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In vitro Antimicrobial Properties of Pluronic F-127 Injectable Thermoresponsive Hydrogel

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Abstract

Pluronic F-127 (PF-127) hydrogel is a versatile biomaterial with promising applications in drug delivery, tissue engineering, and regenerative medicine. PF-127 has antiadhesive activity that prevents bacterial adhesion by creating a hydrated layer on the bacterial surface. This property makes PF-127 suitable for preventing implant-associated infections. In this study, we aimed to evaluate the antibacterial properties of PF-127 using field isolates of *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) and compare them with different antibiotic standards. The antimicrobial potential was assessed using disk diffusion assays with four standard concentrations (20%, 25%, 30%, and 40%). The test microorganisms were inoculated on agar plates, and sterile filter paper disks infused with PF-127 hydrogels were placed alongside standard antibiotic disks. After incubation, the inhibition zones were measured to determine antimicrobial activity. Our results showed that PF-127 lacked intrinsic antimicrobial activity against *S. aureus* and *E. coli* at the tested concentrations. In conclusion, PF-127 hydrogel is a promising neutral carrier hydrogel system for loading antibiotics and antimicrobial compounds. Its unique properties, such as biocompatibility and thermo-responsive behaviour, combined with its antiadhesive activity, make it an ideal candidate for various biomedical applications.

Keywords: Hydrogel, Triblock Copolymer, Drug Delivery System, Poloxamer 407, Synthetic Copolymer, Antibacterial Potential

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INTRODUCTION

Pluronic F-127 (also known as Poloxamer 407 or PF-127) is a hydrophilic thermoreversible hydrogel system that has been used for drug delivery across different routes of administration.[1,2] It is a thermosensitive, biocompatible, and bioabsorbable polymer with great prospects in tissue engineering applications.[3] PF-127 is a non-ionic surfactant made of ethylene oxide and propylene oxide blocks. It is a white, waxy, free-flowing granule that is both tasteless and odourless.[1] The interaction of polyethylene oxide block with water molecules through hydrogen bonds is responsible for its water solubility. PF-127 is currently gaining importance as a system for dermal and transdermal drug delivery.[2] It exists as monomolecular micelles when dispersed in the liquid at low concentrations. However, as the concentration increases, multimolecular aggregates are formed.[2]

Another important characteristic of PF-127 that makes it unique is the thermoreversible properties: fluid state at low temperatures enabling easy administration, and gel state at high temperatures, facilitating the prolonged release of loaded agents.[4] Therefore, PF-127 at 18–50% concentrations will form hydrogel above 10°C and re-liquefy when cooled below this temperature.[5] Furthermore, concentrations above 15% can undergo a reversible thermal transition from micellar liquids to gels facilitating different drug delivery applications.[6]

PF-127 has low toxicity, reverse thermal gelation and good solubilizing properties, so it is used as an efficient drug delivery system.[1] However, thermosensitive PF-127 by itself is not utilized as an antimicrobial agent.[7] Usually, peptides and polysaccharides are combined with PF-127 to enhance their antimicrobial and biological properties.[8,9] It has been used as a drug delivery agent for various anti-neoplastic drugs,[10] lignocaine,[11] and as a dressing material for thermal burn wounds.[12] PF-127 is also used as a carrier for drug delivery in ophthalmology.[13]

PF-127 hydrogel has demonstrated immense potential in the field of biomaterials and drug delivery.[14] Its unique properties, including thermo-responsive behaviour, biocompatibility, and biodegradability, make it ideal for various applications, especially in regenerative medicine. Pluronic F-127 hydrogel is expected to play a crucial role in the development of innovative biomedical solutions.[14] Although several studies have been conducted to incorporate antimicrobial agents into PF-127, it is still not clear whether PF-127 possesses inherent antimicrobial activity at various concentrations that are currently in use.[15,16]

Therefore, the present study was conducted to evaluate the antibacterial properties of PF-127 using the field isolates of *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) and to compare it with different antibiotic standards.

MATERIALS AND METHODS

Pluronic F-127 hydrogel preparation

PF-127 hydrogel was prepared by dissolving the powder (Pluronic® F-127, Sigma-Aldrich, USA) in an ice-cold 0.9% sterile normal saline solution (20% - 20g/100ml, 25% - 25g/100ml, 30% - 30g/100ml, and 40% - 40g/100ml). Once the powder was added to the solution, it was shaken vigorously to dissolve it and kept at 4°C overnight (12 hours) to ensure complete dissolution. The final preparation was filter-sterilized using a 0.22-mm pore-size syringe filter and stored at 4°C under sterile conditions till further use.

