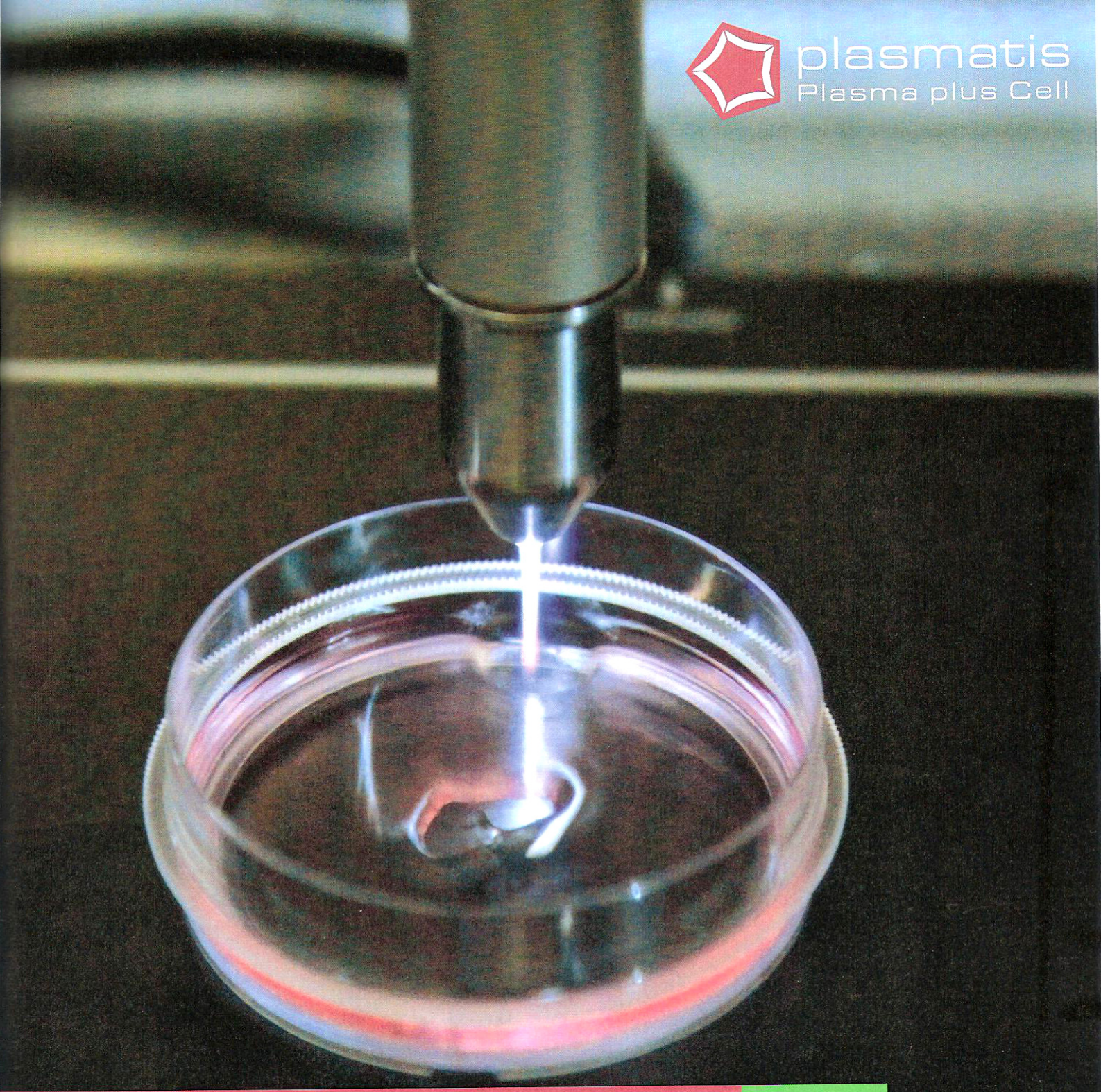

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18th to 20th September 2014

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Decontamination of *Escherichia coli* biofilm by DBD argon jet

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Abstract
Decontamination of bacteria in biofilms by common antibiotics and chemotherapy is difficult due to the higher resistance of biofilm bacterial cells in comparison with their planktonic counterparts. Special cases are biofilms forming on thermo sensitive materials such as catheters, wounds and tissues, because they cannot be sterilized by high temperatures. In our previous experiments, low-temperature plasma in air was able to decontaminate biofilms without damaging a thermo sensitive plastic or human tooth substrate [1,2].

