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Synthesis of Carboxymethyl Chitosan and Coating on Wound Dressing Gauze for Wound Healing

[1]B. Venkatrajah, [2]V. Vanitha Malathy, [1]B. Elayarajah, [3]R. Rajendran and [1]Ram Rammohan
[1]Department of Microbiology and Bioinformatics, CMS College of Science and Commerce, Coimbatore, Tamil Nadu, India
[2]Department of Zoology, Kunthavai Nachiar Government College for Women, Tanjore, Tamil Nadu, India
[3]Department of Microbiology, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Abstract: Wound healing is a long and complex process. To improve wound healing, the wound dressing cotton gauze can be functionalized by imparting moisture holding and antibacterial ability. Moisture is an important factor for wound healing and the absence of microbial intervention can accelerate wound healing process. Direct alkylation method was used to synthesis carboxymethylated chitosan with water solubility, biocompatibility and antibacterial activity. Calcium alginate was used along with modified chitosan as moisture gaining polymeric agent. Pad-dry-cure method was employed to coat both the polymers on cotton gauze surface, which was weaved using 40^s Ne cotton yarn. After coating, the cotton was analysed for its polymer add-on percentage, antibacterial action against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 10229. The persistence analysis of antibacterial activity ensures the polymer withstanding ability on cotton gauze surface. SEM detection of polymers with cotton threads confirms their presence. Wound healing action of the polymer coated cotton gauze was determined using albino rats as animal model.

Key words: Direct alkylation, biocompatibility, antibacterial, calcium alginate, animal model

INTRODUCTION

Fabrics hold an important place in human life, economic status of a country, industrial and medical applications. Its usage in daily life is inevitable and its harbouring ability of dirt and wastes is noticeable. The form a fabric get yarned and weaved can accommodate more particles which act as substrates for microbes to colonise on their surface. Antibacterial fabrics can be a good remedy for retarding microbial growth on fabric surface. A prominent hygiene fabric is essential, especially for the hospitalised immuno-suppressant patients. About 35% of the disease outbreak in hospitals is mainly due to the dissemination of pathogenic microbes in air from various hospital used fabrics. Hospital environment plays a vital role in determining the comfort and health of patients. The prevalence of suspended particles in air can harbour microbes and pollutes the indoor environment. The frequent bacterial species available in hospital indoor environment are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Tambekar *et al*., 2007).

Fabrics with antimicrobial finish must be prominent for hygienic and medical applications. Its major application is to prevent the bacterial growth on textiles. To make a fabric with antimicrobial finish, three component features must be considered. The antimicrobial substance to be coated on fabric surface, the method used to impregnate the antimicrobial substance on the fabric surface and the persisting ability of the antimicrobial action in the fabric after repeated usage. Many substances have been used in view of its antimicrobial ability like, antibiotics, formaldehyde, heavy metal ions, quarternary ammonium salts, phenol and oxidizing agents, herbal formulations, natural biopolymers (Jakimiak *et al*., 2006). Extracts from green tea leaves are used as eco-friendly antimicrobial coating agents on cotton surfaces (Syamili *et al*., 2012). Apart from antimicrobial finish, cotton textiles were functionalized using anizidine a conducting polymer for antistatic applications and as fireproof textile (Issaoui *et al*., 2011). While using antimicrobial agents, their biocompatibility, persistence in fabric surface against the abrasive forces during wearing and withstanding the leaching effect of chemicals during washing have to be considered. A substance which can resist the forces and establish its antimicrobial action effectively can alone give a good antimicrobial finish to fabrics. A marine biopolymer

Corresponding Author: B. Venkatrajah, Department of Microbiology, CMS College of Science and Commerce, Coimbatore, Tamil Nadu, India Tel: +919487508666

extracted from the shells of crustaceans called chitin shows stability to such adverse effects. But exhibits less antimicrobial action. When deacetylated, the formed chitosan from chitin can combat against microbes very effectively. Based on the experiment of Batista *et al.* (2011), the high molecular weight chitosan with less deacetylation percentage exhibits low antimicrobial action against gram negative bacteria than the medium molecular weight chitosan with high deacetylation percentage. Fungal mycelium is also a good source of chitosan. When compared with other fungi, from *Mucor* sp. KN_{03} mycelium higher amount of chitosan was derived (Nadarajah *et al.*, 2001).

Its biological properties includes bacteriostatic and fungistatic and used in wound dressing materials to accelerate wound healing (Shanmugasundaram, 2012). Its mode of action on bacterial cells is by establishing cell surface alterations. Chitosan can alter the cell wall permeability of bacterial cells leading to poor metabolic function. This restricts bacterial growing and colonizing ability (Je and Kim, 2006). Application of chitosan in combination with various other antimicrobial agents increases their antimicrobial nature. The extracted essential oils of thyme and clove were immobilized in chitosan based edible films and analysed. They exhibit enhanced antimicrobial effects than its individual forms (Hosseini *et al.*, 2008).

