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Fabrication of wound dressing with biocompatible polymer stabilized algal based biogenic silver nanoparticles incorporated anti-bacterial antibiotics for the improved antibacterial activity

S. Karthick Raja Namasivayam*, Arockia Mag Flora, S. Nandhini, G. Grishma, U Karthika, N. Divyasri. D. Dharani, and B. Nishanthi

Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India

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ABSTRACT

The high distribution of resistance of a wide range of microorganisms to the diverse range of antimicrobial agents is attracting a great deal of attention. Development of effective and safe drugs based on nano principles are now extensively utilized to fight against pathogenic microorganisms. Among the various route, infection through cross contamination on various objects including wound dressing causes severe health risks in hospitalized patients. In the present study, chitosan polymer stabilized silver nanoparticles incorporated levofloxacin nano drug conjugate was coated on wound dressing by pad dry and the effect of coating on mechanical properties such as tensile strength, bursting strength, air permeability and anti-bacterial activity against human pathogenic bacteria has been carried out. Surface topography of coated wound dressing was characterized by scanning electron microscopy (SEM) which revealed the complete distribution of nano conjugate on the fiber surface with the size range of 70-85nm.Further characterization by Fourier transform infrared spectroscopy (FTIR) showed characteristic modification in the absorbed peaks. Distinct changes in physical properties was not recorded except air permeability. Nano formulation coating showed increased air permeability. Anti-bacterial activity of the nano formulated drug coated wound dressing showed distinct pattern of inhibition.

Keywords: Nano formulation, Wound dressing, Anti-bacterial, Mechanical properties, Inhibition

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INTRODUCTION

Severe bacterial wound infections as gas gangrene and tetanus through open wounds is now being considered as life-threatening illness in the various parts of the world. Wound infection can become a serious concern if injured patients get late definitive care or if the number of injured persons exceeds available trauma care capacity [1]. Hence, wound management is very important to reduce the infection and mortality rate which will lead to develop a more suitable wound dressing material [2]. The use of antimicrobial agents for textiles including wound dressing has also become indispensable to avoid cross-infection by pathogenic microorganisms, to control the infestation by microbes, and arrest metabolism in microbes to reduce odour formation. Recently, an awareness of general sanitation, contact disease transmission, and personal protection has led to the development of antibacterial fibers to

protect wearers against the spread of bacteria and diseases rather than to protect the quality and durability of the dressing [3,4]. Functional finishes on textile fabrics are of critical importance to improve textile products with multifunctional properties such as antibacterial activity, UV protection, and wrinkle free properties. Antibacterial agents were used on textiles thousands of years ago, when ancient Egyptians used spices and herbs as preservatives in mummy wraps. Biocompatible eco-friendly polymer based scaffolds are used for wound dressing and in other fields like tissue engineering, and drug delivery [5].

The research interest for the use of nanotechnology in the textile industry has increased rapidly due to the fact that textile fabrics are some of the best platforms for deploying nanotechnology [6]. During the past few decades there has been an increasing interest in the development of biodegradable nanoparticles for effective drug, peptide, protein, and DNA delivery. Incorporation of the drug into a particulate carrier can protect the active substance against degradation in vivo and in vitro, improve therapeutic effect, prolong biological activity, control drug release rate, and decrease administration frequency[7] (Chen et al, 2011).The rapid expansion of nanotechnology promises to have great benefits for society that increase the residence time of drugs on mucosal membranes and subsequently enhance the bioavailability of drugs with poor oral absorption [8].

With the advent of nanotechnology, new area has developed in the realm of textile finishing. These textiles can be widely used for hygienic clothing, wound healing, and medical applications in hospitals and other places where bacteria present a hazard [9,] (Enhancement of textile materials by nanotechnology is expected to become a trillion dollar industry in the next decade with tremendous technological, economic and ecologic benefits [10].

