Toxicity of Lunar Dust to Human and Other Biological Systems: Enabling Safe Lunar Exploration

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Objective

The authors of this white paper advocate a new Space Biology effort to investigate the fundamental properties of *in situ* lunar dust, with a focus on the enhancement of chemical reactivity and toxicity that arises from the lunar environment, including radiation, particle comminution (fracturing) by micrometeorites, and other processes on the lunar surface. This investigative effort will help to prepare for the safe return of astronauts to the moon and will help to elucidate the nature of the interactions of lunar dust with other biological systems that accompany human explorers.

Introduction

Through the current Artemis program, NASA plans to send astronauts to the lunar south pole by 2024, eventually establishing a permanent presence on the moon[1]. The Artemis program will be an ongoing crewed spaceflight program carried out by NASA with the involvement of commercial spaceflight companies, international partners such as the European Space Agency (ESA), the Japan Aerospace Exploration Agency (JAXA), the Canadian Space Agency (CSA) and the Australian Space Agency (ASA). The Global Exploration Roadmap (ISECG International Space Exploration Coordination Group 2018), with active participation by ESA, represents a blueprint for the next steps for the current and future generation of explorers involving governments, the private sector and academia.

The Apollo experience showed that exposure to dust was an inevitable consequence of lunar surface operations. While a future lunar habitat will almost certainly include an airlock to reduce the entry of dust that has accumulated on suits and equipment during EVA, such activity will likely bring with it lunar dust exposure that will require mitigation. Further, long-duration stays on the lunar surface will likely employ *in situ* resource utilization (ISRU) that will serve to expose non-human organisms to lunar dust (e.g. plant growth and microbes), potentially affecting these systems, as well.

Background

Lunar dust is formed by the continuous micrometeorite bombardment of the lunar surface and is subjected to high energy radiation in the absence of an atmosphere, and a magnetic field. Amorphous material dominates the compositional range of lunar dust: 80% of the fraction below 1 μm is composed of glass[2]. The fraction smaller than 5 μm is rich in impact glass containing deposits of nanophase zero-valent iron[3]. Biological effects of nanophase iron are unknown.

Apollo 14 lunar dust samples have undergone toxicity studies by the *Lunar Airborne Dust Toxicity Assessment Group* (LADTAG), a NASA organization. Sub-optimal storage conditions and lengthy time in storage, however, mean that the samples used by LADTAG had likely lost any "enhanced chemical reactivity" that might have been present when the samples were acquired from the moon. For this reason, studies sponsored by LADTAG probably *underestimate* the true toxicity of lunar dust. Moving forward, experiments designed to provide an accurate, quantitative assessment of the chemical reactivity of lunar dust in its native state are needed, for a re-evaluation of toxicity and for additional studies designed to examine the interaction of lunar dust with other biological systems. Many such studies can likely be performed using either existing, or yet to be developed, lunar dust simulants which have been treated to mimic the reactivity of dust on the lunar surface. This approach should be "cross-validated" by chemical reactivity studies performed on the lunar surface.

Radiation Effects on Minerals and Preliminary Results with Lunar Dust

Although solids and inorganic materials were initially thought to be unaffected by radiation exposure, much evidence indicates that the chemistry of minerals can be altered by radiation. Nuclear reactor radiation, for example, can affect constituents of concrete by interacting with atomic nuclei, breaking chemical bonds, creating crystalline defects and causing other forms of structural disordering.[4,5] Changes in these minerals occur at radiation energy levels that are comparable to the energies of radiative species that are incident on the moon[6], and both crystalline and amorphous materials can be affected. Lunar dust is continuously irradiated by space radiation, so a complete understanding of lunar dust is not possible without an understanding of how lunar dust properties are affected by radiation. All of the components of space radiation (galactic cosmic radiation, solar particle event, solar wind and UV light) have the potential to alter the surface activity of lunar dust, which contacts living tissue. Broadly speaking, electron defects, ion implantation and alteration of the redox state of metal ions are all possible. Some preliminary experiments were performed more than 50 years ago[7] in which the effects of both UV irradiation and solar wind were studied in Apollo samples. Spectroscopic signatures of lunar dust were altered by these exposures, indicating changes in the oxidation state of iron.

Crystalline Silica (Quartz) Effects in the Lungs on Earth: An Important Benchmark for Considering Lunar Dust Toxicity

Respirable crystalline silica dust is one of the most clinically significant pulmonary toxic substances on Earth.[8] Quartz triggers an inflammatory reaction when breathed into the lungs leading to fibrosis (scarring), an irreversible process. Over time—*months to years*—lung capacity is significantly reduced, leading to permanent impairment of lung function and early death. If freshly-fractured quartz (quartz with enhanced chemical reactivity) enters the lungs (e.g. as occurred in the Hawk's Nest Tunnel Disaster), toxicity may be worse, because free-radicals are increased[9,10] and new surface functionalities are formed. While lunar dust cannot be compared directly to crystalline silica (since lunar dust is amorphous), crystalline silica demonstrates that chemical activation can have a profound impact on mineral toxicity.