An annual production of chitin as second abundant biopolymer was estimated as 1×10^{11} tons. Because of biodegradability, biocompatibility, hemostatic and wound healing activity it is used widely in medical field. The drug release profile of chitosan crosslinked with tripolyphosphate was analysed by Phromsopha and Baimark (2010) using gentamicin sulphate antibiotic. A stable and sustained release of the antibiotic was noted which could be advantageous than the burst release of the antibiotic in patients intestine.

Even though chitosan is widely used, its poor or no water soluble property is the major drawback. In its deacetylated form, chitosan is soluble in acids and solvents. Further chemical modifications can make chitosan get dissolve in water (Sugimoto *et al.*, 1998). One such modified chitosan called N,O-carboxymethyl chitosan (NOCC), in its amino and primary hydroxyl sites of glucosamine units (backbone structure of chitosan) possess carboxymethyl substituents. Carboxymethylated chitosan has good water solubility and biocompatility as it fits with the neutral environment of human body (Dolatabadi-Farahani *et al.*, 2006). Similarly, Jideowno *et al.* (2007) modified chitosan with acrylamide for the removal of heavy metal ions from aqueous systems. Their results revealed the high sorption ability of modified chitosan than normal.

Wound dressing material gets the prime importance in wound healing process (Yang and Lin, 2004). The wound gauze with fine absorption of wound exudates at the same time permit evaporation of moisture to certain rate is needed (Liu *et al.*, 2008). It should not have close adherence with the tissues of wound area and creates pain, trauma or additional tissue damage to the patients while removing (Mi *et al.*, 2002). An easily stripped wound dressing material must be developed to reduce the pain suffered by patients when wound dressings are frequently changed (Borkow and Gabbay, 2010).

Due to the haemostatic property, chitosan is used in wound management (Kumar, 2000). Chitosan can faster the wound healing process by decreasing the macrophage prevalence in wounded site (Mi *et al.*, 2001). Alginate, a bio-fibre possesses gel forming ability by ion exchange process. By exchanging the sodium ions of wound exudates with its calcium ions can form gel. This gel forming nature of alginate helps to retain moisture. When a dressing material coated with water soluble chitosan (NOCC) and alginate can accelerate wound healing process (Knill *et al.*, 2004).

In the present study, NOCC and calcium alginate were coated with the cotton fibres of wound dressing material to analyse their wound healing effect. In the previous studies chitosan has been coated as antibacterial finish in cotton gauze but its water insoluble nature can not readily establish its antibacterial effect in wounded sites. Hence as a good alternative, NOCC was applied as antimicrobial coating against the used bacterial strains *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 10229. Sodium alginate can hold moisture in the wound area essential for its healing. The water soluble NOCC can provide immediate antimicrobial effect as it dissolves easily in body fluids prevalent in the wounds to prevent bacterial colonisation.

MATERIALS AND METHODS

Materials: Chitosan with 85% degree of deacetylation and molecular weight of $2X10^5$ was purchased from Sigma Aldrich, USA. Sodium alginate was provided by HiMedia, India. The other chemicals and reagents used were of analytical grade. The standard bacterial cultures (*Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 10229) were obtained from American Type Culture Collection. The cotton yarns of 40^s Ne was procured from Lakshmi Mills Ltd., Coimbatore.

N,O-carboxymethyl chitosan (NOCC) synthesis: N,O-carboxymethyl chitosan (NOCC) was synthesised

following the method of Dolatabadi-Farahani *et al.* (2006). Five grams of chitosan was suspended in 50 mL of isopropyl alcohol and stirred in a magnetic stirrer at room temperature. NaOH solution (10 N) of about 13 mL divided into 5 equal volumes was added to the slurry over a period of 25 min. The alkaline slurry made was stirred for 30 min. Monochloroacetic acid (30 g) divided into 5 equal portions was added with 1 min interval. The temperature was raised to 60°C and continuously stirred for 3 h. The final mixture was filtered and the synthesised NOCC was washed with methanol. After washing, at 60°C the NOCC was oven dried.

Cotton gauze production: The cotton gauze was produced using the yarns procured from spinning mills using gauze bandage loom. The loom has 300 picks min^{-1} capacities and a width of 10-15 cm. Cotton yarns were spun at Fashion Technology Department, Kumaruguru College of Technology and Coimbatore to prepare the cotton gauze.

Properties of cotton gauze: The cotton gauze properties such as ends per inch and picks per inch were measured using the counting glass. The area density was measured using gsm cutter method as per ASTM D3775. Thickness and stiffness of the fabric were measured as per ASTM D 1777-96 and ASTM D 6828 standard methods respectively (Shanmugasundaram, 2012).

