

## FIB/SEM Tomography of Wound Biofilm

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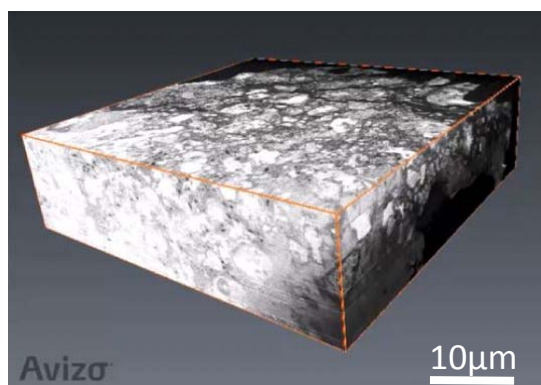
Biofilm has a complex architecture and show high tolerance to host immune responses and antimicrobial agents [1]. Biofilm infection of chronic wounds often makes them very resistant to treatment, and can be a severe health problem in patients with compromised immune systems. Despite the extensive study of biofilm in the past 15 years [2], the pathogenic biofilm structures remain to be characterized. DualBeam FIB/SEM tomography is a suitable tool to investigate three-dimensional (3D) structures of biological materials at intermediate to high resolution. In order to understand the biofilm architecture in animal model and clinical chronic wound, we have studied a porcine pre-clinical wound model involving single-species and multi-species infection as well as chronic wound from a patient using FIB/SEM tomography.

Domestic Yorkshire pigs were subjected to a full-thickness burn (2"x2"). A clinically relevant mixed-species infection was established. Patient tissue was collected from one year old chronic wound. Pig biofilm and patient wound tissue were cut into 200-500µm thick sections. Samples were chemically fixed and *en bloc* stained. After dehydration and infiltration, tissue sections were embedded in durcupan resin and incubated at 60°C for 2 days [3]. The resin embedded tissue was trimmed by ultramicrotome and mounted on an SEM stub. FIB slice and view datasets were collected on a Helios Nanolab 600 DualBeam (FIB/SEM) (FEI, Hillsboro). Images were processed and visualized using MIPAR[4], ImageJ [5], IMOD [6], Chimera [7] and Avizo (FEI, Hillsboro) software packages.

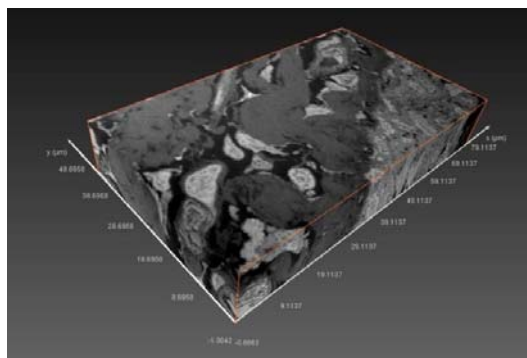
Biofilms formed by aggregated bacteria are encapsulated in extracellular polymeric substance (EPS). Our FIB/SEM tomography results revealed structures of pig biofilm (Figure 1) and patient wound (Figure 2). 3D structures demonstrated the non-uniform distribution of biofilm. Macrophages were found engulfing bacterium. Phagocytes were found squeezing through capillary walls to reach the infection sites. These data are providing unique insights that are improving our understanding of the process of biofilm wound healing.

## References:

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**Figure 1.** 3D volume views of pig biofilm obtained using FIB/SEM tomography



**Figure 2.** 3D volume views patient wound obtained using FIB/SEM tomography