https://doi.org/10.33472/AFJBS.6.6.2024.5271-5282

AFJBS

African Journal of Biological Sciences

ASSESSING CHITOSAN-BASED ELECTROSPUN NANOFIBERS CONTAINING ENVIRONMENTALLY PRODUCED SILVER NANOPARTICLES FOR IMPROVED WOUND HEALING: A COMBINED MECHANICAL AND MICROBIAL ANALYSIS

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Article History
Volume 6,Issue 6, 2024
Received:01 Apr 2024
Accepted : 07 May 2024
doi:10.33472/AFJBS.6.6.2024.5271-5282

ABSTRACT

Wound dressings are described as protective barriers that shield damaged epidermal tissues from the environment and expedite the curative procedure. The incorporation of antimicrobial agents in wound bandages arose from the recognition that bacterial infections were the primary impediment to wound healing. This research aims to comprehensively investigate the mechanical and antimicrobial characteristics of electrospun nanofibers infused with green-synthesized silver nanoparticles, crafted from chitosan. The objective is to deepen our understanding of the interplay between chitosan and silver nanoparticles and their potential in accelerating wound healing. The results indicated that nanofiber membranes composed of CT + PEO + AgNPs exhibited the highest tensile strength, rendering them suitable for practical wound healing applications. Additionally, these CT + PEO + AgNPs nanofibers were found to effectively inhibit bacterial growth, particularly showing enhanced efficacy against *S. aureus* compared to other bacterial strains. Furthermore, these nanofibers demonstrated antifungal activity against a range of fungi, with notable effectiveness against *P. funiculosum* but relatively lower efficacy against *A. fumigatus* and *A. niger*. The potent antibacterial activity of silver nanoparticles in preventing wound infections was underscored by microbiological analysis. This study underscores the potential of these nanofibers to support the body's natural healing processes and mitigate the risk of wound infections. Due to their combination of mechanical strength and antibacterial properties, these materials present a promising option for improving wound healing outcomes.

Keywords: *Electrospinning, antimicrobial activity, tensile strength, chitosan, silver nanoparticles.*

1. INTRODUCTION

The necessity for advanced wound dressings that can last longer before replacement and can quickly cure acute/chronic wounds was demonstrated by the estimated annual expenditure of approximately $25 million on the treatment of chronic non-healing wounds (Srivastava *et al.* 2023). The need for including antimicrobial agents within the wound dressings was brought on by the fact that bacterial infection was the main factor slowing wound healing (Yu *et al.* 2022; Mirhaj *et al.* 2022). It is common knowledge that traditional herbal treatments made from plant extracts can hasten the healing of wounds and these therapies are utilized worldwide (Puri *et al.* 2023). In the scientific literature, reports regarding medicinal plants and their function in promoting several stages of the wound healing process, such as wound contraction, epithelialization, fibroplasia, coagulation, and inflammation, have been widely demonstrated (Kumar *et al.* 2020; Hermosilla *et al.* 2021).

The process of healing a wound is greatly hampered by infections. In actuality, the development of biofilms and microbial growth jeopardize the curative method. So, to stop or get rid of complications, wound bandages must include antimicrobial medications. Due to their therapeutic benefits, cost-effectiveness, lack of side effects, and the rise in antibiotic tolerance microorganisms as a result of the constant use of antimicrobial agents, recently, there appears to be a lot of interest in finding antimicrobial medications made from organic substances (Liu *et al.* 2021; Moeini *et al.* 2020). A constant local administration of antimicrobial agents is also made possible by the inclusion of natural substances into nanofibers, speeding up the healing process (Sahai *et al.* 2021).

A clear, dense covering that protects the injured epithelial layers from the outside environment and speeds up their recovery was described as the wound dressing (RaniRaju *et al.* 2022). In addition to providing protection from pathogens, a wet atmosphere to stop wounds from withering out, an improvement in incision site deterioration, being pervious to oxygen without dehydrating the wound, comfort, and avoiding mechanical trauma are also requirements for wound dressing. One type of suitable dressing used for wound healing is a fibrous sheet containing bioactive chemicals. The extremely porous nanofibrous scaffolds are in the spotlight because of their impressive capacity for nutrient exchange and metabolic exudate clearance, their great ability to prevent microbe infiltration due to micron-sized apertures, and their ease of inclusion of bioactive compounds (Oraon *et al.* 2022; Sahai *et al.* 2020).

When applied to an open wound, chitosan can have a pleasant and relaxing effect since it functions as an analgesic medicine and anti-inflammatory agent in wound care treatment. The first step of wound healing, known as hemostasis, happens when a wound develops when platelets get activated and collect at the wound site to stop bleeding (Rodrigues *et al.* 2019; Feng *et al.* 2021). The ability of chitosan-based membranes to absorb significant volumes of wound exudate and gelatinous films and/or hydrogels with strong adhesive properties to the damaged tissue to halt bleeding occurs under typical wound settings. It is becoming increasingly important to use functional nanofibers with therapeutic and antibacterial properties. Application of the electrospinning process to the production of nanofibers containing medicinally active substances is gaining popularity quickly. Chitosan's antibacterial properties are explained by the positively charged chitosan's interaction with the ions that are negatively charged residues on microorganisms' cell surfaces, which results in significant cell surface modifications and changes in cell permeability (Ardean *et al.* 2021; Rashki *et al.* 2021).