Coating of cotton with NOCC and calcium alginate: NOCC (5.0 g) was dissolved in double distilled water by stirring for 1 h at room temperature. A modified method of Shanmugasundaram (2012) was used to prepare polymer coated cotton gauze. In brief, calcium alginate (2.0 g) was added to the NOCC solution and stirred for 10 min at room temperature. Cotton gauze to be coated was dipped in the NOCC-calcium alginate solution and left overnight. The coated cotton gauze was padded twice with the solution of same concentration to a wet pick of 80%. The padded fabric was dried at 80°C for 5 min and cured at 140°C for 3 min.

Calculating the add-on percentage: The polymer add-on percentage on the gauze surface was calculated following the method of Shanmugasundaram (2012) and was estimated using the following relationship:

$$\text{Drug add-on (\%)} = \frac{W_1 - W_2}{W_2} \times 100 \quad (1)$$

where, W_1 is the weight of polymer coated sample and W_2, the weight of un-coated sample.

SEM analysis: The coated cotton gauze were analysed for the presence of polymer using scanning electron microscope. During sample preparation the cotton gauze specimens to be analysed were mounted on aluminium stubs and using Sputter Coater they were coated with gold and magnified in SEM.

Assay for antibacterial properties: The antibacterial effect of the uncoated and coated cotton gauze was determined qualitatively by agar diffusion plate test using EN ISO 20645:2004 method against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 10229. When effective antibacterial activity was determined against the used bacterial pathogens, AATCC 100-2004 test method was used to analyse reduction in bacterial counts for quantitative determination.

Qualitative antimicrobial determination by EN ISO 20645:2004 method proposed by Erdem and Yurudu (2008) employs a two layered agar plate. The lower layer has sterile culture medium (10±1 mL of Tryptic Soy Agar-TSA) and the upper layer of agar (TSA precooled to 45°C) was inoculated with individual test bacteria (1×10^8 cells). Test gauze specimens (25±5 mm in diameter) were imprinted onto the inoculated TSA using sterile forceps. Agar plates were incubated for 18-24 h at 37°C. Assessment of antibacterial activity was determined by the extent of bacterial growth in the contact zone between the agar and the gauze specimen.

Bacterial growth inhibition zones were calculated using the formula:

$$H = \frac{D - d}{2} \quad (2)$$

where, H is the zone of inhibition in mm, D is the total diameter of specimen and inhibition zone in mm, d is the diameter of specimen in mm.

The antimicrobial activity was quantitatively evaluated against the standard bacterial strain which gets effectively inhibited by EN ISO 20654:2004 method. According to AATCC 100-2004 test method proposed by Pinho *et al.* (2011), the cotton gauze samples both uncoated and coated in a size of 4.25±0.1 cm in diameter were placed in separate 250 mL glass jar with screw cap and inoculated with 1.0±0.1 mL of bacterial inoculum (1×10^5 cells mL^{-1}). After incubation over a contact period of 24 h, 100 mL of sterilized distilled water was added into the jar and stirred vigorously for 1 min. After serially diluting 1 mL of the solution, from every dilution 1 mL of diluted solution was plated on nutrient agar and incubated for 24 h at 37±2°C.

Wash fastness test (AATCC Test Method 124; Version-1996): AATCC test method 124 was used for performing the wash fastness test. This test result ensures the persistence of bioefficacy by bound polymers and herbal extract to cotton gauze after certain number of washes. To evaluate the durability of antibacterial effect and the persistence of coated polymers after washing, the treated cotton gauzes were washed with AATCC Standard Reference Detergent WOB (without bleaching agent). Both uncoated and coated samples were subjected to 3 cycles of consecutive laundering. At the end of the 1st and 3rd laundering cycle, the samples were rinsed with warm water, air dried and tested for antibacterial activity based on AATCC 100-2004 test method.

Bacterial reduction percentage: Colonies of bacteria recovered on the agar plate from both uncoated and coated cotton gauze before wash (AATCC 100-2004 test method sample) and after wash (AATCC 124 test method) were counted and the per cent reduction of bacteria (R) was calculated by the following equation:

$$R\,(\%) = \frac{B - A}{B} \times 100 \quad (3)$$

where, A is the number of bacterial colonies from coated cotton gauze after inoculation over a contact time of 18 h and B is the number of bacterial colonies from uncoated cotton gauze after inoculation at 0 contact time.

Wound healing analysis: The wound healing function of the uncoated and coated cotton gauze was analysed using an animal model. Female Albino rats weighing approximately 150±10 g were left for 2 days at room conditions for acclimatization. A standard pellet diet and water *ad libitum* was maintained throughout the experiment. A minimum of three animals were used as one group for each specimen (uncoated and coated) and for the control drug Teramycin ointment (Pfizer, USA) (Tarun and Gobi, 2012). The study was permitted by Institutional Animal Ethics Committee and was performed according to the international rules relating to animal experiments and biodiversity right. All the animals were anesthetized by intramuscular injection with 0.15 cc of Ketalar. The hairs on the skin of animals were removed and disinfected using 70% ethanol. Circular wound was created on the dorsal interscapular region of each animal by excising the skin with a 10 mm biopsy punch (Diao *et al*., 2008). The cotton gauze samples both coated and uncoated were placed on the wound and the progressive changes in the wounded areas were monitored every other day for 15 days using a camera (Nikon, Japan).

