Biofilms are communities of microorganisms such as bacteria that attach to boundaries separating different phases of matter, such as a solid surface in contact with a liquid (Wimpenny et al., 2000). Characterizing the structure and mechanical properties of microbial biofilms is highly important to understanding their overall function, but current methods are individually insufficient to fully characterize the biofilm and there are large inconsistencies between methods (Böl, 2013). I believe that optical monitoring of the behavior of biofilm under a shear test in real time, rather than observing changes from before and after the test, could lead to a better characterization of biofilm structure and provide an explanation as to why different mechanical testing methods produce different results. I therefore propose to improve the mechanical characterization of biofilms by combining a current testing method, microjet impingement, with rapid three dimensional imaging. I believe that I am well suited for this project because of my experience in Professor Wells’ lab in the Civil and Environmental Engineering department that performs research on the use of biofilms in waste water treatment and my academic knowledge in the field of mechanical engineering.

Biofilms appear in nearly every aspect of life and have a variety of uses, such as water treatment, but can cause major problems in the fields of medicine and industry (Böl, 2013). They are a naturally occurring phenomenon that allows communities of microbes to remain and grow on a surface, in the presence of water and nutrients, and therefore interact and affect their immediate environment (Costerton et al., 1995). After a biofilm is formed and developed, small or large chunks of the biofilm can detach, and then can reattach to another surface, restarting the biofilm growth process (Sauer et al., 2002). Detachment therefore plays a major role in dictating the transport and spread of biofilms and acts as the final step in the biofilm life cycle. To understand the spread of biofilms, then, it becomes necessary to understand how and when detachment occurs. Usually, detachment of biofilm is caused by high mechanical stress caused by changing environmental conditions. (Stoodley et al., 2002a). What is currently unpredictable and unknown are exact detachment stress values of each type of biofilm.

As the biofilm structure evolves, cells begin to produce extracellular polymeric substances (EPS) that vary in structure and material properties depending on the species of microbe and the environment the biofilm is growing in. There are countless combinations of EPS, and this variety causes biofilms to almost always be heterogeneous and difficult to characterize. There are many mechanical characterization tests that induce stress in some way to determine the performance of a test material under said stress. These tests include dropping a plate on the test subject, high speed rotation of the subject, and compression or tension of the subject. However attempts to apply these techniques to biofilm have proven highly inconsistent with each other (Lappin-Scott and Costerton, 1995).

One simple but effective test of the shear and adhesive strength of a biofilm is microjet impingement, which directs a fluid jet perpendicular to the biofilm surface, creating a predictable distribution of stress that varies depending on distance from the center of the jet (Bundy, 2001). If the speed and size of the fluid jet are known, this radial stress distribution allows the stress at any point of the tested biofilm to be determined. Because the fluid jet flow is semi-turbulent, the stress is determined through adjusted laminar flow equations that correct for this less ideal case. These adjustments can be taken from previous literature where they were determined through straight line approximation and regression analysis (Vaishnav, 1983). While microjet impingement has many strengths, this method is limited to viewing the before and after results of testing (Böl, 2013). The most obvious failing of this limitation is that the difference between many small detachments and one large detachment would be indistinguishable. By using a rapid
three dimensional imaging technique, optical coherence tomography (OCT), to observe the biofilm response during microjet impingement, the detachment of the biofilm can be observed in real time which may lead to a more specific and accurate characterization of biofilm structure. This might provide us explanations on why different measurement techniques produce such different results. If successful this new testing method could also be used to compare different types of biofilms, such as those grown in differing environments.

Before performing the experiment, I will need to construct the necessary apparatus to perform microjet impingement test and observe the test with the OCT microscope. This microjet impingement apparatus will be based off of previous experimental methods (Kreth, 2004) and the shear stress will be calculated using information from Vaishnav (1983). This apparatus will consist of a small 0.2 mm diameter needle attached to a long tube, which is itself attached to a pump and reservoir of buffered saline solution. This will supply a steady flow of fluid. The end of the tube and needle will be fastened above the biofilm and the microscope will be mounted above the needle. Constructing this apparatus and making sure that it works and can produce the desired results is projected to take two to four weeks. I will need to design and construct the clamping mechanism, and find a proper arrangement for the nozzle and microscope. Such an arrangement will require the OCT microscope to be close enough to the biofilm to clearly observe any change, and also be unobstructed by the jet impingement equipment which must be held perpendicular above the biofilm. The biofilm will be obtained from the Rotating Annular Reactor (RAR) from the Wells lab, and is currently available for testing.

I will then calibrate the apparatus so that it produces an appropriate stress distribution that will create detachment. Biofilm grown on a rectangular plastic slip in the RAR will be placed in a petri dish containing buffered saline solution which will then be placed under the microjet impingement and OCT apparatus. The needle of the microjet impingement tester will be submerged in this buffered saline solution to prevent splashing and cause the radial stress distribution to behave more predictably and ideally, and I will perform the testing. I will perform initial test trials and observe the OCT data to determine whether or not detachment can be induced and is detectable. If detachment does not occur, I will slowly adjust the conditions of the experimental set up until such results are met. The easiest variation will be distance of the jet opening to the biofilm surface, but other values that I could adjust include needle size and pump speed. Depending on how quickly the apparatus is successfully calibrated and how quickly new biofilm will develop in the RAR, this step may take up to 2 months.

After I set up the apparatus and determine its effectiveness, the characterization of the biofilm can begin. After each test is performed, I will use the location of lesions in the biofilm to determine the corresponding detachment stresses and compare these results to previous results (Böl, 2013). I will also observe the OCT data to link the lesions to specific points in time and to observe the speed at which they form. I will repeat this process at least five times with the same type of RAR biofilm to determine if the OCT information is consistent between testing. This testing and analysis is projected to take 6 weeks.

I believe that I am suited for performing this experiment because of my experience in the Wells laboratory and mechanical analysis. I have been working in the Wells Lab for the past year and am experienced in working with and sampling the reactors as well as designing and constructing simple experimental apparatus. Constructing the microjet impingement tester for an independent project would be the natural next step for my role in the lab. Furthermore, as a mechanical engineer I have taken several classes in fluid dynamics and material and structural behavior which will aid immensely in the analysis and characterization of the biofilm structure.


