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Plasmas for disinfection and healing: an investigation of the underlying mechanisms

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Plasma medicine is an emerging discipline related to the interaction of low-power, atmospheric pressure plasmas with living matter for therapeutic purposes. Initiated by seminal work on disinfection of living tissues, it has evolved into a multi-line research field which is concerned with the interaction of plasma with living matter, both in the form of pathogens and of eukaryotic cells composing the human body. This interaction is mostly due to reactive oxygen and nitrogen radicals produced within the plasma, with possible additional effects given by charged species, electric field and UV emission. Among the applications of plasma medicine, the most developed ones are probably disinfection, where the plasma action is used to get rid of bacteria, fungi and viruses, especially on living tissues, and wound healing, where the plasma constructively interacts with the cell processes, leading to a qualitative improvement and speed up of the healing process. The two applications clearly have a synergic effect in real world applications.

The plasma sources used in plasma medicine are required to operate at low power levels, so as to keep the neutral gas at or near room temperature. This in turn requires a mechanism to extinguish the current prior to arc onset. The two main strategies adopted are either the use of radiofrequency (RF) voltage or the dielectric barrier discharge (DBD) scheme. In this contribution we describe results obtained using a RF plasma source, originally conceived for the treatment of corneal infections [1]. In this source a helium plasma is generated by a high voltage (of the order of 1 kVpp) at radiofrequency (around 5 MHz), applied between two parallel brass grids serving as electrodes. The grids close the ends of two coaxial tubes, the innermost of which carries a helium flow of the order of 2 L/min. The high voltage is applied to the inner grid, whereas the outer one is grounded, granting a high level of electrical safety, even in case of accidental contact with the treated surface. The helium plasma produced between the two grids contains traces of air coming from outside the source, and this drives the production of reactive oxygen and nitrogen species, which are responsible of the observed biological effects.

A thorough study has been performed about the effectiveness of the device in disinfection [2]. Exposure to the afterglow originating from the plasma source successfully inactivated a broad range of microbes, namely Gram-negative and -positive bacteria, fungi, and replicating virus. The plasma treatment reduced the number of colonies with an effect that was related to the treatment time. The plasma treatment was equally effective in inactivating antibiotic-resistant bacteria, such as MRSA. The plasma antibacterial effect increased in association with antibiotics and did not diminish over repeated exposures, suggesting no development of bacterial resistance.

The effectiveness of the plasma treatment applied by the RF source in accelerating wound healing was tested in vitro by studying the activation of fibroblasts, the cells active in the healing process. The tests were performed on cultured human fibroblast-like primary cells. The plasma treatment was found to induce the formation of reactive oxygen species (ROS) in cultured cells. The ROS generated by the plasma treatment increased the expression of a receptor that modulates the inflammatory responses. Plasma exposure promoted wound healing in an *in vitro* model and induced fibroblast migration and proliferation, which were found to be ROS-dependent.

Following these results, an *in vivo* study on animal models was set up. 4×4 cm square wounds were performed under general anesthesia and analgesia on the back of six sheep [3]. The lesions were used to analyze the effects of five different treatments, one of which was the RF plasma, which was applied daily for 2 minutes. The study revealed that the plasma-treated wounds were faster to heal than the control, untreated ones. A strong bactericidal effect of the plasma treatment was evidenced. Intracellular ROS production driven by the plasma was confirmed also in vivo. The plasma treatment was effective in stimulating cell proliferation, led to an anticipated induction of blood vessel formation and gave an anti-inflammatory effect. Concerning skin regeneration, a significantly stronger development of cutaneous annexes (such as hair glands and follicles) was found in the plasma-treated case than in the control case.

As a next step, a new study was designed, aiming at testing an innovative healing approach, intended to combine, for the first time, mesenchymal stem cells (MSCs) and atmospheric pressure cold plasma [4]. Based on clinical, histopathological, and molecular results a synergistic action of the two techniques in sheep was observed. Experimental wounds treated with cold plasma and MSCs showed a slower but more effective healing compared to the single treatment. The combined treatment improved the correct development of skin appendages and structural proteins of the dermis, showing the potential of the dual combination as a safe and effective tool for skin regeneration.

References

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