Development of Bacteriophage Infused Hydrogel for Treatment of Diabetic Foot Ulcer

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**Abstract:** Diabetic foot ulcers (DFUs) represent a significant complication associated with diabetes, frequently resulting in severe infections and limb amputation. Persistent bacterial infections, particularly those caused by Staphylococcus aureus and Klebsiella pneumoniae, impede the wound healing process and necessitate the exploration of alternative therapeutic strategies. This study centers on the development of a bacteriophage-infused hydrogel as a targeted treatment modality for DFUs. The hydrogel was synthesized utilizing polyacrylamide-sodium alginate through radical polymerization, aimed at enhancing bacterial clearance and promoting wound healing. Bacteriophage enzyme extraction was conducted employing a cold acetone precipitation method, thereby ensuring stability within the hydrogel matrix. Antibacterial efficacy was assessed via an agar well diffusion test, which illustrated a significant inhibition zone against multidrug-resistant bacterial strains. The antioxidant potential was evaluated through DPPH and ABTS assays, revealing strong free radical scavenging activity. Structural and molecular stability was confirmed through Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). The findings underscore the hydrogel's dual antibacterial and antioxidant properties, positioning it as a promising therapeutic option for DFUs. By integrating the specificity of phage therapy with hydrogel-based wound care, this study lays the groundwork for an innovative, targeted, and biocompatible solution for chronic diabetic wounds. Further research and clinical trials are imperative to establish its efficacy in practical applications.

**Keywords:** Bacteriophage Therapy; Hydrogel Wound Dressing; Diabetic Foot Ulcer; Antibacterial Activity; Antioxidant Properties.

# INTRODUCTION

One of the most common complications of diabetes that sends diabetic patients to the hospital is diabetic foot. Numerous pathological problems, including neuropathy, peripheral vascular disease, foot ulceration, and infection with or without osteomyelitis, are associated with it [(Harsha & Subramanian, 2022)](https://paperpile.com/c/o75q0E/5LHl6). Most commonly affecting bacteria is staphylococcus. These complications raise the chance of developing gangrene, which ultimately results in limb amputation.[(Ghanaim et al., 2023)](https://paperpile.com/c/o75q0E/uiFf)The Centers for Disease Control and Prevention (CDC) reports that Egypt ranks ninth globally out of the ten nations with the highest prevalence of diabetes, with 7.5 million diabetic people, 15% of whom have acquired a diabetic foot ulcer (DFU) [(Deepika et al., 2022)](https://paperpile.com/c/o75q0E/AZmcK).People with diabetes may not feel a sharp object in their shoe that could puncture the skin and result in a foot ulcer because sensory neuropathy reduces pain and pressure perception.[(Gao et al., 2021)](https://paperpile.com/c/o75q0E/g724)Peripheral vascular disease is primarily caused by atherosclerosis.DFUs frequently experience recurrent or persistent infections [(Solanki et al., 2022)](https://paperpile.com/c/o75q0E/NcP7F). By targeting particular bacterial populations with bacteriophage therapy, the risk of recurring infections may be decreased [(Chidambaram et al., 2022)](https://paperpile.com/c/o75q0E/U0m0w). Particularly in a diabetic setting, atherosclerotic plaque rupture can cause peripheral arterial thrombosis, which can then immediately result in arterial occlusion and lower limb ischemia, which can then cause DFUs [(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/o75q0E/KYqab)..The natural history of diabetic foot ulcers can be broken down into five stages: normal foot (stage 1), high-risk foot (stage 2), ulcerated foot (stage 3), infected foot (stage 4), and necrotic foot (stage 5). This knowledge is crucial for treating diabetic foot ulcers Castillo et al. [2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10148765/#CR12)) Compared to systemic antibiotics, topical therapies like hydrogels are typically easier to administer and more patient-accepted. Treatment regimen adherence may be enhanced by this simplicity of usage. Hydrogels have been used as a vehicle to enable controlled delivery or administration of biologics such as phages to the target site of interest, including woundsIndividuals with diabetes frequently get neuropathy, or damage to their peripheral nerves [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/o75q0E/zJ181)[(Sushanthi et al., 2021)](https://paperpile.com/c/o75q0E/Ad8H9)[(Harsha et al., 2022)](https://paperpile.com/c/o75q0E/W63vT).hydrogels retain a large amount of water within their matrix, they mimic biological tissues and offer the best conditions for containing proteins, living cells, and other biomolecules, which increases their use in the biomedical area. [(Kim et al., 2021)](https://paperpile.com/c/o75q0E/5vJX) Hydrogels, as their name suggests, are intended to help with autolytic debridement, rehydrate escargot, and hydrate wounds.[(Zhang et al., 2024)](https://paperpile.com/c/o75q0E/ZxB4). The water in hydrogels plays a crucial role in the cooling process and aids in keeping the wound's temperature steady [(Neha et al., 2021)](https://paperpile.com/c/o75q0E/XTiIk)[(Maliael et al., 2021)](https://paperpile.com/c/o75q0E/PbLyY)[(Lakshmi, 2021)](https://paperpile.com/c/o75q0E/H2G4I).The water in hydrogels plays a crucial role in the cooling process and aids in keeping the wound's temperature steady. They are flexible and can absorb a lot of water because of their polymeric networks [(Dharman et al., 2021)](https://paperpile.com/c/o75q0E/lsfrw). The benefits of hydrogel include its soft structure and stimulus reactions. . Hydrogels can be utilised to break down slough on the wound surface and are advised for wounds that are dry to slightly exuding [(Tiwari & Jain, 2023)](https://paperpile.com/c/o75q0E/93Ugd). Hydrogels are useful for burns and other painful wounds because of their noticeable cooling and relaxing impact on the skin. Poor mechanical strength, sluggish stability, and rapid deterioration[(Ubaidi & Sumedi, 2023)](https://paperpile.com/c/o75q0E/9GVY)Those who have these chronic, non-healing lesions may develop gas gangrene, an uncommon side effect of diabetic foot ulcers [(Graf et al., 2023)](https://paperpile.com/c/o75q0E/Ia4ZJ). Diabetes mellitus (DM) modifies the mechanisms involved in wound closure, starting with decreased fibrinolysis and an unbalanced cytokine production. In this study, phage resistant bacteria can be eliminated by other phages from the same host due to genetic superiority[(Kifelew et al., 2020)](https://paperpile.com/c/o75q0E/YZ38)Bacteriophages represent a potential solution to this problem. Naturally occurring, bacteriophages infect their target bacteria in a targeted fashion and many can disrupt bacterial biofilms.[(Lee et al., 2023)](https://paperpile.com/c/o75q0E/2Jkc)Phage treatment, which is used to fight and aid in the removal of bacterial infections ranging from skin infections and chronic infections to gastrointestinal diseases, is the primary use of bacteriophages [(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/o75q0E/s6qRW).When treating illnesses that don't respond to antibiotics, phage therapy may be quite helpful.Phage therapy has been successfully used, for instance, to treat the potent staphylococcus bacterial infection known as MRSA disease[(Liu et al., 2024)](https://paperpile.com/c/o75q0E/pkCl).A wide range of bacterial illnesses can be treated using macrophages.Because of its extreme specificity, the cleavage range of bacteriophages is excessively limitedIn 82% of cases, phage treatment proved effective, and in 62% of cases, antibiotic treatment proved effective. Fever, flushing, hypotension, and inflammation are among the side effects of phage therapy.Ensuring the phage biostability and the physical stability of the formulation (solution, suspension, gel, or powder) present a twin challenge in the phage formulation process [(Katyal et al., 2021)](https://paperpile.com/c/o75q0E/NkqGb). Phages, like other proteins, are only partially stable in solution. Phages can be thought of as giant protein complexes encapsulating genetic materials (DNA or RNA) [(Jabin et al., 2021)](https://paperpile.com/c/o75q0E/k6D4U). Naturally, techniques for protein stabilisation must be taken into account; in fact, they have been used in the development of phage treatments.Phage therapy was a promising strategy for DFU because it could reduce or eliminate DFU-MDR bacterial infections while providing several advantages over conventional antimicrobial drugs, including specificity, stability in harsh environmental conditions, and a lack of the cytotoxicity against keratinocytes and fibroblasts that is experienced with antimicrobial molecules since the phage do not infect eukaryotic cells. Furthermore, it replicated at the site of the infection, providing a dynamic therapeutic strategy Phage formulation involves the dual problem of guaranteeing phage biostability and physical stability of the formulation (solution, suspension, gel).During manufacture and storage, the native structure and biological activity of the phages in the formulations must be maintained for the product to be a feasible treatment. To further complicate matters, distinct phages may exhibit varying stability patterns within the same formulation The creation of stable phage formulations for medicinal uses is still a relatively unexplored field of study. Hydrogels have been employed as a means of facilitating the precise administration or transport of biologics, such as phages, to specific target sites, such as wounds .The need for creating effective alternative therapeutic techniques has been brought to light by the growth of multidrug-resistant (MDR) germs, which lead to the failure of antibiotic regimen therapy for DFU (Maciejewska et al. 2018; Cha et al. 2018 In order to reap the benefits of both phages and hydrogels, phage hydrogels have been used to treat and/or prevent multidrug-resistant bacterial infections.Bacteriophage therapy can minimise systemic exposure and lessen negative effects, particularly when it is localised within a hydrogel.

