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Research article

Hemocompatibility and antimicrobial study using chitosan-coated braided silk fibroin fibers

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Abstract: Silk fibers are biomaterials widely employed in various biomedical applications and products used to treat, repair, and replace damaged tissues, alongside being modified with other biomaterials. This study investigates the hemocompatibility and antimicrobial properties of chitosan-coated braided silk fibroin fibers (SFF), and their potential applications in biomedical implants and as sutures in wound dressings. The SFF was braided using a three-strand hand-braiding method and coated with a chitosan solution. The layer-by-layer dipping-coating method was used for coating. The samples were characterized using Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and light microscopy to assess the coating integrity and surface morphology. The SEM revealed swollen, smooth surfaces, which indicated successful chitosan coating, while the XRD patterns confirmed the semi-crystalline nature of the coated fibers with broad peaks typical of biopolymers. Hemocompatibility was assessed using the following coagulation parameters: prothrombin time (PT sec; PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT). The results indicated an improved clotting time compared to the controls, which suggests anticoagulant properties. The antimicrobial activity was evaluated using the agar disc diffusion method against six microorganisms: E. faecalis, B. cereus, S. aureus, E. coli, P. aeruginosa, and C. albicans. The chitosan-coated fibers showed significantly larger inhibition zones than the uncoated controls, thus confirming enhanced antimicrobial efficacy. The study demonstrates that chitosan coating substantially improves the biological property of SFFs, which supports the potential of chitosan-coated SFFs as multifunctional biomaterials suitable for surgical sutures, wound care, and tissue engineering applications.

Keywords: hemocompatibility; silk fibroin fibres; antimicrobial activity; chitosan; scanning electron microscope; X-ray diffraction

1. Introduction

In the field of engineering, biomaterials are used because they have a variety of characteristics that make them compatible with the biological system of tissues [1–3]. The polymers that create these biomaterials can take many shapes, including hydrogels, films, and fibers [4]. Biomaterials, including polymers, can be either natural or manufactured, and their forms require distinct methods of processing and applications due to their numerous desirable properties, including high biocompatibility, low immunogenicity, low bacterial adhesion, and a slow rate of biodegradability [1–6]. Biomaterials are currently used in various applications, including biomedical equipment, other biological products, therapies, repairs, and replacements for damaged tissues and organs. Thrombosis, necrosis, and different reactions can occur when biomaterials come into contact with biological tissues; this is because the host body's defense mechanism is activated against the implanted or foreign material. Because of these side effects, biomaterials must either undergo surface modification or redesign before they can be properly implanted into biological tissues [1–6]. The polymer's surface chemistry affects cell adhesion, migration, proliferation, and differentiation. Surface functionalization or modification is a procedure that gives biomaterials the desired characteristics necessary for practical applications [4,7].

Silk is a continuous strand of fibroin created by the larvae of silkworms such as Bombyx mori (B. mori) as they spin their cocoons. When processed, silk fibers are left with only the fibroin (which gives silk its strength) and the sericin (which acts as a gum). Since natural silk is comprised of two biomacromolecules (fibroin and sericin), it can be used in various contexts, from the medicinal to the technological to the textile. Silk fibroin, a natural protein polymer, has recently seen increased use by researchers in biomedical applications and other domains because of its high mechanical qualities and structural integrity [1,4,6–10]. Silks are a novel family of structural proteins with desirable properties such as biocompatibility, biodegradability, mechanical superiority, an amenability to processing in either aqueous or organic solvents, and chemical modifications for various biomedical settings [7,11–14]. Several investigations have been conducted to increase blood compatibility through silk fibroin surface modifications [4,7]. Research on silk fibroin films was conducted by Jiang et al., who added a layer of zwitterionic phosphobetaine doped with hydroxyl and 8-hydroxy-2-octyl phosphorylcholine. The efforts were fruitful, and the outcomes were encouraging, with zwitterionic phosphobetaine demonstrating a strong nonthrombogenicity in a platelet adhesion test [15]. Additionally, Vepari et al. studied silk fibroin, and their results suggested that polyethylene glycolated silks' surfaces would be important in anti-adhesion and anti-thrombosis tests when applied for biomedical purposes [7].

Chitosan is a cationic polysaccharide of 2-amino-2-deoxy-D-glucan derived from chitin through alkaline deacetylation [1,16–18]. Insect and marine crustacean (e.g., shrimp and crabs) exoskeletons contain this deacetylated chitin. Chitosan's versatility stems from its many desirable qualities, such as its low cost, ease of production, rapid decomposition, low toxicity, and high bioactivity. One of the most appealing aspects of chitosan is the variety of shapes and sizes it may take after being subjected to the right technological procedures [16,17]. The antimicrobial activity, wound healing

ability, biocompatibility, low toxicity, scar prevention capability, and the ability to smooth the surface of various structures are just a few reasons why chitosan, a natural polysaccharide, is so significant as a coating ingredient [1,16,17]. In the medical field, chitosan films have been utilized as tissue and bone engineering scaffolds and as curative wound dressings. When processing silk fibers into sutures, one approach utilized the inclusion of chitosan by surface modification to improve their qualities [18].

This study investigates the antimicrobial effect and hemocompatibility of uncoated braided and chitosan-coated braided silk fibroin fibers (SFF) (Figure 1). The SFFs were braided using physical methods with crocheted needles and highly sterilised for all the analysis. The uncoated and chitosan-coated braided SFFs were characterized using light micrography, Scanning Electron Microscopy (SEM), and X-ray diffraction (XRD) analyses. After a careful search, no study has been published relating to using chitosan to modify the surface of SFFs in recent years or investigating its effect on bacteria and hemocompatibility.

