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History and Future Perspectives for the Evaluation of the Toxicity of Celestial Dust

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HISTORY AND FUTURE PERSPECTIVES FOR THE EVALUATION OF THE TOXICITY OF CELESTIAL DUST

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The Apollo Experience

In 1969 the crew of Apollo 11 successfully landed on the Moon and then returned safely to Earth. The success of Apollo 11 was followed by five more crewed landings (Apollo 12, 14, 15, 16, and 17). Although the missions had slightly different individual objectives, they shared some common objectives, including exploring features on the Moon, examining the lunar environment, and assessing the feasibility of establishing a lunar outpost (Naser, 2019).

All of these Apollo missions were adversely affected by the lunar dust, which established itself early on as a nuisance because of its physico-chemical properties and the associated difficulties in its control and cleanup. With the lack of an atmosphere and in reduced gravity conditions ($1/6$ g), lunar dust is easily lifted from the lunar surface. There are two general classes of dust transport mechanisms: natural (e.g. secondary ejecta from meteor and micrometeoroid collisions with the surface, and electrostatic levitation of dust) and anthropogenic (e.g. astronaut ambulation, rover wheels lifting dust, landing and take-off of spacecraft (Katzan et al., 1991), astronaut falls (Gaier, 2005), or intentional kneeling to better observe the

surface). None of the natural transport mechanisms are expected to transport significant amounts of dust and only the anthropogenic mechanisms seem to have a significant impact on astronaut exposure.

All spaceflight evidence pertaining to the effect of lunar dust on astronauts is anecdotal (Scully et al., 2015) and mission documents have been studied to catalog the possible adverse effects of lunar dust. Some of the adverse effects included visual obscuration, false instrument readings, dust coating and contamination, loss of traction of the rover during an extravehicular activity (EVA), clogging of mechanisms, abrasion of suits, especially gloves, thermal control problems, and seal failures. More specifically, regarding health effects, which is the topic of the present work, astronauts reported that when they returned to the lunar module after EVAs and removed their spacesuits, dust exposure occurred causing eye, throat, and lung irritation. Dust adhered “to everything, no matter what kind of material” with “restrictive, friction-like action” (Cernan et al., 1973). After leaving the lunar surface, any dust in the vehicles began to float in microgravity. Dust found its way into even the smallest openings, and when the Apollo 12 crew stripped off their clothes on the way back to Earth, they found that they were covered with dust. Dust was also transferred from the Lunar Module to the Command Module and caused upper respiratory irritation during the entire trip back to Earth (Gaier, 2005). There was continual inhalation exposure to airborne dust, as well as skin exposure and eye contact from surface contamination on the return journey to Earth (Cain, 2010a). Moreover, the Apollo crews reported that the dust gave off a distinctive, pungent odor, suggesting the presence of reactive volatiles or reactive surfaces on dust particles. Lunar dust induced symptoms of respiratory irritation in some crew members (Cernan et al., 1973) who used expectorants to facilitate clearance of the particles from the upper airways. These effects may be attributed to acute, albeit mild, reactions to dust particles deposited in and cleared from the upper airways (Barratt, 2019). The health effects experienced were heterogeneous and differed in severity and duration. In all cases, the observed symptoms were transient, and no lasting respiratory effects were observed in returning Apollo crew members.

The Need to Investigate the Toxicity of Celestial Dusts

The Future of Space Exploration Will Entail a Dusty Journey into the Unknown

The Apollo lunar flights ended in 1972, but the Moon has remained of great interest to space agencies and scientists worldwide. In 1989, the Space

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Exploration Initiative (SEI) was announced by the United States and committed NASA to returning to the Moon as well as to exploring Mars. This ambitious program slowed, but it regained momentum in 2017 when NASA refocused exploration efforts on the Moon as the starting point to reach Mars and even go beyond (Dunbar, 2018).

This new phase includes the involvement of international and commercial partners. Since transportation to (and from) the Moon requires less energy, time, and cost than that required to reach Mars, the Moon represents the ideal destination to establish a convenient outpost for further space exploration and a test site for examining the human capability to live beyond low earth orbit. Through its current Artemis program (Figure 8-1), NASA envisions sending astronauts to the lunar south pole by 2024 and eventually establishing a permanent presence on the Moon. NASA gained broad international support for the Artemis program from several national agencies and private companies (Potter, 2019). Artemis is now an ongoing crewed spaceflight program carried out by NASA, commercial spaceflight companies, and 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, 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.

Long-duration missions and planetary operations entail numerous risks that must be understood and mitigated to maintain the health and productivity of crew members. Several human spaceflight hazards need to be considered for any exploration mission. A central health concern for future crewed missions is represented by the fraction of lunar soil with a diameter smaller than 20 μm , which is described by the term “lunar dust” (McKay et al., 1991). Based on the Apollo experience, lunar dust caused a plethora of problems for both mechanical systems and crew members, as described above. Thanks to the short time exposure, these symptoms were not long-lasting and did not cause any long-term effects (Scully et al., 2015).

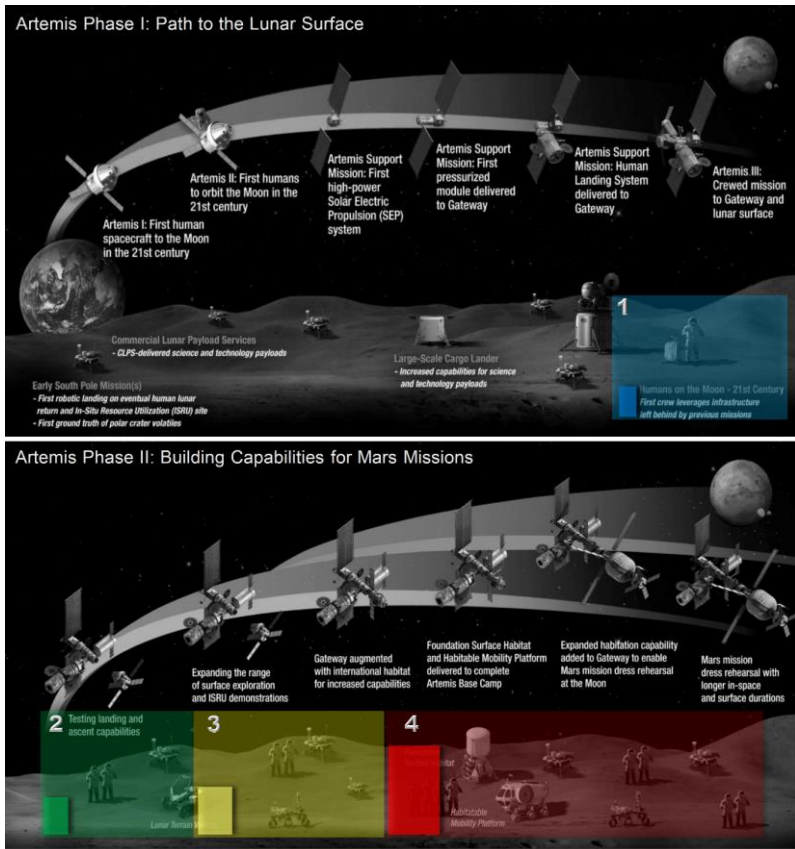


Figure 8-1. Qualitative estimation of lunar dust exposure during the Artemis program, as color-coded intensity bars. Limited crewed activities are expected to occur in Phase I (targeted for 2024) and are destined to increase in Phase II with prolonged human missions (in the period 2025-2029). Phase I activities are planned to use infrastructure left on the Moon's surface by previous uncrewed missions. The first step of Phase II will lead to limited permanent human presence. The degree and duration of exposure to lunar dust is expected to increase as the project matures. Furthermore, the dustiness of the lunar vehicles will significantly increase with the expansion of surface exploration and ISRU demonstrations. With the establishment of surface habitats and the expansion of habitation capability, the astronauts will have to cope with the ubiquitous presence of dust. Adapted from "America to the Moon 2024" (NASA, 2019).

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In the context of future exploration, the astronauts' presence on the lunar surface will initially be limited to missions lasting up to 6.5 days during Phase I of the Artemis program. With the transition to Phase II, the duration of the missions and the number of astronauts involved are destined to increase as well as the duration of their exposure to lunar dust. Figure 8-1 reports ESA's Topical Team on the Toxicity of Celestial Dust's (T3CD) qualitative estimation of lunar dust exposure for each step of the crewed activities during the Artemis program. During the early crewed activities of Phase I that consist of the use of infrastructure left on the Moon's surface by preliminary uncrewed missions (1), exposure is expected to be limited. The first steps (2) of Phase II will be characterized by a limited presence of humans on the Moon, but the extent of their exposure is expected to increase with respect to Phase I due to the spread of dust from landing and ascent activities. Dust exposure will significantly increase at the later stages of Phase II when the residence time of astronauts will increase to potentially beyond one month and the number of EVAs will be significantly higher than during the Apollo program. Moreover, the creation of sustainable infrastructure to explore and sustain human life on the Moon will be achieved by *in situ* resource utilization (ISRU) strategies (European Space Agency, 2018), presenting possible scenarios of inevitable exposure to lunar dust. A logical extrapolation from the Apollo lunar experiences is that critical issues related to dust exposure will occur during a sustained human presence on the Moon. To be prepared for the inevitable exposure and to design appropriate safety measures, lunar dust and its unique properties must be thoroughly investigated from a toxicological perspective.

