Biofilm 7 (2024) 100182

Contents lists available at ScienceDirect

Biofilm

journal homepage: www.sciencedirect.com/journal/biofilm

# Space biofilms – An overview of the morphology of *Pseudomonas aeruginosa* biofilms grown on silicone and cellulose membranes on board the international space station

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ARTICLE INFO

*Keywords:*
Microgravity
Surface topology
Bacteria
Microscopy

ABSTRACT

Microorganisms' natural ability to live as organized multicellular communities – also known as biofilms – provides them with unique survival advantages. For instance, bacterial biofilms are protected against environmental stresses thanks to their extracellular matrix, which could contribute to persistent infections after treatment with antibiotics. Bacterial biofilms are also capable of strongly attaching to surfaces, where their metabolic by-products could lead to surface material degradation. Furthermore, microgravity can alter biofilm behavior in unexpected ways, making the presence of biofilms in space a risk for both astronauts and spaceflight hardware. Despite the efforts to eliminate microorganism contamination from spacecraft surfaces, it is impossible to prevent human-associated bacteria from eventually establishing biofilm surface colonization. Nevertheless, by understanding the changes that bacterial biofilms undergo in microgravity, it is possible to identify key differences and pathways that could be targeted to significantly reduce biofilm formation. The bacterial component of Space Biofilms project, performed on the International Space Station in early 2020, contributes to such understanding by characterizing the morphology and gene expression of bacterial biofilms formed in microgravity with respect to ground controls. *Pseudomonas aeruginosa* was used as model organism due to its relevance in biofilm studies and its ability to cause urinary tract infections as an opportunistic pathogen. Biofilm formation was characterized at one, two, and three days of incubation (37 °C) over six different materials. Materials reported in this manuscript include catheter grade silicone, selected due to its medical relevance in hospital acquired infections, catheter grade silicone with ultrashort pulsed direct laser interference patterning, included to test microtopographies as a potential biofilm control strategy, and cellulose membrane to replicate the column and canopy structure previously reported from a microgravity study. We here present an overview of the biofilm morphology, including 3D images of the biofilms to represent the distinctive morphology observed in each material tested, and some of the key differences in biofilm thickness, mass, and surface area coverage. We also present the impact of the surface microtopography in biofilm formation across materials, incubation time, and gravitational conditions.

The Space Biofilms project (bacterial side) is supported by the National Aeronautics and Space Administration under Grant No. 80NSSC17K0036 and 80NSSC21K1950.

## 1. Introduction

Bacteria can live as two types of populations: planktonic bulks (free-living) or biofilms. Biofilms – the most common type of bacterial population found in nature – are clusters of cells adhered to a surface and/or to each other while embedded in a self-produced extracellular matrix (EM) [1]. These multicellular communities are highly organized and possess unique survival advantages such as increased tolerance to environmental stress due to their protective EM [1–3].

Biofilms have increased antibiotic resistance, which worsens infections and poses a threat to human health [4]. In fact, 60% of all

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https://doi.org/10.1016/j.biofilm.2024.100182
Received 20 November 2023; Received in revised form 22 January 2024; Accepted 4 February 2024
Available online 5 February 2024

**Acronyms/abbreviations**

| | |
|---|---|
| EM | Extracellular Matrix |
| FPA | Fluid Processing Apparatus |
| FDR | False Discovery Rate |
| GAP | Group Activation Packs |
| ISS | International Space Station |
| mAUMg-hi Pi | modified Artificial Urine Media supplemented with glucose and high phosphate |
| PA14 | *P. aeruginosa* UCBPP-PA14 strain |
| PBS | Phosphate Buffered Saline |
| PFA | Paraformaldehyde |
| PI | Propidium Iodide |
| USP-DLIP | Ultrashort Pulsed Direct Laser Interfering Patterning |
| UTIs | Urinary Tract Infections |

microbial infections and 80% of chronic infections have been associated with biofilm formation [2,5], urinary tract infections (UTIs) being one of them [6]. The most common predisposing factor to develop a UTI is the use of catheters [7,8], usually made out of silicone. The inside and surroundings of the catheter, which accumulate urine, are ideal places for bacterial strains like *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* to form biofilms and subsequently establish a UTI [9], in particular in immunosuppressed patients [10].

Spaceflight, with exposure to microgravity and other space stressors, has shown to cause dysregulation in astronauts' immune system [11,12]. Furthermore, microgravity can alter bacterial [13–16] and biofilm behavior in unexpected ways [17,18]. For example, biofilms of *P. aeruginosa* PA14 grown on cellulose membrane were reported in one study to present a column-and-canopy structure in microgravity, not previously observed on Earth [19]. A combination of these microgravity effects poses a risk to astronauts' health and mission success. Despite the efforts to eliminate microorganism contamination from spacecraft surfaces, it is impossible to prevent human-associated bacteria from eventually establishing biofilm surface colonization. Remarkably, the third most likely reason for an emergency medical evacuation from the International Space Station (ISS) is urosepsis [20], sepsis caused by a severe UTI.

Because bacteria will come with us in space exploration, on short- or long-term missions, it is necessary to understand the changes that bacterial biofilms undergo in microgravity to potentially identify strategies for biofilm control [21]. The bacterial component of the Space Biofilms project, performed at the ISS contributes to such understanding by characterizing the morphology and gene expression of *P. aeruginosa* biofilms formed in microgravity with respect to ground controls. Biofilms were grown for one, two, and three days of incubation (37 °C) over six different materials. This manuscript presents the morphology results for biofilms grown on catheter-grade silicone (included in this experiment due to its medical relevance), catheter-grade silicone with ultrashort pulsed direct laser interference patterning (USP-DLIP) (to test microtopographies as a potential biofilm control strategy and their effect on bacterial/surface interaction), and cellulose membrane (to replicate the previously-reported column-and-canopy structure observed on biofilms formed in microgravity).

## 2. Material and methods

### 2.1. Biofilm culture conditions

Biofilms of *P. aeruginosa* UCBPP-PA14 were grown at 37 °C in anaerobic conditions: one set on Earth's gravity and another, equivalent set, in microgravity on ISS. The culture medium for biofilms grown on the materials reported in this manuscript was a modified artificial urine medium supplemented with glucose and high phosphate (mAUMg-hi Pi), a urine-like medium. The composition of mAUMg-hi Pi used in this experiment is described in Ref. [22]), and was adapted from the original recipe of Kim et al. [19,23].

### 2.2. Biofilm formation surface materials

Three materials were tested as biofilm formation surfaces: (*i*) silicone (catheter grade Dow Corning, Cat. Q7-4840), (*ii*) the same silicone with a 3 μm line-like periodic microtopography generated by USP-DLIP (henceforth referred to as silicone DLIP), and (*iii*) cellulose membrane (Millipore, Cat. GSWP01300). The DLIP pattern was applied utilizing a femtosecond pulsed laser source (Spitfire XP by Spectra Physics, 100 fs pulse duration, 800 nm wavelength) and a two-beam interference setup further described in Ref. [24,24]). Pattern periodicity was adjusted to 3 μm to closely fit the size scale of single bacteria cells. Planar patterning was achieved by scanning the surface in a lateral hatching distance of 57% at a fluence of 0.8 J/cm$^2$ and 1 kHz pulse rate, inducing a pattern depth of approximately 700 nm [22]. Silicone was cut into 1 cm$^2$ coupons and the four corners were trimmed to facilitate integration into the spaceflight hardware. Silicone DLIP was cut into 0.635 cm$^2$ coupons (no edge trimming needed). Both, silicone and silicone DLIP, were washed with 1% liquinox (Alconox Inc, Cat. 1201) and sterilized by autoclaving at 121 °C for 30 min. Cellulose membrane coupons came sterile in circles of 1.4 cm diameter that were ready to use. For more details about coupon preparation and integration into the hardware refer to the detailed methodology on [22].

### 2.3. Sample preparation and experiment performance

The sample preparation and performance of the Space Biofilms project, including the biofilms grown in mAUMg-hi Pi relevant to this manuscript, are described in detail in Ref. [22]). In brief, ground and spaceflight samples were prepared using BioServe Space Technologies' Fluid Processing Apparatus (FPA) and Group Activation Packs (GAP) hardware. Each FPA contained one sample and was launched in a three-chamber configuration that allowed to have the sterile growth medium and tested material coupon, the liquid bacterial inoculum in stasis, and paraformaldehyde (PFA) as fixative, in a separate fashion. Loaded FPAs were assembled into the GAPs, launched, and stowed on ISS at 4 °C until experiment activation.