Statistical methods: *In vitro* results of cotton gauze with surface coating formulation was compared statistically with uncoated sample to understand the level of significance using SBSS (ver. 11.0) statistical software. The results were presented as Mean±standard Deviation (SD). Chi Square test was used to compare the mean values of polymer coated cotton gauze with uncoated sample for the determination of antimicrobial activity. Statistical significance was set at $p<0.05$. The wound healing percentage was analysed using One-way Analysis of Variance (ANOVA). The values of $p\leq0.001$ were considered statistically significant.

RESULTS

Properties of cotton gauze fabric: The properties of cotton gauze fabric prepared were listed in Table 1. It can be inferred that cotton gauze prepared has similar specifications to that of the cotton gauze normally used in medical practices.

Synthesis of NOCC: The direct alkylation method of chitosan to improve water solubility was done using monochloroacetic acid as the alkylating agent. The addition of carboxymethyl groups in its amino and primary hydroxyl sites of glucosamine units can modify chitosan and lead to the synthesis of N,O-carboxymethyl chitosan (Fig. 1). The synthesis of modified chitosan can be confirmed by its water soluble ability.

Polymer add-on in cotton gauze fabric: Equation 1 was used to calculate the polymer add-on percentage in cotton gauze fabric. The polymer add-on percentage of cotton gauze is 43.89. After polymer coating, the final weight of cotton gauze gets increased than the initial weight. The difference in the weight of cotton gauze was analysed in triplicate and the mean value was expressed in Table 2. The presence of polymer in the form of thin film of gel between the thread gaps in cotton gauze was focused using stereo-zoom microscope (Fig. 2).

Table 1: Properties of cotton gauze fabric

Properties	Cotton
Ends/inch	45
Picks/inch	24
Fabric areal density (g m^{-2})	63.12
Fabric thickness (mm)	0.037
Flexural rigidity (mg cm^{-1})	
Warp way	121.16
Weft way	127.38

The physical properties of cotton gauze weaved and analysed according to the standards of ASTM standard methods

Table 2: Polymer add-on in cotton gauze

Sample	Weight of uncoated cotton gauze (g)	Polymer add-on (%)
Cotton gauze	10.0	43.89±0.8

An average (±std deviation) of duplicate specimen add-on% were expressed

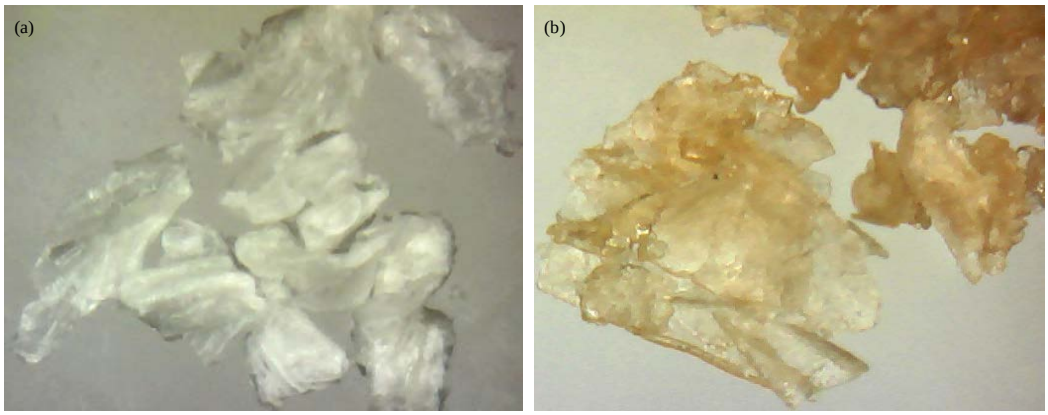

Fig. 1(a-b): Synthesis of N,O-carboxymethylated chitosan; (a) Chitosan and (b) NOCC

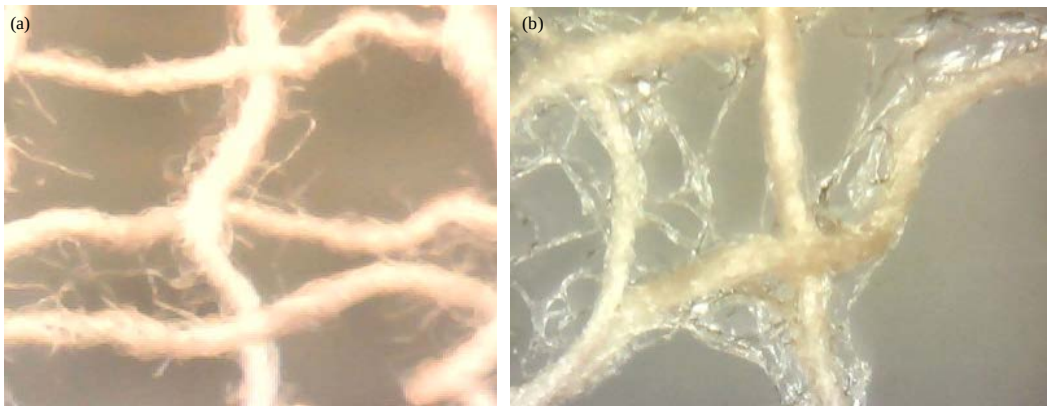

Fig. 2(a-b): Polymer coating on cotton gauze; (a) Normal gauze and (b) Polymer coated gauze

