has been able to accelerate the cure of wounds in human[6]. The therapeutic properties of chitosan are antimicrobial activity against a broad spectrum of microorganisms, alleviating pains and promoting haemostasis and simulated the epidermal cell growth[7,8]. The antimicrobial action of chitosan absorbed onto the wound causing microorganism surfaces, increasing the permeability of the lipid cell membrane and causing death through the loss of essential cell materials. Furthermore, non-toxic, non- allergic, non-adherent and easily removed without trauma biomaterial of chitosan is widely used for many pharmaceuticals and medical fields. Accordingly, chitosan may be suitable to bilayer with silver nitrate for the preparation of effective and long- acting antimicrobial wound dressing. In this

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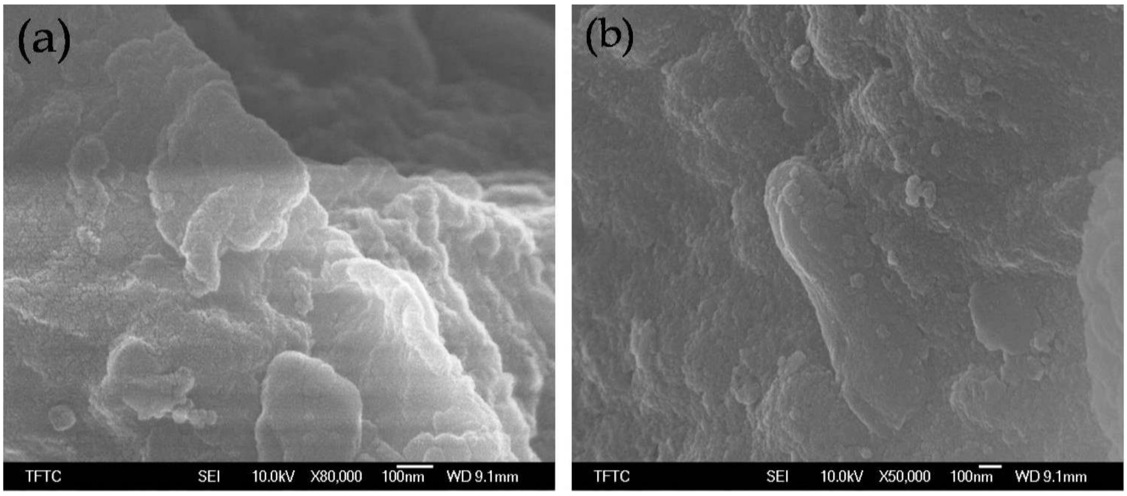

Figure 1: SEM Morphology (a) shows the cross section and (b) shows the surface of the bilateral composites of metal-polymer

