Biofilms in Space

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The impacts of biofilms are well known in the medical, agricultural, commercial, and industrial spaces. It less known that biofilms are undermining many facets of space travel and that their effects need to be understood and addressed for future space missions. Biofilms can damage space crew health and spoil limited food supply. Yet, at the same time, they can benefit plant systems for food growth, nutrient development, and other biological systems that are being explored for use in space travel. Various biofilm removal techniques have been studied to mitigate the hazards posed by biofilm persistence during space travel. Because the presence of biofilms can advance or hinder humanity's space exploration efforts, an understanding of their impacts over the duration of space flights is of paramount importance.

Keywords: biofilms ; space travel ; bacteria ; health ; microbiome ; pathogens

1. Introduction

It is well known that space travel subjects crew members to elevated levels of radiation that are known to increase their risk of mutations and cancer [1][2][3]. While NASA can try to protect their astronauts with shielding materials on spacecraft and spacesuits, bacteria have found a way to successfully adapt to these conditions. It has been demonstrated that bacteria can genetically and physically modify their tolerances to lower earth orbit (LEO) conditions, and one of the main mechanisms for this was the formation of biofilms [4][5][6]. While subjected to microgravity, the bacterial populations within biofilms have evolved modifications to genes and gene expression that allows them to survive in hostile environments while also increasing their virulence and pathogenicity factors [7][8][9][10]. Since astronauts will be exposed to bacterial biofilms during long-term space travel, it is imperative that the space exploration community develop an understanding of biofilm formation, persistence, and the potential mitigation of their hazards.

2. Bacterial Biofilm Adaptation to the Extremes of Outer Space

The physical properties and characteristics of a biofilm are responsible for their protective and persistent nature. A bacterial biofilm is generally comprised of three components: 1) extracellular polymetric substances (EPS), 2) vegetative cells, and 3) bacterial remnants ^[11]. The EPS of a biofilm matrix is a complex mixture of organic material that acts as a structural glue and a physical barrier to disinfectants and antibiotics ^{[12][13]}. The EPS substance is a mixture of carbohydrates, proteins, lipids, and extracellular DNA (eDNA) ^{[12][13]}. The EPS matrix and its role in the pathogenicity and infection mechanisms is understood; however, the risks and impacts associated with biofilms and space travel is in its infancy.

Over the past decade, there has been an increased awareness of biofilms in space-related environments ^{[14][15][16]}. The formation of biofilms on surfaces and the bio-corrosion of space hardware and life-support systems are a significant concern to all space agencies, while also becoming a growing health concern on Earth ^[14]. Most materials found in space craft are incapable of resisting biofilm formation and require continual maintenance to prevent formation. Additionally, critical systems, such as water pipes, air ducts and life support require service to minimize harmful effects from biofilms ^[16]. This is especially true for the International Space Station (ISS) or any other craft designed to support human habitation for prolonged periods of time.

The main limitation to studying space-related biofilm formation is the ability to simulate space conditions. Even with this major hurdle, researchers have been able to utilize crew time on the ISS and have developed equipment that simulates microgravity to advance the study of biofilms in space [17][18]. One such study, presented by Kim et al., demonstrated that *Pseudomonas aeruginosa* formed a denser biofilm when grown under microgravity conditions than a biofilm grown on Earth [19][20]. The researchers were also able to demonstrate that the nutrient and gas diffusion rates within a biofilm grown under microgravity significantly impacted the overall cell density of a biofilm [20].

The microgravity conditions associated with LEO has also been shown to increase virulence factors in both *Salmonella* spp. and *Escherichia coli* ^{[21][22]}. Virulence and pathogenicity factors are tied to a variety of physical, metabolic, and functional gene expression of pathogens ^[23]. For example, flagella, a feature used for movement, is a key morphological feature that is known to be affected during growth under LEO space conditions [19,20]. Along with movement, the flagella has been shown to stimulate innate immunity, needed for the formation of microcolonies, allow cellular invasion, and promote bacterial surface adhesion ^{[24][25]}. For the transcriptome, microgravity has been shown to alter the expression of genes associated with biofilm formation, toxin production and resistance, and sporulation ^[26]. This also raises the question: what would happen if these enhanced pathogens were transported back to Earth following a deep space mission?

