Plasma Interactions With Bacterial Biofilms as Visualized Through Atomic Force Microscopy

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***Abstract*—Bacterial biofilms are microbial communities that are less susceptible to standard killing methods than free-living bacteria. Gas-discharge plasmas were used to treat biofilms for various exposure times. After 5-min plasma exposure, 90% of culturable cells were removed. Atomic-force-microscope images that reveal the sequential changes in cell morphology occurring during plasma treatment are presented.**

***Index Terms*—Atomic force microscopy, microorganisms, plasma applications.**

BACTERIAL biofilms are microbial communities embedded in a matrix mostly composed of exopolysaccharides together with some proteins and nucleic acids [1]. Conventional sterilization and disinfection methods are often ineffective with biofilms because bacteria in biofilms show different properties from those in planktonic life. The use of gas-discharge plasmas represents a novel alternative because plasmas contain a mixture of charged particles, chemically reactive species, and UV radiation, each individually known to be disinfectant/sterilizing agents.

For this paper, gas-discharge plasma was produced by using an Atomflo 250 reactor employing a capacitively coupled electrode design. An atmospheric-pressure plasma jet was generated by using a He flow of 20.4 L/min, a secondary gas flow (N_2) of 0.305 L/min, and an input power of approximately 4.8 W. This same gas treatment, without plasma power, was found to produce no influence on bacteria culturability. Plasma was used to impact, for different exposure times, *Chromobacterium violaceum* bacterial biofilms grown on 96-well microtiter plates [2], [3]. For imaging, bacteria cells were disaggregated from the biofilms by sonication and suspended to produce smears onto glass slides. Atomic-force-microscope (AFM) images were obtained in air in intermittent contact mode.

A series of AFM images in Fig. 1 show cells from biofilms treated with plasma for different exposure times. The top row of images (row A) displays $40 \times 40 - \mu m^2$-area scans of samples treated with plasma for 0 min (control) (column I), 5 min (column II), and 60 min (column III), respectively. At this resolution, a number of bacterial-cell clusters can be seen in the control image, with less recognizably intact clusters in subsequent 5- and 60-min-plasma-exposure images. On closer examination (at higher resolution) in the second row of images (row B), sequential changes in individual bacterial-cell morphology are revealed with progressive increase in plasma-exposure time. The leftmost image of row B shows the control sample to be a cluster of rod-shaped cells, between 1 and 3 μm in length, typical for the morphology of *C. violaceum*. In the middle image of row B, after 5-min plasma exposure, a similar cluster reveals mostly intact cells and a few cells exhibiting defects. For example, the top center cell in that image is devoid of a regular cell structure, and two cells at the bottom of the image are undersized. In the rightmost image of row B, after 60-min plasma exposure, no recognizably intact cells are present, but only bacterial-cell remnants or debris are found. Row C shows images of isolated bacterial cells at yet higher resolution. Distinct from the unaltered control cell of column I in row C, the image of the cell in column II, after 5-min plasma exposure, is that of two oval-shaped regions, a rougher smaller area oval above that of the smoother larger area oval. The relative roughness of the two regions was verified by examining image cross sections (not shown) and analyzing the standard deviation of the surface height. This feature is consistent with cells undergoing damage and was observed in a small percentage of the high-resolution 5-min-plasma-exposure scans. In column III of row C, after 60-min plasma exposure, bacterial-cell remnants are revealed to be extremely rough, and no intact smoother cells (or cell parts) are encountered. These results for the 60-min plasma-treated sample were universal in that no recognizable bacterial cells in any of the 60-min-plasma-exposure images were ever obtained, which were acquired over ten widely separated regions of the sample.

Combining the AFM results with data from several other analysis techniques including fluorescence microscopy, cell viability studies, and ATP and DNA assays [4], a more complete picture emerges and shows that the plasma-treatment process produces interactions that render bacterial cells nonculturable after short plasma-exposure times (e.g., 5 min), but with little change in cell morphology, whereas longer exposure times (e.g., 60 min) result in major cell damage. These findings demonstrate the utility of gas-discharge plasmas as an alternative sterilization method for bacterial biofilms.

