Using Phage in Fighting Infection

By:

Arshia Azizeddin and Fred Yin

Background, Purpose and Hypothesis

The rise of pathogen antibiotic resistance has increased interest for alternatives over the use of conventional antibiotics. Bacteriophage or phage therapy is an alternative form of treatment that is being explored. In phage therapy, a specific phage, through its lytic cycle of infection, is capable of killing a specific bacterium without interacting with the surrounding human tissue or with other harmless bacteria. The virus replicates quickly, so that a single small dose would be enough to destroy the entire bacterial infection.

To study and examine the ability of human pathogens to bind to and invade the human host, a cell line needs to be used to study the bacterium pathogenicity. A cell culture model using immortalized HeLa cells serves as a great model because they are derived from human cervical cancer cells which *E. coli* O157:H7 can easily bind to.

The factors that have to be examined in the application of the phage are concurrent versus post-treatment application of phage and the resultant effectiveness of the phage in eliminating the entire infection.

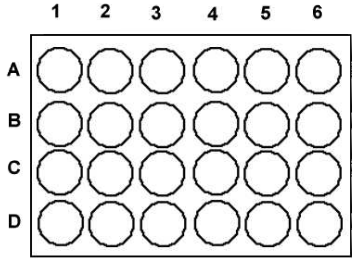
The purpose of this project is to monitor the effectiveness of the phage therapy at different phases of infection in HeLa cells infected with *E. coli* O157:H7 using bioluminescent imaging, thus determining the best time to apply the phage during the infection.

Our hypothesis is that applying the phage concurrent to the start of infection will be the best method of treatment as the phage can destroy most of the bacteria before the infection spreads.

Procedure

- Grew *E. coli* O157:H7 (with lux gene chromosomally integrated) on LB Broth
- Experimentally determined the dilution and amount of direct phage capable of destroying *E. coli* colonies
- Infected Hela cells using the bioluminescent *E. coli* and monitored its process of infection using bioluminescent imaging from the NightOwl Molecular Imager
- Lytic phage was administered concurrent to the *E. coli* infection as a method of treatment and bioluminescent imaging was used to monitor the death of the bacteria
- Washed cells with phosphate buffered saline (PBS) to eliminate *E. coli* that had not adhered to Hela cells
- Hela cells were infected with phage at successively later times after the initial bacterial infection to study the effect of post-infection treatment and bioluminescent imaging was used to monitor the death of the *E. coli*

The following is the template for the plate with 24 wells:



- A1, B1 C1, D1 – positive control (Hela cells + EHEC)

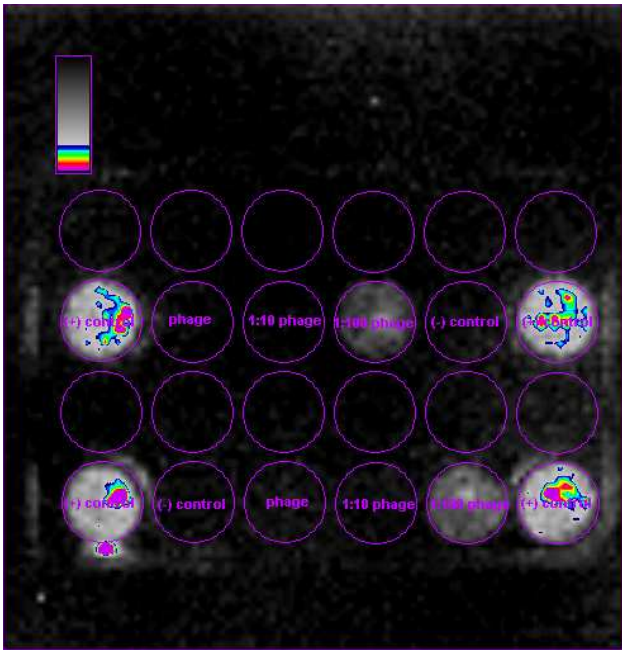
- A2, B2, C2, D2 – negative control (Hela cells)

- 20µl of direct phage was added to every well except for the positive and negative controls in the following times:

- 1 hour before the wash: added 40ul of *E. coli* to each well (except column 2) and infected A3, B3, C3, D3 with phage
- 2 minutes before wash: took picture
- washed cells with PBS
- 1 hour after wash: infected A4, B4
- 3 hours after wash: took picture and infected C4, D4
- 4 hours after wash: took picture and infected A5, B5
- 5 hours after wash: took picture and infected C5, D5
- 6 hours after wash: took picture and infected A6, B6
- 7 hours after wash: took picture

Results/ Observations

Determined the concentration of phage that was most effective against the E. *coli* using 1:10 and 1:100 dilution of phage:



It was evident that 1:10 dilution of phage worked best against the *E. coli* as no light appears in the 1:10 dilution wells while some light still remains in the 1:100 dilutions wells. Therefore, a higher concentration of the phage is most effective against the bacteria and so the actual experiment was carried out with 20µl of direct phage and the following results were obtained:

Concurrent treatment: The data obtained from the NightOwl Molecular Imager illustrated that infecting cells with phage, as a method of concurrent treatment against *E. coli* is very effective. Compared to the positive wells (column 1), the wells infected with phage concurrent to *E. coli* infection (column 3), showed dramatic light loss after only one hour of infection. Since the amount of light is proportional to amount of living *E. coli* colonies and no light could be seen in the 3rd column, it could be said that the majority of the *E. coli* colonies had been destroyed by the phage in wells A3, B3 and C3.

Post-treatment: Infecting cells with phage, as a method of post-treatment against *E. coli* is also very effective. The phage killed about 80% or more of the bacterial colonies in each well after only the first hour.. This is because the light is proportional to the number of live bacterial colonies, so if 80% of the light is gone, that means that about 80% of the colonies are dead. The application of phage at different time periods however, did not seem to be a factor in destroying the bacterial colonies. For example, bacterial deaths one hour after wash in well A4 has about a 3% difference of "light lost" compared to bacterial deaths six hours after wash in A6. In conclusion, it can be said that phage is an effective killer of *E. coli.* The time, however, when *E. coli* is infected with phage is not a factor because the phage is capable of destroying the majority of the *E. coli* colonies after any time period.

Conclusions

From the data, it is evident that phage therapy is an effective method of treatment against *E. coli O157:H7* and would serve as an extremely suitable alternative to antibiotic treatment. The phage were able to destroy about 80%+ of the *E. coli* colonies just one hour after treatment, proving that phage therapy is exceedingly effective

The time of phage application is not an important factor in destroying the *E. coli* colonies: both concurrent and post-treatment work well in killing the majority of the bacterial colonies.

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