Hydrogel phage formulation for combating *Pseudomonas aeruginosa*

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Introduction:

Multidrug-resistant bacterial infections pose a severe public health crisis due to limited antibiotic development, threatening a return to the pre-antibiotic era and incurring substantial human and financial costs. Pseudomonas aeruginosa, a challenging multidrug-resistant pathogen, adds complexity with its drug resistance mechanisms and pathogenic factors.

Bacteriophages, viruses targeting bacteria, offer distinct advantages over antibiotics, such as specificity, replication within hosts, and minimal disruption to natural microflora, supporting their efficacy in preventing and treating bacterial infections, particularly in immunocompromised patients. Hydrogels, biomimetic materials, exhibit potential as a stable and controlled delivery system for phages, creating new avenues for combatting antibiotic-resistant infections. This research aims to evaluate the potential of stable phage hydrogel formulation for controlling Pseudomonas aeruginosa.

Methods:

Preparation of bacterial culture

Luria-Bertani (LB) liquid and solid culture media were prepared by combining tryptone, yeast extract, and sodium chloride in specific ratios, followed by autoclaving. Bacterial inoculation involved using a sample from Urmia University of Medical Sciences' Biotechnology Laboratory, with the bacteria introduced into the liquid culture medium and incubated at 37°C with continuous shaking for 18 hours. Additionally, the bacterial sample was plated on solid culture medium to identify Pseudomonas aeruginosa colonies.

Bacteriophage preparation

The MGS2 phage population was propagated by introducing the phage stock into an overnight bacterial culture, followed by a 24-hour incubation at 37°C with continuous rotation. The resulting mixture underwent centrifugation, and the supernatant enriched with phages was filtered. The filtrate was then combined with PEGNaCl, stored at refrigerated temperatures for 48 hours, and subsequently centrifuged. The resulting precipitate was reconstituted in SM buffer and stored in the refrigerator.

Hydrogel preparation

The hydrogel preparation process involved formulating hydrogels with 2.5% sodium carboxymethyl cellulose in two separate solutions: SM buffer and an acetate solution consisting of anhydrous sodium acetate, sodium chloride, and glacial acetic acid. The preparation was carried out using a Bain-Marie apparatus at 85°C with mechanical stirring.

Viscosity evaluation

Prepared gel formulations (CMC 1.5, 2, 2.5, 3%) were studied for rheological properties using rotating viscometer. The measurements were performed at temperature 25 C (room temperature) at different rotation speed, 0.5-50 RPM.

Release test

The investigation of phage release from the formulated hydrogel involved analyses. For qualitative assessment, hydrogel was placed in individual wells, with two test samples and one control sample in each well. Phage solution were added to each well, along with the acetate buffer. After a one-hour incubation, samples were collected for plaque assays.

Formulation stability evaluation

Three separate 50 ml tubes, each containing 5 grams of the hydrogel and 100 microliters of phage solution with a concentration of 10^8 PFU per gram of hydrogel prepared. These tubes were stored under three different conditions, simulating various environmental temperatures (refrigerator, room temperature, and 40°C) for three months. A reference control group stored the same phage solution in acetate buffer under the same temperature conditions.

To assess the formulation's stability at regular 14-day intervals, samples of each hydrogel were diluted in physiological serum, and a plaque assay was performed using Pseudomonas aeruginosa as the indicator strain.

Formulation efficacy evaluation

For evaluation against laboratory strains, the prepared hydrogel and the phage suspension were introduced into separate wells of a 24-well plate, with each well containing one type of bacterium. A 2% topical erythromycin gel served as a comparative reference. To assess effectiveness against hospital-derived bacterial strains, samples from Motahari and Taleghani hospitals were used, with hydrogel containing the phage added to bacterial suspensions. Absorbance measurements were taken at 30-minute intervals over 7 hours using a Microplate Reader at 540 nm wavelength, with each sample examined four times. Control samples with only the bacterial suspension and a negative control with the formulation and an empty culture medium were maintained for each bacterial sample in the assay.

Results:

Viscosity

The rheological properties of the prepared gels were investigated at room temperature (25°C), and the results were presented graphically in Figure 1. The viscosity (mPa.s) was plotted against different rotational speeds (RPM) of the viscometer. It was observed that the viscosity of the gels increased as the concentration of carboxymethyl cellulose (CMC) increased, with a pronounced viscosity change in the case of CMC at 3%. This increase in viscosity with higher gelling agent concentration is attributed to increased chain interactions as the CMC concentration rises. For CMC at 1.5% and 2%, the viscosity-shearing rate (rpm) relationship exhibited a shallow slope, indicating a more linear behavior, while CMC at 3% and 2.5% showed a steep, non-linear relationship, indicative of non-Newtonian behavior in the gels. Furthermore, CMC at 1.5% and 2% displayed similar viscosity values compared to other formulations. In contrast, the viscosity of CMC at 3% sharply decreased with an increase in shear rate (rpm), demonstrating a shear-thinning behavior. This behavior is attributed to the breakdown of physical cross-links within the CMC 3% gel as the shear rate increases, resulting in a significant reduction in viscosity from 639,000 to 68,200 mPa.s.

