Inflammatory consequences of cold physical plasma-mediated bacterial antigen oxidation

Tim F. Hauptmann[1,2], Daniel Mrochen[1], Barbara M. Bröker[2], Sander Bekeschus[1]
[1]ZIK plasmatis, Leibniz Institute for Plasma Science and Technology (INP) / Felix-Hausdorff-Straße 2 / 17489 Greifswald, Germany
[2]Department of Immunology, University Medicine Greifswald / Sauerbruchstr. DZ7 / 17489 Greifswald, Germany

Motivation

Plasma is described as the fourth state of matter. It is generated by energizing gas up to a critical point at which electrons dissociate from atoms. Thereby plasmas emit electromagnetic radiation, predominately UV radiation and visible light, and contain excited gas molecules, positively and negatively charged ions, free electrons, reactive oxygen/nitrogen species (ROS/RNS) and molecule fragments [1]. This mixture has disinfecting and potentially immunostimulatory effects. Especially with respect to the influence on the immune system the responsible mechanisms are still elusive. Besides the preclinical evaluation of its use in oncology and dentistry, cold physical plasma is routinely used for chronic wound care in the clinical.
In this project, the primary aim was to investigate the immunostimulatory effect of cold plasma treatment. Therefore, the influence of plasma-treated and native bacterial antigens concerning their inflammatory potential was investigated.

Material and Methods

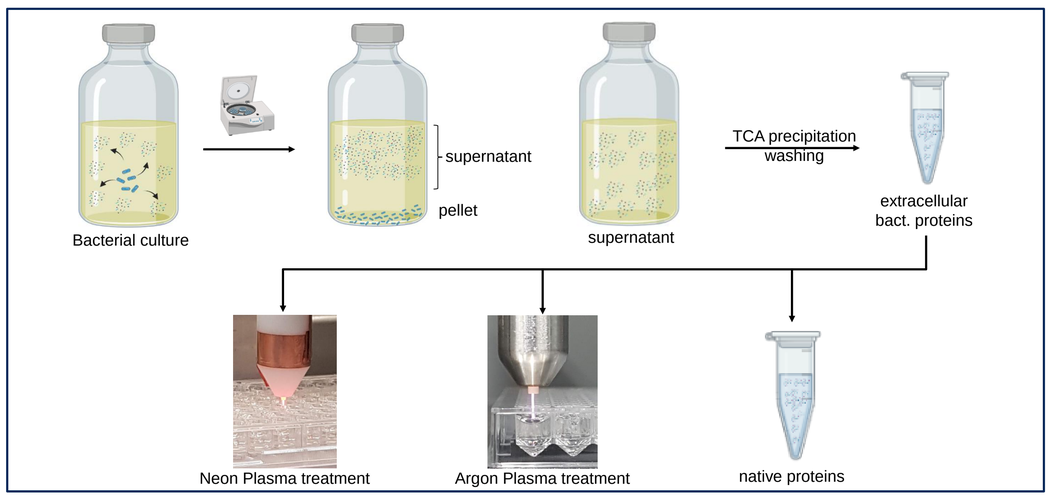

Fig. 1: Separating bacteria (*S. aureus, B. subtilis, P. aeruginosa*) and supernatant by centrifugation. Purification of extracellular bacterial proteins from supernatant by trichloroacetic acid (TCA) precipitation.

