Characterization of *P. aeruginosa* Growth Patterns Under Varying Gravitational Conditions

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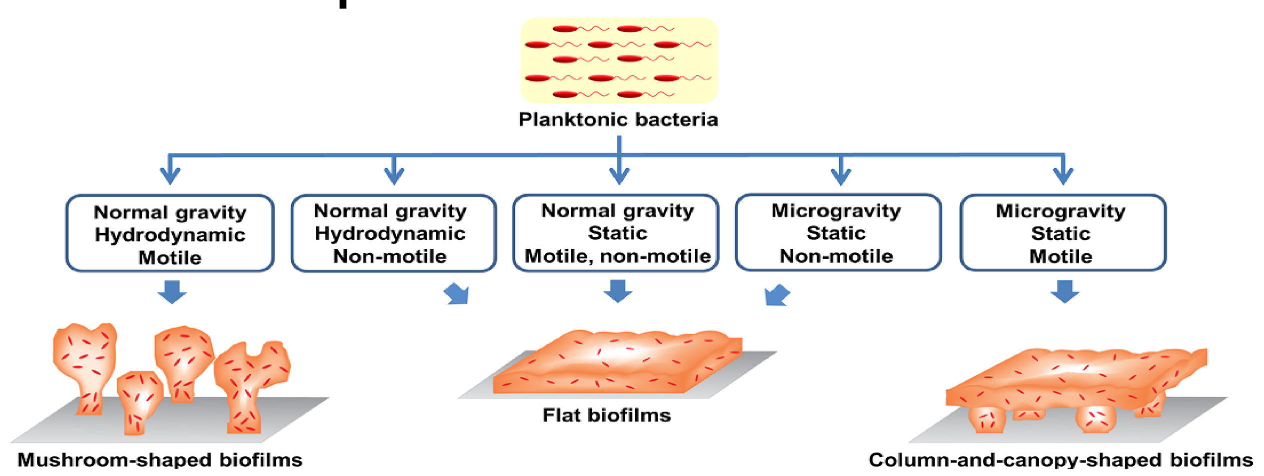
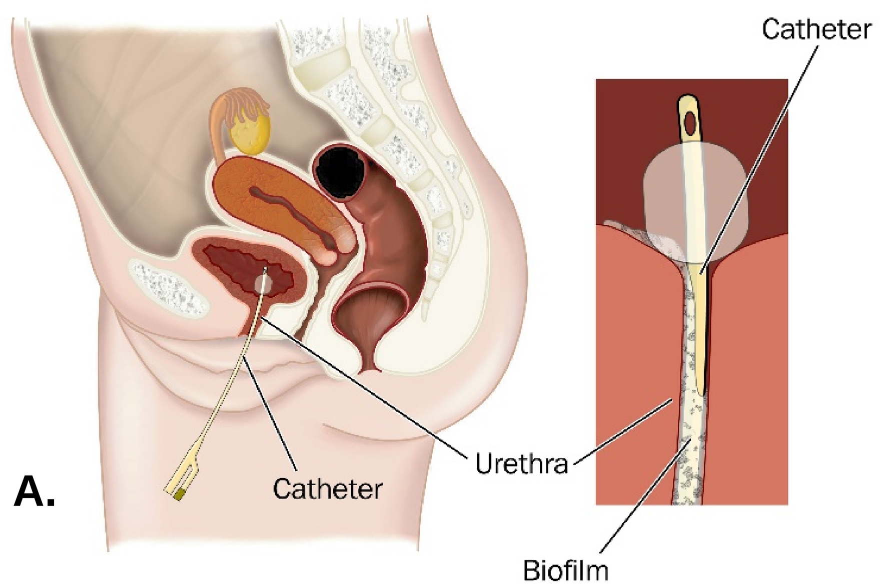
BACKGROUND

- ***Pseudomonas aeruginosa*[1,3,5]**
  - **Responsible for over 11% of all nosocomial infections**
  - **Found in normal gut flora**
  - **Isolated from Apollo 13 and 17 astronauts**