The scientific community has become increasingly interested in using electrospinning to make wound dressings recently (Shahid *et al.* 2021; Yan *et al.* 2019). In addition to the method's ease of routine, adaptability, and flexibility, the nanofiber should have exceptional qualities such a high ratio surface area to volume, flexibility, high porosity, and the ability to absorb wound exudate. These membranes' nanoscale characteristics allow them to mimic the extracellular matrix (ECM) structure, which aids in the adhesion, migration, and explosion of the cells and aids in the regeneration of damaged skin. Furthermore, the tightly connected pore framework and narrow pore dimensions allow for oxygen and water penetration while also preventing pathogen infiltration and the spread of diseases. Additionally, the dormant of these networks to function as sophisticated organically active bandages is demonstrated by the ability to integrate bioactive compounds that actively contribute to the remedial process while assuring their extended discharge to the wound site.

Pure chitosan electrospinning presents difficulties due to the high viscidness of chitosan solutions. Electrospun scaffolds exhibit poor structural stability in aquatic settings and insufficient mechanical characteristics compared to pure chitosan. This study intends to completely examine the antibacterial and mechanical properties of silver nanoparticle-loaded electrospinned nanofibers made from chitosan. While doing this, we hope to learn more about how chitosan and silver nanoparticles combine and how this may contribute to quicker wound healing. The capacity of these nanofibers to tolerate dynamic pressures encountered in a clinical context and to maintain structural integrity during dressing changes depends heavily on their mechanical strength. Additionally, the antimicrobial effectiveness of the silver nanoparticles in the wound dressing will be evaluated in order to determine their capacity to stop infection and enhance overall wound healing results (Mirhaj *et al.* 2022).

2. MATERIAL AND METHODS

2.1. Electrospinning

The first step in the creation of natural silver nanoparticles was the gathering of leaves from therapeutic plants such *Aloe vera*, *Ocimum tenuiflorum* (Holy Basil), *Syzygium cumini* (Jamun), *Ficus carica* (Fig), and *Tridax procumbens*. These leaves were extracted with methanol. 5 mL of each leaf extract were mixed with a 1×10^{-3} M $AgNO_3$ aqueous solution at room temperature to produce AgNPs. Surprisingly, the solution underwent an impressive metamorphosis into a grey-black colour in about 60 minutes, representing the fruitful production of Ag nanoparticles. The production of two separate solutions—an 8% (w/v) polyethylene oxide (PEO) solution and a 3% (w/v) chitosan solution—was required for the subsequent electrospinning phase. After careful optimisation, both solutions were evenly blended using sonication to produce a composite sample known as CT + PEO.

A 4% solution of greenly synthesised AgNPs was added to the CT + PEO polymer solution to generate a composite sample that contains CT + PEO and AgNPs. The resultant nanofibers were collected 15 cm away from the needle after each sample was treated with a pump. Controlled circumstances predominated, with 25°C thermal range and a RH range of 60%. Gravity combined with the electrostatic forces that led to fibre production to facilitate solution flow. Following spinning, an extensive washing of the nanofiber matrix was used to neutralise any acetic acid that might have remained. Finally, a vacuum oven was used to dry the nanofibers for 24 hours so they could be thoroughly characterised.

2.2. Mechanical properties

Uniaxial tensile tests were accomplished on rectangular specimens with sizes of 30 mm in length and 10 mm in breadth in order to characterise the mechanical characteristics of nanofibers in certain circumstances. The evaluations were performed using a ZWICK 1432 tensile tester featuring a twenty Newton weight cell and a controlled elongation rate of 15 mm per minute. The specimens were stretched out over a distance of 10 mm, with a 50% break limit set in advance. These tests were performed under typical environmental conditions, which included a 20°C temperature and a 65%

humidity level. This technique offers an accurate way to gauge the strength of the nanofiber, which is crucial for a variety of applications in industries like engineering, nanotechnology, and materials research. For nanofibers to work optimally in a number of technical and medical applications, it is essential to understand their mechanical properties (Sahai *et al.* 2020).

2.3. Antibacterial activity

A zone inhibition approach was used to examine the antibacterial capabilities of nanofiber membranes. Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* were employed as model microorganisms. Mueller-Hinton agar (MHA) medium, which has a pH of 7.3, was used to cultivate these microbes. Agar media were evenly placed onto the plates to a depth of 5 mm, and then they were left to set up. The microbial suspensions were made using culture broth and a spectrophotometer after adding enough sterile media. 1 x 10^8 CFU/ml of bacterial stock suspension will result from this process. To ensure the confluent growth of the organism, the bacterial solution was streaked across the outermost layer of the culture medium using a disinfected cotton swab.

The three types of nanofiber membranes—CT, CT + PEO, and CT + PEO + AgNP—were sterilized with UV radiation before being put into a petri dish with approximately 10^8 colony-forming units (CFUs)/mL of *S. aureus*, *P. aeruginosa* or *E. coli* and cultured for 24 hours at 37 °C. Each disc's inhibition zone's diameter was measured in order to evaluate its antibacterial effectiveness. After the scaffolds had been incubated, the inhibitory regions around them were measured. In general, this study explored the antibacterial properties of nanofiber membranes and scaffolds made of CT/PEO/AgNPs against several bacterial species.

