

Research Article

Comprehensive Analysis of Silver Nanoparticles incorporated with Polylactic acid (PLA): Antibacterial Activity, XRD Analysis, Cytotoxicity, and SEM Characterization for Health Care Application

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Abstract: This study investigates the integration of silver nanoparticles (AgNPs) into polylactic acid (PLA) to enhance their antimicrobial properties while maintaining biocompatibility. The antibacterial and antifungal activities of the composites were evaluated using disc diffusion and agar diffusion methods against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. X-ray diffraction (XRD) analysis confirmed the incorporation of AgNPs by showing distinct crystalline peaks. Scanning electron microscopy (SEM) was used to characterize the uniform distribution and morphology of the nanoparticles within the PLA matrix. Cytotoxicity was assessed using the MTT assay, measuring cell viability at various concentrations. The antimicrobial tests revealed significant inhibition zones: 12 mm for *E. coli*, 11 mm for *S. aureus*, and 13 mm for *C. albicans*. XRD analysis showed distinct crystalline peaks, confirming the successful incorporation of AgNPs into the composites. SEM characterization demonstrated a uniform distribution of well-defined nanoparticles within the PLA matrix. The MTT assay results indicated minimal cytotoxicity, with cell viability remaining above 94.88% at the highest tested concentration. PLA and silver nanoparticle composites exhibit potent antimicrobial activity and are biocompatible, making them suitable for various healthcare applications. Further research should focus on long-term stability, mechanisms of action, biocompatibility, application-specific optimization, and regulatory compliance.

Keywords: Silver nanoparticles, Polylactic acid, Antimicrobial activity, Biocompatibility, Cytotoxicity

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INTRODUCTION

The use of nanotechnology in healthcare has revolutionized the development of new materials with enhanced properties, particularly in the field of antimicrobial applications. Silver nanoparticles (AgNPs) have emerged as potent antimicrobial agents due to their unique properties and broad-spectrum antibacterial activity. Their integration with biopolymers such

as polylactic acid (PLA) has opened new avenues for creating advanced composite materials for medical applications. Silver has been used for its antimicrobial properties for centuries. However, the advent of nanotechnology has significantly enhanced these properties. AgNPs exhibit high surface area-to-volume ratios, which amplify their interaction with microbial membranes, leading to effective microbial

inhibition [1]. The mechanism of action of AgNPs involves the release of silver ions, which interact with the bacterial cell membrane, generating reactive oxygen species (ROS) and disrupting cellular function, ultimately leading to cell death [2]. Polylactic acid (PLA) is a biodegradable and bioactive thermoplastic derived from renewable resources such as corn starch or sugarcane. PLA is widely used in medical applications due to its biodegradability, biocompatibility, and mechanical properties [3]. The combination of PLA and silver offers a synergistic approach to developing composite materials with enhanced properties.

The antibacterial efficacy of AgNPs, when incorporated into PLA is significantly enhanced. The composite materials exhibit a sustained release of silver ions, which provides prolonged antibacterial activity. Studies have shown that AgNPs embedded in biopolymer matrices effectively inhibit the growth of a wide range of pathogenic bacteria, including both Gram-positive and Gram-negative strains [4]. The incorporation of AgNPs into PLA not only improves the antibacterial properties but also enhances the mechanical strength and thermal stability of the composites [5].

X-ray diffraction (XRD) is a powerful analytical technique used to characterize the crystalline structure of materials. In this study, XRD analysis is employed to determine the crystalline nature of the AgNPs and their distribution within the PLA. The XRD patterns provide information on the phase composition, crystallite size, and degree of crystallinity of the composite materials [6]. The incorporation of AgNPs into the biopolymer matrices can influence the crystallization behaviour of the polymers, leading to changes in their mechanical and thermal properties.

The biocompatibility of AgNPs incorporated into PLA is a critical aspect for their application in healthcare. Cytotoxicity studies assess the potential adverse effects of the composite materials on mammalian cells. It is essential to ensure that the materials exhibit minimal cytotoxicity while maintaining their antimicrobial efficacy. Various *in vitro* assays, such as MTT and LDH assays, are employed to evaluate the cytotoxicity of the composite materials on different cell lines [7]. The findings from these studies provide insights into the safety and potential clinical applications of the AgNPs incorporated into PLA.

Scanning electron microscopy (SEM) is utilized to examine the surface morphology and elemental composition of the composite materials. SEM provides high-resolution images that reveal the distribution and size of AgNPs within the PLA. The morphological characteristics of the composites play a crucial role in determining their mechanical properties and antimicrobial efficacy [8]. The SEM analysis complements the findings from XRD and cytotoxicity studies, providing a comprehensive understanding of the composite materials' structure-property relationships.