Bacterial isolates

One isolate each of *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria), was isolated from the clinical samples obtained from the Referral Veterinary Polyclinic-Teaching Veterinary Clinical Complex, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India.

In vitro antimicrobial potential (Disk diffusion assay - Kirby-Bauer method)

The antimicrobial potential of PF-127 was measured using four standard concentrations (20%, 25%, 30%, and 40%). A standardized inoculum of *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) was prepared by adjusting the turbidity of the bacterial culture with 0.5 Macfarland standard. Muller-Hinton agar was used to perform the assay. The test inoculum was evenly applied over the agar surface using sterile

cotton swabs. This procedure was repeated at least three times. Sterile filter paper disks were infused with different concentrations of PF-127 hydrogels by overnight incubation at 4°C. In addition, standard antibiotic discs such as meropenem 10 mcg, co-trimoxazole 25 mcg, erythromycin 15 mcg, tetracycline 30 mcg, vancomycin 30 mcg, and cefotaxime/clavulanic acid 30/10 mcg were used as reference values.

The hydrogel discs along with the standard antibiotic discs were placed on the agar plate and incubated for 24 h at 37°C. The antimicrobial activities were evaluated according to the Kirby–Bauer method by measuring the inhibition zone diameter (mm).[17] The zone of inhibition was measured, which represents the antimicrobial activity against the test microorganisms. The diameter of the clear zone around the hydrogel disk indicates the level of inhibition. The values were expressed as mean±SD after repeating the experiments three times. The results were interpreted according to the standard criteria described by the Clinical and Laboratory Standards Institute (CLSI). The isolates were classified as sensitive (S), intermediate (I), or resistant (R) according to the reference tables.

RESULTS

In vitro antimicrobial potential of PF-127

The disk diffusion assay using the Kirby-Bauer method was used to assess the *in vitro* antimicrobial potential of PF-127 based on the diameter of the zone of inhibition (Figure 1 and 2). None of the concentrations of PF-127 produced a zone of inhibition when inoculated with *S. aureus* (Figure 2) and *E. coli* (Figure 1) indicating a complete lack of antibacterial activity. The findings indicate that PF-127 does not possess intrinsic antibacterial activity at concentrations ranging between 20% and 40% (Figure 3). Therefore, PF-127 can be used as an ideal neutral carrier hydrogel system for loading antibiotics and other antimicrobial compounds.

In vitro antimicrobial activity of standard antibiotics

The diameter of the zone of inhibition for each antibiotic standard (meropenem 10 mcg, co-trimoxazole 25 mcg, erythromycin 15 mcg, tetracycline 30 mcg, vancomycin 30 mcg, and cefotaxime/clavulanic acid 30/10 mcg) were measured in mm (Figure 3). The values were interpreted based on the standard values set by