Implications of Enhanced Chemical Reactivity

Investigations on terrestrial minerals highlighted a number of features that are relevant for dust toxicity. Oxidative activity of mineral dusts is widely accepted as one of the key factors contributing to lung inflammation. The formation of particle-derived reactive oxygen species (ROS), including superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH), is known to induce oxidative stress in cells, abnormal proliferation, and tissue damage when the amount of ROS overcomes the antioxidant cell defenses. Beyond free-radicals, there is a specific silanol (≡Si-OH) sub-group at the surface of silica, termed "nearly free silanol" (NFS). NFS was recently shown to induce the permeabilization of the lysosome membranes of alveolar macrophages to activate the NLRP3 inflammasome, and to initiate lung inflammation in a rat model.[11] NFS is generated at the surface of crystalline silica during fracturing, and its relative amount is likely modulated by thermal and/or chemical treatments.

Implications in Non-mammalian Systems

Microbes, plants and other biology are expected to play important roles in the new plans for moon exploration and will likely come into contact with lunar dust. Engineered microbes may be utilized for biomining, for example, to extract valuable elements from lunar soil, for Synthetic Biology

purposes.[12–14] Plants will serve as a food source for astronauts and may be grown in lunar soil.[15] Understanding the fundamentals of lunar soil-dust interaction with these non-mammalian biological systems, including the importance of chemical reactivity, will help to ensure that these systems can be successfully deployed.

Earth-based Experimental Approaches to Enable Studies of Toxicity

Simulants, Authentic Lunar Dust and Methods of Chemical Activation

For practical reasons, we recommend that the bulk of the investigations needed to advance our understanding of lunar dust biological effects be Earth-based. This will necessarily involve the use of lunar dust simulants and archived lunar dust. Those materials will need to be "activated" to faithfully mimic lunar dust on the lunar surface.

Simulants provide a practical means to study toxicity. JSC-1a, an amorphous material of volcanic origin, has been used as a simulant for toxicology experiments. JSC-1a, however, lacks nanophase iron and other features of authentic lunar dust. For this reason, participants in the recent NASA Engineering and Safety Center (NESC) workshop on lunar dust (Houston 2020) concluded that "there is a need for a [new] standardized lunar simulant for rigorous assessment of toxicity relevant to upcoming lunar missions." This gap was rated a "top priority" for defining the impact of lunar dust on human health.[16] The authors of this white paper concur with the NESC that a new lunar dust simulant would be highly desirable and would allow higher-quality biological experiments to be performed, especially if a new simulant can be cross-validated against authentic lunar dust.

Authentic Lunar Dust can be used to validate simulant usage. Authentic lunar dust, returned to Earth during the Apollo era, is managed by the ExMAG (the *Extraterrestrial Materials* Assessment *Group* (previously called CAPTEM).[17] Various amounts of materials from the six Apollo missions that returned specimens to Earth are available to be released for research purposes.

Chemical Activation can be achieved by mechanical grinding and radiation. Because the archived lunar dust specimens have lost any chemical reactivity that may have been present at the time the specimens were acquired, re-activation will be necessary, and we have several tools at our disposal to accomplish this task. To partially replicate the effect of dust particle comminution associated with micrometeorite bombardment, mechanical grinding systems can be used. By conducting mechanical-grinding in an inert atmosphere (e.g. argon), we can preserve maximum possible chemical reactivity prior to beginning biological testing. Radiation treatment will be another important modality for chemical activation of lunar dust. Two radiation exposure facilities are available, which allow various types of simulated space radiation, one in the U.S. and one in Europe.[18,19] These facilities provide access to several different beam lines with several different types of energetic particles, to replicate the effects of galactic cosmic radiation and solar particle event radiation. Efforts should also be made to include simulation of the effects of the solar wind. Once chemical activation is established, passivation kinetics and methods to deactivate the dust should be determined.

Lunar Dust Toxicity Testing Using Multicellular Human Lung Mucosa Models

Advanced cellular studies can provide valuable toxicity measurements. The gold standard for human toxicity assessment is *in vivo* studies of inhaled material in rodents, such as those performed by LADTAG. However, such experiments are exceedingly time consuming to perform, and the results carry concerns about inter-species comparability. *In vitro* studies using human epithelial cells present a promising avenue for assessing the acute effects of lunar dust on the lung that will

serve to form a bridge between the chemical activity studies and studies in animals. Physiologically relevant lung-mucosa models with primary human cells cultured at an air–liquid interface are a realistic alternative for pulmonary toxicity testing.[20] Multicellular air–liquid interface models with human primary cells including various epithelial cell types (ciliated cells, goblet cells, club cells and basal cells) and macrophages, provide physiologically relevant airway mucosa models to use for evaluation of health effects of respirable aerosol particles of lunar origin.[21,22] An important feature of these airway wall models is the formation of a thin liquid lining layer including mucus together with the presence of ciliary movement mimicking the mucociliary clearance present *in vivo*. The PreciseInhale® aerosol generator combined with either the Xpose*ALI*® cell exposure module, or a "lung-on-a-chip" device[23], can be used to deliver quantified amounts of aerosols. This approach allows toxicity tests to be completed with a limited amount of material (hundreds of mg). Typically measured outcomes of the exposures are: 1) oxidative stress; 2) inflammatory markers; 3) toxicological endpoints; 4) markers of tissue degeneration) and, 5) ultrastructural morphological changes.