The incorporation of AgNPs into PLA composites holds significant promise for various healthcare applications. These materials can be utilized in wound dressings, surgical implants, and medical devices, where antimicrobial properties are critical to prevent infections and promote healing [9]. The biocompatibility and biodegradability of PLA, combined with the potent antimicrobial activity of AgNPs, make these

composites ideal candidates for developing advanced healthcare materials. Furthermore, the controlled release of silver ions from the composite materials ensures sustained antimicrobial activity, reducing the risk of bacterial resistance.

The objective of this study is to investigate the integration of silver nanoparticles (AgNPs) into polylactic acid (PLA) to enhance their antimicrobial properties while maintaining biocompatibility. This research aims to evaluate the antibacterial and antifungal activities, confirm the incorporation and distribution of AgNPs within the matrices, and assess the cytotoxicity of the resulting composites. The ultimate goal is to determine the potential suitability of these composites for various healthcare applications.

Materials and Methods

PREPARATION OF THE BACTERIAL INOCULUM:

Stock cultures were maintained at 4° C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37°c on shaking incubator and fungal cultures were incubated at 27°c for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours and fungal incubated at 27°c for 3-5 days. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27°c for 3-5 days. These stock cultures were kept at 4°c. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

Disc Diffusion method

The antibacterial activity and antifungal activity of PLA+ Silver treated fabric extract extracts was determined by Well Diffusion method (Bauer *et al.*, 1996).. The 0.5 cm dia of *Acalypha indica* treated fabric was placed into agar media. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on muller Hinton agar plate and potato dextrose agar. well, were put into the agar medium using sterile forceps. plant extract was poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Antibacterial and Antifungal Activity

The antibacterial and antifungal activities of PLA and silver nanoparticle-treated fabrics were evaluated using the disc diffusion method and agar diffusion method. The inhibition zones were measured to determine the effectiveness of the treatments against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.

X-ray Diffraction (XRD) Analysis

XRD analysis was conducted to characterize the crystalline structure of the PLA and silver nanoparticle composites. The samples were prepared by drying the PLA and silver nanoparticle mixture. The XRD patterns were obtained using an X-ray diffractometer, and the resulting data was analysed to determine the crystalline phases present in the composites.

Scanning Electron Microscopy (SEM) Characterization

SEM analysis was performed to examine the surface morphology and size distribution of the silver nanoparticles within the PLA matrix. The samples were prepared by placing a small amount of the composite material on an SEM stub and coating it with a thin layer of gold to enhance conductivity. SEM images were taken at different magnifications to observe the particle morphology and distribution.

Cytotoxicity Assessment (MTT Assay)

The cytotoxicity of the silver nanoparticle-incorporated PLA composites was evaluated using the MTT assay, which measures cell viability based on the reduction of MTT to formazan by mitochondrial enzymes in viable cells.

Materials:

- Vero cell lines (NCCS Pune)
- DMEM medium (HI media)
- Fetal Bovine Serum (Himedia)
- MTT reagent (5 mg/ml, HI media)
- D-PBS (HI media)
- 96-well plate for culturing cells (Nest India)
- T25 flask (Nest India)
- Centrifuge tubes (TARSON)
- Pipettes and tips (TARSON)
- Inverted binocular biological microscope (ICX41)
- 37°C incubator with 5% CO₂ atmosphere (Thermo Fisher)

- 96-well plate reader (Epoch2 Bio Tek)

Procedure:

