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Supplementary Information

Formation of Reactive Nitrogen Species including Peroxynitrite in Physiological Buffer exposed to Cold Atmospheric Plasma

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EXPERIMENTAL PROTOCOL

2 mL of PBS solution at pH 12 (PBS 7.4 alkalinized with NaOH) were treated 10 minutes by He/1%N_2 CAP to maximize the concentration of peroxynitrite formed (see Figure 8 and Table 2). L-tyrosine (≥ 98%, Sigma Aldrich) was added (100 µM final) to the treated sample and the solution was acidified (pH 8 final) by the addition of phosphoric acid (85%, Sigma Aldrich) to shift the reactivity of peroxynitrite toward the formation of nitrogen dioxide NO_2°, which is the true nitrating species.
UV-visible absorption spectroscopy was used to detect the presence of 3-nitrotyrosine, which absorbs in the [250-300 nm] and [400-450 nm] regions at this pH [1-5]. Spectra were recorded (Cary 5000 spectrophotometer) at room temperature in the [250-500 nm] range using a 5 cm-path length quartz optical cell (Hellma).

As shown in Figure S1, peroxynitrite formed in alkaline solution vanishes in presence of L-tyrosine at pH 8, which leads to the appearance of absorption bands at 260-280 and 420-450 nm, fairly corresponding to nitrotyrosine. These results indeed support the hypothesis of peroxynitrite formation, according to its reactivity. However, since the concentration of peroxynitrite initially formed is a few tens of micromolars at most, the 3-nitrotyrosine signal (low absorbance) is weak; moreover L-tyrosine can react with other RONS,[6-8] not only ONOO-, which prevents from its use as a fully reliable probe since a variety of reactive nitrogen species are generated by CAPs.

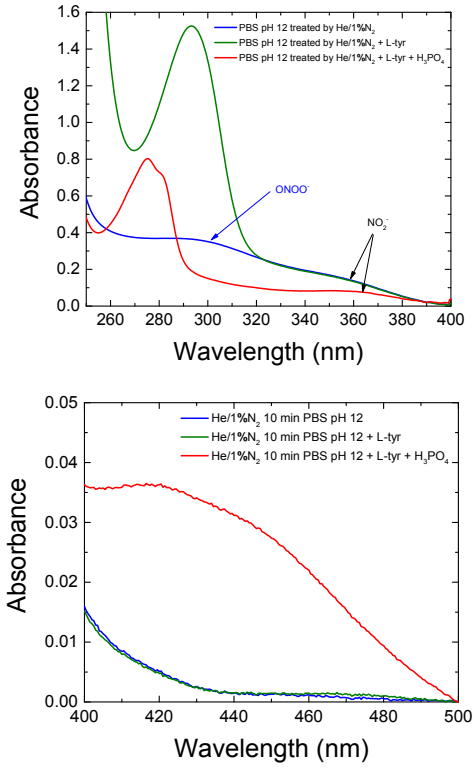

Figure S1. UV absorption spectra of PBS solutions (pH 12) treated by He/1%N_2 CAPs for 10 minutes, before and after the addition of L-tyrosine (100 µM), and followed by an acidification by phosphoric acid (pH 8, final).

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