SOTERIA: SEARCHING FOR ORGANISMS THROUGH EQUIPMENT RECOVERY AT IMPACT AREAS

A MISSION CONCEPT PROPOSAL TO ASSES MICROBIAL FORWARD CONTAMINATION BY RECOVERING LUNAR SPACECRAFT DEBRIS FROM RECENT IMPACT SITES NEAR THE SOUTH POLE.

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I. Accessible spacecraft debris currently on the Moon provides an ideal case study for unanswered astrobiology and planetary protection questions

All spacecraft sent to the Moon have carried viable microorganisms with them, from contamination associated with assembly, test and launch activities on Earth. Since September 1959, approximately 60 missions have delivered upwards of 80 spacecraft, boosters, payloads, rovers, and other structures to the surface of the Moon. Historical measurements and recent mathematical models predict that even after the effect of space exposure and, in some cases, high-velocity impact, most deliver a bioburden of thousands to millions of cells each to the Moon (1, 2). While it is widely assumed that no unsheltered life can indefinitely withstand the harsh physical conditions of the Moon, it is worth examining whether any of the recently delivered Earth biomass remains viable, given that survivors have been reported from multiple space biology exposure studies (3, 4).

Because the Moon's surface environment is sterilizing to life and the Moon itself is not considered a body that will house any indigenous life forms, NASA policy has not required the elimination or even the measurement of microbial contamination on lunar space equipment prior to launch (5, 6). Consequently, contaminated hardware remaining on the moon provides an ideal case study on the outcome of microbial dispersal on pristine worlds. Upcoming Artemis program missions could enable sampling and investigation of this hardware, offering the first ever impact survival data for refining planetary protection protocols (PP) for solar system exploration, as well as unique insight for the field of astrobiology. We propose a two-phased mission where astronauts on the Moon would recover debris from previously crashed spacecraft for return to Earth; subsequently, samples could be analyzed in terrestrial laboratories for viable life and biosignatures (Fig. 1). This sample return mission would be preceded by an imaging mission to obtain high-resolution data on the target sampling site near the Lunar South Pole. We propose the name of **SOTERIA** for this PP mission, in honor of the Greek goddess of safety and delivery from harm.

Figure 1. Proposed mission architecture.

Under COSPAR regulations, SOTERIA is a Category V “unrestricted Earth return” mission. Since the Moon can host no indigenous life, samples may be returned to Earth without concern for back contamination, and potential forward contamination from Earth outward must be mitigated with a “documentation-only” PP plan corresponding to the Moon’s Category II designation. This applies to the outbound leg only and must include pre- and post- launch reports, a post- encounter report, an end-of-mission report, and inventory of all organic compounds onboard in quantities over 1 kg (7), with the goal of preventing contamination of a body other than the Moon that could potentially host indigenous life (i.e., Mars), should an unintentional impact occur (8). Although indigenous life cannot

exist on the Moon, viable terrestrial contaminants could; studying the survival potential of these contaminant organisms will help predict if and how microbes could survive on other astrobiologically-relevant bodies, including Mars and Icy moons. Additionally, current COSPAR bioburden regulations for Category III-IV missions apply to viable aerobic spore-formers only, but SOTERIA will elucidate whether other types of microbes could survive impact, which could potentially widen the definition of “bioburden” for future missions (9). Finally, if SOTERIA does not find viable microbes on the lunar surface but does find dead ones or cellular debris, this could influence contamination control planning for future life detection investigations targeting non-viable organic and biological matter (10).

As one of the three main themes of the 2013-2022 Planetary Science Decadal Survey, the *Planetary Habitats* theme sought to define the conditions required for life and to understand the processes that allow life to arise and persist in the universe. While much progress has been made, many questions remain unanswered, and addressing them must constitute continuing goals in the coming decade. This theme requires sending spacecraft to Mars and other planetary bodies without contaminating them with terrestrial life, and assessing with certainty the provenance of any detected biosignatures. SOTERIA directly addresses these high priority areas in two key aspects, by 1) providing data on the survival of spacecraft-associated organisms in extraterrestrial conditions, directly informing questions of forward contamination, and (2) serving as an opportunity to perform sample collection and analysis for life detection in real planetary field conditions under the necessarily high levels of sterility and contamination control that will be required at other targets in the search for extraterrestrial life.

