Physical mechanisms of plasma assisted wound healing: Production and delivery of active species

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Résumé

The physical mechanisms of plasma assisted wound healing are discussed. Experimental data on plasma production of reactive species and depth of penetration into tissue (in vitro and ex vivo) are presented.

Introduction

In recent years, the range of atmospheric pressure applications for medical and biological purposes is growing fast, and a new field of Plasma Medicine was formed [1-2]. Various types of plasmas, both thermal and non-thermal, are now widely studied for the purposes of blood coagulation, sterilization of living tissues, treatment of various wounds and burns, gastroenterological diseases, and even cancer. This opens up new horizons in both medical and physical sciences, as well as in biomedical and electrical engineering.

Cold atmospheric pressure plasma discharges have been shown to be effective when applied for sterilization and decontamination purposes, wound healing, blood coagulation, and other relevant medical and biological applications. Action of specific charged or neutral active species or radiation is frequently associated with the corresponding specific effect (e.g., anti-inflammatory effect of nitric oxide (NO), and highly oxidative hydroxyl radical and other reactive oxygen species (ROS)). Here we report the results on measurement of simultaneous production of anti-oxidant NO together with oxidative ROS in liquid media and their delivery into agarose gel and tissues by microsecond atmospheric pressure spark discharge.

Materials and methods

In our study we have used DC spark discharge plasma in a pin-to-hole electrode configuration (PHD plasma - Figure 1), previously described in [3] and [4]. The discharge was ignited by applying high positive potential with magnitude of about 4 kV to the central electrode. This resulted in a formation of dense energetic discharge which exists for about 35 μs with average energy of about 1.8 J per pulse.

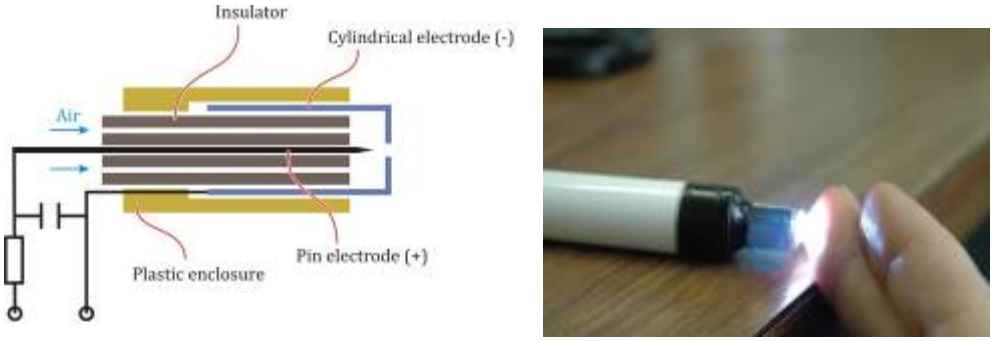

Fig. 1: General schematic of the Pin-to-Hole spark Discharge (PHD) plasma system and a photograph of the discharge in operation.

Measurements of hydrogen peroxide (H_2O_2), and nitric oxide (NO) produced by plasma in phosphate buffered saline (PBS) were done using fluorescent dyes, Amplex UltraRed reagent (Invitrogen), DAF-2 (Cayman Chemical) respectively according to manufacturers' protocols. Superoxide (O_2^-) was measured indirectly by adding superoxide dismutase (Fisher Scientific) into PBS solution containing Amplex UltraRed reagent before the plasma treatment. Fluorescence was measured using an LS55 (Perkin Elmer) fluorescent spectrometer equipped with well plate reader accessory.

Measurements of H_2O_2 and pH penetration into agarose gels (0.6%, 1.5%, 5% of agar) and tissues were done using the same fluorescent dyes. In the case of H_2O_2, the dye was placed in between 1 mm thick agar

slices and incubated for about 15 minutes before the treatment in order to provide presence of the dye in the agar volume; for the pH measurement, the agar gel was prepared by adding fluorescein (Sigma) dye before its solidifying. In order to measure the H_2O_2 and pH in tissue, the dyes were inject using a syringe into a skinless chicken breast tissue to the depth of about 1-2 mm.

