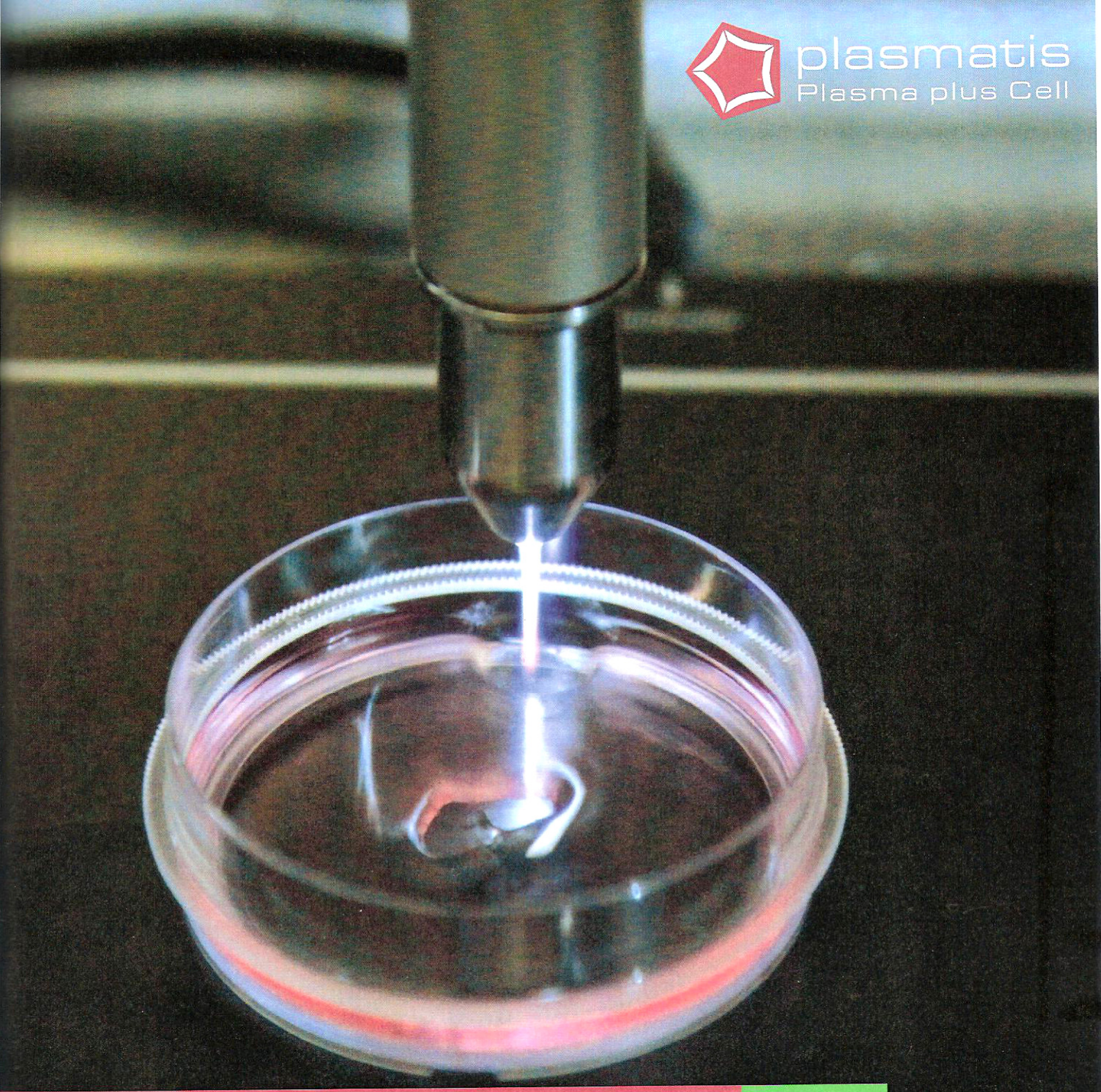

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Measurement of peroxynitrites in plasma treated water solutions

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Abstract

Plasmas generated in air and in contact with liquids generate a number of primary reactive species in the gas phase, which induce formation of secondary reactive species in the liquid phase through the gas-liquid interface. Reactive oxygen and nitrogen species (ROS/RNS) such as hydrogen peroxide H_2O_2, hydroxyl radical $^{\cdot}OH$, nitrites NO_2^-, nitrates NO_3^-, hypochlorite OCl^- and peroxynitrites $ONOO^-$ induce chemical changes in water solutions and various biocidal effects on microorganisms. According to many papers, key species responsible for biodecontamination in plasma treated water solutions or tissue and cell injuries *in vivo* may be peroxynitrites [1]. Peroxynitrites are powerful oxidants that can oxidize many cellular components. The reactivity of peroxynitrites is pH-dependent and *pKa* of $ONOO^-$/ONOOH system is 6.8. The detection of peroxynitrites in generally is difficult because of their high reactivity (very short life time from milliseconds to seconds).

In [2] we attempted to use for the first time a fluorescent dye 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA) to detect peroxynitrites in water solutions treated by transient spark discharge generated in ambient air at atmospheric pressure. Working solutions were differentiated according the content of Cl (phosphate buffered saline, sodium chloride solution), pH (acid pH 3.3 – 3.5, neutral pH 6.8 – 7.4), buffered (PBS, PB) or non-buffered (NaH_2PO_4, NaCl). Because of the possible cross-reactivities with other ROS (H_2O_2, OCl^-, $^{\cdot}OH$, $^{\cdot}O_2^-$) created in plasma treated liquids, we tried to verify the specificity of the 2,7-dichlorodihydrofluorescein diacetate to peroxynitrites. First, we checked the cross-reactivity of H_2DCFDA with various reactive species. The H_2DCFDA dye is the most sensitive to $ONOO^-$ and then less to OCl^-. Fluorescence response of other species like H_2O_2 is not significant. To distinguish which part of the signal is due to which ROS, we used two other fluorescent dyes and some ROS scavengers. We used aminophenyl fluorescein (APF) and hydroxyphenyl fluorescein (HPF). APF measurements with the strongest response to OCl^- showed that not so much OCl^- were formed in our plasma treated PBS samples as we expected. Time measurements of the fluorescent response of H_2DCFDA to $ONOO^-$ (by the bottle) and to treated PBS by transient spark discharge showed the similarity in the time developments. As scavengers, we used ebselen for $ONOO^-$, catalase for H_2O_2, taurine and hypotaurine for OCl^-. First, it seemed, that ebselen was the most specific scavenger for peroxynitrites. After addition of catalase into the treated sample, we observed a significant decrease of the fluorescence signal of H_2DCFDA. If catalase scavenges H_2O_2, which is one of the two key reactants for peroxynitrites formation:

$NO_2^- + H_2O_2 + H^+ \rightarrow O{=}NOOH + H_2O$ [3],

then the decrease of the signal is due to no peroxynitrites present in the treated sample.

We found out that the chemistry pathways at different pH took place in different ways. Because of the presence of different ROS in plasma treated water, the possible cross-reactivities and short half-lives make the detection of specific ROS difficult. Nevertheless, we demonstrated that at least in plasma treated solutions with no Cl presence and therefore no OCl^- created, the H_2DCFDA fluorescence signal is mostly due to peroxynitrites and so this fluorescent spectroscopic method can be use for their measurement under considering some limitations.

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