Among the various metallic nanoparticles ,silver nanoparticles commonly used for nanomedicine production, are reported to be nontoxic to human, but most effective against bacteria, viruses, and other eukaryotic microorganisms at very low concentration They are also effective against tumors with anti-proliferative activity [11].Silver metal and its compound have been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities [12] Silver ions work against bacteria in a number of ways; silver ions interact with the thiol groups of enzyme and proteins that are important for the bacterial respiration and the transport of important substance across the cell membrane and within the cell and silver ions are bound to the bacterial cell wall and outer bacterial cell, altering the function of the bacterial cell membrane thus silver metal and its compounds were the effective preventing infection of the wound [13-15]. Silver nanoparticles have a high specific surface area and a high fraction of surface atoms that lead to high antimicrobial activity compared to bulk silver metal The antimicrobial property allows them to be suitably employed in numerous products such as textiles, food storage containers, home appliances and especially in medical devices Use of silver nanoparticles is in medicine industry as tropical ointments to prevent infection against burn and open wounds is quite effective [16].Taken together, silver nanoparticles may be considered for combination therapy against pathogenic microorganism due to its potential synergistic effect with important antibiotics. These particles can be incorporated in several kind of materials such as cloths. These cloths with metallic nanoparticles are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus* [17]. Stabilization of nanoparticles is necessary for their stability, functionality, and biocompatibility and also essential to preserve the properties of the free nanoparticles and nano conjugates. In the present study, levofloxacin- an anti bacterial

antibiotic highly effective against human pathogenic bacteria has been selected for chitosan mediated stabilization with silver nanoparticles .By the process of chitosan stabilization, the activity of the drug will increase by allowing sustained or controlled release of the drug and thus increase improved anti-bacterial activity [18].In this point of view, chitosan stabilized silver nanoparticles-levofloxacin nano drug conjugate was coated on the wound dressing and the effect of coating on the mechanical properties and anti-bacterial activity has been carried out.

MATERIALS AND METHODS

Reagents and Chemicals

Chemicals and reagents used in the study were of analytical grade. Culture media used in microbial study were of reagent grade purchased from Hi media, Mumbai, India. All the other chemicals used in this study were purchased from Merck, India.

Synthesis of silver nanoparticles

Silver nanoparticles used in the present study was synthesized from cold resistance strain of *Spirullina platensis* biomass as described in our previous work [19]. (Synthesized and purified particles were lyophilized and used for further studies.

Preparation of chitosan stabilized levofloxacin-silver nano drug conjugate (Cs-AgNp-Lf)

In the present study, chitosan was used as stabilizer for the preparation of nano drug conjugate. In a typical procedure of chitosan stabilized nano drug conjugate synthesis, 2.5 ml of silver nano suspension prepared from original stock and equal volumes of 0.01% of chitosan and 0.01% of levofloxacin were suspended in 100ml of deionized water and kept under magnetic –stirring for three hours at 30°C. Slurry thus obtained was lyophilized and stored in screw capped vial. Characterization carried out with Fourier transform infrared spectroscopy (FT-IR) and Scanning electron microscope. FT-IR was carried out with KBr palletized dried sample in the range of 4000-500 cm^{-1} using Bruker OpticGmbH Tensor 27. Particle morphology (Shape and size) and elemental composition was studied by field emission scanning electron microscopy equipped with energy dispersive X-ray analysis (FESEM–EDAX) was performed by SUPRA 55-CARL ZEISS, Germany.

Anti bacterial activity

Anti bacterial activity of nano drug conjugate was studied by micro dilution colorimetric liquid broth assay and agar diffusion assay.

Bacterial strains

Antibacterial activity of nano drug conjugate was tested against *Staphylococcus aureus*, *Streptococcus pyogens*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were obtained from Microbial Type Culture Collection, Chandigarh, India. Respective bacterial strain was maintained on trypticase soy agar (TSA) slant at 4°C.

Inoculum preparation

A loopful of respective bacterial culture was inoculated from the TSA slant into trypticas soy broth, incubated overnight on a rotary shaker (200 rpm) at 35°C. The inoculums were prepared by diluting the overnight cultures with 0.9% sterile saline to a 0.5 McFarland units standard.

Micro dilution colorimetric liquid broth assay

Liquid broth micro dilution assay using chromogenic reagent 3-(4,5-dimethyl thiazol-2-yl)-2-5-dephenyl tetrazolium bromide (MTT). The Minimum inhibitory concentration (MIC) value was defined as the lowest sample concentration that inhibited visible growth of the test bacterium, as indicated by MTT straining.