The Unique Origin and Composition of Lunar Dust

Since the first Apollo astronauts' debriefings, lunar dust toxicity has been one of the major concerns for future lunar exploration, and it became clear during the Apollo experience that lunar dust has an almost uncanny ability to get absolutely everywhere.

Lunar dust is formed by the continuous micrometeorite bombardment of the lunar surface and is subjected to high energy radiation in the absence of humidity and atmosphere. Due to these unique environmental conditions, lunar dust exhibits physico-chemical features uncommon on Earth. Amorphous material dominates the compositional range of lunar dust: 80% of the fraction below 1 μm is composed of glass (Thompson et al., 2010). The fraction smaller than 5 μm is rich in impact glass and nanophase zero-valent iron (np-Fe⁰) (Taylor et al., 2010), which is formed during vapor deposition caused by the flash heating of mineral or glass phases due to

(micro)meteoroid impacts. The abundance of np-Fe⁰ increases as the particle size decreases. The presence of np-Fe⁰ is relatable to the high reactivity of lunar dust (Wallace et al., 2010) and may play an important role in lunar dust toxicity.

Another feature that must be considered is the toxicity of ilmenite – an iron titanium oxide – which might have adverse effects if inhaled because of the presence of iron as well as titanium. Moreover, in the reduced 1/6 g of the Moon, dust easily becomes airborne inside habitats, increasing the risk of inhalation and increasing the fraction of particles that can reach the peripheral lung by escaping the lung clearance mechanism (Darquenne et al., 2013).

Possible Dust Exposure Scenarios

The Apollo experience showed that exposure to dust was an inevitable consequence of lunar surface activity. A future lunar habitat will almost certainly include an airlock with the benefit of reducing the entry of dust that has accumulated on suits and equipment surfaces during EVAs. However, it seems plausible that any EVA activity will likely bring with it dust exposure that will require mitigation. These activities include:

- Routine EVAs, including EVAs for scientific activities and construction, maintenance, and ISRU purposes;
- Transfer from the lunar surface into the lunar habitat; from the lunar surface into a lunar access vehicle; and from the lunar access vehicle into the crew exploration vehicle (CEV);
- Activities during a contingency situation;
- Engineering failure of the dust control systems (e.g. Heat, Ventilation and Air Conditioning (HVAC) filters, electrostatic removal, magnetic capture, etc.). This includes contamination of the inside of the space suit and/or module and habitat after extravehicular activities.

Routes of Human Exposure to Celestial Dusts

The most likely, and very possibly the most consequential, dust exposure is that associated with the inhalation of airborne dust. This route will directly impact the lung epithelium as well as the oropharyngeal and nasopharyngeal regions. It is well-known that terrestrial environmental exposures to inhaled particulate matter pose a significant health risk to humans, and there is every reason to suspect that the same will be true for celestial dusts.

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There are also routes for non-pulmonary exposure:

- Entry of dust into the body through skin penetration. This exposure can occur via a traumatic injury or a penetrating injury resulting in a wound that becomes contaminated with dust. Alternatively, dust can gain entry through minor wounds or abrasions when spacesuits abrade the skin, as current EVA suits have been observed to do. Moreover, if celestial dusts enter the suit interior, as was the case during the Apollo missions, this could serve as an additional source of abrasion or enhance suit-induced injuries (Scully et al., 2015).
- Ocular exposure both during routine exposures, such as the removal of suits following an EVA, and during contingency exposures. Such exposure has the potential to irritate the cornea, the eyelids and lid margins, and the conjunctiva.
- Gastrointestinal exposure. Such exposure can occur acutely as dust is tracked into habitats and potentially contaminates food and food preparation surfaces, in much the same way as pulmonary and ocular exposures might occur. Furthermore, gastrointestinal exposure will very likely be secondary to pulmonary exposure. Most of the dust entering the airways is captured and removed by the mucociliary clearance system, a mucous-covered conveyor belt. This clearance system moves any captured components, in this case dust, to the throat, where those components are swallowed and subsequently disposed of in the gastrointestinal tract. Therefore, as long as there is respiratory or pulmonary exposure, there will also be gastrointestinal tract exposure, even if dust is kept out of food and water.

In the context of prolonged residence on the Moon or other celestial locations there is a likelihood that crews will start growing edible plants *in situ*. This may involve the use of lunar or other planetary soils. In this case, the direct or indirect contamination of plants must be taken into account. Eating vegetables grown in extraterrestrial soils may be a way of directly ingesting celestial dust or toxic ions leached by irrigation water and absorbed by plants. Another concern is represented by the systemic absorption of toxic ions, leached from the soil, absorbed, and concentrated by plants. For example, soluble perchlorate salts, which are believed to have widespread distribution on the Martian surface, ranging from 0.5 to 1% w/w, are easily leached due to their high solubility in water, making it a potential hazard to humans on the red planet (Davila et al., 2013).

Toxicity of Lunar Dust

Lunar Dusts and Lunar Dust Simulants

There are several peculiar physico-chemical features of lunar dust that are likely relevant to toxicity. The lunar regolith was formed in relatively reduced conditions in the absence of atmospheric water and oxygen and continuous micro-meteorite impact events. Furthermore, the continuous bombardment of solar wind implants protons, which radically modify the physico-chemical properties of the dust particle surface. Studies of returned samples have shown that the bulk of this lunar regolith – generally defined as the size fraction below 1 cm of the regolith covering the lunar surface contains a significant amount of reactive dust, including a respirable fraction below 10 μm . Grain size distribution analyses of Apollo lunar soil samples have revealed that between 5 and 20% by weight of lunar soils is in the respirable range (James, 2007).

From a mineralogical point of view, lunar dust is mainly made of impact glass (mostly agglutinitic glass), plagioclase, and pyroxene, which together constitute 70–98% of the dust. Pyroxene and plagioclase are virtually equally distributed in mare dusts, whereas highland dust contains about equal proportions of plagioclase and agglutinitic glass (Taylor et al., 2001a, 2001b, 2010). Minor components include pyroclastic volcanic glass beads and ilmenite and olivine as trace minerals. The abundance of agglutinitic glasses increases with decreasing grain size. The fine fraction of most soils generally contain more than 50% of agglutinitic glass, and the inhalable fraction may contain up to 70% (Taylor et al., 2001a, 2001b, 2010).

Since lunar rocks crystallize in systems with a paucity of free oxygen (negligible partial pressure of O_2), iron at zero-valence state – Fe^0 – represents a stable species and occurs in all lunar rocks as myriads of nanometric iron grains (nanophase zero-valent iron, np- Fe^0) deposited on the rims of agglutinitic glass. Moreover, meteoritic FeNi metal from metal-rich impactors, such as iron meteorites, is also present. Besides this highly reduced form, all remaining Fe is present as Fe^{2+} while virtually no highly oxidized form (Fe^{3+}) occurs. The oxidation state of iron is one of the most relevant geochemical differences between Moon and Earth minerals, where Fe^{3+} dominates mineral chemistry. As a result of this, apparently similar minerals (e.g. ilmenite) on the Earth and the Moon show quite different chemical properties and may present different reactivity towards biomolecules and tissues when inhaled. Several studies on the interaction between toxic minerals and human lungs demonstrated the peculiar role of reduced iron ions exposed at the mineral surface (Weitzman et al., 1984; Kamp et al., 1995; Gazzano et al., 2007; Turci et al., 2011). A second key

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difference is the presence of minerals containing structurally bound hydroxide or water molecules in many terrestrial minerals, which are rare or absent in lunar rocks. Conversely, volcanic glasses on the Moon are generally orders of magnitude more water-rich than their terrestrial equivalents. The different hydration states of the material may have an important impact on inhalation toxicology and warrant further consideration.

The Big Simulant Rush

The ideal material for toxicity studies would be real lunar dust, but with a total mass of Apollo samples being lower than 500 kg, and the dust constituting just a fraction of that, this material is priceless, and only limited quantities are made available for well-planned non-destructive research. This necessitates the use of lunar dust simulants that can be accessed by the wider scientific community. The ideal simulant exhibits high fidelity, and chemical and mineralogical homogeneity. Moreover, it must be easily available and inexpensive to produce and purchase. The features required in a simulant are strictly dependent on the research purposes for which the simulant will be used.

The production of lunar dust simulants started in 1994 with JSC-1, the first lunar soil simulant standardized by NASA. JSC-1 was produced from volcanic tuff/ash mined just north of Flagstaff, AZ (McKay et al., 1994), and it contained abundant volcanic glass (49 wt.%, Hill et al., 2007). Its bulk chemistry resembled some Apollo 14 soils (McKay et al., 1994; Hill et al., 2007). Because of its high glass content, mimicking the high levels of agglutinate glass in lunar soils, this simulant possessed the appropriate lunar geotechnical properties and was originally meant to be used mainly for mechanical engineering purposes. However, McKay and co-workers (1994) stated that JSC-1 exhibited a wider range of physico-chemical features (including bulk chemical composition, mineralogy, particle size distribution, specific gravity, angle of internal friction, and cohesion), which fall within the ranges of mare soil samples. This overestimation of JSC-1 fidelity may have led to the mischaracterization of JSC-1 as representative of all mare soils, which it definitely is not.

