Topical: Studying impacts of gravity on essential microbe-animal interactions in analogue and real nonterran environments

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Abstract

Altered gravity may adversely impact microbe-animal interactions essential for host health, immunity and wellbeing. With the next decades of space exploration posing extreme physiological challenges, disruption of crewmembers' microbiomes by the gravitational continuum is an unknown hazard that requires immediate research focus to mitigate risk. In this paper we discuss current gaps in knowledge and recommend areas of investigation.

1) Introduction

Microgravity impacts many key elements within microbe-animal interactions. The most notable are immune system dysregulation, dysbiosis of the gut microbiome, enhancement of virulence of potential microbial pathogens, and disruption of mutualistic interactions [1-3]. As we look to return to the Moon and towards the first crewed deep spaceflight to Mars in coming decades, astronauts will face greater variation in gravitational environments than previously. It is reasonable to assume this gravitational continuum will affect microbe-animal interactions in ways similar to our current understanding of microgravity. However, little evidence exists for us to predict what these may be. Our ignorance about gravity-influenced microbe-animal interactions presents unacceptable risks to astronaut health, wellbeing, and performance and must be addressed over the decadal period.

The majority of animal-microbial research is performed at two points on this continuum – Earth-based studies at 1g [4-6] and microgravity studies, both simulated and ISS flown at 10^{-6} g [7-9], although some reports involving hypergravity and other magnitudes exist [cf. 10]. Beyond Low Earth Orbit, crews may face diverse gravities, including Lunar gravity at 0.17g and Martian gravity at 0.38g. Neither magnitude has been addressed in animal-microbe studies, introducing profound knowledge gaps for animal-microbial interactions accompanying solar system exploration and habitation. As we advance a space-faring society, we also must advance understanding about the impacts of differing gravities on biological systems, such as animal microbiomes. Composed of lower eukaryotes, prokaryotes, archaea and viruses, the microbiome co-evolved with animals and other hosts, and plays essential roles in, among additional phenomena, host nutrient processing, immunity, development, behaviour, and reproduction [11]. The complex interplay between a host and its microbiome may reflect mutualistic relationships that benefit each other, pathogenic/parasitic relationships that benefit one and harm the other, or commensal relationships that benefit one or the other. Furthermore, these relationships are not restricted to the host-microbe dynamic, but may also arise within harboured microbial communities, where members compete or cooperate with one another for host resources [12]. Functional networks of microbe-microbe and microbe-animal interactions help drive host health and wellbeing, contributing to disease expression.

Impacts on human health are observed for several discrete microbiome populations [13,14], perhaps most notably the large heterogeneous population found in the human gut [15]. Proper maintenance of gut microbiota balance is a major concern during spaceflight due to effects of altered gravity on human physiology [16]. Other earthly commensal microorganisms exhibit finely balanced microbial-animal interactions that render benefits for their host yet also cause disease under certain conditions. Examples of important host protection include skin microbiota capable of preventing skin colonisation of pathogenic bacteria by out-competing them for nutrients and space, or by creating local physiological environments hostile to pathogen growth and more hospitable to beneficial species [17]. Moreover, they produce enzymes and antimicrobial compounds that target other microorganisms [18-20] and modulate the host immune system [21-23]. Research shows too that commensal microbes may turn pathogenic when the overall system is disturbed, leading to disease states which require medical treatment. [14,18,23,24]. Metagenomic studies additionally link the oral microbiome to systemic disease, as well as common oral diseases, such as dental caries, periodontitis and gingivitis [25].

For remote space and extraterrestrial operations, disease caused by disruption of microbe-human interactions can become life-threatening and mission critical. This concept paper justifies and recommends a decade-long strategy that emphasizes analogue Earth and *in situ* space research on animal-microbe interactions with the aim of reducing flight and habitation risks to astronaut health, wellbeing, and performance, thereby maximizing near-term space exploration outcomes, such as that planned for the Artemis program. We further discuss the potential benefits of altered gravity research on animal-microbe interactions to Terrestrial healthcare.

2) Pertinent questions and why they need answers

a) What is the impact of gravitational variation on microbe-host communication mechanisms?