2.4. Antifungal activity

The disc diffusion approach involved a methodical process for measuring the antifungal activity. *Penicillium funiculosum*, *Aspergillus fumigatus*, and *Aspergillus niger* fungi strains were initially cultured and developed to a particular density. To ensure the confluent development of the organism, the microbial suspension was streaked across the exterior of agar plates using an uncontaminated swab of cotton. Then, sterile nanofiber samples (6 mm in diameter) were put onto agar plates that had already been contaminated with the fungi. Following that, these plates were allowed to incubate for a specific amount of time in ideal microbial development circumstances. After standing for 5 minutes, the petri plates were cultured for 2-3 days at 28° C.

A gradient in concentration was created during incubation as the antifungal drug diffused from the discs into the agar. Following development, the diameter of the clear zones of resistance, which represent the inhibition of fungal growth, was measured and compared across various doses of antifungal agents. This gave information about how well the antifungal substance worked against the tested fungi. The experiment was done in triplicate to confirm the precision of the findings, and determined the significance of the detected inhibitory zones.

3. RESULT AND DISCUSSION

3.1. Mechanical properties

Nanofibers were subjected to mechanical tests in both natural and physiologic settings. Each scaffold was tested in triplicate, and the mean findings were reported. As the fibre diameter grew and the porosity shrunk in both cases, the mechanical characteristics improved noticeably. The samples' stresses did not differ significantly from one another, suggesting that their strengths were comparable. The range of elastic modulus variations among the samples also had this similarity.

The breaking modulus of the as-prepared nanofibers was significantly impacted by the addition of AgNPs (Silver Nanoparticles) (Figure 1). With the incorporation of AgNPs, the breaking strength climbed from 7.93 MPa for CT + PEO to 13.82 MPa (Table 1). The probable connections between CT + PEO and AgNPs, which were facilitated by intermolecular contacts, were blamed for the increase in strength of tensile (Hadipour-Goudarzi *et al.* 2014). The nanofiber membranes made of CT + PEO + AgNPs consequently had the maximum tensile strength, indicating that they were appropriate for use in real-world injury repairing applications. It is important to remember that the extracellular matrix of mature tissue produces a milieu where cells can move, multiply, and differentiate into distinct cell types. Therefore, as noted by Vernekar *et al.* in 2016, the scaffold utilised in wound healing should imitate this milieu while taking into account its molecular makeup, durability against hydrolysis, and durability. The cross-linkage of synthetic scaffolds, among other factors, has a substantial impact on how they behave in terms of biomechanical aspects. The resistance to hydrolysis and mechanical strength of nanofibers are improved by adequate and efficient crosslinking (Schiffman and Schauer, 2007).

PEO chains are renowned for their flexibility while chitosan is typically known as a stiff and brittle natural polymer. Consequently, it is anticipated that adding CT (Chitosan) and PEO (Polyethylene Oxide) to mats will increase their flexibility (Angel *et al.* 2022). In scaffolds loaded with nanofibers containing Ag NPs, which were produced using green synthesis, the greatest stretching at tear (highest possible tension stress along the axis of horizontal motion) was noted, indicating the highest degree of flexibility among the fibres. As previously shown, it is well known that the inclusion of nanoparticles may boost the rate of elongation at break (Wang *et al.* 2015).

According to the research, the nanofiber composite containing silver nanoparticles had the maximum flexi power, measuring 13.82 MPa, along with the highest elongation, 19.52%. According to Wang *et al.* in 2015, this result is ascribed to the outstanding crystal hardness of Ag-NPs, which improved the attributes of the fibres due to the suitable increase in the $AgNO_3$ content. This behaviour is probably due to the silver nanoparticles' very small number, even distribution across the polymeric chains, connections with the active sites of CT and PEO, and other factors. As a result, it can be said that mats with a good combination of flexibility and high tensile strength are produced when silver particle content is 0.5%.

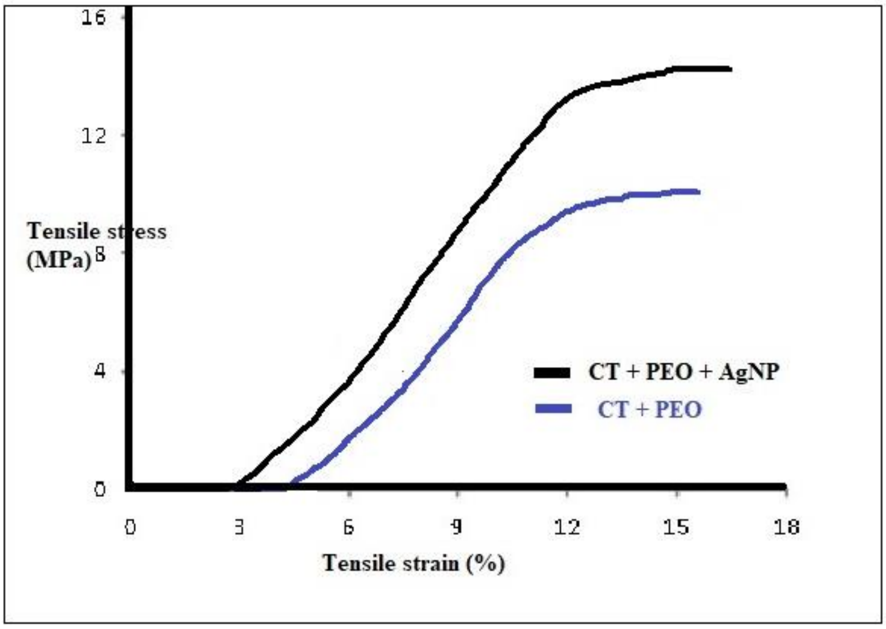

Figure 1: Tensile strength of nanofibers tested.

