Cold Atmospheric Plasma as a Local Therapy for Breast Cancer Bone Metastases: Evidence from 3D Bioprinted Models and SRG Rat Xenografts

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INTRODUCTION: Bone, especially the spine, is a common site of metastasis for breast, lung and prostate cancers. These tumors pose a significant challenge, demanding aggressive treatments like chemotherapy and invasive surgery. To fully remove metastatic lesions, surgical procedures need to extend onto healthy tissue, reducing the probability of remaining malignant cells, but also leaves large voids necessitating reconstruction. Cold plasma therapy, operating at temperatures below 40°C, offers a non-invasive solution by delivering reactive oxygen and nitrogen species (RONS) locally. This type of treatment could theoretically be applied to the tumor resection wound bed just prior to reconstruction. While research shows promising results, the reaction mechanism between plasma and tissues, and proper treatment dosage and reactive species composition to reach the desired effects are still topics of current research. The purpose of this project is to develop and characterize a cold plasma source and investigate its potential in mitigating bone cancer metastasis, hypothesizing its anti-tumor properties. The overall objective is to create a tissue-plasma platform for cold plasma therapy, aiming to control the metastatic spread of breast cancer cells to bone tissue.

METHODS: We developed a platform combining tailored plasma reactivity with a reproducible tumor-stroma bioprinted model. Using a coaxial dielectric barrier discharge source, circular constructs of A1G7 hydrogel (1% alginate, 7% gelatin) were bioprinted with cocultures of MDA-MB-231 breast cancer cells and human bone marrow-derived mesenchymal stem cells (hbmMSCs), mimicking a bone marrow-like microenvironment. CAP effects on metabolic activity and viability were evaluated by AlamarBlue and Live/Dead assays. Mechanistic studies used scavenger rescue experiments with catalase, N-acetyl-L-cysteine (NAC), and L-glutathione (GSH). Hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) were quantified via Amplex Red and Griess assays.
In vivo, MDA-MB-231 cells were implanted into the caudal vertebrae of SRG (Sprague Dawley Rag2 knockout) immunodeficient rats to establish a xenograft model of breast cancer bone metastases. Male rats were used to allow for larger surgical sites, and no sex-based differences are expected because MDA-MB-231 cells are triple-negative and not hormone responsive. The planned sample size was N = 6 per group across four experimental groups. Tumors were allowed to grow for 6 weeks before surgeries involving resection with plasma treatment of 3 minutes and 5 minutes. These treatment groups will be compared with resection alone and resection with doxorubicin treatment. Bioluminescent tumors were imaged using the Newton FT500 system before surgery and at 1 and 2 months after surgery.

RESULTS: CAP showed dose-dependent, selective cytotoxicity toward MDA-MB-231 cells compared with hbmMSCs in both 2D and 3D cultures. H_2O_2 levels decreased significantly after catalase, NAC, or GSH addition, while NO_2^- levels were only slightly reduced by NAC and GSH and remained unchanged with catalase. All scavengers restored viability after CAP, confirming oxidative stress as a primary mechanism of selective cancer cell death. Building on the *in vitro* findings, this study extends the work by establishing the CAP treatment platform in a newly developed SRG rat xenograft model of breast cancer bone metastases. Preliminary *in vivo* results confirmed consistent tumor formation in the SRG rat xenograft caudal vertebrae after 5 weeks, validating the model for subsequent resection and treatment studies.

DISCUSSION AND SIGNIFICANCE: This platform integrates bioprinted tumor-stroma models, mechanistic RONS analysis, and an SRG xenograft model of breast cancer bone metastases to establish a framework for clinically relevant plasma dosimetry. By linking *in vitro* selectivity to an *in vivo* metastasis model, our work lays the foundation for CAP as a personalized, non-invasive therapy to control spinal metastases and support future clinical translation.

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IMAGES AND TABLES:

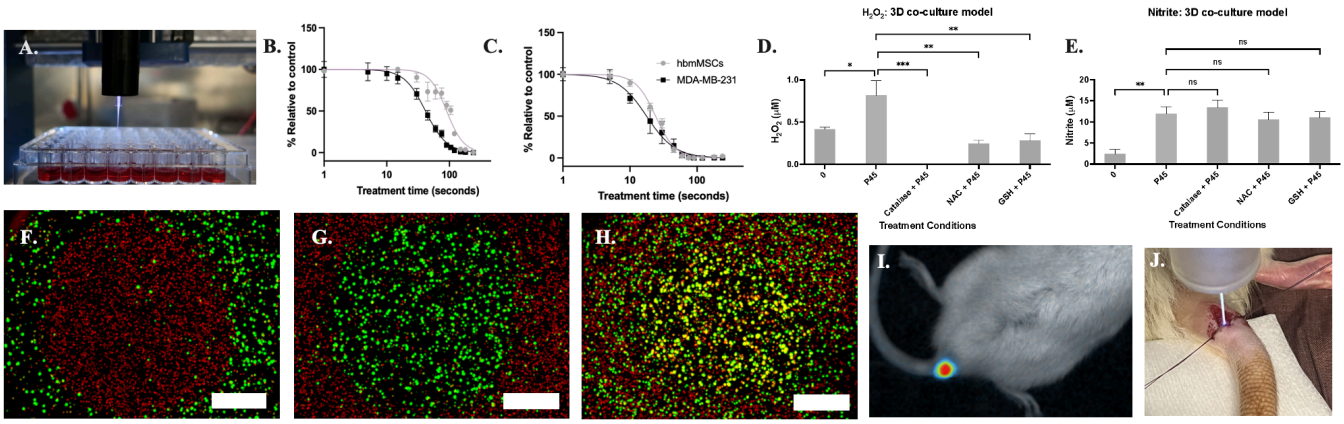

Figure 1. Plasma Treatment Platform. A. Photograph of CAP application during the *in vitro* procedure. The system was automatized using a computer numerical control (CNC) stage, allowing programmable control of treatment duration and positioning for highly reproducible conditions. **B. C.** Dose response of MDA-MB-231 and hbmMSCs cells one day after direct plasma treatment, showing selective cytotoxicity toward cancer cells in 2D culture (**B.**) and 3D culture (**C.**). **D.** Hydrogen peroxide levels after CAP treatment with and without scavengers. **E.** Nitrite levels after CAP treatment with and without scavengers **F.** Live (green)/Dead (red) staining of the bioprinted model after 45 seconds of plasma treatment with MDA-MB-231 cancer cells the central ring and hbmMSCs in the outer ring and the opposite arrangement for **G**. **H.** Live/Dead staining of the bioprinted co-culture after 5 µM doxorubicin treatment. Scale bars: 1250 µm. **I.** Bioluminescence signal of MDA-MB-231 cells implanted in the caudal vertebrae of an SRG rat, five weeks after implantation. **J.** Photograph of CAP application during the *in vivo* procedure immediately following tumor resection.