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# *Ex-situ* fabrication of ZnO nanoparticles coated silk fiber for surgical applications

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## HIGHLIGHTS

- ZnO NPs were hydrothermally synthesized using honey as bioreductant.
- The NPs were coated on silk fiber surface using exhaust method.
- The coated fibers showed better resistance to autoclave sterilization.
- Coated fibers showed antimicrobial activity against *S aureus* till 6 days.
- Coated fibers showed remarkably improved tensile strength (2.2 $N/m^2$).

## ARTICLE INFO

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## ABSTRACT

Bio-hydrothermally synthesized ZnO nanoparticles (NPs) were coated *ex situ* on degummed silk fibers and subjected for X-ray diffraction, Scanning Electron Microscopy, and Energy dispersive spectroscopic characterization. Both coated and/uncoated fibers were sterilized by autoclave and ethylene oxide. Effect of coating and sterilization on mechanical properties of fibers was evaluated measuring its tensile strength. Antibacterial efficacy of coated fibers was assayed against *Staphylococcus aureus*. PXRD of uncoated and ZnO NPs coated fibers fairly differ, with former exhibiting highly amorphous nature and latter showing additional ZnO peaks at 34.42° and 36.41° corresponding to (002) and (101) planes with decreased amorphousness. SEM demonstrated spherical and agglomerated NPs on entire fiber surface and EDS confirmed presence of Zn. Coated fibers had significant improvement in tensile strength, measuring 2.2 $N/m^2$ against 0.82 $N/m^2$ of uncoated silk fibers. Autoclaving affected tensile strength of both coated and uncoated fibers detrimentally though coated fibers showed better resistance. Antibacterial activity of coated fibers was excellent on day 2 (1.9 cm zone of inhibition), which gradually declined by 6th day showing that coated fibers have consistent antibacterial activity against *S. aureus*. Hence, coating with ZnO NPs caused improvement in mechanical properties and antibacterial properties of degummed silk fibers, which could be a potent biomaterial for biomedical applications.

## 1. Introduction

Surgical sutures play a vital role in wound healing by providing close approximation of wounds and facilitating required mechanical support during healing process. Proper closure and stabilization of wound margins in their desired position are critical events that influence success of any surgical outcome [1]. There is plethora of surgical suture materials available both from natural as well as synthetic origin which are either resorbable or non-resorbable. The choice of suture material i.e., type and diameter, depends on location, characteristics and conditions of the tissue to be treated [2]. Until 1970, only naturally occurring suture materials, such as catgut, silk and

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cotton were available [3]. Among them, silk is a natural non-absorbable material that has been used since centuries as suture material because of various advantages it offers. It provides excellent tissue compatibility because of its natural origin, excellent pliability, ease of handling and good knot strength [4]. But one major problem associated with silk is its poor microbial resistance which can prove unfavorable in wound healing.

While considering the material properties of suture materials, any material, which is intended to be developed as surgical suture should undergo the sterilization protocol while retaining sufficient mechanical strength and knot strength to bear the suturing procedure. It was found earlier that different physical and chemical sterilization protocols have various effects on mechanical properties and knot strength of silk sutures. The conventional sterilization processes that are used for medical products include steam (autoclave), chemical (ethylene oxide and formaldehyde), ionizing radiation (gamma or E-beam), low-temperature steam and dry heat (hot air). In an earlier study involving silk sutures that was used for ophthalmic surgeries, it was found that the heat sterilization caused detrimental effects over their mechanical properties [5]. Another study involving the ethylene oxide sterilization, showed slight hydrolysis with changes in molecular weight [6]. To combat these challenges, efforts are being made to develop silk fibers with improved mechanical properties and innate antibacterial activity.

Earlier, researches have been conducted towards improvisation of microbial resistance of silk sutures. Recently, nanomaterials, especially the metal oxides are being used for biomedical applications as they encompass excellent antimicrobial properties. Silver doped bioactive glass powder was used to coat silk surgical suture for biomedical applications [7]. In another study, tetracycline antibiotic was coated on silk sutures and investigated the effect of treatment on properties of silk sutures [8]. In a recent study, polydopamine functionalized silk fibers were coated with silver nanoparticles and their antibacterial activity was assayed against *E. coli* and *S. aureus*, and the doped material was found to have enhanced activity against both the tested microorganisms [9]. On the same line, in the present investigation, biohydrothermally synthesized ZnO nanoparticles (NP) were considered as coating material on degummed silk fibers to evaluate its post sterilization impact over microbial properties and also change in mechanical properties of the coated silk fibers.