Table 1: Tensile and breaking strain percentage of nanofibers.

S. No	Sample	Tensile strength (MPa)	Tensile strain (%)
1	CT	6.48 ± 1.53	14.93 ± 3.7
2	CT + PEO	7.93 ± 1.98	15.23 ± 4.8
3	CT + PEO + AgNP	13.82 ± 2.26	19.52 ± 5.2

3.2. Antibacterial activity

The CT, CT + PEO, and CT + PEO + AgNPs nanofibers were tested for bacterial inhibition against *S. aureus*, *E. coli*, and *P. aeruginosa*. Pure CT nanofiber and CT + PEO were used as blank controls, while CT + PEO + AgNPs nanofiber was utilized as a comparative sample to determine if CTS + PEO + AgNPs nanofibers have potent antibacterial activity. Results clearly demonstrated a region where the bacterial growth was suppressed surrounding the CT/AgNPs nanofibers. As can be observed, pure CT nanofiber and CT nanofiber plus PEO showed decreased bacterial inhibition. On the other hand, it was discovered that CT + PEO + AgNPs nanofibers can stop the growth of bacteria, and that they are slightly more efficient against *S. aureus* than other bacteria (Figure 2).

Here, we have clearly shown that the existence of AgNPs in the fibre affected the inhibition of the proliferation of bacteria. The zones of inhibition for the CT, CT + PEO, and CT + PEO + AgNPs nanofibers against *S. aureus* were 5.8 mm, 14.03 mm, and 18.72 mm, respectively, according to the antibacterial tests. *E. coli*'s zones of inhibition were revealed to be 5.6 mm, 12.85 mm, and 16.32 mm, respectively, while *P. aeruginosa*'s zones of inhibition were shown to be 5.5 mm, 12.73 mm, and 15.97 mm, respectively (Table 2). Gram-positive *S. aureus* was more resistant to the development restraining properties of the nanofiber than Gram-negative *S. aureus*. According to studies, there are alterations between the cell walls of Gram-negative and Gram-positive bacteria that may explain why *P. aeruginosa* and *E. coli* exist (Kharaghani *et al*. 2018; Shalaby *et al*. 2018).

Table 2: Antibacterial activity of experimented nanofibers against pathogens.

S. No	Samples	Inhibition zone diameter (mm)		
		P. aeruginosa	*E. coli*	*S. aureus*
1	CT	5.5 ± 1.3	5.6 ± 1.8	5.8 ± 0.8
2	CT + PEO	13.08 ± 1.9	12.85 ± 1.3	14.03 ± 1.5
3	CT + PEO + AgNP	15.97 ± 2.1	16.32 ± 1.9	18.72 ± 2.2

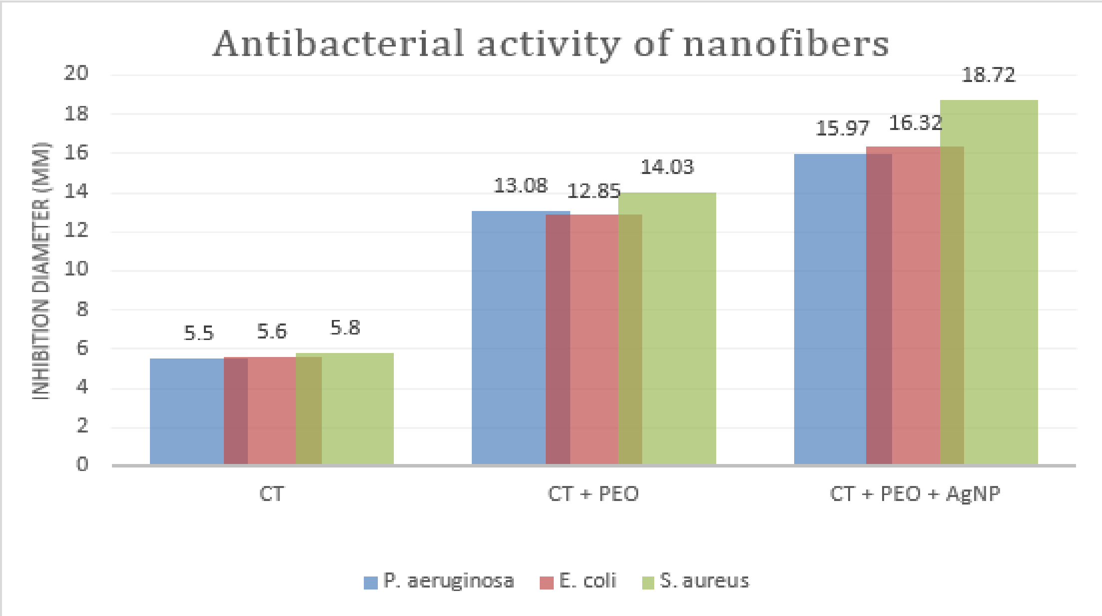

Figure 2: Antibacterial activity.