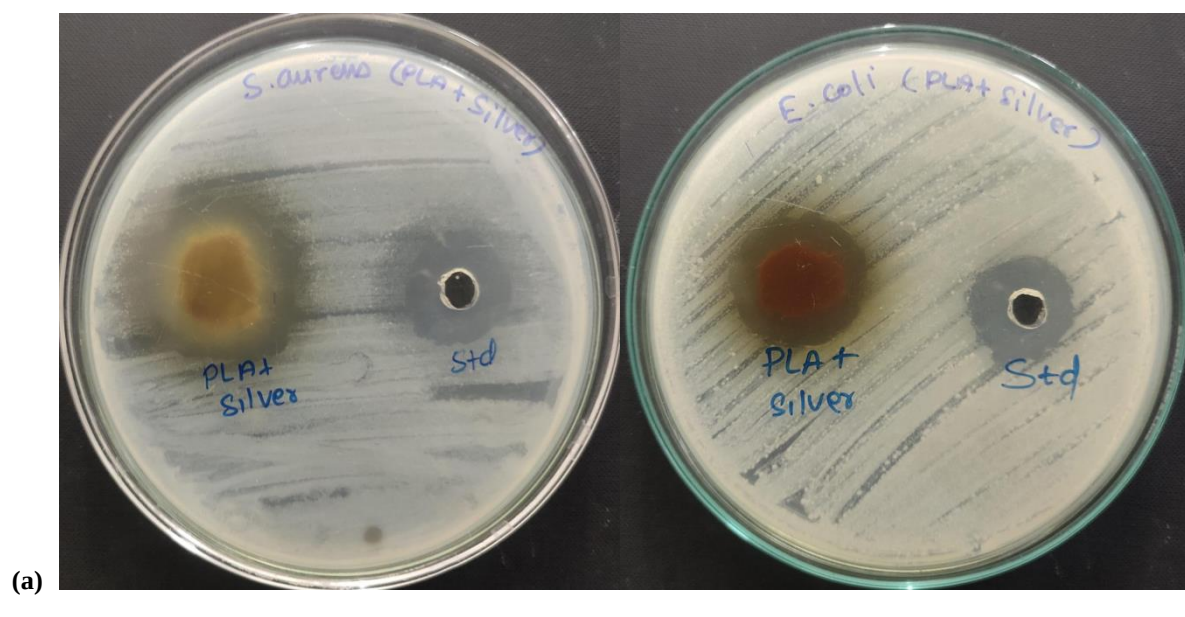
- Vero cells were maintained in DMEM media supplemented with 10% FBS and 1% antibiotic-antimycotic solution.
- Cells were seeded in a 96-well plate at a density of 20,000 cells per well and allowed to grow for 24 hours.
- Appropriate concentrations of the test agents were added to the wells, and the plate was incubated for 24 hours at 37°C in a 5% CO₂ atmosphere.
- After incubation, the spent media was removed, and MTT reagent was added to a final concentration of 0.5 mg/ml.
- Plates were incubated for 3 hours, after which the MTT reagent was removed, and 100 µL of solubilization solution (DMSO) was added.
- The plates were gently stirred to dissolve the formazan crystals, and absorbance was read at 570 nm using a spectrophotometer.

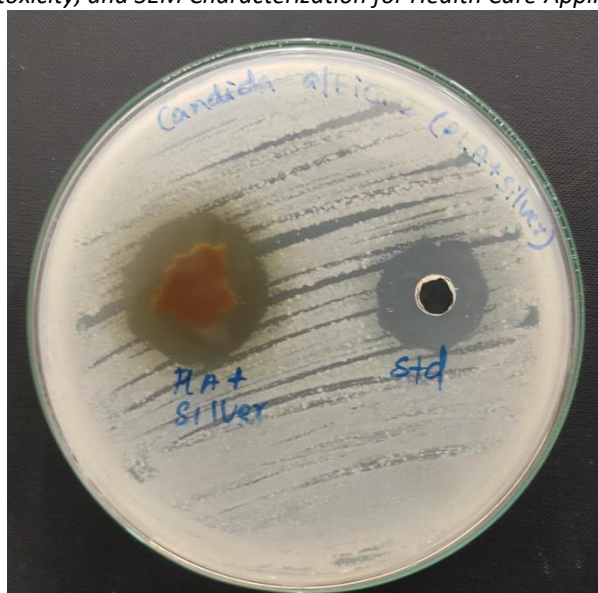
Data Analysis: Data obtained from the antibacterial, XRD, SEM, and cytotoxicity assays were statistically analysed to determine the significance of the results. The mean and standard deviation were calculated for each set of data, and comparisons were made using appropriate statistical tests.

Results

Disc Diffusion Method:

- **E. coli:** The treated fabric showed a 12 mm zone of inhibition.
- **Staphylococcus aureus:** The treated fabric showed an 11 mm zone of inhibition.
- **Candida albicans:** The treated fabric showed a 13 mm zone of inhibition.





(c)

Figure 1. Antibacterial activity and antifungal activity of PLA+ Silver treated fabric extracts (a) The treated fabric showed a 12 mm zone of inhibition with E.Coli, (b) The treated fabric showed an 11 mm zone of inhibition with Staphylococcus aureus, and (c) The treated fabric showed a 13 mm zone of inhibition with Candida albicans.

Agar Diffusion Method:

- The stock cultures of bacteria were inoculated in nutrient broth media and grown at 37°C for 18 hours.
- The agar plates were inoculated with the 18-hour old cultures of bacteria.
- Fabric pieces were placed inside the plates and incubated at 37°C for 24 hours.