In 2005 NASA organized the Lunar Regolith Simulant Materials Workshop with the purpose of establishing requirements for the production and distribution of lunar simulants. The simulants were to be exploited in different branches of research (Sibille et al., 2006) and a “root simulant” needed to be produced. A “root simulant” is a large-volume, homogenized, and fully characterized mare or highland soil simulant that can be used as

the base for future simulants. Derivative additives could be added to the root simulant for specific purposes, including toxicity studies (Sibille et al., 2006).

With the urgent need for lunar simulants, ORBITEC produced 15 tons of simulant JSC-1A. JSC-1A is the mass-produced replica of JSC-1 and ORBITEC offered JSC-1A free of charge to all NASA-funded researchers working on ISRU projects. The simulant rapidly became a common reference in lunar dust research, including toxicity investigations.

In the specific context of toxicology, a simulant demands special processing to properly simulate the peculiar features of the real dust and achieve the required size fraction (namely, $<10\text{ }\mu\text{m}$ for human toxicology studies). The peculiar features of lunar dust are difficult to reproduce. Attempts have been made to produce np-Fe⁰ in JSC-1A by Liu and Taylor (2011), but physico-chemical analyses suggest that Fe was principally present as nano-magnetite with only some minor nano-sized Fe and larger grains of metallic Fe, resulting in a material that was far from anything resembling lunar agglutinitic glass (Liu et al., 2007). Lunar-like simulations of np-Fe⁰ in silica-rich glass were successfully produced in the size range of vapor-deposited glass coatings and in agglutinitic glass by Liu et al. (2007) and Noble et al. (2007). For vapor-deposited glass rims, the technique proposed by Liu et al. (2007) has the potential of being employed for more realistic compositions and for generating thin coatings similar to vapor-deposited glass coatings on lunar soil particles. These represent promising additives to lunar “root simulants.” If surface reactivity is needed for testing purposes, then the Fe⁰ simulant produced by Wallace and colleagues (2010) has been shown to have comparable surface reactivity and oxidative activity to lunar soils.

Besides JSC-1 and JSC-1A, other simulants have been developed over the years. Liu and Taylor (2011) provided an overview of the available simulants in comparison to real lunar soil samples. Since then, additional space agencies and nations interested in future robotic and manned lunar missions have developed their own simulants. To date, these newest simulants have not been subjected to the same wide range of studies as JSC-1 and JSC-1A.

Besides simulants from NASA, well-characterized simulants have been produced by the Chinese Academy of Sciences (CAS). These simulants are mainly intended for engineering studies, and the material description for investigating the toxic properties of the dust, such as mineralogy, particle morphology, and the relative abundance of glassy/amorphous phases, is not readily available. Among these simulants, CAS-1 was obtained by crushing the volcanic scoria (20–40 vol.% of glass) from Sihai pyroclastics at the

Jinlongdingzi Volcano, China (Zheng et al., 2009) to produce an analog of Apollo 14 soil 14163. CAS-1 is essentially a good duplicate of JSC-1 in terms of bulk chemistry. However, the mineral abundance in CAS-1 has not been reported, and CAS-1 does not contain agglutinates or np-Fe⁰. It may however be a good simulant for its geotechnical properties. NAO-1 was produced to mimic Apollo 16 highland soils from a Quni-Zaxiding gabbro from Tibet (Li et al., 2009). Plagioclase was picked from the gabbro and subsequently melted at 1550 °C to form glass, which was mixed with the gabbro and then milled to obtain a particle size smaller than 100 µm. The NAO-1 simulant is similar to JSC-1 in terms of specific gravity but differs from the highland samples. The mean and median particle sizes of NAO-1 are similar to Apollo 17 soils. The morphology and abundance of glass and their relationship with grain size are unknown. The reported chemistry of the plagioclase and bulk-soil chemistry of NAO-1 would seem to make it an approximation for some highland soils. Also, in this case, no np-Fe⁰ is contained within the simulant. Other simulants produced by Chinese scientists include CUG-1A (He et al., 2010, 2011), NEU-1a and NEU-1b (Li et al., 2019), and TJ-1 and TJ-2 (Jiang et al., 2010, 2012). Each of these simulants mimic slightly different characteristics of the lunar soils, allowing specific features of the lunar soils to be studied.

The European Astronaut Centre lunar regolith simulant 1 (EAC-1) has recently been developed by the ESA with the aim of providing a large volume of lunar regolith simulant material. This was developed for research activities at the European Lunar Exploration Laboratory (LUNA), a large training and operations facility that the EAC is building at the German Aerospace Centre (DLR) campus in Cologne, Germany. EAC-1 was thoroughly characterized by Engelschiøn et al. (2020) with a comparison with the most widely characterized simulants (including JSC-1A) and Apollo 17 samples. The findings showed that EAC-1A shares similar physical and chemical characteristics to the lunar regolith, but there are some notable deficiencies and variances. In detail, the cohesion, sphericity, grain size distribution, and major element composition of EAC-1 are comparable to the Apollo 17 samples with the main exceptions of the alkali components, feldspathoids, and the hydrated amphibole and chlorite groups.

These simulants have often been initially developed for the study of specific, frequently engineering-related, aspects of lunar exploration missions (e.g. ISRU activities). In the absence of a well-defined set of universally applied analytical protocols, direct comparisons between the properties of different simulants are difficult. Quantitative figures of merit (FoM) have been developed to compare the physico-chemical properties (considering particle composition, particle size distribution, particle shape

distribution, and bulk density) of ten available lunar simulants with the properties of an Apollo 16 core sample. This enabled an assessment of the potential suitability of the simulants for a range of technical, ISRU, and toxicity studies. Broader applications of this approach seem to have stalled, but it would be useful to apply or further develop quantitative measures of sample suitability when designing dust toxicity studies.

Despite concerns about the applicability and accuracy of the simulants, simulants are, and will remain well into the future, the most accessible method to begin to understand how lunar samples may impact short- and long-term human health. Working with simulants is particularly crucial for methodology testing and experimental optimization in preparation for handling the rare and precious lunar samples. The methodological approaches used in the efforts to study the biological effects of dust – *in vitro* and *in vivo* studies – each start with simulants and then, in the event of promising data, move to experiments with the lunar dust samples. Often, *in vitro* and *in vivo* studies can be done in parallel, each aiming to address a specific biological question.

Studies on the Health Effects of Lunar Dust

Due to its compressed timeline, no research was done on the toxicity of the lunar dust during the Apollo program. In the decades since the program ended, investigations have begun to expand our understanding of the health effects of the lunar samples. In 2005 the Lunar Airborne Dust Toxicity Assessment Group (LADTAG) was founded by NASA and was tasked with defining a permissible exposure limit for the fine respirable airborne lunar dust (defined as particles under 2.5 μm in diameter) as well as determining the ocular and dermal effects of dust exposure. LADTAG undertook ground-based *in vitro* and *in vivo* experiments to achieve this goal.

Dermal Irritation Experiments

Lunar dust's surface properties suggest that it is highly abrasive and there is potential that it could irritate the dermal/water vapor barrier (dermis), leading to dermatitis and/or sensitization of the skin. A transdermal-impedance technique was used to measure the abrasive effect of lunar dust on the skin. This technique measures damage to the dry, outermost layer of the skin, the stratum corneum, which is important for the barrier function of the skin. Pig skin, a high-fidelity model for human skin, was abraded with the lunar soil simulant JSC-1A to test the methodology. Once this approach was proven, pig skin was abraded with Apollo 11, 16, and 17 lunar soil in

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the 43-125 μm size fraction. The preliminary results of these studies showed that JSC-1A and lunar dusts are as abrasive as commercial sandpaper (Jones et al., 2008). The authors concluded that classical skin toxicology studies, including chemical irritancy evaluation and sensitization tests, needed to be performed.

Chemical and Mechanical Eye Irritation Experiments

The chemical and mechanical irritability effect of lunar dust on eyes was carried out by Meyers et al. (2012). The chemical irritability test was done by applying 100 mg of fine (mean particle diameter = $2.9 \pm 1.0 \mu\text{m}$) ground Apollo 14 lunar dust directly to the surface of cultured human keratinocytes, and a commercial kit was used to assess cell viability. This *in vitro* model is globally accepted as a more humane method to do eye irritability testing than testing directly on animal eyes, and it is believed to be a good mimic of the stratified corneal epithelium of the eye. The cell culture results indicated only minimal irritability of the ocular tissue by the dust. To be sure of the results and to assess a larger particle size and a greater number of endpoints, an *in vivo* study was conducted in which three rabbits were exposed to a larger size fraction of unground lunar dust (particles $<120 \mu\text{m}$; median particle diameter = $50.9 \pm 19.8 \mu\text{m}$). The *in vivo* study also showed minimal and transient eye irritation. No special precautions were recommended against ocular exposure to the dust, although in cases where the dust is very thick and becomes irritating, fully shielded goggles could be worn (Meyers et al., 2012), as is common practice when working with terrestrial dusts.

