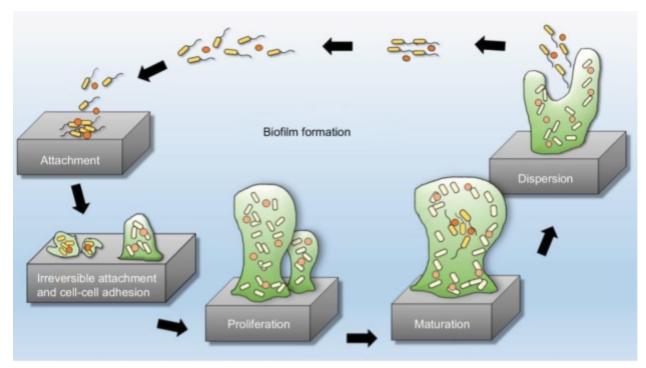


Bleary-eyed, you step into the shower. As the water begins to hit you, your senses awaken and you notice something pink around the drain and on the showerhead. You let the water rush over you and, after a big yawn, pass your tongue over your teeth. They feel slimy and rough and you begin to regret the decision to skip brushing them last night. The pink around the drain and the funny feeling on your teeth are examples of groups of single-celled organisms working in community. You have experienced biofilms.

Biofilms are comprised of bacteria, fungi, or protists that have adhered to a surface. There are five steps to the growth of a biofilm. In the first, planktonic organisms (free-floating/moving microorganisms) attach to a surface, such as your showerhead, teeth, pipes, rocks, or any other moist environment. As these individuals aggregate they begin to produce a slimy material that helps them to adhere more firmly to the surface, making them difficult to remove. This material also protects them from chemicals, such as antibiotics, that could destroy them. The colony of microorganisms grows as individuals reproduce (proliferation) and then matures. Finally, some of the individuals are released from the biofilm (dispersion) and become planktonic to start a new biofilm on a different surface.





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Biofilms aren't unique to our teeth, where we know them as dental plaque, or to our shower drains, where we know them as mold. Biofilms can be found throughout the environment and often cause harm. Medical devices such as catheters are at great risk of developing biofilms that can cause infections in patients. Catheters are tubes made of medical-grade materials that are inserted into the body, often to act as a drain of bodily fluids. Medical implants, such as joint replacements are also at risk for biofilm infection.

Biofilms can even be found on spacecraft! They have been detected on Soviet Salyut, Russian Mir, and American Skylab space stations and are now growing on the International Space Station. They grow in tubing and on other surfaces of the ISS, where they may cause materials to degrade and systems to clog. Just as dangerous, they increase the risks of infection for astronauts.





Biofilm formation inside the condensate plumbing at the inlet to the Russian condensate processor. Image: NASA.

Understanding these biofilms is vital to maintaining health and safety on Earth and on the ISS. Dr. Luis Zea of the University of Colorado – Boulder, is investigating the formation and prevention of biofilms.

Dr. Zea's research focuses on two biofilm organisms – bacteria (*Pseudomonas aeruginosa*) and fungus (*Penicillium rubens*). He is investigating how they grow on a variety of materials and whether or not he can reduce their growth by changing features of these materials. A variety of materials serve as substrates for the biofilms and include cellulose, aluminum, titanium, polycarbonate, quartz, silicone, stainless steel 316 (stainless steel used in building rocket engines). Dr. Zea has changed the surfaces of some of the materials as well, physically altering them by using lasers to carve channels into the substrates that are smaller than the bacterial or fungal cells. In addition, he has added lubricants to some of the textured surfaces to discourage the growth of the biofilms.





Fluid Processing Apparatus. Photo courtesy NASA

Dr. Zea will use a series of Fluid Processing Apparatus (FPA) placed together in a Group Activation Pack (GAP) for the bacterial experiment.



Group Activation Pack. Photo Courtesy NASA



These will be kept at 4°C at launch and will be then incubated at 37°C once the experiment is activated. The experiment will be activated by a crank which will push a growth medium (red in the image) to the bacteria which have been suspended in a medium (blue in the image) that keeps them alive without allowing them to grow, then on to a 1cm² X 1mm thick coupon of substrate material. These will be incubated for 1, 2, or 3 days, then the FPA will be cranked again to move the coupon to a preservative (yellow in the image) known as PFA (paraformaldehyde).

To grow the fungal biofilms, fungal spores will be placed on the coupons of substrates and grown on plates with 24 wells in a chamber at 25°C and 100% humidity for 10, 15, or 20 days. They will then be placed in PFA, just as the bacterial biofilms were, to preserve them until they return to Earth.

To analyze the growth of the biofilms, Dr. Zea is studying the mass, thickness and morphology (surface roughness and structures) of the biofilms. Using confocal laser scanning microscopy, he will measure the height of the biofilm and look for structurally significant columns and canopies of biofilm. Additionally, he will be analyzing gene expression changes in the space flown biofilms as compared to control biofilms being grown on Earth. Previous studies have shown that when microbes are in space, they increase their virulence (ability to cause disease) and may show resistance to antibiotic/antifungal medications. Looking at which proteins are produced by the biofilms will help Dr. Zea and other researchers to see which genes are expressed by the microbes and then to determine which genes are most active in them.

Dr. Zea expects to see thicker biofilms formed in space as compared to Earth. Additionally, he anticipates seeing genes associated with antimicrobial resistance and increased virulence in the space grown biofilms. Finally, he believes that the biofilms grown on coupons with the lubricant impregnated surfaces will be thinner than those grown on the same substrates without the lubricant. He needs your assistance in confirming or refuting his hypothesis.

One of the most fascinating aspects of this research is that Dr. Zea is not a biologist, even though he is studying microbiology in this experiment! Dr. Zea's background is as an aerospace engineer. His path to this research is very interesting and starts with him as a middle school student who was more interested in skateboarding than studying. Check out the <u>interview with Dr. Zea</u> to learn more!