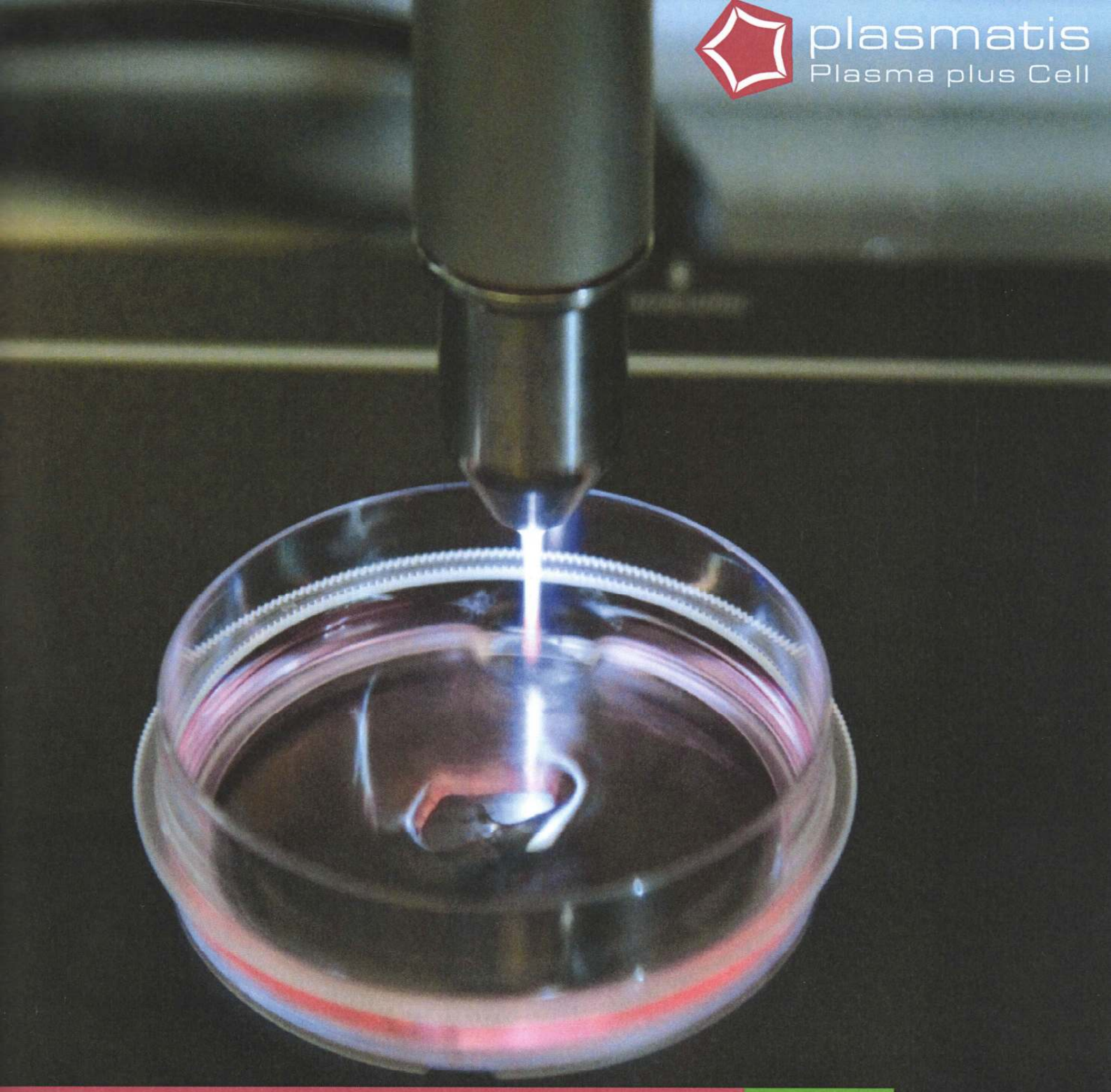

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Measurement of peroxynitrites in water solutions treated by cold transient spark

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Abstract

Plasmas generated in air in contact with water are of the great interest because they produce large quantities of reactive oxygen (ROS) and nitrogen species (RNS) and seem to be the most efficient in bio-decontamination by means of plasma. In [1] we investigated chemical effects in water solutions treated by DC-driven transient spark discharge generated in ambient air and linked them with bactericidal effects in treated bacterial suspensions. Aqueous solutions were electro-sprayed directly through the active zone of the transient spark. Changes of pH, electrolytic conductivity, the concentrations of hydrogen peroxide, peroxynitrites, nitrites and nitrates were measured in phosphate buffered and non-buffered solutions. Acidification and the presence of nitrites NO_2^- and nitrates NO_3^- result from dissolution of nitrogen oxides formed in air plasma by gas-phase reactions of dissociated N_2 and O_2. Under acidic conditions, NO_2^- may decay via reaction with H_2O_2 and proceed to formation of peroxynitrous acid (O=NOOH) or its conjugate base peroxynitrite ($O=NOO^-$), which subsequently decay into the final product NO_3^-. In our first qualitative experiments the relative amounts of peroxynitrites were measured by fluorescence spectroscopy. The concentrations of nitrites, nitrates and peroxides in the treated solutions were correlated with the changes of pH and bactericidal effects. The relative amounts of $ONOO^-$ measured in plasma treated solutions also correlated with the bactericidal efficacy. These measurements indicate an important role of peroxynitrites in bio-decontamination especially under acidic conditions [2]. To verify the peroxynitrites measurements by fluorescence spectroscopy, we checked the cross-reactivity of the used H_2DCFDA dye and the reliability of this assay by using specific scavengers of ROS and RNS.

Results

Aqueous solutions were treated in positive transient spark plasma system with electro-spraying of the treated solution directly through the active discharge zone. We worked with buffered solutions – Dulbeco's phosphate buffered saline (PBS), phosphate buffer without salt (PB) and PB with high salt concentration (1 M NaCl)). When working with scavengers, they were added to the sample after plasma treatment. The presence of peroxynitrites was measured by using a fluorescent dye 2, 7-dichlorodihydrofluorescein diacetate (H_2DCFDA). We investigated the cross-reactivity of the H_2DCFDA dye to various reactive oxygen species, especially hydrogen peroxide, OH radical and hypochlorite anion OCl^- without plasma treatment. We found out that mostly hypochlorite anions OCl^- are responsible for significant fluorescence response of the H_2DCFDA dye. However, this fluorescent response is still much lower than the response of $ONOO^-$. To separate their cross-reactivity, we used specific scavengers of reactive species: taurine for OCl^-, ebselene for $ONOO^-$, katalase for H_2O_2 and 5,5-dimethyl-1-pyrroline-N-oxid (DMPO) for OH radical. We confirmed that ~ 90% of the fluorescent signal of plasma treated solution was due to the signal of $ONOO^-$, OCl^- (PBS contains atomic chlorine) and autooxidation of the dye. Therefore, we could exclude strong influence of another ROS and RNS, e.g. H_2O_2, OH radical, NO_x. In plasma treated solutions without a chlorine content (PB) after addition of scavengers, we detected the signal formed only by peroxynitrites almost two times higher. We also expect that due to the fast decay of peroxynitrites (half life from several milliseconds to seconds), the real concentration of $ONOO^-$ created during the plasma treatment must have been higher than the measured fluorescence intensity. We also found that even in solutions with very high content of chlorine (PB with 1 M NaCl), peroxynitrites were responsible for the most of the fluorescence signal. In summary, we confirmed that fluorescent spectroscopic assay using H_2DCFDA dye is reliable and specific for peroxynitrites measurements.

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