Low pressure plasma inactivation of *Bacillus subtilis* spores: insights into the mechanisms of spore resistance

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Being the most resistant form of a biological system, spores of *Bacillus subtilis* are very resistant against a broad spectrum of sterilization methods and, therefore, are commonly used as a biological indicator in order to verify the functionality of a sterilization procedure. The process of low-pressure plasma sterilization is a promising alternative to conventional sterilization methods as it is extremely fast, efficient and gentle to heat-sensitive materials. Active plasma species contain a high degree of sporicidal UV/VUV-radiation, as well as charged particles and free radicals, which exert detrimental effects on microorganisms. In this study we present novel insights into the key factors involved in spore inactivation by low pressure plasma sterilization using a double inductively-coupled plasma reactor.

In order to standardize the assessment of spore inactivation efficiencies by plasma discharges, an electrically operated deposition device was developed, allowing fast, reproducible, and homogeneous preparation of *B. subtilis* spore in monolayers on surfaces leading to more reliable investigations. We demonstrate that low-pressure plasma discharges of argon and oxygen discharges cause 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 a major factor in in spore resistance towards plasma treatment.

In order to gain a better understanding of the complex molecular mechanisms involved in the inactivation by plasma sterilization processes, we analyzed plasma-induced DNA lesions *in vitro*, identified general and spore-specific DNA lesions, and characterized different DNA repair mechanisms during spore revival after plasma treatment.