These fibres' antibacterial activities have been significantly enhanced by the addition of silver nanoparticles (AgNPs), revealing the flexibility of AgNPs content in regulating these outcomes. AgNPs' deadly properties make them efficient against germs. AgNPs initially cling to the negatively charged surface of the cell membrane, instigating a reduction of membrane permeability and having an effect on the respiratory chain. AgNPs then enter the cell and harm it by cooperating with phosphorus and sulphur, elements found in biological molecules like DNA. AgNPs ultimately cause cell lysis, which results in cell leakage and ultimately results in cell death (Flores-López *et al*. 2019; Liao *et al*. 2019).

The silver nanoparticle-loaded nanofibers in this situation act as vehicles for the carefully timed discharge of silver ions. The findings show that nanofibers loaded with AgNPs have a significant potential for application as biocompatible and antibacterial wound dressings (He *et al*. 2021). Nanoparticles are a good option in combating microbes since they have been shown to have low toxicity in the natural environment and biological cycle (Makvandi *et al*. 2020).

External pathogens like *E. coli*, *S. aureus*, and *P. aeruginosa* can cause an infection at the site of the wound, which can negatively affect the early stages of wound healing through insufficient enhancement of tissue granulation. According to Makvandi *et al*. (2019), advanced wound healing antibacterial materials can reduce the amount of time it takes for a wound to heal by killing the microbes that have developed at the injury site and helping to prevent problems from wounds. Our results indicated the synergistic antimicrobial properties of the manufactured nanofibrous mats against Gram- positive and -- negative bacteria.

Silver nanoparticles have been shown to induce bacterial death through multiple mechanisms. They act on the bacterial cell membrane and wall, hinder DNA and protein synthesis, and disrupt the antioxidant mechanisms (Makvandi *et al*., 2020). In our research, the addition of Ag nanoparticles to chitosan scaffolds displayed a synergistic effect, enhancing their efficiency. This finding aligns with previous studies, such as that by Hu *et al*. (2018), which also testified a synergistic impact of silver nanoparticles. Other research, such as those conducted by Sachdeva *et al.* (2021), have shown that nanoparticles of silver have stronger antimicrobial properties.

In a study by Bagheri *et al*. in 2021, antibacterial Ag-ZnO nanoparticles incorporated into CT/PEO nanofibrous mats exhibited their prospective as efficient wound dressings (Bagheri *et al*., 2022). Lopez-Esparza *et al*. (2016) investigated antimicrobial silver nanoparticles entrenched in PCL nanofiber mats, which showed substantial opposition against both Gram-negative and Gram-positive bacteria, including *P. aeruginosa*, *E. coli*, *K. pneumonia*, *S. pyogenes*, and *S. aureus*. The bactericidal properties of these nanofibers were attributed to their hydrophobicity, resulting from increased fiber diameter, and the anti-adherence activity of silver nanoparticles (Murillo *et al.*, 2023). Additionally, the silver ions (Ag^{+}) they release, can penetrate cells of bacteria and react with substances including phosphorus and sulphur, deactivating proteins by stopping DNA replication as a result. Reactive oxygen species (ROS), which produce unstable molecules with strong antimicrobial effects, are said to be stimulated by AgNPs (Lv *et al.*, 2021).

3.3. Antifungal activity

The aforementioned nanofibers also exhibited antifungal properties against all the tested fungi, with *P. funiculosum* exhibiting the highest effectiveness and *A. niger* and *A. fumigatus* showing the lowest efficacy (Figure 3). The highest inhibition zones are seen in the sample CT + PEO + AgNP, which also contains AgNP. This sample has inhibition zones of 19.87 mm in *P. funiculosum*, 16.52 mm in *A. niger*, and 16.35 mm in *A. fumigatus*, suggesting improved antifungal activity (Table 3). These outcomes can be explained by chitosan's inherent ability to fight off a variety of bacteria and fungi. One of chitosan's most significant biological functions is its ability to inhibit the growth of fungi, and prior research has shown that films made of chitosan can be used to preserve food in packaging and lower microbial populations in medical settings (Hamed *et al.* 2016).

According to Amirabad *et al.*, nanocrystals could enhance Chitosan nanofibers' antifungal activity, specifically against *A. niger*, showing a synergistic effect when these materials are combined. These results could be explained by the influence of nanocrystals on the orientation and immobilization of the nanofibers or by the presence of stabilized and well-distributed Chitosan nanofibers, which would result in a high level of connection between the nanofibers and the fungi (Amirabad *et al.* 2018).

Table 3: Antifungal activity of experimented nanofibers against pathogens.