II. Lunar debris was contaminated with terrestrial microbes – any survivors?

Lunar landers arrive with a significant microbial bioburden

Spacecraft leaving Earth carry microbiological contaminants onboard, including vehicles intentionally and inadvertently impacted into the Moon’s surface. Recent PCR-based studies examining the abundance and diversity of microbial populations in NASA spacecraft assembly clean rooms have consistently found evidence of viable bacteria, archaea, eukaryotes, and viruses in these environments (11, 12). And in earlier decades, regular surveys were conducted on six Surveyor and five Lunar Orbiter vehicles between the years 1959 and 1973, enumerating both vegetative cells and spores (1). From these and other studies, Schuerger et al. (2) estimated that lunar vehicles carry an average bioburden at launch of $\sim 3.89 \times 10^6$ viable spores and vegetative cells per kg of spacecraft dry mass.

Little is known about the effect of high-velocity impact forces (i.e. crashed spacecraft) on microbial survival

The violent forces involved in crash landing on the Moon surface and their possible effect on the spacecraft's microbial population must be considered, since other solar system missions use impacts for “end of life” operations. Moreover, planetary exploration mission failures can also result in uncontrolled high velocity impacts (e.g., the NASA Mars Polar Lander; the ISRO Chandrayaan-2 Vikram lander). Fundamentally, how impact forces reduce spacecraft bioburden is unknown, and the Moon can be used as the first real case study to inform survival outcomes for impacts at other solar system destinations. More research is needed into how kinetic energy and heat dissipate in the process of a spacecraft structure crashing, collapsing and breaking apart (2). Much of our knowledge of microbial impact survival thus far is based on laboratory simulations aimed at constraining the parameters around the potential of lithopanspermia (13), with a focus on spores of *Bacillus subtilis*, given evidence of spores' stress resilience. Individual, unprotected *B. subtilis* spores have shown survival through high velocity impacts up to 229 ± 28 m/s (14). Within spacecraft, survivability might be increased due to cushioning; estimates for *Bacillus sp.* spore survival within rocks at impact velocities

of $1\text{--}5\text{ km}\cdot\text{s}^{-1}$ have yielded survival rates between 0.1% and $<10^{-6}$ of the starting spore densities (13–15). Therefore, microbial spores might be able to withstand substantial impact forces in many cases, but only a study of actual spacecraft samples could provide insight into the integrated effects of the crash dynamics of those complex structures and the biocidal environment on the lunar surface.

Biocidal conditions on the Moon reduce bioburden, but not instantaneously

The Moon possesses no substantial atmosphere, nor a planetary magnetic field, so the surface is exposed to the full influx of galactic and solar radiation. Moreover, lunar surface conditions are extremely harsh, with high doses of ultraviolet (UV) irradiation ($26.8\text{ W}\cdot\text{m}^{-2}$ UVC/UVB), wide temperature extremes (-171°C to 140°C), and low pressure (10^{-10} Pa). Diverse experiments have tested the effects of each of these stressors individually on microbial viability, again often with focus on spore-forming organisms as the most likely source of forward contamination.

