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Antimicrobial Cotton Fibre Coated with UV Cured Colloidal Natural Rubber Latex: A Sustainable Material

Sovan Lal Banerjee^a, Prasad Potluri^b, Nikhil K Singha^{a*}

^aRubber Technology Centre, Indian Institute of Technology, Kharagpur-721302, India.

^bSchool of Materials, The University of Manchester, Manchester, M13 9PL UK

*- Corresponding Authors Email: nks@rtc.iitkgp.ernet.in (Prof. Nikhil K. Singha)

Graphical Abstract

Abstract

This work reports a simplistic approach to develop an antimicrobial coating material based on natural rubber latex (NRL). Here, crosslinking of the NRL (cis 1,4-poly(isoprene)) was carried out utilizing “thiol-ene chemistry” using trimethylolpropane tris(3-mercaptopropionate) (trithiol) as a crosslinker and Irgacure-184 as a photoinitiator under UV irradiation. This process was utilized to prepare an antimicrobial NRL coated cotton fibre via adopting a dip coating method and subsequent curing. Prior to the coating on the cotton fibre and curing of NRL, it was mixed with modified chitosan (Cts) stabilized silver nanoparticles (Ag NPs) at a pH 7.4 (physiological pH). Presence of Ag NPs incorporated cured NRL coating over the cotton fibre, significantly increases its elongation at break up to 25% as compared to the pure cotton fibre (14%). It also increases the hydrophobicity (Water contact angle = 102°) of the fibre. The as-prepared fibre exhibits superior antimicrobial activity against both Gram-negative and Gram-positive bacteria and non-cytotoxic tested over the NIH 3T3 fibroblast cell line which indicates its potential application in the antimicrobial textile.

Keywords: Natural rubber latex, thiol-ene chemistry, chitosan-graft-poly(acrylamide), silver nanoparticles, antimicrobial activity.

Introduction

Human body always has a tendency to be affected by microorganisms like bacteria, virus, yeast, fungus etc.[1, 2] During last few decades, significant research work has been conducted by the researchers to generate antimicrobial coating materials for garments and medical devices that can efficiently inhibit the bacterial attack without affecting the human body.[3] Cotton fibres are generally utilised to prepare textiles for medical applications like healthcare products, non-implantable surgical materials etc.[4, 5] Cotton is a natural fibre having a composition of 1,4-*D*-glucopyranose as a repeating unit.[6] Due to the interesting properties like high sweat absorbing ability and comfort, cotton fibre is extensively used in garment industries as well as in the medical sector. But due to this high water absorbing capacity, the cotton is very susceptible to microbial attack at a specific temperature and moisture content.[7] The main reason behind antibacterial coating is to avoid the injury-induced infections, to suppress the bad odour of the textile materials and to protect the textile materials from deterioration that can cause by the microbial attack.[5, 8] In recent days along with the chemical modifications of the fibre specially ionic modifications [9], researchers use nanotechnology in different fields of applications like coating [10], hydrogel [11], medicine

[12], water purification [13] that can provide substantial effects in antimicrobial action. Among this, Ag NPs attract the significant interest to the researchers compared to the ionic modified fibre due to its tunable microbes inhibitory property depending on the shape and size of the nano-particle [14-16]. Ag NPs can be prepared by using many methods like chemical reduction [17, 18], thermal decomposition [19], UV irradiation [20, 21], plant extract driven reduction [22] etc. Here we have used chitosan as a bio-reducing agent to prepare nanoparticles. Chitosan is a natural polymer consisting of *D*-glucosamine and *N*-acetyl-*D*-glucosamine subunits connected by (1-4) glycosidic bonds [23] and have antimicrobial property depending on the molecular weight of the chitosan [24]. However, chitosan has poor water solubility at pH 7 and in basic pH as its pKa value is 6.5 [25] which is acidic in nature and in many body contact applications like biomedicine, textile, cosmetics etc. it may be detrimental.

In the present investigation, we have mixed the aqueous solution of modified chitosan stabilized Ag NPs to NRL, which is a creamy colloid extracted from *Haevia brasiliensis* tree to prepare a colloidal NRL/Ag NPs. NRL generally contains 45 wt % of rubber molecules (cis-1,4-polyisoprene), 50 wt % of water and 5 wt % of other constituents like protein, carbohydrates and lipids etc. [26]. The latex particles have a size ranging between 0.2-5 μm , consisting of the cis-1,4-polyisoprene core which is covered with a thin layer of adsorbed protein molecules [27]. NRL is widely used in various industrial applications such as tires, automobile, shoes etc. and in biomedical applications such as surgical gloves, dental application, wound dressing patch etc. due to its high mechanical strength as well as angiogenic properties [28, 29] and hydrophobicity. NRL is also used to accelerate skin regeneration [30]. Modified NRL is used for reduction in protein adsorption and platelet adhesion [31]. But it is very much prone to microbial attack due to the presence of proteins. Apart from this, proteins frequently cause problems related to allergic reactions (Type 1 allergy) in contact with the human body and thus increase the tendency of microbial attack. The presence of activators and accelerators, used in the vulcanization of NRL also causes Type IV allergy to the human skin [32]. Few other curing processes like electron beam curing, peroxide-induced curing etc. can be an alternative way but the main disadvantages of these curing processes are high production cost and high operational safety. In this case, we have used, UV-irradiation, a green curing process. UV curing has many advantages over the conventional curing systems used in elastomer as like sulfur or peroxide which require toxic and allergenic accelerators and co-agents [32]. UV-curing is a greener and

