Necessity of Understanding Biofilm Formations in Closed Habitats

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Current ISS Biophysics Research

- **Macromolecular Biophysics (active)**
  - Protein Crystal Growth
    - Real-Time Protein Crystal Growth (RTPCG: Active)

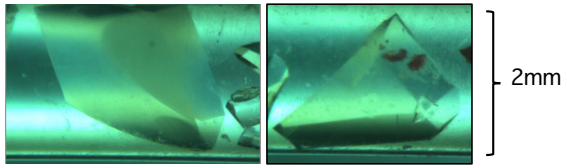

Image Credit: Prof. J Ng, Univ.of Alabama Huntsville

  - Nucleation Precursors in Protein Crystallization (Completed)
    - Explore the effects of solution shear flow on the nucleation of protein crystals, which may be enhanced or suppressed at different rates, including its complete absence, only possible in microgravity.

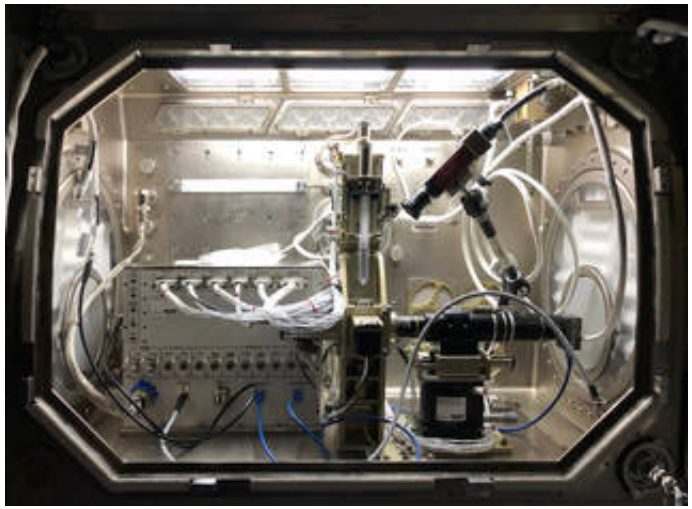
Image credit: NASA/Kevin Depew

  - Amyloid Fibril Formation (RSD: On ISS/Active)
    - Quantify the effects of shear on fibrillization in the bulk and at interfaces (air/water), which can only be performed in the microgravity environment where deleterious effects from sample/chamber surfaces are mitigated in container-less processing.

The Problem – Biofilm Formations

Biofilm formation can degrade materials (incl. clogging piping)
Biofilm can increase pathogenicity

Right: Biofilm formation inside the condensate plumbing at the inlet to the Russian condensate processor
Bottom left: Inside the Russian Functional Cargo Block (Zarya) during Expedition 3 (2001).
Bottom right: fungal contamination on panel in Zarya during Expedition 9 (2004) (Ott, 2018).

Image credit: NASA

Image credit: Roscosmos

Image credit: NASA

Ott, 2018: https://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20180006144.pdf

Biofilms Reference Experiments

Science Definition Team: **S. Gorti** (NASA/MSFC)**, Kevin Sato** (AR)**, Luis Zea** (U. Colorado)**, Robert McLean** (TSU), David Klaus, Ralf Moeller, Frank Muecklich, Louis Stodieck, Jennifer Barrila, Mark Ott, Cheryl Nickerson, Simon Clemett, Mayra Nelman-Gonzalez

Collaboration: Deutsches Zentrum Fuer Luft- Und Raumfahrt E.V (DLR)

ISS Facilities/Hardware: Bioserve for culturing and some imaging, LMM for Epi-Fluorescence Imaging

Objectives: Elucidate mechanisms of gravity-sensing in bacteria by comparing the structure and composition of biofilms generated in microgravity to those grown on earth. Develop knowledge of biophysical mechanisms of material/microorganisms interactions that can influence/inhibit the formation of biofilms on substrates. Characterize biofilm mass, thickness, morphology and the associated gene expression of microbial species growing on different substrate materials. Reference experiments thus need to investigate the growth of biofilms on a range of substrates with varying chemical and physical surface compositions and configurations, respectively. Reference experiments may also investigate the chemical inhibition of biofilm growth on a substrates.

Microgravity Justification: Certain microbes form novel biofilms in microgravity environments (Collins *et al*.).

Application/Impact: Knowledge of biophysical mechanisms of material/micro-organisms interaction that can significantly alter formation of biofilms can result in the development of new class of materials for long duration NASA missions. Development of such materials can also be useful in applications where biofilm formation can be detrimental to human health, particularly in health care industries. Usage of developed materials can also be tremendous cost saving in limiting the number of chemical agents used in removing biofilm formations from substrates.

