In-vitro investigations of different non-thermal atmospheric pressure plasma sources on human keratinocytes

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Physical plasma, the fourth state of matter, is characterized by a mixture of ions, electrons, radicals, electric and magnetic fields and UV-light. Some of these ingredients are already known to cause manifold reactions in mammalian cells. To ensure a safe application of non-thermal atmospheric pressure plasma in medicine the interactions between physical plasma and human cells have to be clarified.

Subject of this study was to investigate the influence of three different non-thermal atmospheric pressure plasma sources on human keratinocytes (HaCaT cells). Adherent HaCaT cells were treated with a surface dielectric barrier discharge (1), a volume dielectric barrier discharge (2) and a plasma jet (3). In order to achieve biological effects different treatment times were necessary. With volume DBD and plasma jet HaCaT cells were treated up to 2 min, surface DBD allowed treatment times from 1-20 min.
To investigate the influence on viability number of adherent cells was counted 24 h after plasma exposition. DNA damage, detected by alkaline single cell gel electrophoresis (Comet assay), was measured subsequently and 24 h after incubation with physical plasma. Further the influence of DBD plasma on the cell cycle was analyzed using flow cytometry (24 h).

24 h after plasma treatment a dose-dependent decrease of number of recovered adherent cells was observed, independently from the plasma source.
Immediately after plasma treatment a dose-dependent increase of DNA damage (parameter: tail intensity by Comet assay) was caused by all plasma treatment regimens, which was diminished after 24 h. With few exceptions (e.g. 60 s treatment volume DBD, 20 min surface DBD) values of tail intensity decreased to level of control cells. An additional change of cell culture medium subsequently after plasma treatment resulted in a higher percentage of recovered cells and in lower DNA damage.
24 h after plasma treatment HaCaT cells showed different distribution pattern of cell cycle stages. All three plasma sources induced a significant raise of the number of HaCaT keratinocytes in G2/M- phase at the expense of G1 phase. An increase of cells in the phase of Sub G1, which is an indicator for apoptosis, was not observed.

In conclusion non-thermal atmospheric pressure plasma caused a dose- and time-dependent influence on viability and DNA of HaCaT cells. An immediate cell culture medium exchange attenuated described effects. The procedure of the cell cycle is also affected by plasma treatment. There are no basic differences in the effects of the three different plasma sources used. Further studies should clarify which plasma component or which combination of components is responsible for described effects.

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