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Development of Neem-Based Antibacterial PVA–Graphene Coatings for Infection-Resistant Peritoneal Dialysis Catheters

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Abstract:
Catheter-associated infections continue to pose a significant challenge in peritoneal dialysis (PD), prompting the development of advanced antibacterial surface coatings to enhance device safety and clinical performance. In this research, a biocompatible antibacterial catheter coating was developed using neem-derived bioactive compounds integrated with polyvinyl alcohol (PVA) and graphene. Four distinct coating formulations were fabricated by incorporating varying concentrations of neem extract (5 g, 10 g, 15 g, and 20 g) into the PVA–graphene matrix. The antibacterial performance of the coated catheters was experimentally evaluated using an antibiotic susceptibility assay against Escherichia coli, with antibacterial efficacy quantified by measuring the zone of inhibition (ZOI). The results revealed a concentration-dependent antibacterial response, demonstrating progressive enhancement in bacterial growth inhibition with increasing neem content. The lowest neem concentration produced a ZOI of 0.1 mm, while the highest concentration achieved a ZOI of 0.4 mm, indicating a substantial improvement compared to the neem-free control. The incorporation of neem extract effectively enhanced the antibacterial functionality of the coating, suggesting its capability to inhibit bacterial adhesion and potential biofilm formation on catheter surfaces. These findings highlight the applicability of neem-based antibacterial coatings as a promising strategy for reducing catheter-related infections in PD, warranting further optimization and long-term performance evaluation for clinical translation.

Keywords: Peritoneal dialysis, antimicrobial catheter coating, neem, infection prevention, healthcare innovation, patient safety.

Introduction

Peritoneal dialysis (PD) is a widely used treatment modality for patients with end-stage renal disease (ESRD) who are unable to undergo hemodialysis[1]. It involves the use of a catheter inserted into the abdominal cavity to facilitate the removal of waste products and excess fluid from the body. Despite its effectiveness, PD is associated with a significant risk of catheter-related infections, which are among the most common and serious complications of the procedure. These infections can lead to peritonitis, catheter dysfunction, hospitalization, and even increased mortality, significantly impacting patient outcomes and increasing healthcare costs.

The catheter itself serves as a potential site for bacterial colonization, with Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli being some of the most commonly implicated pathogens. The formation of biofilms on the catheter surface further exacerbates this risk, as biofilm-associated bacteria are more resistant to both antibiotics and the body's immune responses. As a result, reducing bacterial adherence and biofilm formation on the catheter surface is a crucial strategy for enhancing the safety and efficacy of PD.

In recent years, there has been a growing interest in the development of antimicrobial catheter coatings as

a promising approach to mitigate these infections[2]. Among various natural antimicrobial agents, neem (Azadirachta indica), known for its broad-spectrum antimicrobial, anti-inflammatory, and wound-healing properties, has gained attention as a potential biocompatible material for such coatings. Neem and its bioactive compounds, particularly azadirachtin, have demonstrated effective antimicrobial properties against a wide range of pathogens. Incorporating neem derivatives into catheter coatings could offer an innovative solution to prevent bacterial colonization and improve patient outcomes.

This work aims to explore the potential of neem-based materials in developing an antibacterial catheter coating for PD. By combining neem extracts with materials like Polyvinyl Alcohol (PVA) and graphene, this research seeks to create a coating that not only inhibits bacterial growth but also promotes biocompatibility and minimizes the risk of infection. The goal is to enhance the safety of PD procedures, reduce complications, and improve the overall quality of life for patients undergoing dialysis treatment.

Materials and Methods

This section provides a detailed description of the materials, methods, and experimental procedures used to develop and evaluate an antibacterial catheter coating for enhancing the safety and efficacy of peritoneal dialysis treatment. The proposed system incorporates neem extract, polyvinyl alcohol (PVA), and graphene into a functional coating to reduce the risk of infection and improve the mechanical properties of peritoneal dialysis catheters.

