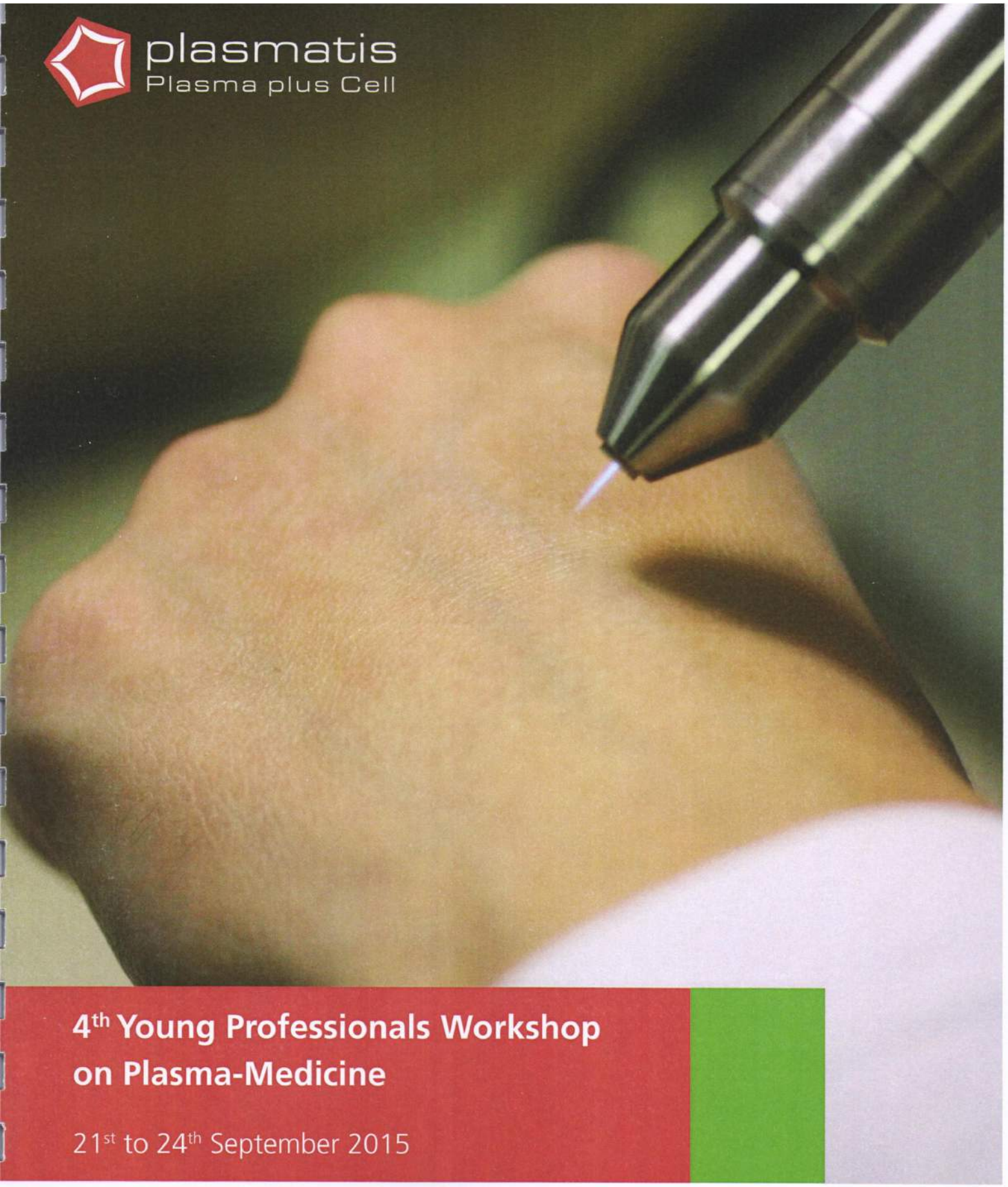
plasmatis
Plasma plus Cell



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Decontamination of *Escherichia coli* biofilm by positive and negative DC corona discharge in air

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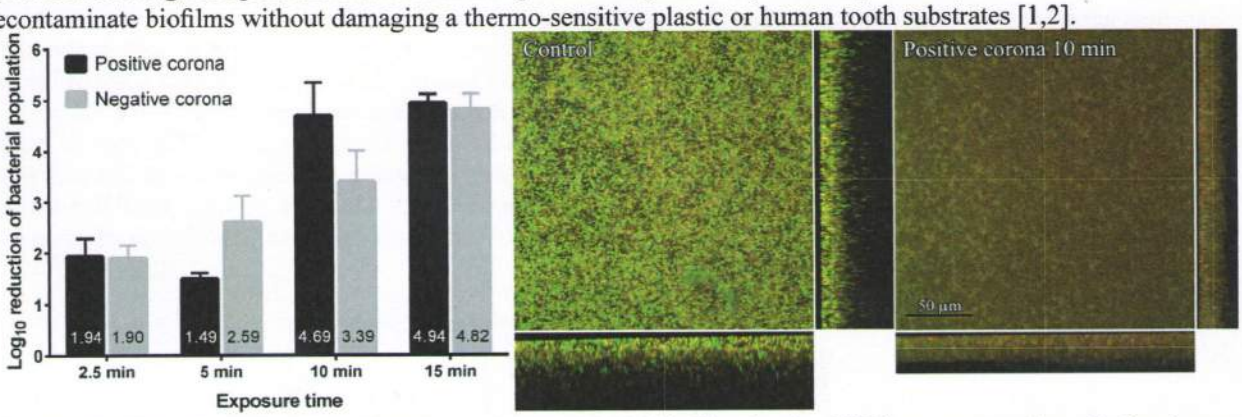
Abstract

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. Biofilms forming on thermo-sensitive materials such as catheters, wounds and tissues represent a high risk, because they cannot be sterilized by established high temperature methods. In our previous experiments, low-temperature plasma in air was able to decontaminate biofilms without damaging a thermo-sensitive plastic or human tooth substrates [1,2].



Decontamination of 48 h-old *Escherichia coli* BW 25113 biofilm (static, 30°C) on cover glass (2×2cm) 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. 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 2.5, 5, 10 and 15 min. Biofilms were dried by dry air flow for 10 min before treatment. After direct plasma treatment biofilm was either stained by fluorescent dyes (BacLight Live/Dead Invitrogen and DAPI) or scraped into solution and resuspended. The biofilm in a solution was serially diluted and 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 fluorescence – bacteria with damaged membrane) and DAPI (blue fluorescence – all DNA in biofilm) was examined by the Confocal laser scanning microscope (OLYMPUS IX81).

Figure 1. Left: the logarithmic reduction of the bacterial population in biofilm after treatment with plasma. Right: optical slice from the middle of the control and plasma treated biofilm (magnification 60×, width of one optical slice is 0.4 µm).

From initialy10^7 cultivable bacteria in biofilm, after 15 min of treatment, we were able to reduce the bacteria population by both positive and negative corona by almost 5 logs. The confocal fluorescent microscopy showed us that that red fluorescence in biofilm increased and cellular structure of biofilm became less visible after plasma treatment for either positive or negative corona: bacterial cell integrity was disrupted.

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References

[1] Z. Kovaľová, K. Tarabová, K. Hensel, Z. Machala, *Eur. Phys. J. Appl. Phys*, **61**, 24306 (2013)
[2] Z. Kovaľová, M. Zahoran, A. Zahoranová, Z. Machala, *J. Phys. D: Appl. Phys*.**47** (22), 224014 (2014)