Assembled GAPs were either kept on Earth as ground controls or sent to the ISS. Samples that went to the ISS, were taken out of cold stowage for experiment activation. The activation happened when the first and second chamber were mixed to introduce the bacteria into the culture medium followed by incubation at 37 °C for 25, 48, or 72 h. After the respective incubation time, termination of the experiment was performed by mixing in the third chamber to fix the samples, followed by transfer to cold stow at 4 °C until processing. Ground samples were performed asynchronously, 2 h after flight samples, to ensure the microgravity timeline was followed as closely as possible.

### 2.4. Morphology analysis

After the spaceflight samples returned to Earth, both sets (flight and ground) were prepared for confocal microscopy, described in detail in Flores et al. [25]. In brief, samples were retrieved from the FPAs, gently washed with phosphate buffered saline (PBS), then nucleic acids were stained with propidium iodide (PI) and lipids stained with FilmTracer FM 1–43 Green Biofilm. The stained samples were imaged using a confocal microscope and the micrographs were used to quantify biofilm mass, thickness, and surface area coverage using the software COMSTAT2 [26–28]. Automatic thresholding was used for the COMSTAT2 analysis of cellulose biofilms, but a manual threshold was input for silicone samples. The manual threshold was obtained by calculating the mean intensity value of the top layers of the biofilm and subtracting two

standard deviations, this was done due to a lack of a clear end of intensity signal of the biofilm over the silicone. Data was non-parametric and statistically compared between groups using Dunn's Test with Bonferroni correction.

## 3. Results and discussion

*P. aeruginosa* biofilms can have different morphologies depending on the surface upon which they grow. This, in turn, can influence their physiology [29–31]. Different morphologies range from biofilms looking as low density porous patches or amorphous mountains on stainless steel [25,32], high density porous mats on plastic [33], an even slimy bulks on silicone [34]. Results show that the surface materials (silicone vs cellulose) and surface topography (silicone vs silicone DLIP) had an effect on *P. aeruginosa* biofilm morphology and confirm that the effect is maintained in microgravity.

A summary of the median values of biofilm mass, thickness, and surface area coverage per material is listed in Table 1. In the following subsections we will discuss the main morphologic characteristics of these biofilms.

### 3.1. Silicone and silicone DLIP biofilms

On Earth, *P. aeruginosa* was capable of establishing biofilms on both silicone and silicone DLIP. For both materials, biofilms looked like a mat covering almost all surface for all days of incubation (Fig. 1). One similarity between biofilms of silicone and silicone DLIP, is the presence of mounts/bulks of high nucleic acid concentration that can rise several micrometres above biofilm surface. This bulks seemed to be formed of intertwined filaments and occasional *P. aeruginosa* cells. It is possible that these nucleic acid structures are formed by lysis of small parts of the biofilm cells, in preparation for vertical growth as the stalk of a mushroom-shaped structure [35,36]. On the other hand, a key difference of silicone DLIP biofilms is the distinct repetitive linear pattern of valleys and peaks that follows the same pattern as the microtopography of the surface (Fig. 2). On silicone the biofilm mat looks mostly smooth with small clumps of cells, giving it a mottled appearance. The biomass and thickness of biofilms on silicone DLIP tend to decrease in time, while for biofilms on silicone there is no clear trend. The biofilm thickness decrease on silicone DLIP could reflect a reduction in nutrient accessibility/penetration in the biofilm structure [37,38] due to the microtopography.

In microgravity, the particular morphology of biofilms as a function of material (silicone and silicone DLIP) was equivalent to what was observed on Earth (Fig. 1). Moreover, the surface of the material was also completely covered with biofilm for all three days of incubation. Despite the overall morphology being similar between biofilms grown on different gravitational regimes, only microgravity biofilms had a trend of increasing biomass (Fig. 3) and thickness (Fig. 4a) in time. This may be indicative of a slow but steady growth that could potentially become significant after 3 days of incubation; therefore, the process of biofilm maturation at 72 h still seems to involve active growth in space but not on Earth. Similar effects of microgravity were observed for planktonic cells, as liquid cultures in spaceflight had a significant increase in concentration compared to Earth (Appendix A). Additionally, biofilms in microgravity on silicone DLIP, seemed to have less variability in surface area coverage than on Earth (Fig. 4b), resulting in a slightly more uniform coverage of all the surface in space. On the silicones, the areas that did not have biofilm formation were focalized and resembled holes in the biofilm mat (Appendix B). The formation of these holes could be due to biofilm detachment events [39] or cell differentiation through directed cell death [40]. Biofilms in microgravity could potentially be experiencing less of these events when growing over silicone DLIP.

There were only two significant differences observed in microgravity biofilms, with respect to the matching Earth controls. The first one was a 46% reduction ($p < 0.05$) of biomass for 1-day old biofilms over silicone DLIP in microgravity (Fig. 3). The second one was a 10% increase ($p < 0.05$) in biofilm surface area coverage for 2-day old biofilms over silicone in microgravity (Fig. 4b). However, these differences were not maintained in time as the biofilms grown in microgravity presented no significant differences with respect to the ground controls at three days of incubation for biomass, thickness, nor surface area coverage.

The selection of the line-like pattern used for silicone DLIP was based on preliminary work that showed these pattern scales and geometries in combination with bacterial morphology of *E. coli* K12 (very similar to the tested Pseudomonas strain) appear to have an effect on bacteria/surface interaction and trigger bacterial responses. Both pattern scale (periodicity) as well as geometry (line-like) contribute to this effect [41,42]. Thus, the line-like pattern was chosen to learn more about bacterial interaction with surfaces and to test if the bacterial response would be the same in a microgravity environment. Moreover, the line-like pattern has shown potential of reducing bacterial adhesion on other materials [41–43] and it was also tested as a potential biofilm control strategy. The periodicity was aimed to be as close as possible to (or lower than) bacterial size, and 3 μm periodicity was the smallest achievable, at the moment, without compromising the height of the structured pattern. There was no significant and maintained difference between biofilms grown on silicone vs silicone DLIP, but there were some temporary changes. On the ground, the thickness of the biofilms on silicone DLIP was reduced with respect to silicone biofilms at day 2 of incubation ($p < 0.001$). While in microgravity, surface area coverage increased on silicone DLIP ($p < 0.05$) with respect to silicone on day 1 of incubation. These differences are non-significant for day 3 of incubation.

Based on our results, the 3 μm periodic line-like patterned microtopography did not reduce *P. aeruginosa* biofilm formation on catheter grade silicone compared to the smooth silicone. The line-like pattern had an effect on the 3D biofilm morphology, but it is possible that the microtopography did not reduce biofilm formation because the lines did not have the appropriate periodicity. A previous experiment performed by Guenther et al. reported that a 6 μm periodic line-like pattern increased attachment of *Staphylococcus epidermidis* [44]. Since we saw no increase or decrease in biofilm formation with 3 μm periodic line-like pattern and increasing the distance between the lines of the pattern could increase biofilm formation, more tests – preferably of smaller

**Table 1**
Median values of biofilm biomass, thickness, and surface area coverage per day and material. Results are presented as median (IQR = Q3-Q1). Biomass, thickness, and surface area coverage were calculated from 3D microscopy images using COMSTAT2. n = 4 biological replicates each imaged in four fields of view. *n = 3 biological replicates, each imaged in four fields of view.

