IDENTIFYING THE LIMITS OF LIFE DUE TO IMPACT-INDUCED MECHANICAL STRESSES ON EXTREMOPHILES. L. Zhao[1,2], C. A. Perez-Fernandez[3], J. DiRuggiero[3], K. T. Ramesh[1,2], [1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, [2]Hopkins Extreme Materials Institute, Johns Hopkins University, Baltimore, MD, [3]Department of Biology, Johns Hopkins University, Baltimore, MD.

Introduction: The possibility of life present on planetary bodies in the solar system has major consequences for the design of missions. Well-informed planetary protection policy is necessary to prevent contamination when exploring bodies, especially with the possibility of sample returns. Specifically, in what conditions can life survive, and what precautions are needed for safe sample return missions? Impact events greatly shape the surfaces of most planetary bodies in the solar system and may have significant ramifications on the introduction and presence of life. Some lifeforms may even survive harsh impact conditions and be transported through the ejecta to other planetary bodies. It is important to understand the limits of the survival of life due to mechanical stresses caused by these impacts.

Previous related work involved impacting bacteria at high velocities [1-3]. However, these experiments are insufficient in determining planetary protection thresholds due to experimental variability and large uncertainties arising from the approaches used [4]. Impact conditions, including pressure and shear stress, vary widely with respect to space and time. Given the system complexity, it is difficult to determine the specific conditions the lifeform was exposed to during a simple hypervelocity impact experiment. Furthermore, despite having a significant role in microorganism structure and behavior [5], the effect of shear stresses has not been directly explored.

Another general challenge associated with this area is the choice of the organisms studied. Previous works were mostly conducted on model bacteria broadly prevalent on Earth [1-3]. However, better organisms to investigate are extremophiles, which are accessible surrogates of extraterrestrial life and an ideal model organism for these experiments due to their adaptations to harsh environments.

This work aims to determine the survival rate of extremophiles subject to dynamically applied pressure and shear stress associated with impact. We present a novel adaptation of the pressure-shear plate impact (PSPI) experiment for microorganism recovery.

Methods: *Model microorganisms.* Since extremophiles are resistant to different stressors, they may better reflect the survivability of potential extraterrestrial life after impact stresses. The bacteria *Deinococcus radiodurans* is specifically selected for its well-known resistance to desiccation, oxidative stress, and ionizing radiation [6], and has been isolated from an established Mars analog environment, the Atacama Desert in Chile.

Pressure-shear plate impact. The PSPI experiment [7] is selected due to its control of the stress states dynamically applied to the specimen, in this case the microorganisms. The experiment involves two parallel flat plates impacting each other, as shown in Figure 1. By altering the tilt of the plates, the ratio of shear stress to pressure experienced by the specimen can be controlled, and the loading duration is determined by wave propagation speeds. Increasing the velocity of the plates increases both the pressure and shear stress experienced by the specimen. During impact, the stress state in the specimen rises to a specific pressure and shear stress, as determined by the velocity, angle, and material properties.

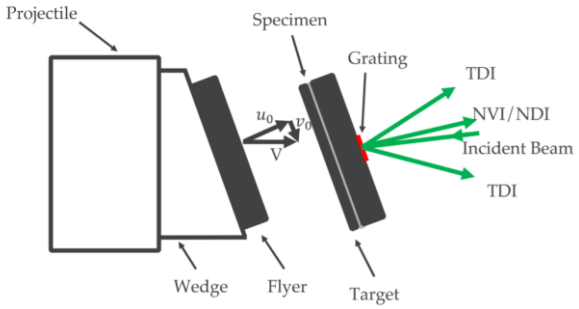

Figure 1. A schematic of the pressure-shear plate impact experiment, where a projectile (left) impacts the target (right) containing the specimen.

In the experiment, the normal displacement interferometer (NDI) and transverse displacement interferometer (TDI) capture the normal particle velocity and transverse particle displacement, respectively, off a diffraction grating on the back surface. With elastic wave analysis, the interferometer data is used to calculate the stress state and strain rate experienced by the specimen as a function of time.

The projectile carries the flyer plate, which impacts a target containing the cells. Figure 2 provides a visualization of the target sandwich configuration. A known number (~10^9) of cells are filtered onto two membranes. To maintain a moist environment, the membranes are placed between two glass fiber filter papers saturated with a saline solution. This specimen configuration is sandwiched between two metal plates. The assembly is then compressed, screwed together, and epoxied to prevent sample desiccation during experimental setup.

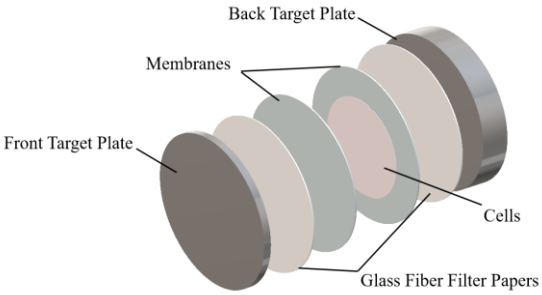

Figure 2. A simplified visualization of the target sandwich configuration that holds the cells.

The plates are manufactured out of S7 steel, selected for its high yield stress and fracture toughness. This ensures that the plates do not fracture after impact and the configuration remains intact. The cells from the membranes can thus be recovered easily after high impact stresses. The steel plates are coated with a thin layer of titanium to prevent oxidation (and the associated toxicity) from the wet environment while still maintaining planar flatness.

Biological analyses. After impact, the target sandwich is collected from the vacuum chamber and opened. The cells are harvested from the membrane through vortexing and elution, and dilutions are prepared for biological analysis.

Cell survivability is quantified using two methods. Colony forming units (CFUs) are counted and compared against a control culture that did not experience the high stresses. However, viable cells may be incapable of forming a CFU. Thus, fluorescence-based LIVE/DEAD assays are performed to stain cells based on cell membrane integrity, and individual cells are counted under a microscope.

Discussion: With proper experimental set up, the stress state the cells experienced can be quantified for a defined set of conditions. From the laser interferometry data, the particle velocity can be calculated, which relates to the multiaxial stress state in the specimen. The microbial survival rate maps to a specific pressure and shear stress governed by experimental parameters. With enough data points, the survivability of a microorganism across a range of conditions can be determined. This set of experiments provides fundamental quantitative information for planetary protection requirements based on impact stresses applied on microorganisms.

The method outlined also has potential for larger scope studies. In future works, the scope of biological analyses will be expanded to study different extraterrestrial surrogate microorganisms, like *Haloferax volcanii*, a halophilic archaeon. The existing workflow may yield a high enough cell recovery from the membrane to perform RNA sequencing. Transcriptomic analyses will elucidate underlying stress responses by cells after experiencing high impact.

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