SEM analysis: SEM analysis revealed that on the NOCC-alginate functionalized cotton gauze, the presence of attached polymer particles were magnified and shown in Fig. 3. The magnification from 100X to 5000X revealed the density of the coated polymers on the surface of cotton threads. At lower magnification, the cotton fibres were observed and in higher magnifications, the polymers adhered on their surface were clearly evident.

Antibacterial property assay: The antibacterial activity analysed by EN ISO 20645:2004 test method of the coated cotton gauze was expressed as zone of inhibition diameter as an average (±standard deviation) of duplicate determination using the Eq. 2. The results tabulated in Table 3 indicate the bioefficacy of the used polymer. The test exhibits good antibacterial activity against *S. aureus* ATCC 6538 and limited effect on *E. coli* ATCC 10229 (Fig. 4). Even though coated cotton gauze was effective against *S. aureus* ATCC 6538 than *E.* coli ATCC 10229, the bacterial reduction percentage was analysed for both the pathogens using AATCC 100-2004. The direct seeding of microbial pathogens on the coated and uncoated cotton gauze enhance the microbial colonisation on the fabric surfaces. Bacterial colonized cotton gauze

Table 3: EN ISO 20645:2004 antibacterial assay method

	Inhibition zone (mm)	
Cotton guaze	*S. aureus* ATCC 6538	*E. coli* ATCC 10229
Uncoated	0 mm and no growth reduction	0 mm and no growth reduction
Coated	3.7±0.2 mm[a]	0 mm and slight growth[b]

An average (±std deviation) of duplicate agar plate values were expressed
[a] Good effect, [b] Limited effect

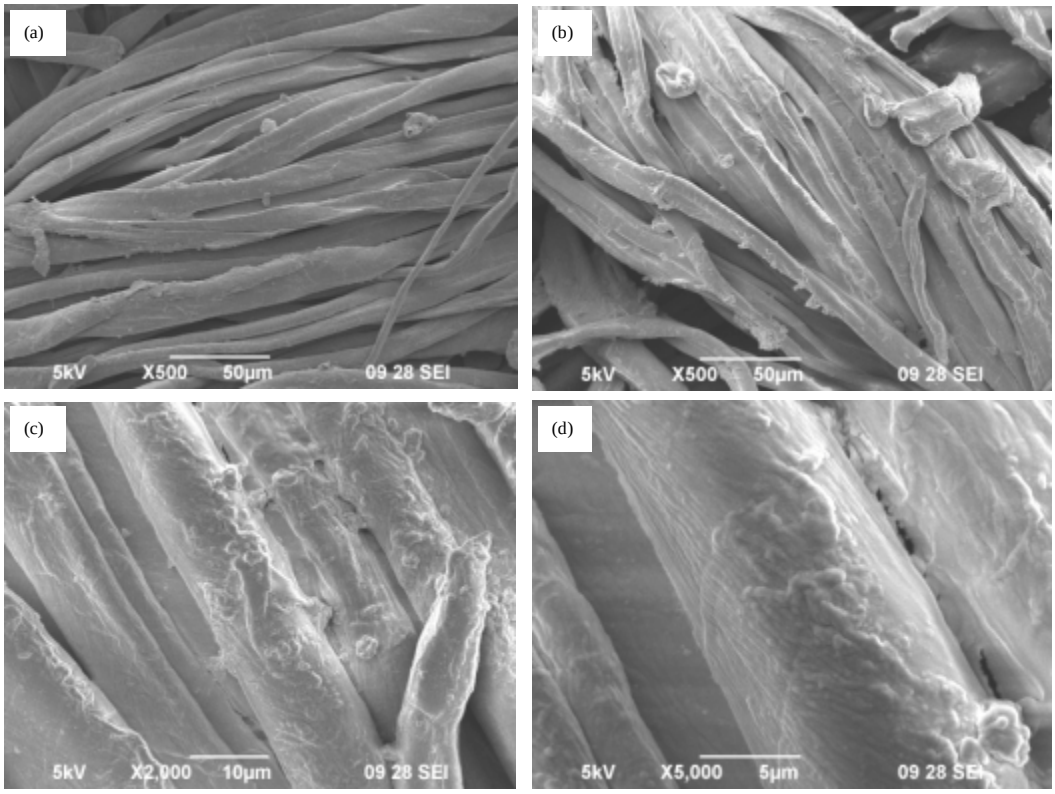

Fig. 3(a-d): SEM images of normal and coated cotton gauze; (a) Normal cotton gauze (X500), (b) Coated cotton gauze (X500), (c) Coated cotton gauze (X2000) and (d) Coated cotton gauze (X5000)