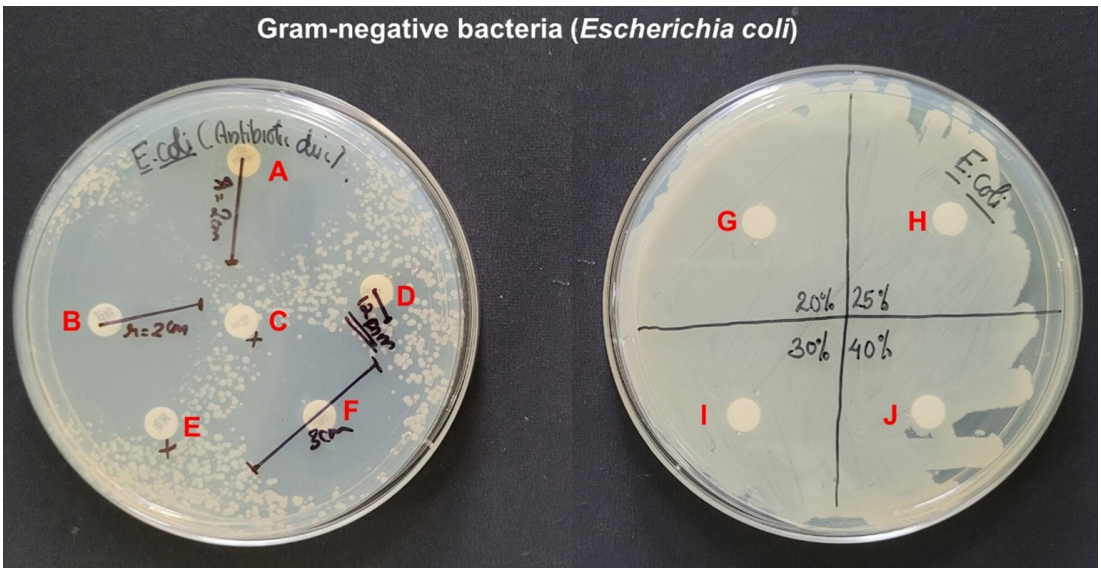

Figure 1. Antibacterial activity of meropenem 10 mcg (A), co-trimoxazole 25 mcg (B), erythromycin 15 mcg (C), tetracycline 30 mcg (D), vancomycin 30 mcg (E), cefotaxime/clavulanic acid 30/10 mcg (F), 20% PF-127 (G), 25% PF-127 (H), 30% PF-127 (I), and 40% PF-127 (J) against *Escherichia coli* (Gram-negative bacteria)

the CLSI. According to the CLSI reference tables, *E. coli* was found to be resistant to vancomycin and erythromycin and sensitive to co-trimoxazole, meropenem, and cefotaxime/clavulanic acid. On the contrary, the *S. aureus* isolate was found to be resistant to erythromycin, tetracycline, and co-trimoxazole as per the CLSI guideline.

DISCUSSION

Pluronic block copolymers are a category of hydrogel that can induce functional alterations at the cellular level.[18] The biological activity of Pluronics is dependent on their ability to incorporate into the cell membranes, thereby undergoing subsequent translocation into the target cells.[18,19] This affects cellular functions, including gene expression, ATP synthesis, apoptotic signal transduction, and drug efflux transporter activity.[19]

The antibacterial activity and biocompatibility of nitric oxide (NO) donor S-nitrosoglutathione (GSNO) releasing PF-127 were evaluated in an *in vitro* study.[20] They demonstrated that the GSNO-hydrogel combination exhibited concentration-dependent cytotoxicity to Vero cell lines and significant antimicrobial activity against *Pseudomonas aeruginosa* with a minimum bactericidal concentration of 0.5 µg/mL. Furthermore, GSNO-hydrogel was found to be non-toxic to Vero mammalian cells and can be used as a delivery system for NO-based antimicrobials. The incorporation of gallic acid into a dual-responsive temperature/pH-based PF-127 hydrogel enhanced the antimicrobial properties and was used for the treatment of atopic dermatitis.[21] The combined application of PF-127 and Ceragenin CSA-13 was reported to reduce the toxic effect of the latter and is indicated for topical applications.[16] The polymeric micelle combination of PF-127 and Cremophor EL was investigated as a drug delivery system for norfloxacin as an antibiotic drug model.[22] Their findings indicated that the micelle combination exhibited good antibacterial activity against clinically isolated bacterial strains and could act as a controlled drug delivery system for hydrophobic antimicrobial drugs.

PF-127 was found to possess antiadhesive activity (abhesive activity) that prevented the adhesion of *S. aureus* and *S. epidermidis* to polymethyl methacrylate.[23] This abhesive activity against staphylococcal adherence was higher when the concentration of PF-127 was increased. A similar finding was also observed when Gram-negative bacteria like *Pseudomonas aeruginosa* were used. PF-127 inhibited the adherence of *P.*