Thermal inactivation of microorganisms can be modeled as an exponential decline in viability with rate dependent on temperature. A temperature of 100°C can incur a $>-6\log_{10}$ reduction in the viability of *B. subtilis* spores in fewer than two Earth days, whereas at 70°C the reduction is only between -3 and -4 logs over 56 days (1, 2). The temperatures experienced by spores or cells on lunar spacecraft debris depend on both geographic location and the location of the organisms within the debris (hardware can offer some thermal shielding). Most experiments testing the effects of space vacuum (10^{-14} Pa) lasting <100 days have found survival of at least 30% (4, 16–20); survival is 10–25% after 327 days, and 1–2% after 6 years, if shielded from UV radiation (21, 22). However, laboratory experiments have found a synergistic interaction between low pressure and high temperature; i.e., spores incubated for 8 hours in the combined conditions of 100°C and $<10^{-4}\text{ Pa}$ saw increased lethality of -6 logs relative to each effect alone (2). Of all relevant biocidal factors, solar UV radiation is likely to be the most potent on exposed surfaces. As with temperature, UV exposure leads to an exponential decline over time in viability of spores of several *Bacillus* strains, with a flux of $9.78\text{ W}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ (lower than that at the Moon's equator) resulting in a decrease of -6 logs in less than 3 hours (23). Like temperature, surface UV flux varies with latitude and longitude, and hardware shielding can substantially reduce lethality (21, 22, 24). Ionizing radiation is another biocidal factor; however, cosmic rays, solar wind particles, and solar particle events have relatively low penetration depth and are therefore relevant only to surface-associated cells and spores, not those within spacecraft interiors (2).

Figure 2. The LMS model (2) predicts bacterial spores might remain in the interiors of recently landed/crashed spacecraft, particularly at polar latitudes where Artemis and CLPS missions are planned. Here, each dot represents one spacecraft with x- and y-coordinates denoting its longitude and latitude, respectively; color indicates the predicted bioburden (total number of viable spores) on deep internal surfaces as of 2019.

The Lunar Microbial Survival model predicts the highest likelihood of surviving microbiota on recently delivered spacecraft debris located near the Lunar South Pole

The above information about spacecraft bioburden at launch and the factors affecting microbial survival were used by Schuerger and colleagues in the Lunar Microbial Survival (LMS) model (2) to predict the abundance of viable microbial life that might still be present on the debris from the 54

missions since 1959 that have landed or crashed vehicles or components on the Moon. Outcomes were calculated separately for external surfaces (exposed to all effects including all ionizing radiation), shallow internal surfaces (in thermal contact to the exterior but shielded from other effects), and deep internal surfaces (shielded from all effects except low pressure) (Fig. 2) (2). The LMS model predicts at least a $-231 \log_{10}$ reduction in viable bioburden for external spacecraft surfaces per lunation at the equator, but only -0.02 logs for deep internal surfaces. At the South Pole, a similar low rate would be expected for permanently shadowed craters, while sun-exposed crater rims at the lunar poles had an inactivation rate predicted at -4 logs per lunation. ***At these rates, it was predicted that the 10 most recently landed lunar spacecraft might all still carry viable microorganisms as of January 2019*** (2). The highest bioburdens were predicted to be on the deep internal surfaces of spacecraft near the Lunar South Pole (Fig. 2); of these, the Chandrayaan-1 Moon impact probe is remarkably close to the locations being discussed for a NASA Artemis missions (see below). ***We therefore propose that some of the Chandrayaan-1 probe debris be collected for return to Earth, to test LMS model predictions and assess the likelihood of forward contamination.***

III. A Lunar South Pole debris retrieval mission should be prioritized in the coming decade

The Moon is not a protected exploration target in PP, but its accessibility and the plans for human missions on the surface offer an opportunity to determine whether spacecraft carrying bioburden can result in viable forward contamination; or, alternatively, if impact forces and subsequent radiation effects can sterilize contaminants. Such knowledge could refine PP approaches for spacecraft heading to other solar system targets, sometimes resulting in hard impacts (purposeful or accidental).

There is precedent for this proposed mission architecture.

Figure 3. Apollo 12 astronauts visit the Surveyor III lander in 1969. From (25).