***P. aeruginosa* in spaceflight demonstrates novel biofilm architecture** (Image Credit: Kim et.al, 2013)

- **Biofilms are ubiquitous[2]**



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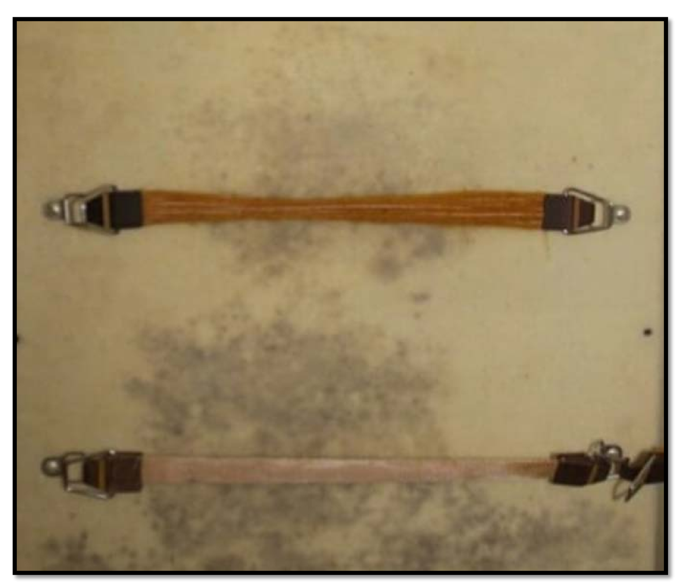


(A) Biofilm development on indwelling medical device (Image Credit: CanstockPhoto) **(B) Biofilm formation seen on the international space station** (Image Credit: NASA)

- **Silicone is a commonly used substrate in both hospital and spaceflight environments**
- **With the growing push for extraterrestrial colonization and long-term spaceflight, there exists a need to identify whether similar changes in morphology occur in varying gravitational environments and whether these changes lend themselves to altered virulence.**