| | Biomass ($\mu m^3/\mu m^2$) | | Thickness (μm) | | Surface area coverage (%) | |
|---|---|---|---|---|---|---|
| | 1 g | μg | 1 g | μg | 1 g | μg |
| **Silicone day 1** | 4.0 (2.4)* | 4.1 (1.4) | 7.6 (12.7)* | 8.0 (2.4) | 99.6 (0.7)* | 99.3 (3.0) |
| **Silicone day 2** | 4.2 (1.3) | 5.0 (1.6)* | 15.6 (6.4) | 10.8 (4.9)* | 98.0 (9.0) | 99.6 (2.1)* |
| **Silicone day 3** | 4.0 (1.9) | 6.3 (4.1)* | 8.9 (3.9) | 13.1 (11.7)* | 99.9 (0.9) | 98.0 (7.7)* |
| **DLIP day 1** | 5.1 (4.1) | 3.1 (1.0) | 12.9 (8.9) | 7.2 (1.9) | 99.7 (0.7) | 99.9 (0.5) |
| **DLIP day 2** | 3.2 (1.8) | 3.0 (2.7)* | 8.1 (3.6) | 9.7 (6.0) * | 99.2 (2.7) | 99.9 (0.2)* |
| **DLIP day 3** | 3.1 (0.9)* | 3.6 (1.9) | 7.4 (6.6)* | 9.2 (5.9) | 98.7 (2.2)* | 99.7 (1.2) |
| **Cellulose day 1** | 3.6 (2.5) | 3.7 (3.3)* | 9.0 (3.5) | 8.5 (3.5) * | 93.0 (7.5) | 99.6 (2.0)* |
| **Cellulose day 2** | 3.4 (2.2) | 1.3 (1.6) | 7.4 (3.8) | 6.0 (2.7) | 95.9 (9.3) | 46.4 (58.7) |
| **Cellulose day 3** | 1.6 (1.5)* | 1.4 (5.2) | 8.2 (6.4)* | 8.6 (7.0) | 64.7 (40.7)* | 41.9 (82.6) |

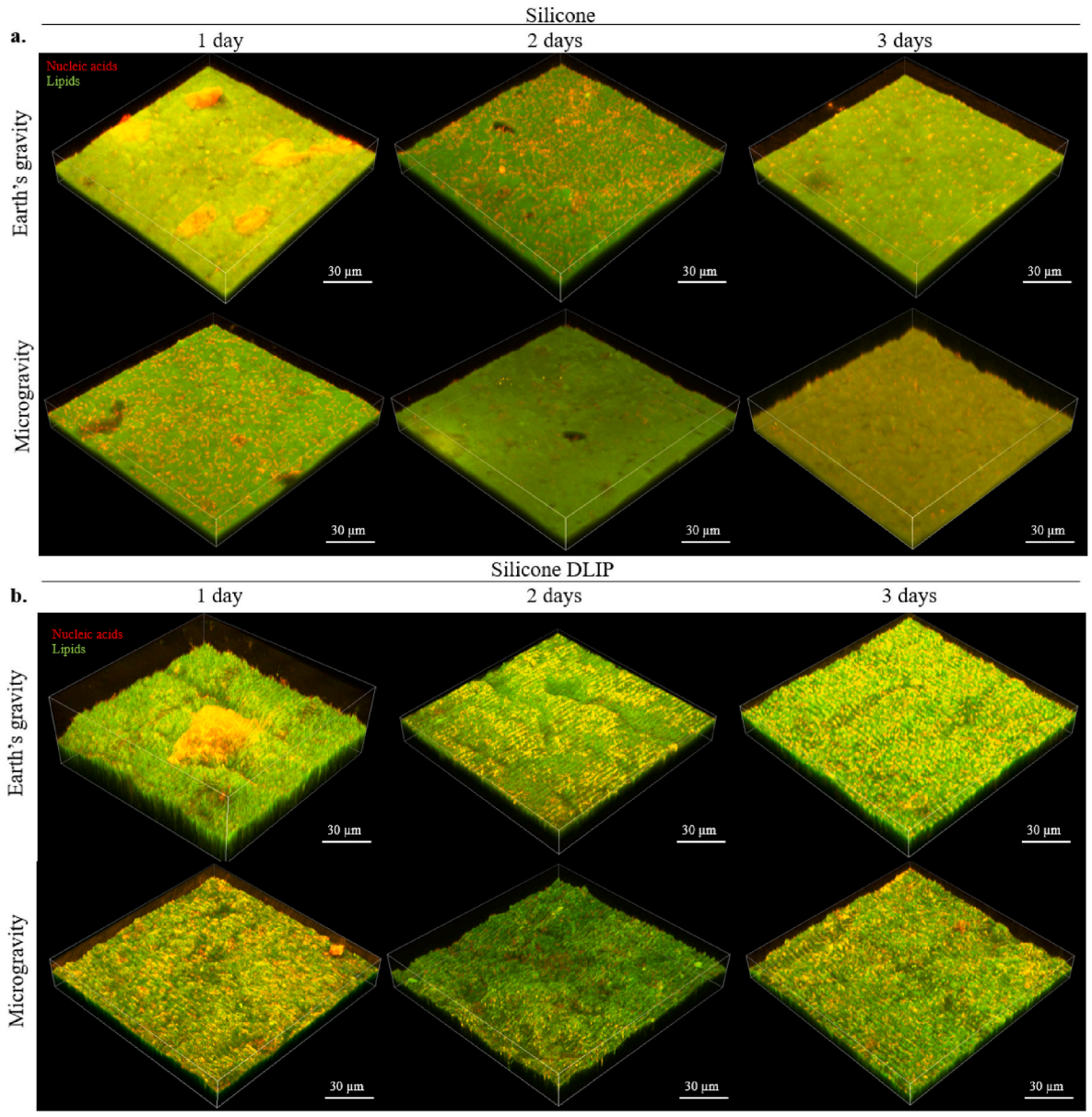


**Fig. 1.** 3D volume view of representative 1, 2, or 3-day *P. aeruginosa* PA14 biofilms grown on Earth's gravity and microgravity for **a.** silicone, and **b.** silicone DLIP. Nucleic acids stained in red, and lipids stained in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

periodicities – are necessary to determine the optimal periodicity (if any) that could reduce biofilm formation. Besides the periodicity of the micropattern, the actual pattern could also be enhanced to have a better biofilm reduction effect. Interestingly, a lamella-like surface pattern (a combination of large lines with smaller perpendicular lines in between) of 6–8 µm of periodicity between large lines and 2 µm periodicity between lamellas has been shown to reduce bacterial adhesion of *Staphylococcus aureus* [44]. More testing is required to determine the effect on *P. aeruginosa* biofilms growing on surfaces with microtopographies of different periodicities and patterns. This is not straightforward as microtopographies have shown multiple results in terms of reducing or promoting biofilm formation. This is believed to be bacterial-strain-, growth-media-, periodicity-, and microtopography-pattern-dependent [42,45–48]. In our particular case, it does not seem to be gravity dependent.

### 3.2. Cellulose membrane biofilms

We replicated the *P. aeruginosa* biofilm experiment conditions used by Kim et al. [19] where the column-and-canopy structure was reported for biofilms grown on cellulose membrane in microgravity. The following were kept constant in between the two experiments.

- Bacterial strain (*P. aeruginosa* PA14)
- Incubation temperature (37 °C)
- Incubation duration (3 days)
- Launch medium (PBS)
- Growth medium (mAUMg-hi Pi)
- Hardware (BioServe's FPAs and GAPs)
- Cellulose membrane (Millipore, Cat. GSWP01300)
- Paraformaldehyde final concentration (4%)
- Nucleic acid stain (Propidium iodide)
- Microgravity samples grown at ISS

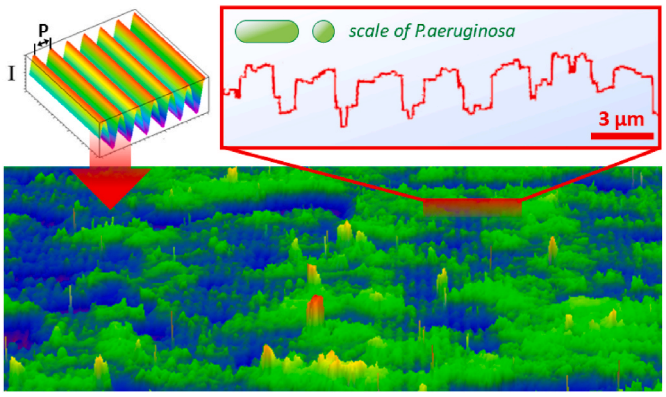


**Fig. 2.** Line-like microtopography of silicone DLIP coupons exhibiting 3 µm periodicity and 700 nm depth in close relation to *P. aeruginosa* single bacterial cell scale resulting from two-beam USP-DLIP. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

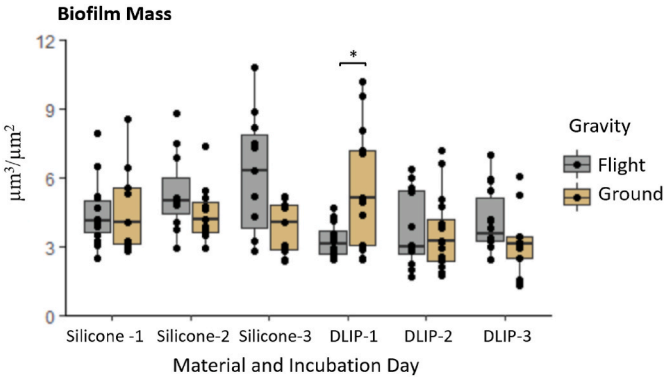


**Fig. 3.** Biofilm mass of *P. aeruginosa* grown over silicone and silicone DLIP. Data as a function of time and gravitational regime. Significant statistical comparisons indicated with horizontal brackets (*p < 0.05). n = 3 or 4 biological replicates as specified in Table 1.

