Intravital Fluorescence Microscopy for the Assessment of Microcirculation and Leukocyte-Endothel Interaction after Application of Tissue Tolerable Plasma in the HET-CAM

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According to current knowledge, low-temperature atmospheric pressure plasma, so-called tissue tolerable plasma (TTP), seems to be a promising therapeutically option for the treatment of chronic wounds [1]. Several in vitro and in vivo studies demonstrated wound healing aspects of plasma treatment [2]. So far, the exact mechanisms and effects on the microcirculation are not elucidated.

The chorioallantoic membrane (CAM) of embryonated hen's eggs represents a vital, vascularized tissue and can be considered as a crossing from *in vitro* and *in vivo*, because tests are performed on living tissue, but not on the embryo itself. Therefore these tests are not classified as animal experiments and in terms of the 3R they may help to reduce animal testing. In previous studies it could be shown in a modified HET-CAM (Hen's Egg Test on the chorioallantoic membrane) that TPP induces aseptic inflammations that are suitable for the modification of chronic inflammations in wounds [3].
By means of intravital fluorescence videomicroscopy it was possible to detect detailed effects of the plasma treatment on the CAM. The method allows the assessment of the microcirculation including the capillary blood flow and the qualitative and quantitative analysis of dose-response relationship. Both haemostatic and vasoconstrictive or-dilatory effects could be assessed. Moreover, the method allows the quantitative analysis of dynamic processes and variables, such as leukocyte-endothelial interaction (LEI) or functional vessel density (FVD).

Method: After 10 days of incubation, the CAMs of fertilized hen's eggs were dissected in microsurgical technique. New is the microscopically assisted puncture of a micro-vessel with a specially made glass micro-cannula (inner diameter 10 microns) using a hydraulic micromanipulator (MO-203, Narishige, Japan) for injection of approximately 5 µl 0.05% rhodamine 6G (Sigma-Aldrich, Germany) as a fluorescent dye for in vivo labeling of autologous leukocytes. Intravital fluorescence microscopy studies to quantify the FGD and LEI were made 5 min after meandering application of TTP (HF plasma jet, neoplasms GmbH, Germany; argon as carrier gas) and the non-activated gas as control.

Results and conclusions: TTP induced a reduction in the FGD while increasing the LEI as a sign of increased immunological reaction. The results are presented in detail in a video clip. The proposed model is suitable for qualitative and quantitative analysis of the effects of tissue tolerable plasma on the functional vessel density (FVD) and the leukocyte-endothelial interaction (LEI) in the vital tissue. It will help to understand the basics of the plasma effects and to develop new therapies for the treatment of chronic wounds by TTP.

References:

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