Space Life Sciences Research Highlights

Spaceflight Promotes Unique Bacterial Biofilm Structure

Many bacteria grow attached to surfaces in complex, three-dimensional communities called biofilms. Although these films are naturally associated with nearly all moist surfaces, some biofilms have been associated with infections, biofouling, and surface corrosion. Space Biology-funded researcher Cynthia Collins has studied biofilm formation during spaceflight. While bacteria are known to grow better in the spaceflight environment than they do on ground, Collins discovered that they also form biofilm structures unlike anything seen on Earth! Such research could lead to better medical treatments for people on Earth and is critical to pave the path for human travel to Mars.

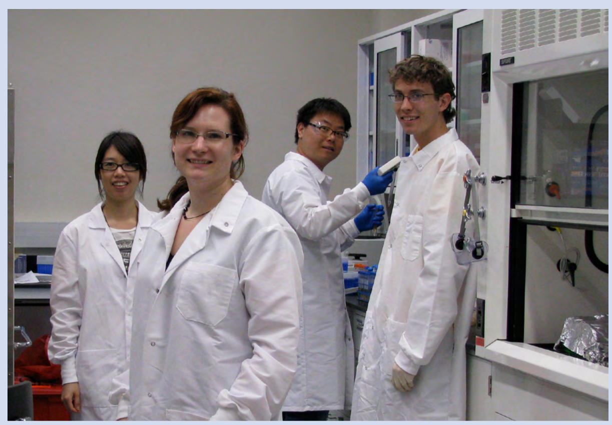
By the time the Russian space station Mir reached the end of its life in March 2001, its quartz windows had been damaged, and some of its metal surfaces had corroded. The culprit was bacterial biofilms. Many bacteria exist in liquids as free-roaming cells, but on surfaces they most often grow in three-dimensional communities called biofilms. Evidence of biofilms has been found in Earth's ancient fossil record, as well as in almost every terrestrial habitat today. They cover your teeth in the morning and contribute to the scum in your shower. "That's really how bacteria exist [on surfaces]," says Cynthia Collins, an associate professor of chemical and biological engineering and of biology at Rensselaer Polytechnic Institute.

Biofilms are important in natural systems, providing many beneficial services, but in other cases, they can be problematic. Biofilms are composed of bacterial cells and a sticky substance (exopolysaccharide) that the cells produce. The structures provide some advantages for bacteria, such as protection from environmental stress. Collins notes that biofilms can form quickly and do their job well, which is why it's so hard to eliminate them from surfaces. As a result, infections can be more difficult for immune systems and antibiotics to defeat when they exist as biofilms.

Collins studies the community behaviors of bacteria and how changes in the environment affect those behaviors. As part of that research, she has sent bacteria on two NASA spaceflight missions and has found the first evidence that spaceflight affects bacterial biofilm formation and is studying molecular mechanisms of how the formation is altered. Collins' research not only highlights the potential for harm from biofilms during spaceflight missions, but could also lead to new ways for dealing with the problems caused by biofilms, both in space and on the ground.

Studying Biofilms During Spaceflight

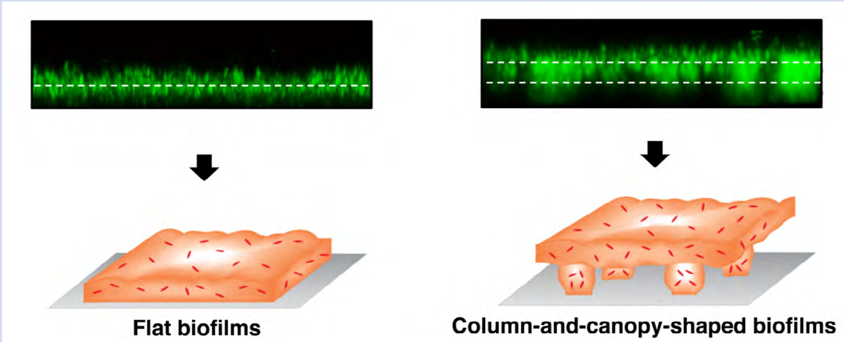
Conducting research in spaceflight is inherently more complex than similar experiments performed on the ground. For one, the experiment is performed by an astronaut, not the researcher. "Handing off your experiment to an astronaut, while amazing, is a little nerve-wracking," Collins says. Beyond that, scientists who design experiments for spaceflight must deal with limitations on the number of samples they can send to the International Space Station (or space or orbit), which means they have to carefully choose the conditions most likely to be informative.