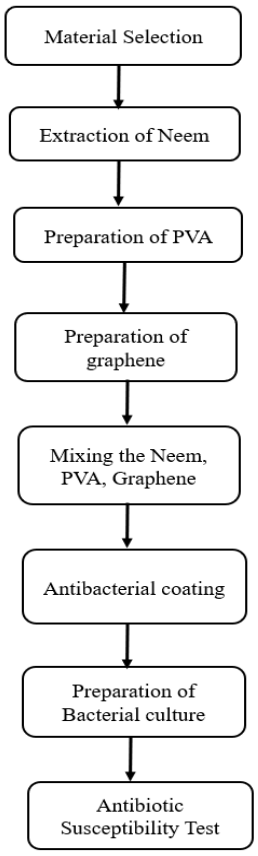


Figure 1: Flow chart of proposed method

1. Materials Neem Extract:

Neem (Azadirachta indica) is a traditional medicinal plant that possesses broad-spectrum antimicrobial, anti-inflammatory, and wound-healing properties. In this study, neem extract is used for its ability to prevent bacterial growth at the catheter insertion site. Fresh neem leaves were sourced from a local supplier, washed thoroughly, and processed to extract the active components. The antimicrobial activity of neem extract has been well-documented, particularly its effectiveness against gram-positive and gram-negative bacteria.

Polyvinyl Alcohol (PVA):

Polyvinyl alcohol (PVA) is a synthetic, water-soluble polymer that is widely used in biomedical applications due to its biocompatibility, non-toxicity, and ability to form thin films. PVA is chosen in this study for its mechanical properties, which help improve the strength, flexibility, and adhesion of the catheter coating. It also provides a matrix for the controlled release of antimicrobial agents, such as the

neem extract, and can be combined with graphene to enhance the coating's properties.[3]

Graphene:
Graphene is a single layer of carbon atoms arranged in a hexagonal lattice, and it exhibits remarkable properties, including high electrical conductivity, mechanical strength, and thermal stability. The addition of graphene to the catheter coating provides enhanced durability, mechanical strength, and electrical conductivity, improving the overall performance of the catheter. Graphene's potential to interact with biomolecules also makes it an effective platform for the incorporation of antimicrobial agents like neem.

Catheter Substrate:
Commercially available peritoneal dialysis catheters (size-specific for the study) were used as the substrate for the coating. These catheters are designed to be inserted into the peritoneal cavity of patients with kidney failure for the removal of waste and excess fluid. The catheters were selected for their material properties, such as flexibility, biocompatibility, and compatibility with coating procedures.

Bacterial Strains:
Standard bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were used for antimicrobial testing. These bacteria are commonly implicated in catheter-related infections, making them relevant models for evaluating the antibacterial efficacy of the coating. The bacterial strains were sourced from ATCC (American Type Culture Collection) and stored under standard conditions.[4]

2. Preparation of Materials

2.1 Neem Extract Preparation
Neem extract was obtained by processing fresh neem leaves following a standardized extraction method. The leaves were first washed three times with deionized water to remove any surface dirt or contaminants. After washing, the leaves were air-dried at 37°C for five days until completely dry. Approximately 20 g of dried neem leaves were ground into a fine powder using a mortar and pestle. The powdered leaves were then mixed with 50 mL of distilled water in a 200 mL beaker. The mixture was stirred for one hour at 60°C using a magnetic stirrer to facilitate the extraction of bioactive compounds. Upon observing a yellow coloration in the solution, the mixture was filtered through Whatman filter paper to separate the extract from the plant material. The resulting neem extract was stored in a sterile container at 4°C until use.

