The genotoxic and cytotoxic impact of lunar dust simulants to A549 cells

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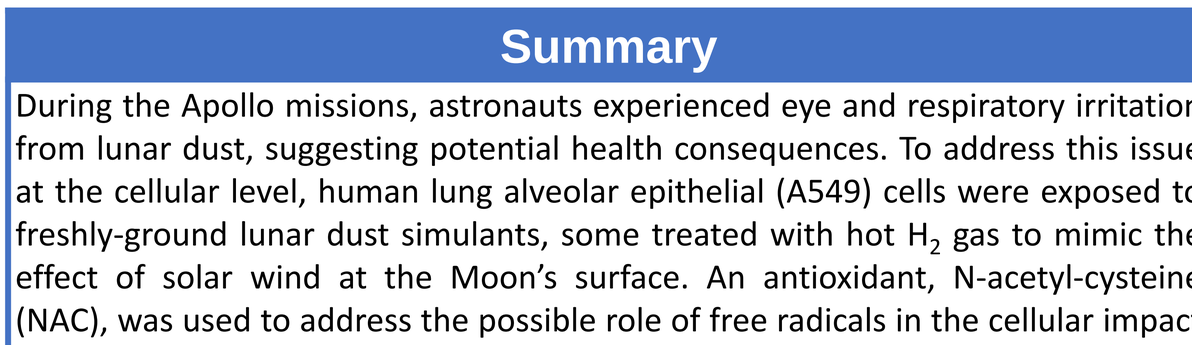
Summary

During the Apollo missions, astronauts experienced eye and respiratory irritation from lunar dust, suggesting potential health consequences. To address this issue at the cellular level, human lung alveolar epithelial (A549) cells were exposed to freshly-ground lunar dust simulants, some treated with hot H_2 gas to mimic the effect of solar wind at the Moon's surface. An antioxidant, N-acetyl-cysteine (NAC), was used to address the possible role of free radicals in the cellular impact of lunar dust simulants. Dose-dependent decreases in cell viability in response to LMS-1 (Lunar Mare Simulant-1) or LHS-1 (Lunar Highlands Simulant) indicated enhanced cytotoxicity of reduced materials (Fig. 1). The integrity of mitochondria was probed using MitoSOX Red, which also showed enhanced toxicity for reduced materials, and protection by NAC (Fig. 2), which was confirmed by direct measurement of cellular O_2 consumption (Fig. 3). A PCR-based assay revealed mitochondrial DNA damage by the simulants, also enhanced by their reduction (Fig. 4). Similar results were found for nuclear DNA damage using the alkaline comet assay (Fig. 5). Our results show oxidative damage induced by exposure to lunar surface dust, protection by antioxidants.



Cell Viability

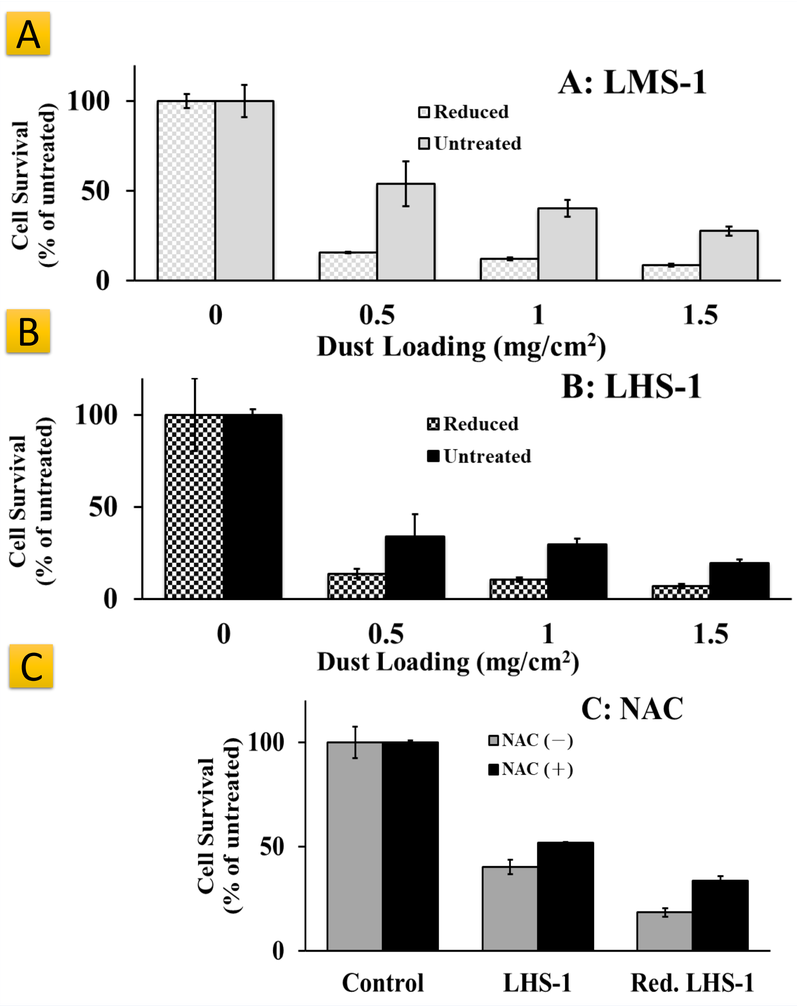


Figure 1. Viability of A549 Cells Exposed to Lunar Dust Simulants. Where indicated, (A) LMS-1 and (B) LHS-1 were first treated with H_2 gas at 900 °C to mimic space weathering by solar wind, and then A549 cells were exposed to the dust samples for 1 h. (C) Before and after 1 h-exposure to LHS-1, A549 cells were incubated with 0.5 mM NAC; 24 h later, viability was scored by trypan blue exclusion (n=3).