Moreover, ZnO NPs have wide application in sunscreens, biosensors, food additives, pigments, antibacterial agents etc [10]. They are known to have significant growth inhibition on *E. coli* and good bacteriostatic activity against *S. aureus*, both of which are pathogenic to humans and cause several infectious diseases [11,12]. The material is a GRAS (Generally Regarded As Safe) material and hence found applicability both in medicine and dentistry. Considering the need and usefulness, ZnO nanoparticles that were biohydrothermally synthesized [13] would augment antimicrobial as well as mechanical properties in silk fibers. The ZnO NPs because of their extremely smaller size are also able to migrate and bind between silk fibers that might bring an improvement in antimicrobial as well as mechanical property by reinforcing the silk fibers.

The current study is intended to examine the effect of coating nano-sized ZnO, on the antibacterial and mechanical properties of *Bombyx mori* silk fibers. Because, one of the disadvantage of silk as suture material is lack of innate antimicrobial property, thus posing a danger of infection along suture line in certain instances, especially those with immune-compromised status. With this gap, we have planned to coat the native silk fibers with biohydrothermally synthesized ZnO nanoparticles [13] to bring an improvement in the mechanical and antimicrobial property of silk fibers, which can be used as potent surgical suture material.

## 2. Materials and methods

### 2.1. Silk material, nanoparticles and bacteria

Bio-hydrothermal synthesis of ZnO nanoparticles and coating of silk fibers was carried out at Centre for Materials Science and Technology, Vijnan Bhavan, University of Mysore, Mysore. Degummed silk fibers of the silkworm, *Bombyx mori* strain - NB4D2 were produced in the Department of Sericulture Sciences, University of Mysore, Mysore. The denier of the silk filament was calculated using the formula,

$$Denier = \frac{Weight\ of\ the\ silk\ filament}{Length\ ofthe\ \ silk\ filament} X\ 9000$$

For the synthesis of ZnO nanoparticles, Zinc acetate (99.9%) was obtained from Alfa Aeser, by Thermo Fisher Scientific, India, NaOH from Loba-chemie Pvt Ltd, Mumbai, Maharashtra, India and ultrapure water of resistivity 18.5 MΩ (Pure labs Q, ELGA, Wycombe, United Kingdom). Pure honey (Dabur India Pvt Ltd., New Delhi, India), which was used as bioreductant was obtained from the local market.

Pure culture of *S. aureus* (MTCC 6908) was obtained from Microbial Type Cell Culture and Gene Banking (MTCC), Chandigrah, Punjab, India. Luria broth and Muller-Hinton agar used in bacteriological studies were obtained from HIMEDIA laboratories, Mumbai, Maharashtra, India.

### 2.2. Sterilization

The physical and chemical sterilization of silk fibers were carried out at JSS Dental College and Hospital. Mechanical testing was carried out at Polymer testing laboratory, Department of Polymer Science Engineering, Sri Jayachamareajendra College of Engineering, Mysore.

### 2.3. Bio-hydrothermal synthesis of ZnO nanoparticles and their characterization

Synthesis of ZnO nanoparticles was carried out following the protocol described elsewhere [14] using pure honey as bioreductant and stabilizing agent under hydrothermal conditions. The characterization of synthesized ZnO NPs was carried out for structural and morphological confirmation using PXRD, UV–Vis spectroscopy, SEM and DLS measurements [14].

### 2.4. Coating of ZnO NPs on silk fibers

Silk degumming is a process mainly involved in removing glue protein (sericin) that encases hard crystalline structure - fibroin and also other impurities. Silk filament reeled from a cocoon using eprouvette was cut into 40 cm length and conditioned at 65% relative humidity and 95 °C for 1.5–2 h. Degumming was carried out at a liquor ratio of 1:40 using standard technique [15]. At the end of the process, test samples were washed for 5 min in 1.5 L hot water and then rinsed for 5 min in 1.5 L cold water. 25 cm of the fibers weighing about 0.003 g were taken for coating process. There were approximately 40–45 filaments in each of the fiber tuft. For coating of ZnO NPs on the surface of fibers, the exhaust method (Mahmud et al., 2017) was employed with suitable modifications, for which a regular bench type water bath shaker was used. 100 ml of 0.5% suspension of biosynthesized ZnO NPs was prepared by dispersing required quantity of NPs in ultrapure water and further sonicating it for 45 min to get complete dispersion. The beaker containing this dispersion was kept in shaker. The vital parameter for successful application of ZnO NPs on the surface of fibers was observed (time, temperature and pH). The silk fibers that was functionalized with sodium alginate was dipped inside the beaker containing ZnO NPs dispersion which was under constant agitation and maintained for 1 h at 40 °C at the optimum pH of 7.7 [16–18]. Further, the NPs coated silk fibers were removed and washed and dried at room

temperature.