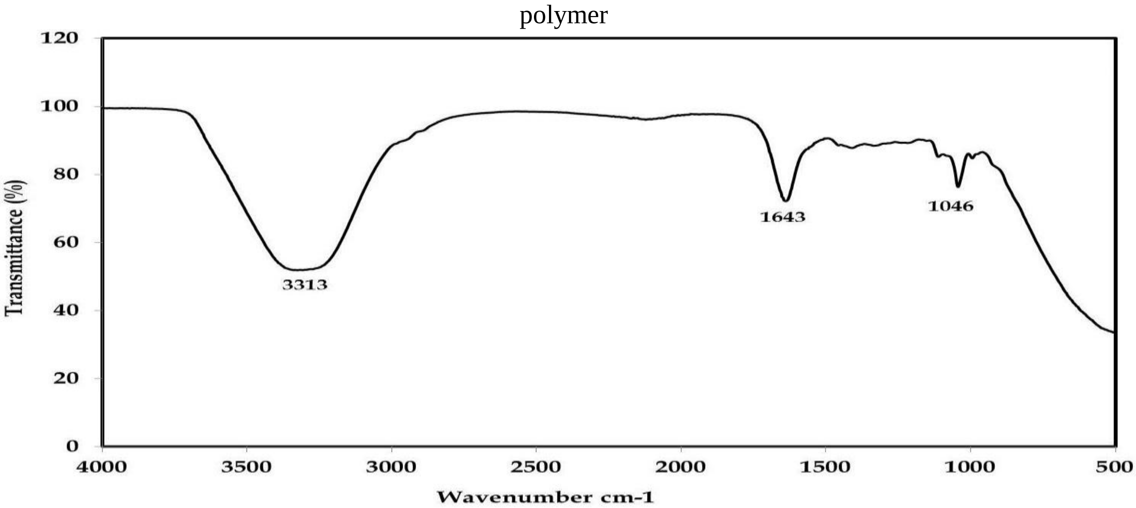

Figure 2: FT-IR spectra of the metal-polymer composites

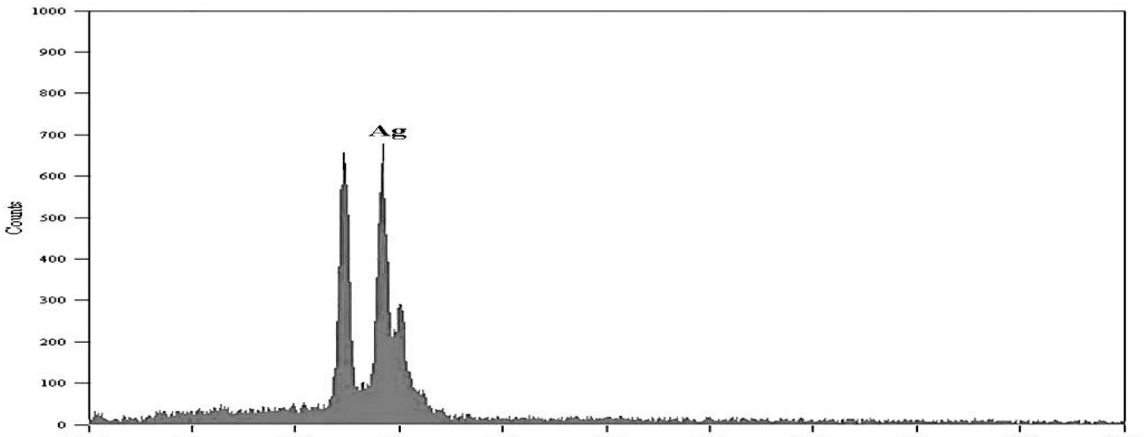

Figure 3: EDAX spectrum of the metal-polymer composites

study the metal based silver nitrate powder is bilayered and consists or coated with the high polymer and natural biomaterial of chitosan. The wound causing bacteria culture *Pseudomonas aeruginosa* , *Bacillus subtitlis, Enterococcus* sp, *Klebsilla pneumoniae*, *Planomicrobium* sp and *staphylococcus aureus* showed prolonged antimicrobial activity and decreased potential silver toxicity by metal-polymer composites. These metal-polymer composites characterised by Scanning Electron Microscopy (SEM), Energy Dispersive X- ray (EADX), Fourier transform infrared (FTIR). The composites activation was measured by agar well diffusion method.