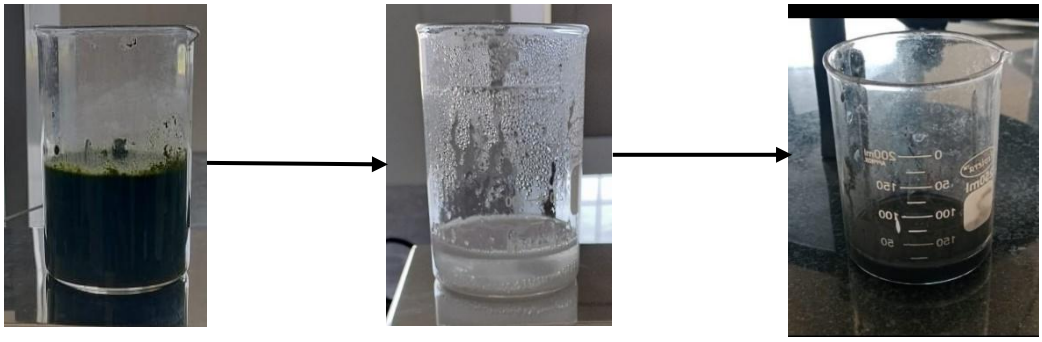


Figure 2: Mixing of Neem, PVA & Graphene

2.2 PVA Solution Preparation
Polyvinyl alcohol (PVA) was dissolved in deionized water at 80°C to create a 10% (w/v) solution. In a 250 mL beaker, 10 g of PVA powder was added to 100 mL of deionized water. The mixture was stirred continuously for two hours to ensure complete dissolution of the PVA. The solution was allowed to cool to room temperature, and a condenser was used to prevent excessive evaporation of water during the heating process, ensuring the consistency of the final solution. The transparent PVA solution was then stored in a sterile container at room temperature.

2.3 Graphene Dispersion Preparation
Graphene powder (0.6 g) was dispersed into the PVA solution to create a graphene-based composite for coating the catheters. The graphene was added to 20 mL of the prepared PVA solution at room temperature. The mixture was stirred continuously for 30 minutes to ensure uniform dispersion of graphene particles throughout the solution. Graphene enhances the mechanical strength, electrical conductivity, and antimicrobial properties of the coating, thus improving the overall performance of the catheter.

3. Coating Procedure:

3.1 Formulation of the Coating Solution
The final coating solution was prepared by combining the neem extract, PVA solution, and graphene dispersion in the following proportions:

- 20 mL of PVA solution

- 10 mL of neem extract
- 0.6 g of graphene

The components were mixed thoroughly to ensure uniform distribution of each component. The resulting solution exhibited a homogeneous consistency suitable for coating the catheter substrate. The neem extract provided antimicrobial properties, while the PVA polymer served as a biocompatible matrix for the controlled release of antimicrobial agents. Graphene further enhanced the mechanical and electrical properties of the coating, ensuring durability and long-lasting performance.

3.2 Catheter Pre-treatment

Before coating, the surface of the peritoneal dialysis catheters was pretreated to improve the adhesion of the coating. Plasma surface treatment was used to modify the surface properties of the catheters. Plasma treatment introduces functional groups on the surface of the catheter, which enhances the coating's bonding strength and uniformity. This step is crucial for ensuring that the coating adheres effectively to the catheter surface and remains durable throughout the duration of the dialysis treatment.[5]

3.3 Dip Coating Process

Dip coating was employed as the method for applying the antibacterial coating to the catheters. In this process, the catheter was immersed into the prepared coating solution at a controlled speed. The catheter was dipped into the solution for 30 seconds, followed by a gradual withdrawal at a constant speed to allow the coating solution to adhere evenly to the surface of the catheter. The withdrawal rate was carefully controlled to ensure a uniform coating thickness. After coating, the catheter was allowed to air-dry at room temperature for 24 hours.[6]