- The diameter of the inhibition zones for each organism was recorded as follows:

- **E. coli:** 12 mm (Figure 1a).
- **Staphylococcus aureus:** 11 mm (Figure 1b).
- **Candida albicans:** 13 mm (Figure 1c).

The results indicate that the PLA and silver nanoparticle-treated fabrics exhibit significant antimicrobial activity against both bacterial and fungal pathogens.

Table 1. Antimicrobial activity of PLA+ Silver treated cloth, Standard Chloramphenicol Fugues- Fluconazole

Organisms Concentration	<i>E.Coli</i>	<i>S. aureus</i>	<i>Candida albicans</i>
PLA+ Silver treated cloth	12mm	11 mm	13 mm
Standard Chloramphenicol Fugues- Fluconazole	10 cm	10 cm	9 mm

The give sample shows the Antimicrobial activity against the Pathogenic Bacteria *E.Coli* and *Staphylococcus aureus* The Fungus *Candida albicans*. The given **PLA+ Silver treated cloth** shows shows good microbial activity depending upon the size of the zone (Table 1).

XRD

XRD analysis was performed to determine the crystalline structure of the PLA and silver nanoparticle composites. The XRD patterns showed distinct peaks corresponding to the crystalline phases of silver, indicating successful incorporation of silver nanoparticles into the PLA matrix (Figure 2).

Peak List:

- **27.9226° 2θ:** Height 75.29 cts, FWHM Left 0.4015° 2θ, d-spacing 3.19538 Å, Rel. Int. 11.80%

- **32.4790° 2θ:** Height 158.99 cts, FWHM Left 0.3346° 2θ, d-spacing 2.75676 Å, Rel. Int. 24.91%
- **38.1185° 2θ:** Height 405.07 cts, FWHM Left 0.2007° 2θ, d-spacing 2.36088 Å, Rel. Int. 63.47%
- **38.3790° 2θ:** Height 638.18 cts, FWHM Left 0.2007° 2θ, d-spacing 2.34546 Å, Rel. Int. 100.00%
- **44.4788° 2θ:** Height 224.26 cts, FWHM Left 0.4015° 2θ, d-spacing 2.03694 Å, Rel. Int. 35.14%
- **46.3990° 2θ:** Height 85.41 cts, FWHM Left 0.4684° 2θ, d-spacing 1.95702 Å, Rel. Int. 13.38%
- **55.0030° 2θ:** Height 33.03 cts, FWHM Left 0.5353° 2θ, d-spacing 1.66952 Å, Rel. Int. 5.18%
- **57.5750° 2θ:** Height 30.04 cts, FWHM Left 0.5353° 2θ, d-spacing 1.60091 Å, Rel. Int. 4.71%
- **64.7259° 2θ:** Height 143.11 cts, FWHM Left 0.4684° 2θ, d-spacing 1.44025 Å, Rel. Int. 22.43%

- **77.5994° 2θ**: Height 166.50 cts, FWHM Left 0.4015° 2θ, d-spacing 1.23035 Å, Rel. Int. 26.09%

These peaks correspond to the crystalline nature of silver, confirming its successful incorporation within the PLA matrix (Table 2).