The only differences were a) ~ 3.5 × $10^6$ CFU in the inoculum compared to Kim et al. of ~ 3 × $10^6$ CFU in the inoculum and b) the addition of the second stain (FilmTracer FM 1–43 Green Biofilm) to tags lipids. The column-and-canopy structure was not observed in our microgravity samples. *P. aeruginosa* biofilms grown in microgravity, as well as the Earth controls, grew as a thin layer of cells scattered through the cellulose membrane with occasional microcolonies and mounts of cells covered with lipids (Fig. 5). Even at three days of incubation, the biofilms did not present the column-and-canopy structure. The differences observed in the dominance of nucleic acids/lipids was not consistent throughout all samples of each condition. Biofilms grown over cellulose membrane did not present any significant difference in terms of biofilm mass, thickness, and surface area coverage for comparisons between microgravity conditions nor between incubation times (Fig. 6).

Even though we replicated as close as possible the spaceflight experiment where the column-and-canopy biofilm structure was described, such structure was not observed in our biofilms. Neither did microgravity induce increased PA14 biofilm formation when cultured in mAUMg-hi Pi. Moreover, our 3-day-old biofilms were less robust (biomass ~1.4 $\mu m^3/\mu m^2$ and thickness ~8.6 µm) than those previously reported (biomass ~5.0 $\mu m^3/\mu m^2$ and thickness ~9.8 µm) [19]. It is important to mention, that in contrast to Kim et al. results where no change in planktonic cell concentration was observed [19], our samples' planktonic cell culture had increased concentration ($p < 0.01$) in

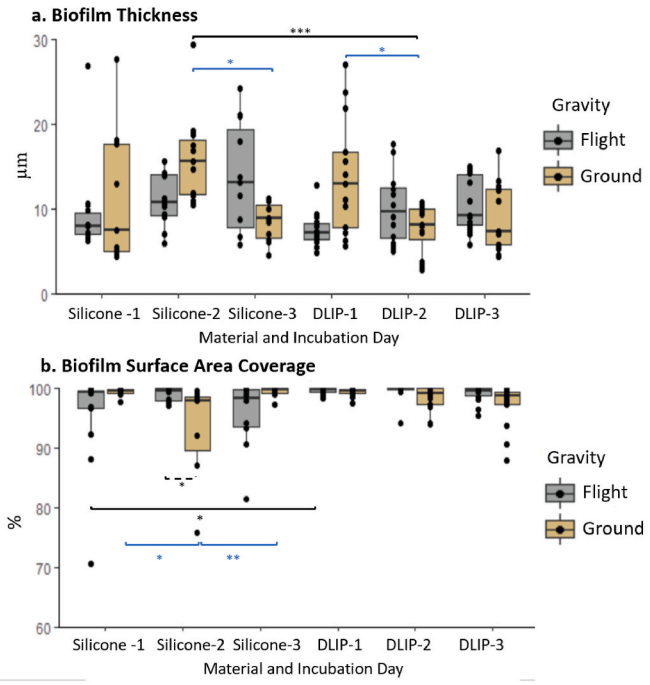


**Fig. 4.** Biofilm **a.** thickness and **b.** surface area coverage of *P. aeruginosa* grown over silicone and silicone DLIP. Data as a function of time and gravitational regime. Statistical significance indicated with horizontal brackets (black for comparisons between silicone and silicone DLIP, blue for comparisons between time points within each material, and dotted for comparisons between gravitational regime). ***p < 0.001, **p < 0.01, *p < 0.05. n = 3 or 4 biological replicates as specified in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

microgravity with respect to Earth (Appendix A). This difference may partially be due to the slightly higher concentration of the bacteria in our initial inoculum.

It remains to be proven or disproven whether with more robust biofilm growth the column-and-canopy structure could have been replicated. However, it should also be considered that the vibrations experienced during reentry and subsequent transport to the sample processing facility, could have been a contributing factor to the column-and-canopy structures reported by Kim et al. Such vibrations could have provided enough shear force to cause the weakly attached areas of the biofilms to detach, resulting in the observed structure ([49–51]; Stoodley, Lewandowski et al., 1999). Nonetheless, it is necessary to perform more tests to characterize the column-and-canopy structure of *P. aeruginosa* in microgravity and to elucidate the mechanisms behind it, should this structure be replicated. If so, it may be preferable to assess this morphology using multiple microscopy techniques, and gene expression and proteomics analyses.

### 3.3. *Microgravity effect on biofilm formation – a review*

In our experiments, microgravity did not induce general form changes (in terms of overall 3D structure) to the biofilms grown in mAUMg-hi Pi nor in LB supplemented with potassium nitrate (results reported in Ref. [25]). The same was true for the biofilms of *P. aeruginosa* PAO1 in microgravity [17]. Nevertheless, when comparing our biofilm results with all the previous spaceflight biofilm experiments, there seems to be a high variability of microgravity effects, even within the same bacterial strain (Table 2).

The biofilm data by itself gave no clear trend or insight on the effects of microgravity on biofilm formation. However, the environment contained not only biofilm cells but also planktonic cells; analysing the balance of both is key to understand the changes as an overall system.

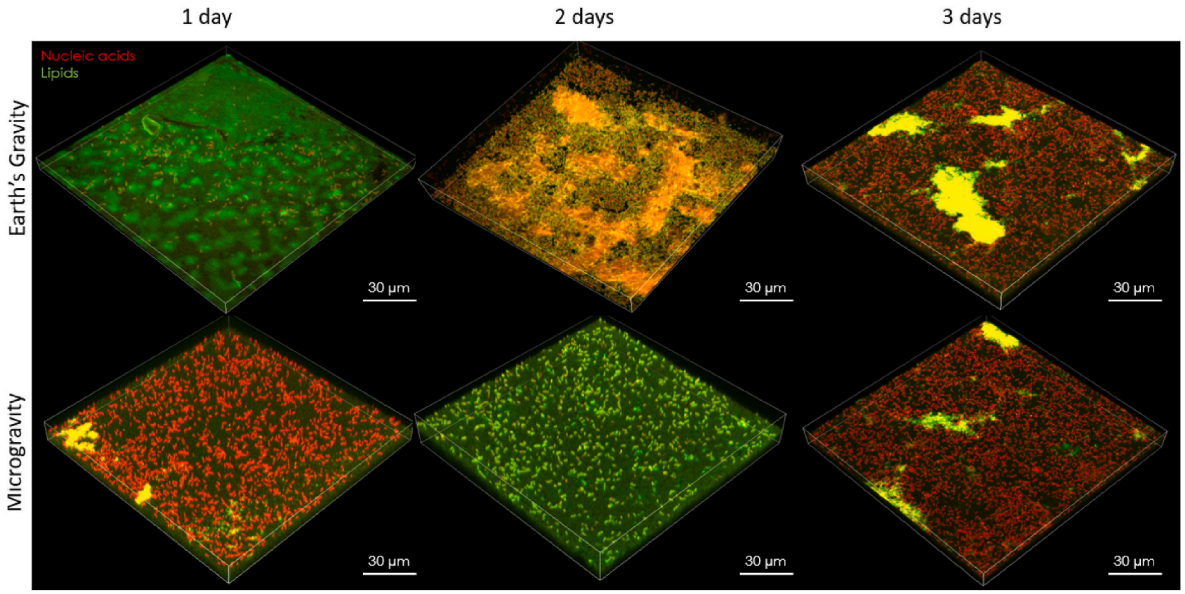


**Fig. 5.** 3D volume view of representative 1, 2, and 3-day *P. aeruginosa* PA14 biofilms grown on cellulose membrane. Images on first and second row represent biofilms grown on Earth's gravity and in microgravity, respectively. Nucleic acids stained in red, and lipids stained in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