Release test

The results obtained from the conducted methodology are presented in Figures 2, focusing on the qualitative assessment of phage release. It is evident from the figures that in both hydrogel samples prepared using SM and Acetate buffers, the phage exhibited effective release from the formulation after one hour of incubation. This release of phage effectively inhibited bacterial growth at the instillation site, as visually demonstrated in the figures. In contrast, the control samples, as anticipated, exhibited no discernible impact on bacterial growth, underscoring the significance of phage release from the hydrogel formulation in preventing bacterial proliferation.

Formulation stability

The investigation into phage stability within the formulated product at various temperatures (2-8°C, 25°C, and 40°C) is presented in Figure 3. The results indicate that at temperatures between 2-8°C, the formulation containing the phage maintains its effectiveness at the highest level for a duration of 12 weeks. However, at 25°C, the phage's efficacy within the formulation significantly diminishes. Notably, at 40°C, the stability of the formulation exhibits a rapid decline, with the phage losing its effectiveness entirely by the end of the second week.

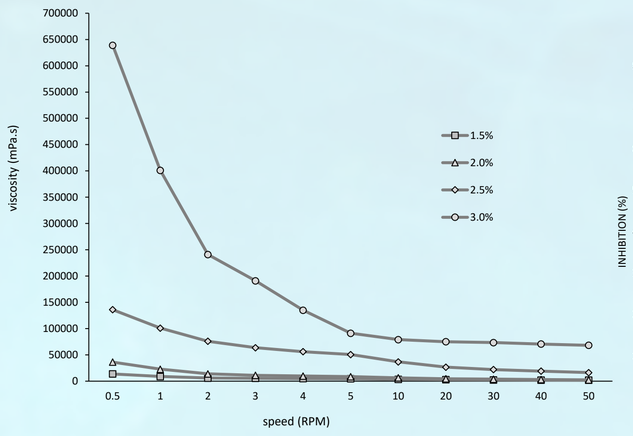

Figure 1: Effect of various concentrations of CMC on viscosity of gels

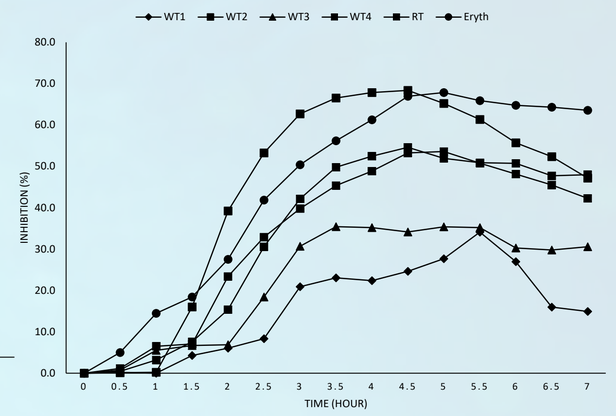

Figure 4 : Inhibition Percentage Over Time (WT: Wild type, RT: Regular Type and Eryth: Erythromycin)

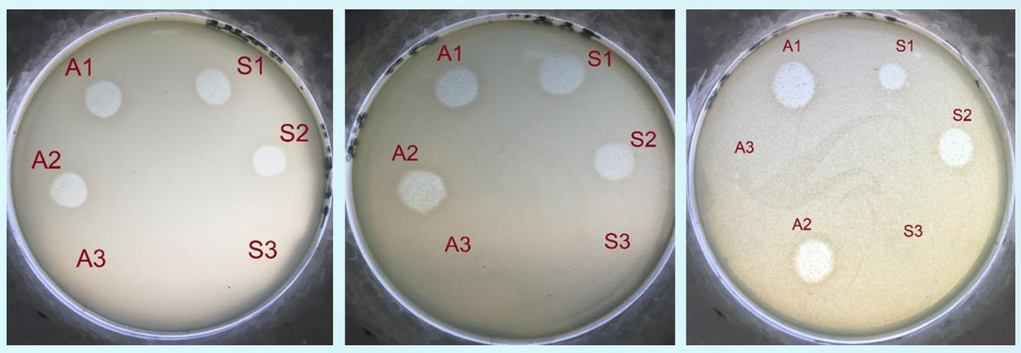

Figure 2. qualitative assessment of phage release. (A: Acetate buffer, S: SM buffer. 1&2: Test samples, 3: Control samples)