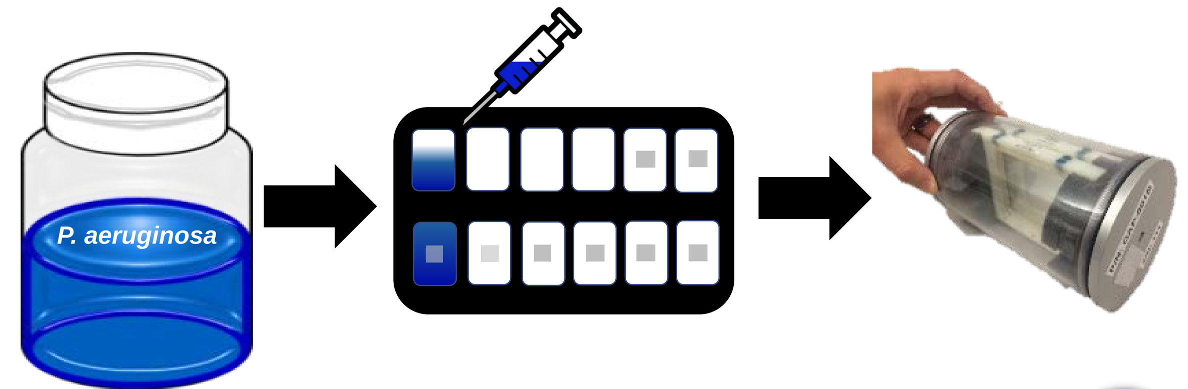
OBJECTIVES

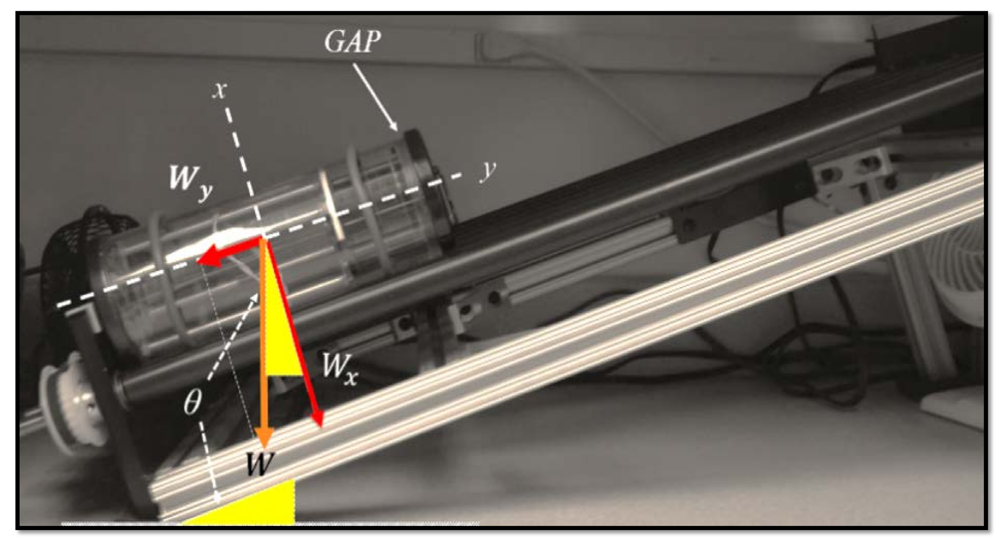
OBJECTIVE 1. Demonstrate planktonic *P. aeruginosa* growth in varying gravitational regimes via colony forming units (CFU) and biomass growth curves over time.

OBJECTIVE 2. Demonstrate *P. aeruginosa* biofilm growth on silicone at varying gravitational regimes via biomass, mean thickness, substratum coverage, and roughness.

METHODS

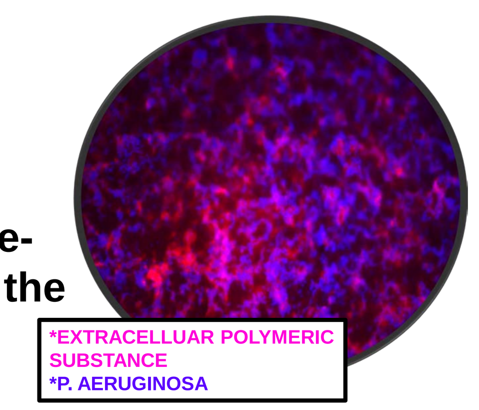
Hydrated P. aeruginosa cultured in 3% TSB was added to a 12 well BioCell loaded with silicone coupons and incorporated inside a Group Activation Pack (GAP) to enable their rotation on the Clinostat.





