

## Abstract

Minor bacterial infections can result in sepsis, a dangerous condition taking the lives of 215,000 Americans each year. Detection of bacteria, especially in low concentration, is a challenging task that can take up to several days. Specifically capturing bacteria from a biological sample would result in a concentrated filtrate that could be analyzed for the presence of bacteria. Here, we explored methods for bacterial capturing using vancomycin and a silicon nitride microfluidic chip. Optical microscopes were used to demonstrate their capturing abilities on various bacteria. Successful identification of contaminated samples would lower the risk of sepsis in patients.

## Background

- In a study of 519 bone marrow transplants, 189 patients were found to have 250 bacteria isolates. [1]
- Vancomycin is an antibiotic that binds a wide range of gram positive bacteria and when presented on a surface, it can be used to capture bacteria.
- Silicon nitride wafers can be fabricated to include about 3µm pores, which are useful in capturing bacteria.

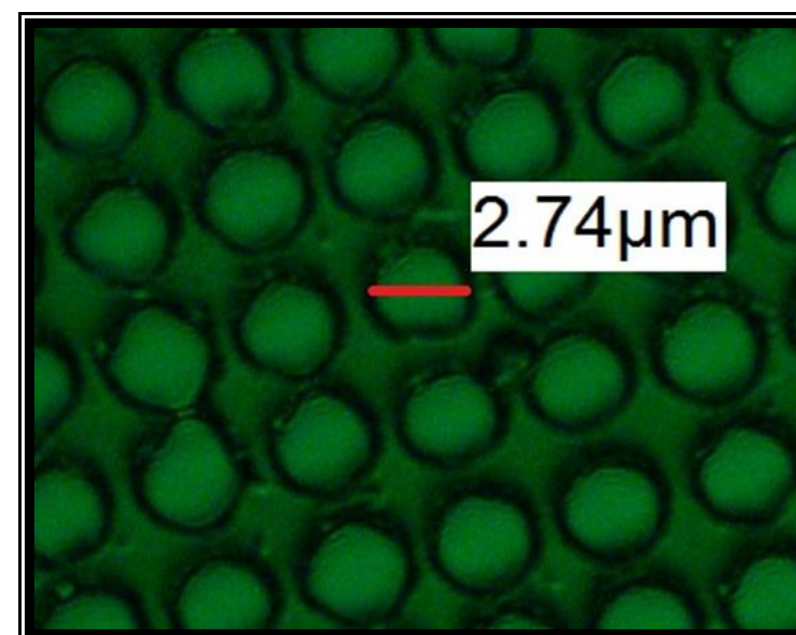


Figure 1. Silicon Nitride wafer at 1000X magnification.

## Method 1

Determining the best method to apply vancomycin to a glass slide for bacterial capturing:

- Glass slides were spin coated with glue and vancomycin at a concentration of 15.6µg/ml.
- A second set of glass slides were spin coated identically, but with an additional layer of vancomycin spin coated on top.



Figure 2. Lactobacillus captured with glue mixture on glass slide.

**Preparation:** Work on bacteria capturing requires making sure that slides are clean, labelled and angled properly. Additionally, to remove bubbles from the glue, the material has to be temporarily left in vacuum.

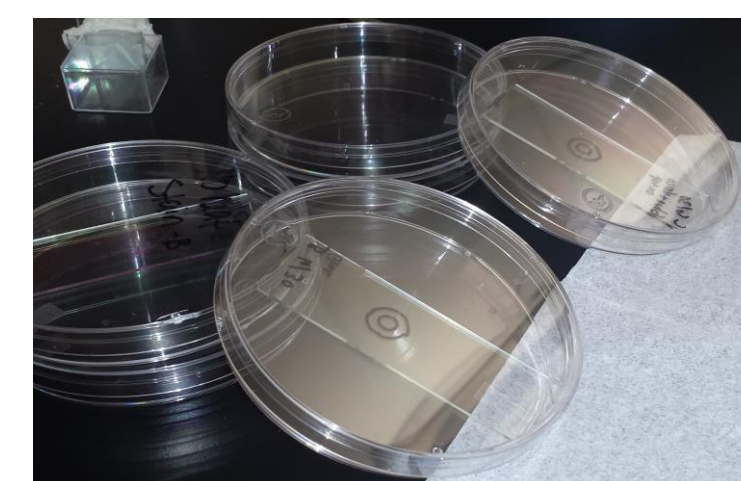


Figure 3. Left: Prepared glass slides ready for bacterial passage.

