Sperm-Powered Antibiotic Releasing Technology for Disruption of Biofilm

Ethan Batiste and Aarya Bandi

1. Introduction and Rationale

Escherichia coli is one of many bacteria species that produce biofilm, a three-dimensional extracellular substance secreted by a colony after it irreversibly attaches to a surface. Biofilm growth is a multi-step process beginning when a bacterial cell attaches to a surface using its 100-500 pili. After this, the bacteria creates a micro-colony, matures to create a biofilm, and cells disconnect from the biofilm (Nandakumar et al, 2013). Biofilms have complex structures, including exopolysaccharides, DNA, and proteins which makes it difficult for antibiotics to reach and eradicate the bacterial target within. In fact, bacteria in biofilms exhibit a 10-1000 times resistance to antibiotics compared to planktonic bacteria. This makes biofilm and the colonies inside of them particularly difficult to eradicate since it requires additional methods of disruption.

A critical reason contributing to the danger of biofilms is their involvement in urinary tract infections (UTIs) which are among the most common healthcare associated infections in the world. Catheter associated UTIs in hospital settings make up more than 70% of cases due to the ease of bacteria buildup, biofilm creation, and the difficulty in sterilizing catheters (Nicastri et al, 2021). Antibiotics are insufficient in sterilization because they slowly diffuse through the complex structural layers of biofilm and the aerobic environment within the biofilm's matrix microenvironment make them completely resistant.

Bull sperm are of great interest in current research to create biological microrobots. We believe bull sperm are uniquely fit to disrupt biofilm because of their biological function, small size, hydrodynamic interactions, and erratic tail movement. Firstly, sperm transport genetic information during sexual reproduction which requires them to penetrate the mucus and cumulus layers before reaching an egg. The cumulus layer surrounding an egg contains approximately 3000 cells in an extracellular matrix (Lin et al, 1994). Similarly, biofilm is made up of an extracellular matrix connecting bacterial cells. The clear similarities between the substances indicate that sperm may be capable of disrupting biofilm.

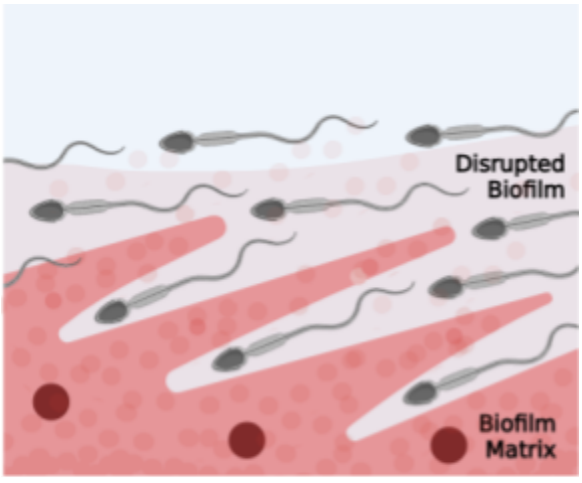

Figure 1: Application of bull sperm to disrupt biofilm allowing antibiotics to have more success.

Secondly, bull spermatozoa are approximately 60-70 μm in length, allowing them to navigate very small spaces beyond urinary catheters that other microrobots may struggle with. Furthermore, hydrodynamic interactions cause bull sperm to accumulate and travel near surfaces. Sperm are pushers causing a hydrodynamic interaction that attracts sperm towards a surface when moving at a nearly parallel angle to it (Elgeti, 2015). The close proximity of the sperm to a wall will cause more frequent impact and greater disruption of the biofilm.

Mammalian sperm tend to carry a net negative surface charge, allowing for bull sperm cells to easily bind to positively charged surfaces (Simon et al, 2016). This pairs perfectly with gelatin particles, which have polyampholyte surface properties. Using gelatin at lower pH allows for its surface to have a positive charge, which can then bind to the sperm cells (Hoque et al, 2015). Gelatin particles have the unique ability to be loaded with antibiotics and other substances for controlled drug release. In tandem with their biodegradability and production of hydrogels that encapsulate and release drugs through hydrolysis or enzymatic degradation, gelatin particles present a versatile method of delivering antibiotics (Varma et al, 2022).