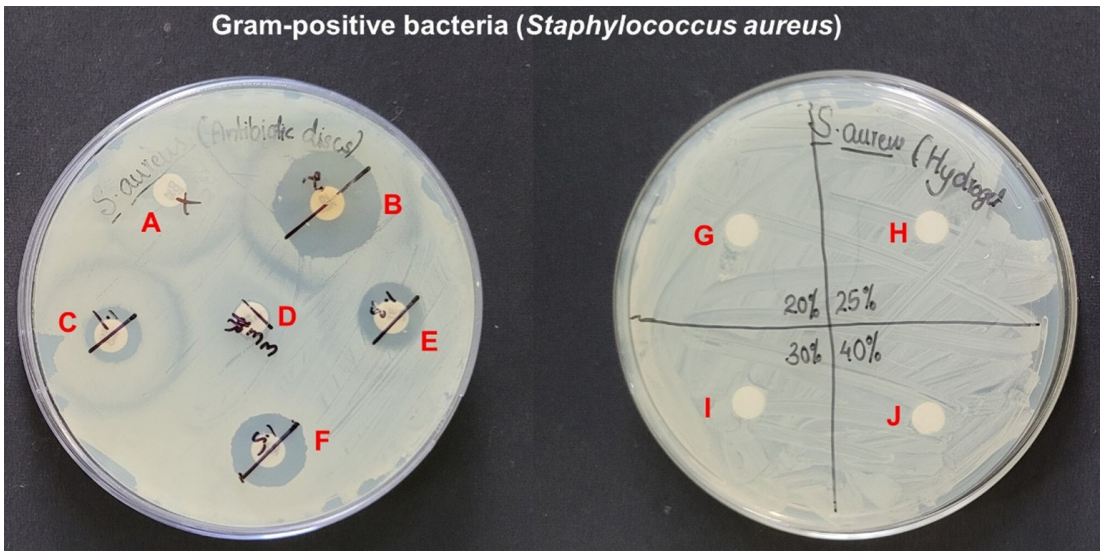

Figure 2. Antibacterial activity of co-trimoxazole 25 mcg (A), meropenem 10 mcg (B), cefotaxime/clavulanic acid 30/10 mcg (C), erythromycin 15 mcg (D), tetracycline 30 mcg (E), vancomycin 30 mcg (F), 20% PF-127 (G), 25% PF-127 (H), 30% PF-127 (I), and 40% PF-127 (J) against *Staphylococcus aureus* (Gram-positive bacteria)

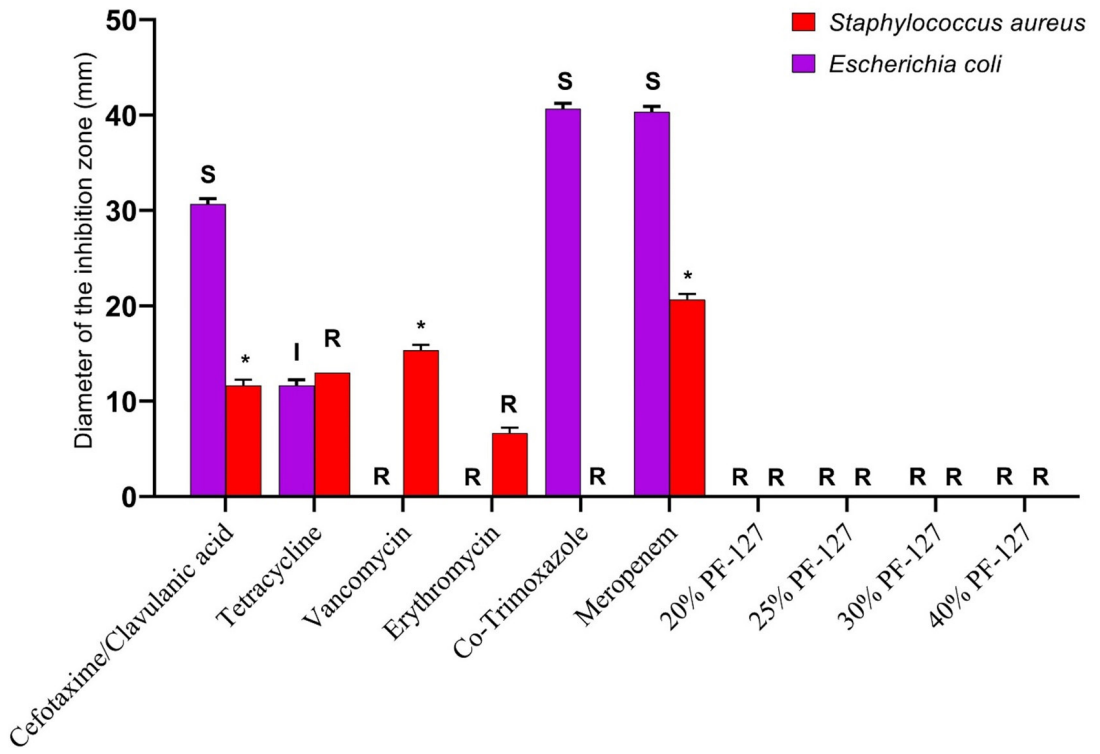

Figure 3. Comparative antibacterial activity of different antibiotic standards (meropenem 10 mcg, co-trimoxazole 25 mcg, erythromycin 15 mcg, tetracycline 30 mcg, vancomycin 30 mcg, and cefotaxime/clavulanic acid 30/10 mcg) with different concentrations of PF-127 hydrogels (20%, 25%, 30%, and 40%) against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). S - Sensitive, I - Intermediate, R - Resistant, and * - Reference value not available in CLSI guidelines

