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## Original article

# Evaluating the use of chitosan coated Ag nano-SeO<sub>2</sub> composite in consolidation of Funeral Shroud from the Egyptian Museum of Cairo



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## ARTICLE INFO

### Article history:

Received 10 May 2014

Accepted 18 September 2014

Available online 7 November 2014

### Keywords:

Polymers nano-composite  
 Chitosan (CCTS) coated Ag-loading  
 nano-SeO<sub>2</sub> composite (CCTS-SLS)  
 Ancient Egyptian linen shroud  
 Investigation methods  
 AFM  
 ICP  
 SEM  
 Selenium dioxide  
 Tensile strength

## ABSTRACT

Polymers nano-composite sciences (PNC) provide us each day with updated contributions enriching the consolidation methodology of ancient materials. This study aims to evaluate the use of cross-linked chitosan (CCTS) coated Ag-loading nano-SeO<sub>2</sub> composite (CCTS-SLS) in the consolidation of a Funeral Shroud from the Egyptian Museum of Cairo. In this study, new linen textile samples were artificially aged to simulate the ancient ones. The new accelerated aged linen textile samples simulated to the ancient one were infested with active strains of fungi and bacteria, which were isolated from the ancient shroud. Both the ancient fibers and the newly prepared linen samples were consolidated with cross-linked chitosan (CCTS) coated Ag-loading nano-SeO<sub>2</sub> composite (CCTS-SLS) and chitosan biopolymer. Various methods and instruments were used to investigate both the treated and untreated samples. The structures of (CCTS-SLS) were characterized by field emission scanning electron microscope (FESEM). The change of the colors ( $\Delta L$ ,  $\Delta a$ , and  $\Delta b$ ), tensile strength and elongation of the untreated and treated linen samples after ageing were assessed. Consolidated and non-consolidated ancient fibers and accelerated linen samples were examined using atomic force microscopy (AFM), scanning electron microscopy (SEM) and scanning tunneling microscopy (STM) and Fourier transform-infra-red spectroscopy (FT-IR) and have been elementally analyzed using inductively coupled plasma (ICP). The consolidated samples were appraised via dynamic thermal analysis (DMTA). The results show that the antibacterial activity of (CCTS-SLS) was affected by the mass ratio of selenium dioxide to chitosan, and by the cross-linking time. The antibacterial activity against *Staphylococcus aureus* sp. strains was studied. Samples treated with (CCTS-SLS) showed excellent properties in comparison to chitosan treated samples. Genetic efficacy of nano CCTS-SLS is different from normal-sized chitosan. The application of cross-linked chitosan (CCTS) coated Ag-loading nano-SeO<sub>2</sub> composite (CCTS-SLS) to the consolidation of the ancient shroud showed good bacterial resistance, enriching the long-term durability of the ancient linen.

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## 1. Research aims

This study aims to evaluate the use of cross-linked chitosan (CCTS) coated Ag-loading nano-SeO<sub>2</sub> composite (CCTS-SLS) in the consolidation of ancient Egyptian linen textiles. Also, this paper aims to evaluate and compare between the effect of normal-sized polymers [chitosan] and hybrid nano-scale ones [cross-linked Chitosan (CCTS) coated Ag-loading nano-SeO<sub>2</sub> composite (CCTS-SLS)],

on the physical properties of ancient linen textiles including the tensile strength, elongation and color changes through examinations implemented on artificially accelerated samples via thermal and light exposure procedures prior to being applied on the shroud. Demonstration of microbial resistance requires a wide scale of appraisal of the polymeric consolidation between chitosan polymer and the innovated synthesized formation of CCTS-SLS; via isolation tests as a powerful tool for identifying subsequent genetic remains on ancient linen.

## 2. Introduction

Polymers nano-composite (PNC) is a widely used term which describes polymers, co-polymers having nanoparticles, or

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**Table 1**  
Specification of linen fabric used.

| Structure   | Colour shade |          |          | Nominal/wt (g/m) | Thread/cm | Linear density |      | % of elongation | Tensile strength |             |
|-------------|--------------|----------|----------|------------------|-----------|----------------|------|-----------------|------------------|-------------|
|             | <i>L</i>     | <i>a</i> | <i>b</i> |                  |           | Warp           | Weft |                 |                  | Warp        |
| Plain weave |              |          |          |                  | Warp      | Weft           | Warp | Weft            |                  |             |
| 1/1         | 52.4         | 1.6      | 12.6     | 108              | 20        | 16             | 20   | 18              | 16               | 28.9 Newton |

nano-fillers, dispersed in the polymer matrix. These PNC can be classified as multi-phase polymeric systems, including composites, blends and forms [1], which play a critical role in enhancing the consolidation materials of ancient textiles which require sensitive conditions of preservation and protection against environmental circumstances which cause harsh physical or chemical change [2], and in addition protection from microbial infection by prokaryotes and eukaryotes.

The required characteristics for the selection of a polymer nano-composite are that it is composed of repeat units, the polymer architecture which relates to the way a branch points, leading to a deviation from simple linear chains, thus supplying the degree of polymerization and persistence time [3]. This real diversity in molecular properties reflects the difference between bulk-unit polymer formations and synthesized nano-ones in co-polymers arrangements and tacticity (relative stereochemistry of chiral centers) as well as the matter which different systems of ionic and hydrogen bonding while forming micro-emulsion blends [4]. Silver-loading nano-SeO<sub>2</sub> antibacterial material has excellent antibacterial and improving activity but suffers from a change in color as a result of the reaction between silver ions and oxygen or sulfur [5]. Chitosan (CTS) is the second plentiful natural biopolymer attracting considerable interest in conservation of ancient sensitive materials due to its biological properties, such as antibacterial activity and genetic efficacy enhancing effect. However, this ideal activity of chitosan is influenced by number of factors including the species of bacteria and fungi, concentration, pH, solvent and molecular weight [6].

In this paper, cross-linked chitosan (CCTS) coated Ag-loading nano-SeO<sub>2</sub> composite (CCTS-SLS) was synthesized by an adsorption cross-linking reaction of monomers and catalytic alkoxides. The shroud was grossly infected with morphogenesis and developing fungal, bacterial and actinomycetes spores forming colonies which infect the linen cells [7], which had to be considered within the polymeric consolidation criteria.

