

## The effects of helium based atmospheric pressure cold plasma (APCP) on wound healing

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Cold plasmas have been proposed for many applications in medicine, such as tissue disinfection and wound healing. Their antibacterial effects have been well demonstrated and depend mainly to the action of reactive oxygen species or ROS, but the molecular mechanisms promoting tissue regeneration in wound healing are not yet fully understood.

We previously reported that the applications of 2-5 min of atmospheric pressure cold plasma (APCP) generated by helium ionization is effective in the inactivation bacteria without any visible changes in cells and tissues (1) and the aim of the presented study is to ascertain if the same doses of APCP are able to promote wound healing.

At this purpose we tested the effects of APCP exposure on dermal fibroblasts viability obtained from 3 different donors by means of MTT and Tunnel test, in presence or absence of a potent antioxidant such as 5mM N-acetylcystein (NAC). To define the cellular response to APCP, we then analyzed by real-time PCR the expression changes of selected genes involved in oxidative stress-related response (OGG1, GPX1) and wound repair (IL1beta, IL6, TNF $\alpha$ , FGF2, TGF $\beta$ , SMAD3,  $\alpha$ -SMA, collagen I) in the plasma treated cells cultures.

Our results demonstrated that in all the three different fibroblasts cultures, 2 min APCP application did not cause any variation of viability 2 h after treatment and increased significantly after 24 h ( $p < 0.05$ ). In fibroblasts of two donors we also found at this time a significant increase of viability of cell exposed for 2 min to APCP when compared to the untreated control. Moreover, NAC pretreatment positively affected the viability of one cell preparations. Evaluation of apoptosis (Tunel test) on all fibroblast preparations treated with 2 min APCP and monitored after 24 h revealed that the number of apoptotic nuclei was comparable to those detected in untreated cells.

RT-PCR analysis of cells two hours after APCP treatment revealed only a transient increasing in GPX1, IL6, FGF2 and TNF $\alpha$  mRNA levels.

In conclusion, cell exposure of 2 min APCP, a dose that inactivates skin pathogens, maintain or increase their viability, but revealed a transitory alteration of the expression of some genes involved in oxidative stress and wound repair.