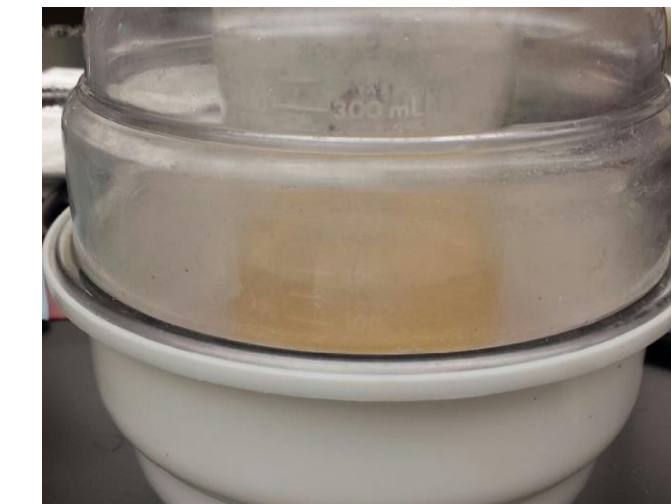
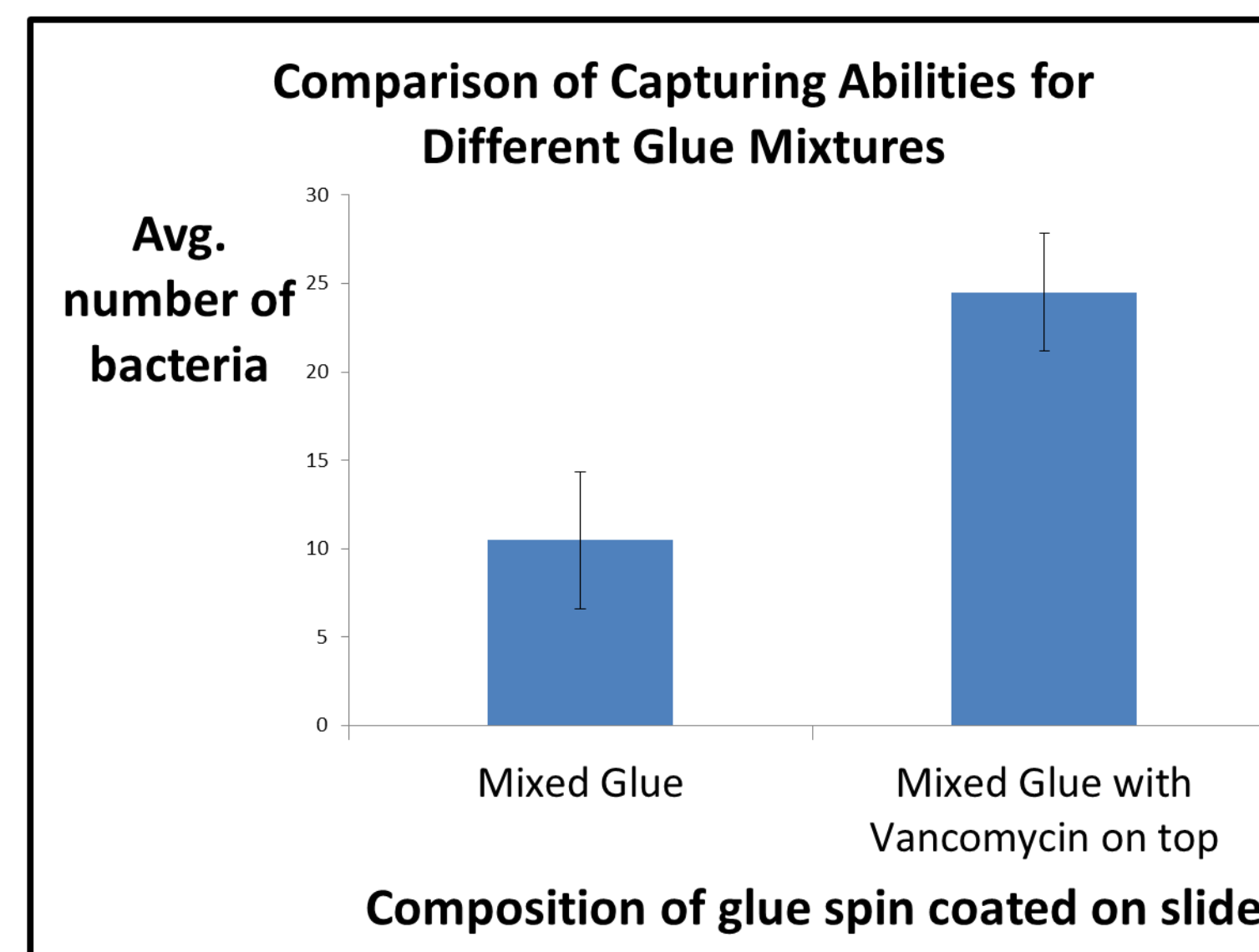


Figure 4. Right: Glue in vacuum for preparation.