cheaper process [33]. It is reported that the product obtained using UV-curing has excellent skin compatibility [34].

Here in, we have used a simplistic approach to prepare hydrophobic and antimicrobial cotton fibre having improved mechanical property compared to the uncoated cotton fibre using a composite latex solution of Cts/Ag NPs/NRL. UV-curing, a green curing technique was utilized to crosslink the NRL using trimethylolpropane tris(3-mercaptopropionate) (trithiol) as a crosslinker via “thiol-ene” reaction. Use of UV-curing system instead of conventional vulcanization systems using sulfur compounds or peroxide compounds makes this system a non-toxic one. To the best of our knowledge, this kind of approach has not been reported for the antimicrobial cotton-NRL composite.

Materials and methods:

Materials used for this work has been explained in the supporting section.

The preparation of the Cts-g-PAAm followed by the synthesis of Cts-g-PAAm stabilized Ag NPs was carried out by following our previously reported work[35] and has been explained in the supporting information.

Preparation of Cts-g-PAAm/Ag NPs incorporated NRL:

The prepared Cts-g-PAAm stabilized Ag NPs (concentration of Ag NPs is approximately 40 μ M) solution was mixed with NRL (40% dry rubber content) in a 50 ml conical flask at different volume ratios of 1:3, 1:1, 3:1 (named as AMNRL-3, AMNRL-2 and AMNRL-1 respectively) to maintain a total batch volume of 10 ml followed by homogenization for 1 h under constant stirring. As pure NRL was hydrophobic in nature, the different volume ratio of Cts-g-PAAm/Ag NPs and NRL was taken to study the change in hydrophobicity of the coating material with variation in Cts-g-PAAm/Ag NPs content. After 1 h, the obtained colloidal solution was characterized by FT-IR, DLS, FESEM, HRTEM and AFM analyses.

Antimicrobial natural rubber latex (AMNRL) impregnation in cotton fibre and subsequent UV curing using thiol-ene reaction:

For the preparation of Ag NPs impregnated cotton fibres, a pre-weighed (100 mg) cotton fibres were immersed into 2 ml of Ag NPs incorporated latex solution (3:1, 1:1, 1:3 volume

ratio of NRL and Cts-g-PAAm/Ag NPs) containing trimethylolpropane tris(3-mercaptopropionate) (crosslinker) (0.032 g, 4 wt% w.r.t the NRL content) and Irgacure-184 (UV curing initiator) (0.032 g, 4 wt% w.r.t the NRL content) for 24 h at room temperature followed by the curing of the coated fibres inside the UV chamber. This process enables a homogenous coating of the latex over the cotton fibres. After that, the cotton fibres were immersed in a buffer solution of pH 7.4 for 1 h to remove the excess unbounded coating material.

Characterizations of the prepared materials have been explained in the supporting information section.

Water uptake study:

About 15 mg of the uncoated cotton fibres and AMNRL coated cotton fibres containing different volume ratios of NRL: Ag NPs (3:1, 1:1 and 1:3) (both cured and uncured) were taken for the water uptake study. The samples were immersed in a pH 7.4 buffer solution at room temperature. The increment in the weight of the dipped cotton fibre was noticed after specific time intervals until the equilibrium was reached. The mole percent uptake of the water (Q_e) by 100 g of the cotton fibres at equilibrium can be expressed as follows-

$$Q_e = \frac{M_e/M_r}{M_i} \times 100$$

Where M_e was the mass of water (g) at equilibrium, M_r was the relative molecular mass of the water (18 g), and M_i was the initial mass (g) of the sample.

Mechanical properties:

The tensile strength of the cotton fibre and Ag NPs/NRL loaded cotton fibre (both cured and uncured) was determined using Universal Testing Machine (LLOYD model no LR10k Plus) at a crosshead speed of 10 mm/min at 25°C and at 65% relative humidity. Ag NPs impregnated, and non-impregnated cotton fibre was cut into a gauge length of 5 cm. All the tensile parameters and % elongation at break were measured using a 10 kg load cell.