Cynthia Collins of Rensselaer Polytechnic Institute studies the behaviors of bacterial communities, including how bacterial biofilms form in altered gravity. Credit: C. Collins

Collins and her colleagues studied two common bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are often found in surface biofilms. These organisms are also opportunistic pathogens. *P. aeruginosa* was the cause of the urinary-tract infection that added complexity to the near disaster on board Apollo 13. Collins' team sent up its experiments on two Space Shuttle missions, STS-132 and STS-135, which allowed them to replicate their findings—and also to learn from their experiences.

At the end of the experiment, some samples were mixed with a fixative so that cells would stop growing. All procedures were replicated on the ground so that the group could compare the biofilms formed during spaceflight with those formed under conditions of terrestrial gravity.



In terrestrial gravity, P. aeruginosa *forms flat biofilms (left). During spaceflight, the bacteria form biofilms with a column-and-canopy structure (right) that looks somewhat like a miniature rainforest.* Credit: C. Collins

When Collins and her colleagues compared motile wild-type strains of space-flown *P. aeruginosa* with samples grown on the ground, they found that "*Pseudomonas* that was grown during spaceflight actually grew more." Not only were there more cells, but the density of growth was greater than in equivalent samples grown on the ground. The real surprise, however, was that instead of being a flat slime on a surface or forming mushroom-like structures, as is commonly seen on Earth under static and flowing fluid conditions, respectively, Collins reported that "what we saw looked much more like a rainforest," with trunk-like columns underneath a big canopy on top. The team reported their findings in 2013 in *PLoS One*.

Collins and her team are still working to determine how these column-and-canopy structures form and what this newly discovered structure means with respect to function of the community, but they have some theories. Flagella-driven motility is necessary to form the mushroom structures on Earth. Non-motile mutant strains form flat films only, whether they were grown on Earth in a flowing or static fluid, or on orbit. Collins thinks the two structured film types (mushroom-like and forest-like) probably use similar biological mechanisms that are somehow linked to bacterial motility and to hydrodynamic conditions. When a motile strain of *P. aeruginosa* was grown in terrestrial gravity, the bacterium produced the mushroom-like structures in flowing conditions and a flat slime in static conditions. However, under the unique static conditions in space it grew in the column and canopy structure.

The bacteria aren't necessarily responding to altered gravity. "They don't have [gravity] sensors on the outside of their walls," Collins notes. But microgravity changes other characteristics of the bacterial environment such as convection, diffusion, etc., to which the microbes are sensitive. These changes not only affected the bacteria growing in biofilms but also the cells that were left suspended in the growth medium.

As part of the *P. aeruginosa* experiment, Collins' team manipulated the nutrient concentration of the growth medium. They found that in a limited-nutrient environment, biofilms formed in spaceflight were thicker and contained more cells than those developed in simultaneous ground controls. "Microbes like a well-mixed system," Collins notes. In spaceflight, motile cells tend to be more uniformly distributed, "so everyone gets a little bit of the nutrients, everyone can divide a few times." In terrestrial gravity, though, other scientists have noted that non-motile cells in suspension tend to settle at the bottom of the container, where the higher concentrations deplete the nutrients and interfere with each other.

Looking Towards Mars

One of the most important factors for improving our understanding of microbes—and their good and bad behaviors—is characterizing how they interact with their environment, how they change the environment, and how the environment changes them. Collins notes that part of that change is microgravity. If we have better knowledge of what happens in spaceflight, "we can improve our fundamental understanding of how microbes behave, and what are the consequences of that behavior."

Such information could, of course, help in designing strategies to fight against hard-to-defeat biofilm-forming pathogens that kill millions of people worldwide every year, and it could help to reduce material degradation or system failure in space due to biofouling. These and subsequent findings could prove vital as humans venture beyond our home planet.

Further Reading

Kim W, Tengra FK, Young Z, Shong J, Marchand N, Chan HK, Pangule RC, Parra M, Dordick JS, Plawsky JL, and Collins CH. Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa*. PLoS One. 2013;8(4):e62437.

Kim W, Tengra FK, Shong J, Marchand N, Chan HK, Young Z, Pangule RC, Parra M, Dordick JS, Plawsky JL, and Collins CH. Effect of spaceflight on *Pseudomonas aeruginosa* final cell density is modulated by nutrient and oxygen availability. BMC Microbiol. 2013;13:241.

Klaus D, Simske S, Todd P, Stodieck L. Investigation of space flight effects on *Escherichia coli* and a proposed model of underlying physical mechanisms. Microbiology. 1997 Feb; 143 (Pt 2):449-55.