The study involved calculating the percentage of inhibition by comparing the absorption ratios of test group samples to their respective control group samples. The inhibition percentage was expressed by subtracting this ratio from 100. Figure 4 represents the results for the first to fourth hospital samples, the laboratory sample, and erythromycin, depicting the inhibition percentage over a 7-hour period. In the case of laboratory bacteria, inhibition commenced after 30 minutes, reaching a peak of 53.5% at 5 hours, followed by a gradual decrease. The first hospital sample showed an increase in inhibition from zero percent after the first hour, peaking at 34.1% around 5.5 hours before declining. In the second hospital sample, inhibition began after the first hour, rapidly reaching 68.3% around 4.5 hours and then decreasing slowly. The third hospital sample exhibited a faster rise in inhibition, reaching a maximum of 35.4% at 3:30 p.m., sustained until about 5:30 p.m., followed by a decline. The fourth hospital sample displayed inhibition starting after 30 minutes, reaching 54.5% around 4.5 hours, with a subsequent gradual decline. In the laboratory sample treated with erythromycin gel, inhibition increased from the start until reaching a maximum value of 67.8% at 5 hours. Thereafter, it slowly decreased until the 7-hour mark. Figure 4 illustrates that erythromycin exhibited a quicker onset and achieved a greater maximum inhibition compared to the phage.

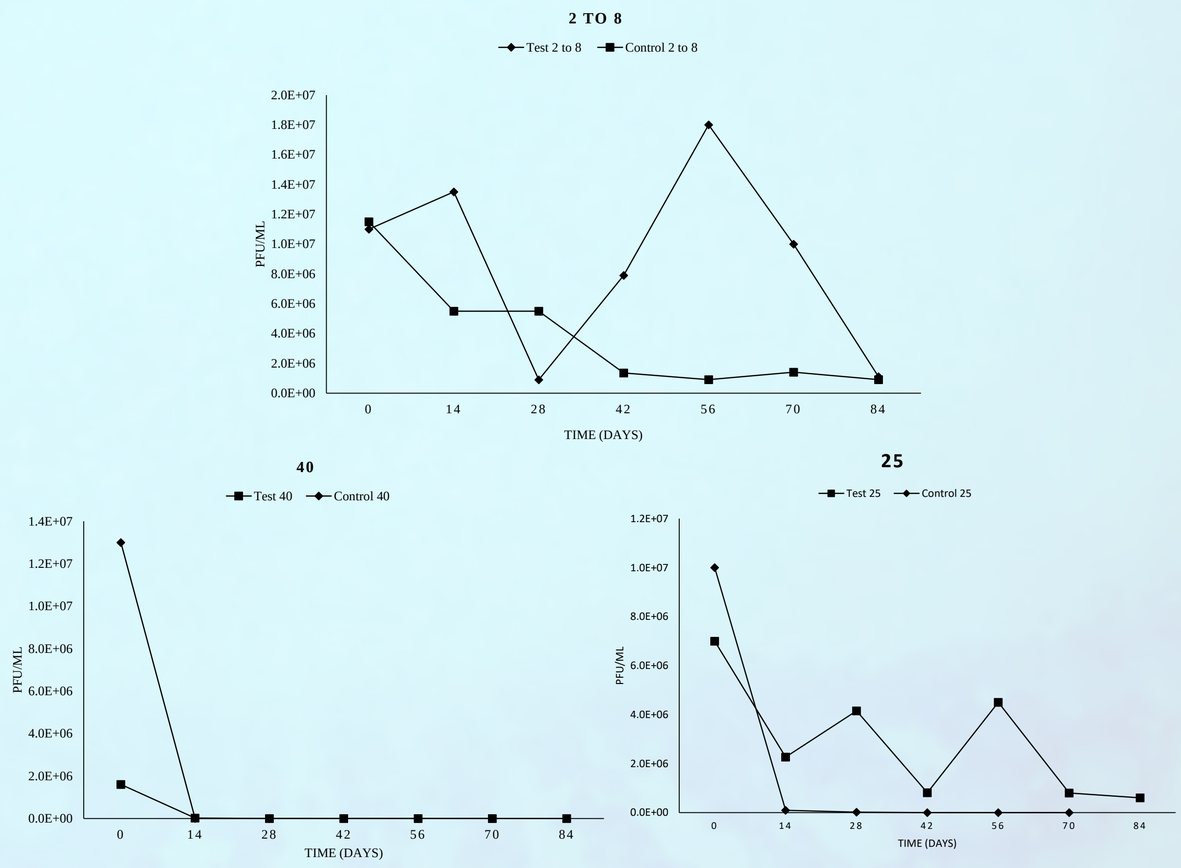

Figure 3: Phage Stability at Varied Temperatures Over 12 Weeks

Conclusion:

This study signifies progress in utilizing hydrogels for phage delivery to combat antibiotic-resistant infections, providing an alternative therapeutic approach to traditional antibiotics. The study reveals hydrogels' potential as a phage delivery system for combating antibiotic-resistant infections, demonstrating effective inhibition of bacterial growth within an hour of incubation. The rheological analysis indicated the suitability of these formulations for biomedical applications.

Keywords:

Pseudomonas aeruginosa- bacteriophage therapy-gel formulation

Refrences:

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