### 3. Materials and methods

#### 3.1. Preparation of a new linen sample similar to ancient ones

Scoured, unbleached linen fabric was used as a simulation for the studied ancient shroud (See Table 1). The fabric was aged by heating at 100 °C for 72 h. Linen textile fabric was cut into 12 × 2 cm (length × width) warp test specimens. The warp strips were produced by unraveling yarns on each side forming 1.5 cm wide strips with a 2.5 mm fringe down each side [8]. A part of the prepared samples were decorated and painted by hand with black paints prepared using ancient materials and methods. Various types of pigments and dyes have been used to paint and decorate linen textiles in ancient Egypt. A part of the model linen specimens were painted and decorated with black carbon ink. The binder was prepared from 59 g of gum Arabic, dissolved in 70 mL of warm water and left to stand for 24 h. Then, 40 g of carbon soot were ground well and mixed with the prepared binder. The filtered ink was used for painting the model linen specimens using a fine brush [9]. Another part of the linen textile sample was decorated with metallic ink. A part of linen textile sample was used without decoration to be used as untreated (control samples). All the prepared samples

(decorated and undecorated) were artificially thermally aged at 100 °C in a precision forced convection oven for 72 h.

#### 3.2. Selected polymers of evaluation

##### 3.2.1. Bulk-sized chitosan (CS)

Chitosan, white mushroom origin (Sigma-Ildrich #740500, DP Mw ~ 120 kDa), a linear polysaccharide produced by deacetylation of chitin is a natural occurring polymer, mainly dissolved in acetic acid solution with various required concentrations [10].

##### 3.2.2. Preparation of nano-loading agent SeO<sub>2</sub> (SLS)

SLS was prepared by adsorption as follows: according to (Krajewska 2004)'s scheme [11], 5 g of nano-SeO<sub>2</sub> powder from Sigma-Aldrich to 50 mL of 0.08 mol/L AgNO<sub>3</sub> solution, 1 mL of coupling agent A-1100 was added, then, the mixture was stirred at 50 °C for 4 h in the dark, with the pH value of the system maintained at 6 by adding nitric acid. The slurry was separated into solid and liquid under vacuum filtration. The separated solid specimen was dispersed into 200 mL of distilled water for washing and then filtered again. The washing and filtration were repeated until no Ag<sup>+</sup> was detected in the filtrate. After that, the specimen was dried at 90 °C to constant weight.

##### 3.2.3. Preparation of chitosan coated Ag-loaded nano-SeO<sub>2</sub> composite (CCTS-SLS)

One gram of chitosan was added to about 200 mL of commonly used solvent dilute acetic acid solution, because chitosan is soluble in acetic acid solution but insoluble in water. The pH value of the solution was maintained at 2.5 by adding 1 mol/L dilute hydrochloric acid with stirring at 25 °C for 0.5 h. After that, according to SLS to CTS mass ratio, SLS was added and stirred at 25 °C for 1.5 h. Dilute ammonia was used to adjust the pH value of the system to 12. The mixture was heated to 65 °C and 0.03 g of 3% glutaric dialdehyde was added dropwise to cross-link the chitosan on the surface of SLS particles. After further heating for a certain time, the product was separated by centrifugation and washed several times with absolute ethyl alcohol. Finally, the CCTS-SLS specimen was vacuum dried at 30 °C for 24 h [12].

#### 3.3. Structure and morphology of synthesized polymeric formation

Morphologies of (CS, CCTS, SLS, CCTS-SLS) samples were characterized by field emission scanning electron microscope (FESEM) Zeiss U9320-A8500 [13].

#### 3.4. Microbial strains procedure

##### 3.4.1. Primary isolations

In this study, cotton swab technique was used to isolate microorganisms from the studied ancient shroud. All processes of fungal isolation and identification were done according to [14–16], while all processes of bacterial isolation and identification were done according to [17–19]. After isolation and identification of the microorganisms, the experimental linen samples were infected with the selected species within the used media through a direct

**Table 2**  
Demonstration of experimental control and treated samples.

| Sample no | Description                            | Category  | Used polymer  |
|-----------|----------------------------------------|-----------|---------------|
| 1         | Plain linen with carbonic ink writings | Untreated |               |
| 2         | Plain linen with carbonic ink writings | Untreated |               |
| 3         | Plain linen with carbonic ink writings | Untreated |               |
| 4         | Plain linen with metallic ink writings | Untreated |               |
| 5         | Plain linen with metallic ink writings | Untreated |               |
| 6         | Plain linen with metallic ink writings | Untreated |               |
| 7         | Plain linen with carbonic ink writings | Treated   | Bulk chitosan |
| 8         | Plain linen with carbonic ink writings | Treated   | CCTS–SLS      |
| 9         | Plain linen with carbonic ink writings | Treated   | Bulk chitosan |
| 10        | Plain linen with metallic ink writings | Treated   | CCTS–SLS      |
| 11        | Plain linen with metallic ink writings | Treated   | CCTS–SLS      |
| 12        | Plain linen with metallic ink writings | Treated   | Bulk chitosan |

incubation period for 15 days in order to be infected with the species affecting the ancient shroud.

#### 3.4.2. Genetic efficacy substratum

It was characterized by applying 16SrRNA, 32SrRNA gene supplying examinations prior to determine the subsequent growth (pattern) of isolated microorganisms. The results were confirmed using internal transcribed spacer regions sequences (ITS), which is suited to characterize the isolations' mycobiomes.

#### 3.5. Treatments

Linen fabric and six samples were treated with both selected polymers (normal-sized chitosan, CCTS–SLS); three samples by the bulk-sized and others by CCTS–SLS in 10% concentration each (See Table 2). The methodology of application required "Reactivation of polymers" method mainly preferred in ancient textiles consolidation [9]. Silkscreen supports reinforced with selected polymers were adhered to the back of the linen samples via paper pulp poultices of polymers' solvent.

#### 3.6. Accelerated ageing

##### 3.6.1. Thermal ageing

Treated and untreated (control) linen samples were artificially thermally aged at 140 °C in a precision forced convection oven (Thiodex-M2000) for 72 h According to (Abdel-kareem, 2004) [8].

##### 3.6.2. Light ageing

For ageing by exposure to light, treated and untreated linen samples were mounted in standard specimen holders and were exposed to light irradiation for 200 h. Irradiation of the samples was carried out using the Atlas Light Fastness Tester. Type of Atlas Fade-Ometer used in this study is XENOTEST®150S+. A light filter was used to simulate light in museums. Exposure conditions were 50 °C and 55% of RH.

