

In vitro and in vivo effects of helium cold plasma on wound healing

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Atmospheric pressure cold plasma (APCP) is a novel tool for tissue disinfection that has also positive effects on wound healing. We recently reported that two minutes of APCP, generated through the ionization of helium gas, exerted an anti-microbial effect without significant damage to cells and tissues (1), effects that may induce also fibroblast proliferation (2). The aim of the present study was to ascertain the molecular changes induced by 2min APCP *in vitro* and *in vivo* using human dermal fibroblasts obtained from four different donors and an *in vivo* mouse skin wound healing model.

Scratch wound healing assay on dermal fibroblasts exposed for two min to APCP showed an improved cell migration, whereas RT-PCR analysis revealed an increased expression of cytokines and growth factors involved in wound healing, such as IL6, FGF2 and TNF α . The *in vitro* effects of APCP exposure on dermal fibroblast viability demonstrated that two min of plasma application did not cause changes in the viability of two cell preparations but induced a significant increase in viability of fibroblasts from the other two donors, when compared to the untreated control ($p < 0.05$). Apoptosis was not altered when analyzed by the TUNEL test in any dermal fibroblast cultures. The *in vivo* wound healing model confirmed that APCP improved tissue regeneration in mice. Expression of inflammatory cells in both control and treated tissues were higher at three days after treatment, suggesting an increased immune defense in the early phases of wound healing. Moreover, a reduction of collagen I staining at 12 days after wounding was observed, compared to both 3 and 9 days after wounding. At 12 days, a complete attachment of regenerated skin was demonstrated by increased staining for laminin, compared to day 9. Furthermore, at 12 days from APCP treatment, we observed a thicker epidermal layer after H&E staining.

In conclusion, our *in vitro* and *in vivo* results showed that APCP maintains or increases cell viability and migration and induces a transitory alteration of the expression of genes involved in wound repair.

References

- [1] Brun P, Brun P, Vono M, Venier P, Tarricone E et al (2012) Disinfection of ocular cells and tissues by atmospheric-pressure cold plasma. *PLoS One* 7:e33245.
- [2] Brun P, Pathak S, Castagliuolo I, Palù G, Brun et al (2014) Helium generated cold plasma finely regulates activation of human fibroblast-like primary cells. *PLoS One* 15:e104397