# MATERIALS AND METHODS

## BACTERIOPHAGE ENZYME EXTRACTION

Bacteriophage was mass cultured in the media along with host cell.After the incubation for 2-3 days the phage lysate was prepared amd enzyme was extracted by cold acetone method.Cold acetone was enclosed to the phage lysate to precipitate the enzyme.Then centrifuged at 7000xg for 15 minutes.After then pellet was collected and suspended in PBS.It was used for further studies

## PREPARATION OF HYDROGEL

Poly acrylamide - Sodium alginate gel was synthesised by radical polymerisation technique.Utilising redox initialising pair of Ammonia persulfate/TEMED along with N,N menthlenedisacrylamide as a cross linear.PAM - SA hydrogel was created by blending acrylamide with sodium alginate in water medium phage enzyme was added into the reaction mixture.After polymerization hydrogel was washed then dried at 60c

## AGAR WELL DIFFUSION TEST

To find out how antimicrobial the materials were, the Agar well diffusion technique was employed. Enterococcus faecalis, Staphylococcus aureus, and mutant Streptococcus strains were injected into nutrient broth. for two to three hours at 37°C incubated. Following incubation, the 0.5 McFarland Standard was used to correct turbidity. Clean petri dishes were filled with Mueller Hinton agar, which had been prepared aseptically. Next, the plates were used to conduct the bacterial lawn culture. We used sterile gel puncture to create four wells, each measuring 10 mm in diameter and 4 mm in depth. Antibiotic discs for the positive control were put in the medium, and dimethyl sulfoxide (DMSO) was added to the well for the negative control. A 24-hour incubation period was then observed for the plates at 37°C. following its incubation diameter of the zone was measured.

