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Original Article

SYNERGISTIC EFFECT OF CHITOSAN AND NANOCRYSTALLINE CELLULOSE ON THE ANTIMICROBIAL ACTIVITY OF ELECTROSPUN POLYLACTIC ACID (PLA) NANOFIBERS

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ABSTRACT

Objective: The world is accelerating to find alternative antimicrobial materials due to the rapid development of antibiotic-resistant bacteria. Electrospinning of polymeric nanofibers can provide an attractive platform of developing bioactive surfaces with improved antibacterial functionality. Purpose: The research aimed to determine how the molecular weight of chitosan as well as the incorporation of nanocrystalline cellulose (NCC) affected the morphology and antibacterial activity of polylactic acid (PLA)-based electrospun nanofibers.

Methods: Nanofibers were prepared through needle electrospinning method. The morphological properties of the prepared nanofibers were determined by the use of the scanning electron microscopy (SEM). PLA was blended with two molecular weights of chitosan (7.5kDa and 15 kDa) in 8.5wt% concentration with or without NCC in the concentration of 8.5 wt% as well. The ASTM E2149-13a dynamic contact method was used to assess the anti-bacterial action against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

Results: Results showed that the low molecular weight chitosan (7.5 kDa) formed smooth and uniform nanofibers (average diameter =169-180 nm), and chitosan of high molecular weight (15 kDa) formed beads with less homogeneous structures. Formulations with chitosan showed good antibacterial properties with growth inhibition of most strains tested being complete or almost complete in 1 h. When NCC was added, there was an improvement in the fiber morphology and an increase inits antibacterial activity, especially with the 7.5 kDa chitosan formulation, which is indicating a synergist effect. In comparison, PLA or PLA/NCC fibers in the absence of chitosan exhibited low or ineffective antibacterial activity.

Conclusion: The optimized Cs/NCC/PLA nanofibers, especially the one that contained low molecular weight chitosan, had a smoother and more uniform nanofibers and high spectrum antibacterial activity. The combination of chitosan and NCC boosts surface activity and membrane disruption of bacteria, which points to the possibility of using these composite nanofibers in biomedical applications as wound dressings and antimicrobial coating.

Keywords: Antimicrobial activity, Chitosan, Nanocrystalline cellulose, Electrospinning, Polylactic acid, Nanofibers

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INTRODUCTION

The development of antibiotic-resistant microorganisms has necessitated the development of alternative antimicrobial measures as a significant health concern in the whole world [1-3]. Nanotechnology has in the recent years come up with new methods of producing new advanced materials with increased antibacterial properties [4]. Polymeric nanofibers in particular have received considerable interest due to their large surface to volume ratio, adjustable porosity and the ability to release drugs or antimicrobial agents in a controlled manner [5-7]. Electrospinning has become an easy and universal method of making nanofibers of custom physicochemical characteristics applicable to biomedical, pharmaceutical and environmental uses [8].

A natural polysaccharide that is made out of chitin is known as chitosan (Cs) and has been extensively researched because of its biocompatibility, biodegradability, and antimicrobial properties [9]. The antimicrobial effect of chitosan is explained by the presence of positively charged amino groups, which have the ability of binding with the negatively charged bacterial cell membranes resulting in the cell permeability disruption and intracellular components leakage [10].

Nonetheless, spinning of chitosan is normally challenging because it is very viscous and insoluble in most organic solvents [11, 12]. Therefore, the addition of chitosan with synthetic polymers like poly lactic acid (PLA) has been suggested to increase the spinnability and mechanical characteristics of chitosan [13, 14]. PLA is a biodegradable polymer, which is biocompatible, and is widely used in biomedical research, such as wound dressing, drug delivery, and tissue engineering [15, 16]. PLA has certain drawbacks such as hydrophobicity and absence of antimicrobial activity, which is restrictive to its utilization in antibacterial applications despite it is offering good mechanical strength and processability. In order to address this shortcoming, different natural polymers and nanomaterials have been added to PLA matrices to improve bioactivity [17-19]. Nanocrystalline cellulose (NCC) is the renewable cellulose source that has an excellent mechanical strength and a large number of hydroxyl groups that can form hydrogen bonds with other polymers [20]. The addition of NCC to polymeric matrices would enhance the mechanical stability, thermal characteristics, and hydrophilicity of the nanofibers [21]. In addition, NCC also offers reactive sites that enable it to interact with other bioactive polymers like chitosan that might enhance the overall antimicrobial activity [21-23].

Polylactic acid (PLA) is considered one of the favorable natural polymers for antimicrobial activities due to its biocompatibility, and environmental sustainability [24]. On the other hand, chitosan is a natural biocompatible polysaccharide with broad spectrum antimicrobial activity [25]. Nanocrystalline cellulose is also considered as one of the most abundant natural polymers used with other polymers for different applications due to its high surface area and favorable interfacial interactions, in addition to the presence of the polar hydroxyl groups in its structure which is able to form hydrogen bonding with other copolymers having the affinity for hydrogen bonding such as chitosan leading to increasing the hydrophilicity of nanofibers. Nanofibers with higher hydrophilicity are expected to have improved antimicrobial activity, and this will open the horizons for such nanofibers to be used in different biomedical applications such as wound healing, scaffolds engineering as well as transdermal drug delivery systems [26, 27].

The objective of the study is to determine how the addition of chitosan with different molecular weights (7.5 kDa and 15 kDa) and nanocrystalline cellulose to PLA-based nanofibers will affect their morphology and antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* with regards to the antibacterial efficacy. This research will focus on examining the impact of incorporating chitosan with different low molecular weights of chitosan with NCC in PLA nanofibers on both the morphological properties as well as the antibacterial activity.