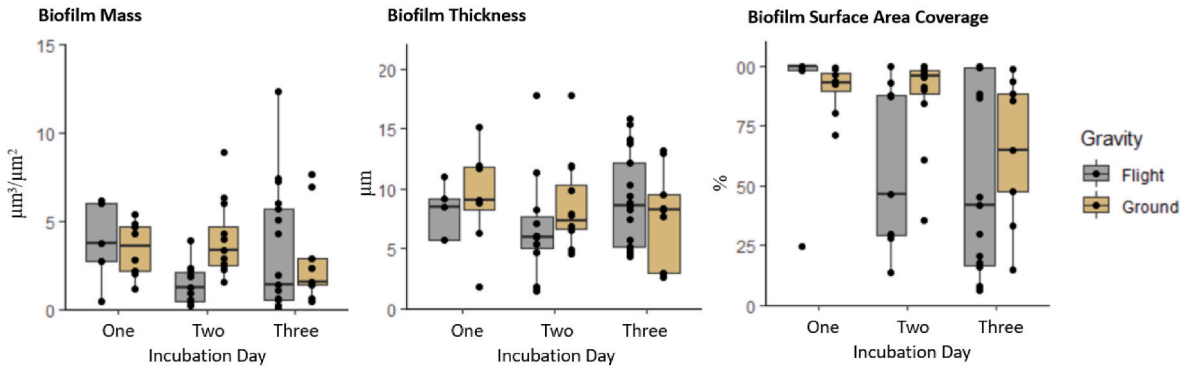


**Fig. 6.** Biofilm mass, thickness, and surface area coverage of *P. aeruginosa* grown over cellulose. Data as a function of time and gravitational regime. No significant statistical differences. n = 3 or 4 biological replicates as specified in Table 1.

Biofilms can have a lower metabolic activity [52] and lower growth rate than planktonic cells [53,54]. Since the later are involved in the first step of the biofilm formation process, planktonic cells have both an advantage of time and metabolism, when it comes to consuming the nutrients available in the environment, compared to the biofilm cells. Considering the planktonic cells in the environment can help us understand the microgravity effects on biofilms.

It is not possible to generalize the microgravity effect on biofilm formation based solely on the results presented in this manuscript. Especially, because previous experiments using the same strain and conditions resulted in a different biofilm response (Table 2). To understand the biofilm changes observed microgravity, the planktonic cell density changes should also be considered. As an effort to explain the different microgravity effects on biofilm formation (increase, decrease, or no change with respect to ground controls) that have been reported across experiments, regardless of the bacterial strain, the model of planktonic cell nutrient-overtake is here proposed (Fig. 7) [55].

In this model, the expected effect of microgravity on biofilms (no change, increases, or decreased formation) will depend on the available nutrients in the media and the planktonic cell growth in microgravity with respect to 1 *g*. For example, when in microgravity, if biofilms are grown in nutrient rich media and the planktonic cells experience increased growth with respect to 1 *g*; then the increased numbers of planktonic cells would deplete the nutrients in the environment faster than the number of planktonic cells at 1 *g*, limiting the nutrients available for the biofilms and resulting in decreased biofilm formation in space. If the biofilms are grown in media with reduced nutrients and the planktonic cell growth is only slightly increased in microgravity with respect to 1 *g*, then the nutrient consumption by the planktonic cells will only be slightly increased to that at 1 *g* and biofilms can still have enough nutrients to achieve same growth as seen at 1 *g* and thus present "no change" in microgravity. But if biofilms are grown in nutrient limited conditions where planktonic cell growth is not increased in microgravity with respect to 1 *g*, nutrients are not rapidly depleted, and biofilms present increased formation in space [55]. This model works under two assumptions. First, that the substratum does not provide nutrition; otherwise, the depletion of the nutrients dissolved in the medium would not be as limiting to biofilms and the cell distribution, biofilm structure, and biofilm dynamics could be altered [56,57]. Second, that the starting amount of nutrients in the medium is the total amount available for the system, meaning that there is no further introduction of nutrients either in a continuous or intermittent manner; otherwise this could influence biofilm dynamics and structures in time [56,58–62].

The planktonic cell nutrient-overtake model proposes a continuous

**Table 2**
Summary of results observed in previous and current biofilm experiments [17–19]. NC = no change. ↑ = slight increased biofilm formation or planktonic cell concentration. ↑↑ = moderate increased biofilm formation or planktonic cell concentration. ↓ = = decreased biofilm formation or planktonic cell concentration. ——— = data not provided or analysis not done. *Experiments performed with gas exchange.

| Bacterial strain | Material surface | 3D form in space | Microgravity effect on biofilm | Medium and nutrient availability | Microgravity effect on planktonic | Reference |
|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* PA14 WT | Cellulose | Column-and-canopy | ↑↑ | mAUMg-hi Pi Reduced & | NC for mAUMg-hi Pi & ↑ for mAUMg | [19,19,23]) |
| PA14 *ΔmotABC* | | NC | ↑ | | | |
| PA14 *ΔpilB* | | Column-and-canopy | ↑↑ | mAUMg Reduced | – | |
| *PA14* WT *-gas** | | NC | NC | mAUMg | – | |
| *PA14 ΔmotABC -gas** | | NC | NC | Reduced | NC | |
| *Burkholderia cepacia* | SS316 | NC | ↑ | Water Limited | NC | [18,18]) |
| | | NC | ↓ | TSB High | ↑↑ | |
| *Pseudomonas aeruginosa* PAO-1 | Polycarbonate membrane | NC | NC | R2A Reduced | – | [17,17]) |
| *Pseudomonas aeruginosa* PA14 | SS316 | NC | ↓ | LBK | ↑↑ | [25,25]) |
| | pSS316 | NC | ↓ | Very high | ↑↑ | |
| | LIS | NC | ↓ | | ↑↑ | |
| *Pseudomonas aeruginosa* PA14 | Silicone | NC | NC | mAUMg-hi Pi | ↑ | This manuscript |
| | Silicone DLIP | NC | NC | Reduced | ↑ | |
| | Cellulose | NC | NC | | ↑ | |

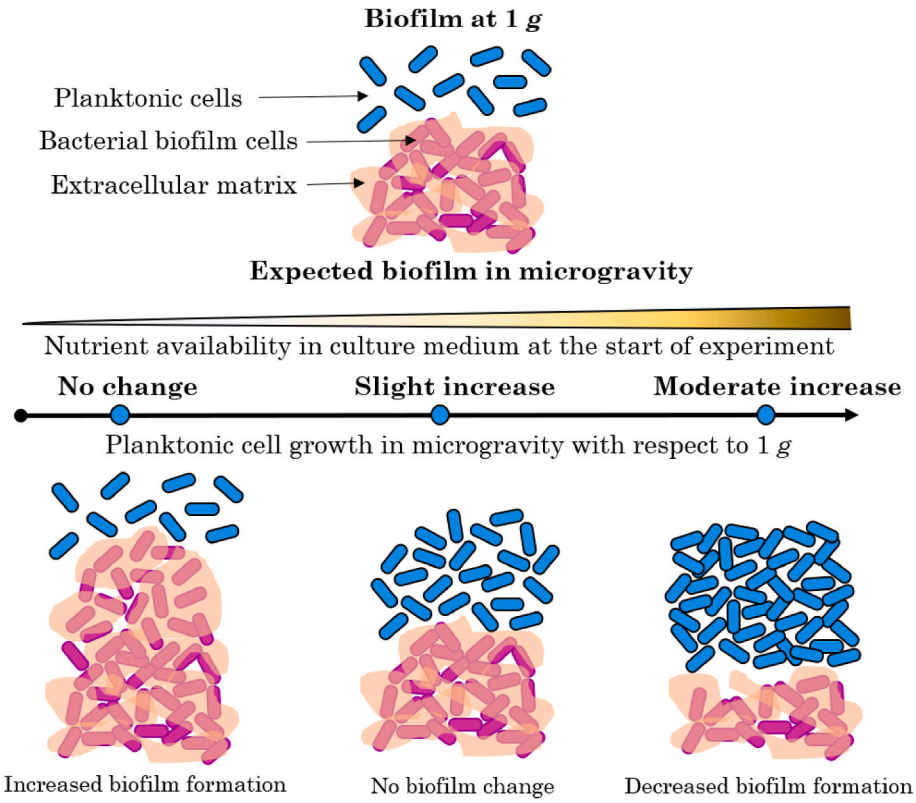


**Fig. 7.** The planktonic cell nutrient-overtake biofilm in microgravity model. This model explains the different effects that microgravity can have on biofilm growth (increase, decrease, or no change) based on the planktonic cell growth in microgravity with respect to 1 *g*, which in turn is dependent on the initial nutrient availability in the medium at experiment's start. The more planktonic cells, the faster the depletion of the nutrients. Model assumptions: substratum does not provide additional nutrition and there is no further introduction of nutrients after experiment start. Taken from Flores (2022) [55].

biofilm formation phenotype based on how fast planktonic cells overtake the nutrients and deplete them and leads to the hypothesis that if present in an environment of limitless nutrients, without depletion of nutrients by planktonic cells, microgravity would increase biofilm formation in all cases.