Antimicrobial assay:

Qualitative analysis of the antimicrobial activity of the cotton fibre and Ag NPs loaded cotton fibre (after curing) was carried out by using the inhibition zone method. A lysogeny

broth (LB)-agar plate was prepared for each sample by taking tryptone (1 g), yeast extract (0.5 g), NaCl (1 g) and water (100 mL) followed by mixing of all ingredients. The pH was adjusted to 7.0. Agar (2 g) was added to the solution before autoclave sterilization. In this study, *Escherichia coli* (*E. coli*) (Gram-negative), *Staphylococcus aureus* (*S. aureus*) (Gram-positive), *Bacillus licheniformis* (*B. licheniformis*) (Gram-positive) were taken as model microbes. Bacterial samples were seeded on the LB-agar plate individually by pour plate technique. For this study, both types of fibres (cotton fibre and Ag NPs/NRL impregnated cured cotton fibres) were cut into small pieces (1mm thick and 1cm length) and placed into the plate followed by incubation at 37°C for 24 h. The antimicrobial activity was tested using a modified agar diffusion assay (disc diffusion method). The presence of the inhibition zone around the fibre on the plate was measured using a zone measurement scale (maximum length of the zone of inhibition was taken).

Determination of minimum inhibitory concentration (MIC):

To determine the MIC of the prepared Ag NPs doped NRL against *S. aureus*, a previously adapted protocol by Wiegand et al. was used with slight modification [36]. We took cured cotton fibre of 1 cm length and 1 mm diameter coated with different compositions of AMNRL (NRL : Ag NPs volume ratios of 3:1, 1:1, 1:3 respectively) in separate test tubes. In each test tube, bacterial suspension in nutrient broth containing 10⁶ colonies forming unit ((CFU)/ml) was added and incubated at 37°C for 24 h. Control tubes were maintained without AMNRL coated cotton fibre. After 24 h, MIC value was determined by observing the visual turbidity of the broth. The test tube with no turbidity is indicating no bacteria growth, and it was taken as MIC.

Morphological analysis of the bacteria:

For the morphological characterization of the bacteria, Log phase bacteria cells were inoculated into fresh LB medium having Ag NPs/NRL coated cotton fibre (1:1 volume ratio) as a positive control and only NRL coated cotton fibre as a negative control. After that, the whole solution was incubated for 3 h at 37°C in a shaking incubator. Bacterial cells were collected via centrifugation and then washed thrice with PBS (pH = 7.4) buffer. The isolated bacterial cells were then fixed with 2.5% glutaraldehyde solution for 2 h, followed by sequential washing with 50, 70, 85, 90, and 100% ethanol for 10 min to dehydrate the whole system. Before SEM imaging, all the samples were gold sputter-coated [37].

***In-vitro* cytotoxicity assay:**

The *in vitro* cell cytotoxicity assay was studied over the NIH 3T3 fibroblast cell line. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colourimetric assay was utilized to monitor the alternation of the metabolic activity of the cells in the presence of the AMNRL as a positive control and only NRL as a negative control. Under the defined condition, oxidoreductase enzyme can reduce the tetrazolium dye MTT, to generate an insoluble purple coloured formazan salt. The obtained formazan salt was dissolved in DMSO, and the O.D was measured at 590 nm to determine the cell viability of the samples. For the MTT assay analysis, Fibroblast cells were seeded into the 96-well plate at a concentration of 1×10^4 cells per well using 180 μ l of Dulbecco's Modified Eagle's Medium (DMEM) having 10% Fetal Bovine Serum (FBS) and 1% antibiotic. Then a phosphate buffer solution (PBS) containing AMNRL (positive control) was added to the culture medium, and the medium was allowed to culture for 48 h in the presence of 5% CO₂ under a humidified atmosphere. The same process was applied to NRL solution, negative control. After that, the culture plate was washed with PBS and the solution of MTT (1 mg/ml) was added to the culture followed by incubation for 4 h at 37 °C. After completion of the incubation, the supernatant was removed, and DMSO was added to dissolve the insoluble formazan salt. The O.D. value of the purple solution was measured at a wavelength of 590 nm. The relative cell viability of the experimented material against the NIH 3T3 fibroblast cell was calculated using the following equation [35]:

$$\text{Cell viability (\%)} = \frac{\text{O. D.}_{590} (\text{sample})}{\text{O. D.}_{590} (\text{control})}$$

Statistical analysis

Mechanical testing, antimicrobial activity and the cell cytotoxicity analyses were carried out in triplicates, and in all case, a triplicate counts $\pm$ standard deviation was reported. To compare the statistical significance of the test samples against the control. student's t-test was carried out.

