Quantifying Bacterial Adhesion of *Listeria monocytogenes to Silicon Nitride Under Water*

Esmeralda Gonzalez, Bong-Jae Park, and Nehal I. Abu-Lail

Department of Biomedical Engineering and Chemical Engineering, The University of Texas at San Antonio, San Antonio TX, 78249

Name: *Esmeralda Gonzalez*
Status: *Junior, Undergraduate Student*
Department: *Chemical Engineering*
Area of Study: *Bioengineering*
USDA/UTSA Mentor: *Dr. Nehal I. Abu-Lail*

WeARE Research Area

Listeria monocytogenes are Gram positive bacteria that can cause an array of diseases that range from fatal to curable. These diseases are largely food-borne. *L. monocytogenes* are also characterized as super bugs as they resist harsh chemical conditions in the environment. To cause diseases, these bacteria form biofilms that enhance their resilience in the environment. The first step of biofilm formation is initial adhesion to a surface. The infection begins when animals or humans consume contaminated food with the bacterium. Since the bacteria survives in soil and water and on food preparing surfaces and food packaging materials, investigating how strongly they adhere to such material will allow for the design of antifouling surfaces that can resist *L. monocytogenes* biofilm formation and ultimately prevent infections.

Motivation or Background

When bacterial infections are concerned, the adhesion of *L. monocytogenes* to surfaces plays a large role in their longevity and effects. Among foodborne bacterial pathogens and according to the Centers of Diseases, Control and Prevention, *L. monocytogenes are* considered the worst foodborne pathogens. This is due to the high transmission, hospitalization and fatality rates associated with them. These bacteria prove most detrimental to pregnant women, their children, older people and the immunocompromised. *L. monocytogenes* can be found in moist environments. They can also survive harsh environments. For example, they can grow under refrigeration, high temperatures, can handle high and low pH levels, as well as antibiotics. *L. monocytogenes* can form resilient biofilms that make treating infections associated with them extremely difficult. With that in mind, we are interested in quantifying how these bacteria attach to surfaces of interest under water in order to assess the abilities of the variable strains to form biofilms and initiate infections. By quantifying the strengths of interactions between bacteria and surfaces, design of antifouling surfaces that can resist these strains become possible.

Objectives

The following are goals of the study:
1. Measure the nanoscale adhesion forces acting between *L. monocytogenes* nine strains that vary in their pathogenicity and a model surface of silicon nitride under water using atomic force microscopy (AFM)
2. Quantify the adhesion energy for the nine strains of *Listeria monocytogenes* that vary in their pathogenicity to silicon nitride under water
3. Compare the adhesion strengths quantified for the nine strains to correlate adhesion and pathogenicity

When completed, the outcomes of our research should provide rationale for the design of antifouling surfaces to *L. monocytogenes*

Methodology

1. AFM adhesion data were collected on imaged bacterial cells of the genius *Listeria* (Figure 1).
2. Measurements were performed with 9 strains that vary in pathogenicity (Table 1) and on gelatin (negative control to which cells attached to).
3. For each strain, measurements were done on cells taken from three independent cultures. For each culture, measurements were done on 5 cells. For each cell, measurements were done on 15 points that covered the entire cell surface. As such, for each strain, 750 curves are to be analyzed.
4. Analyses were started with *Listeria monocytogenes* 874. To perform the analysis, data were adjusted in excel to get a zeroed curve in distance and force like that shown in Fig. 1B.
5. Areas under the curve were calculated for the 225 curves and box plots and histograms that summarize adhesion energies were generated.

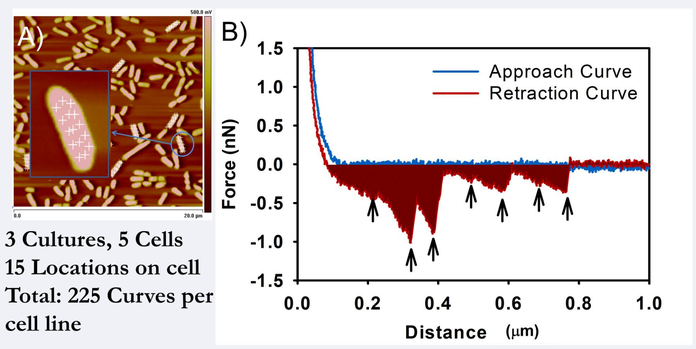

Figure 1: A) AFM images of *L. monocytogenes*. Locations of force measurements on a representative cell are shown. B) the retraction adhesion curve measured. The red shaded area represents the adhesion energy between the cell and silicon nitride in water. Arrows point to adhesion forces.