The model agrees with the data of two of the previous biofilm spaceflight experiments, the experiment of [17] cannot be compared due to lack of information on planktonic cell concentration. It is important to mention that this model does not intend to be perfect, but merely a steppingstone to understand the current results of biofilm formation in microgravity. We acknowledge that there may be more key factors at play in biofilm formation in microgravity and more tests are needed to fully understand. We recommend investigators to develop an experimental design/hardware that could provide a constant supply of

nutrients – without the disruption of the microgravity environment – in order to decipher how big biofilms could grow in near-limitless nutrient conditions in space. Also, to test more rigorously the effect of microgravity in biofilms when in presence of different concentrations of planktonic cells, including motile and non-motile strains.

## 4. Conclusions

*P. aeruginosa* PA14 formed biofilms with different morphologies based on the material surface (silicone, silicone DLIP, or cellulose membrane) and said morphologies were not affected by microgravity. *P. aeruginosa* biofilm structure on silicone DLIP followed the same pattern as the line-like microtopography surface. The 3 μm line-like periodic microtopography over catheter grade silicone did not reduce biofilm formation in microgravity. The column-and-canopy structure previously observed in microgravity was not observed in our experiment. It is possible that the column-and-canopy structure requires a minimal biofilm robustness, which was not achieved in our experiment at 3 days of incubation.

## Funding sources

Luis Zea and Pamela Flores were supported by the National Aeronautics and Space Administration [grant numbers 80NSSC17K0036 and 80NSSC21K1950].

## CRediT authorship contribution statement

**Pamela Flores:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jiaqi Luo:** Writing – review & editing, Resources. **Daniel Wyn Mueller:** Writing – review & editing, Resources. **Frank Muecklich:** Writing – review & editing, Supervision, Resources, Project administration. **Luis Zea:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data available on NASA's Open Science Data Repository (OSDR, https://osdr.nasa.gov/bio/). The microscopy data under study OSD-627 and 10.26030/bp7m-0f62. The data will also be available on NASA's Physical Sciences Informatics (PSI) data repository (https://psi.nasa.gov).

## Acknowledgements

The authors would like to thank the efforts of all who were involved in the success of this project. Thanks to BioServe Space Technologies for providing the hardware for the experiment and for directing space operations. Thanks to astronauts Jessica Meir and Christina Koch for operating the experiment at ISS. Special thanks to Rylee Schauer, Chia-Jui Hsu, Maggie Kolicko, Joella Delheimer, Melissa Dunivant, Jim Wright, James Orth, Lily Allen, Amir Kalani, Anissa Becerra, Zeena Nisar, and Shilpi Ganguly. The authors thank Prof. George O'Toole for making the bacterial strain available.

## Appendix A. (Planktonic cell culture final concentration)

The planktonic cell culture of the biofilm samples was recovered and homogenized for quantification through spectrophotometry. Planktonic cell concentration was higher in microgravity for all conditions when biofilms were grown in mAUMg-hi Pi medium (Fig. 8).

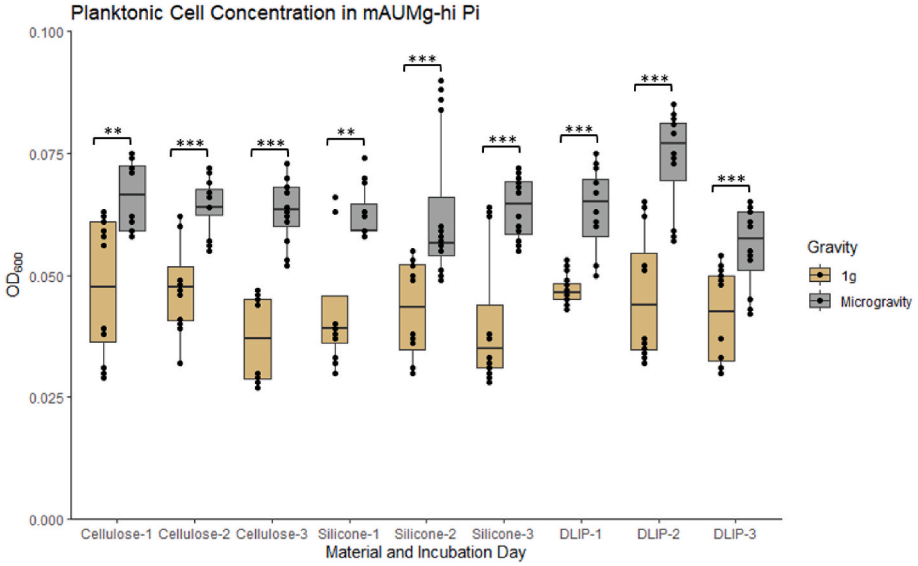


**Fig. 8.** *Pseudomonas aeruginosa* Planktonic cell final OD for samples of biofilm grown in mAUMg-hi Pi. Concentration of planktonic cells plotted as a function of time (1, 2, and 3-day old biofilms) and gravitational condition. Statistical significance specified with horizontal brackets for differences between gravitational regimes (black). DLIP = silicone DLIP. n = 4 biological replicates each with 4 technical replicates. Dunn's test with Bonferroni correction **p ≤ 0.01 and ***p ≤ 0.005.

## Appendix B. (PA14 biofilms on silicones have "holes" without biofilm)

Biofilms that grew over silicone and silicone DLIP covered almost 100% of the surface in all replicates and conditions. The areas that did not have

biofilm were focalized "holes" within the biofilm (Fig. 9). These holes were observed on biofilms both grown on Earth and in microgravity, as well as in samples from all incubation times (1, 2, or 3 days). Even though biofilms on all conditions could present these holes, not all imaged biofilm areas had them.

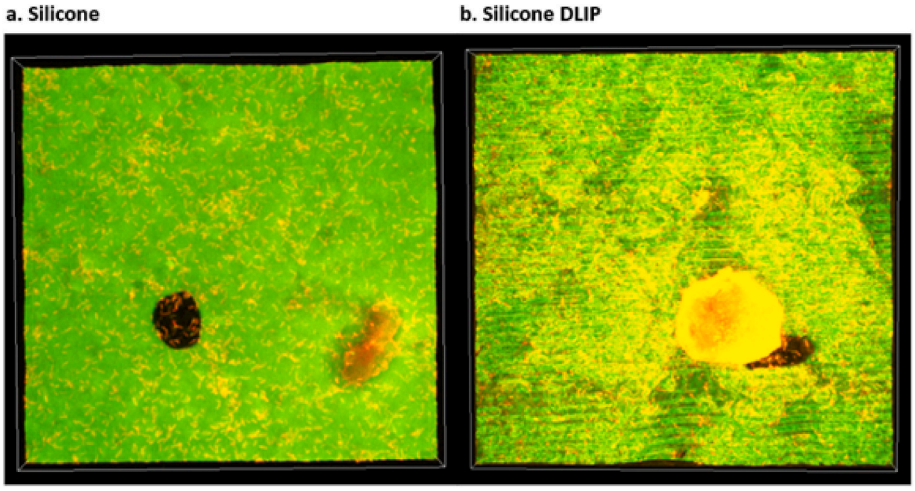


**Fig. 9.** Biofilms of *P. aeruginosa* with "holes" (areas without biofilm formation). The 3D images represent biofilms grown over **a.** silicone and **b.** silicone DLIP for two and one day of incubation, respectively. Nucleic acids stained in red, and lipids stained in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## References


[1] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the Natural environment to infectious diseases. Nat Rev Microbiol 2004;2(2). https://doi.org/10.1038/nrmicro821. Article 2.

[2] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284(5418):1318–22. https://doi.org/10.1126/science.284.5418.1318.

[3] Mah T-FC, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001;9(1):34–9. https://doi.org/10.1016/S0966-842X(00)01913-2.