Results and discussion:

In this investigation, acrylamide was polymerized on Cts surface via grafting from technique using APS as a thermal initiator in an aqueous solution (**Sch. S1**). The obtained grafting % was 74%. The as-prepared Cts-g-PAAm was used to prepare Ag NPs by reducing the aqueous solution of AgNO₃. The resultant solution was then mixed with NRL (containing

trimethylolpropane tris(3-mercaptopropionate, crosslinker and Irgacure-184, UV curing initiator) in different volume ratio. The mixture was applied over the cotton fibre via dip coating method and cured subsequently under UV light. The probable steps of photo-triggered curing of the NRL in the presence of trithiol and Irgacure-184 are schematically represented in **Sch. 1**. In the initiation step, Irgacure-184 undergoes bond cleavage in the presence of the UV light to form the benzoyl radicals as shown in the **Sch. 1**. After that, the curing agent (trithiol) transfers the proton to the formed benzoyl radicals, and as a result, the free thiyl radicals is formed which are responsible for the UV-triggered curing [38, 39]. Here in, we have used a tri-arm thiol that can leads to the crosslinking reaction.

Sch. 1. The probable mechanism of the photo-curing of NRL via thiol-ene reaction.

Fig. 1a showed the ^1H NMR spectra of Cts. From the figure, it was observed that pure Cts showed resonance peaks in between 3.5 ppm – 4.00 ppm due to the presence of **c**, **d**, **e**, **f** protons whereas, resonances at 3.03 ppm was due to the presence of **b** proton. The peak at 2.06 ppm was assigned to the methyl protons of the N-acetyl group [23]. ^1H NMR spectra of Cts-g-PAAm (**Fig. 1b**) was also analysed to confirm the grafting of PAAm unit on the Cts backbone.

212 The appearance of new resonances at 2.05-2.18 ppm and 1.13-1.63 ppm was due to the different
 213 protons of PAAm unit [40].

Fig. 1. ¹H NMR spectral analysis of (a) Cts and (b) Cts-g-PAAm.

216 The grafting of the PAAm chain over the chitosan backbone was also characterized with
 217 FTIR analysis, as reported earlier [35]. The FTIR spectra for Cts and Cts-g-PAAm have been
 218 shown in **Fig. S1(a)** and **Fig. S1(b)** respectively. Few significant changes were observed in the
 219 Cts-g-PAAm/Ag NPs spectrum (**Fig. 2a**) as compared to the Cts-g-PAAm. In case of Ag NPs
 220 containing Cts-g-PAAm solution, the broad absorption band at 3335 cm⁻¹ (-NH stretching) was
 221 decreased and broadened, indicating the binding of Ag NPs with -NH functional groups of Cts-
 222 g-PAAm. In addition, the absorption band at 1669 cm⁻¹ attributed to the carbonyl groups of
 223 amide functionality in a grafted sample was shifted to 1638 cm⁻¹ in the spectrum of Cts-g-
 224 PAAm/Ag NPs. This also supports the binding of Ag NPs with Cts-g-PAAm [41]. **Fig. 2b**, **Fig.**
 225 **2c** and **Fig. 2d** showed the vibrational spectra of NRL, AMNRL-2 and thiol cured AMNRL-2
 226 respectively. For NRL, the cis-1,4-polyisoprene absorption band corresponding to CH wagging
 227 of cis -CH=C(CH₃)- was appeared at 833 cm⁻¹. The absorption bands at 1368 cm⁻¹ and 1444
 228 cm⁻¹ are the characteristics peak for -CH₂ deformation. The absorption band at 1635 cm⁻¹
 229 corresponds to the C=C stretching in cis-1,4-polyisoprene. **Fig. 2b** showed a broad peak at 3454

cm⁻¹ which was due to the presence of hydroxyl group of the moisture present in the latex [42]. But after incorporation of Cts-g-PAAm/Ag NPs into NRL, a broad peak was observed at 3313 cm⁻¹ (**Fig. 2c**); which was due to the presence of –OH and –NH group in Cts. In case of the thiol cured AMNRL-2, a new vibration peak appeared at 1747 cm⁻¹ corresponding to the ester group present in the trithiol based crosslinker, trimethylolpropane tris(3-mercaptopropionate) (**Fig. 2d**). Presence of the ester peak and the decrease in the intensity of 1635 cm⁻¹ corresponds to the C=C stretching in cis-1,4-polyisoprene, indicating the successful curing of the NRL.

Fig. 2. FTIR spectra of (a) Cts-g-PAAm stabilized Ag NPs, (b) natural rubber latex (NRL), (c) mixture of Cts-g-PAAm/Ag NPs with NRL in 1:1 volume ratio and (d) thiol cured NRL having Cts-g-PAAm/Ag NPs in 1:1 volume ratio.