Pulmonary Toxicity Experiments

To study the pulmonary toxicity of lunar dust, *in vivo* intratracheal instillation experiments were first performed in rats (James et al., 2013), followed by nose-only inhalation experiments (Lam et al., 2013) in rats using Apollo 14 dust preparations, and compared with the responses to crystalline silica (strong response control) and titanium dioxide (low response control). Apollo 14 dust was used because it is believed to represent a mix of both highland and mare soil types (Meyer, 2011). Based on multiple biological endpoints, including 19 biomarkers measured in the bronchoalveolar lavage fluid, and tissue histopathology assessments, these experiments demonstrated that the pulmonary toxicity of lunar dust in rats is intermediate between that of titanium dioxide and crystalline silica. Detailed modeling and sophisticated efforts to reconcile all the scientific

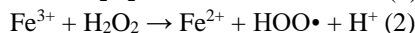
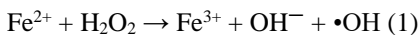
information into a single safe exposure estimate resulted in the recommendation that safe exposure levels have a minimum of 0.2 mg/m³ and a maximum of 0.7 mg/m³. At present, NASA has set a somewhat conservative preliminary permissible exposure limit (PEL) of 0.3 mg/m³ (NASA 2015) to be used in design studies for forthcoming lunar missions in the Artemis program.

Reactivity

Oxidative Reactivity

As observed with a variety of terrestrial particulates, when in contact with biological fluids, many dusts generate free radicals via various mechanisms, including reactive oxygen species (ROS) by the reduction of oxygen, •OH from hydrogen peroxide (Fenton mechanism), and the homolytic rupture of carbon-hydrogen bonds (Fubini et al., 2003). Several surface moieties (i.e. surface sites which may exchange electrons) are associated with these reactions, including unsatisfied valences, poorly coordinated transition metal ions, defects, and electron-donating centers (Andreozzi et al., 2017; Turci et al., 2017). The oxidative activity of mineral dusts is a widely accepted factor contributing to the development of diseases. The formation of particle-driven ROS, including superoxide (•O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (•OH), is the result of the stepwise reduction of dissolved molecular oxygen. When the amount of ROS overcomes the antioxidant cell's defenses, oxidative stress can occur, inducing cell and tissue damage and even death. To our knowledge, the first study on lunar dust and simulants' oxidative reactivity was by Wallace and co-workers (2009), who studied the reactivity of Apollo dust in comparison with a very fine fraction of JSC-1A lunar simulant (JSC-1A-vf) and employing Min-U-Sil quartz as the positive control measuring the production of •OH. The authors tested Apollo dust samples of varied maturity and source (highland versus mare). Aqueous suspensions of mare and highland soils found that highland soils, characterized by lower total FeO contents and less np-Fe⁰, are less reactive than mare soils of the same maturity. Comparisons between ground samples of lunar dust, lunar simulant, and quartz revealed that ground lunar dust is able to produce over three times the amount of hydroxyl radicals as lunar simulant and an order of magnitude more than ground quartz. These results induced the authors to conclude that the production of •OH occurred with the involvement of low redox state iron in its reactivity via the Fenton reaction, shown by equations (1) and (2):

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Even though Wallace et al. (2009) did not measure the H_2O_2 involved in the Fenton reaction, a previous study by Hurowitz et al. (2007) showed that freshly fractured terrestrial basaltic minerals generated H_2O_2 when contacted with water. Wallace and co-workers (2010) further investigated the role of np- Fe^0 in the reactivity of lunar dust and simulants in inducing ROS production. The authors concluded that the reactivity of ground lunar soil can be attributed to the np- Fe^0 . Moreover, initial testing of the decay rate of the ground soils has shown that the half-life of the reactivity is ~ 3.5 h (a potentially important finding that requires further investigation). Finally, the increased reactivity of lunar soil in comparison to lunar simulant has been ascribed to the presence of the unique np- Fe^0 in the agglutinitic glass.

Turci and co-workers (2015) investigated the oxidative reactivity of lunar dust at the molecular level by employing a complementary set of tests, including terephthalate (TA) hydroxylation, free radical release as measured by means of the spin-trapping/electron paramagnetic resonance (EPR) technique, and cell-free lipoperoxidation. The investigation was carried out on JSC-1A-vf in biologically relevant experimental environments. The findings proved that JSC-1A-vf is able to hydroxylate TA in anaerobic conditions, indicating that molecular oxygen is not involved in such a reaction. Spin-trapping/EPR measurements showed that the $\bullet\text{OH}$ radical is not the reactive intermediate involved. The authors proposed that a surface reactivity implying a redox cycle of phosphate-complexed iron via a Fe(IV) state was involved. The role of this iron species was investigated by assessing the reactivity of JSC-1A-vf toward H_2O_2 (Fenton-like activity), formate ions (homolytic rupture of C-H bonds), and linoleic acid (cell-free lipoperoxidation). JSC-1A-vf was active in all tests, confirming that redox centers of transition metal ions on the surface of the dust may be responsible for dust reactivity.

To further clarify the chemical mechanism of ROS generation and the nature of the moieties involved in such reactivity, Kaur et al. (2016) employed simulants JSC-1A, NU-LHT-2M, OB-1, and CSM-CL-S, as well as two simulants, JSC-1A and NU-LHT-2M previously treated, to create high-quality synthetic- Fe^0 -bearing agglutinates. Out of all simulants, CSM-CL-S was found to be the most reactive, followed by OB-1 and then JSC-1A. The authors studied the effect of activation by grinding under an oxidative atmosphere (O_2) and under a vacuum and the effect of the atmosphere on the reactivity operating both under oxygen and nitrogen

(inert) atmospheres. The findings showed that freshly ground dusts were all more reactive than unground dusts. Moreover, the absence of oxygen and water had the effect of increasing reactivity. The results indicate that mechanical stress and the absence of molecular oxygen and water, which are important environmental characteristics of the lunar environment, can lead to an enhanced production of ROS in general. Simulants treated to create agglutination, including the formation of Fe^0 , showed a lower reactivity than untreated simulants. Moreover, reactivity showed a direct correlation with the amount of agglutinitic glass. ROS are formed rapidly when simulants are dispersed in pure water, but the concentration of ROS either stabilizes or decreases over time. In contrast, ROS generation in simulated lung fluid (SLF) is initially slower than in deionized water, but the ROS formation was more prolonged over time. This suggests that in the human lung the production of H_2O_2 is likely sustained for at least hours after inhalation of the simulant, which could lead to chronic inflammation within the lung.

Dissolution

Due to human exploration, water will inevitably come in contact with lunar dust on future missions, especially during long-term stays. Water will be transported along with other mission assets in critical vehicle and habitat life support systems, and it will likely be extracted in quantity through ISRU processing of lunar minerals (Anand et al., 2012). Lunar dust suspended in water leaches metals and other elements for at least months, which suggests that the dust particles in aqueous media will gradually dissolve (Keller et al., 1971). Moreover, dust will come in contact with the water-rich body compartments, especially airways and the gastrointestinal tract, upon astronaut exposure. Since lunar dust, *in situ*, is exposed to intense radiation on the Moon, and particle radiation can disrupt the structure of mineral particles, it is possible that lunar dust is more susceptible to dissolution than terrestrial dusts that are not exposed to radiation. Contact with an aqueous environment can induce dust particle dissolution with the release of potentially toxic ions. In particular, iron or other redox active metal ions may induce the release of $\bullet\text{OH}$ radicals via Fenton reactivity, playing a role in oxidative stress. Furthermore, several transition metal ions that can be released from lunar dust (including Ni, Co, and Cr) can induce allergic responses (Hedberg, 2018; Lidén, 2018), and some of them are classified as carcinogenic (International Agency for Research on Cancer, 1996) or possibly carcinogenic (International Agency for Research on Cancer, 2006). The dissolution behavior of lunar dust was studied by Johnson et al. (1972)

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by employing Apollo 12, 14, and 15 regoliths. The solubility of all the samples was negligible in pure water, whereas high amounts of Al, Fe, Ca, and Mg and Co, Cr, Ni as minor components were observed after 3 h of incubation in an acidic environment (HCl 0.1 M and 1.0 M).

Further, the solubility of particles has effects on biopersistence, which is one of the key players in mineral dust toxicity (Linnarsson et al., 2012). Studies *in vivo* were carried out. Freshly returned lunar dust was injected into tissues of mice and later examined when the animals died naturally in about two years. It was seen in these animals that some particles persisted, but further examination to document the extent of the dissolution or the physical and chemical nature of the surviving particles was not performed (Holland et al., 1973; Johnston et al., 1975).

Effect of Microgravity

Because the lung presents by far the greatest surface area of the body exposed to the environment (ca. 50-80 m²), understanding the pulmonary deposition and subsequent clearance of inhaled dust is important in the context of toxicological effects.