## Method 1 Results

- Average number of bacteria found in four fields of view was used to complete a t-test and compare the two spin coating methods.
- Statistical analysis showed a significant difference between the capturing abilities of the coating methods.



## Method 2 Results

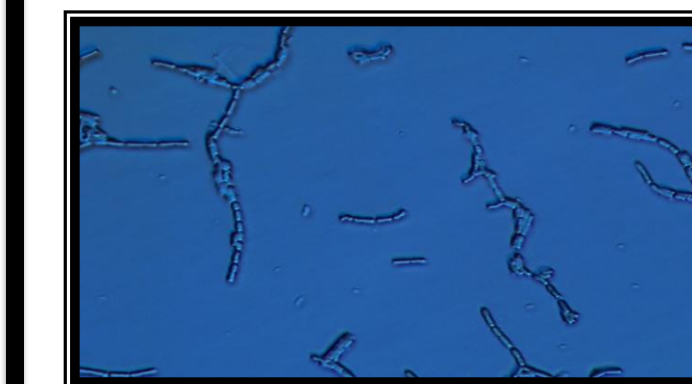


Figure 8. Initial bacterial solution concentration

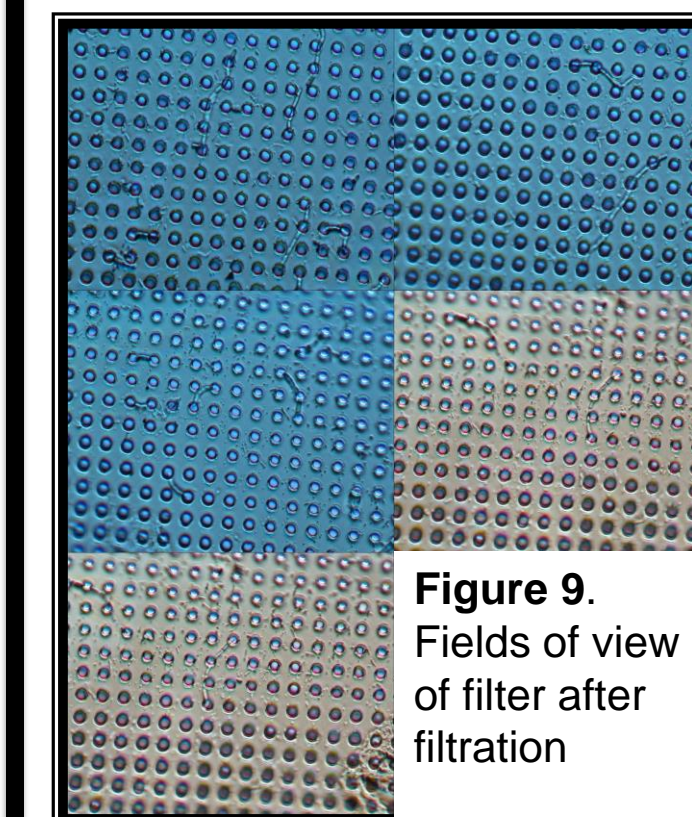


Figure 9. Fields of view of filter after filtration

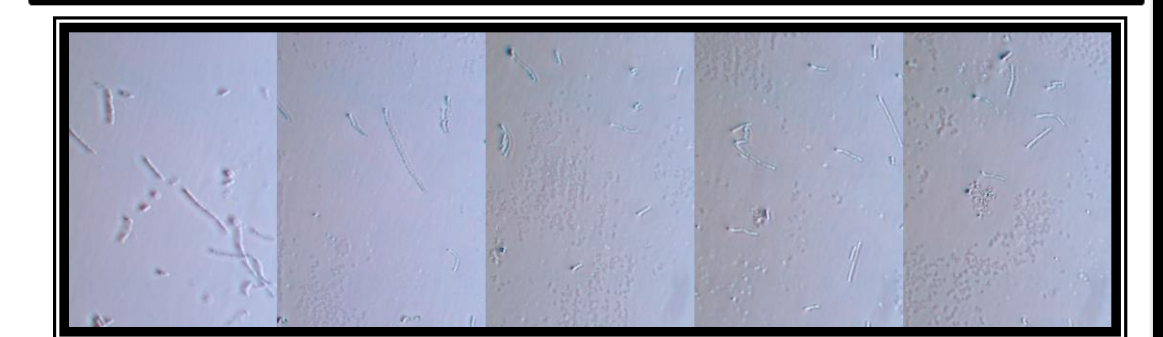
