High-Frequency Ultrasound Characterization of Biofilms

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Abstract: Ultrasound non-destructive evaluation uses high frequency ultrasound to interrogate materials without affecting their performance or structure. The overarching goal of this study is to develop a high frequency, pulse-echo ultrasound system to non-invasively image and characterize biofilms. Analytical techniques used to characterize these biofilms include quantitative evaluation of spectral signature; quantitative evaluation of the integrated backscatter to estimate effective scatterer size; and quantitative evaluation of attenuation. This project will focus on biofilms grown in vitro on coverslips and then on explanted tissue to determine the feasibility of detecting and characterizing parameters such as biofilm thickness, viscosity, density, macrostructure and microstructure. These parameters are needed to understand image properties and design an efficient non-invasive protocol to identify, map the progression over time, and differentiate between single-species and multiple-species biofilms.

Administrative report: This project started on June 1, 2010. The funds requested were used to pay the stipend of Karla Hatfield who worked in the Biomedical and Materials Multimodal Imaging Laboratory throughout the summer.

Preliminary results:
Karla Hatfield joined my lab just at the time when I had purchased a new set of stepper motors and I had decided to update the data acquisition system using LabView.
Over the summer her tasks involved learning LabView which she had never used before; Learn how to use and control a digital oscilloscope; Learn how to control the stepper motors and come up with a graphical user interface that would automate data collection. In the span of three months she did all this and more. She actually started collecting data and designed an interface to display B-scans and C-scans and slicing the data sets at different planes.

Nontypeable Haemophilus influenzae (NTHi) harvested from the middle ear fluid of a patient with acute otitis media were used to grow biofilms. A 50MHz focused immersion piston transducer was used to collect data. Results from the preliminary experiments indicate that:

• We have been able to detect and image differences between the backscatter of a biofilm and a perfect reflector (Figure 1).
• These differences can be quantified in both the spatial domain and the frequency domain.
• In the frequency domain the peak frequency of the spectrum of the dish is at 42 MHz, which represents the resonant frequency of the transducer being used (Figure 2).
• However, the peak frequency of the spectrum of the biofilm is downshifted to 39 MHz, which is an indication of attenuation. This parameter could be used for further characterization (Figure 2).
Figure 1. Left panel: This image is a top-down view of the biofilm. The color bar indicates the relative amplitude, in volts, of the signal at each position. Note the variability which is due to scattering from the biofilm. This can be interpreted as well as a representation of the texture of the biofilm. Right panel: This is a top-down view of the bottom of the dish. The grading from yellow at the bottom of the image to dark blue at the top of the image is an indication of our dish not being perfectly perpendicular to the ultrasound beam propagation. However, when compared to the image on the left one can notice the smoothness of the scattering from the plane surface of the dish.

Figure 2. Comparison of frequency response from biofilm (left panel) and perfect reflector (right panel). The higher frequency oscillations in the signal carry information about the structure of the biofilm.

These preliminary results have led us to design the following experiments:

In support of investigating the possibility of using high frequency ultrasound as a novel methodology to create high-resolution images of non-typeable \textit{Haemophilus influenzae} (NTHi) biofilms will be grown on the rubber bottom of a sterile six-well polystyrene culture plate. Three milliliters of sterile brain-heart infusion medium, supplemented with 5% fildes, will be aseptically added to each well of the plate. Subsequently, 50µl of a 16 to 24 hour culture of NTHi will be added to the experimental wells and incubated under 95% humidity at 37°C for various time intervals ranging from a few hours to several days.

Various stages of biofilm development will be imaged using traditional methods of scanning electron microscopy (SEM) and laser scanning confocal microscopy (LSCM)
as a means of providing an accurate assessment of biofilm development for comparison and validation of the high frequency ultrasound biofilm imaging methodology. Additionally, complete MEM tissue culture media will be used to grow and maintain confluent monolayers of middle-ear epithelial cells (Detroit 562) under 95% humidity, 5% CO2 at 37°C on coated and non-coated circular glass coverslips. After establishment of a cellular monolayer of Detroit 562 cells, the replacement volume of complete MEM, supplemented with 5% filde's, will be added and allowed to incubate at 37°C under 5% CO2 and 95% humidity as a means to ascertain whether or not NTHi can initiate and maturate biofilm growth on middle ear epithelial cells.

The protocol will be repeated for *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Aloiococcus otitidis*, and multispecies. Dr. Robert Osgood from the Medical Sciences department will assist in the creation of the samples.

The approach to this aim will involve the implementation of a high-frequency ultrasound acquisition system. The system will operate in the pulse-echo mode at frequencies in the range of 20 to 50MHz. A single-element focused piston transducer will be mounted in an x-y linear stage, with programmable stepper motors that will allow collection of ultrasound backscatter in a raster mode.

The sequence of imaging and characterization experiments involve:

1. To remove system effects from the data it is necessary to calibrate the acquisition system. This will be accomplished by experimentally determining spatial and spectral characteristics of the acoustic sources.

2. Controlled experiments where biofilms are grown on coverslips that can be measured over time without destroying them. These experiments will allow us to ascertain whether ultrasound is a viable technology for imaging and characterizing biofilms. In this stage we propose to study single-species vs. multiple-species biofilms as they develop and mature. Is there a change in the signature echo that can be used to differentiate these biofilms? Candidates for this study include the major otopathogens of humans: *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Aloiococcus otitidis*. Parametric images can be created using this information.

   Immediately after data collection the biofilm sample will be allowed to continue growing. This imaging protocol will be repeated over the span of several days.

3. Controlled experiments where biofilms are grown on tissue. Depending on availability either excised tissue or tissue-mimicking materials will be used. Characterization of backscatter from tissue and tissue with biofilm on the surface will be conducted and possible differentiating parameters will be determined with the ultimate goal of creating parametric images that can conceivably be used for diagnosis.