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Project Title:	Study of Lunar Dust and Lunar Simulant Activation, Monitoring, Solution and Cellular To	xicity Properties
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Element/Subdiscipline:	HUMAN RESEARCHEnvironmental health	
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Space Biology Special Category:	None	
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Key Personnel Changes/Previous PI:		
COI Name (Institution):	Wallace, William T. (Wyle Integrated Science and Engineering Group)	
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Task Description:	With the plan in place to return humans to the Moon, it is imperative to understand the hazards that may be faced and to determine ways to minimize them. Understanding the effects of lunar dust on both human physiology and mechanical equipment is one of the most pressing concerns, as problems related to lunar dust during the Apollo missions have been well documented. While efforts were made to remove the dust before reentering the lunar module, via brushing of the suits or vacuuming, a significant amount of dust was returned to the spacecraft, causing various problems. For instance, astronaut Harrison Schmitt complained of "hay fever" effects caused by the dust, and the abrasive nature of the material was found to cause problems with various joints and seals of the spacecraft and suits. It is clear that, in order to avoid potential health and performance problems while on the lunar surface, the negative properties of lunar dust must be quenched. Our research will focus on several related areas of research regarding lunar soil: 1) understanding the activation and deactivation processes of lunar soil, as well as how to monitor these processes, 2) understanding the properties of lunar soil in solution (dissolution), and 3) understanding the effects of lunar soil on cellular systems. Initial studies will be carried out using several different materials. Due to the scarcity of pristine lunar soil, tests will be conducted with lunar simulant, JSC-1A-vf, and quartz and titania, which have been used as positive and negative controls, respectively, in toxicological studies. Knowledge of the activation and deactivation processes of lunar soils prior to their transfer to long-term storage. In order to determine methods for dust mitigation on the lunar surface, we must first activate the materials and determine the best methods for deactivation. Additionally, the particles themselves may not require activation in order to be toxic. Therefore, dissolution and cellular toxicity studies will be performed to determine if any t
Rationale for HRP Directed Research	:
Research Impact/Earth Benefits:	The tests and methods used in these studies on lunar dust are applicable to terrestrial materials, such as mineral dusts and nanomaterials. For instance, a method to monitor the reactivity of ground lunar dust could also be used to measure the ability of quartz, a known fibrogenic material, to produce reactive oxygen species. Our reactivity monitoring method has already been adapted for use in a lunar dust reactivity sensor that could be used in the field to help determine when it may not be safe to enter an area (such as near sandblasting operations).
	Work has progressed on all three of the aims described in our task description. Aim 1: Activation and Monitoring
	The lunar soil is subjected to constant bombardment by galactic/cosmic-ray particles, solar ultraviolet rays and energetic ions, and solar-wind protons and alpha particles, in addition to micrometeorite impacts, and all of this occurs in the vacuum of the Moon (10-12 torr). These forces affect the soil to different degrees, with crushing and grinding by micrometeorites being the most energetic in creating a highly reactive product. The production of a multitude of 'dangling' bonds, mostly situated at oxygen atoms in the 40-50 wt% oxygen soil, gives the potential for chemical reactivity with respiratory tissue in humans. The loss of vacuum integrity in the lunar sample containers during the Apollo era ensured that the present lunar samples are not in the same condition as they were on the Moon; they have been passivated by oxygen and water vapor. Hence, there is a need to study the effects of grinding and crushing for the production of chemical reactivity in lunar soil. We have studied these effects in quartz, lunar dust simulant (JSC-1A-vf), and a variety of lunar soils using grinding by mortar and pestle, as this can serve as a first approximation for meteorite bombardment.
	In order to compare the reactivity of lunar dust to terrestrial materials such as lunar dust simulant and quartz, we used the ability of the different dusts to produce hydroxyl radicals in solution as a marker. Due to the transient nature of the hydroxyl radical, we used the terephthalate molecule as a probe. This molecule is non-fluorescent, but will become highly fluorescent when upon reaction with a hydroxyl radical. Additionally, the fluorescence intensity of this product molecule increases linearly with concentration, allowing a determination of the number of radicals produced. Using this technique, we monitored the production of hydroxyl radicals in aqueous solution by ground and unground lunar soil (Apollo 16 soil, 62241). We determined that grinding produced approximately a 10-fold increase in radical production over the unground soil. Grinding of quartz and lunar dust simulant also produced an increase in radical production, but much less than that of the lunar soil. The radical production of the ground lunar soil was approximately 10-fold and 3-fold greater than ground quartz and ground JSC-1A-vf, respectively. The increased reactivity produced for the quartz by grinding was attributed to the presence of silicon- or oxygen-based radicals ("dangling bonds") on the surface. These "dangling bonds" may also play a part in the reactivity of the lunar soil. In order to determine the source of the increased reactivity of ground lunar soil, we ground 8 lunar soils of varying maturity and source (highland or mare) and measured the hydroxyl-radical production. It was determined that there is a direct correlation between the reactivity and the amount of nanophase (3-33 m) metallic iron particles (as a function of soil maturity) in the samples, both highland and mare. Additionally, the highland soils were found to be generally less reactive than the mare soils, a fact explained by the lesser amounts of total iron (and therefore less nanophase iron) in highland samples. As these nanophase iron particles are formed
	Aliquots of the different lunar soils, as well as the lunar simulant and quartz samples, were exposed to humidified air (50% relative humidity, 25 oC) for set time points in order to understand the kinetics of deactivation. After a time exposure, their fluorescence was measured using the terephthalate method. It was found that lunar simulant reached 50% of its initial reactivity in approximately 3 hours, with quartz reaching 50% reactivity in approximately 2 hours. The lunar samples had quite a bit of scatter, with an approximate time to reach 50% reactivity of 3.5 hours with a spread of 1.5 hours.