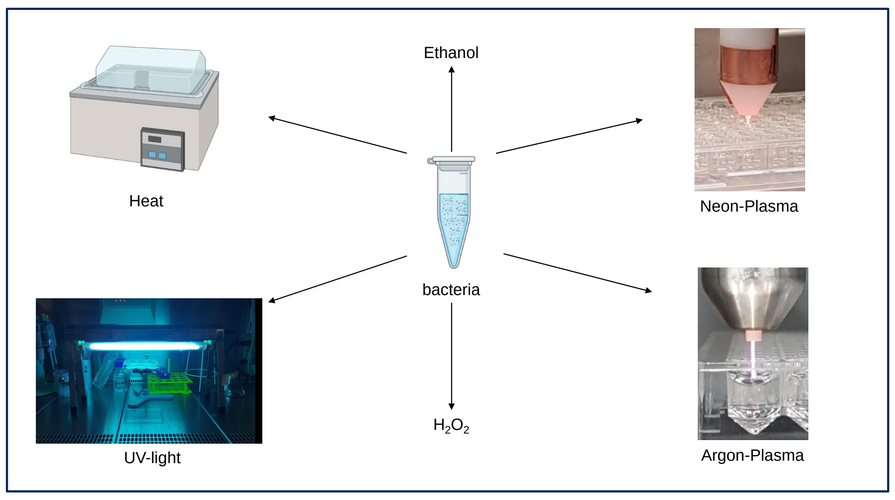

Fig. 2: Lysate preparation of living bacteria

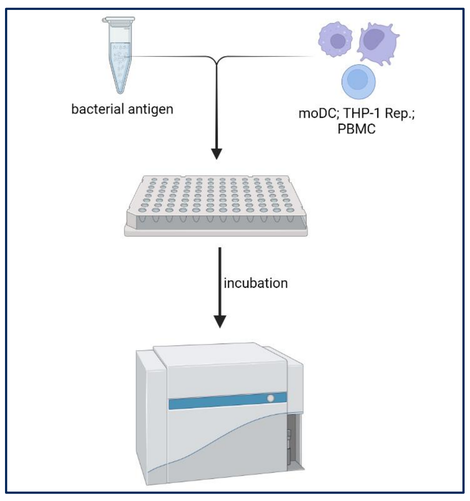

Fig. 3: Incubation of THP-1 Rep; monocyte derived Dendritic Cells; Peripheral Blood Mononuclear Cells with bacterial antigens. Analysis via flow cytometry for cell activation.

Analysis

Influence of bacterial antigens on moDC, THP-1 Rep. and PBMC was measured via flow cytometry. Evaluation via Kaluza Analysis and GraphPad Prism.

Results

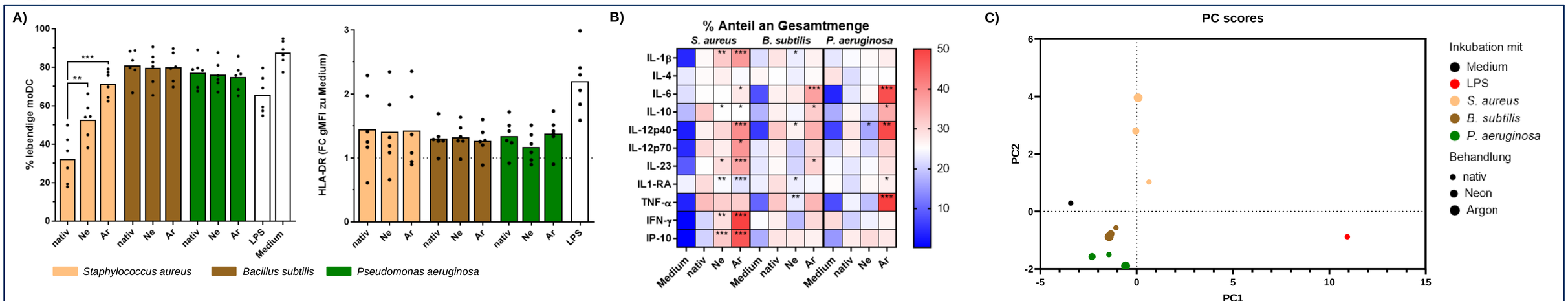

Fig. 4: Incubation of moDC with extracellular bacterial protein: A) cell analysis B) cytokine production C) Principle Component Analysis of cell analysis and cytokine production