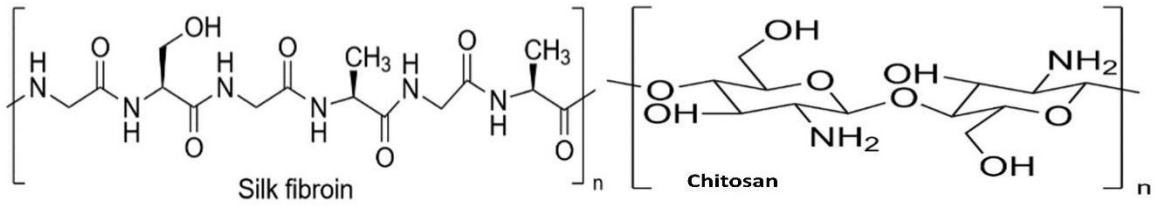

Figure 1. Chemical structure of silk fibroin and chitosan.

2. Materials and methods

2.1. Materials

The silk fibers used were bought from Kirman İplik, Bursa, Turkey. The silk fibers had values of 51.51 brightness, 1.67 N/mm 2 breaking strength, 54.474 Stensby, and 16.66 % max elongation, and had shiny, pure and soft characteristics. The chitosan powder was obtained from Sigma-Aldrich, Turkey. The chitosan powder had an off white to beige and faint brown to light brown color. The appearance (Form) conformed to the requirements of powder and/or chips, deacetylation ≥75 %, viscosity 200–800 cps, c = 1%, and 1% Acetic Acid. 200 mg and 400 mg of chitosan were measured and dissolved in 25 mL of 0.1 M acetic acid in different beakers. The acetic acid solution was prepared fresh and used to dissolve the chitosan, which was later used for to coat the silk fibers. The digital laboratory hot plate magnetic stirrer, magnetic stirring bars, sensitive precision laboratory analytical electronic weighing scale, and all other glassware used were analytical grade.

2.2. Methods

2.2.1. Braiding the silk fibers

The three-strand braiding method was used to braid the silk fibers: the fiber strands were divided into 3 strands and carefully hand-braided to form one strand. Then, they were hand-crocheted to create

a small circle disk of the silk fiber before they were used in the study. The long three-strand braided silk fibers were coated with the chitosan solution and allowed to air dry within 30 minutes to 1 hour intervals for the 200 mg and 400 mg chitosan coating concentrations, respectively.

2.2.2. Preparing the chitosan solution

To prepare the chitosan, the chitosan microparticles were measured using an electronic weighing balance to final measurements of 200 mg and 400 mg. 25 mL of freshly prepared diluted 0.1 M acetic acid was used to dissolve the chitosan within a beaker. The mixtures were placed on magnetic hot plates, magnetic stirring bars were dropped inside the beakers, and the solutions were stirred at a temperature of 60 ℃ and a rotation of 2. The mixtures were allowed to stir for about 20 to 30 minutes to mix homogenously. After the chitosan particles were properly dissolved and a viscous chitosan acetate solution formed, it was allowed to cool down before the three-stranded braided silk fiber dipping process was performed.

2.2.3. Coating the braided silk fibers with chitosan

The layer-by-layer dipping method was employed to coat the braided silk fibers with the chitosan solution. This method involves coating the silk fibers by dipping them in the chitosan acetate solution for 15 to 30 minutes in a beaker to coat and deposit chitosan on the braided silk fibers, and later drying within time intervals of 30 to 60 minutes. The silk fibers were tied around a pipette on one end and hung on the beakers during the dipping and drying process. This procedure was repeated until there was a homogenous coating of chitosan on the braided silk fibers. This process was performed for both concentrations of the chitosan acetate solution, which were used for further investigations.

2.2.4. Measuring the coated and uncoated silk fibers

The thickness or thinness of the coated and uncoated braided silk fibers were measured, and several differences were observed to check whether there were any differences before and after the coating process. An inverted light microscope was used to observe and measure the differences in the diameter of all the braided silk fibers, both for the coated and uncoated braided silk fibers.

2.2.5. Sterilization method for both coated and uncoated samples

The uncoated and coated braided silk fibers were regularly sterilized before every step of the study. The fiber samples were sterilized at the sterilization unit of Near East University Hospital, Lefkosa, using steam sterilization. This relatively simple process kills microorganisms by destroying metabolic and structural components essential to their replication. The samples were exposed to saturated steam at 121 °C for 30 minutes at a pressure of 115 kPa.

2.2.6. Hemocompatibility analysis on coated and uncoated braided silk fibers

The hemocompatibility analysis is a very important test performed on silk fibers to check for coagulation activity in the plasma of blood, where the prothrombin time (PT sec; PT), international

normalized ratio (INR), activated partial thromboplastin time (APTT), and clotting time were measured at 0 minutes, 10 minutes, 20 minutes, 45 minutes, and 90 minutes. These times were used to observe the interaction of the silk fibers, both coated and uncoated, and blood plasma. The STA Compact Hemostasis System was used to analyze the plasma samples and silk fibers in siliconized vacutainer tubes containing 3.2% trisodium citrate. Sodium-citrate anticoagulated whole blood samples were centrifuged at 1500 rpm for 10 min to separate the plasma, platelets, and red blood cells. The plasma was removed and used for the assay. The lengths of the silk fibers were measured between 10 cm and 15 cm. They were cut and dropped into the siliconized vacutainer tubes containing 3.2% trisodium citrate and an anticoagulant tube carrying 2 mL of plasma over various time ranges (i.e., 0 minutes, 10 minutes, 20 minutes, 45 minutes, and 90 minutes). Then, the samples were analyzed by the coagulation machine, and the results were used to understand the interaction between the plasma and coated or uncoated SFFs. The PT (sec), PT (%), APTT, and INR values were used as results markers for the coagulation study. The APTT is the time (in seconds) it takes for blood to clot via the intrinsic and common pathways of the coagulation cascade, which has a normal range of values for 23–35 seconds. The PT values indicate the time (in seconds) it takes for plasma to clot after adding a tissue factor, evaluate the extrinsic pathway of the coagulation cascade, and have a normal range of values of 11–15 seconds. PT (%) is the percentage of normal clotting activity, where a higher % represents faster clotting and ranges between 70–120%. The INR values standardize the PT values obtained during the coagulation study and range from 0.8–1.2.