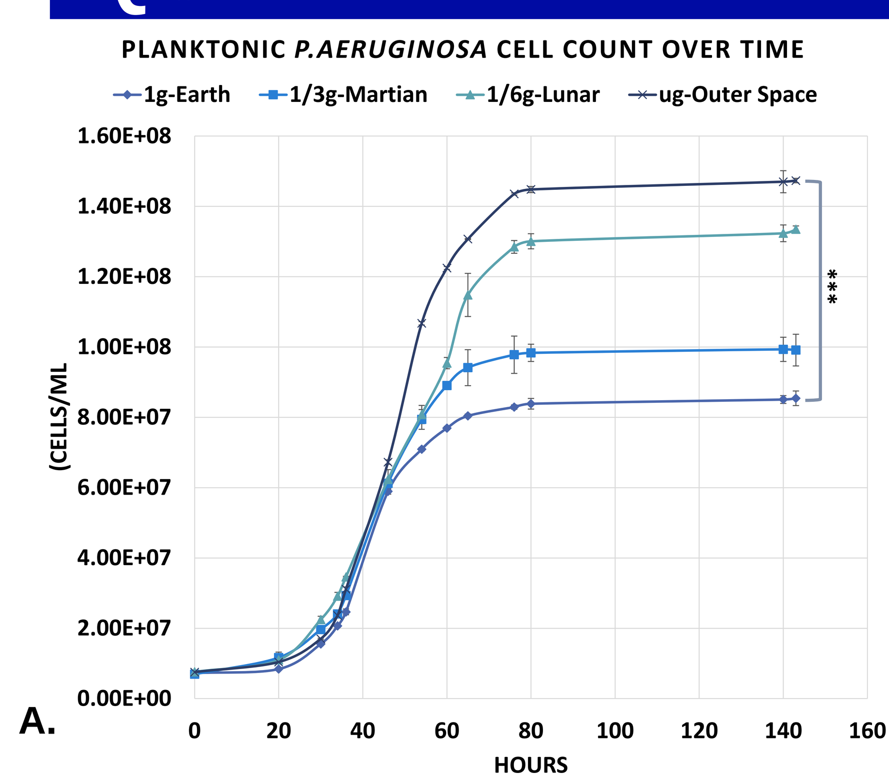
GAP was placed on a clinostat for the simulation of varying gravitational regimes. By continuously rotating the culture vessel, and therefore the gravity gradient, the clinostat can replicate some of the altered extracellular mass transport aspects experienced in actual microgravity.

Planktonic samples were analyzed for cell count and biomass via optical density readings, and silicone samples were analyzed for biofilm biomass, mean thickness, roughness, and substratum coverage via MATLAB and COMSTAT.[4,6] A Brown-Forsythe and one-way ANOVA were used to test for significant differences between the regimes. A post-hoc analysis was conducted to determine significance between each regime individually.



QUANTITATIVE ANALYSIS

A.



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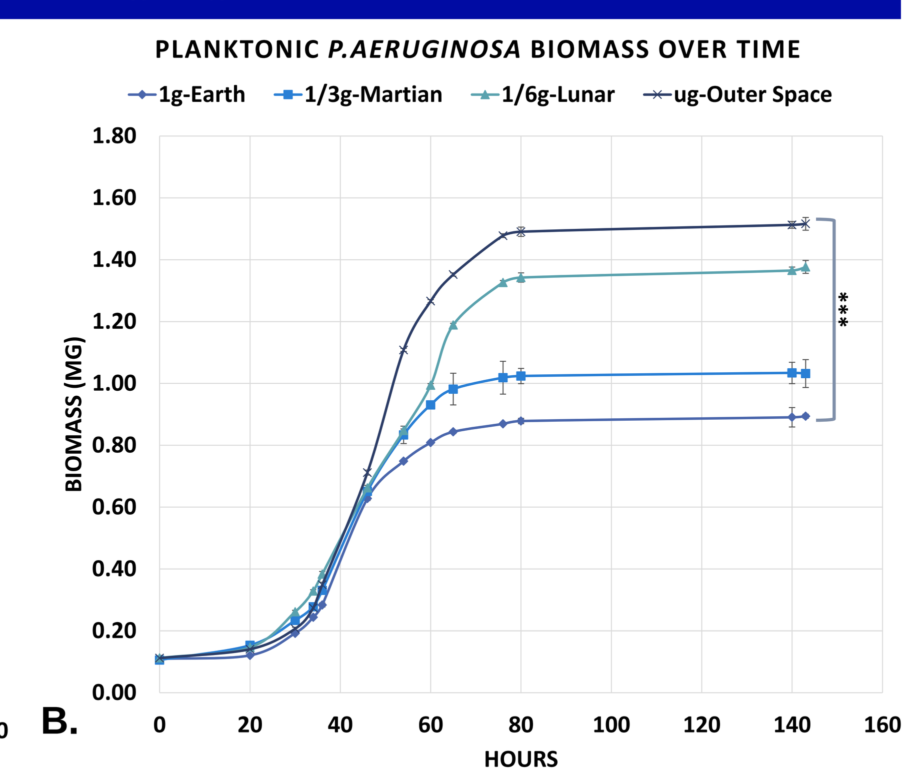

