Lunar Dust and Lunar Simulant Activation, Monitoring, Solution and Cellular Toxicity Properties

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Introduction

During the Apollo missions, many undesirable situations were encountered that must be mitigated prior to returning humans to the moon. Lunar dust (that part of the lunar regolith less than 20 µm in diameter) was found to produce several problems with mechanical equipment and could have conceivably produced harmful physiological effects for the astronauts.[1] For instance, the abrasive nature of the dust was found to cause malfunctions of various joints and seals of the spacecraft and suits. Additionally, though efforts were made to exclude lunar dust from the cabin of the lunar module, a significant amount of material nonetheless found its way inside. With the loss of gravity correlated with ascent from the lunar surface, much of the finer fraction of this dust began to float and was inhaled by the astronauts. The short visits to the Moon during Apollo lessened exposure to the dust, but the plan for future lunar stays of up to six months demands that methods be developed to minimize the risk of dust inhalation. The guidelines for what constitutes "safe" exposure will guide the development of engineering controls aimed at preventing the presence of dust in the lunar habitat.

Due to the lack of an atmosphere, there is nothing to protect the lunar soil from ultraviolet radiation, solar wind, and meteorite impacts. These processes could all serve to "activate" the soil, or produce reactive surface species. However, upon their return to Earth, samples obtained during the Apollo missions were inadvertently exposed to the ambient atmosphere, as the dust caused the knife-edge indium seals of the "rock boxes" to fail.[2] Therefore, in order to understand the possible toxic effects of the reactive dust, it is necessary to "reactivate" the dust.

We have previously developed a method for monitoring the activity of ground lunar soil and lunar simulant.[3] Using the production of hydroxyl radicals in solution as a marker, a fluorescent species is produced that can be measured to determine relative activities. For instance, as can be seen in **Figure 1**, the activity of ground Apollo soil is ~ 3 times that of ground lunar simulant and ~ 10 times that of ground quartz. The development of this test is important, as it is inexpensive and can be transformed into a portable sensor. Electron paramagnetic resonance (EPR) spectroscopy can also be used for this purpose. However, it is costly and bulky, and not appropriate for a simple monitoring system. Even so, EPR can provide collaborative evidence of hydroxyl radical production, as has been shown previously.[4] We present some initial results on the production of radical species in solution using EPR spin-trap experiments.

Placing lunar dust in solution could lead to effects on mechanical and physiological systems, as well as other biological systems. For instance, while it is known that lunar dust is highly abrasive and caused a variety of problems with suits and equipment during Apollo, it is unknown as to how these properties might be affected in the presence of water or other liquids. It is possible that the dust may release minerals (e.g., metallic nanophase Fe) into solution that could speed corrosion or rust. Also, as lunar dust produces hydroxyl radicals (and possibly other reactive oxygen species) in solution, these radicals could also lead to the breakdown of suit or habitat materials. In the body (i.e., in lung solution), the effects could be two-fold. First, if the lunar dust dissolves, it may release an excess of elements (such as zero-valence metallic Fe) that are necessary for bodily functions but only in certain concentration ranges. For lunar dust, the presence of nanophase iron being released into the body is a concern. Secondly, the hydroxyl radicals or other reactive oxygen species produced by the dust in solution could conceivably interact with cells, leading to various problems. A number of previous studies have been performed to determine the various species released by lunar dust and lunar simulant.[5,6] However, there has been no consistent method used for these studies. We have performed systematic studies of the dissolution of both ground and unground lunar simulant in buffer solutions of different pH. These results will also be discussed.

If dust particles of the appropriate diameter (< 3 µm) are inhaled by astronauts, they may make their way into the lower reaches of the respiratory system. Here, there will be interactions with epithelial cells, which could lead to several responses. First, the dust could interact with the cells directly, causing cell death of loss of membrane integrity. Secondly, the dust could cause an inflammatory response by the cells, leading to the production of further reactive species. Several studies have measured the response of cells to quartz and titania exposure.[4,7] In order to understand the possible inflammatory response of alveolar epithelial cells, A549 cells were exposed to various concentrations of lunar dust simulant in cell culture media for 24 and 72 hours. These results will also be discussed.