2.2.7. Antimicrobial susceptibility test using the silk fibers

An antimicrobial susceptibility test was conducted to determine the in vitro activity of the coated and uncoated braided silk fibers. The antimicrobial susceptibility test was performed with six microorganisms: 3 gram-positive bacteria, *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10876), and *Staphylococcus aureus* (ATCC 28923), 2 gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and 1 fungus, *Candida albicans* (ATCC 90028). The disc diffusion method was used, thereby utilizing Mueller-Hinton agar, based on the clinical and laboratory standards protocol for antimicrobial susceptibility testing. For the susceptibility test, the Mueller-Hinton agar was prepared at a concentration of 34.0 g/L and sterilized by autoclaving at 121 °C for 15 minutes, according to the manufacturer's guidelines. Then, the solution was poured into petri dishes to a depth of about 4 mm, left to cool, and placed in the refrigerator and left overnight to fully solidify. Additionally, broth of all the microorganisms was prepared to a standard value of 0.5 McFarland, approximately 10.6 cells/ml, which was measured using the BD Phoenix spec nephelometer. The agar plates were labelled according to the name of the microorganisms to be used, and sterile cotton swabs were used on their surfaces to inoculate them with 10 μL of ID broth containing each microorganism. Moreover, the coated and uncoated crocheted disk fiber samples were carefully placed in the culture media inoculated with each microorganism. The plates were placed at room temperature for 10 minutes and incubated upside down at 37 °C for 24 hours. After 24 hours, the results were readily visible. The diameter of the inhibition zones was measured in triplicate, with their standard error mean values.

2.2.8. Characterization of the samples of the silk fibers

2.2.8.1 Scanning Electron Microscopy Analysis

The SEM analysis was performed at the TUBITAK-Marmara Research Centre at Gebze, Istanbul, Turkey, using an SEM M-JSM-6510 model at an acceleration voltage 10Kv to analyze the surface topography and morphology of the silk braided sutures. The device produces images of the samples by focusing a beam of electrons on them, and the samples are coated with gold to prevent charging.

2.2.8.2. X-ray Diffraction Analysis

The XRD analysis was performed at TUBITAK-MAM Gebze, Turkey, using a Shimadzu XRD-6000 model diffractometer with a Cu Kα radiation source (λ = 1.5405 Å). The diffractometer settings were adjusted based on the sample requirements, with the following X-ray tube conditions: target = Cu, voltage = 40.0 (kV) and current = 40.0 (mA). The Slits had the following conditions: divergence slit = 1.00000 (mm), scatter slit = 1.00000 (mm), and receiving slit = 0.30000 (mm). The scanning was performed with the following conditions: drive axis = Theta-2Theta, scan range = 2.000–69.980, scan mode = Continuous Scan, scan speed = 2.0000 (deg/min), sampling pitch = 0.0200 (deg), and preset time = 0.60 (sec).

3. Results and discussions

3.1. Measurement of the diameter of the silk fibers

The coated and uncoated silk fibers were measured using an inverted light microscope to observe and measure the differences in the braided silk fibers (Figure 2). The values were taken from different regions of the length of the coated and uncoated braided silk fibers. The results showed a clear difference between the coated and uncoated braided silk fibers as compared with the diameter of the single strand of the silk fiber that was not braided (Table 1). The results of the values suggest that the chitosan solution used to coat the silk fibers increased the diameter of the silk fibers, thereby indicating a successful coating process.