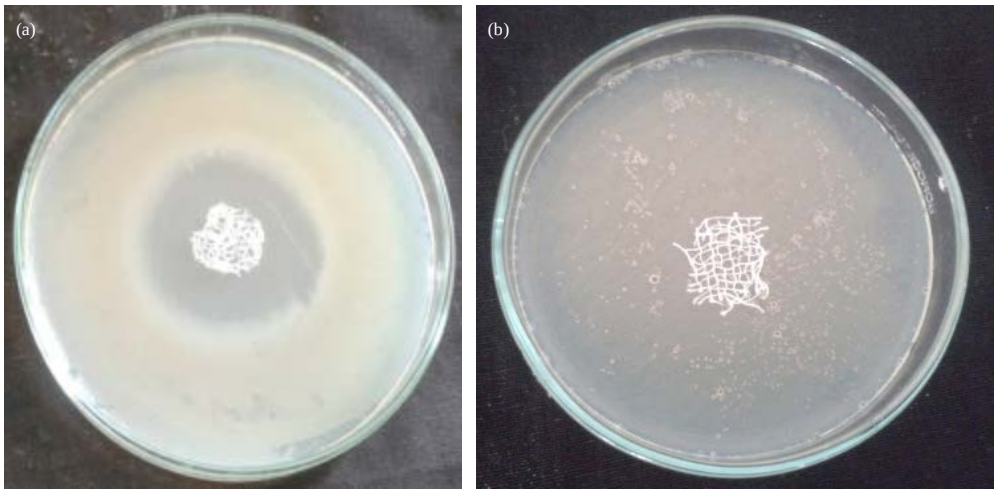

Fig. 4(a-b): EN ISO 20645:2004 antimicrobial testing of coated cotton gauze; (a) Zone of inhibition against *S. aureus* ATCC 6538 and (b) No prominent zone of inhibition against *E. coli* ATCC 10299

when used can cause infection through wounds, also spoil the fabric quality and make persistent odour. Using Eq. 3 the reduction percentage of *S. aureus* ATCC 6538 and *E.* coli ATCC 10229 in uncoated sample was found to

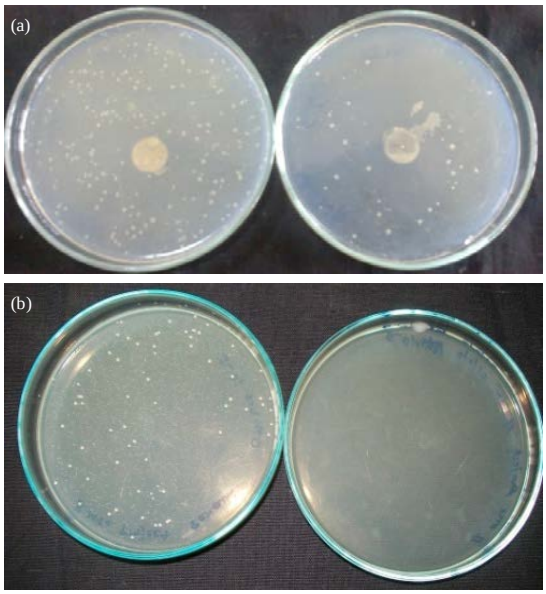

Fig. 5(a-b): Wash fastness test of coated cotton gauze; (a) 10^{-4} dilution plates of *E. coli* and *S. aureus* and (b) 10^{-5} dilution plates of *E. coli* and *S. aureus*

Table 4: Bacterial reduction percentage by wash fastness test

	Reduction of bacteria (%)						
	S. aureus ATCC 6538			*E. coli* ATCC 10229			
Cotton guaze	Before wash	Single wash	Triple wash	Before wash	Single wash	Triple wash	Chi-Square test ($p<0.05$)
Uncoated	0	0	0	0	0	0	$p>0.05$
Coated	92	85	76	80	78	71	$p<0.05$

Bacterial reduction percentage before and after laundering of cotton gauze indicates the persistence of antimicrobial ability of coated cotton

be 0 (Table 4), whereas coated cotton gauze showed 92% and 80% bacterial reduction before wash, respectively.

Wash fastness test (AATCC Test Method 124; Version-1996): The coated and uncoated cotton gauze were washed for 1st and 3rd launderings to determine whether the polymers bound to the fabric would be durable to normal launderings. After washing, the cotton gauze were sterilized and then assayed for antibacterial properties (Fig. 5). Following Eq. 3 the reduction percentage of *S. aureus* (ATCC 6538) and *E. coli* (ATCC 10229) after the first wash was 85 and 78%, respectively. After the third wash of fabric the calculated (Table 4) bacterial reduction percentage showed more than 70% for both the pathogens.