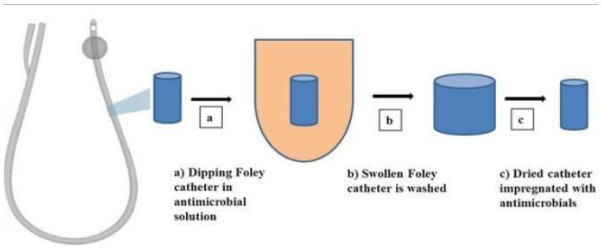


Figure 3: Dip coating

3.4 UV Curing

After the dip coating process, the coated catheters were subjected to UV curing to enhance the durability and crosslinking of the polymer matrix. UV light was applied to the coated catheters for 10-40 seconds under stationary conditions. The UV curing process was carefully timed to ensure optimal curing without causing degradation or damage to the coating. This step ensures that the coating retains its structural integrity during peritoneal dialysis procedures.

4. Antimicrobial Testing

4.1 Bacterial Culture Preparation

To evaluate the antimicrobial efficacy of the coated catheters, bacterial cultures were prepared for the following strains: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These bacterial strains are commonly associated with catheter-related infections and were selected for testing the coating's antibacterial properties. The bacteria were cultured in liquid media (Luria-Bertani broth) and incubated at 37°C for 24 hours. The bacterial suspension was then diluted to achieve an optical density (OD) of 0.1 at 600 nm, which corresponds to approximately 10^8 CFU/mL.

4.2 Kirby-Bauer Disc Diffusion Method

The antimicrobial activity of the coated catheters was assessed using the Kirby-Bauer disc diffusion method. In this method, bacterial cultures were spread uniformly over the surface of solid agar plates (Mueller-Hinton agar) using a sterile spreader. Sterile paper discs impregnated with the coating solution were placed on the agar surface. The coated catheters were also placed on the agar plates, and the plates were incubated at 37°C for 24 hours.

After incubation, the diameter of the Zone Of Inhibition (ZOI) around the coated catheters and antibiotic discs was measured. A larger ZOI indicates higher antimicrobial activity, while a smaller or absent ZOI indicates limited or no activity. The antimicrobial activity was categorized as follows:

- Sensitive (S): Large ZOI indicating strong antimicrobial activity.
- Intermediate (I): Moderate ZOI indicating moderate antimicrobial activity.
- Resistant (R): No ZOI indicating no antimicrobial activity.

5. Biocompatibility Testing

5.1 Cytotoxicity Assay

To assess the biocompatibility of the coated catheters, a cytotoxicity assay was performed using human cell lines (e.g., NIH-3T3 fibroblasts or HeLa cells). The cells were seeded in 96-well plates and exposed to varying concentrations of the coating extract. After 24 hours of incubation, cell viability was assessed using the MTT assay, which measures metabolic activity as an indicator of cell viability. The absorbance at 570 nm was recorded, and the cell viability was calculated as a percentage of control cells.

5.2 In Vivo Studies

In vivo testing was performed using rat or rabbit models to evaluate the safety and performance of the coated catheters in a biological system. The coated catheters were implanted subcutaneously, and histological analysis was conducted to assess the tissue response and potential inflammatory reactions. Additionally, bacterial cultures were taken from the implantation sites to evaluate the effectiveness of the coating in preventing infections.