Experimental procedure

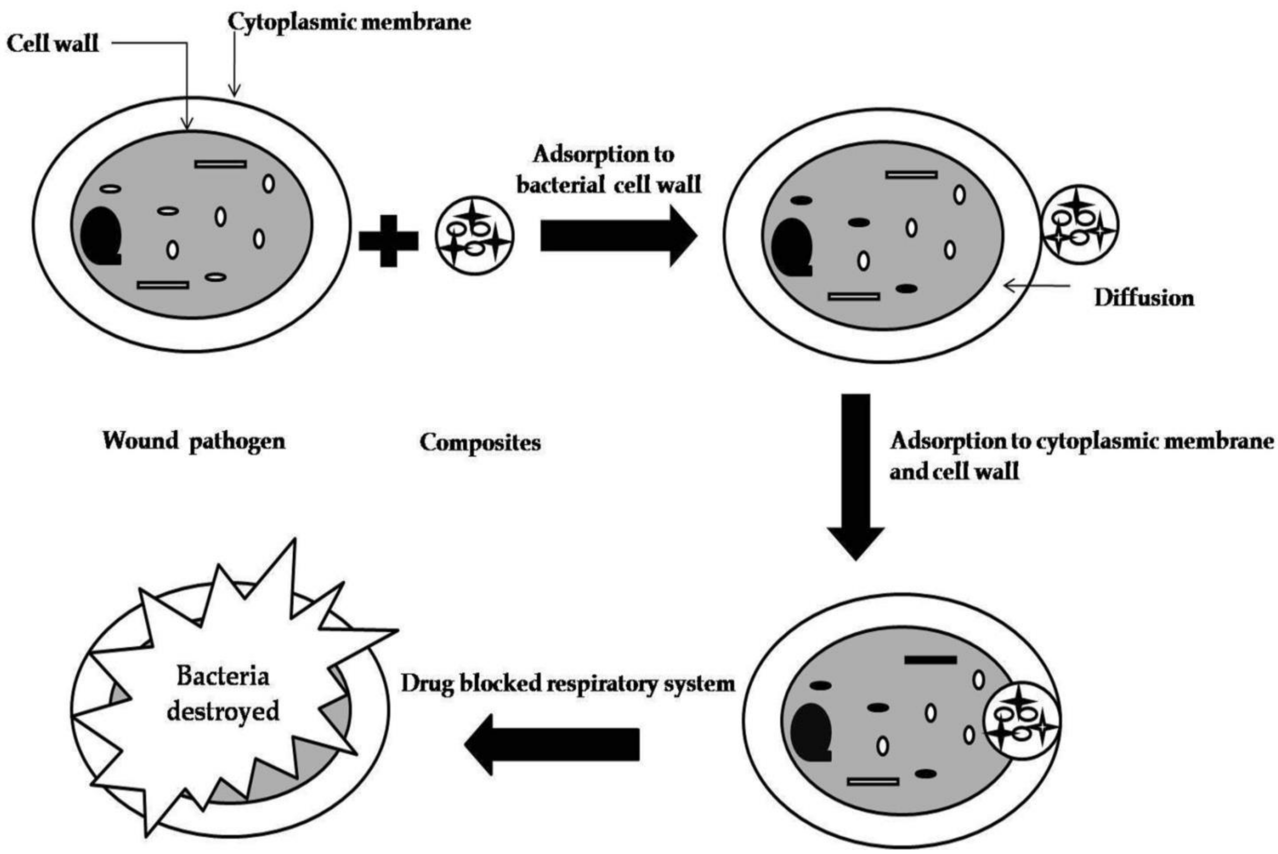

Figure 4: The bilateral composites of metal-polymer release mechanism.