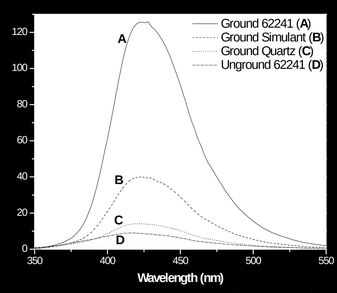

Figure 1: Emission spectra comparing ground and unground Apollo 16 soil (62241) with ground JSC-1A-vf and ground Min-u-Sil 15. The concentration of dust was 3.8 mg/mL.

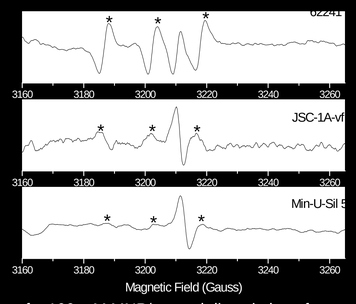

Figure 2: EPR spectra of a 100 mM MNP/acetonitrile solution after exposure to ground quartz (Min-U-Sil 5, bottom), lunar simulant (JSC-1A-vf, middle), and Apollo 16 lunar dust (62241, top). The lunar dust and lunar simulant were ground for 10 minutes, while the quartz was ground for 30 minutes. The asterisks denote the position of the spin adduct triplet.

Materials and Methods

- Test materials: Quartz samples (Min-U-Sil) with diameters below 15 µm were provided by U.S. Silica. Lunar simulant (JSC-1A-vf) was obtained from Dr. James Carter at the University of Texas at Dallas. This simulant was designed to be similar to low-titanium, mature lunar mare regolith with 90% of the particles less than 13 µm . The lunar dust used for these studies was an Apollo 16 soil (62241) provided by Dr. Larry Taylor at the University of Tennessee-Knoxville. This particular sample was a mature highland soil with a size distribution between 3 µm – 450 µm.
- Grinding procedure: 100 mg or less of quartz, lunar simulant, or lunar dust were ground with a mortar and pestle for 10 minutes stopping every 2 minutes to scrape the sides to ensure consistent grinding
- Fluorescence testing procedure: Ground and unground material were added to 15 mL centrifuge tubes containing 2.5 mL of 10 mM terephthalate dissolved in PBS. The mixtures were allowed to interact for 30 minutes before being filtered. The fluorescence spectrum of the filtered solution was obtained using a Perkin-Elmer LS-50B spectrometer.
- EPR testing procedure: For spin trap experiments, 30 mg of sample was added to 100 mM MNP (2-methyl-2-nitrosopropane)/acetonitrile solutions for 10 minutes. Following reaction, the mixtures were filtered and added to EPR tubes for testing.
- Dissolution testing: Buffer solutions of pH 4.0, 5.3, and 6.7 were prepared using citrate and citrate-phosphate buffers. 0.5 mg JSC-1A-vf was added to 20 mL of solution for 72 hours while rotating. The mixtures were filtered and analyzed using ICP-MS.
- Cellular Toxicity: A549 cells were grown in 12-well plates for 72 hours. Treatment media of the appropriate concentration was prepared using serial dilutions and added to the cells for 24 or 72 hours.

Results

EPR (Electron Paramagnetic Resonance)

Quartz, lunar simulant, and lunar soil were ground using a mortar and pestle in order to perform EPR spin-trap experiments. **Figure 2** shows the results produced by these experiments. The triplet marked by the asterisks denotes a spin trap-radical adduct, while the large peak apparent in the bottom spectrum arises from the quartz dewar). It is clear from these results that the level of activity increases in the order: quartz < lunar dust simulant < lunar dust. However, measuring the peak-to-peak splitting produces a value that does not correspond to a radical containing a hydrogen atom. It is possible that the activated species on the lunar dust interact with the acetonitrile to produce a radical other than hydroxyl. In previous studies, Castranova and coworkers studied the effects of ground quartz on alveolar macrophages and used EPR as a monitor of quartz hydroxyl radical production.[4] However, they used a different spin trap (DMPO) in water instead of the MNP/acetonitrile solution used in these studies. The fluorescence studies indicate that hydroxyl is produced by lunar dust in aqueous solution, but future EPR experiments must be performed with DMPO to provide further support.

Dissolution

The solubility of JSC-1A-vf was measured at three pH values in the pH range of physiological fluid. Ground and unground simulant was placed in solution at a concentration of 0.5 mg/mL at ambient temperature and humidity. After 72 hours, the filtered solutions were analyzed for the presence of Si, Al, Fe, Ti, Ni, Cu, Zn, Ca, Mg, K, and Na using inductively-coupled plasma mass spectrometry (ICP-MS). Ni, Cu, Zn, and Na were not found to differ significantly from the control solutions. Other selected results are plotted as a function of pH in **Figure 3**.