The preparation of the Ag NPs using Cts-g-PAAm was monitored via UV-vis spectroscopy. **Fig. 3(i)** showed the UV-vis absorption spectra of Cts-g-PAAm stabilized Ag NPs, NRL and comparison of all the Ag NPs/NRL solutions, containing a different ratio of NRL and Cts-g-PAAm/Ag NPs respectively. The Cts-g-PAAm stabilized Ag NPs showed absorption peak near 425 nm which indicates the formation of Ag NPs in Cts-g-PAAm matrix. It also demonstrates that the formed Ag NPs was spherical in size [43]. Gradual formation of Ag NPs in the presence of Cts-g-PAAm was evidenced by the respective colour change from

light yellow to dark brown (**Fig. 3(i)a**). For NRL, a broad absorption band near $\lambda_{\text{max}} = 239$ nm was observed (**Fig. 3(i)b**); this was the characteristic absorption peak of NRL [44]. UV-vis spectra of the NRL/Ag NPs in a different volume ratio of NRL and Cts-g-PAAm/Ag NPs were also observed. It was noticed that with an increase in the Ag NPs concentration in NRL, absorbance was increased (**Fig. 3(i)c**). This absorption peak was due to the presence of conducting electrons over the nanoparticle surface that exhibits a surface plasmon resonance [45].

Presence of the allergenic proteins traces in NRL was monitored via the ninhydrin colourimetric assay. It was observed that, with an increase in the successive centrifugation step, a decrease in the blue colour intensity was observed (**Fig. 3(ii)**). It is worth mentioning that ninhydrin gives a blue colour solution in the presence of protein. After six successive step of centrifugation, a colourless solution of NRL was obtained and used for further biological applications. NRL used to stabilize via the presence of surface proteins. Proteins, phospholipids and poly(isoprene) unit form a core-shell type structure as shown in **Fig. 3(ii)**, where proteins and the phospholipids acquire the outer shell and poly(isoprene) unit remains in the core. Rapid centrifugation isolates the poly(isoprene) unit as a cream fragment which floats at the upper part of the centrifugation tube. The isolated upper fraction was taken for the ninhydrin test after every step of centrifugation [46].

Fig. 3. (i) UV-vis absorption spectra of (a) Cts-g-PAAm/ Ag NPs, (b) NRL and (c) mixture of NRL and Cts-g-PAAm/ Ag NPs in different volume ratios. (ii) Ninhydrin colourimetric assay to determine the presence of protein residues in the centrifuged NRL.

XRD analysis

The XRD spectra of the materials have been represented in **Fig. S2**. Pure Cts showed diffraction peaks at 2θ of 10.81° and 20.27° (**Fig. S2a**); after grafting of poly(acrylamide) on Cts backbone, a broad peak appeared at 2θ of 20.76° (**Fig. S2b**) which indicates the internal crystalline structure of Cts was affected after grafting. The crystalline structure of Cts might appear due to the presence of hydrogen bonding. For NRL, a broad peak appeared at 2θ of 21° (**Fig.S2c**) due to the amorphous structure of NRL. In Cts-g-PAAm/ Ag NPs, strong peaks appeared due to the presence of Ag NPs at 2θ of 36° and 44° (**Fig. S2d**), which were due to the presence of characteristic (111) and (200) planes in the Ag NPs for its face centred cubic (fcc) structure as reported earlier [47]. Cts-g-PAAm/ Ag NPs mixed NRL was equally characterized

with XRD to establish the presence of metallic silver in the composite colloidal solution. **Fig. S2e** showed the XRD of the Ag NPs based NRL film prepared by a casting method. This Fig. showed prominent peaks for Ag NPs at 2θ of 37.12° , 45.05° , 63.59° and 76.08° . The initial hump near $2\theta=22^\circ$ was due to the presence of amorphous NRL and Cts-g-PAAm in the colloid composition.

Morphological analysis

The surface morphology of Ag NPs incorporated NRL was analysed using FESEM (**Fig. 4**). NRL showed an aggregated structure **Fig. 4(i)a**. The surface morphology of the Cts-g-PAAm stabilized Ag NPs has been shown in **Fig. 4(i)b**. From the figure, it was observed that the polymer stabilized Ag NPs were spherical in shape having a size of 20-30 nm and have core-shell morphology where Ag NPs were uniformly coated with the polymer. When it was mixed with the NRL, an interconnected morphology appeared at a lower concentration of NRL (1:1 NRL: Ag NPs volume ratio) (**Fig. 4(i)c**). The average particle size appeared as 100-150 nm. But with further increase in the NRL content, the thickness of the coating over the nanoparticles was increased resulting in a larger particle size of the nanoparticles (**Fig. 4(i)d**). From the FESEM image, it was also observed that pure NRL formed a smooth surface which was due to the cohesion force of the NRL particles. Elemental mapping of the bulk phase of colloidal Ag NPs/NRL surface (**Fig. 4(ii)a**) confirmed the presence of elemental silver (**Fig. 4(ii)b**). EDX analysis of the sample (**Fig. 4(ii)c**) revealed the existence of the elemental silver in a significant amount. The presence of Ag NPs/NRL over cotton fibre was observed by FESEM analysis (**Fig. 5(i)**). The control cotton fibre exhibits a neat plain spun structure (**Fig. 5(i)a**), whereas NRL/Ag NPs treatment imparted roughness. **Fig. 5(i)b** and **Fig. 5(i)c** indicate that the surface of the cotton fibre became rough compared to the pure cotton fibre after the treatment with NRL/Ag NPs. With an increase in the ratio of NRL in the mixture, the agglomeration of Ag NPs over the cotton surface was observed. **Fig. 5(i)d** showed the thiol cured cotton fibre having a composition of NRL: Ag NPs (1:1 volume ratio).