To effectively disrupt and diminish the presence of biofilm we propose creating Sperm-Powered Antibiotic Releasing Technology for Nanodelivery (SPARTN). SPARTN is a two part solution to eradicating biofilm using bull sperm to penetrate the complex biofilm matrix as well as mechanically bound gelatin particles that will release antibiotics to kill bacteria.

2. Engineering Goals

In this project, we intend to manufacture SPARTN, drug-loaded bull spermatozoa to disrupt biofilm and eradicate bacterial colonies. SPARTN will meet the following criteria:

- Penetration of biofilm's extracellular matrix due erratic tail movements and hydrodynamic interactions drawing sperm towards surfaces.
- Fabrication of gelatin particles that will dissolve to release antibiotics.
- Successful fabrication of SPARTN by mechanically binding gelatin particles to bull sperm.
- Bull sperm and antibiotics will successfully disrupt biofilm without the use of toxic chemicals that could negatively impact patient care.

3. Research Methodology and Procedure

All experiments and research for this project will be completed in Assistant Prof. Veronika Magdanz's lab at the department of Systems Design Engineering, University of Waterloo.

3.1 Biofilm Culturing

- *E. coli* and Lysogeny broth solution will be pipetted into a microfluidic chip, evenly dispersed throughout, then incubated at 37℃ for at least 24 hours to ensure ample biofilm growth along the edges of the chip.

3.2 Bull Sperm Preparation

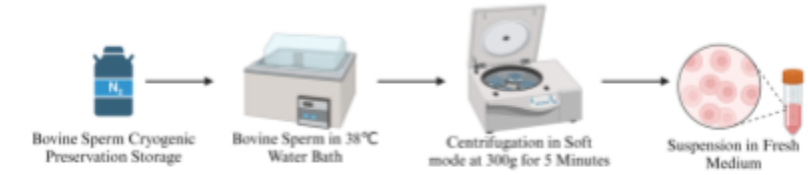

Figure 2: Preparation of Bovine Sperm

Preheat a centrifuge and water bath to 37 degrees celsius for washing and ensuring sperm is kept at appropriate temperature. Place 2 mL of TRIS medium in a centrifuge tube, then place the tube in the water bath. Additional TRIS mediums should also be placed in the water bath for later use. To carry sperm between locations (water bath to centrifuge), a thermos filled with water at 37 degrees celsius should be used. Take sperm straw out of the liquid nitrogen can and place in the thermos for 2 minutes to defrost. Pour sperm into a warmed centrifuge tube with 2 mL TRIS medium. Centrifuge sperm suspension in TRIS for 5 minutes at 200 xg. Ensure soft acceleration and deceleration is on to not agitate the sperm. After centrifuging, remove the TRIS-sperm suspension and place in warmed thermos immediately. Use the 100-1000ul micropipette to remove surfactant. Remove the additional warmed TRIS medium tube from the water bath and pipette an additional 1mL fresh TRIS medium towards the wall of the centrifuge sperm tube. Shake the tube gently to disperse, be careful to ensure sperm stay alive.