MATERIALS AND METHODS

Materials

Nano crystalline cellulose (NCC) is a high crystalline(>70%)natural polymer with rod-shaped structures (10–20 nm wide, 300–900 nm long)used in the preparation of the nanofibers, in this research it was purchased from Sigma-Aldrichand itwas sourced from wood. Different grades of low molecular weight chitosan (LMWC) having an average molecular weight of 7.5 kDa, and 15 kDawith 100%DDA, were prepared in-house, starting from the high molecular weight chitosan (250kDa) using the acid hydrolysis method, the high molecular weight chitosan was purchased from Shanghi Co Ltd, China. Chitosan average molecular weight was calculated using Marck-Houwink equation, and its degree of deacetylations was calculated using the compendial USP method. The copolymer utilized in the preparation of the nanofiber, poly (lactic acid) (PLA) (3052D), was acquired from Unic Technology Ltd. in Taiwan. Conversely, 99.8% of the dichloromethane was acquired from Merck in Germany. The nutrient broth and agar were obtained from HiMedia company. Additionally, reference strains including*Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), and *Escherichia coli* (ATCC 25922) were obtained from Kwik-stik.

Methods

Fabrication of chitosan/NCC/PLA nanofibers using the needle electrospinning system

The spinning blend was prepared by dissolving, PLA, NCC, and chitosan in a suitable amount of Dichloromethane (DCM)at room temperature (25˚C), and left under vigorous stirring for 30 min until a clear solution was obtained.

Before the electrospinning process, the surface tension of the spinning blend was tested at 25 ˚C, using the surface tension analyzer Data physics (model-DCAT9, Germany). The viscosity of the spinning blend was also measured at 25 ˚C using the Brookfield viscometer Model-RST-CC.

The Needle Electrospinning Machine (TL-01) was used to electro spin Chitosan, NCC, and PLA at room temperature [28]. To determine the ideal process parameters required for the electrospinning process, process optimization was required. Throughout the spinning process, the spinning chamber's relative humidity was kept between 45 and 55 percent, best spinning was accomplished by delivering a voltage of 22 kV at spinning blend flow rate of 2 ml/min, tip to collector distance was 25 cm, and a needle gauge of 20G. Aluminum collector was used to collect the electrospun fibers.

Morphology of chitosan/NCC/PLA nanofibers

The generated fibers' morphology was examined using a Hitachi Tabletop TM 3030, Japan, Scanning Electron Microscopy. Following the sputtering of a layer of gold, the samples were placed on aluminum stubs, and the diameters of the electrospun fibers were computed using Image-J software (National Institutes of Health, USA). One hundred fibers chosen from three distinct samples were measured in order to determine the average fiber diameters and diameter dispersion [28].

Anti-bacterial activity

The antimicrobial activity of the fibers was evaluated using ASTM E2149-13a method with slight modificationas the following (Standard Test Method for Determining the Antimicrobial Agents Under Dynamic Contact Conditions) [29, 30]. Antibacterial activity was tested against prototypical Gram positive and Gram-negative bacteria to test the activity of the nanofiber against different group of bacteria to test the efficacy in different cell wall structure and outer membrane of these bacteria. The tested bacteria was *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), and *Escherichia coli* (ATCC 25922). First step was the preparation of bacteria to 1.5×10^8 CFU/ml and then diluted with sterile 0.3 mmol phosphate buffer (KH_2PO_4; pH 7.1) to obtain a 50 ml working suspension of concentration 1.5×10^5 CFU/ml. UV sterilized fiber samples (1 x 1 cm) were moved into flasks and shaken to 1 h at 37 ˚C and 150 rpm. Serial dilutions were done after 1h incubation in 0.9% saline. Viable counting all fiber counting samples were determined after 1 hof incubation. Subsequently, 100 µl if each diluted was spread onto nutrient agar plates and incubated at 37 °C for 24 h, and the colonies count were determined as CFU/ml.

For all tested bacterialstrains, the activity was compared with control group where no fiber was used. The experiment was repeated three times. The bacterial viable count was presented as mean CFU/ml±SD. The growth reduction percentage after 1 h contact was calculated according to the following formula:

$$R\% = \left(\frac{\text{Average viable counts of control} - \text{Average viable count after 1 hr contact}}{\text{Average viable counts of control}}\right) \times 100\% \text{ ------- (1)}$$

Statistical analysis

All the results were expressed as mean± SD for triplicate experiment. One-way ANOVA single factor ($p \leq 0.05$) was used to determine the significance difference of the antibacterial activity compared to negative control.

RESULTS

Characterization of the prepared nanofibers

Surface morphology and fiber size distribution

Blending Cs with both NCC and PLA helped to facilitate its spinnability, and also improved the quality of the nanofibers in some of the prepared formulations. Therefore, 5 wt% PLA was the optimum concentration to be blended with both chitosan and nanocrystalline cellulose. Polymers spinning blends show a surface tension value around 28 mN/m, and viscosity around 25mpas. Sec. The blended polymers together formed neat nanofibers with small diameters.

The composition of each prepared nanofiber is summarized in table 1.

Table 1: Concentrations of Polylactic acid (PLA), Chitosan (Cs), Nano crystalline cellulose (NCC) in the prepared dry nanofiber

Sample ID	% PLA	% Cs	% NCC

F1 (PLA)	100	0	0
F2 (PLA, Cs 7.5kDa)	90	10	0
F3 (PLA, Cs 7.5kDa, NCC)	83	8.5	8.5
F4 (PLA, Cs 15kDa)	90	10	0
F5 (PLA, Cs 15kDa, NCC)	83	8.5	8.5

*Concentrations were calculated in wt/wt %, with total polymer weight of 100g

Fig. 3 to fig. 5 shows the SEM images of chitosan/NCC/PLA nanofibers. Results showed that PLA forms a neat homogeneously distributed nanofibers with an average fiber size of 169.32±2.36 nm.

It can be observed from the fig. that the different molecular weights of chitosan gave nanofibers with different qualities and sizes.