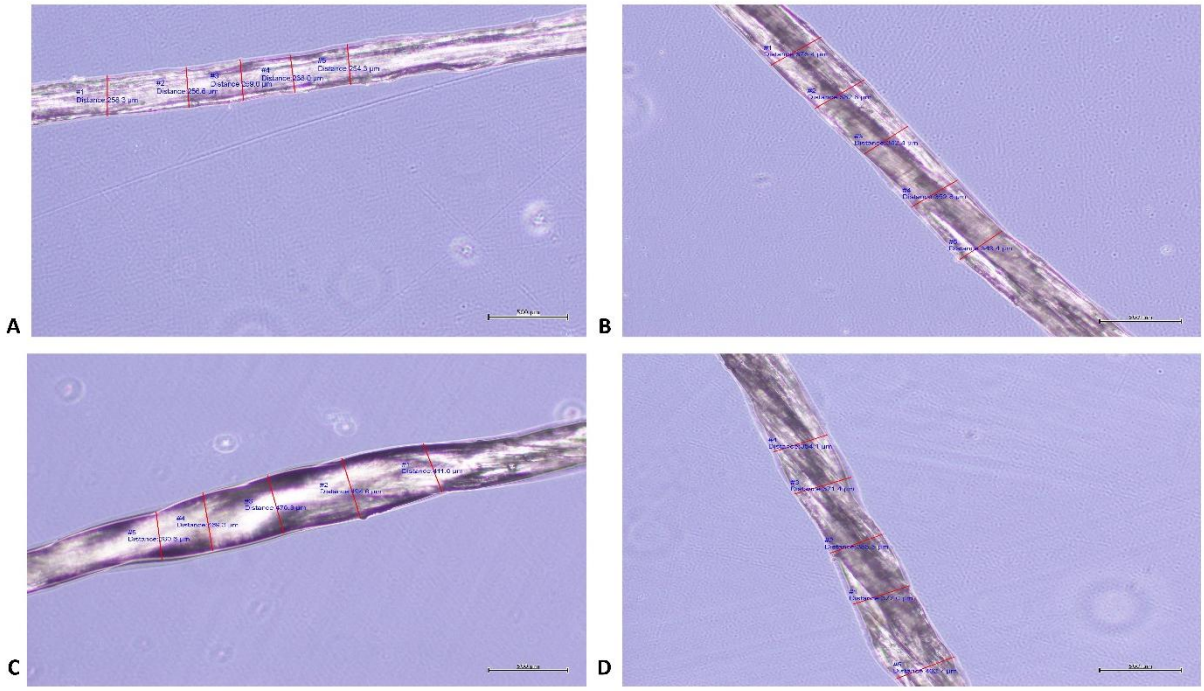

Figure 2. (A) Micrograph of an uncoated single strand of silk fiber with different diameters along its length. (B) Micrograph of uncoated braided silk fiber with varying diameters along its length. (C) Micrograph of 200 mg of chitosan-coated braided silk fiber with different diameters along its length. (D) Micrograph of 400 mg of chitosan-coated braided silk fiber with varying diameters along its length.

Table 1. Showing the differences in the diameter of both coated and uncoated silk fibers.

	Single Fiber(µm)	Uncoated Silk Fiber(µm)	200 mg of Chitosan-Coated Silk Fiber(µm)	400 mg of Chitosan-Coated Silk Fiber(µm)
	258.3	378.4	354.1	411.6
	256.6	382.8	371.4	494.6
	259	342.4	355.5	476.3
	238	352.8	377	469.3
	254.3	343.4	402.7	380.6
Average	253.24	359.96	372.14	446.48

3.2. Scanning Electron Microscopy Analysis (SEM)

The micrographs seen in Figure 3 display the fiber's surface morphology, where the central fiber appears swollen and bulging, with a relatively smooth and thickened surface. Additionally, the fibers show more traditional elongated morphologies but also exhibit surface irregularities, which suggest the treatment or modification of the braided silk fibers with the chitosan solution. The silk fibers show that the coating process is uniform, but with swelling, thus possibly suggesting a

chitosan-type coating that absorbed moisture and expanded on the fibers. The features are more generalized at the X100, X250, and X1000 magnifications (Figure 3), thus indicating the microstructural details of the chitosan particles that coat the braided silk fibers.

