Assessment of OH* generation of lunar dust simulants in biologically relevant fluids as a proxy for the potential human health impacts of lunar dust inhalation Donald A. Hendrix[1] and Joel A. Hurowitz[1], [1]Department of Geosciences, Stony Brook University (donald.hendrix@stonybrook.edu)

Introduction: The Artemis III mission is scheduled to launch and land humans on the surface of the Moon as soon as 2025. Previous studies have found that iron-rich silicate minerals (i.e. olivine, pyroxene), which are commonly found in the lunar mare regions, generate larger measurable quantities of hydroxyl radical (OH*) in solution compared to iron-poor minerals (i.e. feldspar) commonly found in the lunar highland regions [1]. The commonly accepted mechanism for OH* generation is the Fenton reaction, in which the reaction between surficial ferrous iron and solution generates hydrogen peroxide to make OH* [1]. Prior studies [1-2] have recorded the OH* generation of various silicate minerals from greatest to least as follows: olivine, augite, diopside, albite, bytownite, labradorite, and quartz. The OH* has been linked to DNA damage and cancer [3], which is why it is considered an important metric in dust toxicity studies. The oxygen in OH* has an oxidation state of (-1) instead of the usual (-2), hence its high tendency to react and oxidize any biomolecules it comes in contact with, including, e.g., DNA [3].

Statement of the Problem: Up to this point we have worked with terrestrial mineral phases and the lunar simulant Johnson Space Center-1A (JSC-1A). Terrestrial minerals, while similar to those found on the Moon, usually exhibit different chemistries than those found on the lunar surface, are frequently deficient in both Fe and Ca, and contain excess Na, K, and Mg [3]. Metallic iron is also an absent component of terrestrial lunar dust simulants. JSC-1A adequately replicates physical lunar soil properties (i.e. specific gravity, thermal/electrical properties) but does not adequately replicate the chemistries observed in lunar soils. Here, we describe new experimental research aimed at understanding the reactivity of analogue materials that more closely mimic the mineralogy of lunar dust deposits, and account for some of the unique chemical changes that are induced by reductive space weathering processes. This allows for use of lunar dust analogs that are closer to real lunar regolith in many respects, without the need to use precious Apollo samples for destructive analyses. We also assess the reactivity of all lunar simulants in biological fluid simulants of the human respiratory system to understand the chemical mechanisms of oxidative damage due to lunar dust inhalation.

Materials and Methods: We have acquired Lunar Mare Simulant-1 (LMS-1) and Lunar Highland Simulant-1 (LHS-1) from the Exolith Lab at the University of Central Florida. These simulants better replicate the lunar mineralogy of both mare and highlands regions and will give us a better understanding of the reactivity of lunar dusts from these regions. LMS-1 contains approximately 30% pyroxene, 25% glass, 20% plagioclase, and 4% ilmenite. LHS-1 contains approximately 75% plagioclase, 25% glass, and <1% olivine, pyroxene, and ilmenite [5]. These simulants are an improvement to the commonly used lunar simulant JSC-1A due to better replication of mineral abundances, but still exhibit some important chemical differences from lunar soils due to the differing formation conditions of terrestrial and lunar minerals.

One important difference is a lack of metallic nanophase iron (np-Fe^0), which is common in lunar soils due to space weathering. In an attempt to add metallic iron to the samples we have incorporated a simple reduction method described in Allen et al. [6], that involves roasting the simulants at 900°C in a H_2 gas stream. We reduced approximately 3 g of JSC-1A, LMS-1, and LHS-1 using this method.
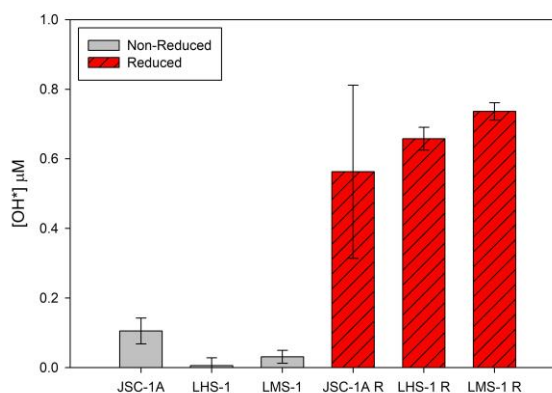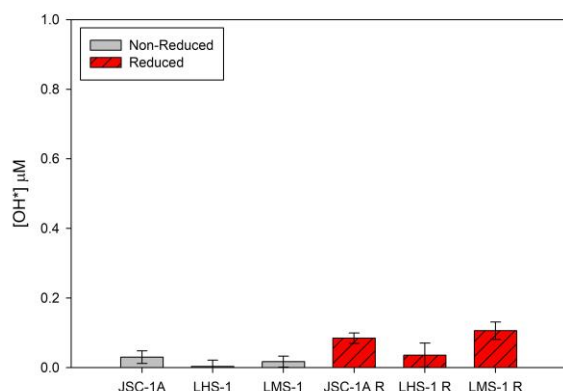

Fig 1. OH* generation of lunar simulants in DI water. R, reduced

OH* generated by all lunar simulants (reduced and non-reduced) were tested in solutions of DI water, simulated lung fluid (SLF), and artificial lysosomal fluid (ALF). SLF simulates the electrolyte and protein rich fluids found throughout the linings of the human lung airways and ALF represents the low pH fluids found inside macrophages, which are cells that clear out bacteria, viruses, and other particulate matter from the human lungs.