#### 3.7. Testing and analysis

Color differences were measured using a Nippon Denshoku-handly colourimeter (NR-3000) PRO Digicolor spectrophotometer. Recorded Coordinator for *L*, *a*, and *b* values were confirmed. Twelve color readings were made and averaged for each sample implying the differences between aged treated and untreated (control) linen samples, expressed as  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$ . Calculations of total color change ( $\Delta E$ ) was achieved by the use of the following equation:  $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$  (DEMOS 2012) [20].

Tensile strength and elongation of treated and untreated linen samples before and after ageing by both polymers were measured using (ASTM D6775) [21] Universal test machine–signal column

5KN. The initial jaw spacing was 50 mm and the test speed was 25 mm/min, temperature 21 °C, relative humidity = 66%.

Surface morphology of treated and untreated samples was investigated using a scanning electron microscope (SEM) type of JEOL-SEM6510 high vacuum.

Atomic force microscope (AFM) XE-3dM (high-resolution metrology) was invested in giving high scanning probe microscopy, which tends to fractions of 1000-nanometer through photodiodes [22], applied on two selected samples treated with CCTS–SLS.

Scanning tunneling microscope (STM)–Doxix osmosis EN81.28 carried out to elucidate equidistant resolution for flax fibrils distribution after treatment with bulk chitosan and CCTS–SLS and thorough demonstration of nano-vascular cells of a linen fiber after treatment with CCTS–SLS.

Fourier transform–infra red spectroscopy (FT–IR) analysis was implemented for treated and untreated samples before and after accelerated ageing realizing equidistant compound structures modified between both, using Bruker IR Spectrometer. A small part of the sample was encased directly in sample holders and spectra were obtained with air as reference representing rapid, sensitive, non-destructive tool to detect oxidative degradation in cellulose textiles [23].

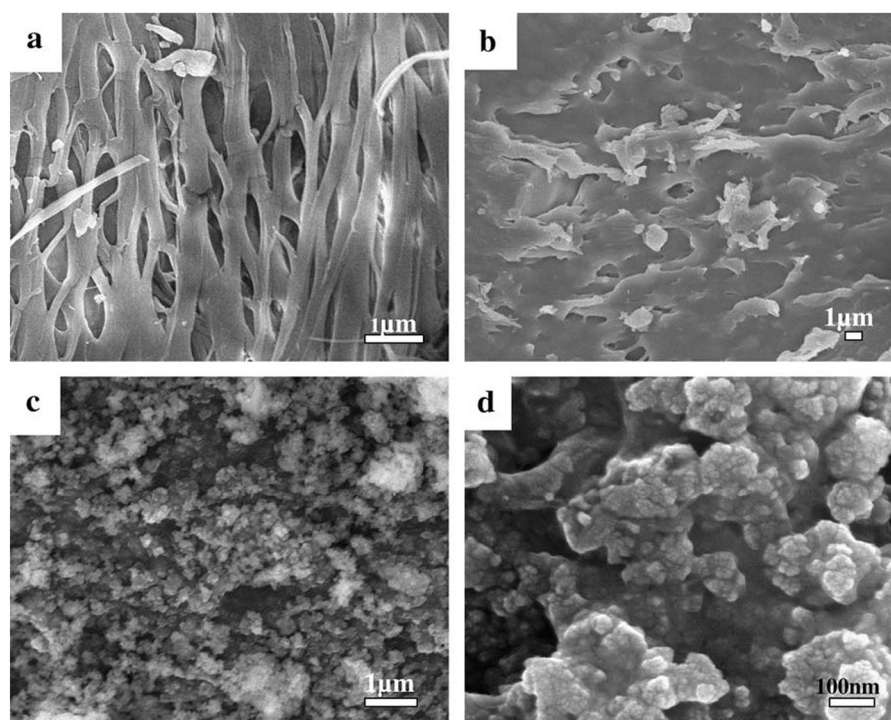
Inductively coupled plasma (ICP–OES), Perkin Elmer as an innovative elemental analysis, low pressure processing as a bias (voltage difference) of cold plasma frequency (6000–10,000 K) reaching  $10^{15} \text{ cm}^{-3}$  of analyzed elements [24], were used used to analyze selected treated sample with CCTS–SLS.

Dynamic mechanical thermal analysis (DMTA), 242-E Artemis, was used to evaluate the degree of polymers deformations with flax treated fibers before and after ageing procedures in comparison with a selected control sample. This criterion represents the conventional case of illustration in the consolidation treatments of fibers.

#### 3.8. Antimicrobial property of samples

The antimicrobial properties of samples were measured by minimal inhibitory concentration (MIC). *Escherichia coli* ATCC 8739 and *S. aureus* ATCC 6538 were selected as indicators of the experimental bacteria, *Luria bertani* (LB).

Broth was used as a growing medium for both the microorganisms *E. coli* and *S. aureus*. The bacteria were grown aerobically in LB broth at 37 °C for 24 h. Five grams of sample powder were placed in 45 mL sterilized phosphate buffer saline (PBS) with the pH value of 5. The suspension was diluted to a series of concentrations by adding PBS solution and placed in 45–50 °C water-bath. Ten millilitres of the above-mentioned antibacterial solution with different concentrations was added into a plate, then, 10 mL of double concentrated MH agar was added under continuous shaking for full mixing [25].



**Fig. 1.** Morphologies of synthesized polymeric formations, (a–d) FESEM images of samples (a) CTS, (b) CCTS, (c) SLS and (d) CCTS–SLS.

Cell solution ( $1\text{--}2\ \mu\text{L}$ ,  $\sim 10^7$  cfu/mL) was taken to inoculate the above-mentioned plate, forming a cell solution quots with a diameter of about 5–8 mm. At last, the inoculated plates were cultivated at  $37^\circ\text{C}$  for 24 h. MIC was determined according to such a standard that the lowest concentration of antibacterial solution needed to prevent visible growth of test microorganism was defined as the MIC against the microorganism. Finally, the inoculated samples were referred to gene testing to confirm the removal of any genetic genomes related to the infecting organisms.

## 4. Interpretation of results

### 4.1. Morphologies of synthesized polymeric formations

The morphologic changes of samples could be clearly observed from the (FESEM) images shown in Fig. 1 a–d. Sample (CTS) took the shape of irregular long strips interconnecting with each other, which is typical of a macromolecular structure because of the straight chain polysaccharide by  $\beta$ -(1,4) glycosidic linkage. Sample (CCTS) was a kind of porous and membranous materials typical for cross-linked polymer. Sample (SLS) was in the shape of superfine powder. Sample (CCTS–SLS) had aggregated particle structures [26].