Results

On Figure 2 the results of plasma production of H_2O_2 and NO in PBS solution are shown. The results of H_2O_2 and pH measurements in agarose gels and tissues are shown on Figure 3.

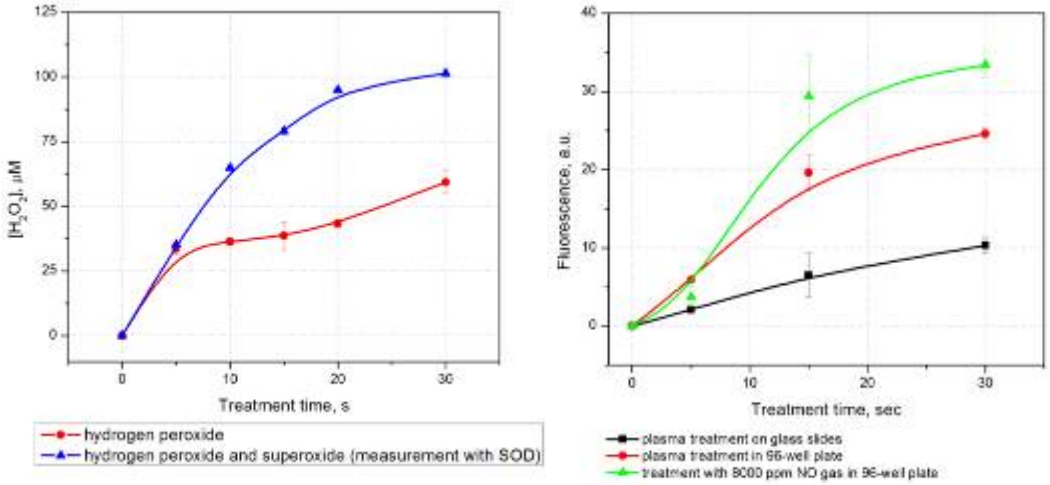

Fig. 2: Pin-to-Hole spark Discharge (PHD) production of H_2O_2 (left) and NO (right) in PBS.

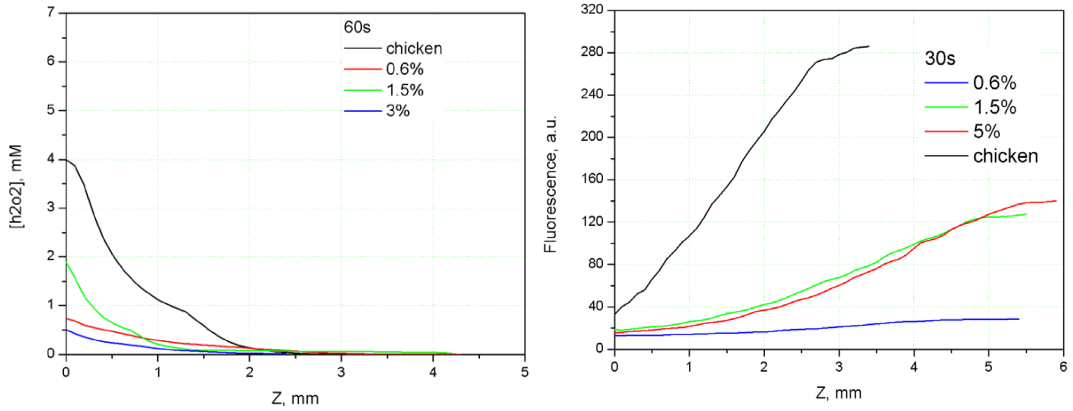

Fig. 3: The profiles of H_2O_2 (left) and pH (right) in agarose gels and tissue after the plasma treatment.

The results show that PHD discharge effectively produces both ROS and RNS species in the treated media, and these species may be delivered into the tissues to the depths of several mm, therefore providing not only surface effects (inactivation of pathogens, first of all), but also therapeutic effects inside of treated tissues.

References

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