### 2.5. Characterization of ZnO NPs coated silk fibers

To confirm the coating of biosynthesized ZnO NPs on the silk fiber surface, PXRD of coated and uncoated fibers was carried out using Rigaku smart Lab–II, CuKα radiation with step size of 0.0001 deg. External morphology of fibers was determined using Hitachi S-3400 SEM and at the same time, elemental composition of fibers was determined using EDS.

### 2.6. Sterilization of silk fibers

As the sterilization protocols are known to alter physical and mechanical properties of surgical sutures, both the coated and uncoated fibers were subjected to autoclave and EtO sterilization (Alliance Elemech ES, Ahmadabad, India). 25 cm of coated/uncoated silk filament was packed in a paper wrap and sealed with autoclave tape. The heat sterilization was carried out using class B (Tuttnauer 2540 EKA, USA) autoclave at 121 °C for 15 min. The chemical sterilization was carried out using Eto sterilizer. The coated/uncoated fibers were sterilized using EtO gas at relative humidity of 60% and exposure time of 6 h followed by ten repeated air flushing cycles/hr to remove the excess ethylene oxide retained.

### 2.7. Determination of mechanical properties

Mechanical properties of both coated and uncoated silk fibers were determined before and after sterilization using autoclave or EtO. The test was performed to determine the breaking strength and maximum load that could be borne by fibers. Coated/uncoated fibers of length 10 cm was mounted on to mechanical grip of UTM as seen in Fig. 1. The lower member of the UTM was pulled at a constant rate of 5 mm/min, until the thread broke. The ultimate tensile strength was determined using the formula;

$$\textit{Ultimate tensile strength} = \frac{\textit{Maximum load}}{\textit{Original cross sectional area}}$$

### 2.8. Determination of antimicrobial activity

Antimicrobial efficacy of ZnO NPs coated silk fibers was determined using agar diffusion test. $1 \times 10^6$ CFU/ml of *S. aureus* fresh liquid culture was prepared in Luria broth by using 0.5 McFarland standard to adjust the turbidity of the solution. 500 µl of this suspension was plated on Muller Hinton agar surface. Further, the ZnO NPs coated fiber that were EtO sterilized and measuring 1 cm was wound circularly and placed on inoculated agar plates and incubated at 37 °C for 24 h. Further, Zone of Inhibition (ZOI) was measured using a Vernier's caliper perpendicular to sutures. The experiment was conducted in triplicates for confirmatory results. The experiment was repeated until 6 days to determine the residual antibacterial activity till no significant inhibition zone existed [19] and threads could no longer be used for repetitive plating due to loss of strength.

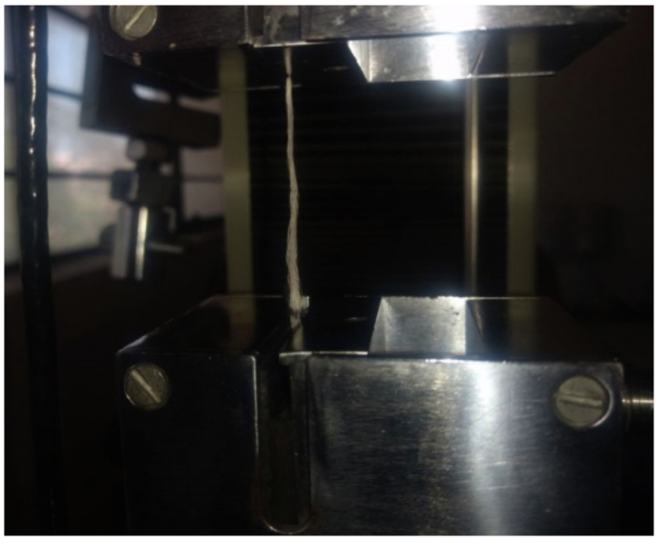

**Fig. 1.** Silk fiber under tensile load in universal testing machine.

### 2.9. Statistical analysis

The data was statistically analyzed using SPSS software by applying one way ANOVA with significance threshold set at 0.05.