### 4.2. Elucidated microbial isolations

Throughout the previous proceeding of isolating microbial (fungal, bacterial) species from the ancient shroud, different species were observed as mentioned in Table 3. Most isolated species incubated were those have the cytoplasmic domination on the plates extending to (59–62C) parameters; enclosing the ribosomes of some bacterial and saprophytic species according to [27]. Commonly prevailed ones observed within these isolations were: *Alternaria tenuissima*, *Trichoderma viride*, *S. aureus*, *E. coli*. These different strains negatively affect the physio-mechanical and durability properties of the shroud so that direct infestation of samples

had occurred as assimilation for the critical condition of the studied shroud.

### 4.3. RNA – genetic plasmid sequences

Referring to the single circular chromosome, the range of isolated microorganisms do not extend 160,000 pairs of endosymbiotic eukaryotes as genes in fungal and bacterial isolations occurred; genomes are usually a single stretch of DNA and although several types of introns do not exist; from that point, 16SrRNA, 32SrRNA testing sequences were demanded relying on plasmids, which are small extra-chromosomal DNAs that contain genes for antimicrobial resistance or virulence factors [28].

The 16SrRNA sequences of *A. tenuissima* and *T. viride* show the healthy communities grown in fiber cells throughout the cellulose DNA transduction as obtained in Fig. 2A. The Dips indicate hyper-variable regions ( $v_1$ – $v_9$ ) and conserved regions representing the affecting chains selected for linen vascular cells.

The internal transcribed spacing (ITS) region structures (Fig. 2B) show the positions of the primers representing the bacterial straits ( $P_1$  to  $P_4$ ). These ITS regions ( $ITS_1$ ,  $ITS_2$ ) showed genetic gap between 18S and 28SrRNA genes, which indicate gradual separation of the gene throughout (antimicrobial) characterized in a very narrow scale “nano” material in which occur replacement

**Table 3**

Demonstration of fungal and bacterial isolations from the studied linen shroud.

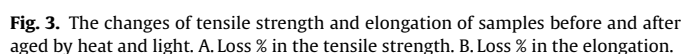
| No | Fungal species                            | Bacterial species                  |
|----|-------------------------------------------|------------------------------------|
| 1  | <i>Alternaria alternate</i> (Fr) Keissier | <i>Bacillus cocci</i> Netcher      |
| 2  | <i>Alternaria tenuissima</i> Kunze        | <i>Myxococcus Xanthus</i> Dontel   |
| 3  | <i>Aspergillus cervinus</i> Neil          | <i>Enterococcus faecalis</i> Heric |
| 4  | <i>Aspergillus fischeri</i> Wehmer        | <i>Staphylococcus</i> Rosenbach    |
| 5  | <i>Chaetomium cochlioides</i> Paisler     | <i>Escherchia coli</i> Migula      |
| 6  | <i>Chaetomium globosum</i> Kunze          |                                    |
| 7  | <i>Penicillium aspertum</i> Shear         |                                    |
| 8  | <i>Trichoderma viride</i> Pers. Ex Fr.    |                                    |



#### 4.4. Colour change

The browning of fabrics represents oxidation of linen to form conjugated unsaturated structures that accelerate the deterioration of linen [3,8]. Results in (Table 5) show that after light ageing, untreated samples seem a bit lighter than treated ones. Samples treated with (CCTS–SLS) show excellent results approximately similar to untreated ones in comparison with those treated by bulk-sized chitosan (CS).

Tensile strength and elongation of untreated and treated linen samples before and after ageing by both heat and light methods were measured. The percentages of losses in tensile strength and



**Table 4**

The color change of untreated and treated linen after heat ageing.

| Sample | Treated/untreated | Used polymer | Color shades |          |          | Color differences |            |            |            |
|--------|-------------------|--------------|--------------|----------|----------|-------------------|------------|------------|------------|
|        |                   |              | <i>L</i>     | <i>a</i> | <i>b</i> | $\Delta L$        | $\Delta a$ | $\Delta b$ | $\Delta L$ |
| 1      | Untreated         |              | 55.6         | 7.59     | 13.23    | –20.6             | 4.2        | 3.1        | 6.5        |
| 2      | Untreated         |              | 54.8         | 6.4      | 12.9     | –14.3             | 6.5        | 2.3        | 5.9        |
| 3      | Untreated         |              | 54.9         | 7.2      | 13.4     | 0.2               | 5.9        | 3.4        | 6.1        |
| 4      | Untreated         |              | 53.6         | 6.9      | 13.43    | –16.1             | 8.1        | 3.2        | 5.7        |
| 5      | Untreated         |              | 54.8         | 7.1      | 13.42    | 0.1               | 6.1        | 3.5        | 6.7        |
| 6      | Untreated         |              | 56.1         | 7.5      | 13.3     | –8.5              | 4.8        | 3.1        | 5.8        |
| 7      | Treated           | Bulk-CS      | 52.5         | 8.9      | 16.7     | –6.4              | 1.5        | 6.4        | 10.5       |
| 8      | Treated           | CCTS-SLS     | 50.6         | 9.8      | 13.43    | 0.1               | 1.5        | 2.9        | 6.0        |
| 9      | Treated           | Bulk-CS      | 54.8         | 7.8      | 14.89    | –3.2              | 2.3        | 4.7        | 9.9        |
| 10     | Treated           | CCTS-SLS     | 57.6         | 8.5      | 15.4     | 0.0               | 0.6        | 5.2        | 5.8        |
| 11     | Treated           | CCTS-SLS     | 54.23        | 8.11     | 14.4     | 0.5               | 1.2        | 7.1        | 6.1        |
| 12     | Treated           | Bulk-CS      | 50.12        | 8.2      | 17.7     | –4.2              | 2.5        | 4.9        | 11.4       |

**Table 5**

The color change of untreated and treated linen after light ageing.

