“Sticky little devils” … improving planetary protection forward decontamination strategies – studies on the spore resistance to low-pressure plasma sterilization & persistence on metallic copper surfaces

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Microbial contamination arising from spacecraft exploration harbors the distinct potential to impact the development and integrity of life-detection missions on planetary bodies such as Mars and Europa. Such missions are subjected to strict regulations. In the context of the planetary protection guidelines, established by the Committee of Space Research (COSPAR) in 1967, it is essential to reduce or eliminate the biological burden on flight hardware prior to launch in order to prevent cross contamination of celestial bodies with environmental or human-associated microorganisms. Depending on type of mission and planetary body, specific planetary protection guidelines are required to clean and sterilize a spacecraft or its components to avoid contamination from terrestrial organisms. The search for extraterrestrial life will rely heavily on validated cleaning and bioreduction strategies to ensure that terrestrial microbial contamination does not compromise the scientific integrity of such missions.

Spaceflight hardware currently undergoes extensive routine cleaning to remove major contaminants, followed by precision cleaning as needed. Nevertheless, several *Bacillus* species have been recurrently isolated from spacecraft assembly facilities, which were resistant to various sterilization treatments such as desiccation, ionizing and oxidizing agents. Commonly used test organisms for cleaning and bioreduction studies are bacterial spores, usually spores of *Bacillus subtilis*, due to their reproducible inactivation response, extensive knowledge on its genetics, physiology and biochemistry as well its ease of use. Spores of *Bacillus subtilis* were used as a bioindicator and a genetic model system to study the sporicidal effects of low-pressure plasma as an active decontamination approach and to evaluate the potential use of antimicrobial copper-containing surfaces. Cold plasmas operated at low-pressure have been successfully applied for the sterilization of medical equipment, packaging in the food industry and surface decontamination. Antimicrobial surfaces are defined as materials that contain an antimicrobial agent (such as silver, copper and their alloys) that inhibits or reduces the ability of microorganisms to grow on the surface of a material. The introduction of antimicrobial surfaces for medical, pharmaceutical and industrial purposes has shown their unique potential for reducing and preventing microbial contamination. In our work, wild-type spores, spores lacking the major protective coat layers (inner, outer, and crust), pigmentation-deficient spores or spore impaired in encasement (a late step in coat assembly) were systematically tested for their resistance to low-pressure argon, hydrogen, and oxygen plasmas with and without admixtures as well as in their contact killing on different metallic surfaces.

Here, we would present an overview of our current understanding on factors involved in the spore resistance to two innovative decontamination approaches, i.e., (i) low-pressure plasma sterilization and (ii) antimicrobial metallic copper surfaces, with special focus on the architecture of the *B. subtilis* spore coat as the major “protective bio-shield”.