Comparing the nanofibers shown in fig. 2 and fig. 4, it is obvious that the lower molecular weight (7.5kDa) formed good quality nanofibers with good distribution and morphology, while chitosan of molecular weight 15kDa formed beaded nanofibers with large number of lumps, beads diameters were approximately measured using the Image J software and it was around 680 nm.

The obtained results helped in concluding that the formation of chitosan nanofibers is influenced by the molecular weight of chitosan, which is in agreement with previously reported studies. It was reported that very low molecular weight chitosan form beads instead of fibres, on the other hand, high molecular weight chitosan form either beaded nanofibers or very large fibres with poor morphology [31, 32].

Once an effect of molecular weight on the morphology and quality of nanofibers was observed, the first thing should be taken into consideration is the conformation changes as the molecular weight changed [33]. It is also well known that the conformation of the low molecular weight chitosan is of linear chains, and as the molecular weight increases the conformation changed to coilly, hellical, and to spherical shapes. as the conformation becomes more complicated then, it is expected that the functional group NH2 will be hidden inside and then will not be available to interact with thewith the oxygen atom in the carboxylic acid functional group available in the PLA structure. So, it could be concluded that for chitosan 15kDa more NH2 groups are hidden with the coily chains of chitosan, then lesser number of NH2 group will be available to interact with PLA so more free chitosan is available which leads to the lower quality of the produced nanofibers. It was concluded by some researchers that molecular weight induced conformational changes of Cs can be associated with possible differences in intra-molecular hydrogen bonds. Likewise, it could be as a result of the difference in the distribution of charges among smaller and larger molecular weight chitosan [34].

In regard to the addition of nanocrystalline cellulose in to chitosan and PLA. It is clear from fig. 3 and fig. 5 that the presence of nanocrystalline cellulose in chitosan/PLA nanofibers resulted in the formation of better-quality nanofibers with better morphology and distribution. Which could be due to the expected interaction between the NH2 groups available in chitosan and the hydroxyl groups (OH-). The presence of the (OH-) group gives a chance to more and more NH2 groups interactions with both PLA and NCC, leading to decreasing the amount of the free chitosan and leading to the formation of nanofibers with better morphology and distribution.

But it is important to mention that the nanofibers of Cs/NCC/PLA prepared with chitosan molecular weight 7.5kDa is of better morphology, distribution, and size compared with those prepared using chitosan of molecular weight 15kDa, which is expected according to what was mentioned above that as the molecular weight of chitosan increases its conformation will become of a coil structure, then more NH2 groups will be embedded inside and then less will be available to interact with both PLA, and NCC. As a result, more free chitosan will be available leading to lower quality nanofibers.

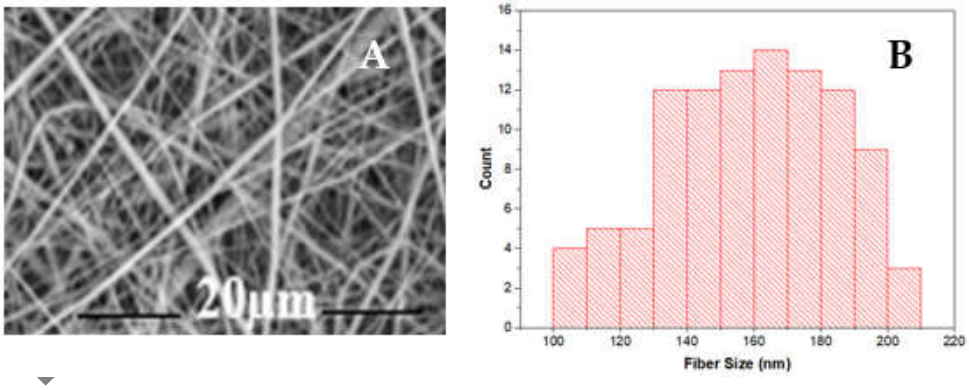

Fig. 1: A: SEM captures with (3K magnification) of F1 (100% PLA) nanofibers, B: Fiber size distribution of F1 (100% PLA) nanofibers

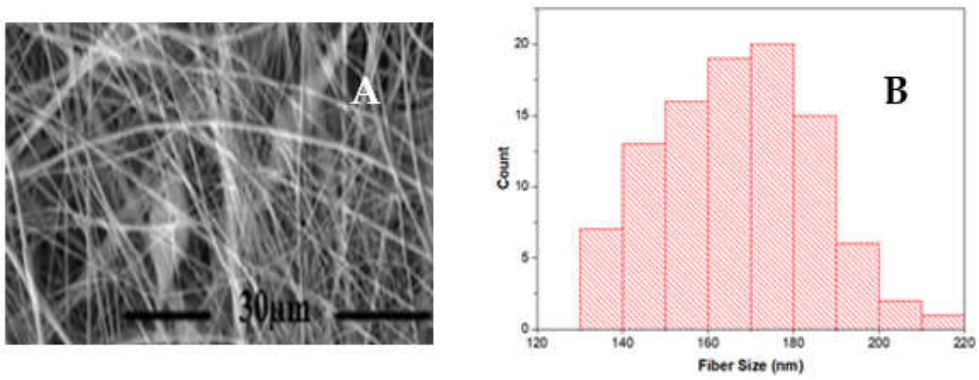

Fig. 2: A: SEM captures with (3K magnification) of F2 (PLA, Cs 7.5kDa) nanofibers, B: Fiber size distribution of F2 PLA, Cs 7.5kDa) nanofibers