| Sample | Treated/untreated | Used polymer | Color shades |          |          | Color differences |            |            |            |
|--------|-------------------|--------------|--------------|----------|----------|-------------------|------------|------------|------------|
|        |                   |              | <i>L</i>     | <i>a</i> | <i>b</i> | $\Delta L$        | $\Delta a$ | $\Delta b$ | $\Delta L$ |
| 1      | Untreated         |              | 61.4         | 1.4      | 11.87    | 3.2               | –0.6       | –0.4       | 4.6        |
| 2      | Untreated         |              | 59.2         | 1.6      | 10.6     | 3.3               | 0.1        | 0.4        | 5.1        |
| 3      | Untreated         |              | 58.9         | 2.4      | 11.0     | 1.3               | 0.42       | 0.6        | 3.8        |
| 4      | Untreated         |              | 53.6         | 6.9      | 13.43    | –2.1              | 0.8        | –2.5       | 2.6        |
| 5      | Untreated         |              | 60.1         | 2.6      | 10.6     | –3.6              | 2.1        | 2.4        | 1.8        |
| 6      | Untreated         |              | 58.6         | 1.9      | 11.0     | 4.0               | –2.5       | 0.8        | 2.6        |
| 7      | Treated           | Bulk-CS      | 61.2         | 1.32     | 12.6     | 3.9               | 0.7        | 1.2        | 4.5        |
| 8      | Treated           | CCTS-SLS     | 59.8         | 1.61     | 11.9     | 1.3               | 0.6        | 1.5        | 2.9        |
| 9      | Treated           | Bulk-CS      | 59.9         | 1.4      | 10.8     | 1.4               | 0.2        | 1.5        | 3.6        |
| 10     | Treated           | CCTS-SLS     | 60.0         | 2.4      | 11.4     | 1.7               | –2.0       | 1.3        | 1.9        |
| 11     | Treated           | CCTS-SLS     | 58.1         | 2.3      | 11.2     | 4.0               | –0.8       | 0.8        | 1.0        |
| 12     | Treated           | Bulk-CS      | 58.9         | 1.6      | 10.8     | 2.1               | 0.6        | 1.0        | 2.6        |

elongation compared with untreated unaged linen samples are presented in Fig. 3.

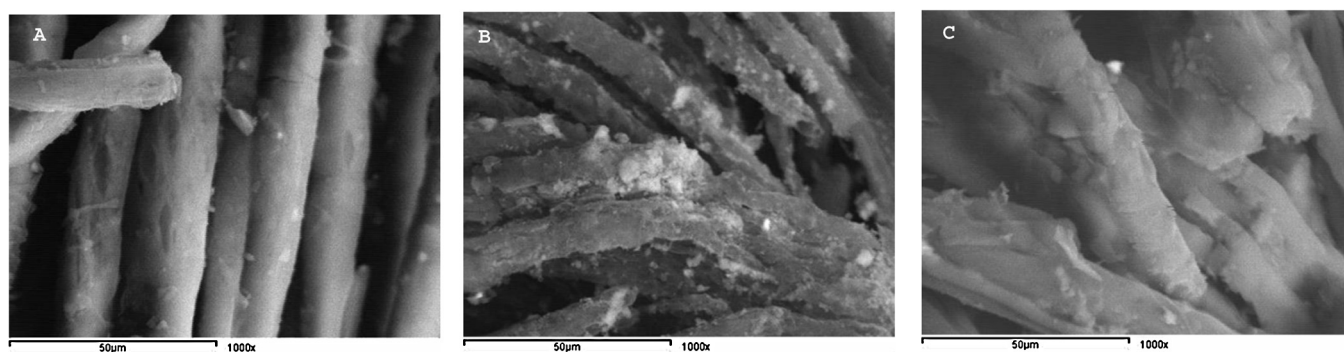
The results of initial characterization (before ageing) show that the treatments increased the tensile strength and elongation of linen samples. Initially, the tensile properties of treated samples with both polymers recorded higher percentages than those untreated. Samples treated with (CCTS–SLS) showed brilliant results compared with those treated by (CS). Generally, samples that were light aged were the weakest followed by thermally aged ones.

After heat acceleration, the results showed that untreated samples had less performance, exceeding lower values to that before ageing. While increasing percentage of treated show satisfactory results with (CS) in samples No. (7,9,12) as shown in Fig. 3, the occurrence of nano-loading  $\text{SeO}_2$  had benefited the fibers' matrices, as shown by the increased the tensile strength as shown in samples (No. 8, 10, 11). After light exposure, linen samples treated

with polymers showed a significant improvement in tensile properties over untreated samples. Also, treated samples with (CCTS–SLS) gave higher performance than those (CS) with vulnerable percentages.

#### 4.6. Scanning electron microscope (SEM)

Observation of the surface morphology of fibers of an untreated sample shows the gradual distribution of a linen fibrils at  $1000\times$  magnification after thermal ageing procedures (Fig. 4). The treated sample with normal-sized (CS) (Fig. 4) show morphological encrustations beneath fibers as a result of narrow extending of supplementary cells of linen than polymers' molecules. The matter, which was disappeared by (CCTS–SLS) consolidation (Fig. 4C), shows good consolidation criteria without encrusting or defiling of polymer formations.



**Fig. 4.** SEM image for selected samples. A. SEM image for distribution of linen fibrils after heat ageing. B. SEM image for linen fibrils after treatment with bulk-CS. C. SEM image for linen fibrils after treatment with CCTS–SLS.

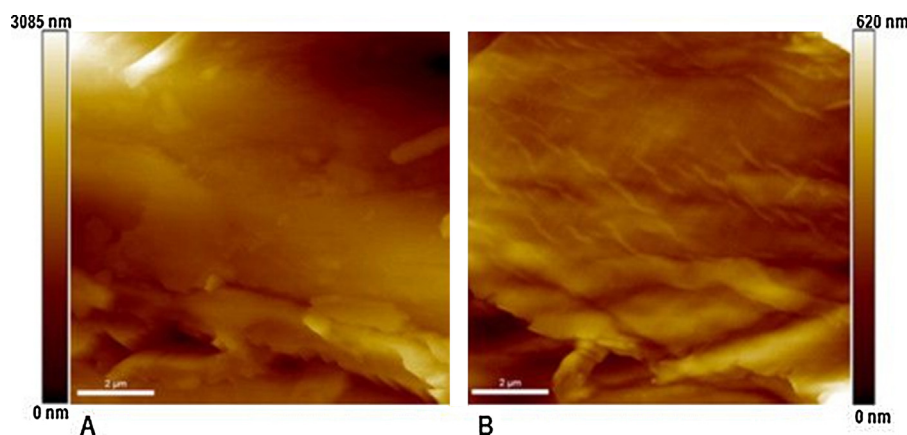


Fig. 5. AFM image of intercellular linen fibrils and polymer morphology after treatment with CCTS-SLS.

#### 4.7. Atomic force microscope (AFM)

For two different samples treated with (CCTS-SLS), Fig. 5 showed 3D-intercellular padding with polymer extending to 120 nano mm in the vascular cell fibril. Although AFM was introduced for high-resolution surface profilometry, the height image records the surface topography exactly only when the tip to sample force is too small to deform the surface. The characterization of (CCTS-SLS) showed excellent coating layer on the linen fiber surface, which appeared too thin and did not disappear the surface of consolidated fibers.

