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BOOK OF ABSTRACTS

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Escherichia coli biofilm decontamination by air DC corona discharges

Zuzana Kovalova[1, 2], Magali Leroy[2], Emmanuel Odic[2], Zdenko Machala[1]

[1]*Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Mlynská dolina, 842 48 Bratislava, Slovakia*

[2]*GeePs | Group of electrical engineering – Paris, UMR CNRS 8507, CentraleSupélec, Univ Paris-Sud, Sorbonne Universités, UPMC Univ Paris 06, 3, 11 rue Joliot-Curie, Plateau de Moulon F-91192 Gif-sur-Yvette CEDEX, France*

A natural form of bacterial growth is in a biofilm. There, bacteria stick together and to the surfaces, protected and surrounded by self-produced extracellular polymeric substance. Protected from adverse conditions, bacteria are less susceptible to common antibiotics and chemotherapy as their planktonic counterparts. Biofilms forming on thermo-sensitive materials such as catheters, tissues, and wounds represent a high risk because they cannot be sterilized by established high temperature methods. Low-temperature plasma represents an alternative for thermo-sensitive surfaces. In our previous experiments in air we were able to decontaminate biofilms without damaging thermo-sensitive plastic or human tooth substrates [1, 2].

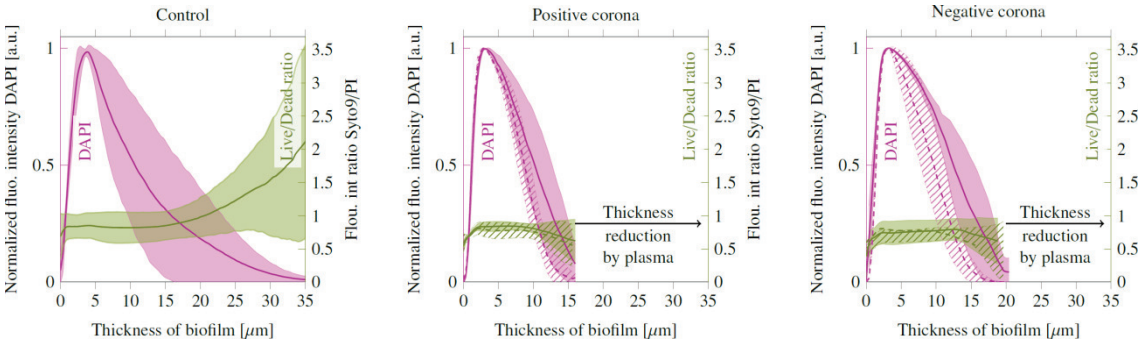

Fig.1: Biofilm fluorescence in confocal laser scanning microscope, dependence on distance from cover slide – bottom of the biofilm. Comparison between control and plasma exposed samples. Normalized fluorescent intensity of DAPI shows thickness of the biofilm (left axis), ratio of green (Syto9) and red (PI) corresponds to live/dead ratio (right axis). 10 min exposure – dashed line with hatched 95% confidence interval a 15 min exposure time – solid line and confidence interval.

Decontamination of 48 h-old *Escherichia coli* BW 25113 biofilm (static, 30°C) on cover glass (2x2cm) was performed by positive streamer and negative Trichel pulse coronas. The discharge chamber in air contains a sharp hypodermic injection needle as a high-voltage electrode opposite to a grounded copper plate at 0.5 cm distance on which samples were placed. Positive streamer corona was supplied with a DC high voltage of 10 kV and electric current pulses were formed with frequency 10-20 kHz. Negative Trichel pulses were supplied by 9 kV DC and current pulses frequency was 1 MHz. Biofilm was exposed to the discharge for 10 and 15 min. Biofilms were dried by dry air flow for 10 min before treatment. After direct plasma treatment biofilm was stained by fluorescent dyes - BacLight Live/Dead Invitrogen and DAPI. Biofilm stained by Syto 9 (green fluorescence – live bacteria), propidium iodide (PI, red fluorescence – bacteria with damaged membrane) and DAPI (blue fluorescence – all DNA in biofilm) was examined by the Confocal laser scanning microscope (OLYMPUS IX81).

After 10 or 15 min treatment in the plasma, the biofilm thickness was reduced significantly. Increase in red fluorescence (bacteria with damaged membranes) was also detected, especially for topmost layers of the biofilm. In comparison with the control biofilm, Live/Dead ratio remained constant for lower layers topmost layers were lost.

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