# RESULTS

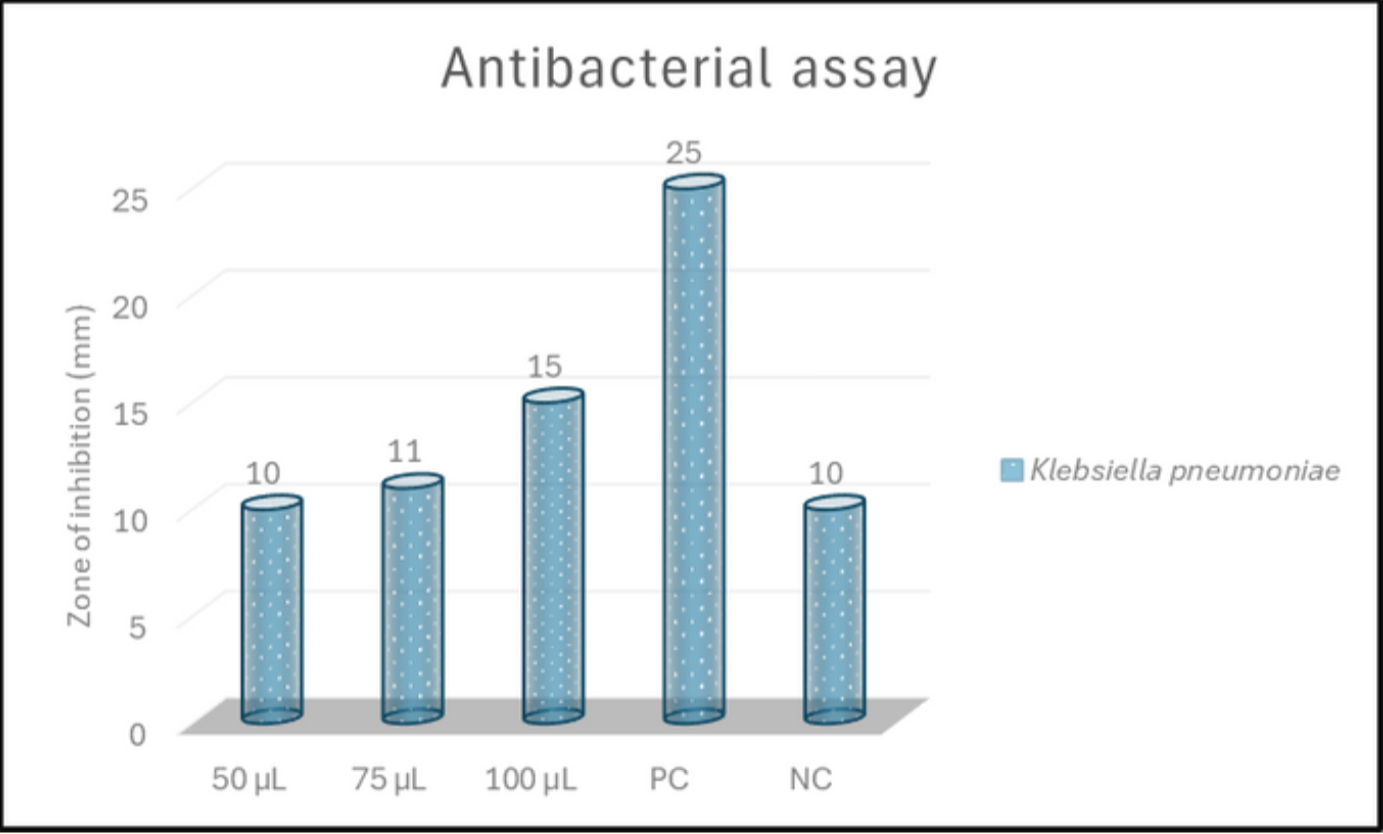


Fig 1: Antibacterial assay

This graph illustrates the results of an antibacterial assay testing the inhibition zones produced by different sample volumes against Klebsiella pneumoniae. The x-axis categorises the samples into various volumes (50 µL, 75 µL, 100 µL) along with positive (PC) and negative (NC) controls. The y-axis quantifies the inhibition zone in millimetres, reflecting the antibacterial activity of each sample.The positive control (PC) exhibits the most significant antibacterial effect, with a zone of inhibition measuring 25 mm. Among the tested sample volumes, the 100 µL sample demonstrates the highest antibacterial activity, producing a 15 mm zone of inhibition. The 75 µL sample shows moderate activity with an 11 mm inhibition zone, while the 50 µL sample has a similar antibacterial effect to the negative control, both yielding a 10 mm inhibition zone. This suggests that the antibacterial activity increases with the sample volume up to 100 µL, beyond which the positive control shows the maximum effect, and the negative control indicates minimal to no activity.

