Effects of low-temperature atmospheric pressure plasma on cell physiology *in vitro*

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The application of physical plasma to living tissue is expected to have many uses in the future, e.g. wound healing by plasma-disinfection, stimulation of tissue regeneration, dental applications, treatment of skin diseases and cancer treatment based on the specific induction of apoptotic processes.[1,2] In contrast, there is little knowledge on how physical plasma interacts with living tissues, in particular with cells. Studying the characteristics of plasma and its interactions with cells *in vitro* is essential.
The experiments were carried out using an argon plasma jet (kINPen®09, INP Greifswald) to gain insights into time-dependent plasma effects on cell attachment, viability and tight junction formation *in vitro*. Murine epithelial cells mHepR1 were suspended in complete cell culture medium and were irradiated with argon plasma (direct approach) for 30, 60 and 120 s. Suspecting that physical plasma may exert its effect via the medium, cell culture medium alone was first treated with argon plasma (indirect approach) and immediately afterwards, cells were added and also cultured for 24 h. Cell morphology and vitality were verified using light microscopy and an enzyme-linked immunosorbent assay. Already after 30 s of treatment the mHepR1 cells lost their capability to adhere and the cell vitality decreased with increasing treatment time. Interestingly, the same inhibitory effect was observed in the indirect approach. Furthermore, the argon plasma-treated culture medium induced large openings of the cell's tight junctions, verified by the zonula occludens protein ZO-1, which we observed for the first time in confluently grown epithelial cells.

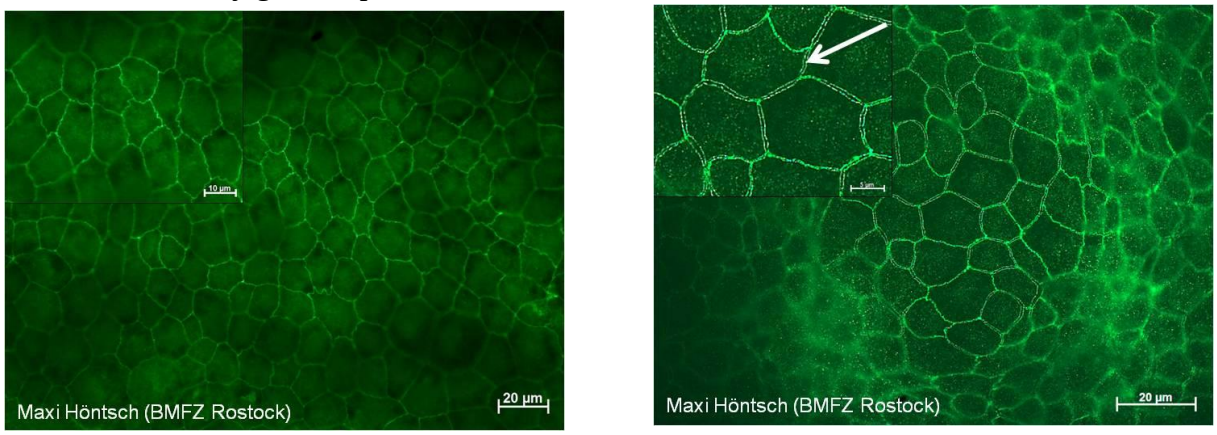

Figure 1: Immunofluorescence staining of the tight junction protein ZO-1 in normal, untreated mHepR1 cells indicating the strong cell-cell contacts at the cell margins, represented by continuous ZO-1 bands (left) and the tight junction protein ZO-1 in confluent mHepR1 cells after 24 h incubation with plasma-treated DMEM. Note the large openings between two cell margins (right, arrow) indicating a loss of the tight cell-cell contacts. AxioObserver.Z1, Carl Zeiss, 63x magnification, bars = 10 (inset) and 20 µm.

[1] Stoffels E., Sakiyama Y., Graves D. B. IEEE on Plasma Science (2008), **36** 1441-55
[2] Vasilets V. N., Gutsol A., Shekhter A. B., Friedman A., Plasma Medicine (2009), **43** 229-32