Results: Reduction of lunar simulants led to substantially larger measurements of OH* in DI water (**Fig. 1**). This implies the potential for lunar dust to generate

large quantities of OH* into solution upon inhalation into the humid environment of the human respiratory system.

Lunar simulants incubated in SLF generated significantly lower quantities of OH* in solution compared to DI water. Reduced simulants generated on average more OH* in SLF compared to non-reduced simulants (**Fig. 2**). Both reduced and non-reduced LHS-1 generated roughly the same levels of OH* likely due to less metallic iron on the surface grains relative to JSC-1A and LMS-1. Simulants incubated in ALF produced the lowest amount of OH* of all three solutions with measurements at or below the detection limit (**Fig. 3**).

Fig 2. OH* generation of lunar simulants in SLF. R, reduced
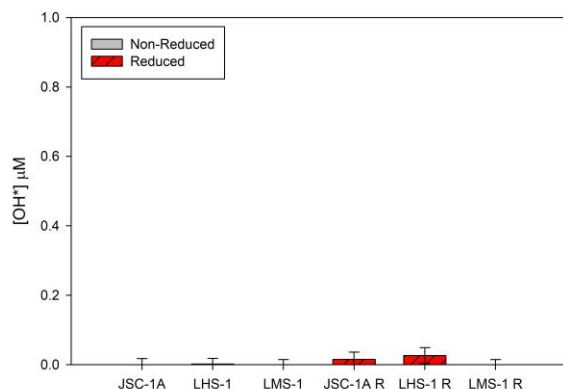
Fig 3. OH* generation of lunar simulants in ALF. R, reduced

Summary: Reduction of lunar simulants has shown that surficial metallic iron leads to large increases in OH* generation. We speculate that metallic iron participates in a Fenton-like reaction that leads to increased observed concentrations of OH*.

Even though less OH* was measured in solutions of SLF and ALF, it cannot be said that less OH* was actually generated by the samples. There is likely a competing effect between the components allowing grains in solution to both inhibit and generate OH*. Other studies have demonstrated the ability of amino acids to interact with OH* in solution [7], which means amino acids may react with OH* at rates much faster than the spin trap compound (DMPO) that we use to measure OH* can. Our work has shown that cysteine, an amino acid and component of SLF, greatly reduces measurable quantities of OH* in solution (data not shown). Citric acid, which is present in ALF at a concentration of 20 g/L, reduces measurable OH* in solution to down near the detection limit (data not shown). It was expected that ALF would lead to large measurable quantities of OH* in solution due to the low pH (approx. 4.5). It is likely that electrolytes may bind to the surfaces of dust grains and inhibit Fenton chemistry from occurring.

These findings have a variety of implications for the human health hazards of lunar dust inhalation. It can mean that inhaled particulate matter inside the human lungs can interact with proteins in lung fluids and oxidize their amino acids which would compromise protein structures. It is also possible that specific components may inhibit the generation of OH* in solution, like citric acid in ALF. This would imply that electrolytes can possibly inhibit the generation of the oxidative radical. It is likely that inhalation of lunar dust would exhibit a competing effect in terms of generating OH* and subsequently inducing oxidative damage.

Suggested Future Work: OH* measurements in all individual components of SLF and ALF including high performance liquid chromatography (HPLC) measurements in all sample components is key in understanding not only which components inhibit measurable OH* generation but what some of the oxidative products of samples incubated in amino acids may look like. HPLC measurements and mass spectrometry measurements for lunar dust simulants incubated with certain proteins, enzymes, and other components found in human lung fluids can give us better ideas for how lunar dust may induce oxidative damage.

References: [1] D. A. Hendrix et al. (2019) *GeoHealth* **3**(1), 28-42. [2] C.J. Horwell et al. (2003) *Environ. Res.* **93**(2), 202-215 [3] M. Dizdaroglu et al. (1991) *Arch. Biochem. Biophys.* **285**(2), 317-324. [4] E. Hill et al. (2007) *GeoHealth* **112**(E2). [5] D.T. Britt et al. (2019) ESF 2019, Abstract #NESF2019-082. [6] C.C. Allen et al. (1994) *J. Geophys. Res.* **99**(23), 173-185 [7] Z. I. Nagy, et al. (1984) *Biochim. Biophys. Acta* **790**(3), 238-250.