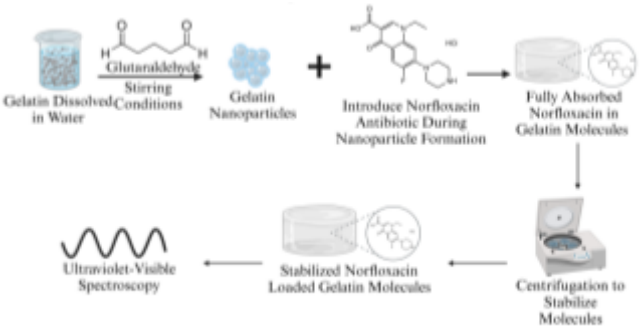

Figure 3: Preparing Norfloxacin Loaded Gelatin Particles

3.3 Drug-Loaded Bull Sperm Fabrication

Prepare 25 mL of DI water at 37 degrees. Add 0.25 g (1.1 % w/v) of type A gelatin to DI and use a magnetic stir set to 37 degrees at 250 rpm for 25 minutes to create a homogenous mixture. Use 2M HCL to correct the pH of the mixture to 2.5 (around 10 µm is needed). Allow to mix for 10 minutes at the same temperature and rpm. While still stirring, add 75 mL of acetone drop by drop. After adding acetone,

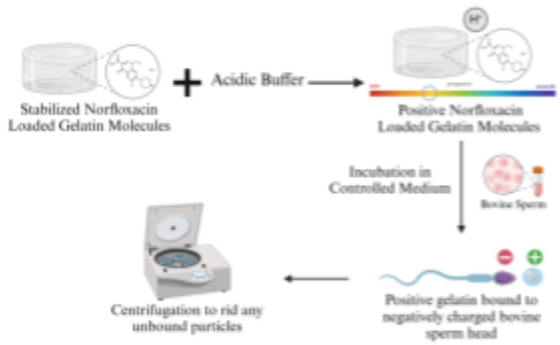

Figure 4: Preparing Norfloxacin Loaded Gelatin Particles

stir for another 10 minutes. Finally, add 100 mL of glutaraldehyde and stir for 20 minutes, at 37 degrees and 250 rpm. Centrifuge nanoparticles at 150 xg with a temperature of 37 degrees for 5 minutes. Afterwards, resuspend the nanoparticles in 1 mL of TRIS for every 5 mL of nanoparticle solution.

3.4 Growing Biofilm in Microfluidic Chip

Grow a culture of K-12 E. coli strain for 12 hours in a rich medium (i.e. Lysogeny Broth). Next, dilute the culture into a fresh lysogeny broth medium at 1:100. Add 100 µL of the diluted bacteria solution into the microchannel in the microfluidic chip. Incubate the microfluidic chip for 4-24 hrs at 37°C. Remove any unbound biofilms after incubation by washing microfluidic chip with sterile PBS. To dye the formed biofilms for fluorescence microscopy, 5 microns of 4 µM (micromolar) Calcein-AM solution and 15 microns of 4 µM (micromolar) must be added to the microchannel and mixed thoroughly. Calcein-AM will stain live bacteria with yellow-green fluorescence (λEX =490 nm, λEM =515 nm) and Propidium Iodide will stain dead bacteria with red fluorescence (λEX =535 nm, λEM =617 nm).

3.5 Introducing NP Bound Sperm to Biofilm

Introduce nanoparticle bound sperm to the microfluidic chip by pipetting into the channel. Note observations of the interaction between the biofilm culture and the NP bound sperm through video and fluorescence microscopy to specifically see interaction, and impact on biofilm respectively.

3.6 Characterization

- A bacterial viability experiment will be conducted using the live/dead stains calcein and propidium iodide. Images will be taken of the bacteria culture in the microfluidic chip illuminated by different wavelengths using fluorescent microscopy.
- To verify if the gelatin has absorbed the norfloxacin, an ultraviolet-visible spectroscopy will be done to identify the presence of the characteristic absorbance of norfloxacin (278 nm).
- A gelatin-to-sperm binding confirmation will be analyzed through microscopy.
- ImageJ, is a free open source program that will be used to quantify the amount of live and dead cells. Fluorescent microscopy images at the optimal wavelengths for calcein and propidium iodide will be taken then uploaded so the data can be effectively interpreted.
- High-speed cameras will be used to video the progression of biofilm disruption.
- To characterize biofilm formation, live-dead staining of biofilms will be used. Propidium iodide and calcein will be used and changes can be observed as biofilm grows.
- Excel will be used to organize data and help present it in a clear and concise manner to show the results of our experiments.