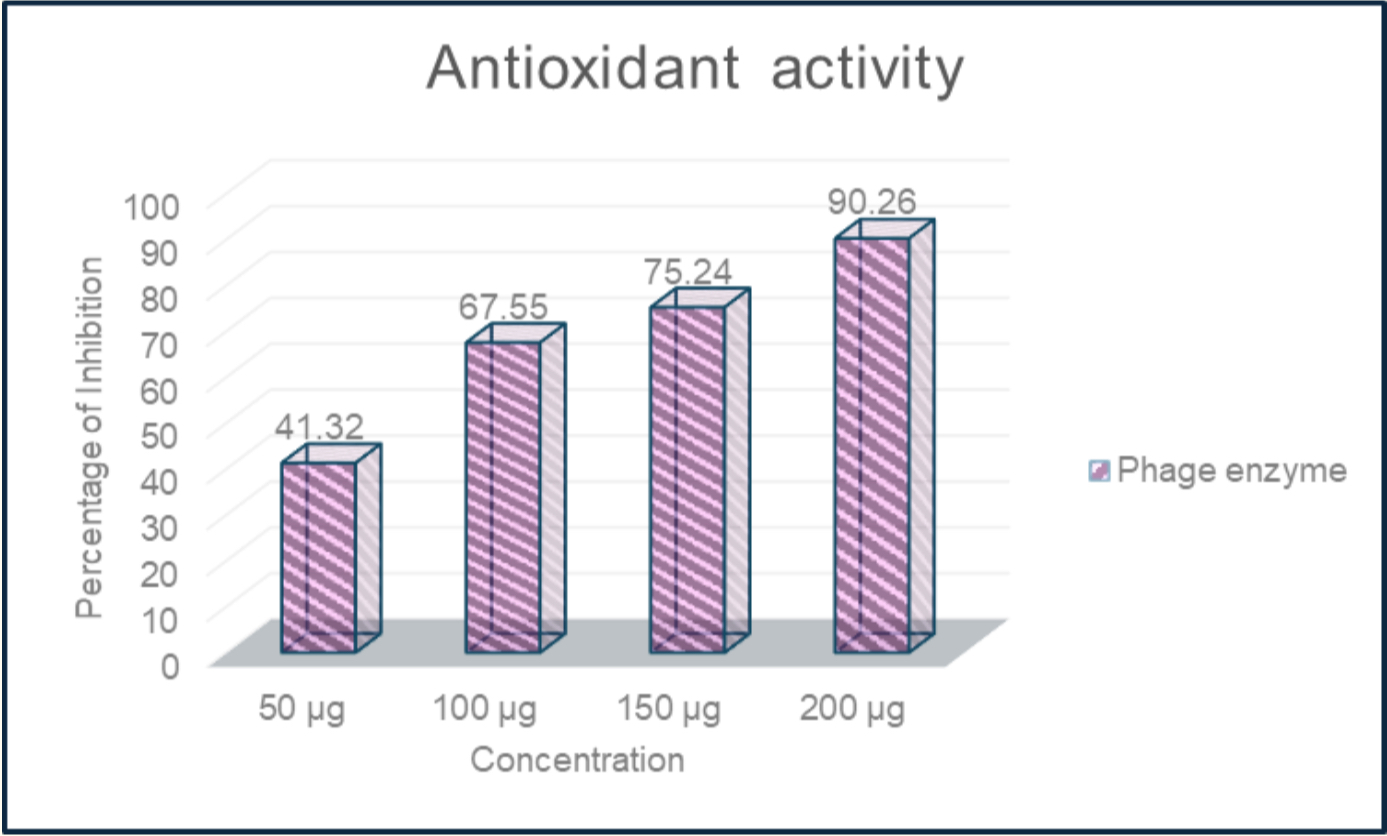


Fig 1: Antioxidant activity

This graph displays the antioxidant activity of a phage enzyme measured at various concentrations, depicted by the percentage of inhibition on the y-axis and the concentration in micrograms (µg) on the x-axis. The data reveals a clear trend of increasing antioxidant activity with higher concentrations of the phage enzyme.At the lowest concentration of 50 µg, the phage enzyme exhibits an inhibition percentage of 41.32%. This indicates moderate antioxidant activity. As the concentration increases to 100 µg, the inhibition percentage rises significantly to 67.55%, demonstrating a marked improvement in antioxidant potential.Further increases in concentration continue to enhance the antioxidant activity. At 150 µg, the inhibition percentage reaches 75.24%, reflecting strong activity. The highest tested concentration of 200 µg shows the greatest antioxidant effect, with an inhibition percentage of 90.26%, indicating very potent antioxidant activity.Overall, the graph indicates a positive correlation between the concentration of the phage enzyme and its antioxidant activity, with higher concentrations resulting in greater inhibition percentages. This suggests that the phage enzyme is effective in scavenging free radicals or inhibiting oxidative processes in a dose-dependent manner.