Wound healing analysis: The albino rats were grouped into 3 comprising 3 individuals each. The wound healing process was analysed by measuring the area of wound closure. The experiment was carried out in triplicate and the mean value of the wound closure area was considered for statistical analysis. From Table 5, it is evident that wound healing action is rapid using coated cotton gauze on wound. The usage of standard ointment is the next best result obtained. The uncoated cotton gauze showed the poor result comparatively (Fig. 6). The result indicates the wound healing and antibacterial function of the biopolymers coated on the cotton gauze.

Table 5: Wound healing test in animal model

	Closure of wound area (mm)			
Wound treating sample	5th day	10th day	15th day	ANOVA ($p\leq0.001$)
Teramycin ointment	8±1	7±1	5±1	$p\leq0.001$
Uncoated	9±1	7±1	6±1	$p\geq0.001$
Coated	7±1	5±1	3±1	$p\leq0.001$

The average mean values of triplicate sample result were used for statistical analysis

DISCUSSION

Chitosan is insoluble in water and hence the structure of chitosan was substituted with carboxymethyl groups instead of amino and primary hydroxyl groups by treating

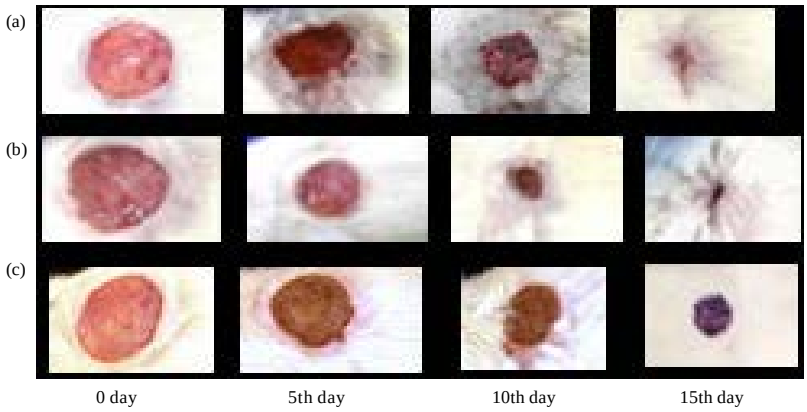

Fig. 6(a-c): Wound healing test in animal model; (a) Teramycin ointment, (b) NOCC-alginate coated cotton gauze and (c) Uncoated cotton gauze

it with monochloroacetic acid to improve its water solubility. The method used to prepare NOCC is direct alkylation method. The use of monohalocarboxylic acid in the form of monochloroacetic acid is the key compound to form N-carboxyalkyl and O-carboxyalkyl chitosan derivatives. The substitution of N- and O- alkyl groups in amino and primary hydroxyl groups of chitosan occurs at high alkali conditions attained by using NaOH along with alkylation by monochloroacetic acid (Mourya *et al*., 2010). The specimen kept under the scanning of electron beam offers the study of their surface morphology under optimal conditions. The coating of polymers on the cotton surface was observed along with the cotton fibres at higher magnifications (Gomes *et al*., 2010). This result ensures the presence of polymers on the sample. During the contact of these polymers by bacterial pathogens, the antibacterial effect can retard the bacterial growth and its colonisation. The physical contact of the coated polymers can induce wound healing by increasing the blood flow and attracting platelets to the site of wound.

Calculating the polymer add-on percentage is important to understand the efficiency of the technique used to coat the polymer on cotton gauze surface. The amount of polymer added to the cotton gauze is responsible for the antimicrobial activity; wound healing function and also the persistence of these activities for long duration. For maintaining the appropriate moisture at the vicinity of wound site, the presence of calcium alginate is essential. The dryness of the wound between the changes of bandages gives more pain and trauma to the patients (Gupta and Saxena, 2011). Alginate by contact with the wound exudates form gel through ion exchange process. The calcium ions of the polymer and the sodium ions in the wound exudate are the reasons for gel formation on the wound surface. The formed gel absorbs moisture and maintains it which is essential for wound healing. Alginate by its gel forming property makes the removal of wound dressing without trauma and reduces the pain experienced by patient. Also it provides a moist environment that leads to rapid granulation and reepithelialisation of wounded tissues (Shanmugasundaram, 2012). For maintaining the antimicrobial activity, NOCC coating in cotton gauze is essential.