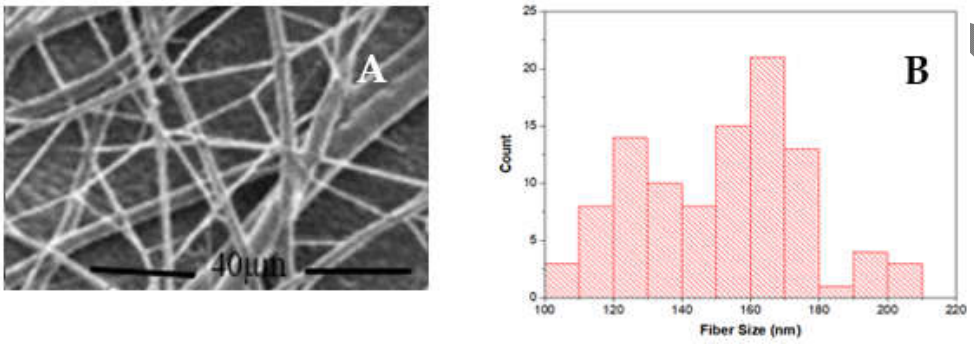

Fig. 3: A: SEM captures with (3K magnification) of F3 (PLA, Cs 7.5kDa, NCC) nanofibers, B: Fiber size distribution of F3 (PLA, Cs 7.5kDa, NCC) nanofibers

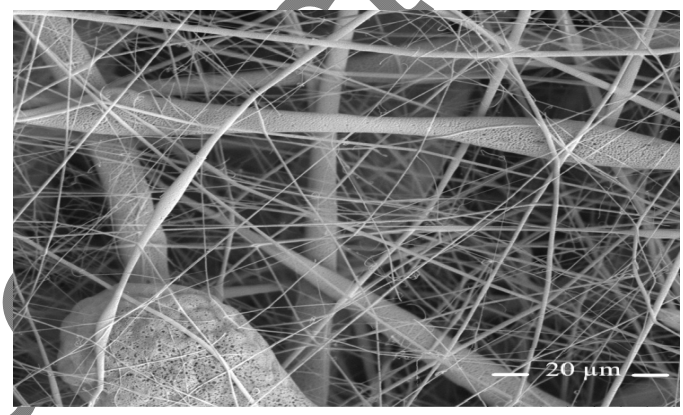

Fig. 4: SEM captures with (3K magnification) of F4 (PLA, Cs 15kDa) nanofibers

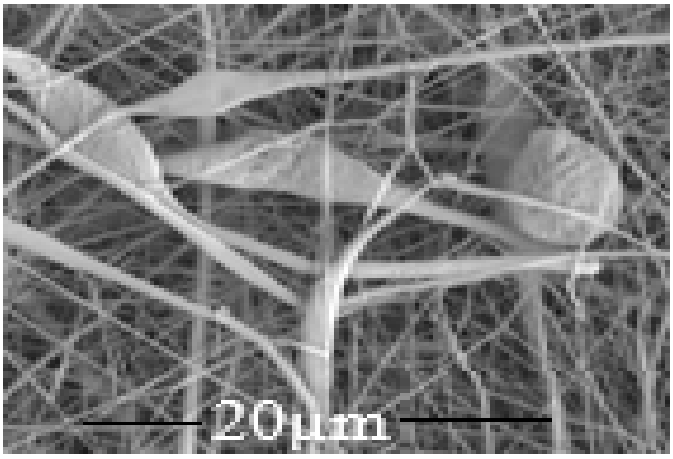

Fig. 5: SEM captures with (3K magnification) of F5 (PLA, Cs 15kDa, NCC) nanofibers

Table 2 summarizes the diameter size of the different prepared nanofibers which was measured using (Image J) software, and fig. (2B, 3B, and 4B) show the corresponding fiber size distribution of the prepared nanofibers. According to diameter size PLA nanofibers (S1) and Cs/PLA nanofibers (S2) it is clear that the addition of chitosan 7.5kDa into PLA increased the average size of PLA nanofibers. On the other hand, a significant difference between Cs/NCC/PLA nanofibers (S3) and Cs/PLA (S2) nanofibers was observed leading to the conclusion that the addition of NCC into Cs/PLA nanofibers helped in the production of nanofibers with good surface morphology, distribution, and size.

Table 2: Average diameter size of the different prepared nanofibers

Nanofiber composition	Average diameter size (nm)±SD
(PLA)	169.32±21.36
(PLA, Cs 7.5kD)	180.92±15.30
(PLA, Cs 7.5kD, NCC)	168.52±12.25
(PLA, Cs 15kDa)	Beaded nanofibers were formed
(PLA, Cs15kDa, NCC)	Beaded nanofibers were formed

*n = 100 nanofiber

Anti-bacterial activity

Against all tested bacterial strains Chitosan 7.5 and 15 showed promising activity with reduction inhibition 100% in most samples after one-hour direct contact with the fiber content when compared with control group for each strain. For *B. cereus* around 100% reduction was observed when the bacteria interacted with chitosan 7.5kDa and 15 kDa the results are summarized in table 3. All the activity was significantly differed than the control group (*p≤0.05*).

Table 3: The antibacterial activity of the nanofiber against *B. cereus*

Test strain *B. cereus*	Growth CFU/ml after 1h contact (mean±SD)	Reduction percentage
Control	13500±350	____
PLA	8800±245*	34.815
PLA/NCC	7967±377*	40.988
PLA/Cs 7.5kDa	No growth*	100.000
PLA/NCC/Cs 7.5kDa	No growth*	100.000
PLA/Cs 15kDa	10±2*	99.926
PLA/NCC/Cs 15kDa	No growth*	100.000

n=3, * p ≤0.05 (antibacterial activity of each nanofiber compared with the control group), For *P. aeruginosa*almost100% reduction was observed when the bacteria interacted with chitosan 7.5kDa and 15 kDa, results are summarized in table 4 with observed lower efficiency of PLA and PLA-NCC without chitosan. All the activity was significantly differed than the control group (*p≤0.05*).

Table 4: The antibacterial activity of the nanofiber against *P. aeruginosa*.