aeruginosa to contact lenses in a concentration-dependent manner.[24] The findings indicate that PF-127 prevents bacterial adhesion (both Gram-positive and Gram-negative bacteria) via a nonspecific mechanism that creates a hydrated layer on the bacterial surface.[23,24] Therefore, PF-127 is ideal for the prevention of implant-associated infections.

Although the abhesive activity of PF-127 has multiple utilities in the biomedical field, they lack intrinsic antimicrobial activity against staphylococci (*S. aureus* and *S. epidermidis*) when used at 4% and 15% concentration.[23] Our study confirmed that PF-127 lacked intrinsic antimicrobial activity against *S. aureus* and *E. coli* at four different concentrations (20%, 25%, 30%, and 40%). The synergism exhibited by the combination of PF-127 and antibiotics is due to a two-step phenomenon. First, the antiadhesive activity of PF-127 decreases the number of adherent bacteria. In addition, the qualitative modification of the bacterial binding site enhances the susceptibility of residual adherent bacteria to antibiotic action.[23]

Other poloxamers such as poloxamer 331 and poloxamer CRL8131 have previously exhibited antimicrobial activity against mycobacteria.[25,26] In addition to exhibiting a synergistic effect with rifampin, poloxamer 331 inhibited the growth of *Mycobacterium avium* complex (MAC) isolates indicating anti-mycobacterial activity.[25] Similarly, another poloxamer CRL8131 exhibited intrinsic anti-mycobacterial activity against *Mycobacterium tuberculosis* and produced synergistic effects when used in combination with antibiotics such as rifampin, isoniazid, and streptomycin.[26]

The unique drug delivery characteristics and thermoreversible nature make PF-127 a promising delivery system that can be used along with various pharmaceutical agents as well as with different routes of administration.[1] The findings

of the present study revealed that PF-127 by itself does not have any antimicrobial properties. However, Pluronic-lysozyme conjugates possess antiadhesive and antibacterial properties. PF-127-lysozyme conjugate has exhibited antibacterial activity against *Bacillus subtilis*.[27] For biomedical and therapeutic applications, the balance between the antimicrobial and viscoelastic properties of PF-127 needs to be considered.[7] A hybrid hydrogel platform was prepared by combining PF-127/chlorhexidine nanoparticles (NP) and chitosan methacrylate-gallic acid (CSMA-GA) polymers possess antimicrobial and antioxidant activities to enhance the antibacterial properties of PF-127.[28] These hybrid hydrogel combinations were found to possess strong reactive oxygen species (ROS) scavenging ability and high antibacterial efficiency during *in vitro* studies, and it promoted angiogenesis and significantly reduced inflammation during the *in vivo* studies. In another study that combined several polymers and ZnO with PF-127, it was observed that PF-127 did not exhibit any antimicrobial or antifungal activities against the various bacterial and fungal strains used.[7]

CONCLUSION

PF-127 hydrogel holds great promise as a versatile biomaterial. Its tunable properties, biocompatibility, and thermo-responsive behaviour make it an attractive candidate for drug delivery, tissue engineering, wound healing, and other biomedical applications. Previous studies have identified the antiadhesive activity of PF-127 against Gram-positive (*S. aureus* and *S. epidermidis*) and Gram-negative (*P. aeruginosa*) bacteria. In addition, poloxamers other than PF-127 have exhibited intrinsic antimicrobial (anti-mycobacterial) activity against MAC and *M. tuberculosis*. However, findings from this study confirm that PF-127 lacks intrinsic antimicrobial activity against *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) at concentrations ranging between 20% and 40%. Therefore, PF-127 can be used as an ideal neutral carrier hydrogel system for loading antibiotics and other antimicrobial compounds due to their antiadhesive property that enhances the susceptibility of microorganisms against the loaded compound.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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