#### 4.8. Scanning tunneling microscope (STM)

Image of fibrils distribution after treatment with (CS) in comparison with sample treated with (CCTS-SLS) (see Fig. 6A and B) shows no signals of precipitation on fibril granular genesis. Nano-vascular cells of a linen fibril treated with CCTS-SLS showed the role of synthesized CCTS-SLS in forming regular adhering

property without precipitation of excess polymerization; in addition to sort and obtain well cross-linkage between linen fibril spillers (Fig. 6C–F).

#### 4.9. FT-IR

Infrared spectra of untreated and treated linen samples before and after ageing by heat method were recorded from  $4000\text{--}400\text{ cm}^{-1}$ . The results showed that there are changes in the IR spectra of all test samples compared with spectra of untreated unaged samples. By comparing results in Fig. 7A and B, it is evident that there are significant spectral changes in regions (from  $1610\text{--}1750\text{ cm}^{-1}$ ) for untreated samples ageing. New bands related to carbonyl functional groups created in the molecular structure from deterioration of the textiles. However, the region from ( $1750\text{--}1600\text{ cm}^{-1}$ ) proved most convenient for monitoring cellulose degradation. This carbonyl functional group could be derived from either aldehyde groups at  $1600\text{ cm}^{-1}$  or carboxylate groups at  $1750\text{ cm}^{-1}$  (Fig. 7C). CCTS-SLS treated sample (Fig. 7D) showed

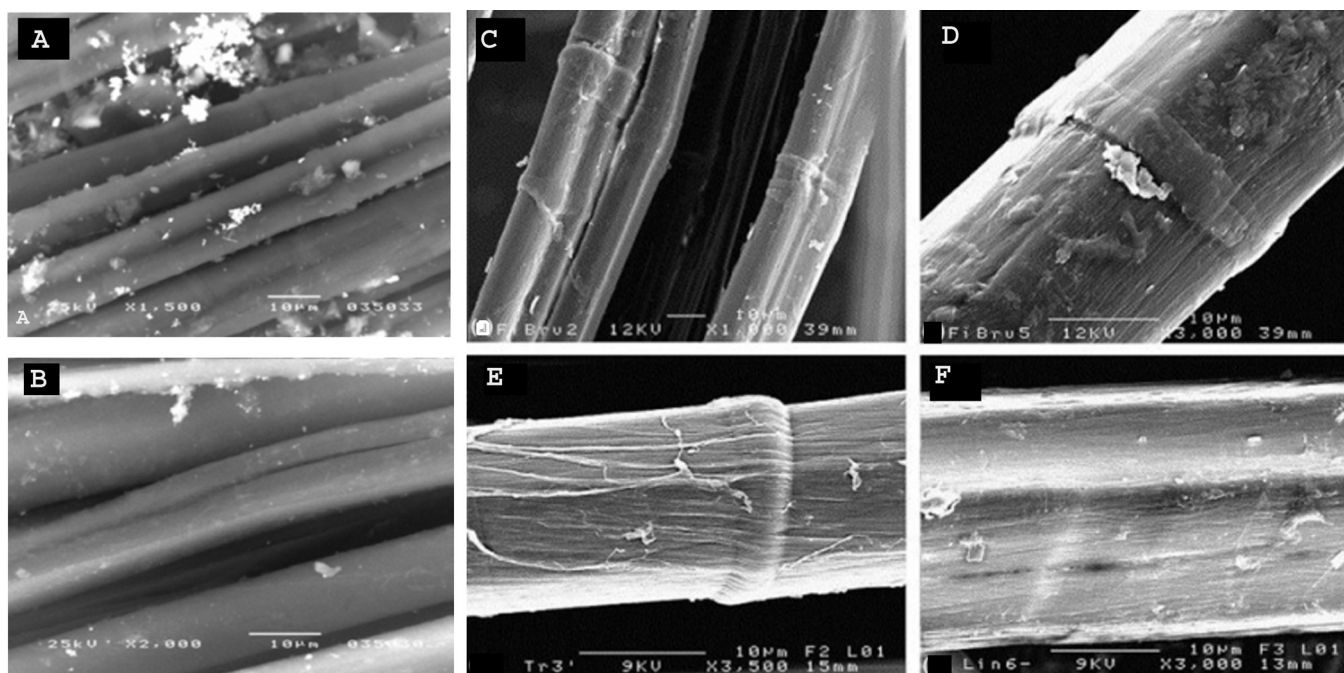


Fig. 6. STM image for selected samples. (A) linen fibril treated with CS and (B) linen fibril treated with CCTS-SLS. C–F. STM image of fibrils treated with CCTS-SLS showing well cross-linkage of linen spillers.