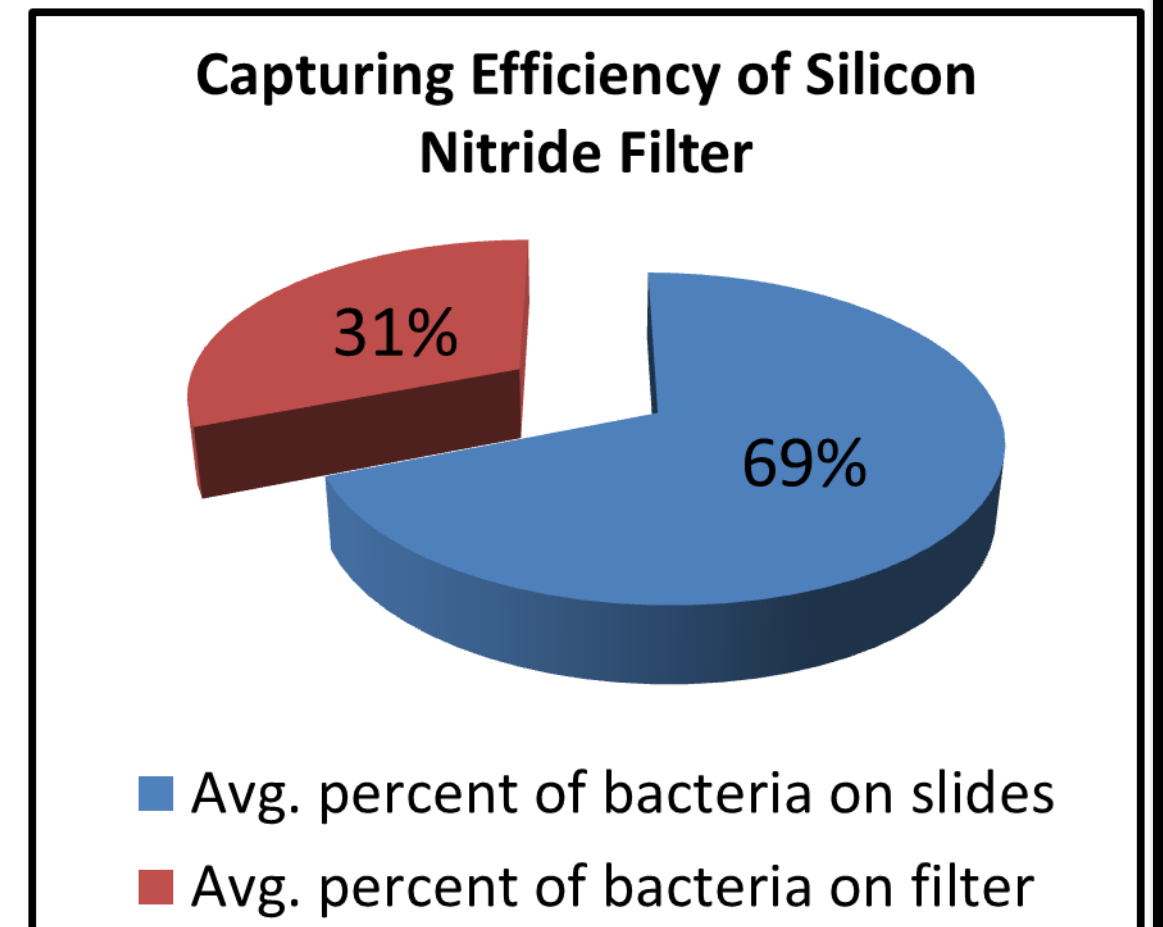


Figure 10. Five fields of view from slide after filtration

## Conclusions

We experimentally demonstrated two bacteria capturing methods. While chemical capturing using vancomycin works great when additional layer is spin coated on top of the special glue, pure mechanical filtration with a porous membrane also allows to concentrate bacteria on a surface. These results are very promising, and the planned future work would be to combine both methods and spin coat vancomycin on top of the filtering membrane.