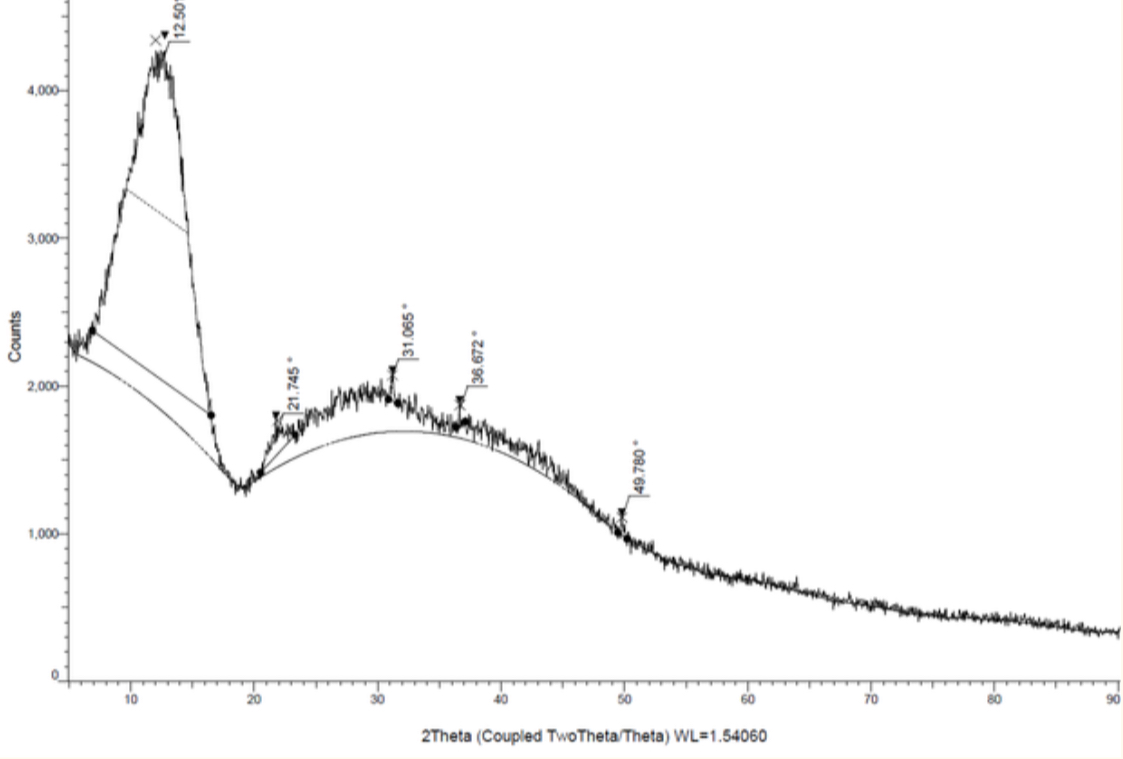


Fig 3: X-ray diffraction

This image represents an X-ray diffraction (XRD) pattern, a technique used to analyse the crystallographic structure of materials. The XRD pattern is plotted with the diffraction angle (2θ) on the horizontal axis and the intensity of the diffracted X-rays (counts) on the vertical axis. The pattern provides crucial information about the material’s crystalline structure by displaying peaks at specific 2θ values.One of the most prominent features in this XRD pattern is the sharp peak at around 12.4°, indicating a significant presence of a specific crystallographic plane within the material. Other notable peaks appear at approximately 21.45°, 31.065°, 36.672°, and 49.780°. These peaks correspond to the angles at which X-rays are constructively interfered by the crystal lattice planes, following Bragg’s Law.The intensity of these peaks, particularly the one at 12.4°, reflects the relative abundance and orientation of the corresponding crystallographic planes. Higher intensity peaks suggest a higher prevalence or better ordering of those planes in the sample. The broad hump and overall background intensity in the pattern suggest the presence of some amorphous material or other sources of scattering that do not produce sharp diffraction peaks.The width and shape of the peaks also provide insights into the material’s properties. Narrow peaks indicate larger crystallites and less strain within the crystal structure, while broader peaks suggest smaller crystallites or the presence of strain. These characteristics help in determining the crystallite size and the presence of defects or disorder within the material.To identify the specific phases present in the sample, one would typically match the observed peak positions with known reference patterns from databases such as the JCPDS. This detailed analysis can reveal the material’s composition and crystallographic structure, providing valuable information for various scientific and industrial applications.

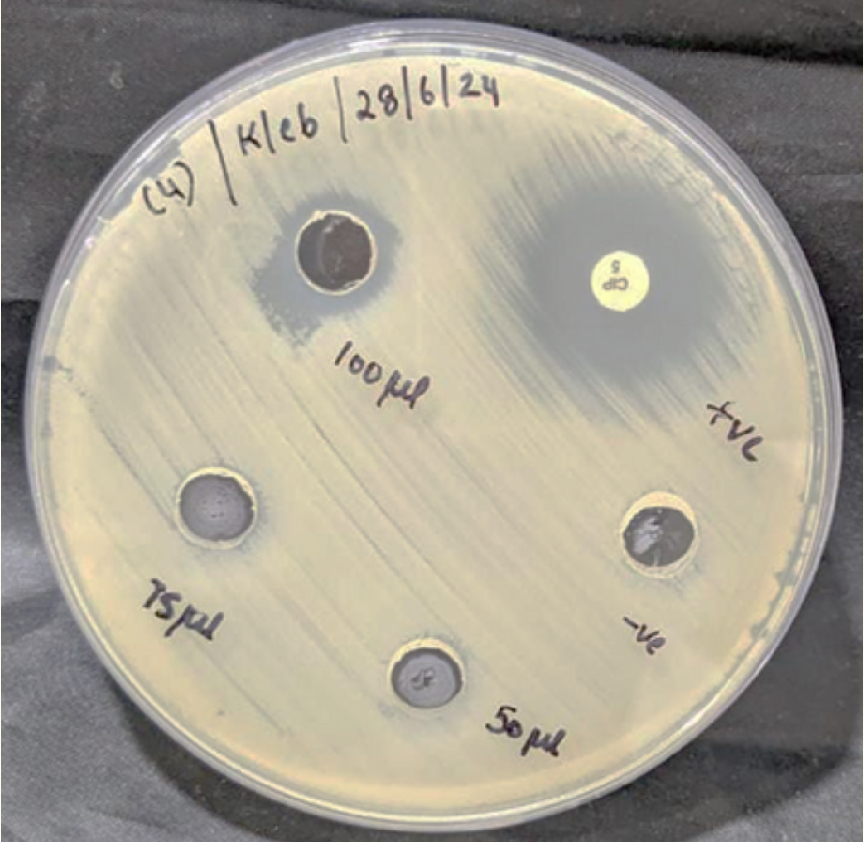


Fig 4: zone

This image shows an agar plate used for an antimicrobial susceptibility test, likely testing the effectiveness of various substances against a bacterial strain, in this case, Klebsiella sp. The plate is divided into sections with different labels indicating the concentration or type of treatment applied.Each section contains a small disc or well where an antimicrobial agent has been applied, indicated by labels such as “50 µL,” “75 µL,” and “100 µL.” These labels denote the volume of the substance applied in each respective area. The clear zones around some of the discs indicate inhibition zones where bacterial growth has been prevented, demonstrating the effectiveness of the antimicrobial agent.

The “+” and “-” symbols likely indicate positive and negative controls:

• +VC: Positive control, typically a known antibiotic to confirm that the bacteria are susceptible.

• -VC: Negative control, possibly a disc without any antimicrobial agent to ensure that bacterial growth occurs in the absence of treatment.

The effectiveness of each treatment is visually assessed by measuring the diameter of the inhibition zones. Larger clear zones indicate greater effectiveness of the antimicrobial agent in inhibiting bacterial growth.