Fig. 4. (i) FESEM image of (a) NRL, (b) Cts-g-PAAm/Ag NPs, (c) AMNRL-1 ((NRL and Cts-g-PAAm/Ag NPs is in 1 : 3 volume ratio) and (d) AMNRL-3 (NRL and Cts-g-PAAm/Ag NPs is in 3 : 1 volume ratio); (ii) (a) FESEM image of Ag NPs/NRL film, used for elemental mapping, (b) elemental mapping image of existing silver in the surface of the film and its (c) EDX spectra.

From the HRTEM images, it was observed that the polymer encapsulated Ag NPs possesses uniform distribution throughout the system, and most of the nanoparticles were spherical in shape which was corroborated with the FESEM image. From the figure, it was observed that high loading of NRL content resulted in an agglomeration of the colloidal mixture (Fig. 5(ii)a). This might be due to the tackiness of NRL in dry condition. Lower content of NRL reduces the inherent cohesion force between the colloidal particles which allow their uniform distribution at the dry condition. In order to verify the crystalline nature of the Ag NPs, SAED (selected area electron diffraction) pattern was studied. The presence of bright circular rings in SAED patterns (Fig. 5(ii)d) confirmed the presence of crystalline Ag NPs in NRL matrix [48]. The blue arrows in the SAED pattern indicates the 111 and 200 plane which is nicely corroborated with the results obtained from XRD.

Fig. 5. (i) FESEM images of (a) pure cotton fibre, cotton fibre coated with (b) AMNRL-2 (NRL and Cts-g-PAAm/Ag NPs is in 1 : 1 volume ratio), (c) AMNRL-3 (NRL and Cts-g-PAAm/Ag NPs is in 3 : 1 volume ratio) and (d) thiol cured AMNRL-2. (ii) HRTEM images of (a) AMNRL-3, (b) AMNRL-2 and (c) AMNRL-1, (d) SAED pattern of Ag NPs present in the Ag NPs/NRL.

In DLS study, though particle size was increased with NRL coating, surface charges prevent the aggregation of nanoparticles. The effective antimicrobial action of Ag NPs depends on the size of the Ag NPs. It is reported that Ag NPs having an average particle size of less than 10 nm can show good antimicrobial action, though the Ag NPs of this range exhibits cytotoxicity [49]. Therefore, it is necessary to characterize the size of the synthesized Ag NPs. **Fig. S3(i)** demonstrated the mean diameters of Ag NPs encapsulated by Cts-g-PAAm and Cts-g-PAAm/NRL at different compositions. With the increase in the NRL content, an increase in the hydrodynamic volume was observed. This could be explained if we consider that the average diameter obtained from DLS study was not for the bare Ag NPs, but it was the diameter of polymer encapsulated Ag NPs. At lower concentration of Cts-g-PAAm encapsulated Ag NPs, more NRL molecules were attached over the surface of nanoparticles, and they could form multiple layers over it, leading to an increase in the water adsorption over the surface of nanoparticles. This causes a higher hydrodynamic diameter of nanoparticles which was clearly observed from the data obtained.

Fig. S3(ii) showed the surface morphology of the Cts-g-PAAm/Ag NPs and NRL encapsulated Cts-g-PAAm/Ag NPs (NRL : Ag NPs volume ratio = 1:1) obtained from AFM analysis. From the image, it was observed that the Ag NPs encapsulated with Cts-g-PAAm were well dispersed. The average particles size was 20-30 nm (**Fig. S3(ii)a**). With the increase in the NRL content, the particle size was increased as observed by the image **Fig. S3(ii)b** (AMNRL-2) and **Fig. S3(ii)c** (AMNRL-1). It is mainly due to the generation of cohesion force between the particles due to the presence of tackiness of the NRL after drying.

