DBD plasma treatment of HaCaT keratinocytes: reactive oxygen species rather than ozone increase integrin expression

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Non-thermal atmospheric-pressure plasma has been shown to influence as first target the cell membrane with its embedded proteins. Such cell surface molecules as integrins, cadherins or the epidermal growth factor receptor (EGFR) are of importance in wound healing and also for development of cancer metastasis. Cold plasma comprises of electrons, positive or negative ions, free radicals [e.g. reactive oxygen species (ROS), ozone] and other excited atoms and molecules. Further, plasma has an optical emission in the UV-region, especially UVB. Each of these components can affect the cells during treatment.

This study focused on measurement of apoptosis, induction of intracellular ROS and cell surface molecules on human HaCaT keratinocytes. Adherent HaCaT keratinocytes were treated with plasma by a surface dielectric barrier discharge in air and argon (1 to 5 min) or with ozone (5 min). Ozone was generated by an Ozonisator and monitored by FT-IR (100, 400, 900 and 1800 ppm). Intracellular ROS, apoptosis (annexin V/propidium iodide staining) and cell surface molecules (α_2-, α_3-, α_4-, α_6-, α_V-, β_1-integrin, E-cadherin, EGFR) were analyzed by flow cytometry 24 h after treatment.

Besides a reduction of cell viability significant intracellular changes were observed. DBD/air plasma for 5 min caused an increased expression of α_2- and β_1-integrin whereas E-cadherin and EGFR expression was not influenced. The effects of DBD/argon plasma were less pronounced. Apoptosis was only increased by DBD/air plasma (5 min) although the proportion of apoptotic cells was rather low. Intracellular ROS detected by the fluorescent dye CM-H_2DC-FDA increased from 6.6 ± 1.1% (untreated control cells) to 18.0 ± 3.2% (5 min DBD/air) and 10.9 ± 1.3% (5 min DBD/argon). A concentration of about 100 ppm ozone was measured above the solid phase during a 5 min DBD/air treatment cycle which had no influence on integrin, E-cadherin or EGFR expression. 1800 ppm ozone caused an increase of α_2- and β_1-integrin whereas all other molecules measured were not affected.

Taken together, the extent of effects depended on the nature of plasma (air vs. argon) and the exposure time of cells to the plasma. Short (≤ 1 min) treatment cycles did neither change cell surface protein expression nor induced apoptosis or intracellular ROS. The effects of plasma on cell membrane proteins observed are rather attributed to induction of intracellular ROS than to generation of ozone.

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