Space Biofilms Team

University of Colorado Boulder — BioServe Space Technologies

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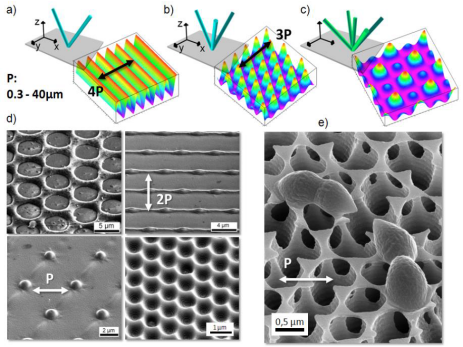
Research Aims

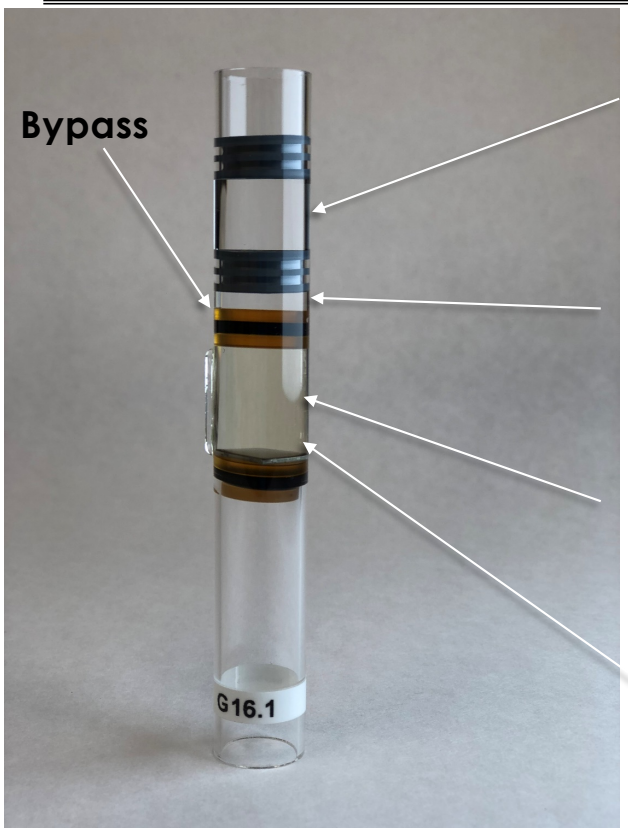
1. To characterize biofilm mass, thickness, morphology and the associated gene expression using different spaceflight-relevant microbial species and substrata materials
2. To elucidate the biomechanical and transcriptomic mechanisms involved in the formation of the "column-and-canopy" biofilm architecture observed in space
3. To characterize potential changes on the parameters being studied and on the genes that confer microorganisms with resistance to oxidative stress, acidity, and antimicrobials
4. To investigate the role of surface topology on biofilm formation



Various Patterned Coupons to be Examined


Incubator for samples

BioServe's FPA in Space Biofilms configuration



Chamber C – Fixative or Preservative (to terminate experiment in space). In our case, we use PFA for microscopy analysis, and RNALater for gene expression studies, both to be performed when samples return to Earth

Chamber B – Bacteria in stasis (separate from the growth medium to activate the experiment in space). In our case, uropathogenic *Pseudomonas aeruginosa*

Chamber A – Growth Medium. In our case, either LB Lennox supplemented with KNO_3 (to simulate wastewater) or modified Artificial Urine Medium (to simulate urine)

Material Coupon – Stainless Steel 316 (SS316), passivated SS316, cellulose membrane, silicone, silicone with nanotopography, and MIT's lubricant-impregnated surface (LIS)

Very Preliminary Flight Sample Comparison

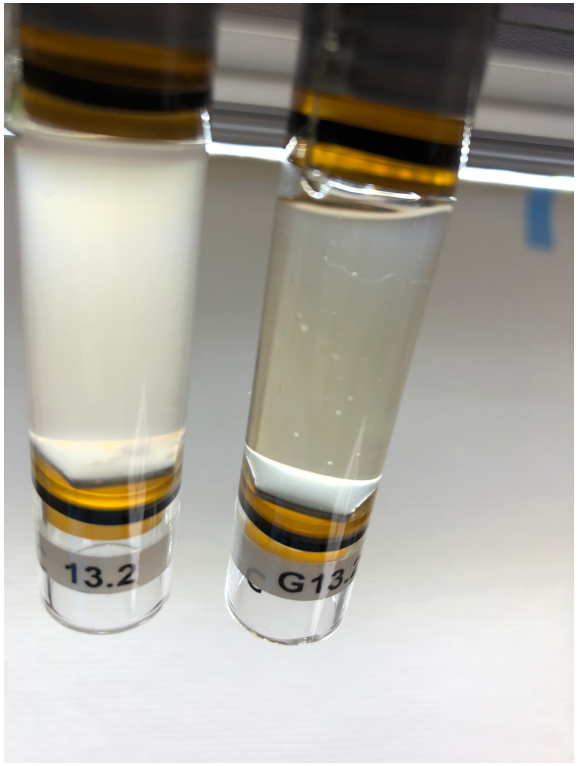


Image Caption: Image of two equivalent samples of *Pseudomonas aeruginosa* cultured in rich medium at 37°C for three days over a stainless steel 316 coupon.

- Two equivalent samples of *Pseudomonas aeruginosa* cultured in rich medium at 37°C for three days over a 310 SS coupon.
- On the left (13.2), the spaceflight sample with turbid culture
- and to the right (G13.2) its matching Earth control with mostly clear fluid.
- While the ground 316 SS coupon looks translucent, prior experiments have demonstrated biofilm formations.
- Growth in the flight samples are considerable, with some sedimentation.
- Cells also appear to adhered to substrate.
- We will know for sure once we do appropriate analysis.
- A strong red-brown hue also observed on substrate.