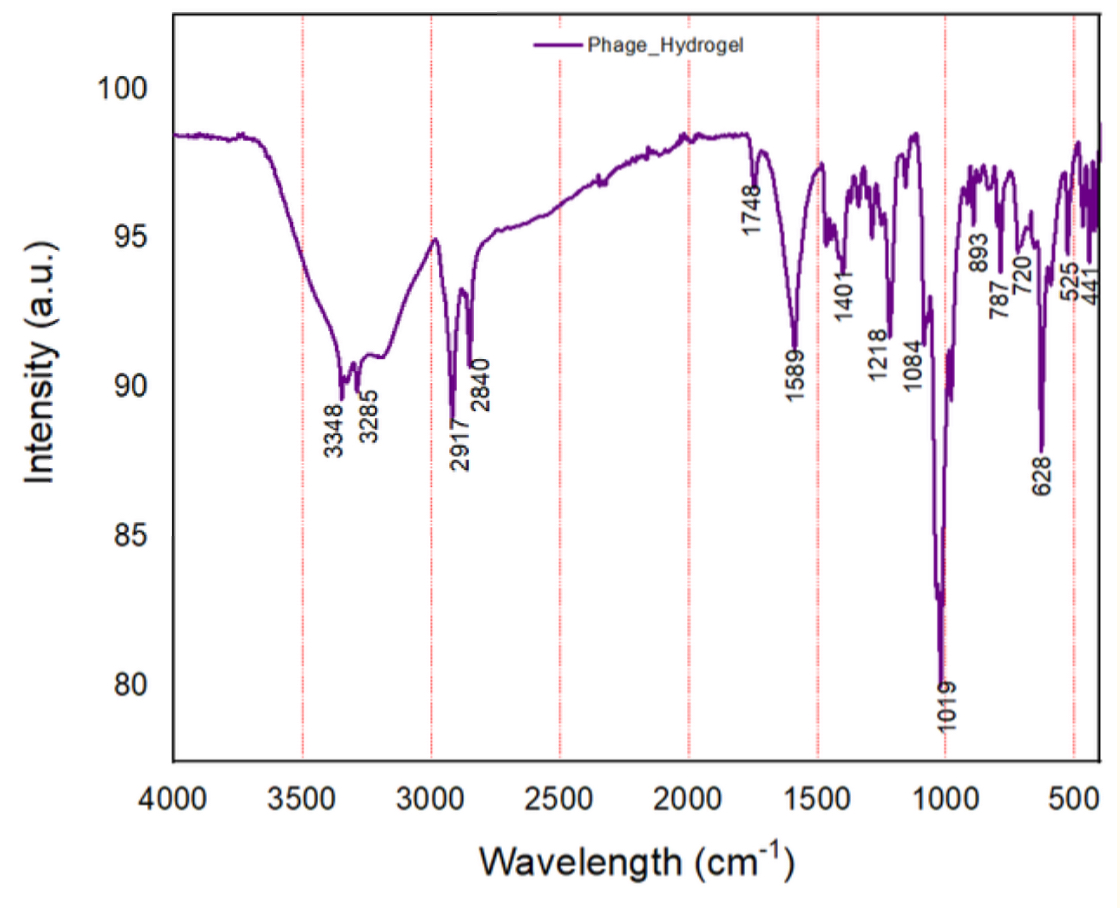


Fig 5: Intensity vs wavelength

This image presents an FTIR (Fourier Transform Infrared) spectroscopy spectrum for a sample identified as "Phage\_Hydrogel." The spectrum illustrates the intensity of infrared light absorbed by the sample across a range of wavelengths, measured in wavenumbers (cm^-1). FTIR spectroscopy is a powerful analytical technique used to identify functional groups within a molecule by examining the characteristic absorption of infrared light.One of the prominent features in the spectrum is the broad absorption peaks at 3348 cm^-1 and 3285 cm^-1. These peaks are typically associated with O-H stretching vibrations, which suggest the presence of hydroxyl groups. Hydroxyl groups are common in hydrogels due to their substantial water content, confirming the hydrogel nature of the sample.Additionally, the spectrum displays peaks at 2917 cm^-1 and 2840 cm^-1, indicative of C-H stretching vibrations. These peaks point to the presence of aliphatic (non-aromatic) hydrocarbons, which are often found in various organic compounds and polymers used to form hydrogels.A sharp peak observed at 1748 cm^-1 corresponds to C=O stretching vibrations, characteristic of carbonyl groups. This suggests the presence of ester or carboxylic acid groups within the hydrogel structure, which could be part of the polymer backbone or crosslinking agents used in the hydrogel synthesis.Other significant peaks include those at 1589 cm^-1 and 1400 cm^-1, which are typically associated with C=C stretching in aromatic rings or N-H bending vibrations in amines. These peaks indicate potential aromatic or amine components in the hydrogel, possibly arising from the incorporation of certain monomers or additives during the hydrogel's formulation. The detailed analysis of these peaks can provide insights into the chemical composition and functionality of the hydrogel.

# DISCUSSION

The development of a bacteriophage-infused hydrogel has shown promising results in addressing bacterial infections and oxidative stress. The hydrogel demonstrated significant antibacterial activity against Klebsiella pneumoniae, a common pathogen in wound infections. The bacteriophages incorporated into the hydrogel effectively target and neutralise this bacterium, potentially reducing the burden of infection in clinical settings [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/o75q0E/2SyAk) . This antibacterial efficacy is particularly valuable in the context of diabetic foot ulcers, where persistent infections can complicate wound healing.Additionally, the hydrogel exhibits enhanced antioxidant properties, which can be beneficial in managing oxidative stress associated with chronic wounds [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/o75q0E/P1pw1). Oxidative stress is a major factor in the pathogenesis of diabetic foot ulcers, leading to tissue damage and impaired healing (Rafi et al., 2024). By mitigating oxidative damage, the hydrogel not only combats bacterial infection but also supports the healing process through its antioxidant activity(Tuluwengjiang et al., 2024).The successful incorporation and stability of the bacteriophages within the hydrogel matrix were confirmed through FTIR and XRD analyses [(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/o75q0E/IKwyF) . Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) provide detailed insights into the molecular interactions and structural integrity of the hydrogel. These analyses validate that the bacteriophages are well-integrated into the hydrogel and remain stable, ensuring their continued efficacy in treating infections. Overall, the combination of antibacterial and antioxidant properties, along with confirmed molecular stability, underscores the hydrogel’s potential as a therapeutic tool for managing complex wounds and infections.