Coating of chitosan stabilized nano drug conjugate

Nano drug conjugate was coated on the wound dressing using pad-dry-cure method [12]/Sterile wound dressing obtained from the health care centre was cut to the size of 10×10 mm, (made up of cellulose fiber) immersed in minimal inhibitory concentration (MIC) of nano drug conjugate suspension followed by curing at respective temperature and time. Residual unbound nano conjugate was removed by washing with sodium lauryl sulphate followed by successive washing with sterile distilled water to remove detergent solution. Washed fabric was air-dried and dried pieces were used for further studies. Characterization of coated fabric was done by scanning electron microscopy and Fourier transform infrared spectroscopy.

Effect of nano coating on mechanical properties

The coated fabrics were examined in the range of basic physical–mechanical parameters followed by its functional properties. The specimen were conditioned at 65 ± 2% relative humidity and 27 ± 2°C before analysis. The tensile strength was carried out in automated materials testing system. The bursting strength was tested on the bursting strength tester by applying the multidirectional load. Simultaneously, air permeability was tested in air permeability tester (Kato Tech. Co. Ltd, Japan. Initially, the air resistance (Pa.s/m) is measured and its inverse gave the air permeability (m/Pa.s). For friction measurements, The results were expressed as coefficient of friction (m), which is the ratio of frictional resistance and the normal load.

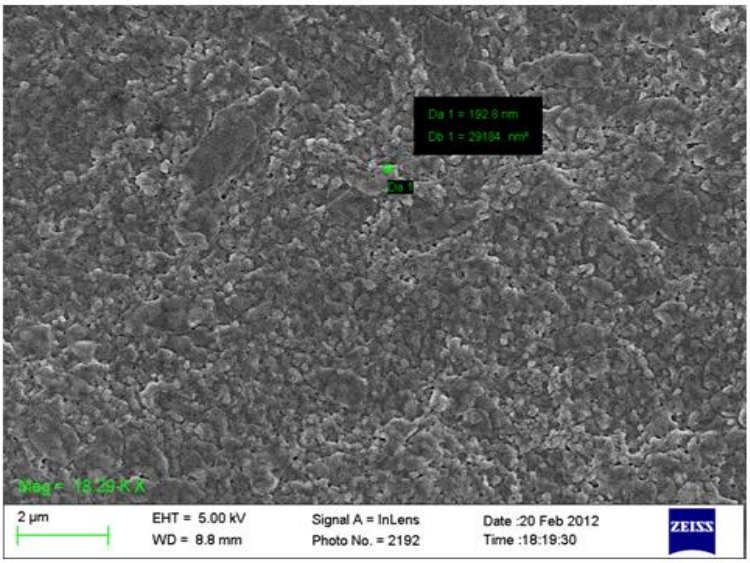
Anti-bacterial activity

Anti-bacterial activity of the coated fabric was done using agar diffusion assay. Bacterial strains and inocula preparation was carried out as described earlier.0.1 ml of the respective bacterial inocula (10^6 CFU/ml) thus prepared was spread on the sterile Muller Hinton agar (Hi media, Mumbai, India) using sterile cotton swabs and the seeded plates were incubated at 37°C for 24 h. After the incubation period, the plates were observed for zone of inhibition.

RESULT AND DISCUSSION

In the present study, silver nanoparticles synthesized from *S.platensis* has been formulated with chitosan coated levofloxacin nano drug conjugate and the prepared nano drug conjugate was coated on the wound dressing. Effect of coating on mechanical properties and anti-bacterial activity was studied. Silver nanoparticles were synthesized from cold resistance strain of *S. platensis* used in the present study was characterized by various techniques as described earlier. Plasmon absorption maxima by UV visible spectrophotometer, particles morphology by SEM ,elemental composition by EDAX, crystallinity and the lattice properties by XRD, functional groups determination by FTIR revealed the synthesized particles were nano dimensional uniform monodispersive particles [19]..Chitosan stabilized levofloxacin-silver nanoparticles conjugate was primarily confirmed by colour change of the reaction mixture from dark brown to pale yellow, scanning electron microscopy analysis and FT-IR The scanning electron microscopy study reveals chitosan stabilized levofloxacin-silver nano drug conjugate as spherical particles with the size range of 70 to 80nm (Figure 1). Such size distribution analysis of antibiotic nanoparticle conjugates confirms that the

particles are well dispersed. FT-IR analysis helps to detect the functional groups, structure of a compound and purity of the sample in a given environment in terms of frequencies of radiation present in the nanoparticles which showed characteristic pattern of absorption peaks (Figure 2).