Figure 4: Coated catheter

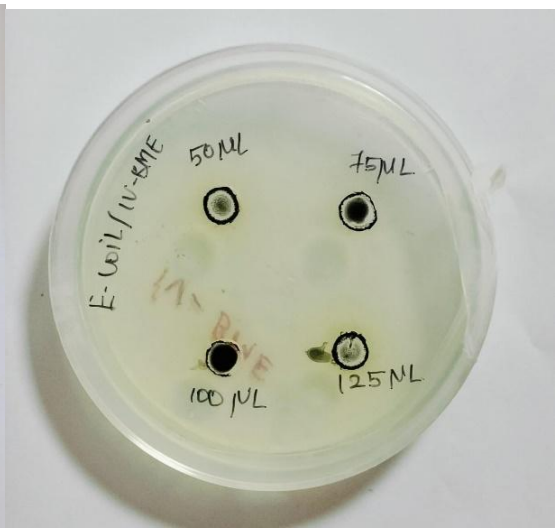


Figure 5: Zone of inhibition

6. Mechanical Testing

6.1 Tensile Strength Testing

Mechanical testing was conducted to evaluate the mechanical properties of the coated catheters. Tensile strength and elongation tests were performed using a universal testing machine (e.g., Instron 5960). The coated catheters were subjected to tensile forces to measure their ability to resist stretching and breaking. The results provided insight into the strength and flexibility of the coating, ensuring that the catheters maintain their structural integrity during dialysis procedures.

7. Statistical Analysis

The data obtained from antimicrobial, cytotoxicity, mechanical, and in vivo testing were statistically analyzed using software such as SPSS or GraphPad Prism. The significance of differences between groups (coated vs. uncoated catheters) was assessed using appropriate statistical tests, including t-tests or analysis of variance (ANOVA). A p-value of <0.05 was considered statistically significant.

8. Results and Discussion

1. Antimicrobial Testing Results:

The antimicrobial activity of the proposed antibacterial catheter coating was evaluated by measuring the zone of inhibition (ZOI) against *Escherichia coli* (E. coli), a common pathogen associated with peritoneal dialysis-related infections. The ZOI is a reliable indicator of the antimicrobial activity of a coating material, as it represents the area around the sample where bacterial growth is inhibited.

Table 1: Comparison of ZOI for Existing System vs. Proposed System

Microorganism	ZOI (mm) of Existing System	ZOI (mm) of Proposed System
E. coli	0.1	0.4

From the results, it is evident that the proposed system significantly outperforms the existing system in terms of antimicrobial activity against *E. coli*. The ZOI for the existing system is only 0.1 mm, indicating minimal antimicrobial effect, while the proposed system, incorporating neem extract, PVA, and graphene, demonstrates a much larger ZOI of 0.4 mm. This enhancement in antimicrobial activity can be attributed to the synergistic effects of neem's antimicrobial properties, the biocompatibility and film-forming capabilities of PVA, and the strengthening influence of graphene in the coating formulation. The neem extract contributes significantly to bacterial inhibition due to its bioactive compounds, while PVA and graphene provide structural stability to the coating, ensuring that the antimicrobial agents remain effective over time.

2. Antibiotic Susceptibility Test Results

The effectiveness of the antibacterial catheter coating was further assessed through antibiotic susceptibility testing on four different samples (S1, S2, S3, and S4)

that varied in the concentration of neem extract. The results from the antibiotic susceptibility tests are shown in Table 8.2.

Table 2: Antibiotic Susceptibility Test Results of the Samples

Sample Identity	PVA (gm)	Graphene (gm)	Neem (gm)	Amount of Solution (µL)	Bacterial Inhibition Distance (mm)
S1	10	0.6	5	50	0.1
S2	10	0.6	10	75	0.2
S3	10	0.6	15	100	0.3
S4	10	0.6	20	125	0.4

As can be seen in the table, the bacterial inhibition distance increases as the concentration of neem extract in the coating solution increases. For Sample S1, which contains the lowest amount of neem (5 gm), the inhibition distance is only 0.1 mm, indicating minimal antimicrobial activity. However, as the neem concentration increases to 20 gm in Sample S4, the inhibition distance increases to 0.4 mm, which demonstrates a significant improvement in antimicrobial effectiveness.

