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Identification of RONS in water induced by air plasmas and their biomedical effects

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Recent advances of biomedical applications of non-thermal atmospheric pressure plasmas show that the biomedical effects are mostly due to reactive oxygen and nitrogen species (RONS) [1]. RONS are very important in biological systems, e.g. in antimicrobial strategies, immune response, or cell signaling pathways [1-3]. Identification of plasma-induced RONS in aqueous media and studying their effects on biomolecules and cell viability/functionality is one of the key directions in plasma medicine.

By controlling the dissipated power, air discharge plasmas can be operated to generate either primarily ozone (O_3) or nitrogen oxides (NO_x), among other species, such as $OH^\bullet$ radicals. O_3 transferred to the liquid, hydrogen peroxide (H_2O_2) formed dominantly from $OH^\bullet$ radicals, and nitrites (NO_2^-) and nitrates (NO_3^-) from NO_x dissolving in water are relatively long-lived RONS in aqueous solutions. They are commonly measured by colorimetric methods using indigo dye for O_3, $TiOSO_4$ reagent for H_2O_2, and Griess reagent for NO_2^-. Low concentrations of NO_3^-/NO_2^- can be precisely detected by ion chromatography [4], while high concentrations by direct UV-vis absorption in the liquid phase.

Unless the aqueous solution is buffered, air plasma treatment leads to its acidification. Under acidic conditions, depending on the actual pH, NO_2^- disproportionate to NO_3^-, both NO_2^- and O_3 react with H_2O_2, and dissolved O_3 naturally decays within minutes. Therefore, for the correct measurements of these RONS, the sampling time after plasma treatment is critical and immediate post-treatment stabilization is often needed to suppress their cross-interactions or decay.

Very short-lived RONS are also of great biomedical relevance. Peroxynitrites ($ONOO^-$/ONOOH) are formed mostly by the reaction of H_2O_2 and NO_2^- under acidic conditions [4-5], however, they quickly dissociate to $NO^\bullet+O_2^{-\bullet}$ (or $NO_2^\bullet+OH^\bullet$) [6]. Their diagnostics by UV absorption or fluorescence spectroscopy is tricky due to their short life time, overlapping absorption with other RNS, and fluorescent probes cross-reactivity with other RONS [4-5].

$OH^\bullet$ radicals are considered the most active ROS but with extremely short life time (~ns). Electron paramagnetic resonance (EPR) using spin traps was shown as a perspective method for their diagnostics, although it is difficult to distinguish the EPR spectra of $OH^\bullet$ and O_2^- [7]. Both $OH^\bullet$ and O_2^- can be transferred from the gas to the liquid and formed by the aqueous reactions, such as ONOOH/$ONOO^-$ acidic dissociation, or Fenton reaction with H_2O_2.

Electrochemical probes are often employed in biochemistry, even on the microscale probing the RONS directly inside a cell [8]. Their potential use for plasma treated liquids is to be explored, accounting for the redox processes of solvated electrons and ions. Besides the specificity of diagnostic methods and aqueous cross-interactions of the measured RONS, mass transfer of the gas-phase RONS into the solution and the roles of specific RONS in the biomedical effects should be considered.

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