Test strain *P. aeruginosa*	Growth CFU/ml after 1 h contact (mean±SD)	Reduction percentage
Control	40333±4189	____
PLA	47167±1554	-16.942
PLA/NCC	38733±2028*	3.966
PLA/Cs 7.5kDa	No growth*	100.000
PLA/NCC/Cs 7.5kDa	No growth*	100.000
PLA/Cs 15kDa	No growth*	100.000
PLA/NCC/Cs 15kDa	243±9*	99.397

n=3,-: indicate increase in growth, * p ≤0.05 (antibacterial activity of each nanofiber compared with the control group), For *E. coli* 100% reduction was observed also when the bacteria interacted with chitosan 7.5 kDa and 15 kDa, the results are summarized in table 5 with observed lower efficiency of PLA and increase in the viable count with PLA-NCC (table 5). All the activity was significantly differed than the control group (*p≤0.05*).

Table 5: The antibacterial activity of the nanofiber against *E. coli*.

Test strain *E. coli*	Growth CFU/ml after 1 h contact (mean±SD)	Reduction percentage
Control	50333±5436	____
PLA	26500±3500*	47.351
PLA/NCC	85667±2054*	-70.199
PLA/Cs 7.5kDa	No growth*	100.000
PLA/NCC/Cs 7.5kDa	No growth*	100.000
PLA/Cs 15kDa	No growth*	100.000
PLA/NCC/C_5 15kDa	No growth*	100.000

n=3,-: indicate increase in growth, * p ≤0.05 (antibacterial activity of each nanofiber compared with the control group), For *S. aureus* the reduction percentages were 95.679 to 98.679 were observed when the bacteria interacted with chitosan 7.5 kDa and 15 kDa the results are summarized in table 6. All the activity was significantly differed than the control group (*p≤0.05*).

Table 6: The antibacterial activity of the nanofiber against S. aureus

Test strain *S. aureus*	Growth CFU/ml after 1 h contact (mean± SD)	Reduction percentage
Control	76000±5000	____
PLA	99667±1144*	-31.140
PLA/NCC	83000±9626*	-9.210
PLA/Cs7.5kDa	1003±97*	98.679
PLA/NCC/Cs 7.5kDa	3283±160*	95.679
PLA/Cs 15kDa	1230±128*	98.381
PLA/NCC/C_5 15kDa	1150±90*	98.400

n=3,-: indicate increase in growth, *p≤0.05 (antibacterial activity of each nanofiber compared with the control group)

DISCUSSION

Knowledge of the intermolecular interactions in the Cs/NCC/PLA system is essential to the explanation of the morphological behavior of the electrospun fibers as well as the antibacterial use. Hydrogen bonding interactions are the main factors in determining the structural compatibility between chitosan (Cs), polylactic acid (PLA), and nanocrystalline cellulose (NCC). The amino groups (–NH 2) of chitosan have the ability to react with the carbonyl groups of the PLA backbone and the large number of hydroxyl groups (–OH) of NCC can serve as the extra site of hydrogen bonding. These numerous points of interaction probably enhance better interactions between polymers, which leads to the increase of fiber integrity and consistency fig. 6.

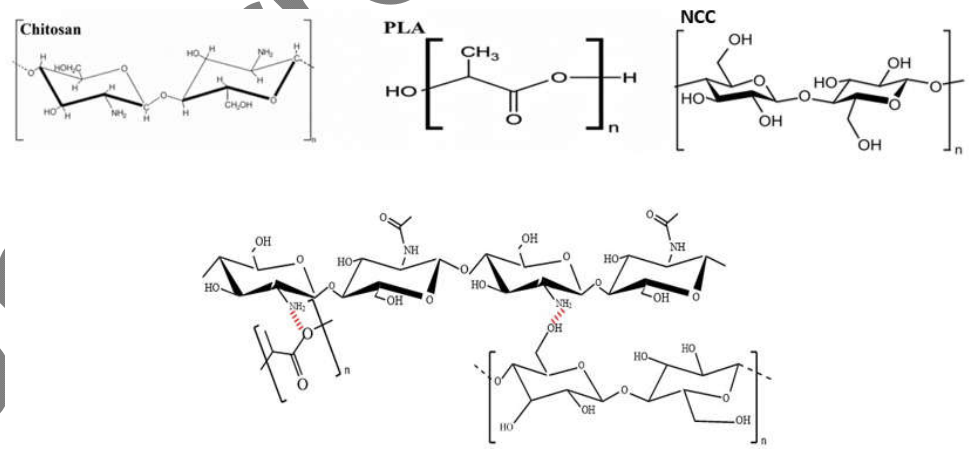

Fig. 6: Chemical structures of Chitosan (Cs), Polylactic acid (PLA), nanocrystalline cellulose (NCC) and their supposed mechanism of interaction (the red dashed lines represent the hydrogen bond)

The results of the SEM indicated clearly that chitosan molecular weight is a decisive factor in the determination of fiber morphology. Low molecular weight chitosan (7.5 kDa) incorporated into PLA resulted in smooth, homogeneous nanofibers with a mean diameter of 169-180 nm on average. However, fibers that were prepared with a high molecular weight chitosan (15 kDa) had bead formation and irregular surface morphology. This result is aligned with other reports about the fact that the higher the molecular weight, the more the chain entanglement and coiling, which can decrease the accessibility of the amino groups to intermolecular hydrogen bonding with PLA[35]. Lower intermolecular compatibility in electrospinning may encourage phase separation and instability of polymer jet, and eventually lead to bead formation [31, 32].

Moreover, the modification of the Cs/PLA system with NCC enhanced the uniformity of fibers and decreased the bead formation. It is probable that the hydroxyl-rich surface of NCC increased the hydrogen bonding interactions within the polymeric matrix, which stabilized the spinning solution and facilitated a higher concentration of the chain alignment during the formation of fibers [28, 35]. This increased interfacial compatibility and so the increased morphology especially in the preparation with 7.5 kDa chitosan and NCC.

