Fluorescein-agarose gel as a tissue model to visualize and measure local CAP-induced acidification

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Abstract: Cold Atmospheric Plasmas (CAPs) have been shown to lower the pH of the treated target. CAP-induced acidification can have beneficial effects on the treated tissue (e.g. disinfection and healing). Conversely a tissue hyper acidification can lead to permanent damages with dramatic consequences. Before starting *in vivo* plasma treatments, preliminary tests should be carried in order to tune the acidifying effect induced by CAP exposition. Here we propose a tissue model that can be a useful tool to visualize and measure pH changes induced by plasma treatment.

Keywords: agarose gel model, pH measurement, fluorescein, plasma jet

1. Introduction

Once in contact with the atmosphere and water, Cold Atmospheric Plasmas (CAPs) initiate complex chemical reactions that lead to Reactive Oxygen and Nitrogen Species (RONS) generation. The produced RONS are mostly responsible for the biological effects observed during and after CAP treatment. Besides their oxidative effect, some CAP-generated species possess also an acidic character. The acidifying effect induced by CAP on treated liquids is widely known [1, 2]. While measuring the pH drop in liquids is relatively easy, the evaluation of the pH on solid surface is more complex. Flat pH-meters permit today to measure the pH on solid surfaces and organs like the skin. However, once applied to the solid target, these flat pH-meters give an average value of the pH on the covered surface. The relatively large surface of the pH-meter electrode does not permit to appreciate pH distribution, especially during a focalized CAP treatment where RONS are concentrated on a small surface. In this work we took advantage of the fluorescein fluorophore whose fluorescence is pH sensitive [3-5] to study the CAP induced acidification in agarose tissue models.

2. Method

In this work we used a modified protocol of our recently published method [6]. Briefly a buffered physiological solution (130mM NaCl, 5mM KCl, 2mM $MgCl_2$, 5.5mM HEPES) was prepared at 6 different pH ranging from 7.4 to 2.5. Fluorescein sodium salt (50µM) and DNA grade agarose (0.02g/ml) were added to each solution and then agarose was gently melted in a microwave. Gels at different pH were poured in 6-well plates in order to obtain a 2 mm thick gel/well. Gels were let to solidify and then kept at 4°C. Before plasma treatment plates were allowed to equilibrate at room temperature. Gel fluorescence was then imaged using the Typhoon™ FLA 9500 biomolecular imager (GE Healthcare Life Science) using an excitation laser at 473 nm and a Y520 emission filter with a wavelength range ⩾ 520 nm. Immediately after the scan, the 6-well plate was exposed to CAP treatments. We treated the gel at initial pH of 7.4 (as generic organ model) and the gel at initial pH of 5.5 (as skin model). Plasma treatment was performed using a Plasma Gun [7] with a negative polarity high voltage pulses having a peak amplitude of −14 kV, full width at half maximum of 2 µs duration. Gels were exposed for 5 min to the thin jet produced at the tip of a thin tapered capillary (1.5 mm internal diameter). Helium was used as feeding gas at a flow rate of 0.5 SLM. After the treatment, the plate was scanned again to image the changes in the fluorescence induced by CAP exposure. 8-bit grey scale images were imported in ImageJ for data analysis. The scan of the 6-well plate before CAP treatment was used to calibrate the fluorescence signal vs the 6 known pH. A calibration curve was thus obtained and used to measure the CAP-induced pH drop. To better visualize the pH variations, grey scale images were converted in

spectrum pseudocolor in ImageJ. pH analysis on the plasma treated surface was done by tracing a straight line passing by the middle of the acidified area and measured values were plotted in a graph (figure 2).

3. Results

Immediately after the CAP treatment, acidified area appears as a darker spot on the green agarose gel. Fluorescein fluorescence is gradually quenched when the pH in the medium decreases. To better visualize the CAP-induced acidification, the 8-bit grey scale images were converted in pseudocolors (figure 1). The spatial pH distribution, measured by tracing a straight line passing through the CAP-exposed surface showed a pH decrease of one unit in the generic organ model, the generic organ model and a slightly higher decrease in the skin model (figure 2). The lower acidification observed in the skin model could be attributed to the HEPES buffering activity having its optimum in the pH range between 8.2 and 6.8.

