## Damage provoked by exposure of human lung cells to lunar regolith simulants

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Abstract. During the Apollo missions, it was reported that the lunar regolith was irritating to the respiratory system. From nasal congestion to irritated eyes, these inflammatory responses occurred after adhered lunar regolith on their space suits was brought into the command module. With the approach of the Artemis program, the potential threat of lunar regolith to astronauts has renewed importance. Reactivity and toxicological studies have focused on how the physical and chemical properties of lunar dust could affect the health of astronauts. This work specifically studied the toxicity of two lunar simulants, Lunar Mare Simulant-1 (LMS-1) and Lunar Highlands Simulant-1 (LHS-1), with and without heat treatment under H<sub>2</sub> gas. Reduction of the ground simulants<sup>1</sup> under hydrogen at 950 °C induced the formation of metallic iron (Fe°) particles<sup>2</sup> and simulated the effects of space weathering. For further identification of the most toxic components of lunar dust, this work also analyzed the toxicity of olivine (an abundant mineral found in lunar dust) by assessing the effect on cell viability, mitochondrial function, and DNA integrity. We used 0.5, 1, and 1.5 mg/cm<sup>2</sup> of LMS-1 and LHS-1 based on our previous testing<sup>3</sup>. Similarly, we used 0.05, 1, 0.2, and 1 mg/cm<sup>2</sup> of olivine with varying compositions. To probe into the cellular response to the materials, we collect cells or the extract after 1 hour of exposure for assessing mitochondrial function or DNA integrity. A 24-hour recovery period for cell viability to more accurately represent the toxic response (apoptosis of the cells). Furthermore, to understand the toxic effects, we also try to interpret our data as a function of surface area since these toxic mechanisms may be dependent on surface area. Exposure to the simulants causes significant cell death. Averagely around 60% for non-reduced lunar dust simulants, and around 90% for reduced ones. Cell viability after olivine exposure was decreased by more than 50% after even only 10% of the concentration of lunar dust simulants. The exposure also affected mitochondrial function with up to 3-fold increase in the fluorescent signal for lunar dust simulants and olivine. The DNA integrity was significantly impaired with only 20-30% intact after exposure to olivine and reduced lunar dust simulants. Our new results4 show that, relative to non-reduced samples, Fe°containing lunar dust simulants have increased toxic effects in terms of cell viability, the function of mitochondria, and DNA damage. Olivine was also proved to contribute to the toxicity of lunar dust simulants with much lower concentration. In conclusion, the damage to cells exposed to lunar regolith may be more severe than most terrestrial ground rock dust particles. Our work provides key information regarding the cellular toxicity, and these data will help better assess occupational health risks to astronauts after exposure to lunar dust. This work can support the potential development of biomarkers and other preventative measures for future lunar explorers.