**NO$_2^-$ and NO$_3^-$ enhance cold atmospheric plasma induced cancer cell death by generation of ONOO$^-$**

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Cold atmospheric plasma (CAP) is a rapidly developed technology that has been widely applied in biomedicine especially in cancer treatment[1]. Due to the generation of various active species in plasma, CAP could induce various tumor cells death and showed a promising potential in cancer therapy. By far it is considered that the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in plasma is the main factors[2]. Among these species, hydroxyl radical (OH), hydrogen peroxydide (H$_2$O$_2$), ozone (O$_3$), superoxide anion (O$_2^-$), nitric oxide (NO), and peroxynitrite anion (ONOO$^-$) are the main components related to biological effects induced by CAPs[3]. ONOO$^-$ is a potent oxidizing and nitrating specie formed from a diffusion-controlled reaction between O$_2^-$ and NO, which could penetrate bilayer lipid membrane and disturb the function of mitochondrion and consequently influence cell metabolism and cause DNA damage leading to cell death[4]. Our previous study also demonstrated the involvement of ONOO$^-$ in the induction of apoptosis by N$_2$/O$_2$ plasma jet[5]. To enhance the biological effects of gas plasma, changing the discharging parameters is the most commonly used method, yet increasing discharging power will lead to a higher possibility of simultaneously damage surrounding tissues.

In this study, by adding nontoxic concentration of additional nitrite and nitrate, we found that more nitrogen supplies benefited for the production of RNS especially ONOO$^-$ and resulted in a better killing effect to cancer cells. We found both NO$_2^-$ and NO$_3^-$ (at two different concentration, 10 µM and 50 µM) could significantly enhance cell viability reduction that was induced by He plasma in myeloma LP-1 cells and leukemia Molm-13 tumor cells. Furthermore, 50 µM of NO$_2^-$ and NO$_3^-$ showed a better synergistic effect with He plasma than that of 10 µM. As demonstrated in our previous study, NO and O$_2^-$ are two of the main substrate to produce ONOO$^-$, so we monitored intracellular and extracellular NO and O$_2^-$ level and found that He plasma treatment could significantly increase extracellular NO and O$_2^-$ level. Our results showed that NO$_2^-$ and NO$_3^-$ could enhance the cytotoxicity of He plasma treatment on myeloma and leukemia tumor cells by the accumulation of ONOO$^-$.

Enhance the killing effect by plasma jet treatment without changing the discharging conditions.

References

![Figure 1: Cytotoxicity of NaNO$_2$, NaNO$_3$ and their synergies with plasma on tumor cells. NO$_2^-$ and NO$_3^-$ could enhance the cytotoxicity of plasma treatment and higher concentration of NO$_2^-$ and NO$_3^-$ showed a better synergistic effect with plasma treatment.](image-url)