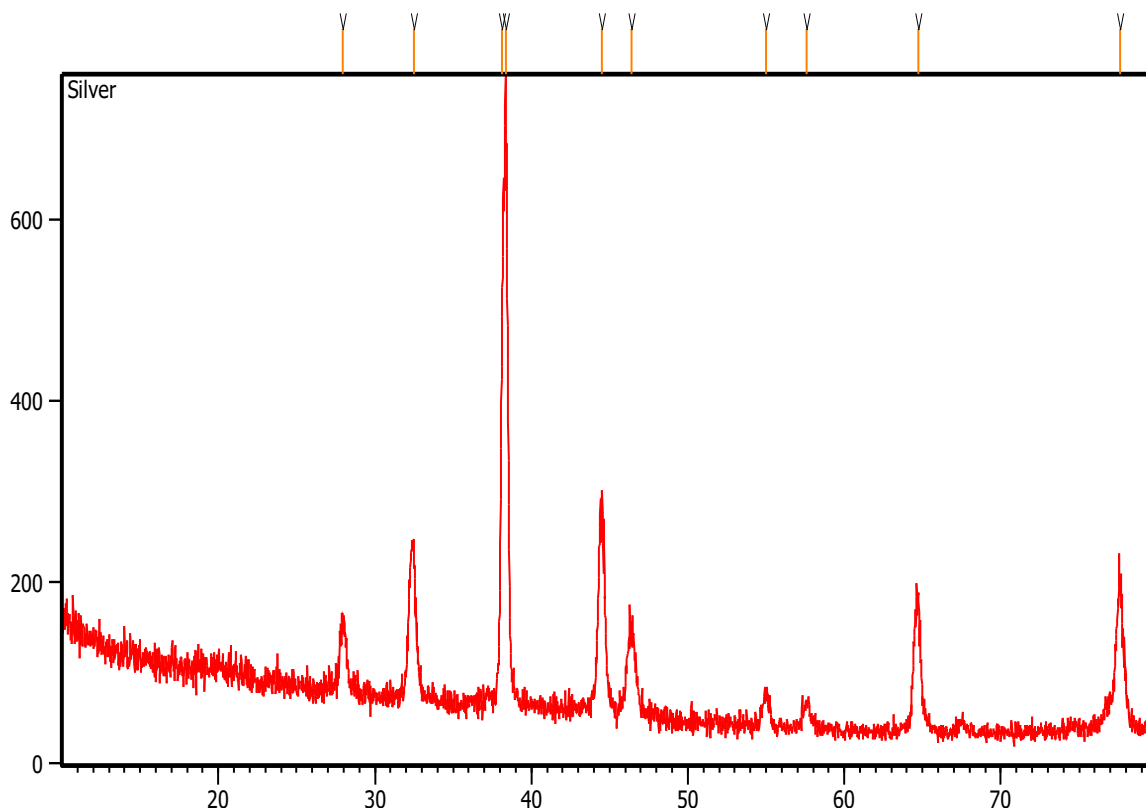


Figure 2. XRD analysis Peak results

Table 2. Peak List

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.9226	75.29	0.4015	3.19538	11.80
32.4790	158.99	0.3346	2.75676	24.91
38.1185	405.07	0.2007	2.36088	63.47
38.3790	638.18	0.2007	2.34546	100.00
44.4788	224.26	0.4015	2.03694	35.14
46.3990	85.41	0.4684	1.95702	13.38
55.0030	33.03	0.5353	1.66952	5.18
57.5750	30.04	0.5353	1.60091	4.71
64.7259	143.11	0.4684	1.44025	22.43
77.5994	166.50	0.4015	1.23035	26.09

SEM Analysis:

SEM images were obtained to observe the surface morphology and size distribution of silver nanoparticles in the PLA matrix. The SEM analysis revealed well-defined, uniformly distributed nanoparticles with an average size of approximately 500 nm.

Observations:

- The particles displayed irregular shapes but maintained chemical homogeneity.

- The uniform morphology observed is due to the presence of interparticle surface connectivity. These morphological characteristics suggest effective dispersion of silver nanoparticles throughout the PLA matrix, which is essential for their antimicrobial efficacy.

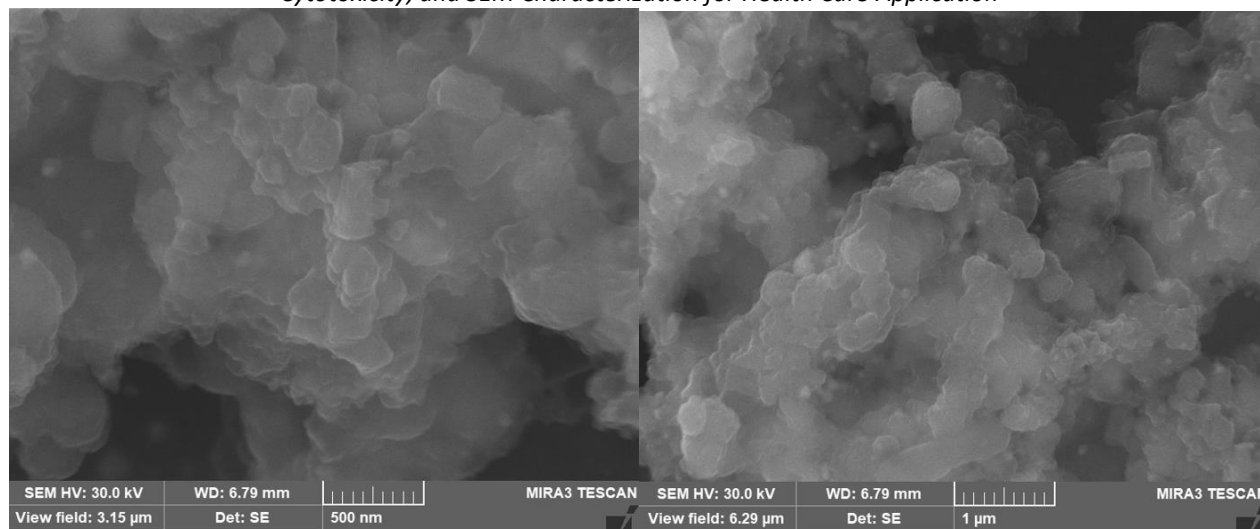


Figure 3. SEM images of nanoparticles

Figure 3 shows the SEM images of nanoparticles at different magnification, which clearly exhibit the nanoparticles like morphology indicate well uniform particles with narrow size distribution lies in the range 6.79 mm of 500 nm and magnification range is 30 kx. Particles seem to have an irregular shape with chemical homogeneity with uniform morphology due to the presence of interparticle surface connectivity

