EPR-detection of reactive species formed in human tissue by electrosurgical argon plasma treatment

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Abstract: This paper discusses the evidence and quantification of spin adducts formed by the reactions of plasma-produced radicals and the spin-trap DMPO both in solution and human tissue. With proceeding treatment time, the overall spin concentrations are changing, but also the relative contributions are variant.
Keywords: plasma medicine; human tissue; electron-paramagnetic resonance; EPR; ESR.

1. Introduction

Non-thermal plasma treatment is a very promising option for the local anti-neoplastic treatment of dysplastic lesions and early-stage intraepithelial cancer. Although new and promising strategies for systemic cancer therapy are being developed, low-invasive and painless treatments to locally control precancerous and early cancer lesions of human tissue gain more and more attention. Amongst those lesions, low or high grade cervical intraepithelial neoplasia (CIN) are treated in clinical studies. CIN is mostly caused by persistent infection with human papilloma virus (HPV) and can progress into invasive cervical cancer (CC) at a later stage. Consequently, there is an urgent need for efficient and low-invasive treatments methods such as plasma medical devices. In the first place, the antiproliferative action arises from the generation of reactive oxygen and nitrogen species (ROS and RNS, respectively), which are subject of this research.

2. Experimental

In the present study, we investigate the amount and distribution of reactive species generated by a non-thermally operated electrosurgical argon plasma source. In order to achieve a medical effect, the presence of ROS and RNS in tissue are of particular interest. This was investigated by spin trapping and electron-paramagnetic resonance (EPR) spectroscopy which was performed in aqueous DMPO solution as well as preputial tissue which resembles cervical tissue in its physiological properties.

For the plasma generation, the Martin–Argon–Beamer-System (MABS, KLSmartin, Tuttlingen, Germany) was utilized, which is designed as an electrosurgical thermal device, but was operated at non-thermal conditions at low power (12 W, distance 7 mm). For the EPR measurements, the MiniScope MS 500 spectrometer (Magnettech GmbH, Berlin, Germany) was utilized. The EPR spectra of known DMPO radical adducts were specifically simulated using the MATLAB toolbox EasySpin and fitted to the measured spectra. It was hereby possible to identify and quantify various radicals. By double integration, both the spin density (spins/g) and the ratios of the different radicals were determined.

3. Results

It turned out that in both, aqueous solution (figure 1) and solid human preputial tissue (figure 2), different oxygen- and carbon-centered radicals are present at increasing concentrations within the first seconds. For plasma treated aqueous solutions, the spin densities of DMPO-OH and DMPO-H were dominant.

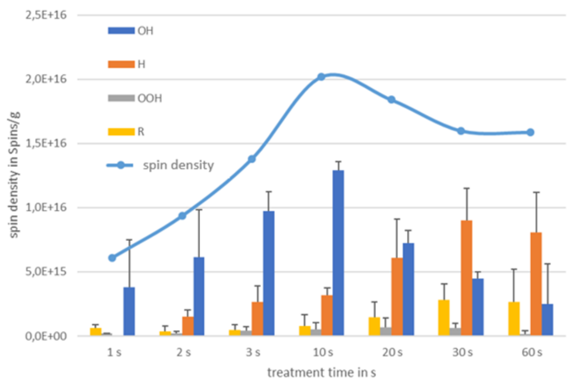

Figure 1: Spin density of different DMPO adducts in aqueous solution after plasma treatment.

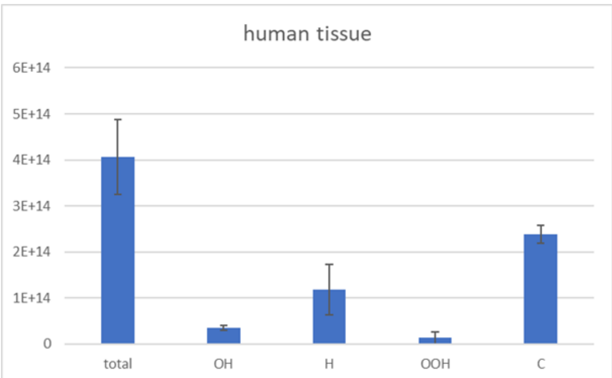

Figure 2: Spin density of different DMPO adducts in human preputial tissue after plasma treatment.

Applying the same treatment to human preputial tissue mostly resulted in the formation of carbon-centered radicals (DMPO-R). At prolonged treatment exceeding 10 seconds, a reduction of the overall spin signal was observed which is attributed to thermal degradation of the spin trap, in accordance with increasing substrate temperature as probed by IR-camera measurements and increasing visible carbonization of the tissue sample. In our presentation, these findings will be compared to those obtained with different plasma medical devices operating with non-thermal plasmas at same experimental conditions.

4. Conclusion

The aim of this study was to characterize the generated radical species by plasma treatment of tissue by a commercial plasma source operated at non-thermal plasma conditions. The given results underline the principle suitability of this technology to obtain high radical concentrations within human tissue, in particular the presence of reactive oxygen and nitrogen species, which are known as active species against cancer cells. The presence of these species generated by an electrosurgical plasma device was proven by EPR for the first time in human tissue and is published in more detail in [1].

The high flexibility of the plasma probes allows treating precancerous and cancerous lesions, such as CIN and other gynaecological neoplasms, as long as unwanted thermal effects are reduced by appropriate and careful operation of the devices. The EPR-based quantification of the generated radical species shows that the non-thermally operated thermal argon plasma sources are suitable for the production of high amounts of radical species, which are the most important mechanism for mediating tissue plasma effects. In plasma treated DMPO solution, the spin densities of DMPO-OH and DMPO-H were dominant. In plasma treated human preputial tissue, carbon-centered radicals DMPO-R were highly abundant. However, due to the high power of common HF electrosurgical units, treatments at steady, non-moving conditions resulted in depletion of the DMPO-X signals as an overdose effect.

5. Acknowledgements

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6. References

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