S. No	Samples	Inhibition zone diameter (mm)		
		P. funiculosum	*A. fumigatus*	*A. niger*
1	CT	7.26 ± 1.2	4.78 ± 0.9	5.5 ± 0.7
2	CT + PEO	12.95 ± 1.8	10.63 ± 1.7	11.03 ± 1.8
3	CT + PEO + AgNP	19.87 ± 2.5	16.35 ± 2.3	16.52 ± 1.9

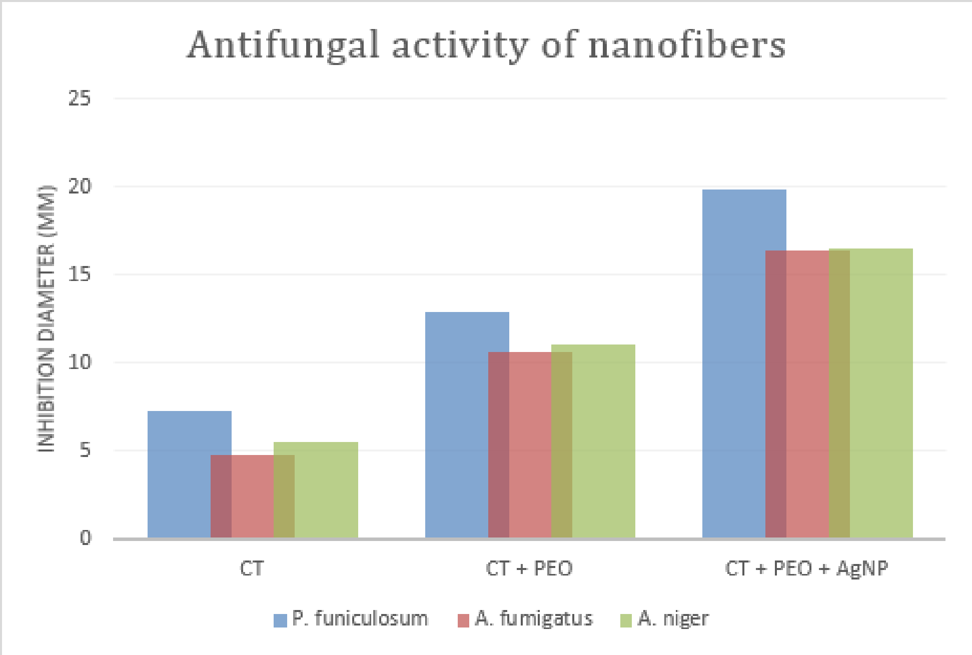

Figure 3: Antifungal activity.

Concerning the antifungal action, fungus growth was inhibited and the fungicidal action was enhanced for some micromycetes, such as *A. niger*, *P. funiculosum,* and *A. fumigatus*, in contrast with other systems when adding silver nanoparticles. It is an extremely pertinent finding given that Aspergillus spp. constitutes one of the primary fungi that cause problems in injuries and burning (Obradovic-Tomasev *et al.* 2014).

4. CONCLUSION

When treating a wound, chitosan-based membranes may effectively absorb significant volumes of wound exudate while hydrogels with high adhesive properties can be put to the injured tissue to halt bleeding. Utilizing antibacterial and therapeutic-capable functional nanofibers is becoming increasingly important. With a specific focus on their usage in wound healing, this work shed significant light on the mechanical characteristics and microbiological effectiveness of electrospun nanofibers made from chitosan that are infused with environmentally produced silver nanoparticles. Assuring patient comfort and clinical application efficacy is their capacity to tolerate mechanical stress and adapt to wound shapes, which is a considerable advantage. The effectiveness of silver nanoparticles in efficiently preventing infection at wound sites was highlighted by the microbiological examination, which showed the substance's strong antibacterial activity. This study emphasizes how these nanofibers could help the body's natural healing processes as well as avoid wound infections. These materials are a good choice for increasing wound healing results due to their combination of mechanical strength and antibacterial qualities. It is imperative to conduct more clinical trials and studies to confirm the products' efficacy, safety, and potential to revolutionize wound care procedures, thereby enhancing patient quality of life and lowering healthcare costs.

DECLARATIONS:

DATA AVAILABILITY STATEMENT

All the data is collected from the simulation reports of the software and tools used by the authors. Authors are working on implementing the same using real world data with appropriate permissions.

FUNDING
No fund received for this project

CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

ETHICAL APPROVAL AND HUMAN PARTICIPATION
No ethics approval is required.