Cytotoxicity report:

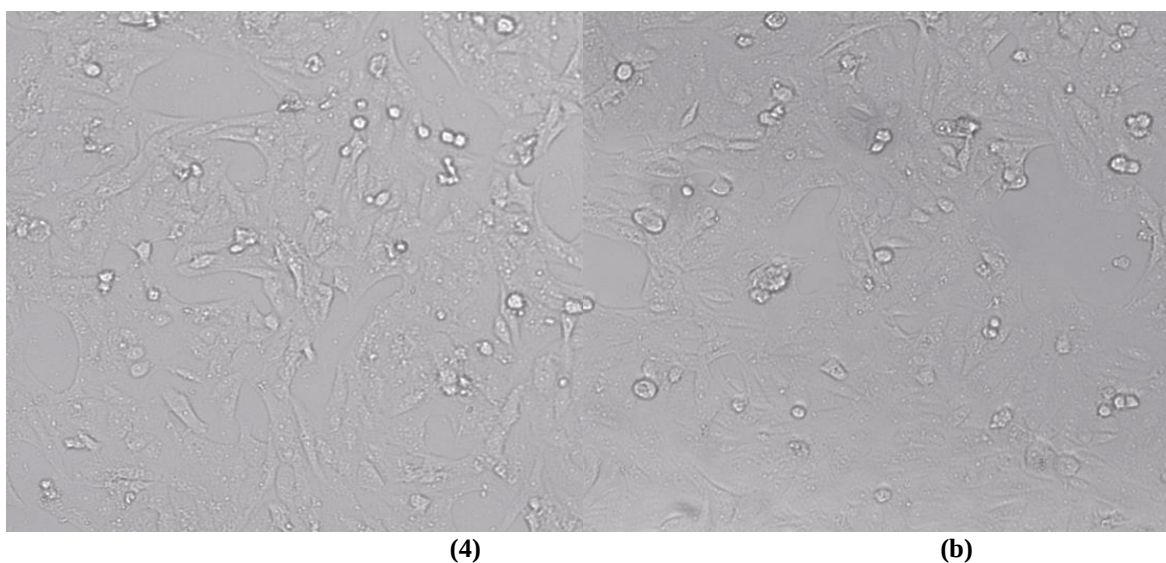
MTT assay procedure

The cytotoxicity of the PLA and silver nanoparticle composites was assessed using the MTT assay with Vero cell lines. The assay measured cell viability after 24 hours of exposure to different concentrations of the composite materials.

Cell Viability Results:

- Untreated Sample: 100% viability
- Sample C - 12.5 μg/ml: 98.66% viability
- Sample C - 25 μg/ml: 97.23% viability
- Sample C - 50 μg/ml: 96.20% viability
- Sample C - 100 μg/ml: 94.88% viability

The results show that the PLA and silver nanoparticle composites exhibit minimal cytotoxicity, with cell viability remaining above 94.88% even at the highest tested concentration of 100 μg/ml. This indicates that the composites are biocompatible and suitable for potential biomedical applications.