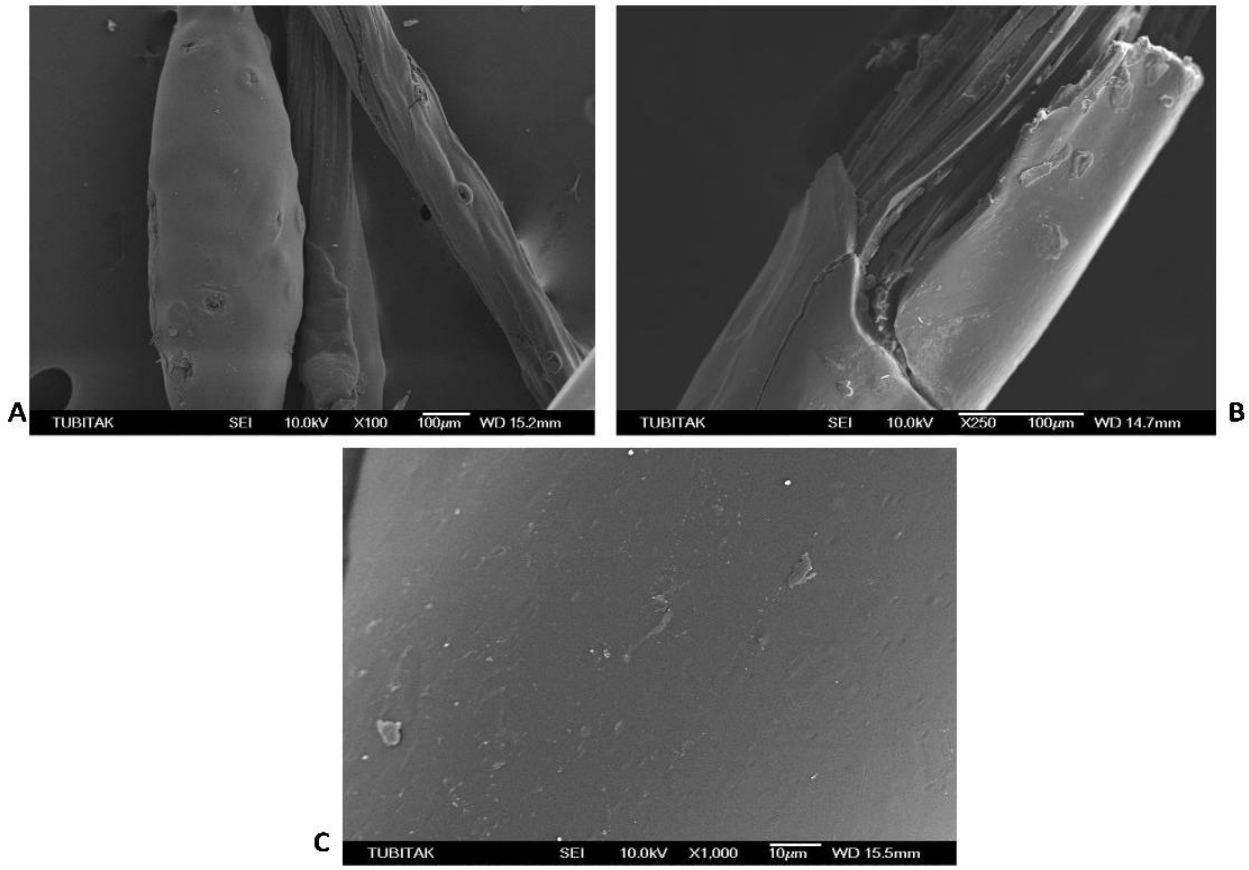

Figure 3. (A) SEM micrograph of 100 μm coated silk fiber at X100 magnification. (B) SEM micrograph of 100 μm coated silk fiber at X250 magnification. (C) SEM micrograph of 10 μm coated silk fiber at X1000 magnification.

3.3. X-ray Diffraction Analysis (XRD)

The result of the XRD analysis suggests that the main peak is centered around $2\theta \approx 21°$, with smaller peaks at 9°, 12°, 14°, 17°, 23°, and 25° (Figure 4). There were additional broad features that were observed around 10° and 25°–30°, and we see the overall intensity gradually drops beyond 30°, with no sharp peaks at higher angles. The broadness of the peaks, especially at 20°, suggests that the material is either amorphous or semi-crystalline rather than highly crystalline, which is typical for biopolymer materials such as silk fibroin, chitosan, or coated polymeric fibers, which often exhibit amorphous halos in the 15°–25° range. Moreover, the results suggest that the braided silk fibers were coated with the chitosan solution. The chitosan-coated braided silk fibers typically displayed a broad diffraction peak near 20°, which corresponds to either a disordered β-sheet or amorphous regions. The β-sheet peak observed around 20° (2θ) remained prominent in both coated and uncoated silk fibroin fiber samples. There was a slight broadening and intensity reduction of peaks in the coated

silk fibroin fiber, which indicates a potential decrease in crystallinity or a more amorphous structure due to the coating.

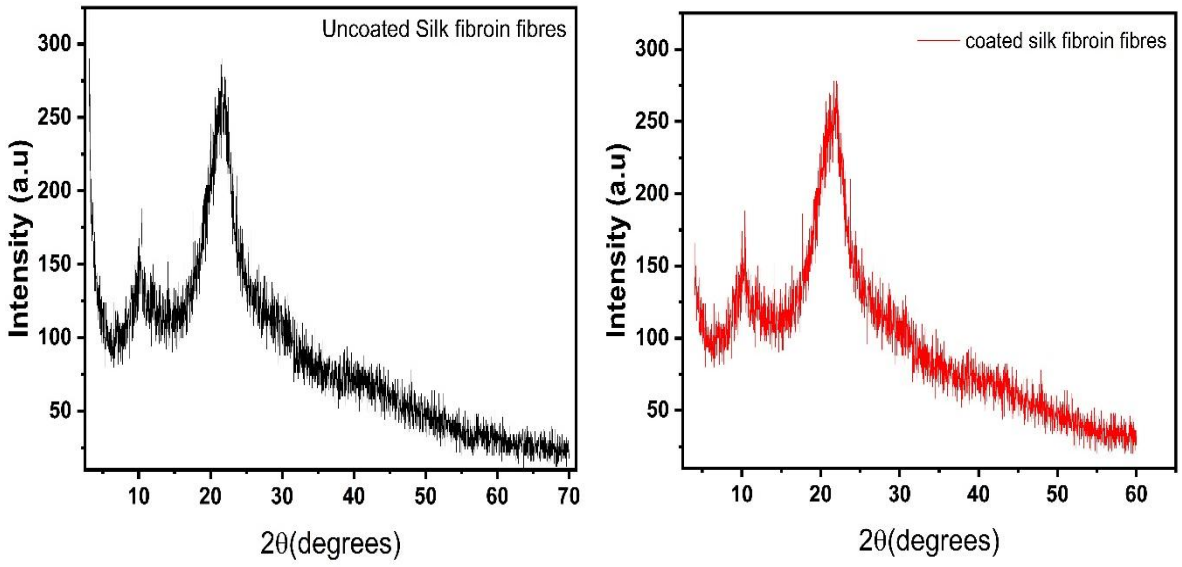

Figure 4. X-ray diffraction study performed on the uncoated and coated silk fibroin fibers.

3.4. Antimicrobial Susceptibility Assay

The results of the antimicrobial susceptibility assay showed the zones of inhibition after 24 hours of incubation. The uncoated silk fibers served as the negative control group, which showed zones of inhibition against all the gram-positive and gram-negative bacteria and fungus utilized for the susceptibility tests. The zones of inhibition of the uncoated silk fibers were smaller. The table's results suggest the silk fibers' natural antimicrobial properties (Figures 5 and 6). The chitosan-coated braided silk fibers (i.e., the positive control group) showed larger zones of inhibition against all the gram-positive and gram-negative bacteria and fungus, which were used more than the uncoated silk fibers (Table 2). The zones of inhibition observed in cultures of both gram-positive and gram-negative bacteria and fungus were consistently larger for the chitosan-coated groups. The antimicrobial mechanism is likely attributed to the cationic nature of chitosan, which disrupts microbial cell walls, thus inhibiting growth and proliferation. Table 2 and Figures 5 and 6 show the diameters of the inhibition zones in triplicate with their standard error mean values.

