HYDROPHOBIC SURFACES OF SPACECRAFT COMPONENTS ENHANCE THE AGGREGATION OF MICROORGANISMS AND MAY LEAD TO HIGHER SURVIVAL RATES OF BACTERIA ON MARS LANDERS.
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Introduction: Inorder to minimize the forward contamination of Mars, spacecraft are assembled under cleanroom conditions that require several procedures to clean and sterilize components. Surface characteteristics of spacecraft materials may contribute to microbial survival on the surface of Mars by protecting spores from sterilizing agents, including UV irradiation. The primary objective of this study was to evaluate the effects of surface characteristics of several spacecraft materials on the survival of *Bacillus subtilis* spores under simulated Martian conditions.

Methods: Endospores of *Bacillus subtilis* HA-101 were grown in a liquid sporulation medium, washed, and concentrated according to the procedures of Mancinelli and Klovstad [1] and Schuerger et al. [2]. Monolayers of *B. subtilis* were prepared on spacecraft materials by depositing 1.25 x 10^6 endospores in 100 µl of sterile deionized water (SDIW) to the upper surfaces of 1-cm^2 coupons. Eight spacecraft materials were used for these studies and included: two brands of uncoated aluminum 6061, graphite, astroquartz, chemfilm- (i.e., alodine-) treated aluminum, clear-anodized aluminum, black-anodized aluminum, and stainless steel. Spacecraft materials were UV-sterilized for 1 hr prior to the deposition of monolayers by exposing coupons to a Hg-lamp (254 nm) at an intensity of 6.5 W m^{-2}. Microdrops of suspended endospores of *B. subtilis* were dried onto spacecraft materials at 25 ^oC overnight in either a laminar flow hood or microbial incubator.

Monolayers of *B. subtilis* were then exposed to Martian conditions of pressure (8.5 mb), temperature (-10 ^oC), high CO_2 atmosphere, and irradiated with a Mars-normal UV-VIS-NIR spectrum [2]. The simulations were conducted within a low-pressure Mars chamber at the Kennedy Space Center, FL (KSC), as described elsewhere [2]. Monlayers were exposed to 1 min or 1 hr of Mars-normal UV irradiation adjusted to simulate clear-sky conditions on equatorial Mars (*tau* = 0.5) at the mean orbital distance of Mars. Mars simulations lasted 4 hrs total elapsed time from intial evacuation of room air from within the Mars chamber to repressurization of the chamber. In addition, bacterial monolayers on all eight spacecraft materials were coated with gold and imaged with SEM.

Results: When exposed to 1 min of Mars-normal UV, the numbers of viable *B. subtilis* spores were reduced 3-4 decades for both brands of Al 6061, stainless steel, chemfilm-treated Al, clear-anodized Al, and black-anodized Al. In contrast, bacterial suvival was reduced only 1-2 decades for monolayers on graphite and astroquartz when bacterial spores were exposed to 1 min of Mars-normal UV irradiation.

When bacterial monolayers were exposed to 1 hr of Mars-normal UV irradiation, no viable bacteria were recovered from both brands of Al 6061, stainless steel, chemfilm-treated Al, clear-anodized Al, and black-anodized Al. In contrast, bacterial suvival was reduced 2-3 decades for monolayers on graphite and astroquartz when bacterial spores were exposed to 1 hr of Mars-normal UV irradiation. Thus for both the 1-min and 1-hr exposures, significantly greater numbers of *B. subtilis* endospores survived on the hydrophobic surfaces of graphite and astroquartz than on the hydrophilic surfaces of the other spacecraft materials.

SEM images of the bacterial monolayers revealed that endospores of *B. subtilis* formed large aggregates of multi-layered spores on the hydrophobic graphite and astroquartz materials but not on the other six spacecraft materials (Figure 1). Although many endospores were observed to have been deposited within pits on aluminum 6061 and within cracks on stainless steel and chemfilm-treated Al, adequate levels of UV irradiation were able to penetrate these surface features and inactivate the resident populations of *B. subtilis* endospores.

Conclusions: The higher survival rates for endospores of *B. subtilis* on graphite and astroquartz were attributed to the formation of large mulit-layered aggregates of spores in which the lower layers were protected from UV irradiation by the overlying cells. Aggregates of endospores formed on the hydrophobic surfaces of graphite and astroquartz but did not form on the hydrophyllic materials. Furthermore, shallow pits and cracks on spacecraft materials were not deep enough to prevent the inactivation of endospores deposited within these features. Results indicate that the surface characteristics of spacecraft materials may contribute to the survival of microorganisms when vehicles are landed on Mars and exposed to direct and diffuse UV irradiation. Of the factors tested, hydrophobicity was more important than the pitting and cracking of surfaces in determing the survival of *B. subtilis* endospsores under Martian UV irradiation.