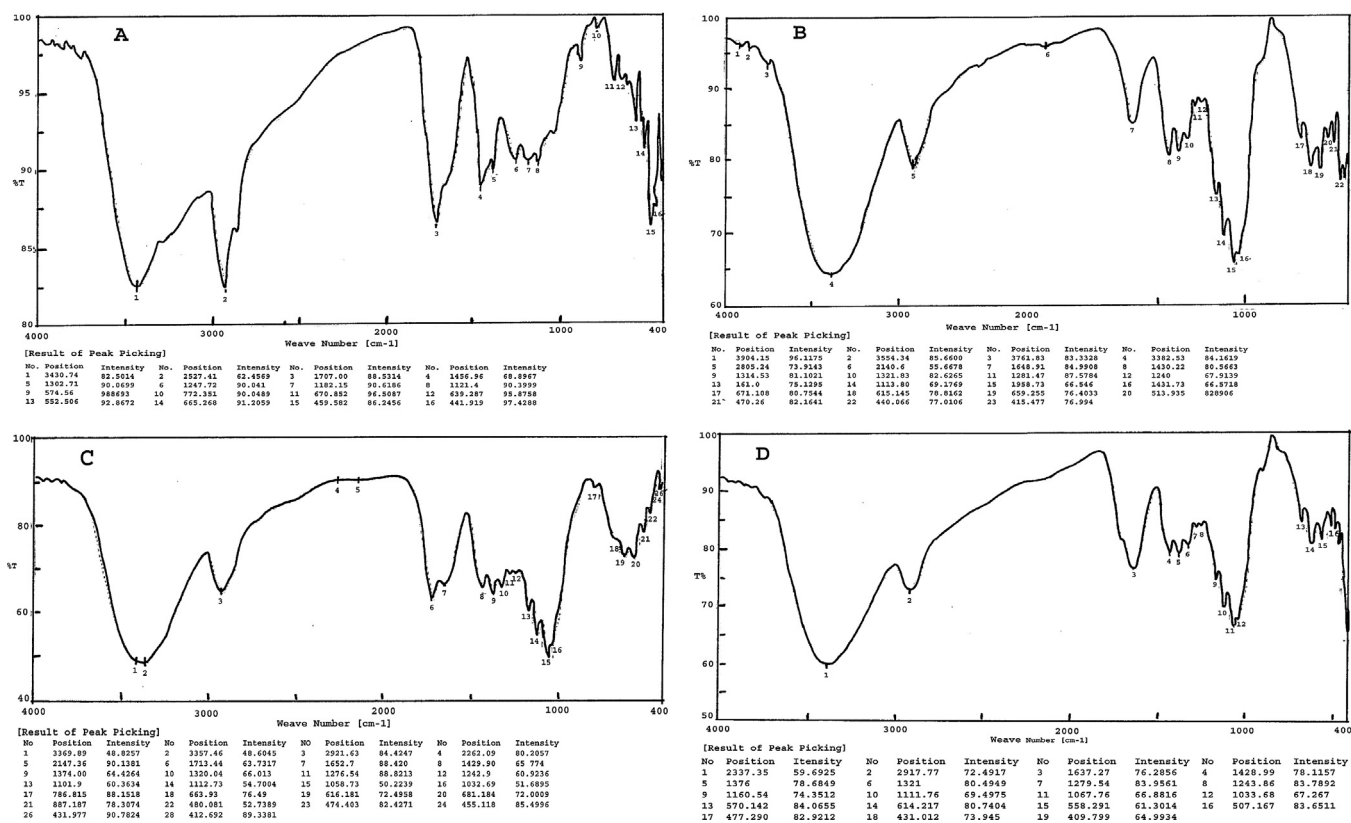


Fig. 7. FT-IR spectra of selected linen samples. (A) Untreated linen sample before ageing. B. Linen sample treated with CS before ageing. C. Untreated linen sample after ageing. D. Linen sample treated with CCTS-SLS after ageing.

reduction of carboxylic acid groups at  $3500\text{ cm}^{-1}$  as an indication of micro-metabolic inhibition caused by Ag-loaded nano-SeO<sub>2</sub> influence in the polymer formation.

#### 4.10. Inductively coupled plasma (ICP-OES)

For a selected treated sample with CCTS-SLS showed accurate elemental analysis, we could realize of elements giving measurements for the polymer wt% reaching 68 wt% very close that of CS, which means that CCTS-SLS occur at higher volume and weight for the consolidated fabric (Fig. 8).

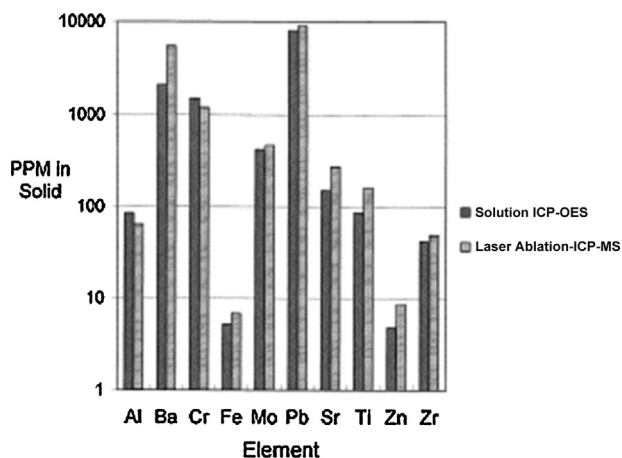


Fig. 8. ICP elemental analysis of selected treated linen sample with CCTS-SLS.

#### 4.11. Dynamic mechanical thermal analysis (DMTA)

This was used to characterize the polymer deforming properties within several stages as shown in Fig. 9 as follows:

- FH30: showed CS treated sample before heat ageing as the spectrum shows two characteristic stages for decomposition. The first stage starts at  $35 \pm 50^\circ\text{C}$  and end at  $91 \pm 50^\circ\text{C}$  with weight loss of  $8 \pm 0.3\%$ . This could be attributed to the moisture content of the untreated and treated linen fabrics;
- FH5: indicated CS polymer after heat ageing forming correspondence and higher sequence to 12d/NM indicating complete deformations of chitin saccharide molecules as a result of heat.

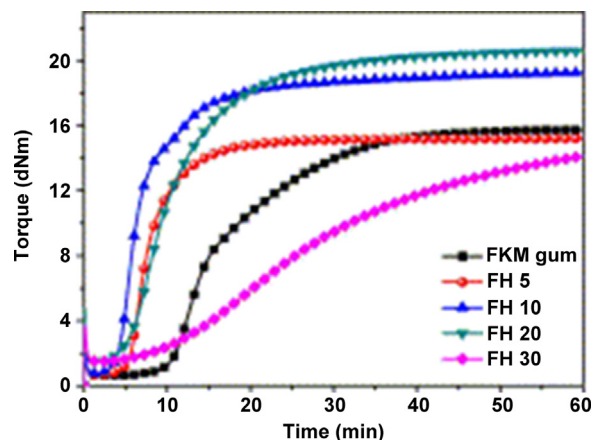


Fig. 9. DMTA analyses for demonstration of used polymers degradation.