The deposition of aerosols in the human lung occurs through a combination of inertial impaction, gravitational sedimentation, and diffusion. For 0.5 to 5 µm diameter particles under resting breathing conditions, the primary mechanism of deposition is sedimentation, and therefore the fate of these particles is markedly affected by gravity (Darquenne, 2014). The first experimental study of aerosol deposition in altered gravity was carried out by Hoffman and Billingham by employing 2 µm diameter particles for gravity (g) levels ranging from 0 to 2 g. There was an almost linear increase in deposition with increasing g level (Hoffman et al., 1975). Subsequent studies by Darquenne et al. (1997) have shown this to be broadly correct for particles in the size range of 0.5 to 3 µm. Thus, in lunar gravity (½ g), the deposition of particles is less than in 1 g due to the reduced sedimentation rate. However, the reduction in sedimentation means that particles that would normally be deposited in the medium- and small-sized airways in 1 g remain in suspension, and are then able to be transported to the peripheral lung where they eventually deposit through sedimentation in the smaller peripheral air spaces, or through the effects of diffusion. The effect of reduced gravity on deposition was studied by Darquenne and Prisk (2008) in six subjects on the ground (1 g) and during short periods of lunar gravity (½ g). In this study, the deposition of boluses of aerosolized monodisperse polystyrene latex particles (0.5 and 1 µm diameter particles) administered to six healthy subjects was examined. While deposition was

reduced in lunar gravity compared to normal gravity, the penetration volume required to achieve a given level of deposition was greater in $\frac{1}{6}$ g than in 1 g, indicating that the peripheral deposition of particles was enhanced in lunar gravity (Darquenne et al., 2008).

Another potential influence on dust deposition is that of the density of the cabin or spacesuit gas. This is likely to be quite different to that on Earth, with the proposed lunar habitat atmosphere having a gas density about $\frac{1}{2}$ that of sea-level air and with EVA suit atmospheres even less dense. The deposition of particles in conditions approximating the lunar habitat atmosphere showed a minor effect of gas density, with the key finding that gravity, and not gas properties, is the main factor affecting aerosol deposition in the lung (Darquenne et al., 2013).

Knowing the site of particle deposition in the lung has important implications for toxicological studies. Particles that deposit in the large central and medium-sized airways are rapidly removed from the lung by the mucociliary clearance system with clearance times of hours to days. However, particles that deposit in the peripheral lung do so beyond the reach of the mucociliary clearance system (Darquenne et al., 2013). Thus, the residence time of particles deposited in the lung periphery is much longer (weeks to months). This difference may have important implications as the longer the contact time between the tissue and the particles, the greater the potential of deposited particles to induce lung damage.

Measurements of the rate of clearance of deposited particles in the lung under conditions of altered gravity have never been made. However, direct observation of the site of the deposition of inhaled particles allows inferences to be made regarding clearance times. In humans, the absence of gravity caused a smaller portion of 5 μm particles to deposit in the lung periphery than in the central airways of the lung. For 5 μm diameter particles, deposition is dominated by inertial impaction, a mechanism most efficient in the large- and medium-sized airways. In the absence of gravity, sedimentation (which is more efficient in the smaller airways) was eliminated, allowing the large inhaled particles to stay in suspension and subsequently be exhaled. In contrast, for fine particles ($\sim 1 \mu\text{m}$), both aerosol bolus inhalations in humans and direct studies in rats show that particles deposit more peripherally in reduced gravity than in 1 g (Darquenne, 2014). Thus, it is likely that while overall deposition in the lung may be reduced in low gravity, those particles that are deposited will be those in the smaller size fractions (likely $< 2 \mu\text{m}$) and will be deposited in the more peripheral regions of the lung. This will result in prolonged residence times in the lung, serving to raise their potential for causing toxicological effects.

Future Perspectives

Developing New Simulants for Long-term Toxicity Assessment

The University of Central Florida (UFC) maintains the online Planetary Simulant Database (www.simulantdb.com) of all known regolith simulants (past and present) and their compositional information. Few of these are recorded as dust simulants, and hazardous or toxic properties are typically actively reduced for the purpose of safer human handling. Hence, there is a clear need for developing new dust simulants for long-term toxicity assessment.

Considerations for Future Lunar Simulants

Future lunar dust simulants for toxicity assessment will initially require the production of the sub-20 μm dust size fraction, including accurate recreation of the particle size distribution curve of real lunar dust. The sub-2.5 μm size fraction, which will be essential for *in vivo* and *in vitro* toxicity studies, should also be thoroughly characterized for size and crystallo-chemistry. These two fractions account for around 20 wt.% and 2 wt.% respectively of the lunar soil (Park et al., 2008; Cooper et al., 2010). It is important to accurately represent the particle size distribution, as this is not only related to surface area and reactivity, but can also have a significant bearing on pulmonary deposition and distribution, as well as affecting clearance and translocation (Nakane, 2012). When developing simulants for specific applications, it is desirable to derive it from a “root simulant” to aid standardized methods for future replication (Carter et al., 2004; Sibille et al., 2006). The continual gardening process through micrometeorite bombardment occurring on the surface of the Moon sees the delicate glassy rinds rich with nanophase metallic iron (np-Fe⁰) preferentially concentrated into the finer fraction (e.g. Taylor et al., 2001b). This concentration of np-Fe⁰ within the finer fraction is also a function of its vapor deposition process being surface-area dependent (Noble et al., 2001). Hence, developing a dust simulant for toxicity assessment is not merely a case of grinding a “root simulant” down to fine respirable size. Key additive components are also required, including the agglutinitic silicate glasses that constitute around 50-80 wt.% of the dust size fraction, as well as the np-Fe⁰ (McKay et al., 1991; Taylor et al., 2001b). The latter will ideally be synthesized utilizing the processes of Yang Liu et al. (2007) and Noble et al. (2007). Other key properties relating to toxicity assessment are particle shape, texture, crystallinity, and reactive surface areas in relation to particle size. One of

the most relevant, yet unexplored, discrepancies between all currently available “root simulants” and real lunar dust is the effect on the surface chemistry of high energy space radiation.

The most frequent particle size for the <2.5 μm fraction is the 0.1 to 0.2 μm range, with an overall smooth decrease in particle size observed down to around 20 nm (Park et al., 2008; McKay et al., 2015), which creates a large surface area. The highly vesicular nature of the glassy agglutinates that dominate the comparatively larger regolith fraction (McKay et al., 1991) is all but absent in the <2.5 μm size fraction of an Apollo sample (14003, 96), appearing as mostly smooth amorphous glasses (McKay et al., 2015). This may be a factor as to why lunar dust is less toxic than ground quartz (Lam et al., 2010; McKay et al., 2015) and is an important consideration for the development of the finest fractions of lunar dust simulants. None of the main larger-volume lunar simulants currently and historically available reproduce the highly irregular particle shapes of many real lunar soils. It has been noted that particle shape and shape distribution with size is particularly hard to reproduce in simulants (e.g. Taylor, 2010). Given that this may be a significant parameter affecting surface area and toxicity, it may, however, still be worth addressing. The compositional trend of increasing np-Fe⁰ with diminishing particle size in bulk regolith (e.g. McKay et al., 1991) stands true down to 2 μm , as does the decreasing trend of MgO and FeO, and increasing Al₂O₃ (plagioclase feldspar) that has previously been noted for the <20 μm fraction (McKay et al., 2015). This chemical trend is attributed to a combination of diminishing mafic minerals, such as olivine (plus pyroxene), with a comparative increase in plagioclase (e.g. Cintala et al., 1992) with the <20 μm dust fraction. Conversely, the trend for bulk regolith coarser than 20 μm trends toward an increase in both the mafic minerals and plagioclase components with decreasing grain size, and a steady decrease in lithic fragments (Papike et al., 1982). The compositional variation below 20 μm may be better reflected with crystalline mineral additives when deriving simulant dusts for toxicity assessment from root regolith simulants. Accurate representation of surface area and nanophase iron content would benefit assessments involving the activation and monitoring of dust, such as that conducted by Wallace et al. (2009).

Near-term human missions, such as Artemis, and longer-term sustained activities at the lunar surface are largely targeting polar locations for the science and resource potential offered by polar water ice and other cold-trapped volatiles. The polar region is dominantly highland terrain, and it would therefore be prudent at this stage in time to also focus on a high-fidelity highland dust. Currently the best authentic examples of highland that we have are samples in the Apollo collection from the Apollo 16

landing site, with additional compositional information provided by lunar meteorites.

Considerations for Future Martian Simulants

Mars' surface dust has only been studied remotely by robotic missions. There are different geological processes that have acted on the surface regolith on Mars when compared to the geological processes on the Moon, including physical erosion by wind and water and chemical weathering by fluids and oxidants (see e.g. Cannon et al., 2019 and references therein). Among the 100 μm particle size range (i.e. the detection limit of the Spirit rover), grains appear to be rounded and agglutinates are absent. This is in stark contrast to the equivalent size range regolith on the Moon and is attributed to wind alteration on Mars, and supported by an observed difference between this rounded surface dust and underlying coarser regolith (McGlynn et al., 2011). The wind also acts to homogenize the fine-grained dust at the surface on a global scale (Yen et al., 2005; Schuerger et al., 2012; Downs et al., 2015) with the most common silicate minerals being feldspar, pyroxene, and olivine, similar to the composition of basaltic Hawaiian volcanic ash. This dust is highly oxidized, contains nanophase iron oxides, and is rich in salts (Morris et al., 2006).

