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# "Killing them softly" … challenges in the Bacillus subtilis spore inactivation by plasma sterilization

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The elimination of bacterial endospores is absolutely essential in numerous fields, ranging from hospital hygiene, the food processing industry, all the way to the space industry. A major goal of space exploration is the search for signatures of life forms and biomolecules on other planetary bodies and moons in our solar system. The transfer of microorganisms or biomolecules of terrestrial origin to critical areas of exploration is of particular risk to impact the development and integrity of life-detection missions.1 Plasma sterilization is a promising alternative to conventional sterilization methods for spaceflight purposes. Due to their extraordinary resistance properties, spores of the Gram-positive bacterium *Bacillus subtilis* are used as biological indicators for decontamination studies to identify the relevant mechanism that leads to the rapid bacterial inactivation.1,3 Here, we present novel insights into the key factors involved in spore inactivation by low pressure plasma sterilization using a double inductively-coupled plasma reactor. (2,4)
In order to standardize the assessment of inactivation efficiencies by plasma discharges, an electrically driven spray deposition device was developed, allowing fast, reproducible, and homogeneous preparation of *B. subtilis* spore monolayers. We demonstrate that plasma discharges caused significant physical damage to spore surface structures as visualized by atomic force microscopy. A systematic analysis of *B. subtilis* spores lacking individual coat and crust layers - the first barrier to environmental influences – revealed the coat to be one of the contributing factors in the spore resistance to plasma sterilization. (2-4)
Furthermore, we identified spore-specific and general protection mechanisms and DNA repair pathways during spore germination and outgrowth after plasma treatment, leading to a better understanding of the complex molecular mechanisms involved in the inactivation by plasma sterilization processes.

**References:**

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(2) Fiebrandt et al. 2017. Plasma Phys. Control. Fusion 59:014010.
(3) Raguse et al. (2016a) Appl. Environ. Microbiol. 82:2031-2038.
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