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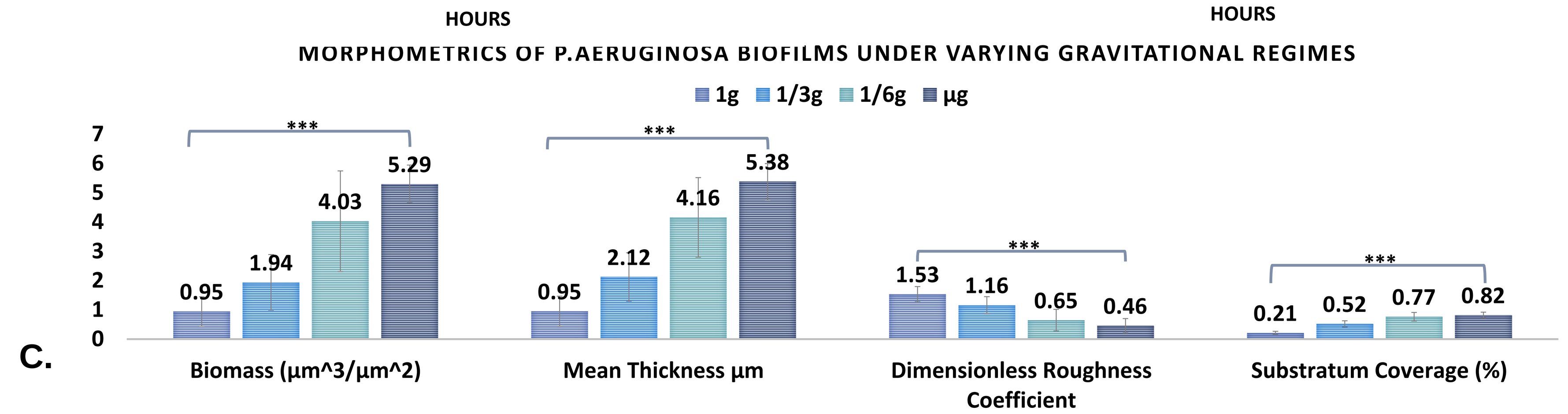
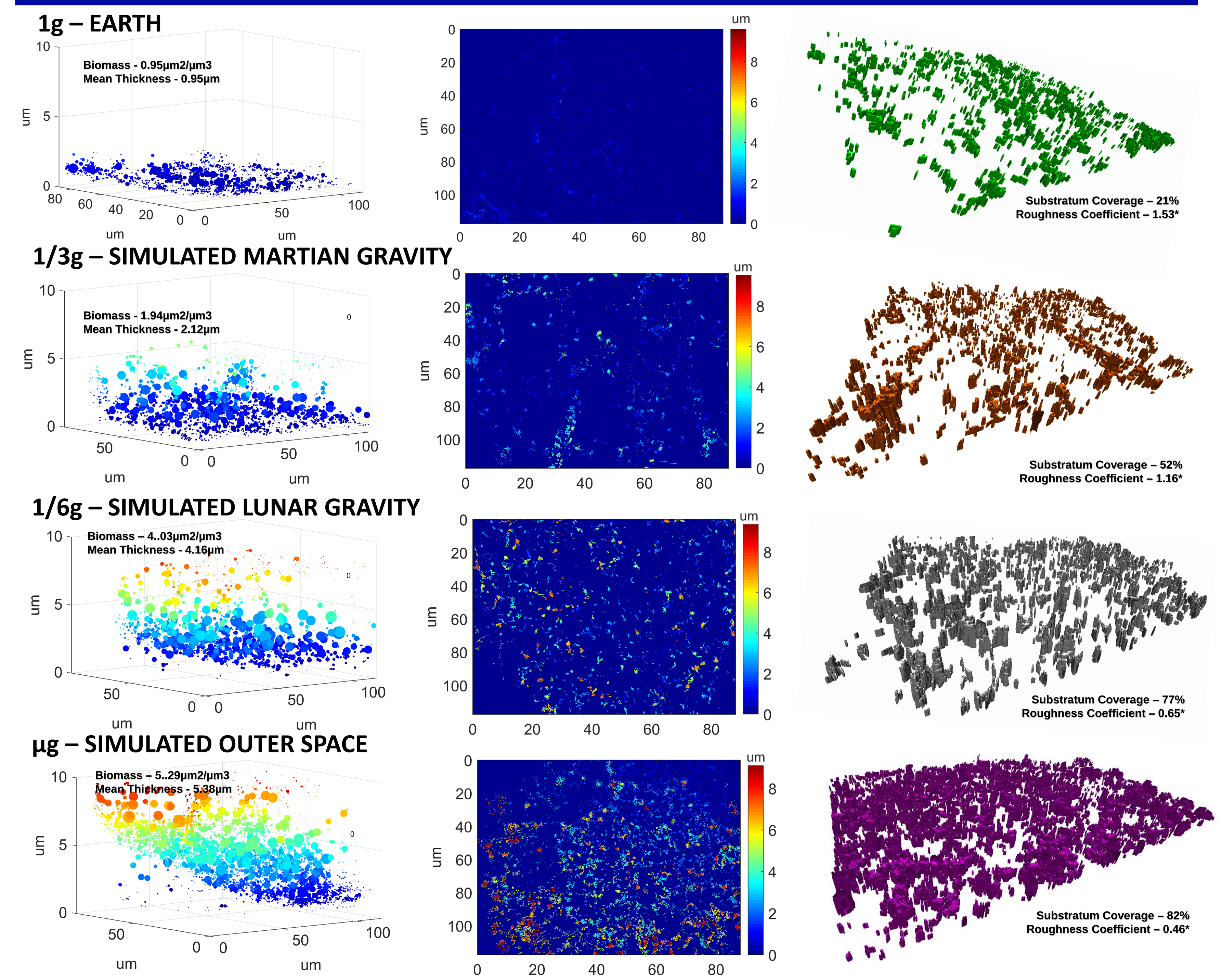