## Reference

1. Berjan A. Collin, Helen L. Leather, John R. Wingard, and Reuben Ramphal "Evolution, Incidence, and Susceptibility of Bacterial Bloodstream Isolates from 519 Bone Marrow Transplant Patients", Clinical Infectious Diseases 2001 33: 947-953.

## Method 2

Determining the capturing ability of the silicon nitride wafer:

- Diluted bacterial solution was passed through silicon nitride membranes and onto a glass slide.
- Bacteria on filters and slides were counted to determine capturing ability of filter.

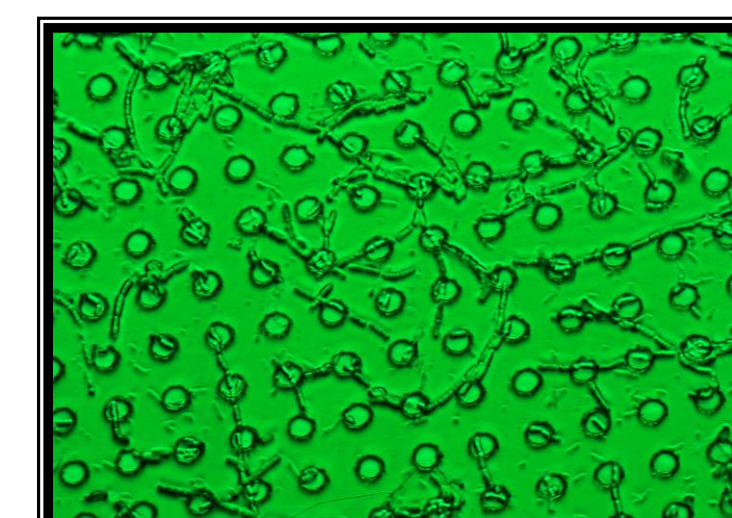


Figure 5. Lactobacillus bacteria filtered with silicon nitride wafer

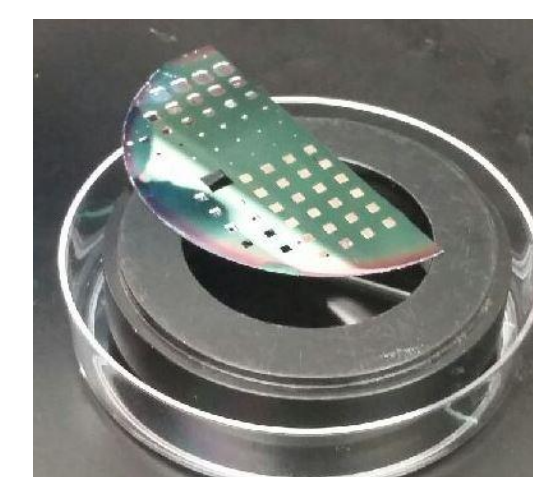


Figure 6. Silicon nitride wafer with 3 micron pores.

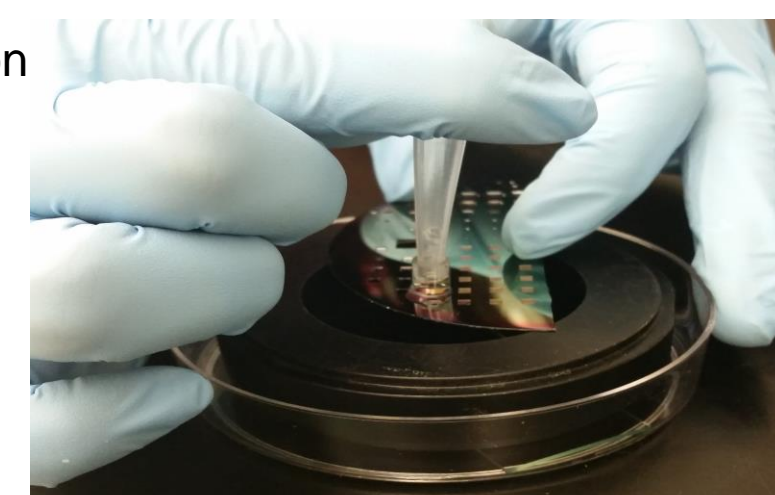


Figure 7. Set up for passing bacterial solution through wafer.

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