Mechanical properties:

Fig. 6(a) and **Table S1** illustrated the mechanical properties, such as tensile strength, modulus and elongation at break (%) of uncoated and Ag NPs/NRL modified cotton fibre. The cotton fibre (control) showed 14.6% elongation at break whereas, after coating with NRL/Ag NPs composite mixture, elongation at break increased to 19%. With an increase in the NRL content, all the properties were improved drastically compared to the control one. Stress at break of the untreated cotton fibre was 16.6 N/mm² whereas, for AMNRL treated cotton fibre containing 1:1 volume ratio of NRL and Cts-g-PAAm/Ag NPs showed stress at break of 35.3 N/mm². This is due to the sufficient wetting of the cotton fibre with AMNRL; which was observed in the FESEM pictures (**Fig. 5(i)b** & **Fig. 5(i)c**). Presence of NRL inside the microfibrillar space of the cotton fibre imparts elasticity to the fibre which increases the elongation. Along with this, the presence of Cts-g-PAAm reduced Ag NPs in the NRL matrix, occupied the space present between microfibers in the cotton fibre that increases the strength of the fibre compared to the control one. So the presence of both NRL and Cts-g-PAAm/Ag NPs in the cotton fibre showed a synergistic effect that improved the tensile modulus and elongation at break properties. Upon curing of the coated latex via thiol-ene reaction, a further increment in the elongation at break (25%) was observed. Modulus of the system was also increased compared to the uncured system as observed from the **Fig. 6(a)**. Thiol crosslinking enhances the integrity of the system that imparts the higher modulus to the system as well as helped in significant elongation before the break as compared to the control cotton fibre. The obtained tensile data has been summarized in **Table S1**.

Fig. 6. (a) Stress-strain plot of the uncoated cotton fibre and cotton fibre coated with AMNRL-2 and thiol cured AMNRL-2; **(b)** water uptake study.

Water uptake study

Fig. 6b showed the mol % of water uptake by the uncoated cotton fibre, and the modified NRL coated fibre at room temperature. From the graph, it was observed that cotton fibre showed 22 mol% uptake of water. Whereas, NRL coated fibre showed only 10 mol% of water uptake. The capillary action was the main reason behind the water absorption by the cotton fibre. In the case of uncoated cotton fibre, as the surface of the fibre was in direct touch with the water surface, the amount of water absorption by the fibre was high compared to the NRL coated cotton fibre. The formation of the hydrophobic layer by the dried NRL (hydrophobic material) over cotton fibre restricted absorption of the water by the fibre. The

presence of hydrophobicity over the NRL coated fibre was ascertained by measuring water contact angle. It was found that the presence of Cts-g-PAAm encapsulated Ag NPs in NRL phase, raised its hydrophilicity. As a result water uptake by the cotton fibre increased with the content of Cts-g-PAAm/Ag NPs in AMNRL. It was observed that after curing with thiol, the water uptake drastically reduced (16% to 10%) in case of an AMNRL coated cotton fibre having a composition of 1:1 volume ratio of NRL: Ag NPs. Upon curing the capillary force exerted by the coating has been drastically reduced as mobility of the coating is restricted upon curing.

Antimicrobial assay:

The antimicrobial activity of the synthesized Cts-g-PAAm stabilized Ag NPs, and Ag NPs containing NRL coated cured cotton fibre was studied against *Escherichia coli* as a model Gram-negative bacteria and *Staphylococcus aureus* and *Bacillus licheniformis* as a model Gram-positive bacteria. From the **Fig. 7(i)**, it was observed that NRL/Ag NPs coated cured cotton fibre showed higher activity over the microbes compared to the control uncoated cotton fibre. This was due to the presence of smaller-sized Ag NPs that have high surface area and better contact with the microbes [50]. According to Morones et al., smaller size Ag NPs were more active and could readily attach to the bacterial cell as well as it could also efficiently penetrate through the cell wall using pinocytosis process [51]. Amro et al. reported that the antimicrobial action of the Ag NPs was due to the accumulation of the nanoparticles over cell wall which generates “pits” on the wall that results in lysis of the cell [52]. Many research studies reveal that Ag NPs can be converted to Ag^+ by respiratory enzymes present in the bacterial cell. The Ag^+ ions made an electrostatic attraction with the negatively charged cell wall and results in cell lysis [53-55]. The formed Ag^+ ions bind with the purine and pyrimidine base pairs resulting the disruption of the H-bonds between base pairs leading to the DNA dissociation. Along with this Ag^+ ions decrease the intracellular thiol levels in *S. aureus*, disturbing the bacterial thiol-redox homeostasis process resulting bacterial cell death. The probable mechanism of antimicrobial activity has been schematically represented in **Sch. 2**. The obtained zone of inhibition value has been shown in **Fig. 7(i)d**.

Fig. 7. (i) Antimicrobial activity of (a) control cotton fibre (control) and cotton fibre coated with (b) AMNRL-1, (c) AMNRL-2 and (d) AMNRL-3 compositions respectively, (e) zone of inhibition value; (ii) MIC determination assay of (a) control sample (pure cotton fibre), cotton fibre coated with (b) AMNRL-1, (c) AMNRL-2 and (d) AMNRL-3, tested against *S. aureus* bacteria; (iii) morphology of (a) untreated *S. aureus* bacteria and (b) AMNRL treated *S. aureus* bacteria; (iv) WCA analysis over (a) NRL, (b) AMNRL-2, (c) AMNRL-1 and (d) thiol cured AMNRL-2.

