Cold atmospheric plasma treatment of chronic wounds—investigation of the effects of reactive oxygen and nitrogen species on fibrosis-related cellular signaling pathways

J. Letellier-Bao[1], C. Boudoux[1], D. Rosenzweig[2], A. Philip[2], S. Reuter[1]
[1]*Physics Engineering, Polytechnique Montréal, Montreal, Canada*
[2]*Department of Surgery, McGill University, Montreal, Canada*

Abstract: Plasma provides a tool for controlling the composition of reactive species applied to a wound. In this work, a dual-regime plasma source is developed that allows changing the plasma from a reactive oxygen to a reactive nitrogen species regime. Each is expected to influence cell communication differently. By studying their effects on cells, we can come to tailor the plasma reactivity to provide an adapted treatment to wounds. A special focus is set on signalling pathways in fibrotic illnesses.

1. Introduction

A novel treatment for chronic wounds is cold atmospheric plasma. Non-equilibrium plasmas generate highly reactive species which can induce locally confined redox-chemistry in tissues. Reactive oxygen and nitrogen species are known to play a vital role in cell signaling and influence a range of mechanisms implicated in all phases of wound healing—inflammation, vascular formation, proliferation, remodeling of scar tissue [1]. Reactive species have dual functions depending on the healing phase, their concentrations, etc. This is why a good characterisation of the plasma composition is essential. Plasma composition was simplified to the hypothesis of 2 regimes which could have dual effects on fibrosis—oxygen regime vs nitrogen regime. Reactive oxygen species are known to be pro-inflammatory by increasing the oxidative stress in the environment of the cells, and reactive nitrogen species have anti-inflammatory behavior [2].

2. Methods

A dual-regime argon-driven plasma jet is developed by modifying plasma environment and the mixture in the feed gas [3] to produce opposing chemistries: 1% N_2 in Ar + N_2 curtain yielding a NOx chemistry, termed RNS-plasma and 1% O_2 in Ar yielding an Ox chemistry, termed ROS-plasma. Absorption and emission spectroscopy techniques are used to measure key species density in the plasma, determining the regime the plasma operates at (e.g., UV-absorption spectroscopy at 254nm is used for quantifying ozone). Biological experiments are conducted on hTERT fibroblasts treated with each regime, analyzing cell behavior, protein expression and chemical compositions as a response to modulated plasma treatment.

3. Results and Discussion

Figure 1 compares ozone production of different plasma regimes, measured with absorption spectroscopy, which allowed to define opposing regimes (circled in yellow). Emission spectroscopy and FTIR allow to confirm presence of Nx species in the RNS-plasma and Ox species in the ROS-plasma. Metabolic activity and LIVE/DEAD biological assays on cell culture show comparable activity under both regime treatments. Protein expression of collagen Type 1 is shown to vary under plasma treatment compared to control and a chemical analysis shows that the cells react differently to the two plasma regimes

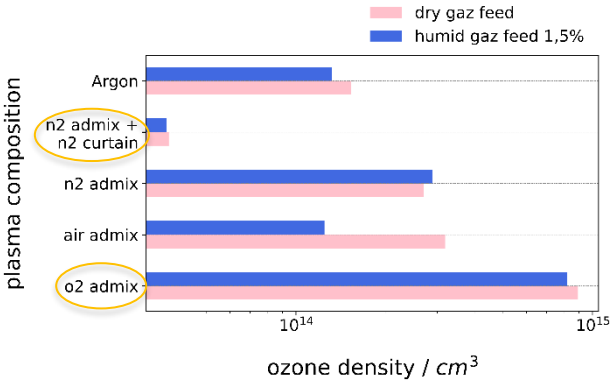

Fig. 1. Ozone production with different plasma compositions. RNS-plasma and ROS-plasma compositions are circled in yellow.

4. Conclusion

Investigating signaling pathways under dual plasma regime treatments have the potential to yield an insight into the specific role of reactive species in cell communication. This will deepen the understanding of the physiology of chronic wounds as a function of the redox environment, paving the way to a personalized plasma treatment technology.

Acknowledgement
This work is supported by NSERC and TransMedTech Institute grants.

References

[1] S. K. Dubey et al., Process Biochem. 112(12) (2021)
[2] D. Feibel et al., Biomedicines, 11(5) (2023)
[3] B. Schmidt et al., Plasma Processes and Polymers, 13(11), 1120-1127 (2016)