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Bioefficacy of Biopolymer in Biomedical Cotton Finish against Nosocomial Pathogens

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ABSTRACT

The marine crustaceans shells are collected for their richness in chitin, a natural polymer when chemically deacetylated can form a derivative called chitosan, which is a promising material for biomedical applications on account of its biocompatibility, biodegradability, cellular binding capability, antimicrobial activity and wound healing effect. Extraction of chitin involves demineralization and deproteinization. In crude form, chitin has a highly ordered crystalline structure with poor solubility and low reactivity. The chitin structure can be modified by removing the acetyl groups by means of a chemical hydrolysis in concentrated alkaline solution at elevated temperature to produce a deacetylated form. When the fraction of acetylated amine groups are reduced to 40-35%, the resultant co-polymer, (1 → 4)-2-amine-2-deoxy-β-D-glucan and (1 → 4)-2-acetamide-2-deoxy-β-D-glucan, is referred to as chitosan. Blending of reactive chitosan to fabrics using an exhaust reactive dyeing method can be performed to prepare the biomedical cotton fabrics. AATCC-100 standard test method was done for quantitative antimicrobial evaluation of coated cotton fabrics against selected nosocomial pathogens. Wash fastness test (AATCC-124 test method) results ensured the bioefficacy of bound chitosan to fabrics and the persistence of antimicrobial activity to the number of washes. Colonies of bacteria recovered on the agar plate for both untreated and treated of the washed and unwashed fabrics was used to analyse the reduction percentage of bacteria.

Introduction

Chitosan is obtained by the hydrolysis of chitin and until now, many researchers have examined chitosan as a promising material for biomedical applications on account of its good biocompatibility, biodegradability, cellular binding capability, antimicrobial activity and wound healing effect (Yun Ok Kang, 2009). The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years (Jayakumar, *et al.*, 2007). Two main mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan. The second mechanism involves the inhibition of the RNA and protein synthesis by permeation into the cell nucleus (Williams, 2005).

The medical industry is challenged by the presence of microorganisms and the negative effects they cause. Deterioration, defacement and odours are all dramatic effects which occur from the microbial contamination of surfaces as varied as carpeting and medical non-woven fabrics (Syamili, *et al.*, 2012). These surfaces can also act as a microbial "harbour", as most offer ideal environments for the proliferation of microorganisms that are harmful to buildings, textiles and humans. The ability to make surfaces resistant to microbial contamination has advantages in many applications and market segments. This is especially true in medical markets where many products have contributed a degree of aseptic sophistication beyond that required of consumer products (James W. Krueger, 2003).

The transfer of gram-positive bacteria, particularly multi-resistant *Staphylococcus aureus* among patients is a growing concern (Phaechamud Thawatchai and Charoenteeraboon Juree, 2008). One critical aspect of bacterial transfer is the ability of the microorganism to survive on various common hospital surfaces. Surfaces used in medical applications have unique microbial problems and their control is a complex task (Gadi Borkow and Jeffrey Gabbay, 2010). The microbiological integrity of surfaces has been the object of numerous studies ranging from bacterial loading of carpeting to the evaluation of the barrier properties of non-woven fabrics (Alice N. Neely and Matthew P. Maley, 2000).

Advanced medical textiles are significantly developing area because of their major expansion in such fields like wound healing and controlled release, bandaging and pressure garments, implantable devices as well as medical devices, and development of new intelligent textile products (Majeti, N.V., and Ravi Kumar, 2000). Present day society is undergoing changes such as ageing of the population, increase of life span of individuals especially in Europe and US, various situations and hazards of human activity and civilization including transport accidents, chemical materials, fire, cold, diseases, sports. Such factors stimulate the rapid movement of wound care product market with the requirement of novel technique and technologies to develop modern textile materials and polymers (Liu, *et al.*, 2008). The present study was focused on

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such aspects like blending chitosan derived from chitin and blending chitosan to cotton textile for the production of biocompatible bio-medical textiles with antibacterial activity against nosocomial **pathogens.**

Materials and Methods

Crab shells were powdered, depolymerized by treatment with 3-5% of aqueous NaOH solution and the calcium was removed by treatment with an aqueous 3-5% HCl solution at room temperature to afford a white or slightly pink precipitate of chitin. The N-acetylation of chitin was done by treatment with an aqueous 40 – 45% EtOH solution and the precipitate was washed with H_2O. The crude sample was dissolved in an aqueous solution of 2% acetic acid. The remaining was insoluble material and was removed from the clear supernatant solution. This was neutralized with sterile distilled water to afford a purified sample of chitin as white precipitate.

Preparation of Chitosan

Chitin (4gm) was deacetylated with 50% (w/v) NaOH at a solid to liquid ratio of 1:20 and temperature regimes of 100ºC, 120ºC and 140ºC and in each case; the time taken to obtain soluble chitosan in 1% acetic acid was noted. The chitosan was then filtered through filter paper washed with distilled water and then neutralized with ethanol. The separated white precipitate was left to dry at room temperature and weighed.

