Gas plasma-mediated ROS-delivery and toxicity in pancreatic cancer cells using 3D hydrogel models

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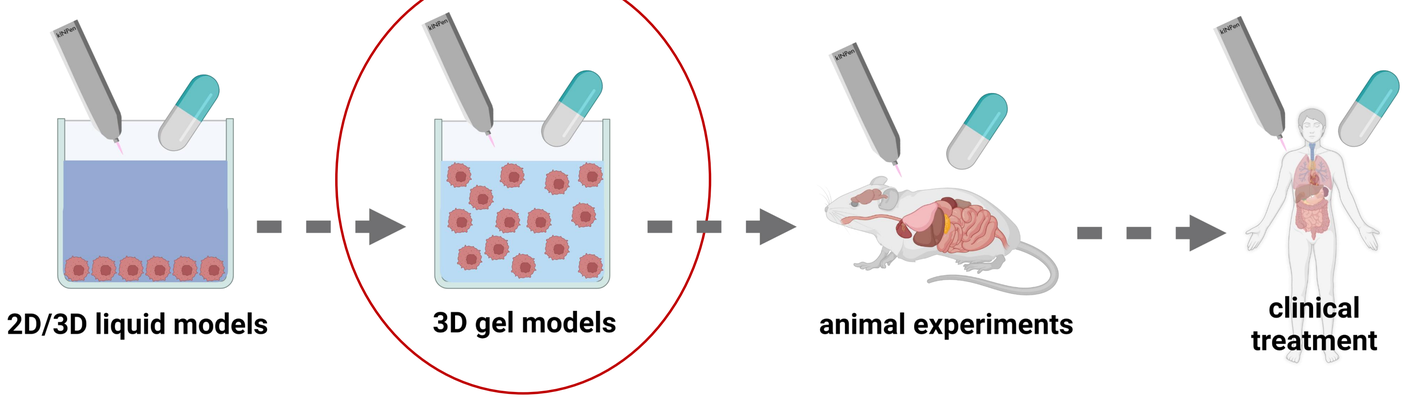
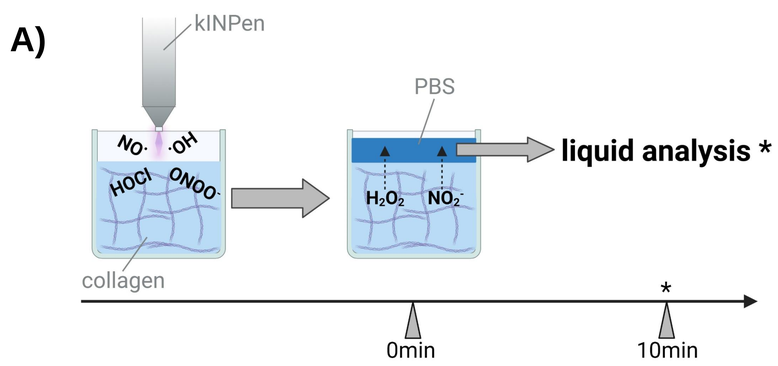
MOTIVATION



Medical gas plasma therapy has been successfully applied to many types of cancers in preclinical models and also palliative treatment regimes in head and neck cancer patients [1-2]. This innovative treatment approach generates a versatile mix of reactive oxygen (ROS) and nitrogen species (RNS) that have been linked to a variety of antitumor effects. However, directly tracing ROS/RNS trajectories into tissues remains challenging and limits transfer of knowledge gained in 2D models *in vitro*. In this study, 3D characterization of ROS delivery and their biological impact was coupled to spatial evaluation of tumor toxicity in hydrogel models to overcome this gap.

A – ROS DELIVERY INTO HYDROGEL

long-lived ROS / RNS (H_2O_2, NO_2^-)



