Microbial Response to Extreme Impact Stresses. L. Zhao[1,2] C. A. Perez-Fernandez[3], J. DiRuggiero[3,4], K. T. Ramesh[1,2,4], [1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD (lilyzhao@jhu.edu), [2]Hopkins Extreme Materials Institute, Johns Hopkins University, Baltimore, MD (ramesh@jhu.edu), [3]Department of Biology, Johns Hopkins University, Baltimore, MD, [4]Department of Earth and Planetary Science, Johns Hopkins University, Baltimore, MD

Introduction: Recent and upcoming space missions, including the DART and the MMX mission, have again raised questions about the survival of life following meteorites' impact, their role in the formation of life, and the possibility of panspermia. Perhaps more pertinently, understanding the limits of life in conditions similar to impacts is crucial to plan missions for collecting extraterrestrial samples. To explore the possible presence of life, their spread through planetary bodies, and possible survival, precautions are needed to prevent contamination.

Although there have been previous works related to studying the survival of microorganisms to high- and hypervelocity [1-3], they are insufficient for assessing the effects of normal and shear stress on microorganism survival [4]. Since impact conditions (including temperature and stress) vary complexly with space and time, nuanced experiments with microorganisms are necessary to decouple the exact effect of impact stresses on survival. Additionally, terrestrial microorganisms found in extreme environments can thrive in harsh conditions that better reflect the habitats in space and would be suitable for these experiments.

To better understand the mechanisms that allow microorganisms to survive extreme mechanical stresses, molecular analysis is needed. Therefore, maximizing the amount of genetic material following impact is also critical in experimental design.

First outlined at the 2022 Lunar and Planetary Science Conference [5], experiments are currently underway to identify the limits of survival and the cellular response of *Deinococcus radiodurans* to a range of pressure and shear stresses similar to that experienced in impacts.

Methods: To decouple the effects of varying pressure and shear stress, the pressure shear plate impact (PSPI) experiment [6] was selected for use to induce impact stresses on *D. radiodurans*. In the experiment, two flat parallel plates, with a thin layer of bacteria in between, are impacted by a third flat parallel plate. Based on the angle of impact, impact velocity, and material properties of the plates, the pressure and shear stress experienced by the bacteria can be estimated using wave analysis. Interferometry, which measures the particle velocity history, can confirm the stress experienced and show the loading duration of the stress. Varying the impact velocity and angle of impact will also alter the magnitude of stress and ratio of pressure to shear stress, respectively.

D. radiodurans was selected in this study for its resistance to multiple extreme conditions, including desiccation, oxidative stress, and ionizing radiation [7]. Previous experiments have also been conducted on its survival following exposure to the ISS, which makes it a relevant microorganism to study for astrobiology research [8].

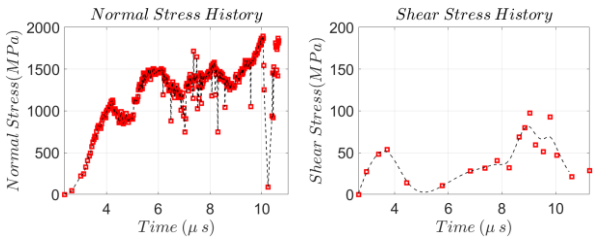

Figure 1. An example of the evolution of normal stress and shear stress over time for a PSPI experiment. The loading duration of maximum stresses can be measured from the plots.

Following an experiment, the microorganisms are recovered and allowed to grow under optimal conditions to quantify their survival by counting the number of colony forming units (CFUs). The survival is quantified by comparing the CFUs after the experiment with proper controls. The CFU count is also normalized by the percentage of bacteria recovered after the experiment, which is calculated using cell counting by fluorescent microscopy.

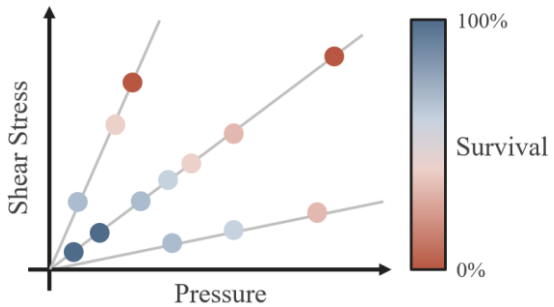

Figure 2. A schematic illustrating how the survival of *D. radiodurans* may look like across a range of pressure and shear stresses following experiments conducted. The gray line indicates experiments conducted at the same angle of impact.

With the current experimental setup, we recovered enough bacterial cells to do survival, TEM imaging, and transcriptomic analysis to explore cellular damage and response to pressure.

Results: Interferometry in the experiment captured the stress history and evolution over time, as shown in Figure 1. This accurately confirmed that the microorganisms are experiencing pressure and shear stresses for microseconds.

Following many experiments across different pressures and shear stresses, the survival of *D. radiodurans* could be quantified in the pressure vs. shear stress space. An example of what it may look like is shown in Figure 2.

Membrane integrity and cell viability were also affected by high pressure. Cells of *D. radiodurans* presented possible membrane modifications after the impact that could indicate that the cells are no longer viable (Fig.3).

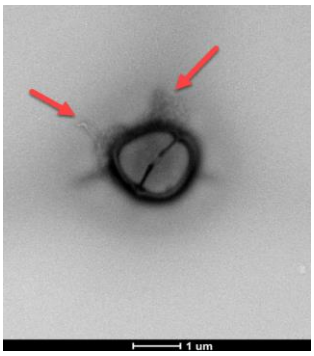

Figure 3. TEM image of *D. radiodurans* cells exposed to high dynamic pressure. Red arrows point to possible damage in the membrane.

We were able to extract a sufficient yield of RNA from the cells exposed to the experiment for RNA-seq analysis. However, the RNA quality (as measured by the RNA Integrity Number (RIN) in Figure 4) seems to also be affected by the stress. Nucleic acid degradation under stressful conditions has already been documented in molecular and astrobiology research [7,8].

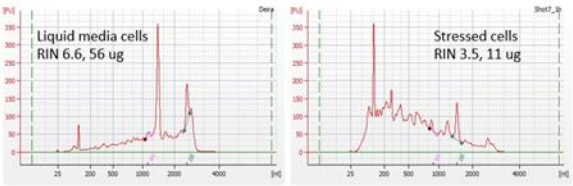

Figure 4. RNA quality and yield for *D radiodurans* cells. Stressed cells present less RIN than that of cells in exponential growth (RIN 6.6 vs. 3.5).

Experiments and data analysis are currently underway to identify the mechanisms and limits of survival of *D. radiodurans* to a range of pressures and shear stresses.

Discussion: Survival of *D. radiodurans* can be quantified across a range of pressure and shear stresses, and sequencing data analysis is being conducted to elucidate the underlying molecular response. Our data on the limits of life will help to understand how microorganisms could survive under panspermic scenarios and redefine planetary protection policies.

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References: [1] Burchell, M. J. et al. (2001) *Icarus, 154,* 545-547. [2] Fajardo-Cavazos, P. et al. (2005) *Astrobiology, 5,* 726-736. [3] Fitzmaurice, B. C. et al. (2017) *Icarus, 239,* 1-7. [4] Persat, A. et al. (2015) *Cell, 161(5),* 988-997. [5] Zhao, L., et al. 53rd Lunar and Planetary Science Conference. Abstract #2577. [6] Klopp, R. W. et al. (1985) *Mech. Mater.* [7] Daly, M. J. (2009) *Nat. Rev. Microbiol., 7,* 237-245. [8] Kawaguchi Y., et al. *Front Microbiol*. 2020