Decontamination of 48 h-old *Escherichia coli* BW 25113 biofilm (static, 30°C) on cover glass (2×2cm) was performed by DBD argon jet in argon atmosphere (atmospheric pressure). Alternating high-voltage was connected to a blunt stainless-steel electrode (pk-pk 8 kV argon, 11.7 kV argon + water vapour, 30.2 kHz), placed in the axis of 10 cm long Pyrex glass tube. Two grounded copper strips connected with copper mesh were on the outside surface of the glass tube. Discharge was ignited between the HV electrode and grounded copper strips. Two slm of argon were flowing inside the tube of the jet or 760 ppm of water vapour was admixed to the argon flow. The biofilms were placed 1 cm from the jet nozzle and dried for 5 min by argon flow before treatment in plasma. After direct plasma treatment biofilm was either stained by fluorescent dies (BacLight Live/Dead *Invitrogen* and DAPI) or scraped into solution and resuspended. Resuspended biofilm was serially diluted, solution spread on agar plates and bacterial colonies were counted after 24 hours at 37°C. Biofilm stained by Syto 9 (green fluorescence – live bacteria), propidium iodide (red stain – bacteria with damaged membrane) and DAPI (blue fluorescence – all DNA in biofilm) was examined by the Confocal laser scanning microscope (*Leica TCS SP8X*).

The experiments on planktonic bacteria showed significant increase of biocidal effect while using argon with water vapour as working gas (4 logs reduction) in comparison to dry argon (2 logs) within 10 min from the initial load of 10^7 bacteria. So for the biofilm experiments, mostly argon with water vapour was used. 20 min biofilm exposure to the plasma caused 6 logs reduction from the initial load – obtained from cultivation of biofilm. Confocal microscopy of treated and untreated biofilm showed reduction in the thickness of biofilm by 2/3 after plasma treatment and increase of red fluorescence (Fig 1.)

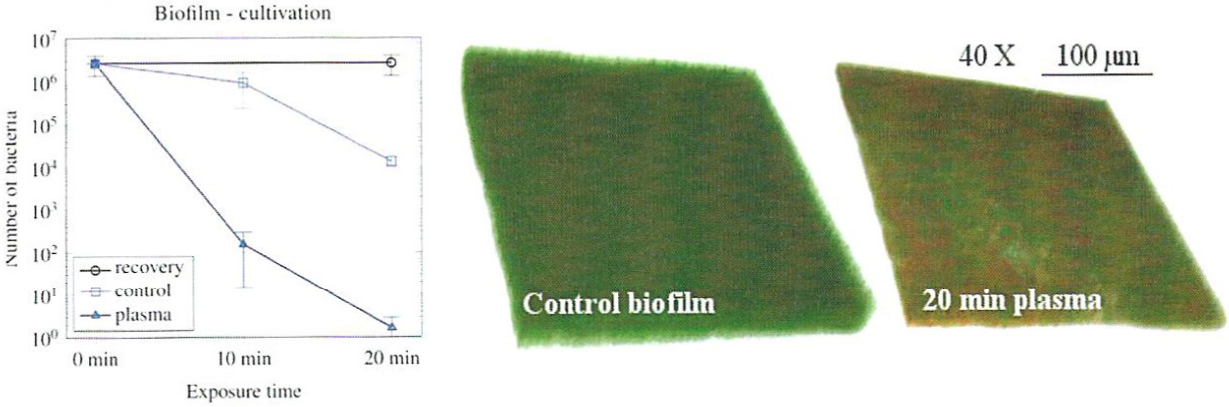

Figure 1.: Survival curve of biofilm cultivation after two different exposure times. 3D projection of biofilm fluorescence without treatment and after 20 min of plasma treatment.

Low-temperature plasma of DBD argon jet in argon atmosphere is able to reduce viability and thickness of biofilm.

Acknowledgement: This work was supported by French government scholarship and by Slovak Research and Development Agency APVV-0134-12.

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