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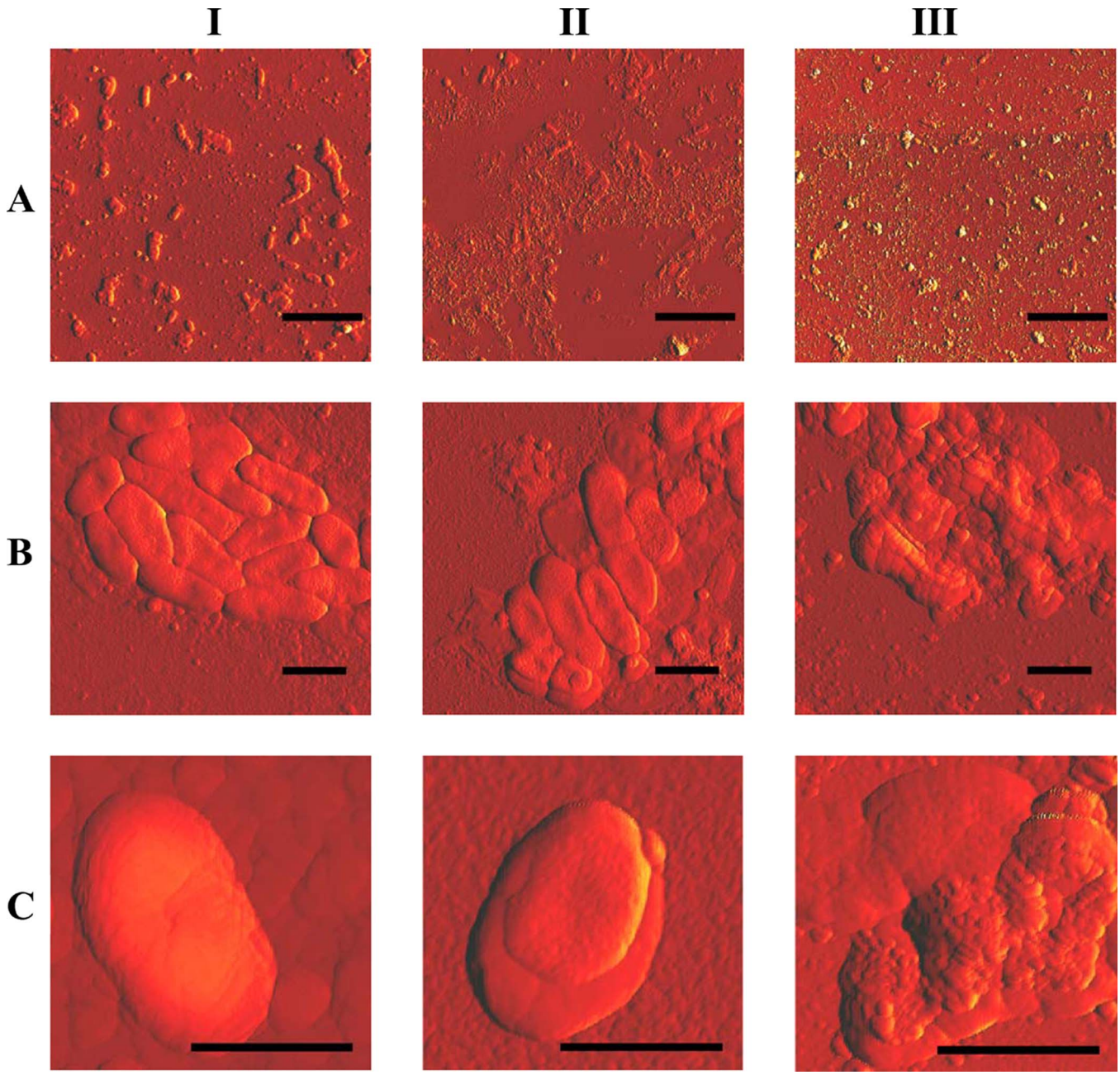

Fig. 1. AFM images of *C. violaceum* bacterial cells exposed to gas-discharge plasma for 0 min (column I), 5 min (column II), and 60 min (column III). Row A consists of $40 \times 40 - \mu m^2$-area scans (bar length, 10 μm), row B consists of $5 \times 5 - \mu m^2$-area scans (bar length, 1 μm), and row C consists of $2 \times 2 - \mu m^2$-area scans (bar length, 1 μm).

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