## 3. Results and discussion

### 3.1. Bio-hydrothermal synthesis of ZnO nanoparticles and their characterization

Natural honey, a rich source of carbohydrate, has high nutritious value and documented as world's oldest food source [20]. The major constituents of honey are fructose and glucose and several vitamins, minerals, amino acids and trace amounts of flavonoids, which contribute to its reducing (antioxidant) nature [21,22]. The polysugars present in honey performs the dual action of stabilization and reduction of metallic ions [23].

The hydrothermal technique is known to have excellent control over the size and morphology of nanoparticles [24]. Combining the advantage of both the methods, the nanoparticles of ZnO were synthesized. The synthesized nanoparticles were 49 nm in size and spherical in shape and their formation was confirmed by UV spectroscopy and PXRD as cited in [24].

### 3.2. Physical/morphological characterization of coated and ZnO NPs coated silk fibers

#### 3.2.1. PXRD

The phase composition of samples was confirmed by XRD results. Fig. 2A shows XRD pattern of uncoated degummed silk fibers which explicit its crystal structure [25]. The pattern appears amorphous depicting prominent organic phase [26]. Fig. 2B exhibit the XRD pattern of ZnO NPs coated silk fibers. The graph is characterized by absence of wide amorphous peak, with more prominent and sharp peaks indicating the phase change due to incorporation of ZnO NPs. Presence of enhanced peaks at 34.42° and one more at 36.41° corresponding to 2Θ values of (002) and (101) that confirms the presence of ZnO (JCPDS 36–1451) as supported from previous literature [27] and also the EDS results mentioned in subsequent section.

#### 3.2.2. SEM and EDS

SEM images of uncoated silk fibers, presenting with fine and translucent uniform fibers that are randomly connected and having smooth surface as seen in Fig. 3A and B. This morphology of degummed silk fibers is supported by earlier literature [28]. The silk fibers that were treated with ZnO NPs showed agglomerated as well as discrete NP that were present throughout the surface of silk fibers as seen in Fig. 3C and D. At the same time, the density of loading ZnO NPs on fiber surface was quite high, which also got reflected in the results of antibacterial activity against *S. aureus*.

The EDS spectra further confirmed the successful doping of ZnO NPs on the surface of silk fibers. A total of 0.5% of weight of Zn was found in EDS spectra of coated silk fibers (Fig. 4 and Table 1).

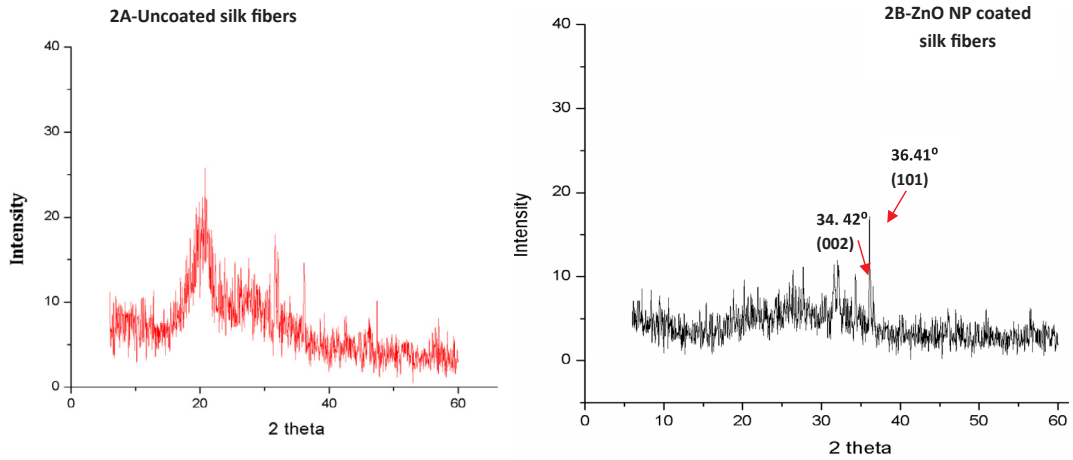


**Fig. 2.** A - X-ray diffractogram of uncoated silk fibers with broad amorphous peak, B - Diffractogram of ZnO NP coated silk showing changed phase with prominent 2Θ at (002) and (101) plane.