In situ Lunar Surface Dust Measurements and Sample Return

The special characteristics of the lunar surface environment require *in situ* measurements of dust reactivity, as well as a new effort to return lunar dust in a pristine state for ground-based detailed chemical analysis and toxicity assessments. The latter can be used to validate and further improve the design of future dust toxicity research based on the use of new lunar dust simulants. Sample return from multiple lunar sites (or *in situ* measurements from multiple sites) should be prioritized, in order to understand how lunar dust varies from site to site, which could affect toxicity.

A simple-to-perform method that provides easy-to-interpret results of lunar dust reactivity in situ on the lunar surface is required. A suite of instruments should be carried on the first Artemis lunar lander missions to provide sample context (size distribution, particle shape, mass, and mineralogical/chemical information) as well as a measure of dust reactivity. A fluorometric assay based on the conversion of the terephthalate anion (TA, non-fluorescent) to 2-hydroxyterephthalate (TA-OH, strongly fluorescent) upon exposure to ROS could be readily exploited to obtain the reactivity information required.[24,25] To translate the fluorescence signal to a usable quantitative reactivity assessment, information about the size distribution and particle shapes/roughness (together yielding a measure of sample surface area) is required. This information could be obtained *via* a combination of optical and atomic force microscopy based on the Mars Phoenix lander heritage. An alternative method would be the direct measurement of specific surface area via the B.E.T method.[26] Mineralogical/chemical information of sample particle composition could be obtained using Raman/LIBS (laser-induced breakdown spectroscopy) instrumentation, to assess the influence of the composition of the dust on reactivity. The latter may be important as the mineralogical makeup of Artemis landing sites may differ significantly from those of Apollo landing sites.

Pristine sample return (small volumes/mass only) should be a component of the earliest Artemis missions (or any other lunar surface mission) and should include a means to sample lunar dust obtained from a site distant from the landing site (and thus avoiding the effects of rocket plumes). Top-soil samples should be collected in containers designed to be sealed on the lunar surface under non-reactive atmosphere (e.g. argon positive pressure) and should maintain those conditions for transportation back to Earth. Such samples need to be analyzed in terrestrial laboratories to close the loop between the *in situ* measurements on the lunar surface and studies of the toxicity of lunar dust simulants on Earth. Analysis of the reactivity of returned samples and direct comparison to *in*

situ data will quantify the effect of sample return. Both archived Apollo lunar dust and simulants should be subjected to mechanical milling in a non-oxidative atmosphere to simulate micrometeorite impacts on the moon. Ion implantation and transition metal ion reduction should be carried out at radiation facilities to mimic the effects of solar wind. Simulants should be compared to recently-returned pristine lunar dusts by the same TA assay, and the results compared to those obtained *in situ*. Direct comparison of the reactivity of lunar dust on the lunar surface with reactivity of both activated simulants and activated archived lunar dust will provide information necessary to directly address the existing knowledge gap highlighted in the recent NESC workshop[16].

Summary

The authors of this white paper advocate a new Space Biology initiative in lunar dust research, directed at furthering our understanding of the biological consequences of *in situ* lunar dust, by considering the effects of chemical reactivity caused by space radiation and mechanical fracturing. This renewed effort will serve to extend the results of NASA's previous effort (organized under LADTAG) and will be relevant not just to the human system but to Synthetic Biology technology and plant systems that will likely accompany human explorers. The proposed studies are focused on "do-ability" and the return of valuable information that will support NASA's Artemis Program and the moon exploration plans of other space agencies. The proposed research effort is directed at delivering results that will produce actionable information that can be utilized to set standards for lunar dust exposure and, where necessary, to implement mitigation strategies to prevent deleterious effects of potentially highly-reactive lunar dust. The proposed studies are structured to require limited on-the-moon activity, and modest sample return. Further, the largely ground-based studies can begin immediately and adjustments to the experimental approach can be made as new information becomes available in early phase Artemis missions.

Recommendations

R1	*Lunar mission*: Acquire the mineralogical and chemical context of lunar dust samples
R2	*Lunar mission*: Measure the reactivity of lunar dust in aqueous media as radicals that are generated per unit surface area of lunar dust
R3	*Lunar mission*: Return pristine lunar dust samples to permit cross-validation studies of re-activated Apollo era lunar dust, and simulants
R4	*Earth based*: Develop new lunar dust simulants as recommended by the NESC
R5	*Earth based*: Determine the ability of returned lunar dust to form ROS when in contact with water, and compare to simulants after (re-)activation, and determine passivation kinetics in habitable environments.
R6	*Earth based*: Use human lung mucosa models to determine the toxicity of dust simulants that have been cross-validated against lunar dust reactivity *in situ.*
R7	*Earth based*: Use human lung mucosa models to determine the toxicity of activated archived lunar dust, activated simulants, and pristine lunar dust.

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