INVESTIGATION OF THE CHEMICAL STABILITY OF PLASMA-ACTIVATED SOLUTIONS

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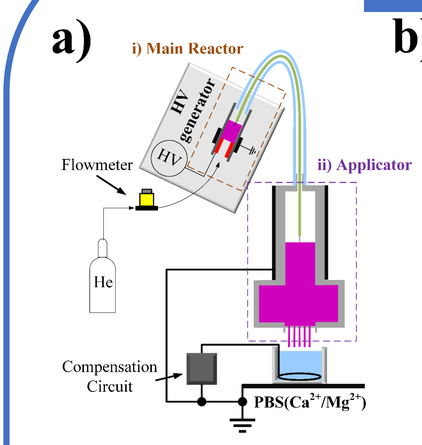
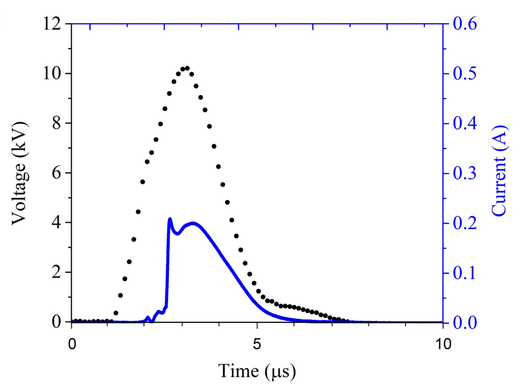
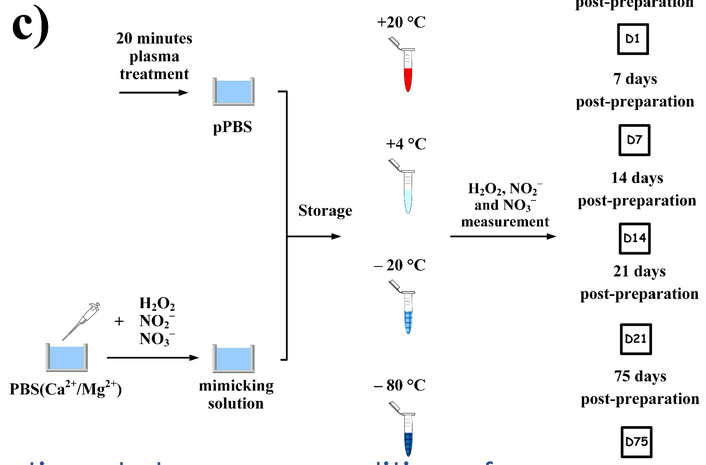
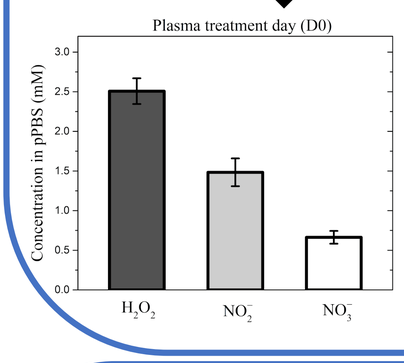
Experimental setup and Overview









Aim: Investigation of the pertinent storage conditions for plasma-treated PBS(Ca^{2+}/Mg^{2+}), pPBS, to retain its cytotoxic characteristics that correspond to the synergistic effect of H_2O_2, NO_2^-, NO_3^- and the pH of the solution [1]