Communication between microbes and their host is complex and not fully understood. To coordinate behaviour across their population, fungi, bacteria and archaea use the release of messenger molecules into their environment. Detection of signalling molecules, termed quorum sensing, influences metabolism, growth, biofilms and virulence of microbes of their own species and others [26] and also incurs host responses [27]. Additional communication events between microorganisms and their hosts include signalling systems triggered by other microbially-produced molecules (e.g. carbohydrates, metabolites, RNA) [28-30] and the modulation of microbial behaviour by host-released molecules, such as metabolites, hormones, immunomodulatory proteins, and nucleic acids [31]. Furthermore, at the organism level, microbially produced metabolites crucially affect vital host processes, influencing immunity, metabolism and brain function [21,32,33]

Microgravity perturbs molecular physiology during spaceflight [34,35], so one may reasonably hypothesize that this disruption extends to molecular physiology governing communication between host and microbiome, increasing risk to crew health through altered communications with their microbial symbionts [1,36-38]. What remains unknown is how Lunar or Martian gravity impact these signalling systems, or what effects occur when moving along the gravity continuum over the course of a deep space mission (i.e. Earth to LEO to Lunar Gateway to Mars). Will signalling systems be compromised? If so, is it through reduced production of signalling molecules, impaired molecule detection by the receiving cell/tissue, or changed signalling (physico)chemistry, computations, and diffusion patterns and rates? How might gravity variations cause these effects? These and more questions highlight the need to improve our knowledge, at molecular and systems levels of study, about the influence gravity has over complex communication systems and health risks.

b) How does the gravitational continuum impact biofilm formation, virulence and antimicrobial resistance?

Microorganisms often produce protective structures called biofilms to attach themselves to surfaces and shield themselves from their environment. Composed of polysaccharides, proteins, eDNA and microbiota, biofilms are the main lifestyle of microbial taxa [39] and contain cosmopolitan populations of diverse species across kingdoms [40]. The tremendous heterogeneity of biofilms helps protect their inhabitants from a range of host and ambient environments while permitting access to nutrition. Biofilms are commonly compared to tissue of metazoa [41], with channels for nutrient supply [42], cells with different metabolic activity [43] and co-ordination of cellular behaviour by extracellular signalling mechanisms [44,45]. Considering pathogenic microbe-animal interactions, the protective properties of biofilms retard immune cell access, chemical decontaminants, antimicrobial treatments and mechanical removal [46]. Biofilms also promote expression of virulence genes [47], increase mutation rates in residing cells, and increase horizontal transfer of antimicrobial resistance genes [48,49]. Protective qualities of biofilms also extend to noncellular microbes, as pathogenic viruses may harbour in biofilms without loss of infectivity due to immunoresponse, antivirals or decontamination methods [50].

Most biofilm studies in microgravity, both simulated and actual, have investigated single species biofilms [51]. Such compositions are unrepresentative of prevalent polymicrobial biofilms, hindering mechanistic appraisal of biofilm formation, structure and protection across the gravitational continuum. This gap in understanding raises many questions. Do biofilms become more or less protective? How might biofilms change? Are biofilm mutation and horizontal gene transfer rates accelerated or slowed? Is virulence modified by gravitational variation, and how can we mitigate or suppress the mechanisms involved in virulence? As biofilms are ever-present, flight crews face infection risks from biofilms produced by their own harboured microbiota (e.g. wound associated biofilms, urinary tract biofilm infections, biofilm enhancement of reactivated virus infections) as well as from biofilms in the spacecraft environment [52]. So, it is essential that these questions are answered and better next-generation technologies developed and deployed to counteract that risk.

c) What happens to interkingdom population dynamics, and metabolic interdependency along the gravity continuum?

Microbiome ecologies are defined by their habitat and interactions between community members via networks of cooperation and competition [53]. Microbes release metabolites into the extracellular environment, where they are utilised by other community members, which in turn produce other metabolites in a web of interdependence [54]. This metabolic exchange drives co-occurrence of species and division of labour within the microbiome [55]. Such intra-community behaviour is very complex and our understanding of associated population dynamics is poor, especially in the context of gravitational variation. We do know that microgravity can change production of microbial metabolites [56]. Yet, we do not know if these metabolic alterations affect microbial population dynamics. Do microbial population dynamics shift along the gravitational continuum? If so, what drives changes, perhaps transitions in the host environment or adaptations within the harboured microbes that then impact the surrounding community? Research must be prioritized to gain more detailed insights regarding the effects of gravity on microbial community dynamics, as such findings will contribute to critically maintaining astronaut health, wellbeing, and performance on long-term space missions.

d) What changes occur in the microbiome due to gravitational variance?