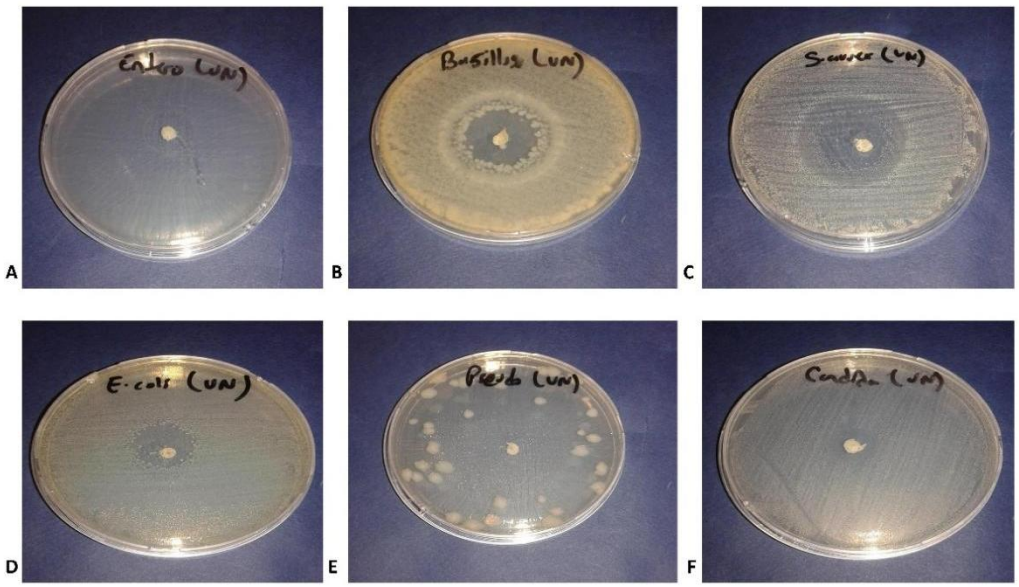

Figure 5. Inhibition zones for the uncoated braided SFF samples against the gram-positive and gram-negative bacteria and fungus. A, B, and C display the petri dishes for the gram-positive bacteria, D and E display the petri dishes for the gram-negative bacteria, and F displays the petri dish for the fungus.

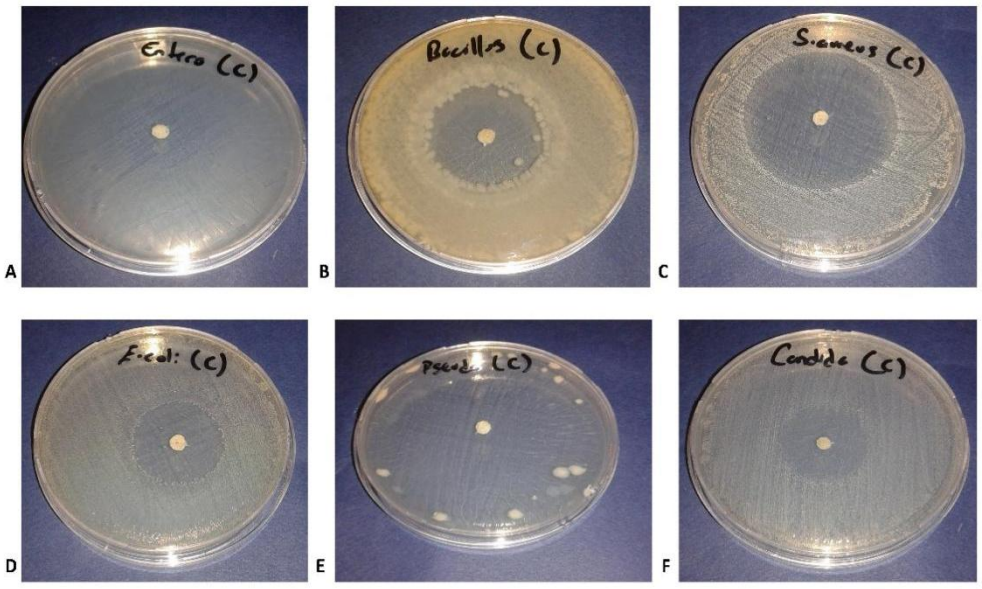

Figure 6. Inhibition zones for the coated braided SFF samples against the gram-positive and gram-negative bacteria and fungus. A, B, and C display the petri dishes for the gram-positive bacteria, and Dand E display the petri dishes for the gram-negative bacteria, and F displays the petri dish for the fungus.

Table 2. Ratio differences in mm as a result of the inhibition zones formed by the antimicrobial effect of both the coated and uncoated silk fibers.

Micro-organisms	Uncoated Silk Fibers(mm)	Coated Silk Fibers(mm)
E.faecalis	16 ± 0.09	38 ± 0.21
B.cereus	25 ± 0.15	36 ± 0.23
S.aureus	36 ± 0.22	50 ± 0.31
E.coli	19 ± 0.11	35 ± 0.18
P.aeroginosa	55 ± 0.32	66 ± 0.42
C.albicans	20 ± 0.12	31 ± 0.21

3.5. Hemocompatibility Assay

The hemocompatibility assay was performed to determine the anticoagulant activity of the uncoated and chitosan-coated silk fibers. The PT, APTT, and INR markers were taken to check the anti-coagulant activity of these fibers. These were used to determine the hemocompatibility of the silk fibers through various clotting times (i.e., 0 minutes, 10 minutes, 20 minutes, 45 minutes, and 90 minutes). The results were compared with the normal standard clotting time or values for a healthy person (Table 3). After the plasma samples were centrifuged, the coated and uncoated silk fiber samples were evaluated for their blood compatibility using the aforementioned hemocompatibility assays. The plasma samples without fibers were used as controls. The results from the uncoated braided silk fiber (Figure 7) were around the same standard values for APTT, PT (%), PT, and INR, though some had values less than the standard values. The results of the hemocompatibility tests (Figure 8 and Figure 9) showed that silk fibers coated with chitosan could alter the blood clotting times, which suggests an anticoagulant action in the coated samples because their APTT, PT, and INR values were consistently greater than those of the uncoated samples and control plasma. This characteristic is especially beneficial for biomaterials in blood contact applications since it lowers the risk of thrombosis and encourages a safe circulation system.