During the Apollo 12 mission, astronauts retrieved a portion of the Surveyor III's camera during a surface EVA in November 1969 (Fig. 3) (25). Back on Earth, Mitchell and Ellis (1971) reported that a single pure culture of *Streptomyces mitis* was recovered from circuit board foam insulation that was deeply embedded within the camera body. These results suggested that the *S. mitis* cells had survived launch, space travel, and 2.5 years of the harsh lunar conditions described above (26). However, photographic and video evidence, recently re-analyzed, show researchers very clearly handling the lunar hardware without following currently acceptable sterility procedures (27). Additional observations furthered skepticism about the survival claim, including the fact that concurrent analysis on wire cables of the Surveyor III were unable to isolate any surviving microbial species, and that *S. mitis* was among the organisms isolated from the crew during microbial testing (27, 28). Similar analysis should be repeated

using debris retrieved from other landing/impact sites. With more modern, stringent, and sensitive microbiological methods, assessment of recovered microbial cells and spores from lunar spacecraft debris could be achieved with a lower chance of false positives or negatives compared to the effort by Mitchell and Ellis (1971).

Recent image analysis identifies Chandrayaan-1 Moon Impact Probe as an optimal target near the Lunar South Pole

India's Moon Impact Probe, part of the Chandrayaan-1 mission to the Moon, was launched from Sriharikota, India on 22 October 2008, released by the orbiter, and braked to descend to the surface on 14 November 2008, impacting near the south pole while imaging a swath down the 14° E meridian from the equator to the pole. The exact coordinates of impact have been uncertain until recently. Initial attempts to locate the impact site involved matching MIP descent images (29) with the best available map of the time, the Clementine global mosaic, and following them down to the impact site; locating the final images taken near the pole was challenging. The most specific published impact location was 89.76° S, 39.40° W (320.60° E), reported by ISRO to the United Nations in 2009 (30).

Figure 4. Descent track and probable landing site of the Chandrayaan-1 Moon Impact Probe, inferred from descent images.

(229.7° $\pm$ 4.0° E). The longitude uncertainty is largely along track and dependent on the spacecraft altitude. This location is on the Earth-facing flank of the Connecting Ridge, one of the most illuminated points on the Moon and one considered for an early NASA Artemis Program landing, for example, by Coan and colleagues (32)). This MIP impact location is 1500 m from Coan's proposed landing site.

More recent, high-resolution polar mosaics have been produced from Lunar Reconnaissance Orbiter Narrow Angle Camera (NAC) images; these enable unambiguous location of the last few MIP images. Figure 4A shows the MIP groundtrack with frames on a base map constructed from LRO data. The last few MIP frames were taken from ISRO (2011) and those preceding it were taken from ISRO video sequences. The final frames pass west of the previous published location and the pole, ending with frame 3105 (Figure 4B) on the eastern wall of a crater earthwards of the high ridge connecting the Shackleton and De Gerlache rims ("Connecting Ridge"). Although 3110 images were taken, frame 3105 is the last one presently known. The dataset is not part of ISRO's formal lunar data release.

Using these resources, the LRO topography, MIP image locations and estimated spacecraft altitudes based on the footprint dimensions of the images (31), an impact site can be estimated. The impact apparently occurred at 89.44° $\pm$ 0.01° S, 130.3° $\pm$ 4.0° W

In addition to the Chandrayaan-1 MIP, we have identified three other high-priority target locations near the South Pole: the NASA Lunar Prospector orbiter (crashed 31 January 1999 at 87.5° S/42.3° E), the NASA LCROSS shepherding spacecraft and Centaur upper stage (crashed 9 October 2009 at 84.7° S/311° E), and the ISRO Chandrayaan-2 Vikram lander (crashed 6 September 2019 at 70.9° S/

22.8°E). These sites might serve as alternatives or complements to the Chandrayaan-1 site. The NASA spacecraft would avoid the requirement, posed by the Chandrayaan craft, for diplomatic negotiation with India to obtain cooperation on crash site identification and permission for use of the debris. However, as they lie within permanently shadowed regions, they are more difficult to access.