According to EN ISO 20645:2004 standard test method's evaluation, ≥1-0 mm inhibition zone and no growth under specimen were accepted as effective, whereas 0 mm inhibition zone and slight growth were evaluated as limited effect (Erdem and Yurudu, 2008). Agar diffusion test is a preliminary test to detect the diffusive antimicrobial finish. Antibacterial activity of tested gauze against Gram positive cocci was effective than Gram negative rod. This resistance may be due to the complicated cell wall arrangement of Gram negative bacteria. On the other hand, the Gram positive cocci have a simple cell wall structure in which the cytoplasm membrane has a rigid peptidoglycan layer composed of networks with plenty of pores, which allow foreign molecules to enter the cell with ease. Since chitosan is comprised of membrane-active microbiostatics can exhibit inhibitory effect of disrupting the cell membrane permeability (Je and Kim, 2006).

The seeded bacterial inoculum on gauze surface coated with NOCC and alginate can exhibit interference on their colonizing ability. The resistant bacterial pathogen shows no changes in their morphology even when exposed to antimicrobial agents. The bacterial morphology has a known importance in their analysis.

The antibiotics used against the bacterial pathogens can influence morphological changes indicating its antibiotic resisting capability. When exposed to antibiotics, bacteria shows significant morphological changes like variations in size and shape. Many bacterial cells show a smaller size than usual and others are abnormally larger. The change in their morphology directly indicates its antibiotic resisting capability. Structural changes of bacterial cells are more evident when exposed to bacteriostatic doses of antibiotics than under the effect of natural antimicrobial agents like chitosan (Gomes *et al*., 2010).

The difference between the reduction percentage of uncoated and coated cotton gauze were highly significant. This indicated that the chitosan bound to the cotton fabric, exhibited its antibacterial activity and its property was not affected even when mixed with calcium alginate.

The results of wash fastness test provided the evidence for the persistence of antimicrobial activity of the polymer coated cotton gauze after washes. According to the AATCC standards, less than 50% of bacterial reduction is considered to be insignificant and more than 70% of bacterial reduction is acceptable (El-tahlawy *et al*., 2005). The results of the analysed specimen showed >70% of bacterial reduction. This ensures the resisting ability of the coated polymers on cotton gauze against the abrasive force while laundering. The persistence of the antimicrobial activity after 3rd wash shows the capability of the polymer coating method used in this study.

The wound closure ability of the test specimens in animal models were compared with the standard ointment every other day (Tarun and Gobi, 2012). The wound healing effect by polymer coated test specimen was observed in accelerated form by checking the closure of wounded area. The test specimen coated with calcium alginate along with chitosan helps to maintain moisture than the uncoated test specimen (control). The presence of alginate polymer leads to the formation of gel through ion exchange process. The gel formation is due to exchange of ions between sodium in the wound exudate and the calcium in polymer that influence moisture prevalence essential for wound healing (Shanmugasundaram, 2012). The bioactive property of chitosan affects macrophage prevalence in the wound site for improved wound healing. The other properties like bacteriostatic and fungistatic are particularly useful for wound treatment. The presence of chitosan will provide antibacterial effect which helps the wounded cells to regenerate without any microbial intervention. Hence, the presence of both sodium-calcium ions along with alginate and chitosan accelerate wound healing effect. Due to the presence of the moisture, the removal of cotton gauze in every dressing was easy and patients experience no pain and trauma. Dried cotton gauze could damage the new tissue layer formed while removing and this was avoided as it was not sticking with the wound. Healing of wound in accelerated fashion because of the antibacterial action of chitosan and moisture retaining property of alginate are the reasons behind wound healing in animal model treated with coated cotton gauze.

Wound healing in the used animal model exhibits a complex process involving series of changes. The wound was associated with slight inflammation leading to haemostasis and clot formation in the initial hours. In the first week of wound healing, fibroplasia and neovascularization occurs. This leads to the formation of granulation and re-epithelialization of tissues in the second week. At the end of second week, new extracellular matrix and tissue remodelling takes place for the wound to get heal (Diao *et al*., 2008).

CONCLUSION

Biopolymers chitosan and calcium alginate were used in this study for rapid wound healing process. As chitosan is not a water soluble polymer, chemical modification of carboxymethylation can yield N,O-carboxymethyl chitosan with high water solubility. The cotton yarns of 40^{s} Ne was used to weave wound dressing cotton gauze. The pad-dry-cure method was used to coating the biopolymers on the cotton gauze surface. The polymer add-on percentage showed efficient coating of polymers by the cotton gauze. The functionalized cotton gauze was analysed for its antibacterial activity against the bacterial pathogens *S. aureus* (ATCC 6538) and *E. coli* (ATCC 10229). The qualitative agar diffusion method and the quantitative AATCC 100-2004 methods were used to analyse the antibacterial efficiency of the coated cotton gauze. The ability of coated polymers to resist the abrasive force by laundering procedure was analysed using wash fastness test. The presence of calcium alginate enhances moisture retention in wound gauze which is essential for wound healing. This was analysed using animal models with created wounds and wound healing was observed in accelerated fashion on polymer coated cotton gauze. The alginate can form gel by ion-exhange process with wound exudates and readily soluble NOCC can avoid the microbial interference. These protective action leads to the rapid closure and healing of wounds.

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