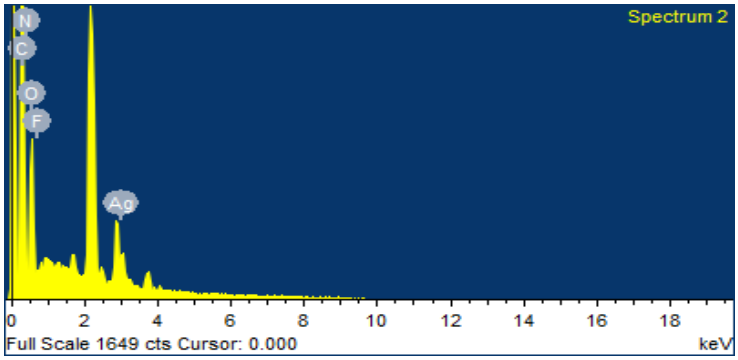


Figure-1: SEM (a) and EDAX spectra (b) image of nano drug conjugate

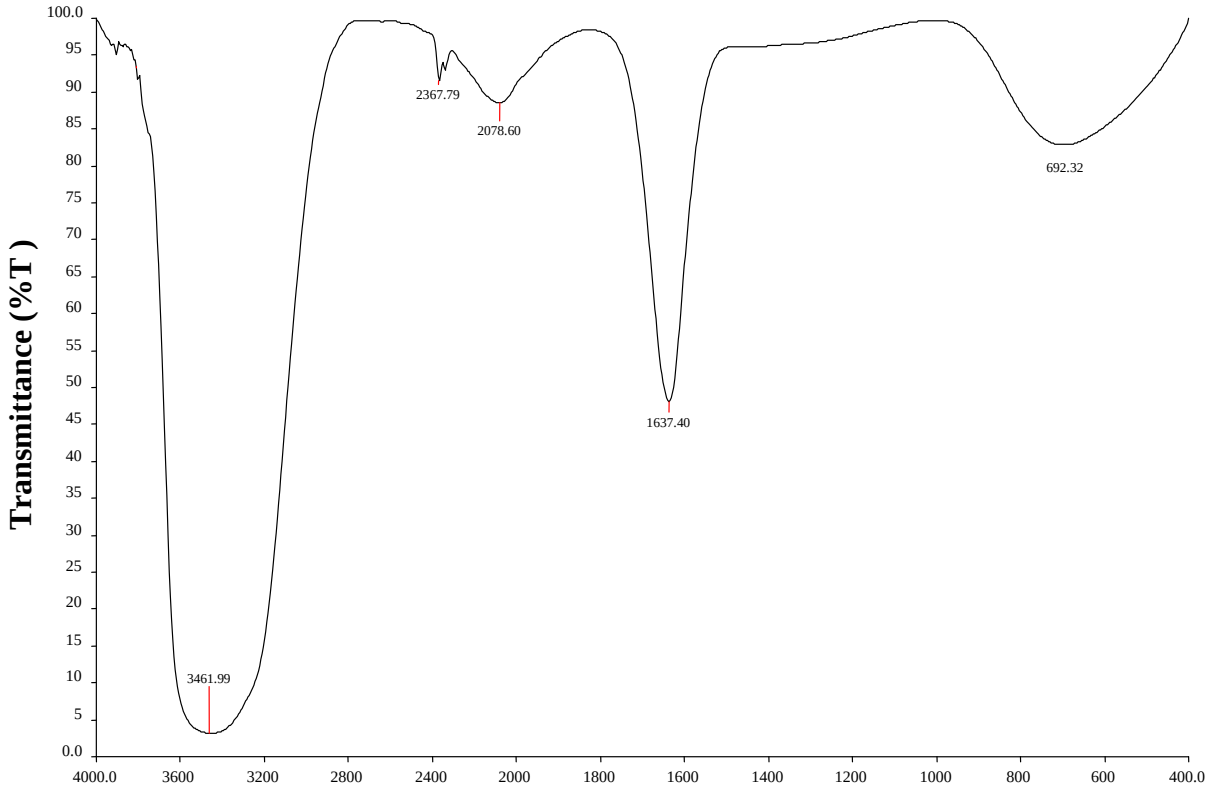


Figure-2: FTIR spectra of nano drug conjugate

Synthesized nano drug conjugate was coated on the wound dressing by pad dry cure method. Characterization of the coated material was carried out by SEM and FTIR. Surface topography of the wound dressing studied by SEM showed uniform dispersion of the nano conjugate particles finely embedded on the fibre surface with the size range of 70-85nm (Figure 3). Further confirmation by FTIR analysis. When the FTIR spectra of control and nano drug conjugate coated dressing were compared, it is clear that all the absorbed peaks were changed upon nano drug conjugate coating (Figure 4).

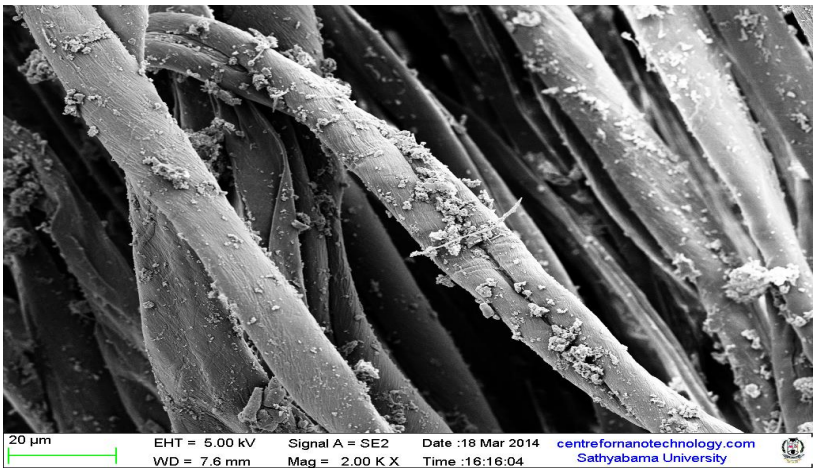


Figure-3: SEM image of nano drug conjugate coating on wound dressing

(a) *Stap.aureus*

(b) *Strep.pyogenes*

(c) *P.aeruginosa*

(d) *K.pneumoniae*

Figure-4: Zone of inhibition of nano drug conjugate coated wound dressing against pathogenic bacteria

Effect of nano drug conjugate on the mechanical properties clearly revealed distinct changes in mechanical and anti-bacterial properties (Table 3-6). Nano coating revealed increased air permeability. Air permeability plays a major role in the function of textile materials used to provide an indication of the breathability of coated fabrics. Uniform distribution and nano size of nano conjugate improved air permeability and hence its breathability. But, nano coating did not cause any change in bursting strength. coefficient of friction is the parameter most frequently used to evaluate degrees of smoothness or roughness, so it is important to match this quantity with the tactile feel of the fabrics studied [2]. Earlier studies on friction mostly dealt with the frictional properties of cotton fibres and chemically finished fabrics. frictional properties of the nano drug conjugate coated dressing has been shown in table which reveals friction was significantly lower than control (P=1%) because of the nano size and uniform distribution of the nano drug conjugate. Antibacterial activity of cotton fabrics impregnated with silver nanoparticles against *Staph.aureus* synthesized from *Fusarium oxysporum* was reported Tensile strength, bursting strength and air permeability was studied to determine nano drug conjugate mediated influence on fabric mechanical properties.