Blending chitosan with cotton textiles using reactive dye binding method

The fabric from a commercial producer used as the uniform by the hospital personnel was the test fabric for the study. The white coloured fabric was 100% cotton, tight-weave denim-like fabric, which was commercially scoured and bleached. The fabric was cut into large squares, approximately 12cm x 12cm and ironed to remove wrinkles. Cut into 4.25 cm of length and width, the fabric samples were sterilized in an autoclave using a dry cycle.

Reactive dye binding method

An exhaust dyeing method was used to bind the freshly neutralized chitosan to the tested cotton fabric. The dye bath was prepared by adding 0.5 ml of Triton-X-100 (octylphenol ethoxylates), 75 g of sodium sulphate, and 6.5 g of the chitosan in 1.2 L of deionized water. Three, 20g weighed squares of cotton fabrics were submerged in the dye-bath and heated to 60°C. After 30 min of incubation, 12 g of Sodium hydroxide dissolved in 100 ml of deionized water was added. Then the temperature was raised to 80°C, and the fabrics were heated for another 30 min. After 30min the fabrics were rinsed in deionized water and heated for 10 min at 80°C in deionized water, then rinsed and kept in a convection oven at 105°C until dried.

Bioefficacy of biomedical cotton fabrics

Assay for antibacterial properties (AATCC 100 Method; Version-1999)

The antimicrobial activity was quantitatively evaluated against the standard strains of *Staphylococcus aureus* and *Escherichia coli* according to AATCC 100 test method. The fabric samples both treated and untreated with 4.25 ± 0.1 cm in diameter were placed in a 250 ml glass jar with screw cap and absorbed 1.0 ± 0.1 ml of bacterial inoculum. After incubation over contact periods of 24 hrs, 100 ml of sterilized distilled water was added into the jar and vortex vigorously for 1 min. The solution was then serial diluted from 10^{-1}, to 10^{-8}. The diluted solution was plated on a nutrient agar and incubated for 24 hrs at 37 ± 2°C.

Wash fastness test (AATCC Test Method 124; Version-1996)

AATCC Test Method 124-1996, proposed in the reference quoted by Chun & Gamble (2007) was used for performing the wash fastness test. The test result ensures the bioefficacy ability of the bound chitosan to cotton fabrics and the number of washes it can withstand in the textile.

AATCC Test Method 124. Version-1996

Wash condition Version 1996	
Cycle	Normal/Cotton Sturdy
Wash water temperature	60 ± 3ºC
Rinse water temperature	Less than 29ºC
Water level	18 ± 1gal
Agitation speed	179 ± 2 spm
Wash Time	12 minutes
Spin Speed	630-660 rpm
Final spin cycle	6 minute

To evaluate the durability of antibacterial effect after washing, the treated fabrics were washed according to AATCC 124-1996 test method with AATCC Standard Reference Detergent WOB (without bleaching agent). One cycle of laundering by this method is equal to five typical careful hand launderings at temperature of 40 ± 3ºC.All the treated samples were subject to 3 cycles consecutive laundering. At the end of the 3rd cycle, the samples were rinsed with warm water & air dried and tested for antibacterial activity based on AATCC 100 method (mentioned above).

Bacterial Reduction Percentage of chitosan blended cotton Colonies of bacteria recovered on the agar plate for both untreated and treated fabrics before and after washing were counted and the reduction percentage of bacteria (R) was calculated by the following equation

$$R\ (\%) = (B - A) \times 100 / B \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, A is the number of bacterial colonies from treated specimen after inoculation over 24 hr. contact period and B is the number of bacterial colonies from untreated control specimen after inoculation at 0 contact time.

Result and Discussion

The crude powder of crab shell was treated with acid, solvent and water for neutralizing the pH and removing the calcium and other unwanted minerals. The resultant powder showed neutral pH after many washes with water and was colloidal in nature with pale white colour. The deacetylated colloidal chitin prepared was done using NaOH and the solubility of this resultant chitosan powder was done at different high temperatures. The time taken for dissolving the chitosan was indicated in table-1. The complex biopolymer chitin was depolymerized using saturated salt solution and the unwanted calcium was removed by treating with aqueous HCl solution. Solvent treatment was used to N-acetylate the chitin and was neutralized with water wash. The chitin was de-acetylated using high concentrated salt solution for preparing chitosan. Its solubility checking was done at different high temperatures for finding the time it takes to dissolve in 1% acetic acid.

Table 1. Time taken for dissolving chitosan.