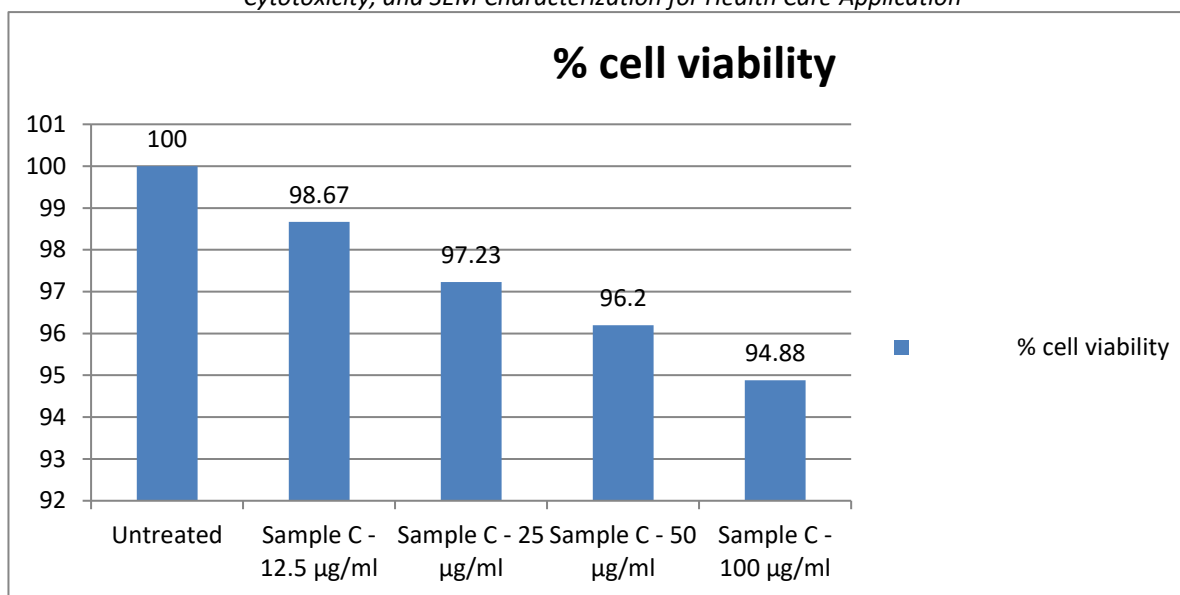


Figure 4 Cytotoxicity (a) Control, (b) 100 µg/ml and (c) % of cell viability.

The given extract Shows no toxicity for the Human Vero cells. The Higher concentration of the 100 µg/ml of PLA+ Silver extract shows 94.88 % of cell viability as shown in Figure 4c.

Discussion

The integration of silver nanoparticles (AgNPs) into biodegradable polymers such as polylactic acid (PLA) and chitosan has shown promise in enhancing antimicrobial efficacy while maintaining biocompatibility and environmental sustainability. This study aimed to comprehensively analyze the antibacterial activity, X-ray diffraction (XRD) analysis, cytotoxicity, and scanning electron microscopy (SEM) characterization of these composites. The results demonstrated significant antimicrobial activity, successful incorporation of AgNPs, uniform particle distribution, and minimal cytotoxicity, indicating the potential of these materials for various applications in healthcare and beyond. The antimicrobial efficacy of the PLA and silver nanoparticle-treated fabrics was evaluated against common pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*) and the fungus *Candida albicans*. The disc diffusion method and agar diffusion method were employed to assess the inhibition zones.

The results from the disc diffusion method revealed that the treated fabric exhibited considerable antimicrobial activity. Specifically, inhibition zones of 12 mm for *E. coli*, 11 mm for *Staphylococcus aureus*, and 13 mm for *Candida albicans* were observed. These findings indicate that the incorporation of silver nanoparticles significantly enhances the antimicrobial properties of PLA, making it effective against a broad spectrum of pathogens.

The agar diffusion method corroborated these results, showing similar inhibition zone diameters. This consistency across different methods reinforces the reliability of the antimicrobial activity observed. The substantial inhibition zones suggest that the silver nanoparticles effectively disrupt bacterial and fungal cell walls, leading to cell death. These results align with

previous studies that have demonstrated the potent antimicrobial properties of silver nanoparticles due to their ability to release silver ions, which interact with microbial cell membranes and intracellular components [1].

The enhanced antimicrobial activity observed in this study highlights the potential of PLA and silver nanoparticle composites in applications where antimicrobial properties are crucial, such as in medical devices, wound dressings, and food packaging. The effectiveness against both bacteria and fungi broaden the scope of potential applications, making these materials versatile and highly functional.

XRD analysis was employed to investigate the crystalline structure of the PLA and silver nanoparticle composites. The XRD patterns exhibited distinct peaks corresponding to the crystalline phases of silver, confirming the successful incorporation of AgNPs into the PLA matrix.

The prominent peaks observed at 27.92°, 32.48°, 38.12°, 38.38°, and 44.48° 2θ values are characteristic of silver nanoparticles. These peaks indicate the presence of crystalline silver within the composite material, which is essential for maintaining the antimicrobial efficacy of the nanoparticles. The sharp and well-defined peaks suggest a high degree of crystallinity, which is beneficial for the stability and durability of the composites.

The presence of crystalline silver within the PLA matrix is crucial for its antimicrobial activity. The crystalline structure facilitates the sustained release of silver ions, which are responsible for the antimicrobial effects. The XRD analysis provides valuable insights into the structural properties of the composites, which directly influence their functional performance.