4. Risk and Safety

4.1 Hazardous Biological Agents

Live bacteria cultures, specifically *E. coli* (Biosafety Level I) and bull sperm (Biosafety Level I), will be handled throughout the experiment in Dr. Magdanz's lab. Safety equipment such as lab coats, gloves, and safety glasses will be worn at all times in the Biosafety II lab. We will be trained to handle materials and carry out experiments, while following WHMIS 2015. Biosafety guidelines will be followed in respect to the disposal of *E. coli* and bull sperm. Any materials containing *E. coli* will be treated with bleach then placed in the liquid bio-waste bin. Bull sperm will be rinsed down the drain.

4.2 Potential Risks

During experimentation, potential risks include the handling and preparation of bull sperm and the toxicity experiment to analyze results. Firstly, bull sperm is cryogenically preserved in a container filled with liquid nitrogen. We will be trained to handle the bull sperm and will be using proper equipment including lab coats, gloves, and safety goggles. Secondly, the propidium iodide stain is carcinogenic. To ensure safety during experimentation we will follow WHMIS 2015 by avoiding breathing in the substance and having adequate ventilation in the lab.

A thorough risk assessment will be completed by finding all possible risks in our lab and procedures. After this, we will evaluate the likelihood of the risk impacting us and what exactly it is caused by. Finally, we will create procedures to circumvent the risks and complete our experiments in a safe manner. In the event we are exposed to a dangerous substance, we will follow the proper protocol by reporting the incident and taking the proper steps.

Bibliography

Figures created using BioRender.com.

Abebe G. M. (2020). The Role of Bacterial Biofilm in Antibiotic Resistance and Food Contamination. *International journal of microbiology*, 2020, 1705814. https://doi.org/10.1155/2020/1705814.

Elgeti, J. et al. (2015). Physics of microswimmers—single particle motion and collective behavior: a review. Rep. Prog. Phys. 78. https://doi.org/10.1088/0034-4885/78/5/056601.

Hoque, M.E., et al. (2015). Gelatin Based Scaffolds for Tissue Engineering - A Review. *Polymers Research Journal*. https://www.researchgate.net/publication/272748016_Gelatin_Based_Scaffolds_For_Tissue_Engineering_-_A_review#pf5.

Lin, Y. et al. (1994). A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg. The Journal of cell biology, 125(5), 1157–1163. https://doi.org/10.1083/jcb.125.5.1157.

Magdanz, V. et al. (2019). Sperm-particle interactions and their prospects for charge mapping. *bioRxiv.* https://doi.org/10.1101/624510.

Nandakumar, V. et al. (2013). Characteristics of bacterial biofilm associated with implant material in clinical practice. Polym J 45, 137–152. https://doi.org/10.1038/pj.2012.130.

Nicastri, E. et al. (2021). Healthcare Associated Urinary Tract Infections. *International Society for Infectious Disease*. https://isid.org/guide/hospital/urinary-tract-infections/#:~:text=CAUTI%20accounts%20for%20approximately%2070,in%20Intensive%20care%20units%20respectively.

Simon, L. et al. (2016). Optimization of microelectrophoresis to select highly negatively charged sperm. *Journal of assisted reproduction and genetics*, 33(6), 679–688. https://doi.org/10.1007/s10815-016-0700-x.

Varma, A.K., & Arora, N. (2022). Design, Development and Statistical Optimization of Acyclovir-Loaded Protein Nanoparticles. *International Journal Of Pharmaceutical Sciences And Research,* 453-458. https://ijpsr.com/bft-article/design-development-and-statistical-optimization-of-acyclovir-loaded-protein-nanoparticles/.

Yao, M., Simoes, A. (2023). Urodynamic Testing and Interpretation. *Stats Pearls*. https://www.ncbi.nlm.nih.gov/books/NBK562310/.