The antimicrobial findings greatly assert that chitosan is the key bioactive ingredient of the nanofiber system. PLA/NCC and PLA fibers did not demonstrate significant or even consistent antibacterial activity, but all the formulations containing chitosan demonstrated a significant reduction in bacteria in one hour of dynamic contact. The antimicrobial effects of chitosan are well explained by electrostatic interactions of positively charged amino groups located in chitosan with negatively charged bacterial cell membranes that result in membrane destabilization, membrane permeability, intracellular content leakage, and eventual cell death [36, 37].

Interestingly, when NCC was incorporated together with chitosan, it increased the antibacterial activity in the majority of the strains tested. In spite of the fact that NCC is not a compound with inherent antimicrobial activity, it seems to be a structure enhancer. The dispersion of chitosan into the PLA matrix, the reduced aggregation of chitosan, and the higher exposure of active amino groups on the surface of fibers [38], can be enhanced by the increased surface area and hydrophilicity of NCC. These functional groups will be more readily available, which probably increases the strength of electrostatic interactions at the fiber-bacteria interface leading to the enhanced growth of bacteria. Besides, hydrogen bonding of NCC and chitosan can stabilize reactive amino sites and keep them accessible to interact with membranes [28].

Also, the antibacterial response of different bacterial strains varied. One hundred percent or close to a hundred percentage inhibition was obtained with *B. cereus*, *P. aeruginosa*, and *E. coli*, with slightly lower percentages of reduction observed with *S. aureus*. This behavior may be attributed to variations in the bacterial cell wall architecture. Gram-negative bacteria have a negatively charged lipopolysaccharide-rich outer membrane that has the potential to increase the electrostatic attraction of positively charged chitosan [39-41]. Conversely, Gram-positive bacteria like *S. aureus* possess a more solid peptidoglycan and teichoic acids that can partially restrict polymer entry and determine membrane permeability [42, 43]. Samokhin *et al.* (2025)described a comparable strain-specific change in the activity of chitosan, which had a greater impact on *P. aeruginosa* than on *S. aureus* [43]. However, the results of this research support the possibility of Cs/NCC/PLA nanofibers as a multifunctional antimicrobial agent.

One interesting observation was that sometimes the growth of bacteria increased when using PLA or PLA/NCC fibers in the absence of chitosan. This phenomenon may be caused by more than one aspect. Under some circumstances, the degradation of PLA can lead to the release of lactic acid oligomers that can be used as a source of carbon. In the same way, NCC has hydroxyl-cellulose frameworks that can sustain bacterial metabolism [44]. Moreover, surface roughness of nanofibers can be increased, which can potentially increase bacterial adhesion, and the dynamic contact conditions applied in ASTM E2149-13a can enable nutrient diffusion to the bacterial cells [45]. All this could be the reason of the negative reduction percentages of certain control formulations. Notably, the growth of bacteria was always suppressed after the addition of chitosan to the system, which strengthens its leading antimicrobial activity.

In general, the optimized formula that comprised PLA, NCC and low molecular weight chitosan (7.5 kDa) resulted in the best balance between morphological homogeneity and antibacterial activity. The synergistic effect of this hybrid system seems to be due to the fact that its polymer compatibility is increased, functional groups become more exposed to the surface, and the electrostatic interaction between this hybrid system and bacterial membranes is enhanced. The results are consistent with the past studies by Asadzadeh *et al.* (2024), who revealed that PLA-based nanocomposites that include chitosan and cellulose components have enhanced antimicrobial characteristics and biomedical potential [46]. The present study, in concurrence with their results, advocates that chitosan-based composite nanofibers can be used in biomedical applications, including wound dressings, tissue scaffolds, antimicrobial coatings, and as a possible food-packaging material.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates that the incorporation of chitosan and nanocrystalline cellulose into PLA nanofibers significantly influences both fiber morphology and antibacterial performance.

All chitosan-containing formulations exhibited strong antibacterial activity, achieving complete or near-complete bacterial growth inhibition within one hour of contact. The addition of NCC enhanced fiber uniformity and improved surface exposure of active amino groups, contributing to a synergistic antibacterial effect.

Future studies should focus on comprehensive mechanical characterization to evaluate tensile strength, flexibility, and structural stability of the fabricated nanofibers. In vitro cytotoxicity and biocompatibility assessments are also necessary to confirm their suitability for biomedical applications. Furthermore, long-term antimicrobial durability studies and controlled release investigations would provide deeper insight into sustained antibacterial performance. Evaluation in relevant biological or wound-healing models may further validate their clinical potential.

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Nil

AI DISCLOSURE

No artificial intelligence (AI) or AI-assisted tools were used in the writing, data analysis, or creation of fig. for this manuscript.

AUTHORS CONTRIBUTIONS

Suha M. Abudoleh is the principle investigator who wrote, revised, formatted, and submitted the manuscript to the journal.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Tangden T, Carrara E, Hellou MM, Yahav D, Paul M. Introducing new antibiotics for multidrug-resistant bacteria: obstacles and the way forward. Clin Microbiol Infect. 2025;31(3):354-59. doi: 10.1016/j.cmi.2024.09.025. PMID 39374649.
2. Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA et al. Antimicrobial resistance: impacts, challenges, and future prospects. Journal of Medicine Surgery and Public Health. 2024;2:100081. doi: 10.1016/j.glmedi.2024.100081.
3. Hemeg HA. Nanomaterials for alternative antibacterial therapy. Int J Nanomedicine. 2017;12:8211-25. doi: 10.2147/IJN.S132163. PMID 29184409.