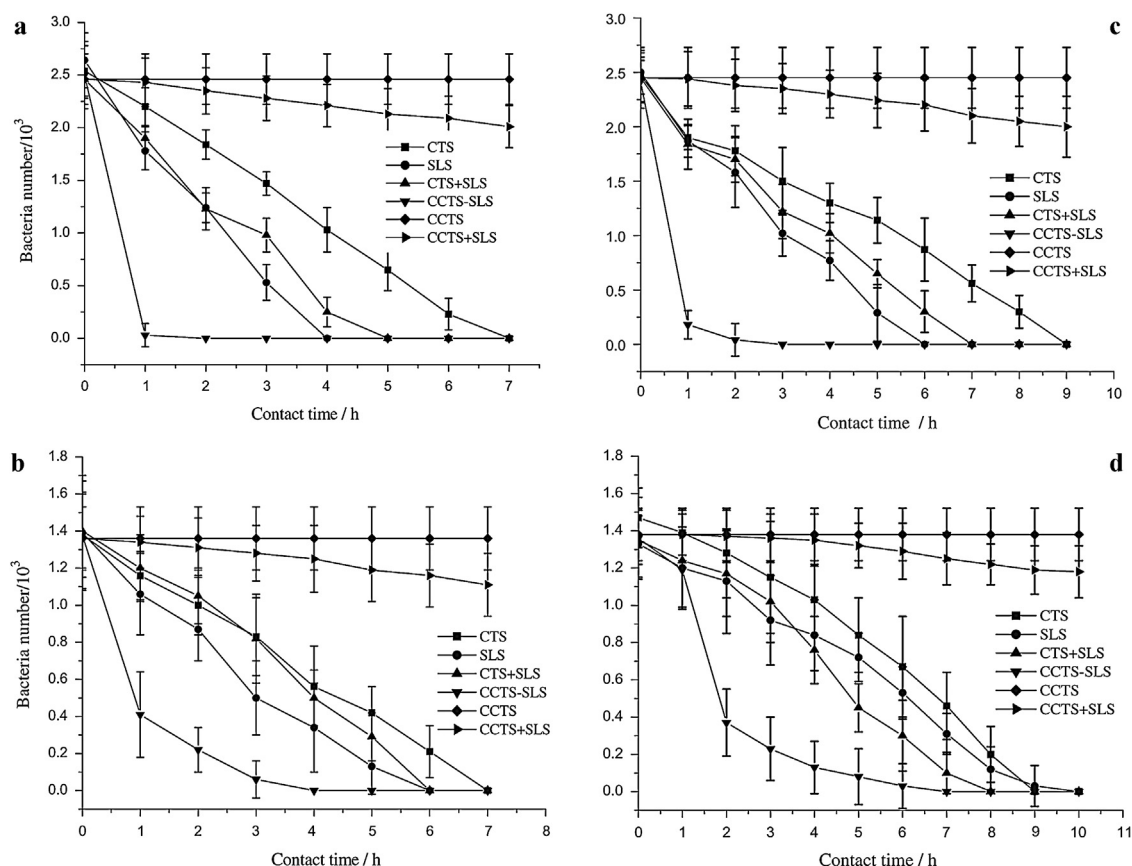


Fig. 10. Time course of antimicrobial activity of 5 mg samples against (a) *E. coli*, (b) *S. aureus*, (c) *Alternaria tenuissima* and (d) *Trichoderma viride*.

This result is in agreement with (Lei, Bi's, 2007) [26] previous studies;

- FH10: showed CCTS–SLS polymer before ageing;
- FH20 characterized CCTS–SLS after ageing seems to retain its properties without critical deformations, which demonstrates the benefits of its usage.

Consequently silver catalysts performed cross-linking on polymeric nano-molecules preventing them from ageing deformations. FKM represented the average standards of tested polymers in contact with their potential intensities.

#### 4.12. Antimicrobial activity of CCTS–SLS

Many factors could influence minimal inhibitory concentration tests (MIC) of CCTS–SLS as mass ratio of isolated species to the polymer concentration. Typical values of cell parameters were recorded in Fig. 10 A and B, representing the time course of 5.0 mg samples against *E. coli* and *S. aureus* so that, if calculated as CTS ratio 1.0 to concentration of CCTS–SLS 1% with cross-linking time 1.5 h. This gave the MIC values of 1000 µg/mL and 1250 µg/mL against *E. coli* bacteria and *S. aureus* as far as breaking down –NH groups by Ag<sup>+</sup> ions and H<sup>+</sup> ions; steric hindrance of NH<sub>2</sub> groups in the cross-linked polymer inhibits the complex reaction of amino groups in CCTS with silver ions, resulting in their complete reduction in the sample treated with CCTS–SLS. Genomic isolations implemented on fungal strains did not give any correlation to the concentration of CCTS–SLS as observed in Fig. 10C and D, showing the time course of 5.0 mg samples against *A. tenuissima* and *T. viride*, and though lack of giving the same inhibitory results showing the high genetic existence of *T. viride* as for the lack of cross-linkage with its genomic biochemical compounds.

The results for the time course of the antimicrobial activity are quite different between bacterial and fungal species, indicating better antibacterial effect obtained against *E. coli* and *S. aureus*. For two dosages of CCTS–SLS, the contact time required to kill all the fungal and bacterial species decreased from 3 to 2 and from 7 to 4 h, respectively, confirming that CCTS–SLS had more powerful antibacterial activity.

#### 5. Consolidation of the 18th Dynasty Funeral Shroud

From all the previous experimental studies, the synthesized CCTS–SLS polymer was chosen as having the better physio-mechanical, chemical and antimicrobial properties. The ancient linen shroud dated to Hatnofer (Senenmut's – chief minister at Queen Hatshepsut's era – mother) about 1850 BC from plain texture linen (1/1), having carbon and metallic inked inscription from Book of the Dead.

According to Abdel-Kareem et al., 2008 [9], reactivation of polymers method was chosen as a practical way of implementation for the synthesis of the polymer nano-composite (CCTS–SLS). Silkscreen supports have been used as an incipient support filled with the used polymer (concentration 10%) dissolved in acetic acid and dried at room temperature. Then, the shroud was placed face down. The previously treated silkscreen support was laid over the back of the shroud, making sure that the warp is correctly aligned. Paper pulp poultices of diluted acetic acid was used to reactivate the synthesized polymer so as to the adhering force be limited between the support and the behind surface of the shroud, thus, there is no penetration of the adhesive into the upper surface of the ancient linen. The method gave excellent results without suffering any penetration of the polymer onto the fabric and thus achieving a successful consolidation process.

## 6. Conclusions

Cross-linked chitosan coated Ag-loading nano-SeO<sub>2</sub> composite was prepared by cross-linking CTS on the surface of SLS particles.

The mass ratio of SLS to CTS, acetic acid concentration and cross-linking time during cross-linking reaction affected the antibacterial activity of the CCTS–SLS.

CCTS–SLS, as an excellent, stable non-staining adhering polymer showed only one drawback in increasing the weight and volume of the consolidated fabrics as a result of the critical mass ratios of Ag<sup>+</sup> and H<sup>+</sup> catalysts used in addition to nano-selenium dioxide.

CCTS–SLS does not show satisfactory results as an anti-fungal bioinhibitor in comparison with its antibacterial activity as for the lack of concision of its mass ratio to the fungal strains mycobiomes.

CCTS–SLS, as an excellent, stable non-staining adhering polymer can be used safely used in consolidation of ancient Egyptian linen textiles.

## Acknowledgment

I am deeply grateful to Chris Cooksy, DHA Group, UK for his revising the language of this article in its great final format.

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