SEM analysis was conducted to observe the surface morphology and size distribution of the silver nanoparticles within the PLA matrix. The SEM images revealed well-defined, uniformly distributed nanoparticles with an average size of approximately

500 nm. The particles displayed irregular shapes but maintained chemical homogeneity and uniform morphology.

The uniform distribution of silver nanoparticles is critical for ensuring consistent antimicrobial activity throughout the composite material. The irregular shapes of the nanoparticles may enhance their interaction with microbial cell membranes, thereby improving their antimicrobial efficacy. The presence of interparticle surface connectivity observed in the SEM images suggests that the nanoparticles are well-integrated within the PLA matrix, which is essential for maintaining the structural integrity and functional performance of the composites.

The SEM characterization highlights the importance of nanoparticle dispersion in achieving optimal antimicrobial properties. The well-dispersed nanoparticles ensure that a maximum surface area is available for interaction with microbial cells, thereby enhancing the overall efficacy of the composites.

The cytotoxicity of the PLA and silver nanoparticle composites was evaluated using the MTT assay with Vero cell lines. The assay results indicated that the composites exhibited minimal cytotoxicity, with cell viability remaining above 94.88% even at the highest tested concentration of 100 µg/ml.

The high cell viability observed in this study suggests that the PLA and silver nanoparticle composites are biocompatible and safe for potential biomedical applications. The minimal cytotoxicity is particularly important for applications involving direct contact with human tissues, such as in wound dressings and medical implants.

The MTT assay results align with previous studies that have reported the biocompatibility of silver nanoparticles at low concentrations [9]. The high cell viability observed in this study further supports the potential of these composites for safe and effective use in various biomedical applications.

The minimal cytotoxicity observed in this study is attributed to the controlled release of silver ions from the nanoparticles. The slow and sustained release of silver ions ensures that effective antimicrobial concentrations are maintained without causing significant harm to human cells. This balance between antimicrobial efficacy and biocompatibility is crucial for the successful application of these composites in healthcare settings.

The results of this study have significant implications for the development of antimicrobial materials for healthcare applications. The enhanced antimicrobial activity, confirmed by the disc diffusion and agar diffusion methods, suggests that PLA and silver nanoparticle composites can effectively prevent and control infections. This is particularly relevant in the context of increasing antibiotic resistance, where alternative antimicrobial strategies are urgently needed.

The successful incorporation of silver nanoparticles, as demonstrated by XRD analysis, ensures that the antimicrobial properties of the composites are maintained over time. The high degree of crystallinity observed in the XRD patterns indicates that the composites are stable and durable, making them suitable for long-term applications.

The SEM characterization highlights the importance of nanoparticle dispersion in achieving optimal antimicrobial efficacy. The well-dispersed nanoparticles ensure that the

composites provide consistent and reliable antimicrobial protection, which is essential for preventing infections in clinical settings.

The minimal cytotoxicity observed in the MTT assay supports the safe use of these composites in biomedical applications. The high cell viability indicates that the composites are biocompatible and can be used safely in contact with human tissues. This is particularly important for applications such as wound dressings, where prolonged contact with human skin is required.

Future Research Directions

While the results of this study are promising, further research is needed to fully understand the potential and limitations of PLA and silver nanoparticle composites. Future studies should focus on the following areas:

- **Long-term Stability and Efficacy:** Investigate the long-term stability and antimicrobial efficacy of the composites under various environmental conditions to ensure their durability and effectiveness in real-world applications.
- **Mechanisms of Action:** Explore the mechanisms of antimicrobial action of the silver nanoparticles to better understand how they interact with microbial cells and how their efficacy can be optimized.
- **Biocompatibility and Safety:** Conduct in vivo studies to assess the biocompatibility and safety of the composites in animal models. This will provide valuable insights into their potential for use in human healthcare applications.
- **Application-Specific Optimization:** Optimize the formulation and processing conditions of the composites for specific applications, such as wound dressings, medical implants, and food packaging. This will ensure that the composites meet the specific requirements of each application.
- **Regulatory Compliance:** Evaluate the composites for compliance with relevant regulatory standards and guidelines to facilitate their commercialization and use in healthcare settings.

Conclusion

This study demonstrates the significant antimicrobial efficacy, structural integrity, and biocompatibility of PLA and silver nanoparticle composites. The results indicate that these composites have the potential to be used in various healthcare applications where antimicrobial properties are essential. Further research and development are needed to fully realize their potential and to optimize their performance for specific applications. The findings of this study contribute to the growing body of knowledge on the use of silver nanoparticles in biodegradable polymers and highlight their potential for addressing the challenges of antimicrobial resistance and infection control.

Author Contributions:

Funding:

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Conflicts of Interest: The authors declare no conflict of interest.

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