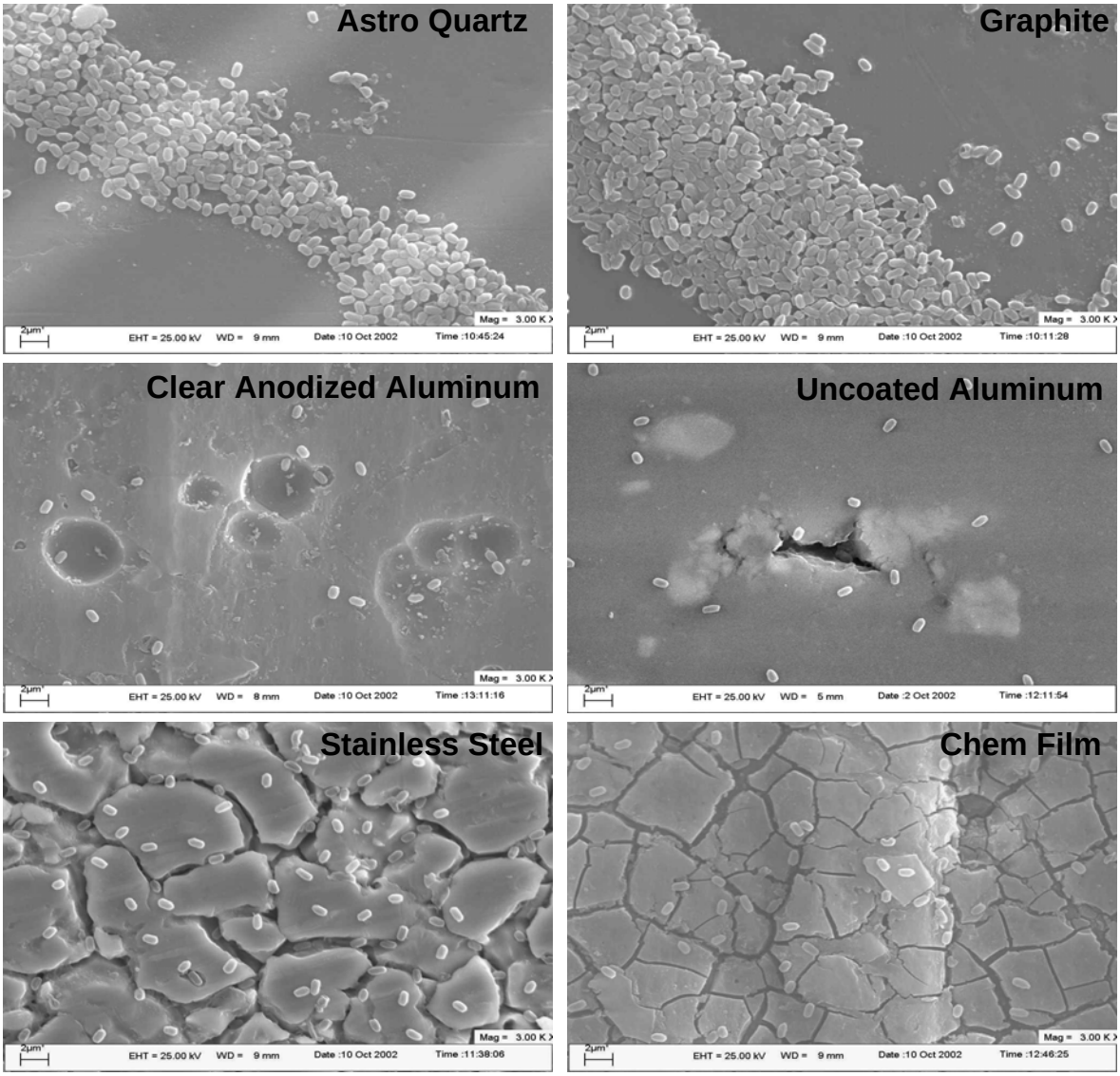

Figure 1. Spacecraft materials were selected based on their use on the Mars Exploraiton Rovers Spirit (successfully landed in Gusev Crater on January 3, 2004) and Opportunity (scheduled to land on Meridiani Planarum on Janaury 25, 2004).

References: [1] Mancinelli, R. L. and Klovstad, M. (2000) *Planetary Space Sci*., 48, 1093-1097. [2] Schuerger A. C. et al., (2003) *Icarus*, 165:253-276.