How much is “enough”? – strategies to monitor plasma-bio interactions for plasma endpoint detection

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Abstract: We investigated strategies to monitor plasma-bio interactions in real-time and *in situ* to provide input data for plasma endpoint detection. Different bio-electrochemical sensors were tested for feasibility in de-ionized water, buffer solutions, cell culture medium with and without cells, and finally in a murine wound model *in vivo*. The results indicate that bio-electrochemical sensors are a suitable strategy for plasma endpoint detection.

1. Introduction

Plasma medicine offers treatment alternatives for diseases from wound healing [1] to cancer treatment [2] and viral infections [3]. There has been no consensus reached for a definition of a “plasma dose” and current treatments rely on empirical observations. Furthermore, the extent of clinical efficacy cannot be evaluated until several days after treatment. Only a few methods have been successfully used for real-time plasma control (e.g., temperature measurements, deposited power, and optical emission spectroscopy) [4,5]. Here, we report a proof-of-concept for plasma endpoint detection based on *in situ* real-time measurements *in vitro* and *in vivo*. We established real-time detection methods enabling safe and effective treatment regimens useful for medical cold atmospheric plasma (CAP) applications.

2. Methods

A microsecond pulsed volume dielectric barrier discharge (DBD) is used [6]. A copper electrode is covered with a 2 mm thick Al_2O_3 layer serving as dielectric. The DBD is powered by a self-built power supply controlled by a microcontroller. The power supply can be operated up to 25 kV_{pp} with pulse frequencies up to 1 kHz. The studies presented here were performed at 300 Hz. A variety of bio-electrochemical sensors (ZPS WIR-000-00163, Zimmer & Peacock, Norway) were tested for feasibility to perform real-time measurements *in situ.* The bio-electrochemical sensors were attached to the microcontroller which was used to either perform open-circuit potentiometry or cyclic voltammetry, depending on the analyte to be detected. In conjunction with low-pass filters, real-time measurements of the small current and voltages were enabled.

3. Results and Discussion

Figure 1 shows an example of the plasma endpoint detection when using H_2O_2 as analyte of choice. The controller turns off the plasma device once a set target concentration is reached. The results depicted are a proof-of-concept in phosphate-buffered saline (PBS) with an arbitrarily chosen endpoint of 0.175 V, corresponding to 39.8 µM. *In vitro* tests with a scratch assay in keratinocytes have shown a dose-dependent increase in H_2O_2 as well as oxidation-reduction potential (ORP), correlating with an increase in mitochondrial superoxide production. In a murine wound model, similar trends in real-time and *in situ* H_2O_2 and ORP measurements were observed. The *in vitro* and *in vivo* results demonstrate the feasibility of the real-time measurements to monitor plasma-bio interactions.

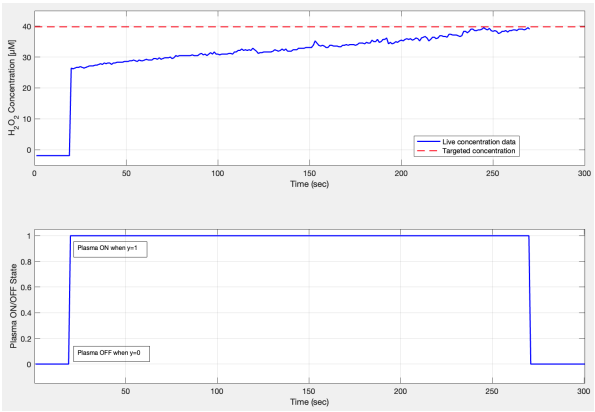

Fig. 1. Plasma Endpoint Detection. The controller turns off the plasma device once a set concentration (here 0.175 V = 39.8 µM of H_2O_2, dashed line) is reached.

4. Conclusion

Bio-electrochemical sensors to measure H_2O_2 and ORP can be a strategy to monitor plasma-bio interactions for plasma endpoint detection. The sensors can be used *in situ* and for real-time measurements, allowing to control CAP delivery for medical applications such as wound healing.

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