REFERENCES

1. Amirabad, L.M., Jonoobi, M., Mousavi, N.S., Oksman, K., Kaboorani, A. and Yousefi, H., 2018. Improved antifungal activity and stability of chitosan nanofibers using cellulose nanocrystal on banknote papers. *Carbohydrate polymers*, *189*, pp.229-237.
2. Angel, N., Li, S., Yan, F. and Kong, L., 2022. Recent advances in electrospinning of nanofibers from bio-based carbohydrate polymers and their applications. *Trends in Food Science & Technology*, *120*, pp.308-324.
3. Ardean, C., Davidescu, C.M., Nemeş, N.S., Negrea, A., Ciopec, M., Duteanu, N., Negrea, P., Duda-Seiman, D. and Musta, V., 2021. Factors influencing the antibacterial activity of chitosan and chitosan modified by functionalization. *International Journal of Molecular Sciences*, *22*(14), p.7449.
4. Bagheri, M., Validi, M., Gholipour, A., Makvandi, P. and Sharifi, E., 2022. Chitosan nanofiber biocomposites for potential wound healing applications: Antioxidant activity with synergic antibacterial effect. *Bioengineering & translational medicine*, *7*(1), p.e10254.
5. Bürgers, R., Eidt, A., Frankenberger, R., Rosentritt, M., Schweikl, H., Handel, G. and Hahnel, S., 2009. The anti-adherence activity and bactericidal effect of microparticulate silver additives in composite resin materials. *Archives of Oral Biology*, *54*(6), pp.595-601.
6. Feng, P., Luo, Y., Ke, C., Qiu, H., Wang, W., Zhu, Y., Hou, R., Xu, L. and Wu, S., 2021. Chitosan-based functional materials for skin wound repair: Mechanisms and applications. *Frontiers in Bioengineering and Biotechnology*, *9*, p.650598.
7. Flores-López, L.Z., Espinoza-Gómez, H. and Somanathan, R., 2019. Silver nanoparticles: Electron transfer, reactive oxygen species, oxidative stress, beneficial and toxicological effects. Mini review. *Journal of Applied Toxicology*, *39*(1), pp.16-26.
8. Hadipour-Goudarzi, E., Montazer, M., Latifi, M. and Aghaji, A.A.G., 2014. Electrospinning of chitosan/sericin/PVA nanofibers incorporated with in situ synthesis of nano silver. *Carbohydrate polymers*, *113*, pp.231-239.
9. Hamed, I., Özogul, F. and Regenstein, J.M., 2016. Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. *Trends in food science & technology*, *48*, pp.40-50.
10. He, C., Liu, X., Zhou, Z., Liu, N., Ning, X., Miao, Y., Long, Y., Wu, T. and Leng, X., 2021. Harnessing biocompatible nanofibers and silver nanoparticles for wound healing: Sandwich wound dressing versus commercial silver sulfadiazine dressing. *Materials Science and Engineering: C*, *128*, p.112342.
11. Hermosilla, J., Pastene-Navarrete, E. and Acevedo, F., 2021. Electrospun fibers loaded with natural bioactive compounds as a biomedical system for skin burn treatment. A review. *Pharmaceutics*, *13*(12), p.2054.

12. Hu, M., Li, C., Li, X., Zhou, M., Sun, J., Sheng, F., Shi, S. and Lu, L., 2018. Zinc oxide/silver bimetallic nanoencapsulated in PVP/PCL nanofibres for improved antibacterial activity. *Artificial cells, nanomedicine, and biotechnology*, *46*(6), pp.1248-1257.
13. Kharaghani, D., Jo, Y.K., Khan, M.Q., Jeong, Y., Cha, H.J. and Kim, I.S., 2018. Electrospun antibacterial polyacrylonitrile nanofiber membranes functionalized with silver nanoparticles by a facile wetting method. *European Polymer Journal*, *108*, pp.69-75.
14. Kumar, H., Jain, S. and Shukla, K., 2020. Evaluation of alfalfa (Medicago sativa) leaves for wound healing activity. *Journal of Pharmacognosy and Phytochemistry*, *9*(5), pp.1164-1169.
15. Liao, S., Zhang, Y., Pan, X., Zhu, F., Jiang, C., Liu, Q., Cheng, Z., Dai, G., Wu, G., Wang, L. and Chen, L., 2019. Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant Pseudomonas aeruginosa. *International journal of nanomedicine*, pp.1469-1487.
16. Liu, J., Wu, D., Zhu, N., Wu, Y. and Li, G., 2021. Antibacterial mechanisms and applications of metal-organic frameworks and their derived nanomaterials. *Trends in Food Science & Technology*, *109*, pp.413-434.
17. López-Esparza, J., Espinosa-Cristóbal, L.F., Donohue-Cornejo, A. and Reyes-López, S.Y., 2016. Antimicrobial activity of silver nanoparticles in polycaprolactone nanofibers against gram-positive and gram-negative bacteria. *Industrial & Engineering Chemistry Research*, *55*(49), pp.12532-12538.
18. Lv, H., Cui, S., Yang, Q., Song, X., Wang, D., Hu, J., Zhou, Y. and Liu, Y., 2021. AgNPs-incorporated nanofiber mats: Relationship between AgNPs size/content, silver release, cytotoxicity, and antibacterial activity. *Materials Science and Engineering: C*, *118*, p.111331.
19. Makvandi, P., Ali, G.W., Della Sala, F., Abdel-Fattah, W.I. and Borzacchiello, A., 2019. Biosynthesis and characterization of antibacterial thermosensitive hydrogels based on corn silk extract, hyaluronic acid and nanosilver for potential wound healing. *Carbohydrate polymers*, *223*, p.115023.
20. Makvandi, P., Wang, C.Y., Zare, E.N., Borzacchiello, A., Niu, L.N. and Tay, F.R., 2020. Metal-based nanomaterials in biomedical applications: antimicrobial activity and cytotoxicity aspects. *Advanced Functional Materials*, *30*(22), p.1910021.
21. Mirhaj, M., Labbaf, S., Tavakoli, M. and Seifalian, A., 2022. An overview on the recent advances in the treatment of infected wounds: Antibacterial wound dressings. *Macromolecular Bioscience*, *22*(7), p.2200014.
22. Moeini, A., Pedram, P., Makvandi, P., Malinconico, M. and d'Ayala, G.G., 2020. Wound healing and antimicrobial effect of active secondary metabolites in chitosan-based wound dressings: A review. *Carbohydrate polymers*, *233*, p.115839.
23. Murillo, L., Rivero, P.J., Sandúa, X., Pérez, G., Palacio, J.F. and Rodríguez, R.J., 2023. Antifungal Activity of Chitosan/Poly (Ethylene Oxide) Blend Electrospun Polymeric Fiber Mat Doped with Metallic Silver Nanoparticles. *Polymers*, *15*(18), p.3700.
24. Obradovic-Tomasev, M., Popovic, A., Vuckovic, N. and Jovanovic, M., 2014. Mixed fungal infection (Aspergillus, Mucor, and Candida) of severe hand injury. *Case Reports in Infectious Diseases*, *2014*.
25. Oraon, R., Rawtani, D., Singh, P. and Hussain, C.M. eds., 2022. *Nanocellulose Materials: Fabrication and Industrial Applications*. Elsevier.
26. Puri, A., Sahai, N., Ahmed, T. and Saxena, K., 2023. 3D bioprinting for diagnostic and therapeutic application. *Materials Today: Proceedings*.