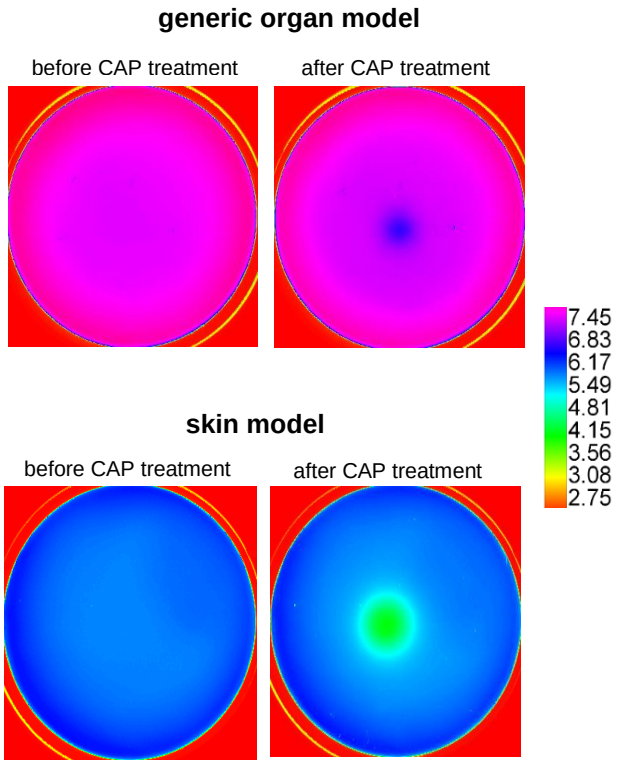

Figure 1. Visualization of the CAP-induced acidification on the fluorescent agarose gel models. To better visualize the acidified area, 8-bit images were converted in spectrum pseudocolor in ImageJ

4. Conclusions

In this work we propose a novel technique useful to evaluate and measure the CAP-induced acidification on agarose-gel tissue models. Agarose-gels with initial pH of 7.4 or 5.5, mimicking respectively a generic organ or the outer skin pH, were used to evaluate the acidifying effect of plasma treatment. These models can be useful during CAP treatment planning, especially when a focalized treatment should be administered. Controlling the local induced acidification can help to avoid hyper-acidification that can cause chemical burn on the CAP exposed tissue.

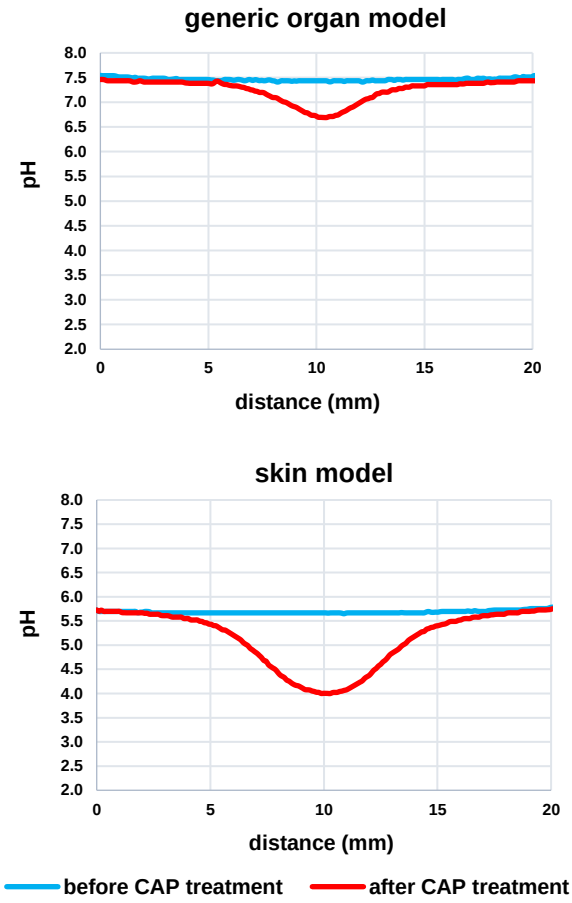

Figure 2. Plotted graphs of pH measured along a manually traced straight line passing through the CAP-exposed surface

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6. References

[1] Brisset J.-L., et al., Acidity control of plasma-chemical oxidation: applications to dye removal, urban waste abatement and microbial inactivation. *Plasma Sources Sci. Technol*. 20 034021 (2011).

[2] Oehmigen, K., et al., The Role of Acidification for Antimicrobial Activity of Atmospheric Pressure Plasma in Liquids. *Plasma Processes and Polymers*, 7(3- 4): p. 250-257. (2010)

[3] Emmart, E.W., Observations on the absorption spectra of fluorescein, fluorescein derivatives and conjugates. *Archives of Biochemistry and Biophysics*, 73(1): p. 1-8. (1958)

[4] Leonhardt, H., L. Gordon, and R. Livingston, Acid-base equilibriums of fluorescein and 2',7'-dichlorofluorescein in their ground and fluorescent states. *The Journal of Physical Chemistry*, 75(2): p. 245-249. (1971)

[5] Martin, H., et al., Sodium fluorescein as a retinal pH indicator? *Physiological Measurement*, 26(4): p. N9. (2005)

[6] Busco G., et al, Cold atmospheric plasma-induced acidification of tissue surface: visualization and quantification using agarose gel models. *J. Phys. D: Appl. Phys.* 52 24LT01 (2019)

[7] Robert, E., et al., Characterization of pulsed atmospheric-pressure plasma streams (PAPS) generated by a plasma gun. *Plasma Sources Science and Technology*, 2012. 21(3): p. 034017.