### *3.3. Determination of mechanical properties*

Table 2 shows the tensile strength exhibited by different groups of silk fibres. The ultimate tensile strength of fibers under the given peak load was determined using UTM, by pulling it at a constant speed of 5 mm/min until the sample broke. Experiments were performed in triplicates and the average of ultimate tensile strength was expressed in $N/m^2$. The peak load that was taken differed between samples. The control samples (unsterile uncoated silk fibers) showed lowest tensile strength of $\sim 0.82\ N/m^2$, whereas the ZnO NPs coated unsterile silk fibers showed $\sim 2.2\ N/m^2$ tensile strength at the peak load of 2.2 kg. This result indicates that *ex situ* coating of ZnO NPs on the surface of silk fibers have positively brought an improvement in its mechanical properties. The polymeric molecules of silk fibers are reinforced by nano sized ZnO NPs, which probably has acted as cross linking agent or filler [29] increasing the resistance of coated fibers under pulling load. The micro-structural evidence for the same is contemplated from the SEM images (3.2.2, Fig. 3C), wherein the two brins (silk fibroin) in a bave that contribute equally and independently to tensile load bearing ability [30] were cross linked by ZnO nanoparticles. Thus, tensile strength of ZnO NPs coated fibers undoubtedly enhanced due to cross-linking of smaller sized ZnO nanoparticles, which is unique feature of the present study. Besides, degumming process - removing the gummy substance (sericin) - allow migration and binding of ZnO NPs with ease and facilitate cross-linking of two brins that enhances the tensile strength of ZnO-NPs linked silk fiber.

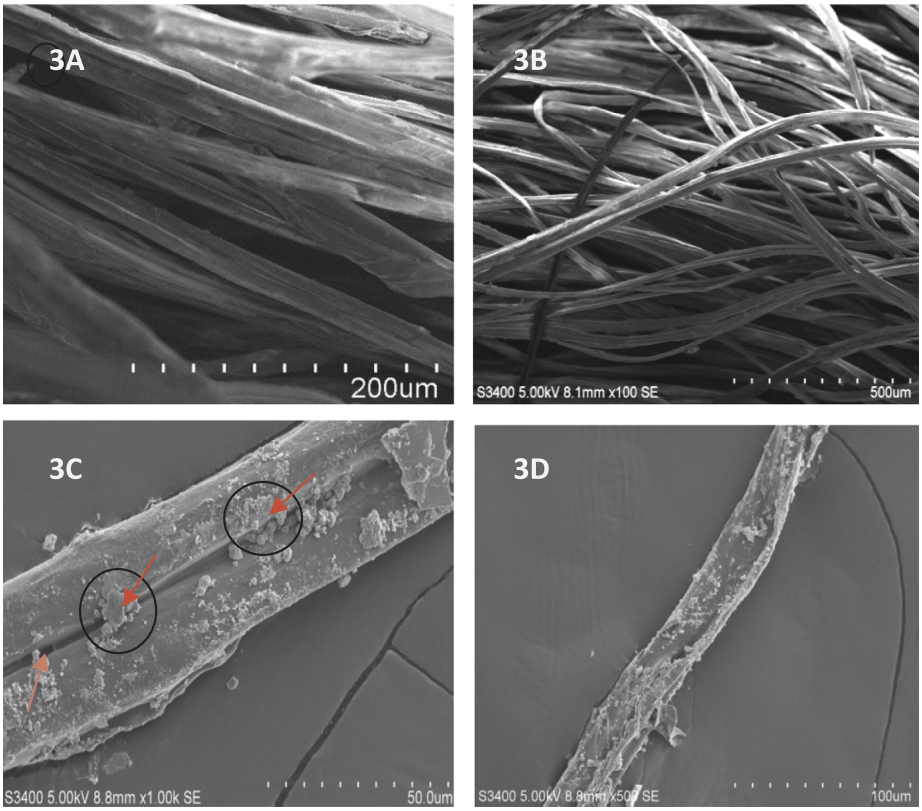


**Fig. 3.** A & B – SEM of uncoated silk fibers with long network like structure and with clear appearance; C & D - SEM of ZnO NP coated silk fiber with uniform distribution of nanoparticles.