Model *L. monocytogenes*

Strain*	Serovar	LD50	Source	
15313	1	$>1.2\times10^{11}$	Rabbit	Avirulent
HCC25	4	3.5×10^{10}	Catfish kidney	
19118	4e	7.8×10^{9}	Chicken	Intermediate
19112	2	1.6×10^{9}	Human	
19115	4b	6.0×10^{8}	Human	Virulent
1002	ND	5.2×10^{8}	Pork sausage	
874	ND	$<8.0\times10^{7}$	Cow brain	
EGDe	½ a	$<1.1\times10^{7}$	Guinea pig	
51776	4b	NA	Dairy	

Increase in Pathogenicity

- Prof. Mark Lawrence (Mississippi State University) for providing few *L. monocytogenes* strains
- Prof. Doug Call for providing (Washington State University) for providing few *L. monocytogenes* strains

* Liu, D., et al., *Journal of Medical Microbiology*, 2003, 52, **1065-1070**

LD50 is the bacterial lethal dose required to kill 50% of mice in animal study

Table 1. Summary of the model *L. monocytogenes* strains investigated

Results

- The *Listeria monocytogenes* 874 is the first data set to be completely analyzed.
- The average adhesion energy taken for 75 curves measured for each culture were -209.4, -222.7, and -111.9 AJ.
- When all 225 curves were averaged, the overall adhesion energy of the *L monocytogenes* 874 was -163.6 AJ.
- The distribution of the adhesion energies are shown in the histogram below (Figure 2)
- As can be seen from the histogram, the energies are normally distributed.
- The histogram allows us to see that the most frequent Energy is in the -185 to -137 AJ range. This is supported by the interquartile range of the box plot which demonstrates half of the data is located within a range of -108 to -203 AJ. Both ranges support the idea of the value of -163.6 being an accurate average for the data.
- We can see an indication of an outlier in the histogram which was confirmed by the box plot an outlier of -374.388AJ.
- Figure 3 shows the distribution in the data in the form of a box plot. The boundaries of the box are the first and third quartiles in the data, the line is the median and the error bar is the standard deviation in the data.
- As can be seen from Figure 3, the data are heterogeneous
- The outlier observed in Fig. 2 pushes the overall average in a greater negative direction but is directly combated with the right-handed skew in the histogram. This skew can also be seen in the box plot as we contain a larger 75th percentile which is in the less negative direction.

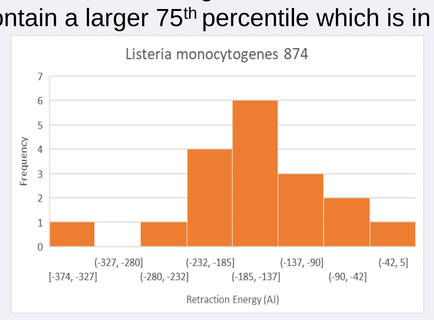

Fig. 2 : Histogram of *L. monocytogenes* 874 data

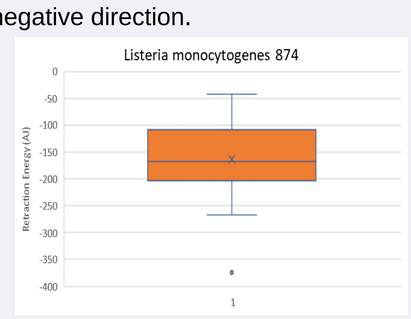

Fig. **3:** Box Plot for *L. monocytogenes* 874 data

Skills and Experience

I used my experience in Matlab to aid in the use of a new coding language of visual basic application (VBA) and through this research am gaining experience in VBA. This experience extends to the use of Macros in excel which is involved in learning the VBA.

I also use my mathematical and statistical experiences to interpret and analyze the data. The use of real data also strengthens these skills. I have had that only previously in a simplified classroom setting. This helps with understanding the significance of certain attributes found in graphs and tables and in deciding what is important.

What I Learned

Working with an abundance of data in excel, I had to learn to manipulate the data using Macros. Some of the data had to be hand moved as the data changed drastically between points of measurement so that had to be learned and processed quickly. What could be done in macros had to be learned from scratch having no previous experience. Having never worked with macros or VBA this was a challenge, but I was able to automate many aspects of the data processing such as the transfer of data to excel, the addition of tables and graphs to every sheet, and the use of the trapezoidal formula in achieving the energy retraction.

Future Plans

Considering the energy curves and analysis for only the *Listeria monocytogenes* 874 is completed at the moment, I will continue to process the data for the 8 other strains and the gelatin. I do plan in continuing improving and speeding up the data movement process so more time can be spent on analyzing the collected data. This will hopefully give more time to display and interpret the data in more significant terms.

This continuation will allow us to process to the ultimate goal of aiding in the efforts to create and design antifouling surfaces for these strains of bacteria

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