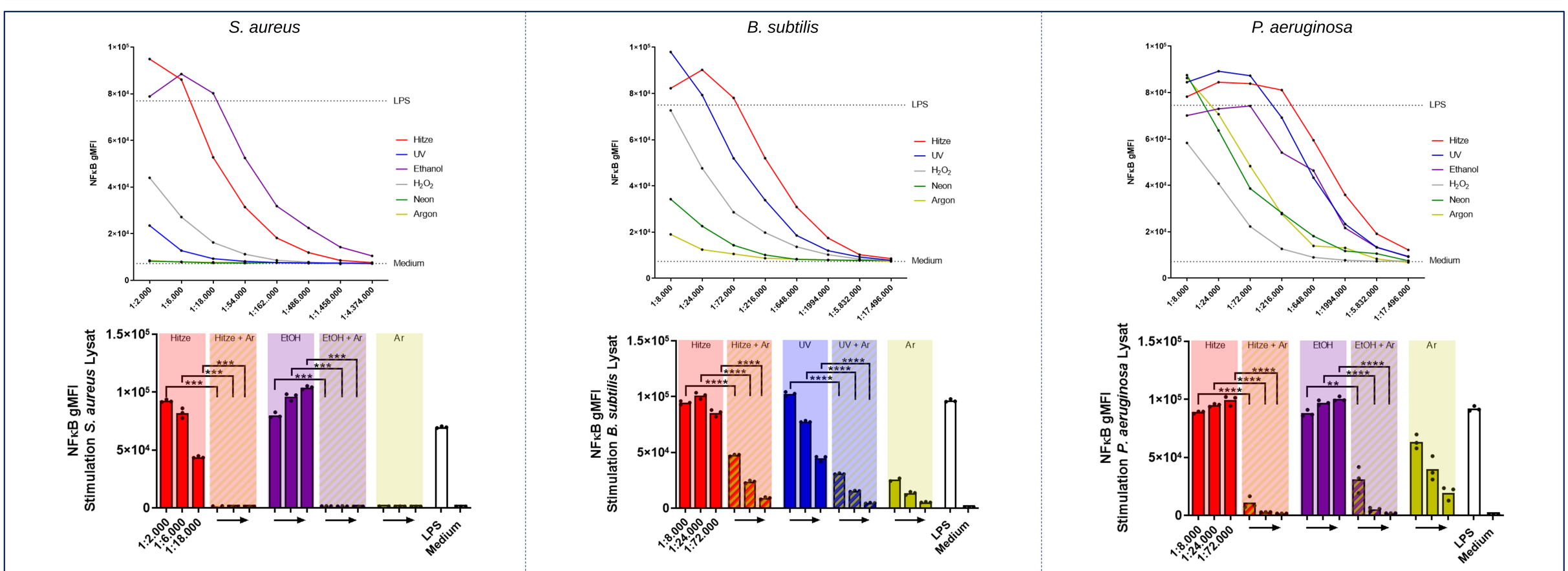

Fig. 5: Incubation THP-1 Rep. with:
S. aureus lysate: single inactivation & combined inactivation

B. subtilis lysate: single inactivation & combined inactivation

P. aeruginosa: single inactivation & combined inactivation

Conclusion and Outlook

A first step to figure out whether and how plasma might alters immune cell activation against bacteria was to investigate the effect of plasma treated bacterial proteins.
We compared the effects of H_2O_2 and plasma generated bacterial lysate on THP-1 Rep. cells and PBMC to observe the effects on the adaptive immune system.
The different lysate methods , conditions and bacterial species were found to have an influence on the stimulatory effect. However, plasma showed no negative effects.
The results and established methods of protein modification and lysate production offer potential for future projects. These can analyze the optimum conditions of plasma treatment with a stepwise adaptation of the methods.

References:
[1] Bernhardt, T et. al. (2019) *Oxidative Medicine and Cellular Longevity*

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