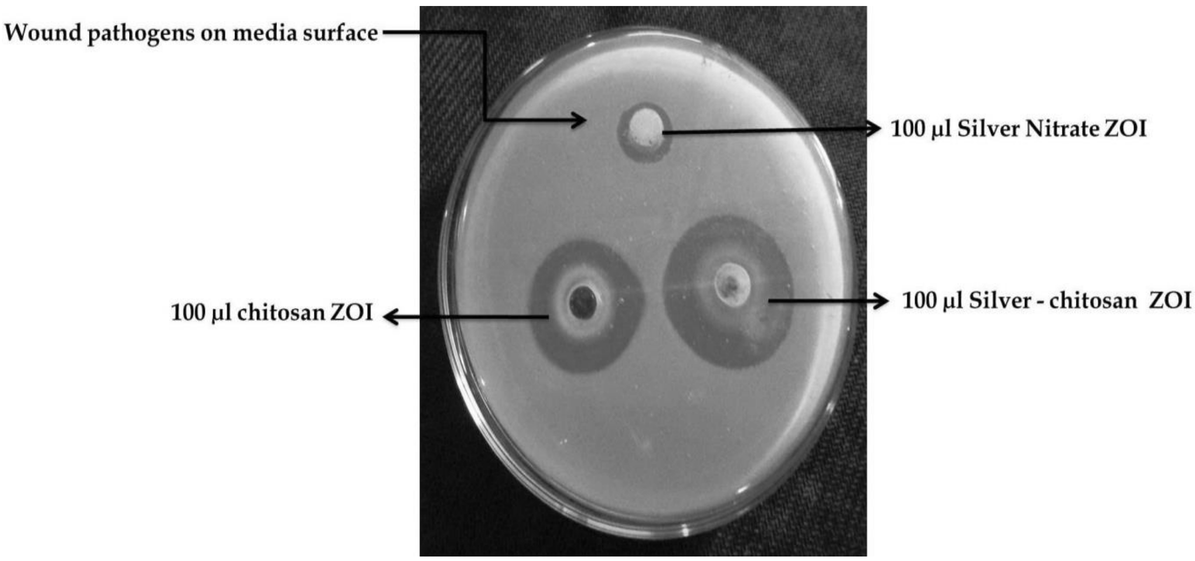

Figure 5: Antimicrobial action of silver, chitosan and silver-chitosan composites

Chemicals

Chitosan obtained from crab shells by deacetylated product of chitin. Silver nitrate powder purchased from of near medical shop. Muller hinten agar and Acetic acid purchased from Hi-Media, Mumbai. The wound causing pathogens *Pseudomonas aeruginosa (10145), Bacillus subtilis* (3053), *Enterococcus* sp (RMAA), *Klebsilla pneumoniae* (MAA), *Planomicrobium* sp (MCRA) and S*taphylococcus aureus (12600)* were purchased from MTCC.

Preparation of metal-polymer composites dressing

5 gram of chitosan was dissolved with stirring in distilled water containing 1% acetic acid. Insoluble substances were removed by filtration. The Silver nitrate was incorporated with chitosan by dispersing market powder in chitosan solution (1 wt %) using homogenization. The silver-chitosan bilateral composites used for antimicrobial activity against wound causing microorganisms.

Characterization studies

The metal-polymer bilateral composites were prepared and heated in hot air oven with circulating hot air until for dry the powder form. Furthermore, the dry powders were

Table 1: Antimicrobial activity of chitosan against wound infecting microorganisms

S. No	Wound Pathogenic bacteria	Concentration of chitosan	Zone of inhibition (mm in diameter)
1.	*Bacillus subtilis*	50 µl	20.93±0.066
		100 µl	24.56±0.233
2.	*Enterococcus sp*	50 µl	20.16±0.166
		100 µl	25.66±0.333
3.	*Pseudomonas aeruginosa*	50 µl	19.66±0.333
		100 µl	26.73±0.266
4.	*Klebsiella pneumoniae*	50 µl	19.86±0.133
		100 µl	23.33±0.667
5.	*Planomicrobium sp*	50 µl	20.33±0.667
		100 µl	22.16±0.601
6.	*Streptococcus aureus*	50 µl	20.66±0.333
		100 µl	27.66±0.333

± Standard Deviation

characterized by SEM were performed using a Philips model CM 200 instrument to study the morphological nature of the metal/polymer coated powder. The EDAX analysis was performed to verify the elements of the metal-polymer composites powder. The FTIR measurements (Perking-Elmer) were carried out to identify the responsible functional group of the metal-polymer composites powder.

In vitro antibacterial studies

The inhibition zone of the silver nitrate powder incorporated with chitosan solution was measured on agar plates inoculated with wound causing strain using the inhibition zone test, using 50 µl and 100 µl concentrations. Silver is released from chitosan solution into the inoculation the medium to inhibit the growth of wound causing *Pseudomonas aeruginosa, Bacillus subtilis, Enterococcus sp, Klebsilla pneumoniae, Planomicrobium sp* and *Staphylococcus aureus* bacterial culture. After 24 hrs incubation, the inhibition zones around the well of silver nitrate incorporated chitosan composites were compared with that of blank chitosan without silver nitrate and blank silver nitrate without chitosan.