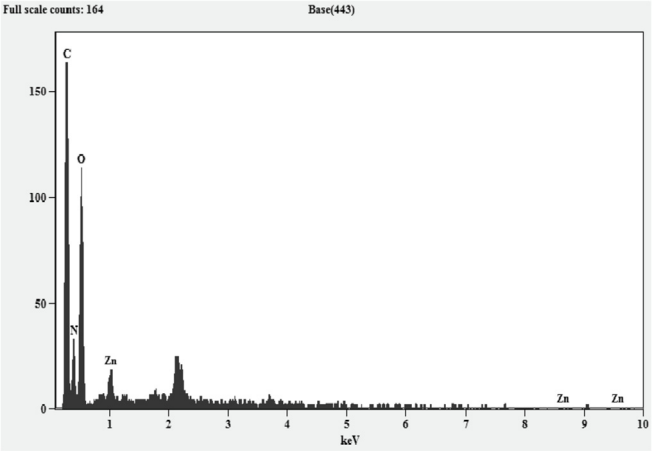


**Fig. 4.** EDS spectra of ZnO NP coated silk fibers having presence of Zn as a detectable element in the spectra.

**Table-1**
Weight percentage of individual element distribution in ZnO NPs coated silk fiber.

| Element Line | *Weight %* | *Weight % Error* | *Atom %* |
|---|---|---|---|
| *C K* | 26.87 | ± 0.62 | 31.38 |
| *N K* | 38.50 | ± 2.33 | 38.56 |
| *O K* | 34.15 | ± 0.99 | 29.95 |
| *Zn K* | 0.48 | ± 0.31 | 0.10 |
| *Zn* L | – | – | – |
| *Total* | 100.00 | | 100.00 |

At the same time, effect of heat and chemical sterilization on tensile strength of coated and uncoated silk fibers was also analyzed. From the results of the test, it was evident that heat (autoclave) caused detrimental effects on tensile properties of both coated and uncoated silk fibers with slight improvement seen in coated fibers. Autoclaving results in critical structural rearrangement affecting degradation and mechanical properties [31,32]. An improvement in tensile strength of ZnO NPs coated fibers after sterilization may be due to cross-linking established by smaller sized ZnO NPs [33] (Table 1). This feature is due to combined impact of ZnO NPs and silk fiber, because, ZnO basically has high heat capacity and conductivity along with high melting temperature [34], while silk shall be heated in an oven at 110 °C, which disintegrate at 170 °C [35,36]. So, ZnO-NPs linked silk fibers shall be a much better material for surgical applications.

### 3.4. Determination of antimicrobial activity

As indicated earlier (2.2), 0.5% suspension of ZnO NPs was used for coating on the surface of silk fibers. The antimicrobial activity assay was carried out for several days (from day 1–6) until the fibers lost all their strength. The fibers were EtO sterilized before inoculation. The uncoated sutures did not show any antimicrobial activity, whereas the ZnO NPs coated fibers showed a consistent antimicrobial activity,

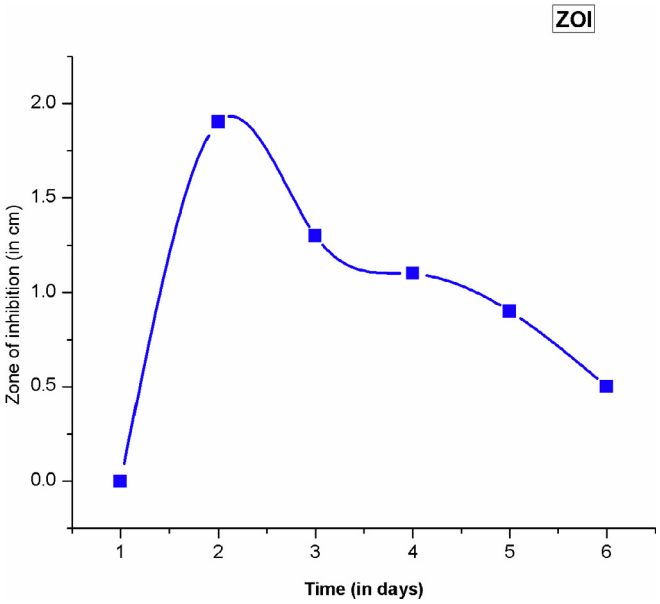


**Fig. 5.** Antibacterial activity of ZnO NP coated silk fibers against *S. aureus*.

though declining from 1.9 mm (on day 2) to 0.5 mm (on day 6). The experiment could not be continued further as the fibers lost all their strength for transferring to plating due to silk-protein degradation. Fig. 5 shows the graphical representation of ZOI caused by ZnO NP coated silk fibers. Thus, ZnO NPs coated fibers developed in the present investigation shall be a bio-degradable potent suture for surgical applications.