Of particular significance for toxicity is the high concentration of global perchlorate salts, measured in the regolith at 0.5 to 1 wt.%, which is several orders of magnitude greater than that for soils on Earth (Davila et al., 2013). Perchlorate anion can interfere with normal thyroid function by competitively inhibiting iodide uptake, reducing thyroid hormone production and further affecting normal metabolism, growth, and development of organisms (Wolff, 1998; ATSDR, 2008). If plant species are irrigated naturally or artificially with water containing perchlorate, uptake will occur, including uptake into edible portions of the plant. Cucumber, lettuce, and soybean demonstrated their potential to take up perchlorate from contaminated sand. There was a significant perchlorate concentration burden for cucumber and lettuce (Yu et al., 2004). Perchlorate accumulation was detected in edible portions of several garden plants, although with a lower bioconcentration. Another study indicated that perchlorate was selectively partitioned in chinaberry and mulberry trees, with leaf concentrations of 1.3-5.0 mg/kg of dry weight and fruit concentration of 0-0.5 mg/kg of dry weight (Tan et al., 2004). Nitrates are also present (Stern et al., 2015). The adsorption of H_2O_2 into the regolith may also be occurring as a result of H_2O_2 production induced by electrostatic fields generated by charged particles in dust storms (Atreya et

al., 2006; Scully et al., 2015). These are all considerations for additives to a “root simulant” for the toxicity assessment of Martian dust.

Similarly to the Moon environment, the $\frac{3}{8}$ g Martian gravity will serve to increase the fraction of particles that can reach the peripheral lung, escaping the lung clearance mechanism (Darquenne et al., 2013). Before developing a more accurate simulant for toxicity purposes, knowledge of particle size distribution, charge state, component solubility, porosity/surface to volume ratio, and textures are other factors of Martian dusts that need to be determined, beyond just Martian soil composition. There are numerous Martian simulants that have been produced, with many of them honed specifically for the testing and development of new analytical instruments for the Mars 2020 rover. These instruments will inform on the aforementioned properties and hazards posed by Martian dust, including XRF and ultraviolet RAMAN for analyzing fine-scale elemental and mineralogical compositional, and an array of atmospheric sensors that will also measure radiation, and dust size and shape (www.mars.nasa.gov/mars2020). Furthermore, the Mars 2020 mission will cache collected samples on the surface of Mars for future retrieval and return to Earth.

Currently, none of the available Martian simulants have the perchlorates included, precisely because of their toxic nature. The new MGS-1 simulant presents a possible viable “root simulant” for starting to develop a toxicity simulant that can be spiked with perchlorates. The MGS-1 simulant is created by mixing pure minerals together (Cannon et al., 2019) in the proportions based on the Curiosity rover’s measurements of the Rocknest soil in Gale Crater (e.g. Bish et al., 2013; Achilles et al., 2017).

Using this approach aims to avoid the tendency for simulants to gain water through interaction with the terrestrial atmosphere (i.e. via absorption or adsorption), which appears to be the case for JSC Mars-1 and MMS simulants that are derived from hydrothermally altered volcanic material (Allen et al., 1998; Peters et al., 2008). For example, JSC Mars-1 contains approximately 20 wt.% water (Allen et al., 1998), whereas 1.5- 2.0 wt.% water has been measured in the upper layer of Martian regolith at the Rocknest location by the Curiosity rover (Jun et al., 2013; Archer Jr et al., 2014). Unknown, poorly crystalline/amorphous material comprises approximately 20 wt.% of the Rocknest soil and cannot be explained by any single component. Separate experimental analyses have led to the inference that this portion may be a mixture of basaltic glass, nanophase oxides such as ferrihydrite, and sulfate species. These are all being included in MGS-1 (see Cannon et al., 2019 and references therein). MGS-1 and all alternative Martian simulants are cataloged in the online Planetary Simulant Database

(www.simulantdb.com). JSC Mars-1 and MMS are largely no longer available outside of NASA.

***In situ* Analyses and Authentic Dust**

Toxicity assessment using authentic dust may be possible after the successful demonstration of techniques utilizing simulants and after notable efforts to scale down experiments for smaller sample masses (Taylor et al., 2016). Such analyses will require the necessary preparation to separate a representative dust or respiratory fraction from bulk regolith samples, and where desired, the reactivation of surfaces. With regard to separation, dry sieving is only effective typically to the 45 μm size range, after which wet sieving or gravitational settling techniques using water, Freon, or alcohol tend to be applied (e.g. Basu et al., 2001; Park et al., 2008). For toxicity studies, not only is it less favorable to be exposing the particles to potentially chemically altering liquids, but such separation processes are estimated to require a starting bulk regolith mass on the order of kilograms to attain a few grams of the $<2.5 \mu\text{m}$ -sized fraction (McKay et al., 2015). This is just not feasible when using such rare material.

Alternative separation processes were applied by the LADTAG consortium to study Apollo 14 sample 14003 (McKay et al., 2015), which was taken to represent a mix of both highland and mare type soils (Meyer, 2011). A combination of jet mill crushing, involving self-collision between particles, and cyclone extraction conducted under an ultra-pure nitrogen environment was deemed an appropriate separation method. The resultant dust compared relatively well to the considerably smaller mass of “native respirable dust” that had been extracted using cyclone extraction alone, albeit slightly less rich in the nano-phase iron component than the native dust (McKay et al., 2015). The subsequent *in vivo* and *in vitro* experiments utilizing the separated respiratory dust are discussed elsewhere in this paper and described in full by James et al. (2013) and Lam et al. (2013).

Given that surface reactivity is such an important factor relating to toxicity studies, it is vital that *in situ* studies are conducted at the lunar surface prior to the sustained presence of humans. Another approach may be to specifically target lunar dust samples as part of future sample return missions. Should this be deemed an important step for human space exploration, the development of sample collection, containment, and curation methods that best preserve surface reactivity in returned lunar dust will need to be investigated in the near future.

High-energy Activation of Lunar Dust

The effects of space radiation on lunar dust is an important gap in our understanding of lunar dust toxicity. Space radiation interacts with lunar dust and can alter its chemical properties. Radiation exposure on the lunar surface is much higher than on Earth because the Moon has no atmosphere and a weak magnetic field. Components of the space radiation spectrum can therefore interact with dust: UV, solar wind, acute solar particle events, and sustained exposure to galactic cosmic rays.

These effects have been known since the Apollo 11 mission. Loftus et al. (2008) reviewed the work by Hapke et al. (1970) in which the effect of UV irradiation of Apollo 11 samples induced changes in the optical properties of lunar dust (reflectance spectra, absorption spectra). The authors attributed the phenomenon to the probable oxidation of Fe^{2+} to Fe^{3+} . Additional studies of energetic photon effects done with X-rays showed that the absorption spectrum was affected in the 4.5 eV energy range, again indicating changes in the oxidation state of iron (Hapke et al., 1970). The re-examination many hours after x-radiation evidenced some reversibility of these changes, although detailed passivation studies were not performed. The irradiation of Apollo 11 lunar dust samples with low energy protons, to mimic the solar wind, resulted in changes in the visible and IR reflectance spectrum, indicating changes in the chemistry of lunar dust, of similar magnitude to the effects of UV exposure (Hapke et al., 1970).

Solar wind is a low-energy stream of charged particles composed mainly of protons along with trace proportions of heavy elements including O^{7+} and ^3He (Killen et al., 2012). Solar wind interacts with the lunar surface inducing the implantation of ions. Furthermore, the intense radiation and particle radiation can disrupt the structure of mineral particles. For this reason, it is possible that the dissolution behavior of lunar dust is different from terrestrial dusts that are not exposed to radiation. Disruption of the mineral structure could indeed affect the dissolution of lunar dust in an aqueous environment. One of the first studies on solar wind implantation in lunar dust was carried out by Bibring et al. (1974), who studied the combined effects of collision and ion implantation into micron-sized lunar dust grains (namely lunar minerals extracted from an internal chunk of lunar igneous rock 15065) with a high-voltage electron microscope (HVEM). They exposed the sample to high fluxes of low-energy ions, including H, D, ^{13}C , N, Ne, Ar, Kr, Xe, and Pb nuclei. The observation of micron-sized grains either naturally exposed to space environmental parameters on the lunar surface or artificially subjected to space simulated conditions strongly suggests that such events could drastically modify the mineralogical

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composition of the grains and considerably ease their aggregation during collisions at low speeds.

The disruption of the mineralogical structure of lunar dust particles by high energy radiation bombardment may influence the dissolution rate of lunar dust. The bioavailability of metal ions (primarily iron) could be increased following high energy radiation bombardment. This could exert a dual yet contradictory effect, as has been observed in some inhaled terrestrial particles. On the one hand, high solubility can determine a low biopersistence of inhaled particles; on the other hand, the release of toxic ions at high local concentrations can induce acute inflammation or other toxic effects.

Coronal mass ejections from the Sun interrupt the solar wind and inject into the interplanetary system high fluxes of protons with energies up to a few hundreds of MeV. These solar particle events can deliver very high doses, even lethal doses, for unprotected crews. Exposure to solar particle events can also alter the chemical properties of the lunar dust, potentially making the dust surface more reactive when in contact with human tissue.