Oxygen Consumption Rate

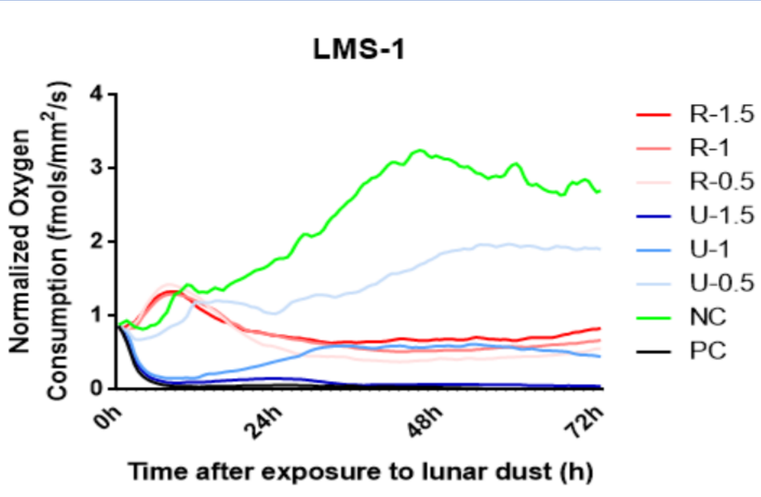


Figure 3. Oxygen Use by Simulant-exposed A549 Cells. RESIPHER (LUCID Technologies) was used to measure real-time O_2 consumption by cells in culture. Reduced (R) and untreated (U) LMS-1 (n=4) showed dose-dependent disruption of respiration, more for the former. NC, cells not treated; PC, positive control.

MitoSOX Red Assay

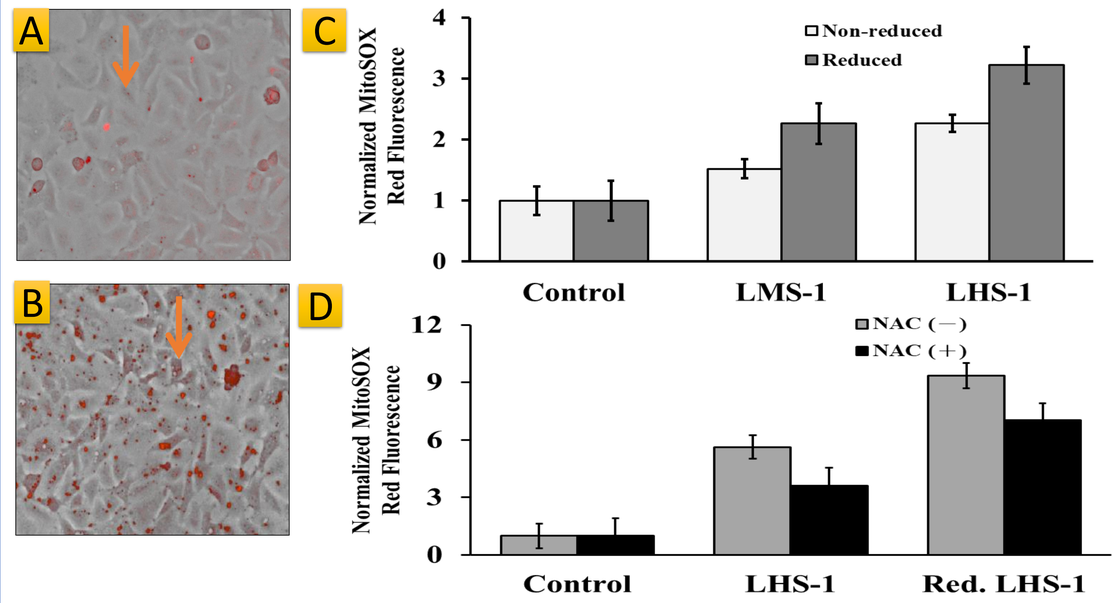


Figure 2. MitoSOX Red Fluorescence in Simulant-exposed Cells. (A) Untreated A549 cells. (B) A549 cells exposed to LHS-1, leading to oxidized MitoSOX Red in mitochondria (see arrows). (C) Quantified MitoSOX Red fluorescent signals. (D) Protection by 0.5 mM NAC incubation.