Fig. 7(ii) showed the MIC assay acquired for *S. aureus* bacteria. From the image, it was observed that in case of negative control (broth containing uncoated cotton fibre), a significant amount of turbidity was formed due to the high proliferation of bacteria (**Fig. 7(ii)a**). But broth solution containing NRL/Ag NPs (1 : 1) coated cotton fibre, amount of proliferation of bacteria

was less (**Fig. 7(ii)c**) and it was also monitored that cotton fibre having high loading of Ag NPs (NRL/Ag NPs 1:3 volume ratio) showed maximum efficiency as we know that antibacterial efficiency is a dose-dependent phenomenon (**Fig. 7(ii)d**).

As observed from the FESEM image **Fig. 7(iii)**, it was found that *S. aureus* bacteria are round in shape and has smooth cell wall surface. But after the treatment with AMNRL coated cotton fibres, the integrity of the cell wall was damaged severely. As a result, the size and shape of the cells changed drastically (wrinkled) and round shaped morphology was disrupted due to the leakage of the intercellular components [56, 57].

Sch. 2. (a) Schematic representation of the preparation of AMNRL coated cotton fibre and its antimicrobial activity; **(b)** Probable mechanism of antimicrobial activity by a polymer coated Ag NPs.

Water contact angle (WCA) measurement can reveal the hydrophilicity and wettability of a given surface. Lowering of the contact angle was observed with increase in the hydrophilic character of the surface. **Fig. 7(iv)** showed the picture of water droplet over the polymer film and the water contact values. It was observed that with increase in the Ag NPs content in NRL matrix, hydrophilicity of the system also increased, resulting in a drastic decrease in contact angle from NRL coating (97°) (**Fig.7(iv)a**) to Cts-g-PAAm/Ag NPs incorporated NRL (NRL : Ag NPs = 1 : 1) coating (80°) (**Fig.7(iv)b**) to Cts-g-PAAm/Ag NPs incorporated NRL (NRL : Ag NPs = 1 : 3) coating (65°) (**Fig.7(iv)c**). The obtained result illustrated the lowering of surface tension due to the presence of hydrophilic Ag NPs [58]. In contrary, the thiol cured AMNRL having a composition of NRL : Ag NPs = 1 : 1, showed a WCA value of 102° (**Fig.7(iv)d**). The increase in the WCA value might be due to the curing of the NRL matrix that increases the integrity of the coating material as well as the presence of hydrophobic crosslinker decreases the hydrophilicity of the whole system.

In-vitro cytotoxicity assay

The tendency of agglomeration of Ag NPs due to its high surface to volume ratio makes it a more toxic for the human cells. [59] To reduce the toxicity, in our case, Ag NPs was coated with the bio-polymeric layers as explained earlier. The cytotoxicity of the prepared system was studied through MTT assay against NIH 3T3 fibroblast cell line. From **Fig. 8**, it was observed that- NRL (**Fig. 8a**), Ag NPs incorporated NRL (1:1 volume ratio) (**Fig. 8b**) and thiol cured Ag NPs incorporated NRL (1:1 volume ratio) (**Fig. 8c**) did not show severe cytotoxicity against fibroblast cells. In our system, Ag NPs present at a concentration of $20\ \mu\text{M}$. The presence of Ag NPs at a concentration of $100\ \mu\text{M}$ or less is non-toxic to fibroblast cell [60]. The absorbance value of the MTT assay test has been shown in **Fig. 8d**. It was observed that all the compositions have shown more than 75% cell viability. It was also observed that the presence of chitosan in NRL enhances the cell viability.

Fig. 8. *In-vitro* cytotoxicity assay against NIH-3T3 fibroblast cell line.

Conclusion:

In conclusion, Ag NPs were prepared *in situ* using polyacrylamide (PAAm) grafted chitosan as a polymeric reducing agent. The as prepared Cts-g-PAAm/Ag NPs was blended with natural rubber latex (NRL) at pH 7.0. DLS study concluded that with an increase in the NRL amount, hydrodynamic radius of the formed Ag NPs increased. SAED and XRD pattern proved the presence of crystalline Ag NPs which had an fcc crystal structure. The synthesized colloidal solution was coated over the cotton fibre and subsequently cured via UV-irradiation method using photoinitiator and a trithiol. It was observed that the presence of silver nanoparticles incorporated NRL as a coating material improved the mechanical properties of cotton fibre as well as its hydrophobicity. Interestingly, the Ag NPs based NRL solution showed excellent antibacterial activity against both Gram-positive and Gram-negative bacteria. Integrating all these properties, it can be concluded that these treated cotton fibre can have a potential application in biomedical applications.

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