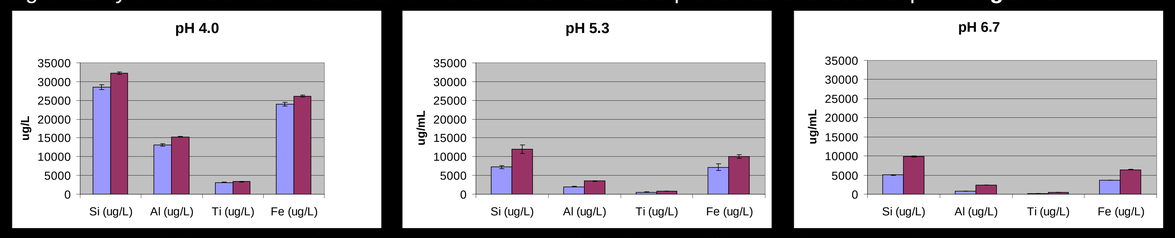

Figure 3: Change in concentration of various elements measured by ICP-MS after placing JSC-1A-vf in buffer solutions of different pH for 72 hours. Maroon: ground, Blue: unground. The concentration used for all tests was 0.5 mg/mL.

Lowering the pH of the buffer solutions and grinding the simulant are both found to increase the amount of material leached into solution. Each of these results may seem intuitive, but they could have important ramifications for astronauts. The presence of fractured species arising from meteorite impacts would be expected to cause the same effects as grinding. Additionally, physiological pH normally lies on the alkaline side of neutrality (~ 7.4); In certain portions of the cells, pH may approach 4.0. Previous work has shown that lowering the pH to 7.4 using phosphate-buffered saline leads to a further decrease in leaching of unground JSC-1A-vf. Therefore, dissolution of dust is more likely to be a problem in intracellular systems. However, further experiments are required to determine if this is also true of lunar soil. Also, fluids containing salts and surfactants present in the body will also provide a great deal more information regarding possible dissolution concerns regarding lunar dust.

Cellular Toxicity

A549 alveolar epithelial cells were tested for cytokine production after exposure to culture medium containing ground JSC-1A-vf. These cytokines are known to participate in the inflammatory response of the body to foreign antigens. **Figure 4** shows the effects of concentration and time on the production of IL-6 and IL-8 by A549 cells. These initial studies indicate that both exposure time and concentration play a role in the production of these cytokines. Further studies will be performed on quartz, for which toxic effects are known, and lunar dust to determine any possible differences in toxicity. Additionally, future studies will be performed on BEAS-2B bronchial epithelial cells in order to observe any differences in toxicity in this area of the respiratory tract.

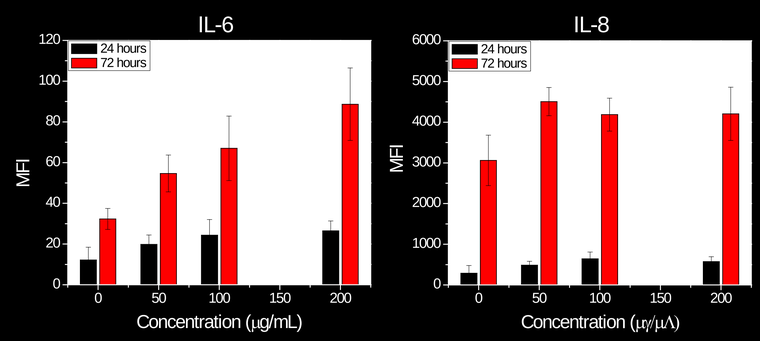

Figure 4: Production of IL-6 and IL-8 by A549 alveolar epithelial cells exposed to ground lunar simulant, JSC-1A-vf

Conclusions

This work has shown the effects of grinding on the activation level of lunar dust, the changes in dissolution properties of lunar simulant, and the production of cytokines by cellular systems. Grinding of lunar dust leads to the production of radicals in solution and increased dissolution of lunar simulant in buffers of different pH. Additionally, ground lunar simulant has been shown to promote the production of IL-6 and IL-8, pro-inflammatory cytokines, by alveolar epithelial cells. These results provide evidence of the need for further studies on these materials prior to returning to the lunar surface.

Acknowledgements

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