Planktonic *P. aeruginosa* demonstrated significantly different (p<0.01) (A) final cell counts and (B) biomass values between each gravitational regime, with μg demonstrating the greatest values and 1g demonstrating the lowest values. (C) Biofilm morphometrics demonstrated clear patterns of growth with decreasing gravitational regime. Biomass, thickness, and substratum coverage followed an increasing trend with decreasing gravity and biofilm roughness demonstrated decreasing trend with decreasing gravity.

3D BIOFILM ANALYSIS



***P. Aeruginosa* demonstrates significant growth under decreasing gravitational conditions. (Left Column) Centroid plot of cells within biofilm with larger circles indicating areas of greater cell density. (Middle Column) Overview of biofilm thickness density with warm colors indicating areas of greater thickness. (Right Column) Biofilm isosurface render demonstrating approximate substratum coverage and roughness.**

CONCLUSIONS

- ***P. aeruginosa* proliferated with greater capacity in both simulated Lunar and Martian environments, but the simulated microgravity environment was by far the worst with final cell concentrations increasing by over 72% compared to 1g controls.**
- **Gravitational regime plays a role in the formation of *P. aeruginosa* biofilms and altering architectural characteristics. Most notably, the loss of microcolonies - indicated by decreasing roughness coefficients and increasing substratum coverage – was observed as simulated gravity was reduced.**
- **Silicone loses its biofilm resistive capacities with decreasing simulated gravity.**
- **Further studies are needed to elucidate the underlying mechanisms of these changes and determine if any of these changes may have detrimental effects to long-term space flight missions and eventual Lunar and Martian colonies.**

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*Complete list of references available in written manuscript