METABOLIC ACTIVITY IN RELATION TO BACTERIAL VIABILITY INDUCED BY TRANSIENT SPARK DISCHARGE

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Transient spark discharge plasma generated in ambient air was applied to liquid solutions of planktonic *S. aureus* and *E. coli* bacteria circulating through the discharge zone. Metabolic activity and bacterial viability induced by direct plasma treatment in different types of liquids and bacterial growth phases were evaluated and correlated with concentrations of reactive species in liquids. Plasma treatment of *E. coli* / *S. aureus* in saline solution for 10 min resulted in a decrease in metabolic activity by 68 / 66% and bacterial population by 2.5 / 2.8 log, respectively.

1. Introduction

Multiple applications of cold atmospheric plasma (CAP) and plasma-activated water (PAW) in biomedicine are getting more attention in recent years. They require comprehensive and in-depth research of the complex mechanisms of the interaction of cold plasmas with biological systems at all levels. The understanding of basic mechanisms of plasma effects on living cells is one of the main preconditions to develop systematically innovative methods for bio-decontamination. One of the major factors that influences inactivation efficacy of CAP is the background environment (e.g. type of liquid) [1]. The liquid-phase processes have been identified to be the main key to determine detailed pathways of plasma-cell interactions. Liquids and bacterial suspensions after treatment by CAP become antimicrobial and these effects can be attributed to the generation of redox-active species (reactive oxygen and nitrogen species, RONS) [2]. Interestingly, the stable end-products in different plasma-treated liquids (non-buffered and buffered water or saline solutions, cell culture media) are more or less the same (hydrogen peroxide, nitrite and nitrate) which suggests general pathways of chemical reactions triggered by plasma [3][4]. Despite this, bactericidal effect varies depending on the type of a liquid [5]. The objective of this work was to investigate the effect of transient spark discharge plasma on planktonic *Escherichia coli* and *Staphylococcus aureus* in culture media, buffered and saline solutions and establish the correlation between their metabolic activity and viability.

2. Materials and methods

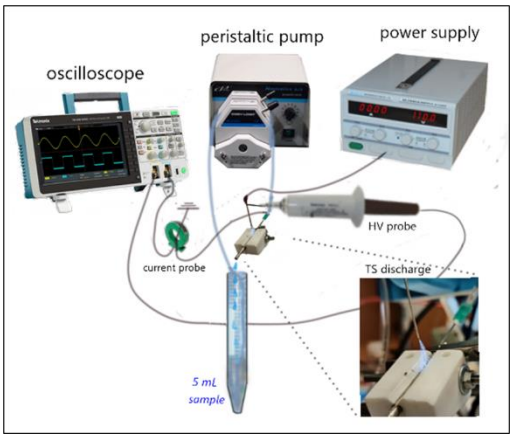

Fig. 1. Experimental Setup

Transient spark (TS) discharge (DC-driven self-pulsing repetitive streamer to spark transition discharge) was operated in ambient atmospheric pressure air in a direct contact with a bacterial suspension (volume = 5 mL initial $C_n=10^7$ CFU/mL) circulated by a peristaltic pump through the discharge plasma zone (Fig.1). The discharge was operated at the constant applied voltage ~14 kV and pulse repetition frequency ~ 2 kHz, and plasma treatment times 5 and 10 min.

The standard strains of Gram-positive *S. aureus* CCM 3953 and Gram-negative *E. coli* CCM 3954 bacteria were used and treated by the plasma during their stationary/exponential phase of growth. After exposition to plasma, changes in metabolic activity by MTT reduction assay and bactericidal effect by colony forming unit (CFU) enumeration method were analysed. All experiments were performed in three different solutions: culture media (Mueller Hinton Broth, MHB), saline solution (saline) and phosphate-buffered saline solution (PBS). In addition to biological analysis, the accumulation of RONS (nitrites NO_2^-, nitrates NO_3^-, hydrogen

peroxide H_2O_2) in the solutions was measured by UV-VIS absorption spectroscopy [6]. Changes in pH, electrical conductivity, temperature and ORP of treated liquids were monitoring during experiments, too.

3. Results

TS discharge resulted in an effective inactivation (up to 3 log reduction) of *S. aureus* and *E. coli* in physiological saline solution. On the other hand, it had almost no effect on the viability in PBS and culture media. Increasing plasma treatment time did not cause stronger bactericidal effect. Once the plasma was turned off the bactericidal effects continued and this delayed effect resulted in a complete inactivation of *E. coli* after 1 hour, and 5.05 log reduction of *S. aureus* after 2 hours incubation.
Metabolic activity was not in a correlation with viability of bacteria as it decreased with increasing treatment time for both *S. aureus* and *E. coli* (Fig. 2) in all three types of liquids. Trend of metabolic activity suppression in different growth phase of *E. coli* in saline solution was detected: in the stationary phase, activity was suppressed by 50% in 5 and 10 minutes, whereas in the exponential phase, 5 minutes resulted in 57% suppression, and 10 minutes in 68%.
Measurements of RONS showed concentration of H_2O_2 in deionized water, buffered and saline solutions increased with increasing treatment time. The maximum accumulation of NO_2^- was obtained in buffered solution. In deionized water and saline solution predominance of NO_3^- was observed.

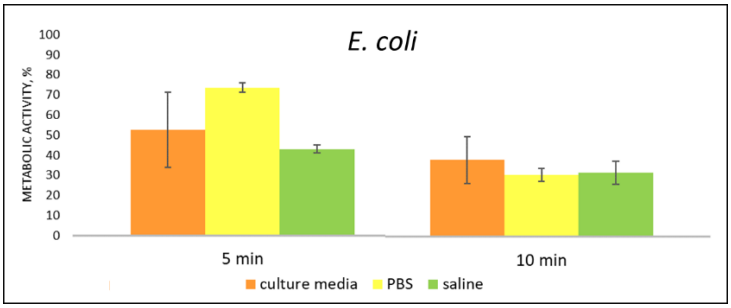

Fig. 2. Metabolic activity of *E. coli* after plasma treatment in culture media (Mueller Hinton Broth, MHB), PBS (phosphate-buffered saline) and saline solution (NaCl, 0.85%).

4. Conclusion

Reduced metabolic activity and the presence of RONS did not cause bactericidal effect against *E. coli* and *S. aureus* in PBS and culture media after TS discharge treatment. RONS in combination with acidic environment promoted bactericidal effect in saline solution. The findings prove that plasma treatment can result in a significant bactericidal efficacy that depends on type of a liquid and less on the bacteria growth phase. The mechanisms of specific action of RONS on individual components of bacteria (cell membrane, DNA, proteins) should be investigated thereafter. Study of CAP interaction with bacterial cells will allow us to obtain additional knowledge required to embody the full potential of cold plasma in biomedicine.

Acknowledgment

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5. References

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