Finally, the issue of sustained exposure to galactic cosmic rays is largely unexplored. Even if galactic cosmic rays induce low radiation doses compared to solar particle events, they are very energetic and include a small but significant component of heavy ions. Galactic cosmic rays can penetrate the soil much deeper than solar wind, and the heavy ions can produce more significant chemical modifications (Durante et al., 2011).

Passivation Kinetics and Chemical Endpoints

An important factor in designing a future lunar habitat and mitigation procedures is determining a method by which to “deactivate” reactive lunar soil. A simple method to determine this deactivation time was proposed by Wallace et al. (2010) by subjecting ground lunar dust samples to conditions of known temperature and humidity (25 °C and 50% relative humidity) and then measuring the production of •OH by the terephthalate assay (TA). The time required to reach one half of the initial reactivity was ca. 220 min. The decay values did not seem to correlate with the maturity or origin of the soils (mare versus highland). Even after one week of deactivation, the tested soil (67461) did not return to its unground value. This finding was observed on all samples tested, as well as the highland soil sampled during Apollo missions. Hendrix et al. (2019) studied the reactivity of JSC-1A and several mineral components occurring in lunar regolith by detecting HO• radicals by Electron Paramagnetic Resonance spectroscopy coupled with spin trapping techniques. Some information on passivation kinetics was found

by these authors by measuring HO• from freshly pulverized augite, one of the mineral components of lunar mare regolith, after being exposed to the air for increasing periods of time. The capability of augite to release HO• decreased as a function of the time of exposure to the air similarly to that observed for quartz in the same experimental conditions. This suggests that a deactivation process induced by an oxidative environment occurred. The information provided by this study is limited to only one mineral component, and the humidity and temperature conditions are not reported.

However, it should be noted that it is still unknown if “deactivated” soil will have any detrimental *in vivo* health effects (such as the production of H₂O₂) if it is inhaled by astronauts.

Description of Biological Endpoints

Cellular Endpoints

Cellular studies using 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 *in vivo*-like lung-mucosa models with primary human cells cultured at the air-liquid interface are becoming a realistic alternative for pulmonary toxicity testing (Upadhyay et al., 2018). The use of such micro-physiological systems offers a unique opportunity for the direct deposition of particles of different origins onto a semi-dry apical cell surface consisting of mucus and beating cilia, a situation that mimics the deposition of particles onto the airway surface *in vivo* (Ji et al., 2017). These multi-cellular airway wall models can be co-cultured with innate effector cells (macrophages) which enable studying cell-to-cell interactions and crosstalk between cells that are present in human lungs (Ji et al., 2018).

The features of the micro-physiological systems not only mimic the *in vivo* situation but also avoid the constant concern of species differences when using animal models. Lung anatomy, cellular composition, or molecular responses in animal models significantly differ from humans. For instance, chronic bronchitis and chronic obstructive pulmonary disease are characterized by excessive mucus production. However, bronchial glands in mice and rats are anatomically localized only in the proximal trachea, making it difficult to reproduce these disease entities. Therefore, a debate has arisen in the last decade regarding the predictive value of mouse models in inflammatory diseases.

The use of *in vitro* models has been established, which aim at improving our understanding of pathophysiological processes and to provide novel and more reliable experimental systems for toxicological studies. The use of our established multicellular air-liquid interface models, which are considered as the next level advancement to mimic communications occurring between different cell types, are comparable to the *in vivo* situation. Hence, multicellular air-liquid interface models with human primary cells including various cell types such as various epithelial cell types (ciliated cells, goblet cells, club cells, and basal cells) and macrophages are expected to be the most physiologically relevant airway mucosa models to use for the evaluation of health effects of ultrafine particles of different origins. Further, another important feature in 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*. Therefore, these multicellular air-liquid interface models provide

high-fidelity models of *in vivo* lung mucosa with comparable tissue morphology and function to that seen *in vivo*, including extensive cell-cell interaction.

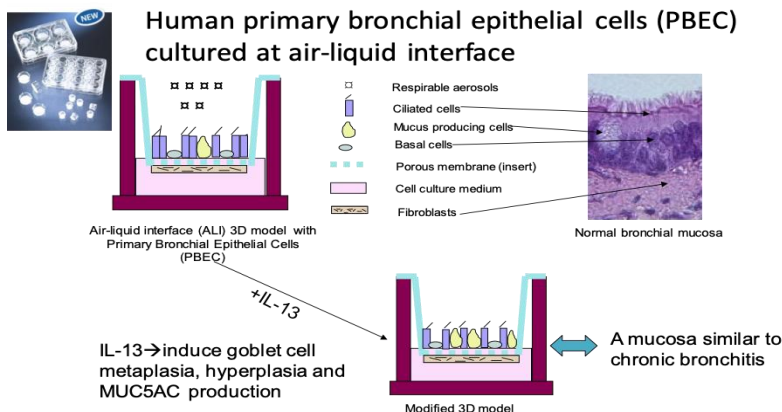


Figure 8-2. Normal and chronic bronchitis-like mucosa. Gerde and Palmberg have successfully established both normal and interleukin-13 (IL-13) induced chronic bronchitis-like multicellular bronchial mucosa models (Ji et al., 2017, 2019), and have exposed those models to different particles like carbon nanoparticles, diesel particles, and gases (aldehydes and diacetyl) (Ji et al., 2017, 2018, 2019; Dwivedi et al., 2018; Thimraj et al., 2019).

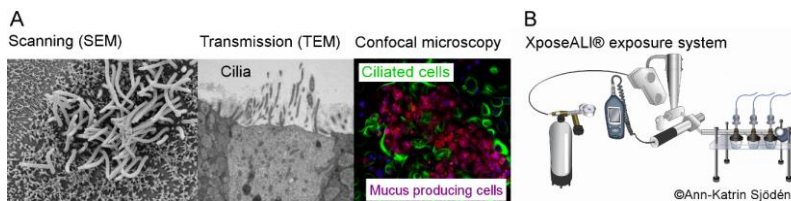


Figure 8-3. Bronchial mucosa model and exposure system. Microscopic details of the bronchial mucosa model (A) and an overview of the XposeALI® exposure module (B), which utilizes the PreciseInhale® aerosol delivery platform.

Figure 8-3A illustrates our established bronchial models with ciliated cells, mucus-producing cells in scanning and transmission electron microscopy (SEM and TEM) and confocal microscopy (Upadhyay et al., 2018). Figure 8-3B is the XposeALI® module that we routinely use to expose bronchial

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and alveolar mucosa models to aerosolized particles (adapted from Anna Stenholm's thesis).

***In Vivo* Endpoints**

Studies in animals have been the mainstay of inhalation toxicology assessment. In the context of lunar dust, the current permissible exposure limit set by NASA came as a direct result of the studies of rats exposed to aerosolized ground lunar material delivered via a nose-only inhalation technique performed by LADTAG (James et al., 2013; Lam et al., 2013). They assessed toxicity via both histopathological changes in the lungs and over a dozen inflammatory markers in the bronchoalveolar lavage fluid. Chinese scientists have also recently investigated the pulmonary and cardiovascular effects of the exposure of Wistar rats to several Chinese simulants (Sun et al., 2018, 2019a, 2019b).

Going forward, similar studies will likely be required to address the issue of whether the dust present on the lunar surface has a higher toxicological potential than samples curated for over 40 years, which may have different surface chemistry. *In vitro* exposure models will need to be complemented with rodent exposures to the same dust aerosols for investigating corresponding *in vivo* endpoints. Unfortunately, the techniques used in the LADTAG studies (rats, aerosolized exposure, exposures of many days) present significant problems in terms of future studies. Any studies performed using actual lunar material will be constrained by the availability of such material, especially if sample return or curated pristine samples are to be used.

An alternative to the method used by LADTAG is the recently developed PreciseInhale® aerosol delivery platform (Figure 8-3B), which is suitable for both *in vitro* (Figure 8-3B) and *in vivo* (Figure 8-4) exposures. This platform can be used for the delivery of the same aerosols to different exposure modules *in vitro* and *in vivo*, enabling the comparison of various toxic endpoints with a minimum level of translational errors in dosage between the modules.

In preliminary studies the lunar dust surrogate sample JSC-1a-vf was aerosolized with the DustGun generator of the PreciseInhale® platform. Aerosol at a concentration of 2.5 mg/L with a mass median aerodynamic diameter of 2.5 μm (GSD=1.9) was consistently generated. Intratracheally intubated rats were exposed to this aerosol during spontaneous breathing and reached a deposited dose of dust in the lungs of 1.2 mg (SD=4%, n=4) within about 20 minutes of exposure time. The substance utilization in terms of lung deposited amounts as a fraction of spent amount was approximately

1%. This is lower than during intratracheal instillation, but considerably higher than during nose-only tower exposures (Fioni et al., 2018). In both the *in vitro* and *in vivo* exposure modules, highly reactive dust samples can be kept under inert conditions until shortly before the exposure of the cells or animal to the aerosol.