	Aim 2: Solution Properties
Task Progress:	There is no standard protocol for experiments aimed at understanding the solubility and dissolution characteristics of mineral dusts. We developed a protocol using lunar dust simulant (JSC-1A-vf). Initial tests showed that an increase in pH occurred when simulant was placed in water. Therefore, it was determined that buffer solutions must be used in order to remove one variable in the dissolution experiment. Buffer solutions (citrate or citrate-phosphate) were prepared at physiologically-relevant pHs of 4.0, 5.3, and 6.7. Dust concentrations of 0.5 mg/mL were added to the solutions and

	rotated for 3 days to ensure constant suspension. After filtering the solutions, concentrations of silicon, aluminum, titanium, iron, copper, nickel, zinc, sodium, magnesium, calcium, and potassium were measured using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and compared to concentrations from solutions containing no dust. At all pHs, the concentrations of nickel, copper, zinc, and sodium were not significantly higher than the controls. However, increased amounts of the other elements were measured in the filtered solutions, with the more acidic buffer solutions being more effective at leaching the measured species from the dust. Grinding of the dust prior to placement in the buffer solutions led to consistently higher concentrations of the species of interest.
	In order to determine the effects of lung fluid on dissolution kinetics, lunar dust simulant was placed in a modified Gamble's solution (used in the literature to mimic lung fluid) of pH 7.38 under the conditions described above. After measurement using ICP-MS, it was found there was a significant change in the concentrations of silicon, aluminum, titanium, and iron when dust was added to the lung fluid simulant. Additionally, there was a considerable increase in the concentrations found in the lung fluid simulant/lunar dust simulant mixture (pH 7.38) in comparison to those in the phosphate buffer/lunar dust simulant mixture (pH 7.4).
	Future work will include a study of the dissolution properties of a lunar dust sample in buffer solutions of selected pH as well as a time-course study of the dissolution of lunar dust simulant in selected buffers.
	Aim 3: Cell culture
	It has been shown previously that freshly ground quartz is more harmful to cellular systems than aged quartz. The ability of ground quartz to oxidize lipids is directly correlated to the number of radicals produced in solution and to the freshness of the silica. We aimed to compare the ability of lunar dust simulant to produce inflammatory mediators in cellular systems, as well as effects on viability, with that of quartz.
	For viability testing, A549 alveolar epithelial cells and BEAS-2B bronchial epithelial cells were grown and exposed to ground and unground quartz and lunar simulant for 24 hours at concentrations = $1000 \ \mu g/cm2$. Viability was tested using the trypan blue exclusion method. In both cell lines, exposure to unground quartz led to a significant loss in viability at concentrations = $200 \ \mu g/cm2$. In contrast, concentrations of at least $500 \ \mu g/cm2$ of unground lunar dust simulant were required to lead to any noticeable loss in viability. When exposed to ground quartz and lunar dust simulant, A549 cells began to show a decrease in viability at lower concentrations of dust, but the loss in viability at higher concentrations was less than that of the unground samples. Similar results were also seen for BEAS-2B cells.
	The production of the inflammatory mediators IL-6 and IL-8 was used as a marker of cellular stress. Similar to the viability testing, cells were plated and allowed to grow for 72 hours prior to exposure to quartz and lunar dust simulant. After 72 hours, dust concentrations = $1000 \ \mu g/cm^2$ were added to the cells for 6, 24, 48, and 72 hours. The supernatants were removed and tested for the presence of IL-6 and IL-8. For both cell lines, some increase in the cytokine concentration was seen after addition of both ground and unground dust. The cytokine concentrations found for the controls were often high, and the cytokine concentrations found at longer time points and dust concentrations did not follow any expected trends. In general, these cells seem to be quite robust with regards to exposure to quartz and lunar dust simulant at the physiologically relevant concentrations used in our studies.
Bibliography Type:	Description: (Last Updated: 12/20/2011)
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