In situ Toxicological Investigation of the Lunar Dust Reactivity to Design New Simulants and Activation Protocols for Astronaut Health

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Background

Artemis programme success relies on several ambitious Science Goals that include the need for “investigating and mitigating exploration risk to humans”. Since the Apollo era, lunar dust toxicity has been one of the major concerns for future lunar exploration. The Artemis III mission to the Moon’s South Pole will again expose the two-member crew to lunar dust. The peculiar lunar environmental conditions may radically modify the physico-chemical properties of the dust particle surface this in turn may enhance the reactivity of particulates encountering biomolecules and tissues upon inhalation. Low-valance state iron (Fe^{2+} and Fe^{0}), which is thermodynamically favoured by the non-oxidizing lunar environment, occurs in all lunar rocks. Nanophase iron (np-Fe^{0}) is generated during impingement of micrometeorites on the lunar surface and occurs in the form of nanometric grains that are deposited on the rims of agglutinitic glass. Furthermore, the lunar dust is continuously irradiated by space radiation (cosmic ray, solar wind events) that may induce electron defects, ion implantation and alteration of the redox state of metal ions.[1]

Several studies on the interaction between toxic minerals and human lungs demonstrated the peculiar role of reduced iron ions exposed at the mineral surface of inhaled particles.[2] Specifically, the presence of np-Fe^{0} is one of the causes for the high reactivity of lunar dust[3] and is thought to be responsible for the lunar dust inflammatory effect. Oxidative activity of mineral dusts is widely accepted as one of the key factors contributing to lung inflammation. The formation of particle-derived reactive oxygen species (ROS), including superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet OH$), is known to induce, when the amount of ROS overcomes the antioxidant cell defenses, oxidative stress in cell, abnormal proliferation, and tissue damage.

The assessment of the impact of lunar dust on human health needs to take into account the enhanced reactivity of lunar dust in the experimental design of toxicological researches. Recently, the second

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NASA Engineering and Safety Center (NESC) workshop on lunar dust (Houston 2020) gave clear indications that “there is a need for a standardized lunar simulant for rigorous assessment of toxicity relevant to upcoming lunar missions” and rated this gap in the dust assessment as a “top priority” for defining the impact of lunar dust on human health.

Why should in situ studies on lunar dust reactivity be considered a priority?

Samples from Apollo missions have been extensively characterized and their toxicity assessed by LADTAG (Lunar Airborne Dust Toxicity Assessment Group). Nevertheless, Apollo dust samples returned to Earth were not in the enhanced reactive chemical conditions induced by the lunar environment, when astronauts were, and again will soon be, exposed to them. Current knowledge has been established using those materials and carries with it a considerable uncertainty due to the modifications that likely occurred to the toxicity-relevant physicochemical properties of particle surfaces. As a consequence, the *in situ* study of the reactivity of lunar dust will be required to design ground-based studies that reactivate the dust to restore the effects of the peculiar lunar environment. Experiments designed to provide an accurate quantitative assessment of the chemical reactivity of lunar dust in its native state will serve as a reference point to study the mechanisms involved in the lunar dust surface reactivity in terrestrial laboratories and ultimately to understand their potential toxicity. Moreover, *in situ* measurements can be designed to provide critical data so that lunar dust samples can be returned, transferred and manipulated appropriately in subsequent and more complex laboratory studies, including physico-chemical characterization, and *in vitro* and *in vivo* toxicological screenings.

To be performed on the lunar surface a simple-to-perform method that provides easy-to-interpret results is required. A fluorimetric assay based on the conversion of the terephthalate anion (TA, non-fluorescent) to 2-hydroxyterephthalate (TA-OH, strongly fluorescent) upon exposure to ROS could be readily exploited to obtain the information required.[4] The surface reactivity of lunar dust quantified *in situ* by means of the TA experiments will be used to design new simulants for health studies and activation protocols for *in vitro* and *in vivo* toxicological studies. Specifically, mechanical milling in a non-oxidative atmosphere will be used to simulate micrometeorite impact on the Moon and ion implantation and transition metal ion reduction will be carried out at high energy radiation facilities to mimic the effect of solar wind. The simulants will be compared to real lunar dusts by the same TA assay, and the results compared to those obtained *in situ*. The direct comparison of the reactivity of lunar dust on the lunar surface with the reactivity of both activated simulants and activated curated lunar dust will provide information necessary to directly address the existing knowledge gap highlighted in the recent NESC workshop.

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