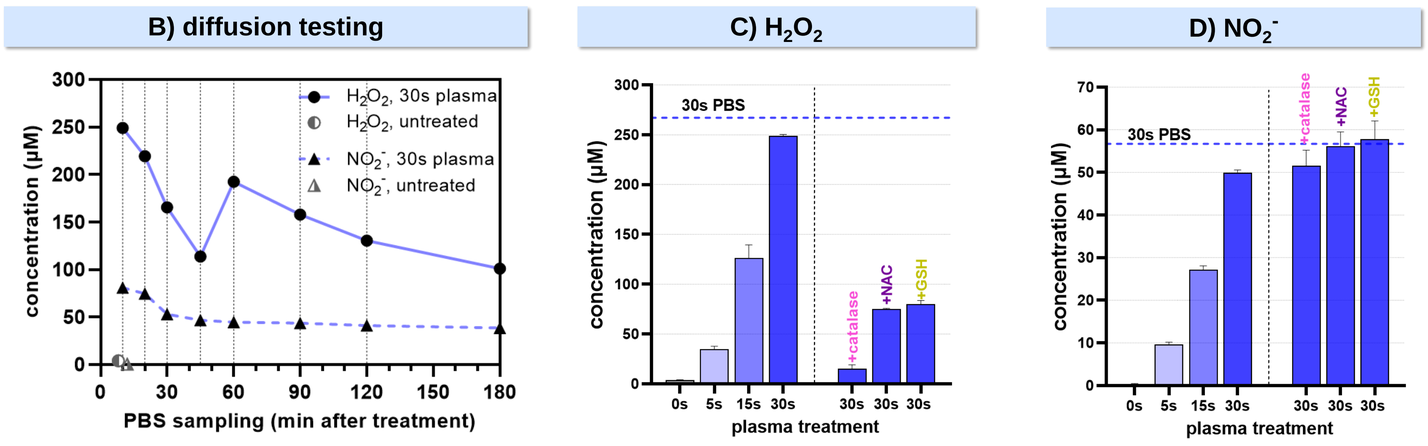
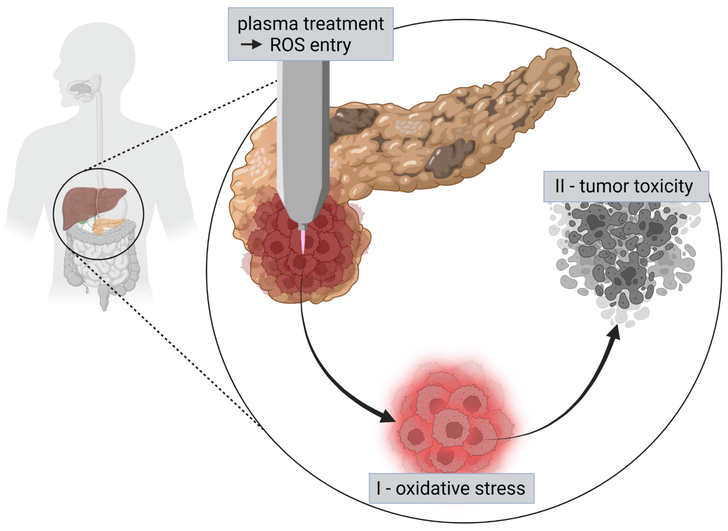


Fig. 1: ROS / RNS profile in gas plasma-treated collagen hydrogel compared to PBS. [3]
A) Schematic overview of treatment procedure for quantification (after **B)** titrating the time point of PBS sampling, 10min incubation time was chosen) of **C)** hydrogen peroxide (H_2O_2) and **D)** nitrite (NO_2^-) after gas plasma exposure for 1s, 5s, 15s, and 30s in presence or absence of antioxidants (catalase, n-acetylcysteine (NAC) and glutathione (GSH)). The blue line indicates the analyte concentrations in PBS at equivalent treatment conditions.

