Electron microscopy imaging of *S. mutans* biofilm profiles

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Bacterial Biofilms

Bacteria biofilms are colonies of cells and protective EPS.

Biofilm growth depends on conditions including nutrient concentrations.

Top-down SEM imaging provides information about substrate coverage but not thickness or features.

Can electron microscopy be used to obtain high resolution images of biofilm structure and formation?

Can electron microscopy be used to quantitatively determine the effect of sucrose concentration on biofilm formation and thickness?
Methods

Specimen Preparation
- Centrifuge tube
- 5 mL THY broth
- Inoculate with S. mutans
- Incubate 24 hours
- Petri dish
- Titanium coated substrate
- Add bacteria solution
- Add sucrose to concentration
- Incubate 24 hours
- Aspirate
- Fix biofilm with Methacarn
- Dehydrate with ethanol

Specimen Imaging
- Critical point dry and sputter coat the biofilm
- Specimen mounting spring
- Biofilm
- Titanium coated substrate
- Specimens imaged with FEI Helios in UK EMC
Profile images of *S. mutans* biofilms

Fragile cell features and EPS structures can be imaged.

Heights and formation of structures can be determined.

Varying the focus point allows us to image features away from the specimen edge.
Profile images of *S. mutans* biofilms

Heights of bacteria mounds depend on the concentration of sucrose.

Heights of bacteria mounds agrees with substrate coverage in top-down imaging.

Sucrose is necessary for biofilm growth, but too much sucrose hinders biofilm growth.

0 mM sucrose  75 mM sucrose  750 mM sucrose
Imaging Biological Specimens

Air drying can cause damage to biological specimens

High voltage and long dwell times can damage specimens