# CONCLUSION

​​The results of this study underscore the significant potential of bacteriophage-infused hydrogels as a novel and effective treatment for diabetic foot ulcers. This innovative hydrogel not only demonstrates robust antibacterial activity against Klebsiella pneumoniae, a prevalent pathogen in diabetic wounds, but also exhibits enhanced antioxidant properties. The dual action of combating bacterial infections and reducing oxidative stress addresses two critical issues that frequently impede wound healing in diabetic foot ulcers. The ability of the hydrogel to target and neutralis harmful bacteria while simultaneously mitigating oxidative damage offers a comprehensive approach to managing these complex wounds.Furthermore, the detailed molecular characterization of the hydrogel, as confirmed through Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analyses, reinforces the reliability of this therapeutic strategy. These analytical techniques verify that the bacteriophages are successfully incorporated into the hydrogel matrix and maintain their stability over time. This molecular validation is crucial, as it ensures that the hydrogel will continue to function effectively in a clinical setting, providing sustained therapeutic benefits.Overall, the promising results from this study highlight the potential for bacteriophage-infused hydrogels to become a valuable tool in the management of diabetic foot ulcers. By addressing both infection and oxidative stress, this hydrogel offers a targeted and effective treatment option that could significantly improve wound care outcomes. The encouraging findings suggest that further development and clinical evaluation are warranted to fully explore and optimise this novel approach for broader medical application.