The impact of LEO conditions on the phenotypical characteristics of microorganisms has and continues to be studied to further understand what impact space travel will have on the microbial population about spacecraft. Aboard the Shenzhou VIII spacecraft, a strain of *Klebsiella pneumonia* was found to have conferred enhanced antibiotic resistance during the mission ^{[27][28]}. Interestingly, mutations continued to occur after returning to Earth ^{[27][28]}. The authors demonstrated that the mutations improved at least nine virulence/pathogenicity functions of the strain—including, but not limited to, oxidation-reduction capability and biofilm formation. Schiwon and colleagues demonstrated that over 75% of the *Staphylococcus* and *Enterococcus* species studied on the ISS demonstrated antibiotic resistance ^[29]. The research group postulated that most of the pathogens were normal human microflora, likely originating from the crew and cargo and that the LEO microgravity environment and the constant low-dose radiation exposure promoted the mutations. The increased pathogenicity and virulence factors of the human microbiome illustrates a serious challenge for long-duration space travel. There is no pre-flight "sterilization" process for crew members and their cargo that would limit microbial contamination and mutation during space flight ^[30]. To further emphasize the point, it should be noted that the standard 3-week quarantine procedures for crew members were ineffective at removing and/or limiting exposure to microorganisms that were exposed to LEO conditions ^{[31][32]}.

The phenomenon known as anhydrobiosis has also been associated with bacterial biofilms in space. Contrary to the belief that water is needed to sustain bacterial life, many studies have shown that upon drying, certain bacteria are able to exist in a suspended state with little metabolic activity. Upon rehydration, the organisms are reactivated ^{[33][34]}. An experiment by Billi et al. demonstrated that dried biofilms of *Chroococcidiopsis*, when compared to their multi-layer planktonic counterparts, were able to recover faster after exposure to Mars-like conditions ^[35]. It was speculated that the drying process protected the organisms by minimizing the impact of free radicals and other reactive species that are present in Martian environments. Similar to bacteria suspended in anhydrobiosis, bacterial spores, when comprised in a biofilm, have also been shown to survive exposure to outer space conditions. Horneck et al. demonstrated that over the span of 6 years in outer space, *Bacillus subtilis* spores survived on the bottom layer of a biofilm ^[36]. A protective mechanism similar to anhydrobiosis is believed to protect bacterial spores from free radicals and cosmic galactic radiation.

3. Potential Benefits

Not all biofilms have negative impacts for space travel. In fact, biofilms may provide us with clues on how we as humans can tolerate space flight. For example, Rettberg et al. used a biofilm "dosimeter" to determine if adequate UV radiation was being experienced by astronauts on their space missions to produce adequate vitamin D ^{[42][43]}. The results from the biofilm-based studies indicated that the amount of vitamin D synthesis was inadequate and oral supplementation or sunlamp UV exposure on long-duration missions was recommended. These recommendations are now routinely used during space flight.

It has been proposed that biofilms formed via bacterization could be used to promote competitive ecologies within space systems ^[38]. Intentional bacterial seeding has also been proposed for environmental remediation and human health on Earth ^{[44][45][46][47][48]}. This idea has been proposed and studied, though it is in its infancy, for space-based applications. For example, Ichikawa et al. describes a biofilm reactor experiment used on spaceflight missions, which uses bacteria to clean up the nitrogenous byproducts produced by aquatic organisms ^[49]. Other applications may involve the seeding of beneficial bacteria in waste reactors, on various food production systems, and even seeding the astronaut's intestinal tract prior, during, and after space flight. While these ideas are noteworthy, the interplay between space and bacterial colonization needs further exploration. This is especially true since the long-term effect of radiation on beneficial bacteria has not been studied.

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