B - TUMOR CELL RESPONSE



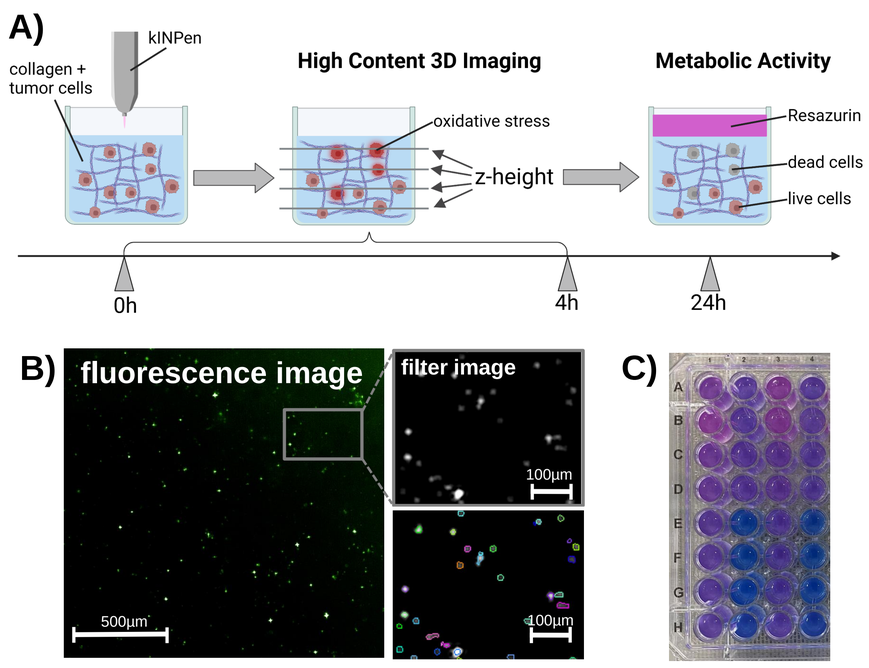
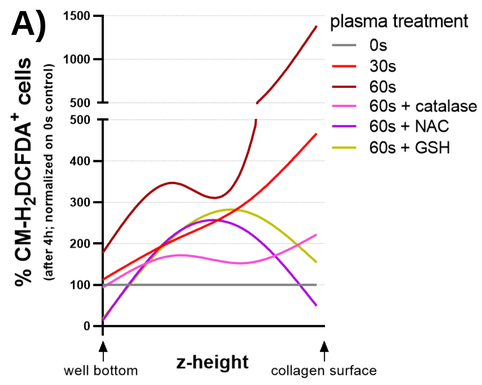


Fig. 2: Experimental setup and image analysis
A) Tumor cells were seeded into a collagen hydrogel, which was exposed to argon plasma for 30s or 60s. Cellular oxidation and tumor toxicity were evaluated during a z-resolved high content imaging time-lapse fluorescence microscopy. Metabolic activity of tumor cells was assessed after 24h. **B)** Algorithm-driven analysis of tumor cells after high content imaging. **C)** Representative image of the resazurin assay for evaluation of metabolic activity.

I – OXIDATIVE STRESS



II – TOXICITY

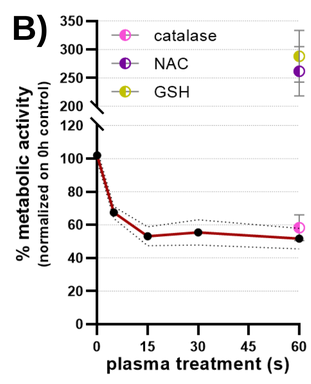
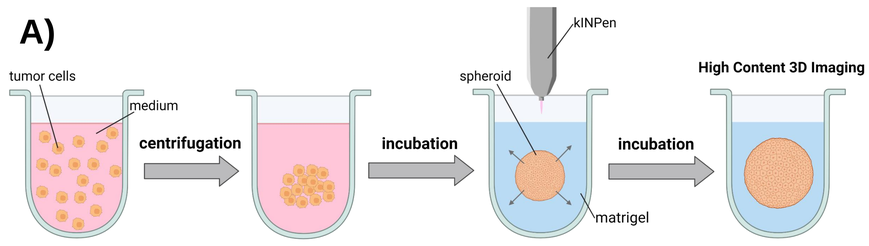
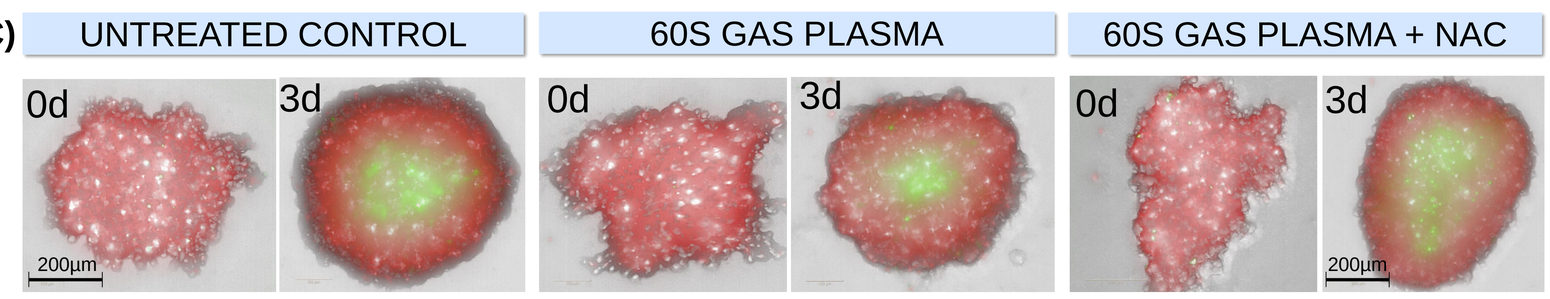