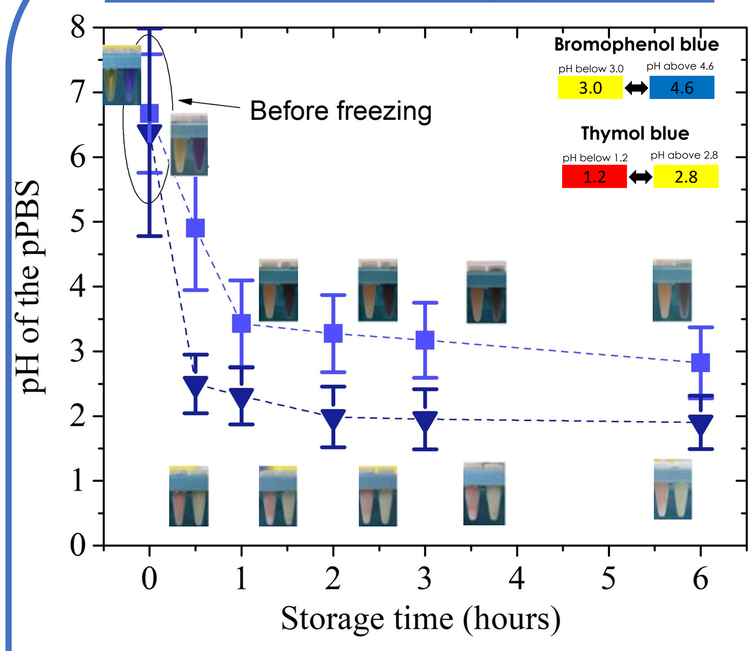
- ✓ Which storage conditions allow the concentration of these reactive species to be preserved?
- ✓ Do the results of these storage conditions correlate with the pPBS anti-cancer effects?

Theoretical approach [2]

$$-\frac{d[H_2O_2]}{dt} = -\frac{d[NO_2^-]}{dt} = +\frac{d[NO_3^-]}{dt} = +k[H^+][H_2O_2][NO_2^-]$$

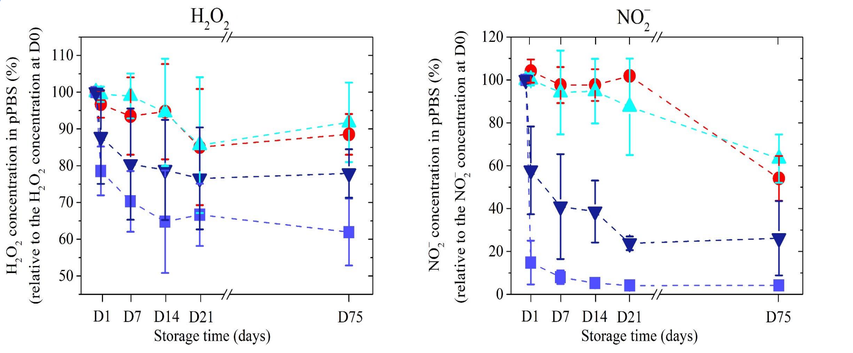
$$H_2O_2 + NO_2^- + H^+ \rightarrow NO_3^- + H^+ + H_2O$$

Experimental approach



Freezing of the solution (at **-20 or -80 °C**) results in its temporary acidification (when in frozen state)

Plasma-treated solutions

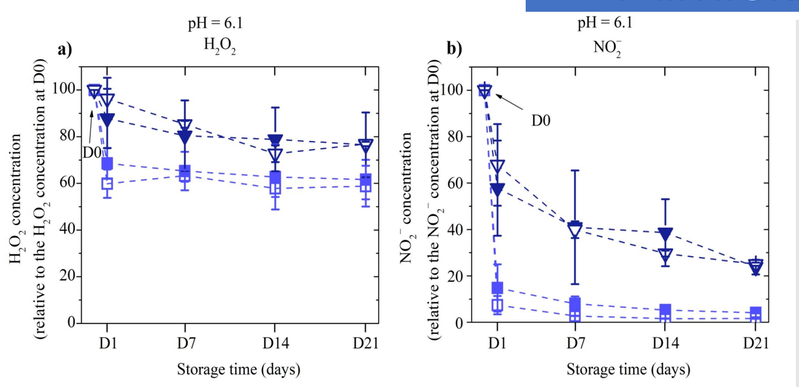


The **pH** of all the solutions (when in liquid form) remain relatively **stable** (max SD = ± 0.41) after 75 days of storage independently of the storage temperature

