Cold Plasma-Based Redox Therapy for Bone Tumor Growth Control and Bacteria Inactivation

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INTRODUCTION: Bone, especially the spine, is a common site of metastasis for breast, lung and prostate cancers. Treatments for these tumors rely on heavy doses of chemotherapeutic agents and invasive surgical procedures. To fully remove metastatic lesions, surgical procedures need to extend onto healthy tissue which reduce the probability of remaining malignant cells. This difficult procedure often requires bone reconstruction and graft, but also leaves high risks of open wound infection. Cold plasma therapy is a novel type of therapy which could greatly assist surgical intervention by delivering locally and non-invasively highly reactive oxygen and nitrogen species. This technology operates at temperatures lower than 40 °C and can selectively modify the apoptosis dynamics of the tumors by modulating the cell-to-cell communication pathway. Similarly, the pulsed electric fields that initiate the reactivity create conditions where bacteria undergo electroporation, which enhance the antimicrobial effect of the redox treatment. While research shows promising results, the reaction mechanism between plasma and tissues, and proper treatment dosage and reactive species composition to reach the right effects are still topic of current research. The aim of this project is to create and characterize a cold plasma source and its effect on mitigating the bone cancer metastasis as well as the spread of bacterial infection.

METHODS: We have developed a platform combining tailored plasma reactivity through a kHz coaxial dielectric barrier discharge source and a highly reproducible circular bone tissue model. The bone tissue model was bioprinted (Cellink BioX) using cell-laden hydrogel made of 1% alginate and 7% of gelatin (A1G7). Thus, a multi-well plate was generated with identical "breast-to-bone" metastasis as a coculture model of MDA-MB-231 and hbmMSCs cells. Similarly, a direct plasma treatment method is also used on *E. Coli*. cultivated in LB-Miller media has been suspended in PBS and treated by plasma for different duration. The colonies were counted after each plasma treatment time. For diagnostic purposes, highly sensitive methods such as optical emission spectroscopy has been combined with electrical energy dissipation measurements and by fiber optic temperature measurements. Liquid reactive species of oxygen and nitrogen (RONS) are measured by UV-VIS colorimetry achieving selectivity through the combination of scavengers, colorimetric probes and RONS selectivity from the gas phase. For each set of plasma treatment at different parameters (i.e. energy, gas composition, distance and time of treatment), metabolic activity through Alamar blue assays at day 1, 2 and 3 and live/dead measurements are used to detail the biological response of the tumor cells.

RESULT SECTION: Preliminary results have shown that A1G7 cell-laden hydrogel could be bioprinted with reproducible results in a model of coculture MDA-MB-231 cancer cells and hbmMSCs/fibroblasts. Cell migration over a period of 30 days showed the viability of the bioprinted model. Plasma showed a selective antitumoral effect on MDA-MB-231 cancer cells over hbmMSCs healthy cells in 2D culture. Moreover, the dose response of our inner and outer part of the 3D bioprinted model show an IC50 of cancer cells of 11.49 sec compared to 43.35 sec for the healthy cells, which suggests a selective antitumoral effect of plasma in 3D culture. A direct plasma treatment method on *E. Coli* has also shown that a 2.5 minutes treatment at 0.5 cm of distance decrease significantly the bacterial activity with effects similar to a dose response curvature. Colorimetric assays have also confirmed that long-lived species (H_2O_2 and NO_2^-) can be tailored through the energy, the distance, the duration of treatment and the composition of the atmosphere around the plasma.

DISCUSSION: In essence, our innovative interaction platform enables us to create chemistry that was previously only supplied endogenously by biological system, such as singlet and atomic oxygen, and observe the response on infected tumorous material as a mean to develop a novel type of redox-based treatment. The use of a bioprinted model not only ensures reproducibility and high control over tissue modeling techniques, but also to investigate in detail the migration of a tumor core to a stromal outer layer. With the combination of a tailored plasma jet, this platform becomes extremely relevant for exploring new therapeutic avenues using exogenous RONS and developing a more personalized approach for current non-invasive treatments. Dose response for direct and indirect plasma treatment and 2D characterization of reactive species in the tissue model still need to be completed in order to assess the efficiency of the treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: With the help of the developed platform, we will be able to address a fundamental question of plasma-based therapy: *can specific redox pathways be addressed and used for precision medicine*. The results of the will be extremely helpful for a transfer of the technology to clinical applications. The main outcome of the project will be to find a route to a) develop a new type of healing or prevention of infectious diseases after surgery and b) reduce the growth of metastasis in bone cancer.

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IMAGES AND TABLES:

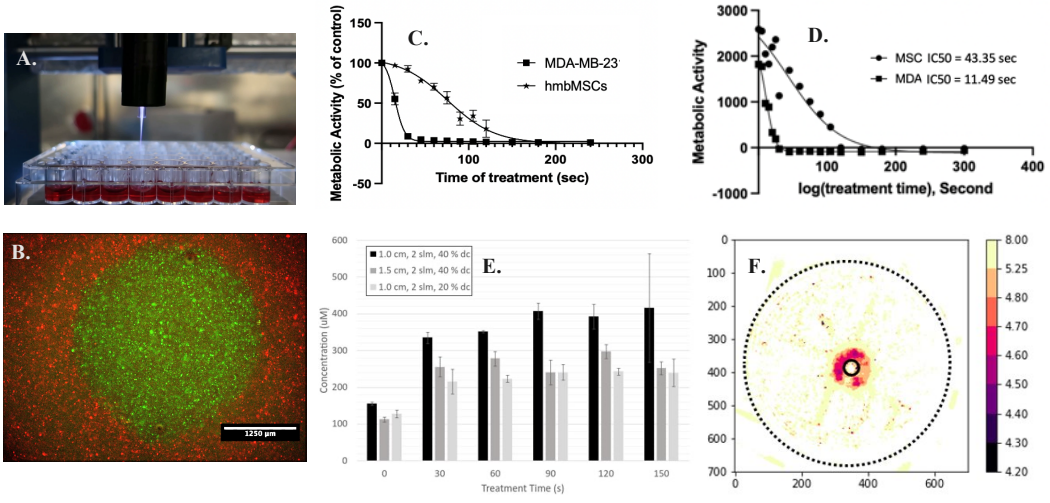

Figure 1. Plasma Treatment Platform. **A.** The plasma jet has been automatized with the help of a computer numerical control (CNC). Through only programming, we ensure control over the time and duration of treatment creating high reproducible conditions. **B.** The bioprinted model contains a 3D coculture of cancer cells (MDA-MB-231 + GFP) in the inner ring and healthy cells (fibroblasts + mCherry) in the outer ring in A1G7 hydrogel. **C. D.** Dose response of MDA-MB-231 and hbmMSCs cells after one day of direct plasma treatment shows a selective effect on cancer cells in both 2D culture (**C.**) and 3D culture (**D.**). **E.** Colorimetric assays of nitrite on hydrogel for parameters of energy, time and distance of treatment. Those parameters can influence the chemical production of the plasma jet, which ensure conditions where tailoring is possible. **F.** pH distribution of plasma treatment on a 5 cm wide hydrogel disk. This pH was taken with a fluorescence technique for 3 min of treatment.