Fig. 3: Plasma treatment induces intracellular oxidative stress and cytotoxicity in pancreatic carcinoma cells (Panc01) seeded into collagen hydrogel.
A) Increase of intracellular ROS levels (number of cells with positive fluorescence signal for CM-H_2DCFDA) in relation to gas plasma treatment time was partially rescued in presence of catalase, NAC, and GSH in Panc01 cells. Spatially-resolved evaluation of intracellular ROS levels indicated higher oxidation in upper regions of the collagen matrix.
B) Decrease of metabolic activity is rescued in presence of NAC and GSH but not catalase. Gas plasma exposure showed a treatment time-dependent toxicity gradient.

C – COMPLEX 3D TUMOR MODELS





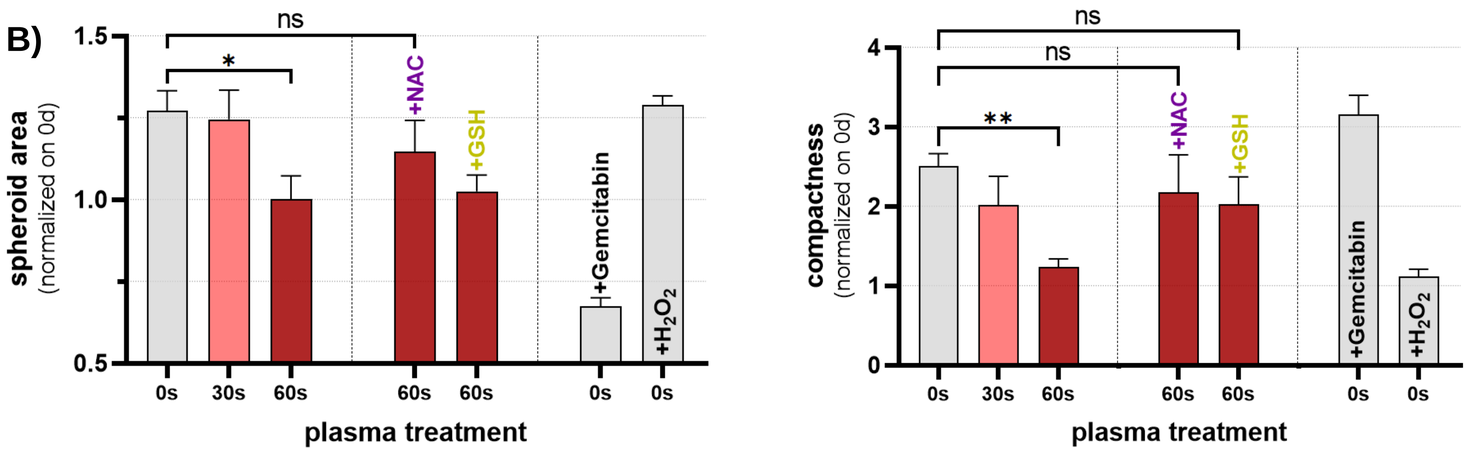


Fig. 4: Gas plasma treatment reduced spheroid growth and compactness while inducing cytotoxicity in tumor spheroids of pancreatic carcinoma cells (Panc01) seeded into biological hydrogel.
A) Tumor cells were seeded into cell medium and allowed to form compact spheroids after centrifugation and incubation. After adding matrigel hydrogel, gas plasma treatment was performed and following parameters were analyzed. **B**) ROS scavengers NAC and GSH partially rescued gas plasma-mediated reduction of spheroids growth and compactness. H_2O_2 induced profound spheroid degradation after oxidative stress while Gemcitabin seemed to induce spheroid shrinkage.

References:

[1] S. Bekeschus, et al. (2020). Advanced Science
[2] H.R. Metelmann, et al. (2018). Clinical Plasma Medicine
[3] L. Miebach, **M. Hagge**, S. Bekeschus (2023). Bioengineering

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