27. Rani Raju, N., Silina, E., Stupin, V., Manturova, N., Chidambaram, S.B. and Achar, R.R., 2022. Multifunctional and Smart Wound Dressings—A Review on Recent Research Advancements in Skin Regenerative Medicine. *Pharmaceutics*, *14*(8), p.1574.
28. Rashki, S., Asgarpour, K., Tarrahimofrad, H., Hashemipour, M., Ebrahimi, M.S., Fathizadeh, H., Khorshidi, A., Khan, H., Marzhoseyni, Z., Salavati-Niasari, M. and Mirzaei, H., 2021. Chitosan-based nanoparticles against bacterial infections. *Carbohydrate polymers*, *251*, p.117108.
29. Rodrigues, M., Kosaric, N., Bonham, C.A. and Gurtner, G.C., 2019. Wound healing: a cellular perspective. *Physiological reviews*, *99*(1), pp.665-706.
30. Sachdeva, A., Singh, S. and Singh, P.K., 2021. Synthesis, characterisation and synergistic effect of ZnO nanoparticles to antimicrobial activity of silver nanoparticle. *Materials Today: Proceedings*, *34*, pp.649-653.
31. Sahai, N., Gogoi, M. and Tewari, R.P., 2021. 3D printed chitosan composite scaffold for chondrocytes differentiation. *Current medical imaging*, *17*(7), pp.832-842.
32. Sahai, N., Saxena, K.K. and Gogoi, M., 2020. Modelling and simulation for fabrication of 3D printed polymeric porous tissue scaffolds. *Advances in Materials and Processing Technologies*, *6*(3), pp.530-539.
33. Schiffman, J.D. and Schauer, C.L., 2007. Cross-linking chitosan nanofibers. *Biomacromolecules*, *8*(2), pp.594-601.
34. Shahid, M.A., Ali, A., Uddin, M.N., Miah, S., Islam, S.M., Mohebbullah, M. and Jamal, M.S.I., 2021. Antibacterial wound dressing electrospun nanofibrous material from polyvinyl alcohol, honey and Curcumin longa extract. *Journal of Industrial Textiles*, *51*(3), pp.455-469.
35. Shalaby, T., Hamad, H., Ibrahim, E., Mahmoud, O. and Al-Oufy, A., 2018. Electrospun nanofibers hybrid composites membranes for highly efficient antibacterial activity. *Ecotoxicology and environmental safety*, *162*, pp.354-364.
36. Srivastava, G.K., Rodríguez, S.M., Fadilah, N.I.M., Markey, G., Hao, D.L.Q., Shukla, P., Fauzi, M.B. and Panetsos, F., 2023. Progress in Wound Healing Products Based on Natural Compounds, Stem Cells and MicroRNA-Based Biopolymers in the European, USA, and Asian Markets: Opportunities, Barriers, and Regulatory Issues.
37. Vernekar, V.N., James, R., Smith, K.J. and Laurencin, C.T., 2016. Nanotechnology applications in stem cell science for regenerative engineering. *Journal of Nanoscience and Nanotechnology*, *16*(9), pp.8953-8965.
38. Wang, X., Cheng, F., Gao, J. and Wang, L., 2015. Antibacterial wound dressing from chitosan/polyethylene oxide nanofibers mats embedded with silver nanoparticles. *Journal of biomaterials applications*, *29*(8), pp.1086-1095.
39. Yan, X., Yu, M., Ramakrishna, S., Russell, S.J. and Long, Y.Z., 2019. Advances in portable electrospinning devices for in situ delivery of personalized wound care. *Nanoscale*, *11*(41), pp.19166-19178.
40. Yu, R., Zhang, H. and Guo, B., 2022. Conductive biomaterials as bioactive wound dressing for wound healing and skin tissue engineering. *Nano-micro letters*, *14*, pp.1-46.