RESULT AND DISCUSSION

The goal of this study is to prepare a polymer coated silver nitrate powder enhance and fit the basic requirement of a wound dressing. The preparation of chitosan coated silver nitrate as a functional wound dressing result and discussions are below.

Characterization of metal/polymer coated dressings

Figure 1 (a) shows the cross section and (b) shows the surface of the silver- chitosan composites. SEM image confirmed that the morphology of silver- chitosan composites. These silver ions get aggregated with each other and chitosan. Silver ions dispersed uniformly in chitosan solution so it has a smooth surface. Figure 2 shows the FTIR of the silver-chitosan were characterized to obtain the information on its composition. The FTIR both characteristic peaks of of the silver-polymer the strong and broad peak at 3313 cm^{-1} were attributed to O-H stretching contains alcohols and phenol functional groups respectively[9]. and sharp peak near 1653 cm^{-1} was designated as an C=C stretching vibration responsible to alkenes functional group. The narrow band at 1048 cm^{-1} resulted from the C-O stretching vibration alcohols, carboxlic acids esters and ethers functional groups are present[10]. These peaks results provide the conformation of silver- chitosan composition. Figure 3 shows The EDAX analysis of silver nitrate wound dressing cream coated by chitosan polymer confirmed the presence of silver in the chitosan mixture, mostly the reduction of silver nitrate to release silver ions, the formation of intense peak Ag crystalities atomic ratio at 3kev related to silver element and other signals like C, O, Mg, Si and Cl confirms the presence of elemental compounds of chitosan.

Mode of action on metal - polymer dressings

The release of silver from chitosan was investigated to evaluate their drug release characteristics. The silver nitrate dissolved by water and release silver ions as well as nitrate from chitosan. The dissociation of silver nitrate and the release of nitrate and silver ion depend on chemical equilibrium of the ionic interactions between nitrate and silver ions, and chitosan solution. The killing mechanism of microorganisms by silver ions 1) Firstly, silver ion prohibit ATP synthesis via binding to the ATP synthesis enzyme molecules in the cell wall, 2) When enters silver ion to the cell it's binds with DNA and RNA, leading to the DNA denaturation, there by inhibiting cell division and replication of pathogens. 3) Finally, silver ions blocked the respiratory system of microorganisms in the cytochrome oxidase and NADH- succinate-dehydrogenase region[11]. These steps are reported to the mode action of antimicrobial activity of the silver ions[12]. But silver ions are highly toxic to keratinocytes, fibroblasts[13]. and mostly deposited or bound at the wound surface which may be toxic to the tissue of skin and Enders the blood quickly[14]. Burke reported[15] that higher concentration of silver is toxic to tissue and may be a bearing to organs. So compare to metal polymers are powerful candidates, harmless and high antimicrobial activation on wounds. Chitosan has a better antimicrobial agent when compare to chitin[16]. The exact antimicrobial mechanism of the chitosan and their derivatives is still

Table 2: Antimicrobial activity of silver nitrate powder against wound infecting microorganisms.

S. No	wound pathogenic bacteria	Concentration Of silver nitrate	Zone of inhibition (mm in diameter)
1.	*Bacillus subtilis*	50 µl	11.06±0.882
		100 µl	16.83±0.166
2.	*Enterococcus sp*	50 µl	12.33±0.166
		100 µl	15.33±0.667
3.	*Pseudomonas aeruginosa*	50 µl	14.50±0.120
		100 µl	18.66±0.333
4.		50 µl	10.23±0.145
	Klebsiella pneumoniae	100 µl	14.83±0.166
5.	*Planomicrobium sp*	50 µl	9.33±0.333
		100 µl	17.76±0.145
6.	*Streptococcus aureus*	50 µl	10.23±0.145
		100 µl	14.83±0.166

± Standard Deviation

Table 3: Antimicrobial activity of metal-polymer composites against wound infecting microorganisms.