[4] Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010;35(4):322–32. https://doi.org/10.1016/j.ijantimicag.2009.12.011.

[5] Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA. Bacterial biofilm and associated infections. J Chin Med Assoc 2018;81(1):7–11. https://doi.org/10.1016/j.jcma.2017.07.012.

[6] Nickel JC, Costerton JW, McLean RJC, Olson M. Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections. J Antimicrob Chemother 1994;33(suppl_A):31–41. https://doi.org/10.1093/jac/33.suppl_A.31.

[7] Azevedo AS, Almeida C, Melo LF, Azevedo NF. Impact of polymicrobial biofilms in catheter-associated urinary tract infections. Crit Rev Microbiol 2017;43(4):423–39. https://doi.org/10.1080/1040841X.2016.1240656.

[8] Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J Clin Diagn Res : J Clin Diagn Res 2012;6(9):1478–82. https://doi.org/10.7860/JCDR/2012/4367.2537.

[9] Ahmed I, Sajed M, Sultan A, Murtaza I, Yousaf S, Maqsood B, Vanhara P, Anees M. The erratic antibiotic susceptibility patterns of bacterial pathogens causing urinary tract infections. EXCLI Journal 2015;14:916–25. https://doi.org/10.17179/excli2015-207.

[10] Tolkoff-Rubin NE, Rubin RH. Urinary tract infection in the immunocompromised host: lessons from kidney transplantation and the Aids epidemic. Infect Dis Clin 1997;11(3):707–17. https://doi.org/10.1016/S0891-5520(05)70381-0.

[11] Mann V, Sundaresan A, Mehta SK, Crucian B, Doursout MF, Devakottai S. Effects of microgravity and other space stressors in immunosuppression and viral reactivation with potential nervous system involvement. Neurol India 2019;67(8):198. https://doi.org/10.4103/0028-3886.259125.

[12] Rooney BV, Crucian BE, Pierson DL, Laudenslager ML, Mehta SK. Herpes virus reactivation in astronauts during spaceflight and its application on Earth. Front Microbiol 2019;10. https://www.frontiersin.org/articles/10.3389/fmicb.2019.00016.

[13] Aunins TR, Erickson KE, Prasad N, Levy SE, Jones A, Shrestha S, Mastracchio R, Stodieck L, Klaus D, Zea L, Chatterjee A. Spaceflight modifies Escherichia coli gene expression in response to antibiotic exposure and reveals role of oxidative stress response. Front Microbiol 2018;9. https://doi.org/10.3389/fmicb.2018.00310.

[14] Horneck G, Klaus DM, Mancinelli RL. Space microbiology. Microbiol Mol Biol Rev : MMBR (Microbiol Mol Biol Rev) 2010;74(1):121–56. https://doi.org/10.1128/MMBR.00016-09.

[15] Zea L, Prasad N, Levy SE, Stodieck L, Jones A, Shrestha S, Klaus D. A molecular genetic basis explaining altered bacterial behavior in space. PLoS One 2016;11(11):e0164359. https://doi.org/10.1371/journal.pone.0164359.

[16] Zea L, Larsen M, Estante F, Qvortrup K, Moeller R, Dias de Oliveira S, Stodieck L, Klaus D. Phenotypic changes exhibited by E. coli cultured in space. Front Microbiol 2017;8. https://www.frontiersin.org/article/10.3389/fmicb.2017.01598.

[17] McLean RJC, Cassanto JM, Barnes MB, Koo JH. Bacterial biofilm formation under microgravity conditions. FEMS (Fed Eur Microbiol Soc) Microbiol Lett 2001;195(2):115–9. https://doi.org/10.1016/S0378-1097(00)00549-8.

[18] Pyle BH, McFeters GA, Broadaway SC, Johnsrud CK, Storga RT, Borkowski J. Bacterial growth on surfaces and in suspensions. In Biorack on spacehab. Biological experiments on shuttle to Mir missions 03, 05, and 06. European Space Agency (Brochure) SP-1222, 1999.

[19] Kim W, Tengra FK, Young Z, Shong J, Marchand N, Chan HK, Pangule RC, Parra M, Dordick JS, Plawsky JL, Collins CH. Spaceflight promotes biofilm formation by Pseudomonas aeruginosa. PLoS One 2013;8(4):e62437. https://doi.org/10.1371/journal.pone.0062437.

[20] Law J, Cole R, Young M, Mason S. *NASA astronaut urinary conditions Associated with spaceflight*. 87th annual scientific meeting of the aerospace medical association. 2016, April 24. Atlantic City, NJ, United States, https://ntrs.nasa.gov/search.jsp?R=20150020958.

[21] Zea L, McLean RJC, Rook TA, Angle G, Carter DL, Delegard A, Denvir A, Gerlach R, Gorti S, McIlwaine D, Nur M, Peyton BM, Stewart PS, Sturman P, Velez Justiniano YA. Potential biofilm control strategies for extended spaceflight missions. Biofilms 2020;2:100026. https://doi.org/10.1016/j.bioflm.2020.100026.

[22] Flores P, Schauer R, McBride SA, Luo J, Hoehn C, Doraisingam S, Widhalm D, Chadha J, Selman L, Mueller DW, Floyd S, Rupert M, Gorti S, Reagan S, Varanasi KK, Koch C, Meir JU, Muecklich F, Moeller R, Zea L. Preparation for and performance of a Pseudomonas aeruginosa biofilm experiment on board the International Space Station. Acta Astronaut 2022. https://doi.org/10.1016/j.actaastro.2022.07.015.

[23] Kim W, Tengra FK, Shong J, Marchand N, Chan HK, Young Z, Pangule RC, Parra M, Dordick JS, Plawsky JL, Collins CH. Effect of spaceflight on Pseudomonas aeruginosa final cell density is modulated by nutrient and oxygen availability. BMC Microbiol 2013;13(1):241. https://doi.org/10.1186/1471-2180-13-241.

[24] Müller DW, Fox T, Grützmacher PG, Suarez S, Mücklich F. Applying ultrashort pulsed direct laser interference patterning for functional surfaces. Sci Rep 2020;10(1):3647. https://doi.org/10.1038/s41598-020-60592-4.

[25] Flores P, McBride SA, Galazka JM, Varanasi KK, Zea L. Biofilm formation of Pseudomonas aeruginosa in spaceflight is minimized on Lubricant Impregnated Surface. Npj Microgravity 2023;9(1):1–14. https://doi.org/10.1038/s41526-023-00316-w.

[26] Heydorn A, Nielsen AT, Hentzer M, Sternberg C, Givskov M, Ersbøll BK, Molin S. Quantification of biofilm structures by the novel computer program COMSTAT. Microbiology 2000;146(Pt 10):2395–407. https://doi.org/10.1099/00221287-146-10-2395.

[27] Heydorn A, Ersbøll BK. *Comstat 2*. Welcome to the comstat 2 homepage. 2015. http://www.comstat.dk/.

[28] Vorregaard M. *Comstat2—a modern 3D image analysis environment for biofilms* [Master's Thesis. 2008. Technical university of Denmark (DTU)], http://www2.imm.dtu.dk/pubdb/pubs/5628-full.html.