S. No.	Temperature	Time required to dissolve chitosan
1.	100ºC	4 min
2.	120ºC	3.5 min
3.	140ºC	2.75 min

The exhaust dye reactive method used for the fixation of biopolymers to cotton fabrics was done at the hot condition in the presence of a surfactant. The surfactant, Triton-X 100 showed much efficiency over chitosan for the firm binding and stability with cotton fibres. The exhaust dyeing reactive method proposed by Chun, D., *et al*, (2007) was followed for the fixation of biopolymers to cotton fabrics. The surfactant

used called Triton-X 100 showed much efficiency over chitosan for the firm binding and stability of them with cotton fibres (Fig.1 & 2)

Fig 1. Uncoated cotton fabric

Fig 2. Chitosan coated cotton fabric

Initial testing determined whether the reactive biopolymers would covalently bond to the cotton fabric and impart antibacterial properties to the fabric. A pilot test was done with chitosan using both *Staphylococcus aureus* and *Escherichia coli* as challenge bacteria (table-2 & 3). Using equation.1, the reduction percentage of *S. aureus* and *E. coli* in untreated fabric was found to be 0, whereas with fabric treated with reactive chitosan showed 60% and 76% bacterial reduction against *S. aureus* and *E. coli* respectively (table-4). The difference between the reduction percentage of control and the chitosan treated swatches were highly significant. This indicated that the chitosan got bound to the cotton fabric, and its antibacterial activity was not affected.

Table 2. Bioefficacy of Chitosan coated cotton against *S. aureus*.

S. No	Samples	Number of colonies			
		10^{-2}	10^{-3}	10^{-4}	10^{-5}
1	Untreated (cotton)	TNTC	TNTC	TNTC	74
2	First wash	TNTC	TNTC	TNTC	75
3	Third wash	TNTC	TNTC	TNTC	75
4	CoatedCottton	97	78	56	34
5	First wash	78	69	45	26
6	Third wash	45	37	29	19

Table 3. Bioefficacy of Chitosan coated cotton against E. coli

S. No	Sample	Number of colonies			
		10^{-2}	10^{-3}	10^{-4}	10^{-5}
1	Untreated (cotton)	TNTC	TNTC	TNTC	94
2	First wash	TNTC	TNTC	TNTC	95
3	Third wash	TNTC	TNTC	TNTC	95
4	Coated Cotton	93	80	66	54
5	First wash	71	68	47	30
6	Third wash	61	59	39	28

The large swatches of treated and untreated cotton fabric were washed 1 and 3 times to determine whether the chitosan bound to the fabric would be durable through normal washing and be persistent. After washing, the cotton swatches were sterilized and then assayed for antibacterial properties. Using equation.1, the bacterial reduction percentage after the third wash of fabric with chitosan showed 80% and 76% bacterial reduction against *S. aureus* and *E. coli* respectively (table-4). This indicated that the chitosan can withstand standard washes and without losing its antimicrobial activity can make the textile a biomedical fabric.

Table 4. Bacterial Reduction Percentages.

S. No	Samples	Reduction of bacteria (%)			
		S. aureus		*E. coli*	
		Single wash	Triple wash	Single wash	Triple wash
1	Untreated sample	0	0	0	0
2	Treated sample	>73.3%	>80%	>68.97%	>75.86%

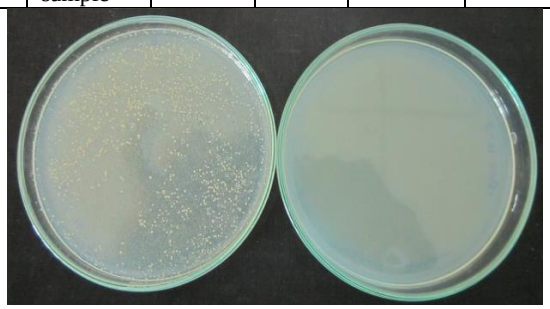

Fig 3. Wash fastness test of coated cotton gauze Dilution plates of *E. coli* processed with uncoated and coated cotton fabric

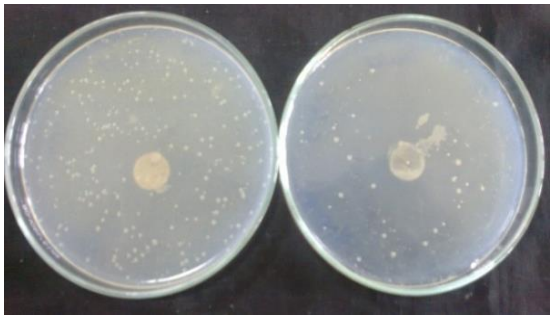

Fig 4. Wash fastness test of coated cotton gauze Dilution plates of *S. aureus* processed with uncoated and coated cotton fabric

Our present study findings agree well with the experimental data reported by Chun and Gamble (2007) and Pranee Rattanawaleedirijin *et al.*, (2008) suggesting that the biopolymers could be prepared as reactive dyes that can covalently bind to cotton fabrics. Biopolymers treated cotton fabrics displayed the antibacterial properties that persisted through 3 launderings.

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