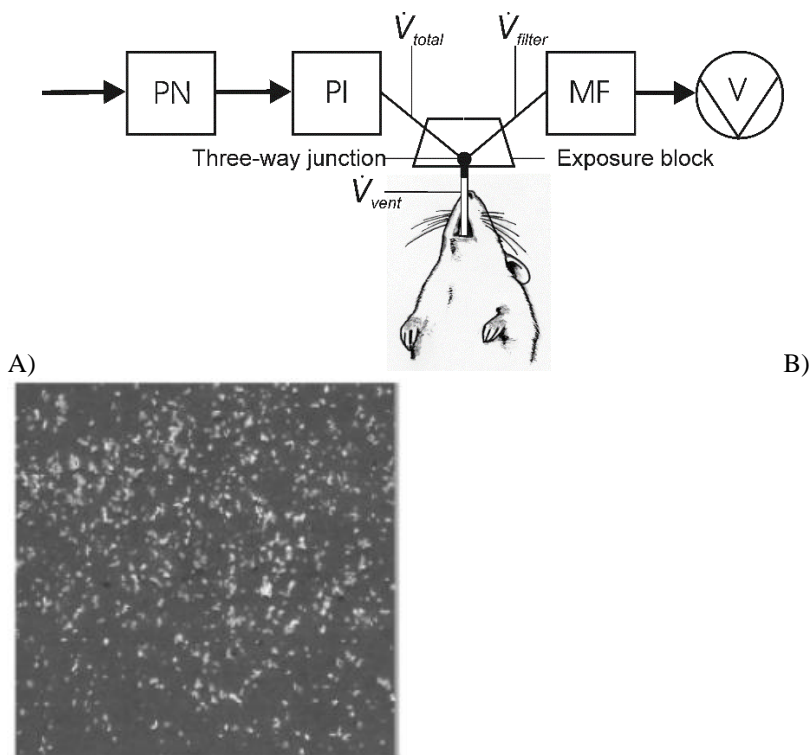


Figure 8-4. Configuration of the intratracheal single rodent exposure set up. A schematic of the exposure system for the lung phantom exposures: PN, the pneumotachograph; PI, the PreciseInhale® exposure platform (see Figure 8-3B); MF, the end filter; V, the vacuum pump; \dot{Q}_{total} , the exposure airflow; \dot{Q}_{vent} , the ventilation airflow, generated by the lung phantom ventilated with the rodent ventilator; \dot{Q}_{filter} , the constant component of the exposure airflow. The balance of the airflow streams at the three-way junction is expressed as $\dot{Q}_{total} + \dot{Q}_{vent} + \dot{Q}_{filter} = 0$. Scanning electron microscopy micrograph of aerosolized JSC-1a-vf collected on a membrane filter (frame size 500 μm).

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While simulant usage may be a viable alternative to the use of actual lunar material, the aggressive timeline of the Artemis program (first boots on the Moon in 2024) means that studies will need to be performed rapidly. A study design that allowed for a single exposure of an animal to lunar dust with subsequent readout of the response would be ideal. The highly robust immune system of rodents compared to humans presents a challenge here. However, a lung-selective knockout of vascular endothelial growth factor in mice presents a possible solution.

These mice show a much higher sensitivity to cigarette smoke than other mice (Lee et al., 2019). As such, lunar dust (or a high-fidelity lunar simulant) could potentially be delivered in a single exposure using intratracheal inhalation via an aerosol delivered from the PreciseInhale® platform. This would serve to bypass the highly effective dust filtration system of the rodent nose, allowing a single high dose to be delivered to the lung. The high sensitivity mice would permit experimental designs using a single exposure. Minimal amounts of material would be required, allowing studies with actual lunar material to be performed if desired. Following a single dose, animals can be studied at various time points using both bronchial alveolar lavage (multiple time points if required) and a histopathology assessment of the tissues (terminal endpoint). Such an approach would allow for both rapid throughput and minimal material requirements.

Long-term Exposure – A Major Gap in our Knowledge

During the Apollo crewed missions, exposures to lunar dust were uncontrolled and brief but sufficient to cause acute health effects (Cain, 2010). However, future lunar missions will be of much longer duration, ranging from surface stays of about 6.5 days in the early phase of Artemis to more than a month in later phases. Thus, the potential for ongoing episodic exposure to lunar dust will likely increase as crews will be performing repeated surface EVAs, each with the potential for exposure to dust.

Extrapolating human health effects from long-term animal or cellular exposure studies is fraught with difficulty. Therefore, it seems likely that an ongoing medical surveillance program for the crews will be needed. Such a program could readily include the provision of the capability to perform both forced spirometry as a standard (but rather insensitive) means of detecting the pulmonary effects of the dust, but also more sensitive means of detecting pulmonary inflammation such as exhaled nitric oxide levels. Both technologies are compact in nature, meaning they could be deployed

to the lunar habitat, and both have been successfully used on the ISS in measuring the effects of long-term exposure to microgravity on the lung and any issues relating to the ISS environment.

Eye Irritation/Toxicity

The current literature on eye irritation and toxicity is limited to one paper on the ocular effects of real lunar dust. The paper by Meyers et al. (2012) is excellent preliminary work, but it reports only studies on Apollo 14 lunar dust (a low-titanium mare lunar dust). Dust from the highlands area of the lunar surface has a substantially different mineral content, and therefore, these results may not be representative of that dust nor of dust from exotic locations such as the areas in the basins of craters near the poles. Future work would likely be done using the well-established *in vivo* human keratinocytes culture system.

Cardiovascular Effects

It is well-accepted that air pollution affects people with cardiovascular disease (Rajagopalan et al., 2018), and there is literature that suggests airborne dusts do the same (Querol et al., 2019). It could be prudent to understand what the cardiovascular effects of lunar dust exposure could be in an effort to understand the full human health effects of lunar dust exposure.

Recommendations

Since 2010, the ESA Topical Team on the Toxicity of Celestial Dust (T3CD) working group has involved researchers from academia and space agencies across a broad spectrum of technical backgrounds. T3CD is currently charged with identifying the most challenging questions related to the toxic effects of celestial dust on humans and suggesting approaches to address these questions. In this contribution, T3CD and supporting topical experts have reviewed the current knowledge on the determinants of dust toxicity, the composition and size of lunar dust, and all aspects related to its toxicity. The group has identified a number of knowledge gaps that need to be addressed in an effort to constrain the required extent of mitigation activities protecting astronauts from the potentially toxic effects of lunar and Martian dust.

Pertaining to the issue of the radiation activation of lunar dust and its toxicological implications, T3CD recommends that a broad multi-agency,

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multi-national effort be undertaken to perform the needed ground-based studies, using archived lunar dust samples. Adequate experimental techniques and resources are available to effectively close this important knowledge gap and to pave the way for a safe, sustained human presence on the Moon.

Further, T3CD recommends a range of future studies (as detailed above) using ground-based, irradiated lunar simulants to unravel the toxicity of lunar and Martian dusts in their real environment and foster safer crewed exploration of celestial bodies.

Conclusion

Since the first Apollo astronauts' debriefings, the ubiquitous presence of lunar dust and its potential toxicity has been one of the major concerns for lunar exploration. Such concern prompted NASA to form the Lunar Airborne Dust Toxicity Advisory Group (LADTAG) in 2005. After extensive *in vitro* and *in vivo* testing, LADTAG was able to recommend a safe exposure estimate for lunar dust particles, and at present, NASA has set that value as the preliminary permissible exposure limit (0.3 mg/m^3) to be used in design studies for forthcoming lunar missions in the Artemis program. The program plans call for several phases with increasing potential exposure to lunar dust, both in terms of quantity and time, as the residence time of humans on the lunar surface increases. Further, the number of astronauts will grow as the Artemis program proceeds, raising the possibility of toxic effects in some. Particular attention must be devoted to designing those IRSU activities that exploit lunar rocks and soils. The main activities that could expose astronauts to airborne lunar dust have been ranked as follows: i) routine EVAs, including EVAs for scientific activities and construction, maintenance, and ISRU purposes; ii) astronauts' transfers between the lunar surface, lunar habitat, lunar access vehicle, and crew exploration vehicle (CEV); iii) activities during a contingency situation; and iv) engineering failure of the dust control systems.

The main route of exposure to lunar dust is certainly inhalation. However, the new prolonged exposure scenarios require that other non-pulmonary exposure routes are taken into consideration. These include but are not limited to: i) skin penetration; ii) ocular exposure; iii) gastrointestinal exposure; and iv) indirect exposure to toxic soil contaminants through edible plants.

The new planetary exploration phases envisaged by the Artemis program will require the availability of a new generation of celestial dust simulants, specifically designed to consider the new long-term exposure. In

particular, a “root simulant” (perhaps more than one), mineralogically similar to celestial soil, will need to be adapted to toxicological studies by considering: i) particle size distribution; ii) the occurrence of crystalline and amorphous phases that are not present in Earth materials; and iii) the effect of space radiation on the chemical reactivity and solubility of the crystalline and amorphous phases.

The analysis of the currently available simulants highlights the need for experiments that will deliver the necessary information to design toxicologically relevant simulants. *In situ* quantification of the surface reactivity of the lunar dust should ideally be carried out on the lunar surface prior to the long-term sustained presence of humans.

Looking forward to lunar and Martian exploration objectives, additional *in vitro* and *in vivo* studies are urgently needed to expand the understanding of the effects of short- and long-term exposure to celestial dust on human health. Ideally this work should have begun before the next humans put their footprints on the Moon, and ultimately on Mars, to keep our crews safe.

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