PCR Assay

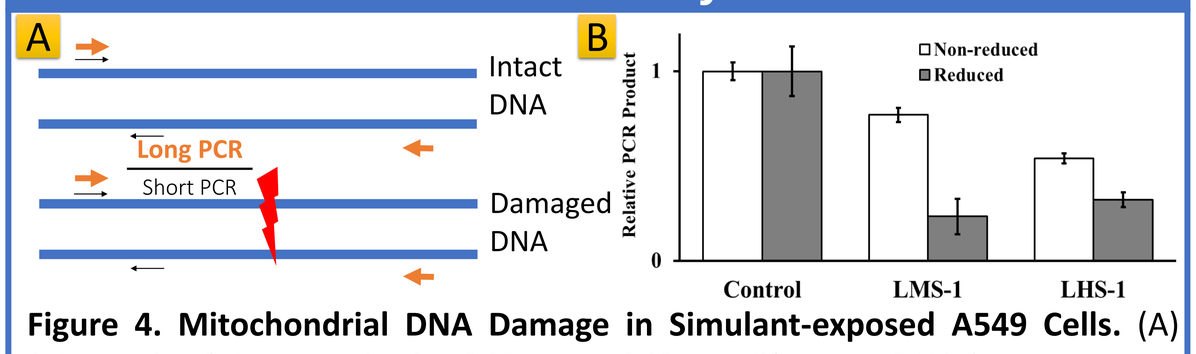


Figure 4. Mitochondrial DNA Damage in Simulant-exposed A549 Cells. (A) Schematic of the quantitative PCR assay. PCR amplifies two DNA fragments: the black (thin) arrows indicate amplification of a short DNA fragment, while **bold orange arrows** indicate a larger region, with a much higher probability of interference from DNA damage (**red bolt**) than for the short PCR. Cells were exposed to the lunar dust simulants for 1 h, and their DNA immediately isolated for the PCR assay. The ratio of the PCR products reports mitochondrial DNA integrity (*lower* ratios indicate *more* damage) (B) The normalized PCR product after exposure to lunar dust simulants.

Alkaline Comet Assay

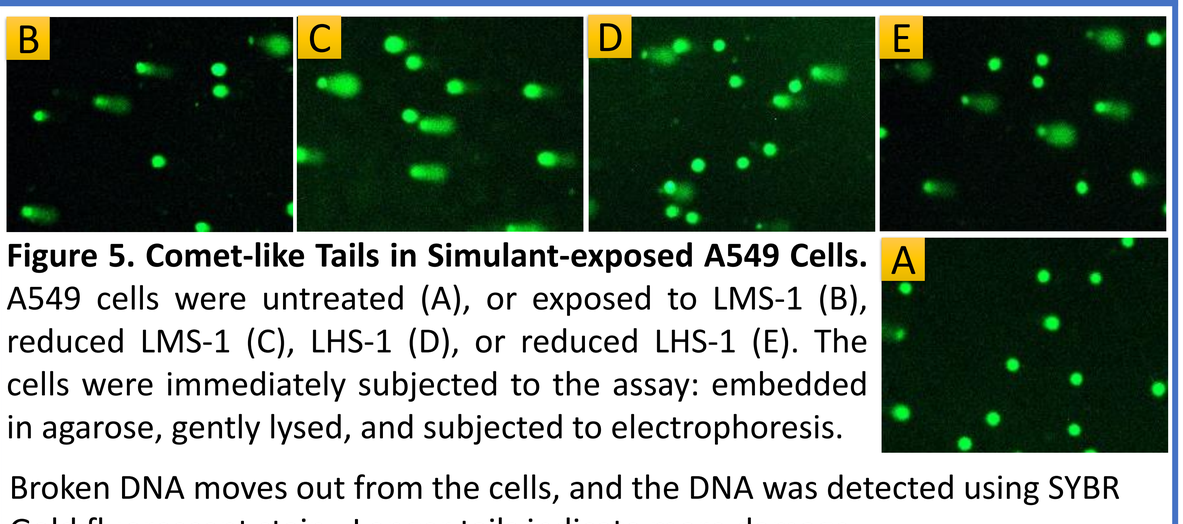


Figure 5. Comet-like Tails in Simulant-exposed A549 Cells. A549 cells were untreated (A), or exposed to LMS-1 (B), reduced LMS-1 (C), LHS-1 (D), or reduced LHS-1 (E). The cells were immediately subjected to the assay: embedded in agarose, gently lysed, and subjected to electrophoresis.

Broken DNA moves out from the cells, and the DNA was detected using SYBR Gold fluorescent stain. Longer tails indicate more damage.

Conclusion

Exposure to LMS-1 and LHS-1 causes significant cytotoxicity, genotoxicity, and mitochondrial dysfunction in A549 cells. The chemical reduction of both materials enhanced these effects, but NAC can boost the cell defense and thus alleviate such toxic effects. Our results suggest possible biomarkers to monitor the potential impact of ROS on explorers provoked by lunar regolith exposure.

References

[1] Caston, R. et al. (2018). GeoHealth, 2(4), 139–148.
[2] Ayala-Torres, S. et al. (2000). Methods, 22(2), 135–147.

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