SOTERIA Phase 1: Image

Before sending astronauts to recover samples, a precursor imaging mission is required to map out the Chandrayaan-1 debris field and potentially also the Lunar Prospector, LCROSS, and Chandrayaan-2 impact sites. This mission could be carried out by lunar orbiters with high-resolution cameras, and/or future Commercial Lunar Payload Services (CLPS) landers.

SOTERIA Phase 2: Gather

SOTERIA's second phase would consist of collecting debris from the impact site and returning it to Earth for microbiological analysis. Only a few kilograms of material would be required, and the required crew training would be minimal. Unlike other surface science investigations, an untrained eye could likely identify metallic spacecraft debris amongst lunar rocks/regolith, and crewmembers could place samples in pre-sterilized containers, eliminating additional steps for maintaining aseptic conditions. If lack of a Lunar Terrain Vehicle (LTV) precludes execution during the first Artemis mission, this phase could be carried out on future crewed missions. Moreover, if the LTV can be remotely operated and equipped with a motorized arm, the LTV could recover and bag the debris and transport it to a crew-accessible location for pickup by the next crewed mission. An added advantage of this procedure could be the lowered possibility of contamination by crew handling.

Earth-based analyses should be diverse

Returned spacecraft debris samples could undergo a suite of microbiological assays in modern laboratories. We propose analysis for enumeration of culturable organisms, detection of non-culturable viable organisms, and analysis of biosignatures that would pose a contamination risk to detection of novel life on other planets (Table 1).

Table 1. Recommended assays to be carried out on returned lunar samples

Purpose	Assay	Description
Enumeration of culturable spores & cells	Swab- or wipe-rinse sampling & culturing in Trypticase Soy Agar	Procedures from NASA Handbook for the Microbial Examination of Space Hardware. (33)
DNA-based identification of all viable organisms, including novel species, and viable-but-not-culturable (VBNC)	Viability PCR: treatment with propidium monoazide to inactivate nonviable DNA, followed by 16S rRNA gene amplicon or metagenome sequencing	Verified by NASA Planetary Protection researchers to distinguish between viable and non-viable organisms in spacecraft assembly cleanrooms (11, 12, 34). Untargeted assay.
High-sensitivity DNA-based identification of known microorganisms	DNA microarray	Verified by NASA Planetary Protection researchers for identification of low-abundance microorganisms in spacecraft assembly cleanrooms, with higher sensitivity than PCR. (35)
Visual observation of intact microbial cells	Scanning electron microscopy	Previously used for analysis of biogenic signatures on meteorites, e.g. (36)
Detection of biogenic organic compounds	Deep UV resonance Raman and fluorescence spectrometry	Similar to <i>in situ</i> analysis by SHERLOC on Mars Perseverance rover for biosignature detection
Identification of biogenic organic compounds (e.g., lipids, amino acids)	Organic extraction followed by gas chromatograph-mass spectrometry	Standard laboratory methods for analysis of biological compounds, as in (37)

Controlled exposure experiments could complement the recovery of debris collection

Because many factors of the initial bioburden of the debris are unknown, a complementary exposure mission experiment could carry a known density of microbial cells, including several different species within diverse materials and shaped objects, to the Moon for controlled exposures. This type of experiment would help with interpreting results from any recovered debris, and validation of the LMS model. The science payloads could be simply deployed during Artemis landings and collected at a later time point for concurrent laboratory analysis on Earth alongside debris assessments.

Even a negative result would be valuable

Data gathered from genuine space artifacts would fill a unique and important knowledge gap in astrobiology, planetary exploration and space biology. The proposed SOTERIA mission takes advantage of a natural experiment: hardware from previous missions readily accessible on the Moon. The feasibility of this mission, particularly its proximity to planned Artemis landing sites near the Lunar South Pole, makes it especially promising. ***Recovering live microorganisms is not the goal of this mission concept; a negative result, in which even the most sensitive microbiological assays fail to detect viable cells, would also be valuable to the planetary science community by helping to place current COSPAR PP regulations and procedures in perspective with data derived from another world for the first time in the history of spaceflight.***

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