From the Table 1-4 it is clear that tensile strength of the fabric in warp-direction was found to be reduced. But distinct changes was not recorded in fabric weft direction and the percentage of strain was reduced in both warp and weft directions. Nano coating revealed increased air permeability. But, nano coating did not cause any change in bursting strength. coefficient of friction is the parameter most frequently used to evaluate degrees of smoothness or roughness, so it is important to match this quantity with the tactile feel of the fabrics studied.

Table: 1. Effect of nano drug conjugate coating on the tensile strength of wound dressing

Treatment	Av. Breaking load (kg)		Strain (%)	
	Warp	Weft	Warp	Weft
Control	26.1	22.1	21.1	18
Nano drug conjugate	17.3[a]	21.3	12.3[a]	11.2[a]
Column carries alphabet is statistically significant at 5 % level by DMRT				

Table: 2. Effect of nano drug conjugate coating on the air permeability [m/(kPa.s)

Treatment	air permeability [m/(kPa.s)
Control	11.23
Nano drug conjugate	15.21 [a]
Column carries alphabet is statistically significant at 5 % level by DMRT	

Table: 3. Effect of nano drug conjugate coating on the burst strength (kg/cm^2)

Treatment	Burst strength (kg/cm^2)
Control	7.21
Nano drug conjugate	7.02
Mean value is not statistically significant by DMRT	

Table: 4. Effect of nano drug conjugate coating on coefficient of friction of wound dressing

Treatment	fabric to fabric		fabric to metal	
	Warp	Weft	Warp	Weft
Control	2.121	2.034	0.542	0.541
Nano drug conjugate	2.142	2.043	0.582	0.589
Mean value is not statistically significant by DMRT				

Anti-bacterial activity of the nano drug conjugate was studied by determination of minimum inhibitory concentration (MIC) using MTT assay.MIC of the nanoparticles against the tested bacterial strains was studied by broth dilution method. MIC values of nano drug conjugate against all the tested bacterial strains were lesser than free antibiotic. It can be seen that nano drug conjugate showed high antibacterial efficacy (Table 5). Agar diffusion assay was employed to evaluate anti-bacterial activity of the nano drug conjugate. All the tested bacteria were found to be susceptible. Among the tested bacterial strains, maximum inhibitory effect was recorded in *Pseudomonas aeruginosa* and *Staphylococcus aureus* with 30.0 and 29.0 mm of zone of inhibition. It is also observed that the zone of inhibition of *Streptococcus pyogenes* and *Klebsiella pneumonia* was very lesser than *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Similar finding was recorded in coated fabric treatment Table 6.

Table: 5. MIC (mg/ml) and MLC (mg/ml) of nano drug conjugate against the tested bacterial strains

Treatment	Tested bacteria	MIC	MLC
Free AgNps	*Staphylococcus aureus*	0.512	0.621
Free levofloxacin		0.92	0.987
Free chitosan		0.0	0.0
Nano drug conjugate		0.121[a]	0.081[a]
Free AgNps	*Streptococcus pyogenes*	4.421	5.213
Free levofloxacin		1.211	1.012
Free chitosan		0.0	0.0
Nano drug conjugate		0.991[a]	0.934[a]
Free AgNps	*P.aeruginosa*	5.812	5.213
Free levofloxacin		4.012	5.012
Free chitosan		0.0	0.0
Nano drug conjugate		1.121[a]	.213[a]
Free AgNps	*K.pneumoniae*	9.121	8.021
Free levofloxacin		3,210	4.012
Free chitosan		0.0	0.0
Nano drug conjugate		2.123[a]	2.213[a]
In column, mean carrying the alphabet is statistically significant at 5 % level by DMRT			

Table: 6. Zone of inhibition (mm) of nano drug conjugate against tested human pathogenic bacteria

Tested bacteria	Zone of inhibition (mm) Nano drug conjugate	Coated dressing
Staph.aureus	29	31.5
Strep.pyogenes	27	29.4
P.aeruginosa	30	31
K.pneumoniae	23	23.4
Mean values are not statistically significant by DMRT		

CONCLUSION

Design of anti microbial wound dressing based on biocompatible nanoparticles will be highly recommend in medicine to fight against skin infection causing bacteria known to cause high mortality. Toxicity studies will be studied to confirm the biocompatibility in future, development of wound dressing will form a new revolution in medicine.

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