4. AlQurashi DM, AlQurashi TF, Alam RI, Shaikh S, Tarkistani MA. Advanced nanoparticles in combating antibiotic resistance: current innovations and future directions. JNT. 2025;6(2):9. doi: 10.3390/jnt6020009.
5. Hans R, Talukdar S, Tyagi P. Blended polymeric nanofibres for antimicrobial activity. J Chem Health Risks. 2025;15(4):2661-68.
6. Morie A, Garg T, Goyal AK, Rath G. Nanofibers as novel drug carrier — an overview. Artif Cells Nanomed Biotechnol. 2016;44(1):135-43. doi: 10.3109/21691401.2014.927879, PMID 25016918.
7. Duan X, Chen HL, Guo C. Polymeric nanofibers for drug delivery applications: a recent review. J Mater Sci Mater Med. 2022;33(12):78. doi: 10.1007/s10856-022-06700-4, PMID 36462118.
8. Al-Enizi AM, Zagho MM, Elzatahry AA. Polymer-based electrospun nanofibers for biomedical applications. Nanomaterials (Basel). 2018;8(4):259. doi: 10.3390/nano8040259, PMID 29677145.
9. Desai N, Rana D, Salave S, Gupta R, Patel P, Karunakaran B, et al. Chitosan: a potential biopolymer in drug delivery and biomedical applications. Pharmaceutics. 2023;15(4):1313. doi: 10.3390/pharmaceutics15041313, PMID 37111795.
10. Yilmaz Atay H. Antibacterial activity of chitosan-based systems. In: Jana S, Jana S, editors. Functional chitosan. Singapore: Springer Singapore. 2020. p. 457-89. doi: 10.1007/978-981-15-0263-7_15.
11. Li B, Qiu L, Zhang J, Liu S, Xu M, Wang J, et al. Solubilization of chitosan in biologically relevant solvents by a low-temperature solvent-exchange method for developing biocompatible chitosan materials. Int J Biol Macromol. 2024;254(3):127950. doi: 10.1016/j.ijbiomac.2023.127950. PMID 37951431.
12. Yacob N, Talip N, Mahmud M. Idayu-Mat-Sani NA. Samsuddin NA, Ahmad Fabillah N. Determination of viscosity-average molecular weight of chitosan using intrinsic viscosity measurement. *J NuclRelat Technol*. 2013;10:39–44.
13. Syed MH, Khan MM, Abdullah N, Zahari MA. Effect of drug loading on sustained release through needleless electrospun nanocellulose/chitosan/polylactic acid based drug delivery system. Int J Biol Macromol. 2025;329(1):147843. doi: 10.1016/j.ijbiomac.2025.147843. PMID 40992474.
14. Jeevitha D, Amarnath K. Chitosan/PLA nanoparticles as a novel carrier for the delivery of anthraquinone: synthesis, characterization and in vitro cytotoxicity evaluation. Colloids Surf B Biointerfaces. 2013;101:126-34. doi: 10.1016/j.colsurfb.2012.06.019, PMID 22796782. colsurfb.2012.06.019.
15. da Silva D, Kaduri M, Poley M, Adir O, Krinsky N, Shainsky-Roitman J, et al. Biocompatibility, biodegradation and excretion of polylactic acid (PLA) in medical implants and theranostic systems. Chem Eng J. 2018;340:9-14. doi: 10.1016/j.cej.2018.01.010, PMID 31384170. cej.2018.01.010.
16. Santoro M, Shah SR, Walker JL, Mikos AG. Poly(lactic acid) nanofibrous scaffolds for tissue engineering. Adv Drug Deliv Rev. 2016;107:206-12. doi: 10.1016/j.addr.2016.04.019, PMID 27125190. addr.2016.04.019.
17. Mendonça CJ, Dantas LR, Soni JF, Tuon FF. Antimicrobial action of a biodegradable thermoplastic impregnated with vancomycin for use in 3D printing technology. Braz Arch Biol Technol. 2024;67:e24231110. doi: 10.1590/1678-4324-2024231110.
18. Moya-Lopez C, González-Fuentes J, Bravo I, Chapron D, Bourson P, Alonso-Moreno C, et al. Polylactide perspectives in biomedicine: from novel synthesis to the application performance. Pharmaceutics. 2022;14(8):1673. doi: 10.3390/pharmaceutics14081673, PMID 36015299.
19. Bischof S, Bušac T, Ivanković T, Rolland du Roscoat S, Lukic B, Kovačević Z. PLA-based green antimicrobial and flame-retardant biocomposites reinforced with *Sida hermaphrodita* fibers. Coatings. 2025;15(5):595. doi: 10.3390/coatings15050595.
20. Trache D, Tarchoun AF, Derradji M, Hamidon TS, Masruchin N, Brosse N, et al. Nanocellulose: from fundamentals to advanced applications. Front Chem. 2020;8:392. doi: 10.3389/fchem.2020.00392, PMID 32435633.
21. George J, Sabapathi SN. Cellulose nanocrystals: synthesis, functional properties, and applications. Nanotechnol Sci Appl. 2015;8:45-54. doi: 10.2147/NSA.S64386. PMID 26604715.
22. Zhang Y, Liu Y, Li R, Ren X, Huang TS. Preparation and characterization of antimicrobial films based on nanocrystalline cellulose. J Appl Polym Sci. 2019;136(8):47101. doi: 10.1002/app.47101.
23. Alavi M. Modifications of microcrystalline cellulose (MCC), nanofibrillated cellulose (NFC), and nanocrystalline cellulose (NCC) for antimicrobial and wound healing applications. e-Polymers. 2019;19(1):103-19. doi: 10.1515/epoly-2019-0013.
24. Shao L, Xi Y, Weng Y. Recent advances in PLA-based antibacterial food packaging and its applications. Molecules. 2022;27(18):5953. doi: 10.3390/molecules27185953, PMID 36144687.
25. Goy RC, De Britto D, Assis OB. A review of the antimicrobial activity of chitosan. Polimeros. 2009;19(3):241-47. doi: 10.1590/s0104-14282009000300013.
26. Kargarzadeh H, Huang J, Lin N, Ahmad I, Mariano M, Dufresne A, et al. Recent developments in nanocellulose-based biodegradable polymers, thermoplastic polymers, and porous nanocomposites. Prog Polym Sci. 2018;87:197-227. doi: 10.1016/j.progpolymsci.2018.07.008.
27. Norizan MN, Shazleen SS, Alias AH, Sabaruddin FA, Asyraf MR, Zainudin ES et al. Nanocellulose-based nanocomposites for sustainable applications: a review. Nanomaterials (Basel). 2022;12(19):3483. doi: 10.3390/nano12193483, PMID 36234612.
28. AlJbour ND, Beg MD, Gimbun J, Zahari MA. Fabrication of blended chitosan nanofibers by the free surface wire electrospinning. Pharmacia. 2023;70(3):465-73:e98122. doi: 10.3897/pharmacia.70.e98122.
29. Woskowicz E, Łożyńska M, Kowalik-Klimczak A, Kacprzyńska-Gołacka J, Osuch-Słomka E, Piasek A, et al. Plasma deposition of antimicrobial coatings based on silver and copper on polypropylene. Polimery. 2020;65(1):33-43. doi: 10.14314/polimery.2020.1.5.
30. Dumitrescu I, Iordache OG, Mocioiu AM, Nicula G. Antimicrobial functionalization of textile materials with hydrophobins and Ag/ZnO composite nanopowders. Ind Textila. 2013;64(6):303-12.
31. Koski A, Yim K, Shivkumar S. Effect of molecular weight on fibrous PVA produced by electrospinning. Mater Lett. 2004;58(3-4):493-97. doi: 10.1016/s0167-577x(03)00532-9.
32. Deitzel JM, Kleinmeyer J, Harris D, Beck Tan NC. The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer. 2001;42(1):261-72. doi: 10.1016/s0032-3861(00)00250-0.
33. Qun G, Ajun W. Effects of molecular weight, degree of acetylation and ionic strength on surface tension of chitosan in dilute solution. Carbohydr Polym. 2006;64(1):29-36. doi: 10.1016/j.carbpol.2005.10.026. carbpol.2005.10.026.
34. Tsaih ML, Chen RH. Effect of molecular weight and urea on the conformation of chitosan molecules in dilute solutions. Int J Biol Macromol. 1997;20(3):233-40. doi: 10.1016/s0141-8130(97)01165-3, PMID 9218172.
35. Aljbour ND. Electrospun nanofibers of chitosan/nanocrystalline cellulose/polylactic acid with improved morphology and distribution. J Phys Conf Ser. 2025;3003(1):012002. doi: 10.1088/1742-6596/3003/1/012002.
36. Kiskó G. Natural control of food-borne pathogens using chitosan. Microorganisms. 2025;13(9):2036. doi: 10.3390/microorganisms13092036, PMID 41011367. [Book reference — publisher info missing].
37. Akdaşçi E, Duman H, Eker F, Bechelany M, Karav S. Chitosan and its nanoparticles: a multifaceted approach to antibacterial applications. Nanomaterials (Basel). 2025;15(2):126. doi: 10.3390/nano15020126, PMID 39852740.
38. Syed MH, Khan MM, Zahari MA, Beg MD, Abdullah N. Developing nanocellulose-loaded chitosan–polylactic acid biofilms for green food packaging. Green Mater. 2024;13(5):396-406. doi: 10.1680/jgrma.24.00057.
39. Guarnieri A, Triunfo M, Scieuzo C, Ianniciello D, Tafi E, Hahn T, et al. Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect Hermetia illucens. Sci Rep. 2022;12(1):8084. doi: 10.1038/s41598-022-12150-3, PMID 35577828.

40. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiol Rev. 1985;49(1):1-32. doi: 10.1128/mr.49.1.1-32.1985, PMID 2580220.
41. Asaftei M, Lucidi M, Cirtoaje C, Holban AM, Charitidis CA, Yang F, et al. Fighting bacterial pathogens with carbon nanotubes: focused review of recent progress. RSC Adv. 2023;13(29):19682-94. doi: 10.1039/d3ra01745a, PMID 37396836.
42. Hosseinnejad M, Jafari SM. Evaluation of different factors affecting antimicrobial properties of chitosan. Int J Biol Macromol. 2016;85:467-75. doi: 10.1016/j.ijbiomac.2016.01.022, PMID 26780706. ijbiomac.2016.01.022.
43. Samokhin Y, Varava Y, Diedkova K, Yanko I, Korniienko V, Husak Y, et al. Electrospun chitosan/polylactic acid nanofibers with silver nanoparticles: structure, antibacterial, and cytotoxic properties. ACS Appl Bio Mater. 2025;8(2):1027-37. doi: 10.1021/acsabm.4c01252, PMID 39810459.
44. Deng Y, Pan J, Yang X, Yang S, Chi H, Yang X, et al. Dual roles of nanocrystalline cellulose extracted from jute (*Corchorus olitorius* L.) leaves in resisting antibiotics and protecting probiotics. Nanoscale Adv. 2023;5(23):6435-48. doi: 10.1039/d3na00345k, PMID 38024324.
45. Colli-Gongora PE, Moo-Tun NM, Herrera-Franco PJ, Valadez-Gonzalez A. Assessing the effect of cellulose nanocrystal content on the biodegradation kinetics of multiscale polylactic acid composites under controlled thermophilic composting conditions. Polymers. 2023;15(14):3093. doi: 10.3390/polym15143093, PMID 37514482.
46. Asadzadeh F, Ghorbanzadeh S, Poursattar Marjani A, Gholami R, Asadzadeh F, Lotfollahi L. Assessing polylactic acid nanofibers with cellulose and chitosan nanocapsules loaded with chamomile extract for treating gram-negative infections. Sci Rep. 2024;14(1):22336. doi: 10.1038/s41598-024-72398-9, PMID 39333220.