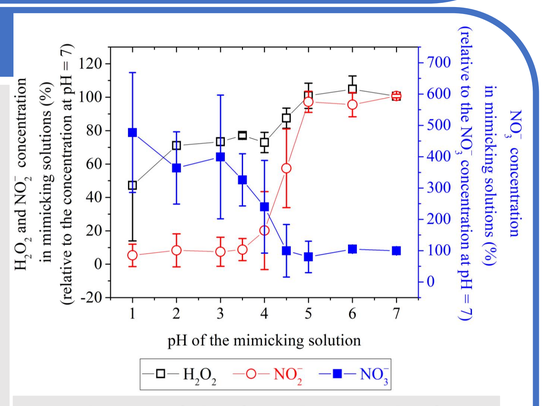
- ❖ pPBS is chemically **stable** when stored at **+4 and +20 °C**
- ❖ At **-20 and -80 °C**, the concentration of **H_2O_2 and NO_2^- decreases** while the concentration of **NO_3^- increases**

Hypothesis: when pPBS is frozen, H_2O_2 oxidizes NO_2^- converting it to NO_3^-

Mimicking solutions

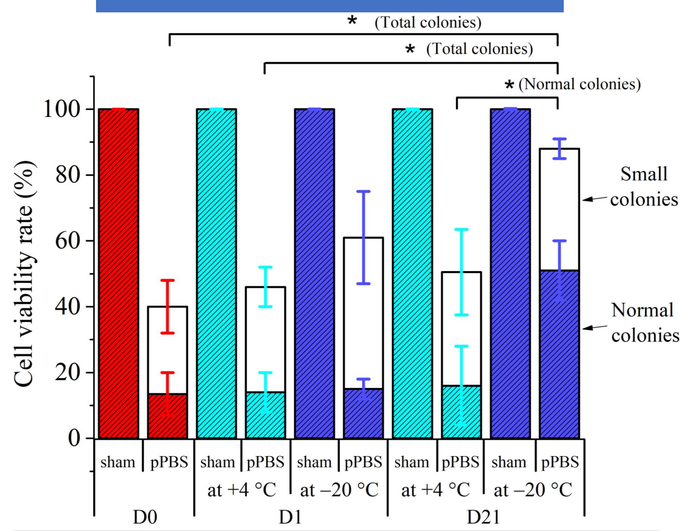


Mimicking solutions containing only **H_2O_2, NO_2^-, NO_3^- and acidic pH** (6.15) behave similarly to pPBS in terms of the time evolution of the concentration of the reactive species



NO_2^- oxidation by H_2O_2 in acidic environment

Application on cancer cells



pPBS preserves its cytotoxic effect for 21 days when stored at +4 °C but not when stored at -20 °C

Conclusions & Perspectives

- ❖ **H_2O_2, NO_2^- and NO_3^- concentrations in pPBS are**:
1. **Stable** when stored at **+4 or +20 °C** for at least **21 days**
2. **Not stable** when stored at **-20 or – 80 °C** even after the first day of storage
- ❖ The **variation** of the **reactive species concentrations** (decrease for H_2O_2 and NO_2^-, and increase for NO_3^-) is **more significant** when the solution is stored **at – 20 °C than at – 80 °C**
- ❖ **pPBS preserves its cytotoxic characteristics, *in vitro***, when stored **at +4 °C but not** when stored **at – 20 °C**

- ➢ Other storage conditions, such as light exposure and packaging, should be appraised
- ➢ Investigate the mechanisms that lead to the differences between storage at -20 and -80 °C

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References:
1. Sklias, K., Santos Sousa, J. & Girard, P.-M. Role of short- and long-lived reactive species on the selectivity and anti-cancer action of plasma treatment in vitro, *Cancers* **13**, 615 (2021)
2. Anbar, M. & Taube, H., Interaction of Nitrous Acid with Hydrogen Peroxide and with Water, *J. Am. Chem. Soc.* **76**, 6243 (1954)

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