Basically, due to the hygroscopic nature of the silk fibers microbial infection is not uncommon as the porous hygroscopic structure retains moisture and nutrients, which is favorable environment for microbial development [37,38]. *S. aureus* is one of the common microbes attacking the silk while being pathogenic to humans causing skin and mucous membrane infections [39]. As observed from previous studies, the antimicrobial activity of ZnO NPs is directly proportional to decrease in their size and ZnO NPs of 40–50 nm size have caused effective inhibition of *S. aureus* [40]. It was proven earlier that ZnO NPs have better inhibitory effect over gram positive bacteria due to structure of bacterial cell wall, cell physiology, metabolism and degree of contact [41]. In our study, we could observe that ZnO NP coating present on silk fibers effectively inhibited the growth of microbe up to 6 days and covering a wide area of 1.9–0.5 cm. Normally, the sutures are removed after 5–10 days of surgery depending on the anatomic location [42] and as the silk sutures are mainly indicated in suturing of mucosal and intertrigenous areas, which are very rich in blood supply leading to early wound healing, ZnO NPs coated silk fibers could be considered as suture biomaterials for such areas [43].

Taken together, we could infer that coating of bio-hydrothermally synthesized ZnO NPs on silk fibers has brought certain changes that could be well utilized in clinical applications. In the current study, we have assessed the antimicrobial activity of ZnO NPs coated silk fibers against a representative organism of wound infection, the *S. aureus*,

**Table-2**
Tensile strength of different groups of silk fibers.

| Test groups | Peak load at break (in Kg) | | | | Ultimate Tensile strength ($N/m^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Before autoclave | After autoclave | Before EtO | After EtO | Before autoclave | After autoclave | Before EtO | After EtO |
| Control (uncoated fibers) | 0.82 ± 0.006 | 0.30 ± 0.12 | 0.82 ± 0.0 | 0.79 ± 0.006 | 0.81 ± 0.00 | 0.30 ± 0.05 | 0.80 ± 0.05 | 0.79 ± 0.006 |
| Test group(ZnO NP Coated fibers) | 2.20 ± 1.00 | 1.77 ± 0.58 | 2.20 ± 0.20 | 2.17 ± .208 | 2.21 ± 0.35 | 1.16 ± 0.05 | 2.23 ± 0.05 | 2.20 ± 0.006 |
| F-value | 572.16 | 1853.087 | 142.830 | 131.742 | 4744.892 | 33800.00 | 91164.500 | 90312.500 |
| Sig. | 0.000** | 0.000** | 0.000** | 0.000** | 0.000** | 0.000** | 0.000** | 0.000** |

similar antimicrobial potency of ZnO NPs - silk fibers against other multiple drug resistant organisms is warranted. At the same time, it has opened ample scope for the comparative evaluation of mechanical properties of ZnO-silk fibers with other existing suture materials for adequacy of tensile property and knot strength.

### 3.5. Statistical analysis

The results of tensile strength measurement (peak load at break and ultimate tensile strength) and antimicrobial activity against *S. aureus* was determined using one way ANOVA using SPSS software. Results of mechanical testing showed that for both the variables i.e., peak load at break and ultimate tensile strength, the p value remained $< 0.001$ and hence the study is statistically significant. Similarly the results of antimicrobial activity of coated fibers remain significant with p value less than 0.001.

## 4. Conclusion

The present study was performed to explore the possibility of using bio-hydrothemally synthesized ZnO NPs as antibacterial coating on the surface of degummed silk fibers from *B. mori* cocoons, which do not have inherent resistance to *E. coli* [44]. The ZnO NPs coated fibers exhibit significant antibacterial activity against *S. aureus* in sustainable manner up to 6 days. Besides, *ex situ* coating confirmed through PXRD, SEM and EDS results revealed coated fibers posses a better mechanical strength than uncoated fibers by the virtue of cross linking of smaller sized ZnO nanoparticles with two brins of a bave. Thus, it could be concluded that incorporation of ZnO NPs on degummed silk fibers not only enhances mechanical strength and heat stability but also antibacterial properties of native silk fiber. Thus, ZnO NPs - silk fibers shall be considered for fabricating antibacterial sutures with high tensile strength for surgical applications.

## Conflicts of interest

None.

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