Table 3. Normal standard clotting time and values for a healthy person without thinning drugs.

APTT (seconds)	PT (seconds)	PT (%)	INR
23.6–35.2	11.5–15	70–120	0.80–1.20

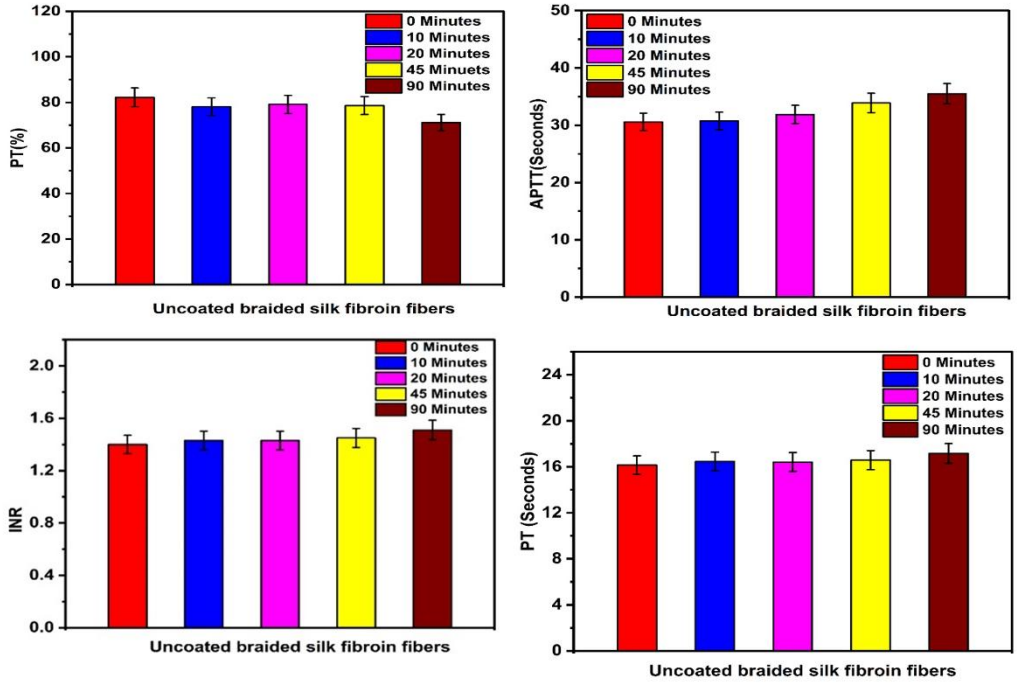

Figure 7. Hemocompatibility graph of the uncoated braided silk fibers for APTT, PT (%), PT, and INR.

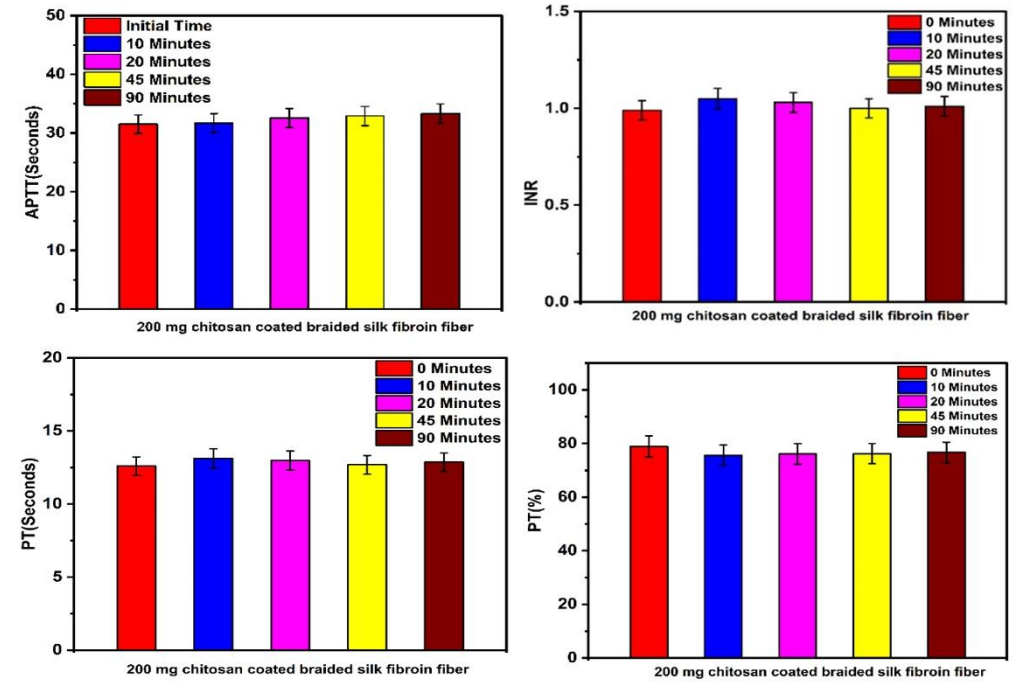

Figure 8. Hemocompatibility graph of the 200 mg chitosan-coated braided silk fibers for APTT, PT (%), PT, and INR.