# refereces

1. [Ajay, R., Rakshagan, V., Queenalice, A., Vinothkumar, S., Ravivarman, C., & Saravanadinesh, P. (2022). Effect of triazine comonomer substitution on the structure and glass transition temperature of monomethacrylate-based resin polymer: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 202–207.](http://paperpile.com/b/o75q0E/s6qRW)
2. [Ajay, R., Sasikala, R., Rakshagan, V., Raghunathan, J., LalithaManohari, V., & Baburajan, K. (2022). Evaluation of cytocompatibility of thermopolymerized denture base copolymer containing a novel ring-opening oxaspiro comonomer. *World Journal of Dentistry*, *13*(2), 127–132.](http://paperpile.com/b/o75q0E/KYqab)
3. [Ajay, R., Suma, K., Sasikala, R., Rakshagan, V., Baburajan, K., & Kalarani, G. (2022). Evaluation of linear dimensional stability of monomethacrylate-based dental polymer containing a novel tricyclic diacrylate cross-linker using a novel surface-level index technique. *World Journal of Dentistry*, *13*(6), 568–573.](http://paperpile.com/b/o75q0E/P1pw1)
4. [Balaji Ganesh S, & Sugumar, K. (2021). Internet of Things—A novel innovation in dentistry. *Journal of Advanced Oral Research*, *12*(1), 42–48.](http://paperpile.com/b/o75q0E/IKwyF)
5. [Chidambaram, S. R., George, A. M., Muralidharan, N. P., Prasanna Arvind, T. R., Subramanian, A., & Rahaman, F. (2022). Current overview for chemical disinfection of dental impressions and models based on its criteria of usage: A microbiological study. *Indian Journal of Dental Research : Official Publication of Indian Society for Dental Research*, *33*(1), 30–36.](http://paperpile.com/b/o75q0E/U0m0w)
6. [Deepika, B. A., Ramamurthy, J., Girija, S., & Jayakumar, N. D. (2022). Evaluation of the antimicrobial effect of Ocimum sanctum L. oral gel against anaerobic oral microbes: An in vitro study. *World Journal of Dentistry*, *13*(S1), S23–S27.](http://paperpile.com/b/o75q0E/AZmcK)
7. [Dharman, S., Kumar, R., Shanmugasundaram, K. (2021). Ecofriendly Synthesis, Characterisation and Antibacterial Activity Of Curcumin Mediated Silver Nanoparticles. *International Journal of Dentistry and Oral Science*, 2314–2318.](http://paperpile.com/b/o75q0E/lsfrw)
8. [Gao, D., Zhang, Y., Bowers, D. T., Liu, W., & Ma, M. (2021). Functional hydrogels for diabetic wound management. *APL Bioengineering*, *5*(3), 031503.](http://paperpile.com/b/o75q0E/g724)
9. [Ghanaim, A. M., Foaad, M. A., Gomaa, E. Z., Dougdoug, K. A. E., Mohamed, G. E., Arisha, A. H., & Khamis, T. (2023). Bacteriophage therapy as an alternative technique for treatment of multidrug-resistant bacteria causing diabetic foot infection. *International Microbiology: The Official Journal of the Spanish Society for Microbiology*, *26*(2), 343–359.](http://paperpile.com/b/o75q0E/uiFf)
10. [Govindaraj, A., & Dinesh, S. P. S. (2021). Effect of chlorhexidine varnish and fluoride varnish on White Spot Lesions in orthodontic patients- a systematic review. *The Open Dentistry Journal*, *15*(1), 151–159.](http://paperpile.com/b/o75q0E/2SyAk)
11. [Graf, S., Thakkar, D., Hansa, I., Pandian, S. M., & Adel, S. M. (2023). 3D metal printing in orthodontics current trends, biomaterials, workflows and clinical implications. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/o75q0E/Ia4ZJ)[10.1053/j.sodo.2023.01.001](http://dx.doi.org/10.1053/j.sodo.2023.01.001)
12. [Harsha, L., Navaneethan, R., Acid, T., & Acid, C. A.-A. (2022). CITRIC ACID-AN VITRO STUDY. *International Journal Clinical Dentistry*, *15*(3), 413–419.](http://paperpile.com/b/o75q0E/W63vT)
13. [Harsha, L., & Subramanian, A. K. (2022). Comparative assessment of pH and degree of surface roughness of enamel when etched with five commercially available etchants: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 181–185.](http://paperpile.com/b/o75q0E/5LHl6)
14. [Jabin, Z., Nasim, I., Vishnu Priya, V., & Agarwal, N. (2021). Quantitative Analysis and Effect of SDF, APF, NaF on Demineralized Human Primary Enamel Using SEM, XRD, and FTIR. *International Journal of Clinical Pediatric Dentistry*, *14*(4), 537–541.](http://paperpile.com/b/o75q0E/k6D4U)
15. [Katyal, D., Subramanian, A. K., Venugopal, A., & Marya, A. (2021). Assessment of Wettability and Contact Angle of Bonding Agent with Enamel Surface Etched by Five Commercially Available Etchants: An In Vitro Study. *International Journal of Dentistry*, *2021*, 9457553.](http://paperpile.com/b/o75q0E/NkqGb)
16. [Kifelew, L. G., Warner, M. S., Morales, S., Vaughan, L., Woodman, R., Fitridge, R., Mitchell, J. G., & Speck, P. (2020). Efficacy of phage cocktail AB-SA01 therapy in diabetic mouse wound infections caused by multidrug-resistant Staphylococcus aureus. *BMC Microbiology*, *20*(1), 1–10.](http://paperpile.com/b/o75q0E/YZ38)
17. [Kim, H. Y., Chang, R. Y. K., Morales, S., & Chan, H.-K. (2021). Bacteriophage-Delivering Hydrogels: Current Progress in Combating Antibiotic Resistant Bacterial Infection. *Antibiotics*, *10*(2), 130.](http://paperpile.com/b/o75q0E/5vJX)
18. [Lakshmi, T. (2021). Medicinal value oral health aspects acacia catechu-an update. *International Journal Dentistry Oral ScienceVolume*, *8*, 1399–1401J.](http://paperpile.com/b/o75q0E/H2G4I)
19. [Lee, C., Mayer, E., Bernthal, N., Wenke, J., & O’Toole, R. V. (2023). Orthopaedic infections: what have we learned? *OTA International : The Open Access Journal of Orthopaedic Trauma*, *6*(2 Suppl), e250.](http://paperpile.com/b/o75q0E/2Jkc)
20. [Liu, K., Wang, C., Zhou, X., Guo, X., Yang, Y., Liu, W., Zhao, R., & Song, H. (2024). Bacteriophage therapy for drug-resistant Staphylococcus aureus infections. *Frontiers in Cellular and Infection Microbiology*, *14*, 1336821.](http://paperpile.com/b/o75q0E/pkCl)
21. [Maliael, M. T., Subramanian, A. K., & Srirengalakshmi. (2021). Effectiveness of a fluoride-releasing orthodontic primer in reducing demineralization around brackets – a systematic review. *Orthodontic Waves (English Ed.)*, *80*(4), 218–223.](http://paperpile.com/b/o75q0E/PbLyY)
22. [Neha, N., Maiti, S., & Jessy, P. (2021). Adhesion microflora role denitrifies colour stability provisional crowns: in-vitro study. *Int J Dentistry Oral Sci*, *8*(8), 3805–3809.](http://paperpile.com/b/o75q0E/XTiIk)
23. Rafi, D. M., Lakshmi, T. V., Shirley, C. P., Ravivarman, G., & Senthilkumar, G. (2024, April). Improving Prostate Cancer Diagnosis with Weakly Supervised Learning and Radiology-Confirmed Negative MRI Data. In 2024 International Conference on Inventive Computation Technologies (ICICT) (pp. 1183-1188). IEEE.
24. [Sabarathinam, J., & Madhulaxmi, R. (2021). Development anti inflammatory antimicrobial silver nanoparticles coated suture materials. *Int J Dentistry Oral Sci*, *8*(3), 2006–2013.](http://paperpile.com/b/o75q0E/zJ181)
25. [Solanki, L., Shantha Sundari, K. K., Muralidharan, N. P., & Jain, R. (2022). Antimicrobial effect of novel gold nanoparticle oral rinse in subjects undergoing orthodontic treatment: An ex-vivo study. *Journal of International Oral Health: JIOH*, *14*(1), 47.](http://paperpile.com/b/o75q0E/NcP7F)
26. [Sushanthi, S., Doraikannan, S., Indiran, M., & Rathinavelu, P. (2021). *Rajeshkumar S. Vernonia Amygdalina*. 3330–3334.](http://paperpile.com/b/o75q0E/Ad8H9)
27. [Tiwari, A., & Jain, R. K. (2023). Comparative evaluation of White Spot lesion incidence between NovaMin, probiotic, and fluoride containing dentifrices during orthodontic treatment using laser fluorescence - A prospective randomized controlled clinical trial. *Clinical and Investigative Orthodontics*, 1–8.](http://paperpile.com/b/o75q0E/93Ugd)
28. Tuluwengjiang, G., Rasulova, I., Ahmed, S., Kiasari, B. A., Sârbu, I., Ciongradi, C. I., & Samaniego, S. S. C. (2024). Dendritic cell-derived exosomes (Dex): Underlying the role of exosomes derived from diverse DC subtypes in cancer pathogenesis. Pathology-Research and Practice, 254, 155097.
29. [Ubaidi, M., & Sumedi, S. (2023). Effect of hydrogel as autolysis debridement media on Wound Healing in Diabetic Ulcer Patients in holistic homecare. *Journal of Complementary Nursing*, *2*(1), 139–142.](http://paperpile.com/b/o75q0E/9GVY)
30. [Zhang, X., Ning, F., Chen, Y., & Dong, C.-M. (2024). All-in-one polysaccharide hydrogel with resistant vascular burst pressure and cooperative wound microenvironment regulation for fatal arterial hemorrhage and diabetic wound healing. *International Journal of Biological Macromolecules*, 132736.](http://paperpile.com/b/o75q0E/ZxB4)

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