Evaluation of the Toxicity of Lunar Dust Exposed to Simulated Space Radiation: Perspective from an ESA Topical Team

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Introduction: In the next few years, NASA, ESA and other space agencies will embark on renewed crewed exploration of the Moon, to advance the ability of humans to live and work in extreme environments, and to answer broad scientific questions related to the history of the Solar System. In particular, NASA's Artemis Program, with involvement from ESA, other international partners, and commercial partners, calls for multiple Moon landings, and, eventually, a surface outpost, to enable a sustained human presence on the Moon.

The Apollo Experience. From 1969-1972, NASA astronauts landed on the surface of the Moon six times (Apollo 11, 12, 14-17). During these missions, astronauts were exposed to lunar dust as a result of crew member entry into the lunar modules with spacesuits covered with lunar dust from extravehicular activities. A variety of acute respiratory tract symptoms were experienced by the crew, especially in reduced gravity when lunar dust would rise from the floor into the cabin atmosphere, as the lunar module lifted off the surface of the Moon. These were short-term exposures—just a few days at most—too short to expect any significant findings from the standpoint of pulmonary effects.

NASA Studies of Apollo14 lunar dust toxicity. In 2005, with future lunar missions in mind, NASA formed the *Lunar Airborne Dust Toxicity Assessment Group* (LADTAG), to study the toxicity of authentic lunar dust using samples returned to Earth during the Apollo era. The primary purpose of LADTAG was to evaluate the pulmonary toxicity of lunar dust[1]. To this end, rats underwent inhalation exposure to Apollo 14 lunar dust as well as control materials, such as titanium dioxide and crystalline silica (30d exposures), followed by testing for evidence of pulmonary inflammation and fibrosis. Lunar dust was found to be less toxic than crystalline silica, but more toxic than titanium dioxide. These experiments, however, were not designed to evaluate three important effects of the lunar environment that could potentially increase the chemical reactivity of lunar dust: UV light exposure, solar wind exposure and space radiation exposure. In light of these knowledge gaps, our understanding of the toxic effects of lunar dust in the lungs is substantially incomplete [2,3].

The effects of space radiation-induced chemical activation of lunar dust—an important gap in our understanding of lunar dust toxicity. Abundant evidence indicates that mineral dusts and the parent materials from which they are derived are sensitive to irradiation, including components of space radiation, such as high-energy protons[4–9]. The effects of radiation on these materials include electronic defects, ion implantation and degradation effects, all of which are indicative of chemical changes. Actual lunar samples, returned to earth during the Apollo era, have been subjected to radiation treatment as well, and show evidence of chemical changes that could have significant toxicity implications in the lungs [10,11].

Prospective experimental design: To safely sustain the presence of humans on the Moon, evaluation of lunar dust toxicity in the lung must be completed. The toxic properties of lunar dust are likely dependent on changes induced during high-energy irradiation on the Moon's surface. To gain insight into the full toxic potential of lunar dust, we must: i) activate lunar dust (or analogues) using high-energy irradiation to induce surface states similar to that of real lunar dust as it actually exists on the Moon; ii) evaluate the kinetics of high-energy states induced by activation; iii) define a set of tests to evaluate chemical surface reactivity of activated dust; iv) evaluate the cytotoxicity of activated Moon dust on human primary lung epithelial cells. We propose to undertake the following set of experiments.

i) *High-energy activation of Lunar dust.* Lunar dust analogues and lunar dust will be irradiated at the SIS18 synchrotron of the GSI Helmholtz Center in Darmstadt (Germany), the ESA reference facility for ground-based galactic cosmic ray simulations. Samples will be exposed to 1 GeV/n Fe-ions, a proxy of the heavy ion component of cosmic rays. The range in Moon regolith at this energy is approximately 10 cm. Graded doses will be used in the range 0.1-10 Gy to simulate short- and long- time dust exposure to radiation. Chemical analysis can start from a few minutes after irradiation for the low dose samples up to a few hours for those exposed to high doses, any delay being due to radioactive activation.

ii) *Passivation kinetics and chemical endpoints*. Cosmic ray-activated dusts will be aged in different

atmospheres (from ultra-dry N_2 to synthetic air with dosed water vapor) to study the passivation kinetics of irradiated surfaces in terms of chemical and toxicological endpoints. Surface redox reactivity of pristine and activated dust samples will be measured via cell-free tests. Lipid oxidation, formation of reactive oxygen species (ROS) and carbon centered radicals, lysis of liposomes and red blood cell hemolysis measurements will be used to clarify the mechanism of interaction with and damage to biological molecules [12].

iii) *Description of biological endpoints.* Cell exposure, and, ultimately, *in vivo* rat exposure experiments to assess inflammatory responses to particle deposition over short (hours) and long-term (weeks to months) will be carried out to assess the toxicity of pristine, activated and passivated lunar dusts.

iv) *Dust delivery*. The Dustgun generator (PreciseInhale™) will be used to de-agglomerate and deliver lunar dust aerosols for biological experiments. Advanced respiratory cell cultures will be exposed to these aerosols at the air/liquid interface; both multicellular bronchial and alveolar mucosa models will be studied, including immunocompetent cells. After exposure, immunologic, inflammatory, and toxic readouts will be measured, including oxidative stress, apoptosis and imaging (TEM, SEM, and confocal microscopy)[13-15].

Future perspectives: The ESA Topical Team on the Toxicity of Celestial Dusts (T3CD) is charged with addressing the most challenging questions related to lunar dust health effects. To address the issue of radiation activation of lunar dust and its toxicological implications, T3CD advocates that a broad multi-agency, multi-national effort be undertaken to perform the needed ground-based studies, using archived lunar dust samples. Adequate experimental techniques and resources are available to effectively close this important knowledge gap, and to pave the way for a safe, sustained human presence on the Moon.

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