[29] Kempes CP, Okegbe C, Mears-Clarke Z, Follows MJ, Dietrich LEP. Morphological optimization for access to dual oxidants in biofilms. Proc Natl Acad Sci USA 2014; 111(1):208–13. https://doi.org/10.1073/pnas.1315521110.
[30] Picioreanu C, van Loosdrecht MCM, Heijnen JJ. Mathematical modeling of biofilm structure with a hybrid differential-discrete cellular automaton approach. Biotechnol Bioeng 1998;58(1):101–16. https://doi.org/10.1002/(SICI)1097-0290(19980405)58:1<101::AID-BIT11>3.0.CO;2-M.
[31] Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in Pseudomonas aeruginosa biofilm development. Curr Opin Microbiol 2007;10(6):644–8. https://doi.org/10.1016/j.mib.2007.09.010.
[32] Santos Rosado Castro M, da Silva Fernandes M, Kabuki DY, Kuaye AY. Modelling Pseudomonas fluorescens and Pseudomonas aeruginosa biofilm formation on stainless steel surfaces and controlling through sanitisers. Int Dairy J 2021;114: 104945. https://doi.org/10.1016/j.idairyj.2020.104945.
[33] Hammond A, Dertien J, Colmer-Hamood JA, Griswold JA, Hamood AN. Serum inhibits P. aeruginosa biofilm formation on plastic surfaces and intravenous catheters. J Surg Res 2010;159(2):735–46. https://doi.org/10.1016/j.jss.2008.09.003.
[34] James GA, Boegli L, Hancock J, Bowersock L, Parker A, Kinney BM. Bacterial adhesion and biofilm formation on textured breast implant shell materials. Aesthetic Plast Surg 2019;43(2):490–7. https://doi.org/10.1007/s00266-018-1234-7.
[35] Allesen-Holm M, Barken KB, Yang L, Klausen M, Webb JS, Kjelleberg S, Molin S, Givskov M, Tolker-Nielsen T. A characterization of DNA release in Pseudomonas aeruginosa cultures and biofilms. Mol Microbiol 2006;59(4):1114–28. https://doi.org/10.1111/j.1365-2958.2005.05008.x.
[36] Petrova OE, Schurr JR, Schurr MJ, Sauer K. The novel Pseudomonas aeruginosa two-component regulator BfmR controls bacteriophage-mediated lysis and DNA release during biofilm development through PhdA. Mol Microbiol 2011;81(3): 767–83. https://doi.org/10.1111/j.1365-2958.2011.07733.x.
[37] Lydmark P, Lind M, Sörensson F, Hermansson M. Vertical distribution of nitrifying populations in bacterial biofilms from a full-scale nitrifying trickling filter. Environ Microbiol 2006;8(11):2036–49. https://doi.org/10.1111/j.1462-2920.2006.01085.x.
[38] Suarez C, Piculell M, Modin O, Langenheder S, Persson F, Hermansson M. Thickness determines microbial community structure and function in nitrifying biofilms via deterministic assembly. Sci Rep 2019;9(1). https://doi.org/10.1038/s41598-019-41542-1. Article 1.
[39] Hunt SM, Werner EM, Huang B, Hamilton MA, Stewart PS. Hypothesis for the role of nutrient starvation in biofilm detachment. Appl Environ Microbiol 2004;70(12): 7418–25. https://doi.org/10.1128/AEM.70.12.7418-7425.2004.
[40] Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, Koch B, Givskov M, Kjelleberg S. Cell death in Pseudomonas aeruginosa biofilm development. J Bacteriol 2003;185(15):4585–92. https://doi.org/10.1128/JB.185.15.4585-4592.2003.
[41] Müller DW, Lößlein S, Terriac E, Brix K, Siems K, Moeller R, Kautenburger R, Mücklich F. Increasing antibacterial efficiency of Cu surfaces by targeted surface functionalization via ultrashort pulsed direct laser interference patterning. Adv Mater Interfac 2021;8(5):2001656. https://doi.org/10.1002/admi.202001656.
[42] Siems K, Müller DW, Maertens L, Ahmed A, Van Houdt R, Mancinelli RL, Baur S, Brix K, Kautenburger R, Caplin N, Krause J, Demets R, Vukich M, Tortora A, Roesch C, Holland G, Laue M, Mücklich F, Moeller R. Testing laser-structured antimicrobial surfaces under space conditions: the design of the ISS experiment BIOFILMS. Frontiers in space Technologies, vol. 2; 2022. https://www.frontiersin.org/articles/10.3389/frspt.2021.773244.
[43] Rosenkranz A, Hans M, Gachot C, Thome A, Bonk S, Mücklich F. Direct laser interference patterning: tailoring of contact area for frictional and antibacterial properties. Lubricants 2016;4(1). https://doi.org/10.3390/lubricants4010002. Article 1.
[44] Guenther D, Valle J, Burgui S, Gil C, Solano C, Toledo-Arana A, Helbig R, Werner C, Lasa I, Lasagni AF. Direct laser interference patterning for decreased bacterial attachment. Laser-Based Micro- and Nanoprocessing X 2016;9736:216–24. https://doi.org/10.1117/12.2216065.
[45] An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. 2002. https://doi.org/10.1002/(SICI)1097-4636(199823)43:3<338::AID-JBM16>3.0.CO;2-B.
[46] Ploux L, Ponche A, Anselme K. Bacteria/material interfaces: role of the material and cell wall properties. J Adhes Sci Technol 2010;24(13–14):2165–201. https://doi.org/10.1163/016942410X511079.
[47] Tebbs SE, Sawyer A, Elliott TSJ. Influence of surface morphology on in vitro bacterial adherence to central venous catheters. Br J Addiction: Br J Anaesth 1994; 72(5):587–91. https://doi.org/10.1093/bja/72.5.587.
[48] Whitehead KA, Colligon J, Verran J. Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. Colloids Surf B Biointerfaces 2005;41(2–3):129–38. https://doi.org/10.1016/j.colsurfb.2004.11.010.
[49] Dunsmore BC, Jacobsen A, Hall-Stoodley L, Bass CJ, Lappin-Scott HM, Stoodley P. The influence of fluid shear on the structure and material properties of sulphate-reducing bacterial biofilms. J Ind Microbiol Biotechnol 2002;29(6):347–53. https://doi.org/10.1038/sj.jim.7000302.
[50] Paul E, Ochoa JC, Pechaud Y, Liu Y, Liné A. Effect of shear stress and growth conditions on detachment and physical properties of biofilms. Water Res 2012;46 (17):5499–508. https://doi.org/10.1016/j.watres.2012.07.029.
[51] Stoodley P, Cargo R, Rupp CJ, Wilson S, Klapper I. Biofilm material properties as related to shear-induced deformation and detachment phenomena. J Ind Microbiol Biotechnol 2002;29(6):361–7. https://doi.org/10.1038/sj.jim.7000282.
[52] Walters MC, Roe F, Bugnicourt A, Franklin MJ, Stewart PS. Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. Antimicrob Agents Chemother 2003;47(1):317–23. https://doi.org/10.1128/AAC.47.1.317-323.2003.
[53] Drenkard E. Antimicrobial resistance of Pseudomonas aeruginosa biofilms. Microb Infect 2003;5(13):1213–9. https://doi.org/10.1016/j.micinf.2003.08.009.
[54] Llama-Palacios A, Potupa O, Sánchez MC, Figuero E, Herrera D, Sanz M. Proteomic analysis of Fusobacterium nucleatum growth in biofilm versus planktonic state. Molecular Oral Microbiology 2020;35(4):168–80. https://doi.org/10.1111/omi.12303.
[55] Flores Ayuso DP. Effects of spaceflight on morphology and gene expression of biofilms of Pseudomonas aeruginosa grown over different spaceflight- and medically-relevant materials. In: ProQuest dissertations and theses; 2022 [Ph.D., University of Colorado at Boulder], https://www.proquest.com/docview/2760166802/abstract/53958E596A041D9PQ/1.
[56] Romaní AM, Giorgi A, Acuña V, Sabater S. The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams. Limnol Oceanogr 2004;49(5):1713–21. https://doi.org/10.4319/lo.2004.49.5.1713.
[57] Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, Burmølle M, Stewart PS, Bjarnsholt T. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat Rev Microbiol 2022;20(10). https://doi.org/10.1038/s41579-022-00767-0. Article 10.
[58] Siri M, Vázquéz-Dávila M, Bidan CM. Nutrient availability influences E. coli biofilm properties and the structure of purified curli amyloid fibers. 2023.09.07.556686 bioRxiv 2023. https://doi.org/10.1101/2023.09.07.556686.
[59] Stanley NR, Lazazzera BA. Environmental signals and regulatory pathways that influence biofilm formation. Mol Microbiol 2004;52(4):917–24. https://doi.org/10.1111/j.1365-2958.2004.04036.x.
[60] Stoodley P, Boyle JD, DeBeer D, Lappin-Scott HM. Evolving perspectives of biofilm structure. Biofouling 1999;14(1):75–90. https://doi.org/10.1080/08927019909378398.
[61] Stoodley P, Lewandowski Z, Boyle JD, Lappin-Scott HM. Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: an in situ investigation of biofilm rheology. Biotechnol Bioeng 1999;65(1):83–92. https://doi.org/10.1002/(SICI)1097-0290(19991005)65:1<83::AID-BIT10>3.0.CO;2-B.
[62] Stoodley P, Dodds I, Boyle Jd, Lappin-Scott Hm. Influence of hydrodynamics and nutrients on biofilm structure. J Appl Microbiol 1998;85(S1):19S–28S. https://doi.org/10.1111/j.1365-2672.1998.tb05279.x.