S. No	Wound Pathogenic bacteria	Concentration Of silver-chitosan composites	Zone of inhibition (mm in diameter)
1.	*Bacillus subtilis*	50 µl	30.86±0.133
		100 µl	34.66±0.333
2.	*Enterococcus sp*	50 µl	29.83±0.441
		100 µl	33.73±0.266
3.	*Pseudomonas aeruginosa*	50 µl	32.33±0.333
		100 µl	36.56±0.233
4.	*Klebsilla pneumoniae*	50 µl	31.8±0.115
		100 µl	31.66±0.333
5.	*Planomicrobium sp*	50 µl	29.93±0.066
		100 µl	31.93±0.066
6.	*Streptococcus aureus*	50 µl	30.33±0.333
		100 µl	35.76±0.145

± Standard Deviation

unknown, but separate active mechanisms have been proposed. The mechanism based on an electro static model interaction between negatively charged microbial cell membranes are attracted by positively charged amino group of chitosan molecules[17]. and this interaction leads to the leakage of proteinaceous and other intracellular constituents. The chitosan mainly act on the outer surface of the microorganisms and interacts with the membrane of the bacteria cell to alter the cell permeability. In lower concentrations also chitosan able to bind the negatively charged bacterial surface to cause agglutination, while at a higher concentrations, the large number of the positively charged chitosan molecules may have communicate with a positive charge to the microorganism surfaces[18]. The silver – chitosan composites release mechanism with wound causing pathogens showed in figure 4. This mechanism result of chitosan composites can reduce a rapid diminishing of microorganisms on an infected wound and finally blocked the respiratory system of wound causing microorganisms. Silver impregnated with polymer of chitosan provides the antimicrobial efficiency with a sustained release of silver ions[19]. The silver-chitosan fabulous material to minimize the transmission mechanism of infective agents, and enhance or facile application of antimicrobial activity to improve the health care[20]. Silver nitrate powder has a high concentration of silver, more absorbed by wound beds but no residual activity of dressings when compared to polymer coated dressings. These new metal- polymer composites wound dressing should maintain a moist environment the wound interface, allow gaseous exchange, remove exudates and act as a barrier to microorganisms. The benefits of metal-polymer composite dressings would be an advantage for the treatment on infected wound repair in a mild way and regeneration though it's the antimicrobial efficacious.

In vitro antimicrobial studies

The contact antimicrobial property of chitosan at table 1, silver nitrate at table 2 and polymer (chitosan) metal (silver) composites result showed in Table 3. The observation of results was investigated by using an agar well diffusion method. The high value diameter of the zone of inhibition for the chitosan- silver composites is 37 mm in 100 µl and 32 mm in 50 µl against *Pseudomonas aeruginosa*, whereas that of silver nitrate is 18 mm in 100 µl and 14 mm in 50 µl against *Pseudomonas aeruginosa* and chitosan is 27 mm in 100 µl and 20 mm in 50 µl against S*taphylococcus aureus* when compare to other five wound causing microorganisms. This result indicates that the chitosan-silver composites has a more effective contact antimicrobial property than silver nitrate. Silver nitrate slowly releases silver ions as an antimicrobial agent, and the diffusion of silver ions might be blocked by the formation of secondary compounds. Figure 5 shown in the silver nitrate, chitosan and bilateral composites (metal- polymer) activation antimicrobial activity. In addition, the polymer of chitosan provides high surface area to contact with six wound causing microorganisms. Furthermore, the silver nitrate has a low antimicrobial property when compared to chitosan by disrupting cell membranes. Therefore, the chitosan - silver composites has a superior contact antibacterial property to that of silver nitrate.

CONCLUSION

This work should focus on the development of new metal-polymer bilateral wound dressing materials. The range of chitosan application has been extended to include many fields such as artificial organs, drugs, health care products, wound healing, food, textile and water treatment, etc. The use of silver-chitosan wound dressings also can promote for wound healing and decrease the risk of silver absorption in comparison with silver nitrate dressings. In addition, silver-chitosan wound dressings are made from available materials and require this production which is an economical, efficient and eco-friendly process. Finally this metal-polymer bilateral dressing composites perform better than the clinically approved silver based burn creams and powders. So we believe that these dressings will have immense use wound dressing in medical field for their efficient antimicrobial functions.

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