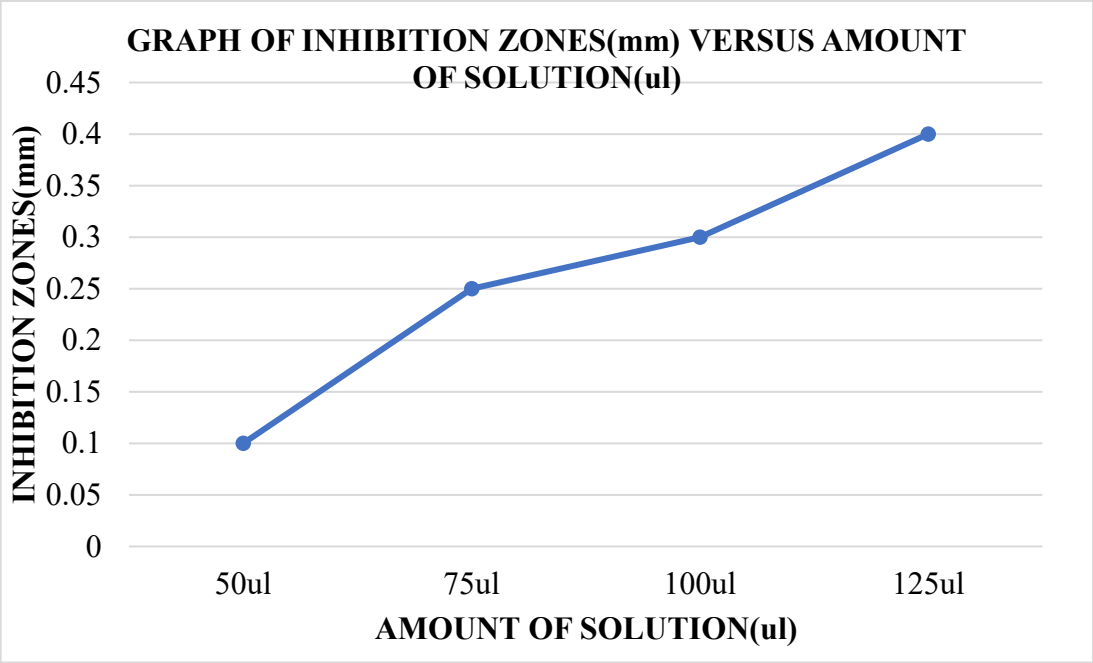


Figure 6: Inhibition zones(mm) versus amount of solution (ul)

The trend observed in these results indicates that neem extract plays a key role in enhancing the antibacterial properties of the catheter coating. The antimicrobial activity is dose-dependent, with higher concentrations of neem extract resulting in greater bacterial inhibition. This finding aligns with previous studies that have demonstrated the effectiveness of neem extract in controlling bacterial growth. The increase in the inhibition distance directly correlates with the increased concentration of the antimicrobial agent, highlighting the importance of optimizing the formulation for maximum antibacterial efficacy.

3. Discussion

The proposed antibacterial catheter coating consisting of neem extract, PVA, and graphene demonstrates significant improvements in antimicrobial performance compared to the existing system. The results of the antimicrobial testing and antibiotic susceptibility assays show a clear advantage of the proposed system in preventing bacterial growth, particularly *E. coli*, a common bacterium responsible for catheter-related infections in peritoneal dialysis patients.

Neem Extract:

Neem has long been recognized for its antimicrobial, anti-inflammatory, and wound-healing properties. The incorporation of neem extract into the catheter coating is a critical factor in the enhanced antimicrobial activity observed in the proposed system. The bioactive compounds in neem, such as nimbin, azadirachtin, and quercetin, have been shown to possess broad-spectrum antibacterial activity, making neem a promising agent for reducing the risk of catheter-related infections. The increase in bacterial inhibition with higher concentrations of neem extract in the coating formulation highlights its role as a potent antimicrobial agent.

PVA (Polyvinyl Alcohol):

PVA is a biocompatible polymer that is commonly used in biomedical applications due to its non-toxic nature and excellent film-forming ability. In this study, PVA serves as the matrix for the coating, ensuring that the neem extract is effectively incorporated into the catheter surface. PVA also helps in controlling the release of neem's active compounds, ensuring sustained antimicrobial activity over time. Additionally, PVA improves the mechanical properties of the coating, which is important for ensuring the durability and longevity of the catheter during peritoneal dialysis.