The microbiome of an animal is a dynamic entity, changing over time and under the influence of different environmental conditions [57]. Spaceflight alters the composition of various microbial communities of animals [58] and environmental factors, such as circadian rhythm [59,60], diet [61] and stress [62,63] that drive microbiome makeup. These environmental factors indirectly affect hosts via microbiome action, which may impair wound healing [64] and cause mood, anxiety, psychosis, and motor disorders [21,22,24,65,66]. Recent scientific debate highlights the lack of understanding about how microbiome flux influences neuropsychiatric state, behaviour and immunity [67]. We need to know how environment-driven microbiome changes may be altered in gravity conditions other than 1g. Are the mechanisms of change the same in different gravity magnitudes? Are effects on microbial communities potentiated or dampened, and what does that mean for the host? What happens when different environmental factors are combined, and what countermeasures can be taken to suppress unwanted environment-driven alterations in microbiomes? These questions need to be answered in order to preserve microbiome homeostasis and host functions.

e) What microbiome-directed therapies could be employed to support health and wellbeing along the gravitational continuum?

As on Earth, personalised microbiome-directed therapy may be used to modulate microbial community status, although exact approaches may differ due to gravitational conditions. Operation of target host systems may be bolstered by positive host-microbe interactions, such as through use of probiotic or replacement microbes [21,68]. Effects of probiotic organisms in simulated microgravity nonetheless remain inconclusive [69-71]. Further research on the use of probiotic microbes in microgravity is clearly required to reach a definitive answer. In contrast to purely probiotic activity, therapeutic strategies may be adopted that exploit microbial mechanisms to regulate microbiota populations, most notably phage therapy and quorum quenching. Bacteriophages (phages) are bacteria-specific viruses that kill their host under certain circumstances and continue to be investigated for their use against multidrug resistant infections [21,22,72]. But, few details exist about their effects in microgravity. Quorum quenching, the production of molecules that disrupt quorum signalling [73], may be a more suitable alternative treatment solution. Although untested in microgravity settings, targeting quorum signalling, and therefore microbe-microbe and microbe-host communications, may modulate both microbial network and host responses. Such therapies are in their infancy and much is unknown about their potential application in supporting health and wellbeing along the gravitational continuum. Further study is necessary to establish the feasibility of their use.

3) Potential benefits to terrestrial healthcare and other applications

Understanding how microbe-animal interactions support crew health during long-term and deep space missions is directly applicable to healthcare. Firstly, the reversible effect of microgravity on the human body makes it a unique tool in medical research, with physiological effects similar to that of those caused by aging, immunological impairment and dysbiosis [15,74]. In effect, analogue and real astronaut crews model extreme physiological conditions which perturb microbiota and host health, allowing developed and deployed space-related countermeasures to be directly translated to strategic healthcare applications on Earth for populations enduring perilous physiological conditions accompanying famine, drought, extreme temperatures, infectious disease outbreaks, civic instability, and other crises. Secondly, research on microbe-animal interactions along the gravitational continuum will improve understanding of the molecular mechanisms of these systems, aspects of which could be taken and applied to similar molecular systems in other contexts (e.g. microbe-plant interactions, biofouling, etc.) Knowledge could be further utilised in synthetic biology, biomanufacturing processes and microorganism-driven *in situ* resource utilisation.

4) Summary and recommendations

Coming decades will witness establishment of a Lunar gateway and manned Mars missions, as we evolve into a space-faring society. But, conditions under which crews will live for months to years at a time have anticipated deleterious effects on their health. Current countermeasures and risk mitigation are insufficient to support long-term deep space missions, so new technologies and methodologies need to be developed to ensure crew health is supported and maintained. One consideration is development of countermeasures involving microbe-animal interactions. Many gaps in our understanding exist about these interactions and how they are essential to the health and wellbeing of host organisms. It is thus crucial that research be prioritized to assess these interactions over the next decade. We recommend the following:

- Identifying key beneficial microbe-animal interactions and molecular processes in both microbial communities and host that contribute to maintaining optimum function of the immune system, nervous system and gut.

- Developing methods that can be used to support microbial networks and preserve mutualistic interactions.

- Investigating impacts of the gravitational continuum on microbial communities, including effects on beneficial microbes and their mutualistic interactions and on the pathogenicity and virulence of pathogenic and commensal microbes.

- Identifying and developing appropriate space-deployable, clinically relevant biological systems and high-performance computing and empirical technologies for modelling microbe-animal interactions (e.g. wound models, polymicrobial biofilm models, animal models, and standardised methodology of their use).

- Development of imaging and quantification technologies for monitoring and measuring gravitational impact on microbe- animal interactions (e.g. biomarkers/probes sensitive to gravitationally-induced physiological changes, real-time whole animal imaging).

- Development of computational models and simulations for analysis and prediction of microbe-animal interactions

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