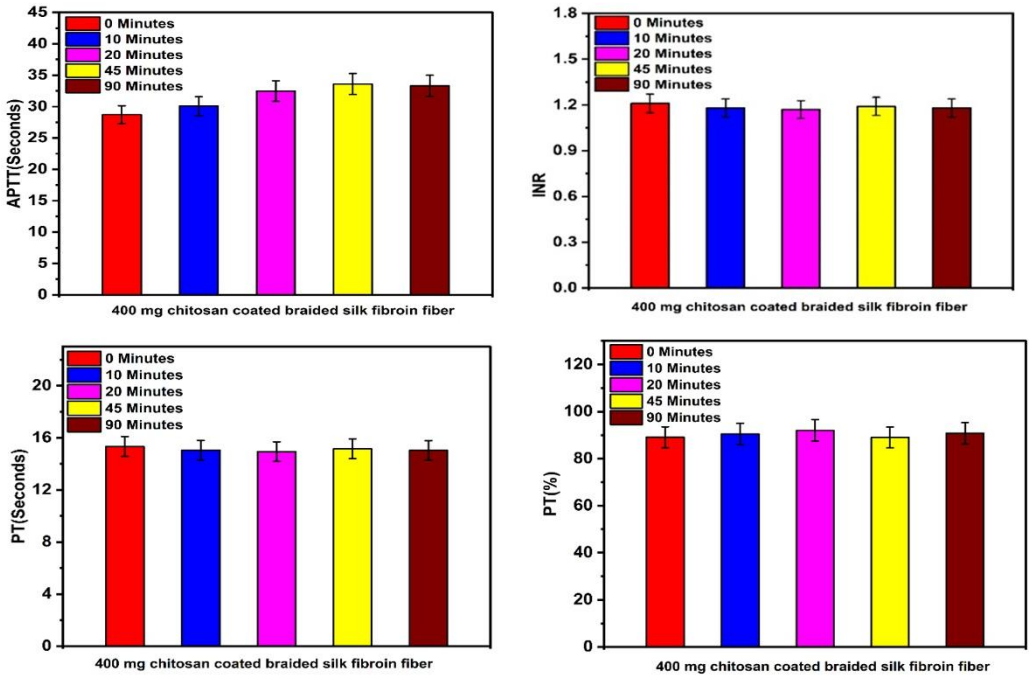

Figure 9. Hemocompatibility graph of the 400 mg chitosan-coated braided silk fibers for APTT, PT (%), PT, and INR.

4. Limitations of the study

The results from the study suggested and demonstrated the potential property of chitosan-coated braided SFFs as antimicrobial and hemocompatible biomaterials. However, several limitations should be highlighted, which will help in the future interpretation of the study. First, the in vitro method of the antimicrobial and hemocompatibility assay may not completely represent the in vivo biological assay when applied due to the human body's complexities and how the cells interact with biomaterials. There is a need to validate the coated fiber's use in clinical applications. Second, the antimicrobial susceptibility assay used against selected bacterial and fungal strains was only performed across 24 hours, and the assay did not explore the long-term effect of the chitosan-coated silk fibers or their effect under stress. Additionally, the long-term stability and degradation of the coated silk fibers were not explored. Lastly, the study only used only two concentrations of chitosan, which makes it difficult to determine and optimize a concentration that will achieve maximum effect in the study.

5. Conclusions

This study's results demonstrate that applying a chitosan coating to braided SFFs significantly enhances their functional properties, particularly their hemocompatibility and antimicrobial activity. The successful integration of chitosan into the silk matrix was evident from morphological and structural analyses. The SEM imaging showed a uniform, swollen fiber surface indicative of coating absorption and adhesion. The XRD analysis confirmed the semi-crystalline structure of the coated fibers, which was characterized by broad peaks common to biopolymer materials such as chitosan

and silk fibroin. The hemocompatibility assays revealed that the chitosan-coated silk fibers modulated blood clotting times. The APTT, PT, and INR values for the coated samples were consistently higher than those of the uncoated samples and control plasma, thus suggesting a potential for anticoagulant activity. This property is particularly advantageous for biomaterials intended for blood-contact applications, thereby reducing the risk of thrombosis while promoting safe interactions with the circulatory system. Additionally, the coated silk fibers displayed a significantly enhanced antimicrobial efficacy. The zones of inhibition observed in cultures of Gram-positive and Gram-negative bacteria and a fungus were consistently larger in the chitosan-coated groups. The antimicrobial mechanism is likely attributed to the cationic nature of chitosan, which disrupts microbial cell walls, thus inhibiting growth and proliferation. These properties make the chitosan-coated SFFs promising candidates for biomedical use, especially in suture materials and wound healing devices where infection control and blood compatibility are critical. This study establishes the utility of chitosan-coated SFFs as a dual-function biomaterial with robust antimicrobial and hemocompatible features. The techniques are simple, reproducible, and cost-effective, thus making them viable for future clinical and industrial applications in biomaterials engineering.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

All authors declare no conflicts of interest in this paper.

Authors contributions

Pwadubashiyi Coston Pwavodi: Investigation, Writing- Original draft preparation, Methodology, Validation. Pwadubashiyi Coston Pwavodi: Supervision, Writing - Review & Editing. Pwadubashiyi Coston Pwavodi: Resources, Writing - Review & Editing. Pwadubashiyi Coston Pwavodi: Formal analysis, Data collection. Pwadubashiyi Coston Pwavodi: Conceptualization, Writing - Review & Editing: Pwadubashiyi Coston Pwavodi

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