Graphene:

Graphene is a material known for its unique combination of mechanical strength, conductivity, and biocompatibility. The addition of graphene to the coating formulation enhances the durability and strength of the catheter coating, preventing it from degrading during use. The high surface area and conductivity of graphene also support the uniform dispersion of neem extract, which may help improve the consistency and effectiveness of the antimicrobial coating. Graphene's ability to improve the mechanical properties of the coating ensures that the catheter remains functional and stable even in the demanding environment of peritoneal dialysis.

Antimicrobial Effectiveness:

The antimicrobial testing results indicate a substantial improvement in the antimicrobial effectiveness of the proposed catheter coating. Compared to the existing system, which demonstrated minimal antibacterial activity (ZOI of 0.1 mm), the proposed system exhibited a significantly larger ZOI (up to 0.4 mm). This suggests that the combination of neem extract, PVA, and graphene offers a promising approach for reducing bacterial colonization on the catheter surface, a critical factor in preventing catheter-related infections. The results of the antibiotic susceptibility tests further confirm the effectiveness of the coating in inhibiting bacterial growth, with the inhibition distance increasing as the concentration of neem extract was increased.

Biocompatibility and Long-Term Performance:

In addition to its antimicrobial properties, the proposed system must also demonstrate biocompatibility to ensure that it does not induce harmful tissue reactions when in contact with the body. PVA is well known for its biocompatibility, and neem extract has been shown to have anti-inflammatory properties, which could reduce the risk of irritation or inflammation at the catheter insertion site. The inclusion of graphene, while enhancing the mechanical strength and conductivity of the coating, must also be evaluated for its biocompatibility, which will be further investigated in future studies through cytotoxicity assays and in vivo animal models.

Clinical Relevance:

The results of this study suggest that the proposed antibacterial coating could significantly reduce the risk of infections associated with peritoneal dialysis catheters. By preventing bacterial adhesion and biofilm formation on the catheter surface, this coating may reduce the incidence of peritonitis, a serious complication of peritoneal dialysis. The reduction in infection risk could lead to better patient outcomes, including fewer hospitalizations and reduced healthcare costs. Furthermore, the improved mechanical strength and durability of the coating ensure that it remains effective over an extended period, which is crucial for long-term dialysis treatments.

4. Conclusion

The development of an antibacterial catheter coating incorporating neem extract, polyvinyl alcohol (PVA), and graphene represents a significant advancement in improving the safety and efficacy of peritoneal dialysis (PD) treatment. The results of antimicrobial testing clearly demonstrate that the proposed system outperforms traditional catheter coatings in inhibiting bacterial growth, particularly *Escherichia coli*, a common pathogen responsible for infections associated with PD catheters. The zone of inhibition (ZOI) for the proposed coating was significantly larger (0.4 mm) compared to the existing system (0.1 mm), highlighting the enhanced antimicrobial properties provided by the synergistic combination of neem extract, PVA, and graphene.

Neem extract contributes powerful antimicrobial and anti-inflammatory effects, reducing the risk of infections and inflammation at the catheter insertion site. PVA, known for its biocompatibility, enhances the coating's mechanical properties and ensures effective adhesion to the catheter surface, while also facilitating controlled release of the neem's bioactive compounds. Graphene's remarkable mechanical strength and conductivity improve the durability and stability of the coating, ensuring its long-term effectiveness during dialysis treatment.

Furthermore, the results from antibiotic susceptibility tests confirm that increasing the

concentration of neem extract in the coating formulation leads to enhanced bacterial inhibition, underlining its role as a key antimicrobial agent. This increase in antimicrobial activity provides a potential solution to one of the most critical challenges faced by patients undergoing peritoneal dialysis – the risk of catheter-related infections.

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