Hypoxia-Inducing Cryogels for Preclinical Anticancer Drug Screening

J. Sinoimeri[1], T. Colombani[1], S. A. Bencherif[1,2,3]

[1]Department of Chemical Engineering, Northeastern University, Boston, USA; [2]Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, USA; [3]Sorbonne University, UTC CNRS UMR 7338, Biomechanics and Bioengineering (BMBI), Compiègne, France

Statement of Purpose: Hypoxia, defined as low oxygen tension, is a characteristic feature of solid tumors and a hallmark of aggressive cancers.[1] The rapid growth of tumors often results in the development of a hypoxic microenvironment. Metabolic adaptation to hypoxia leads to tumor cell growth and invasion, resistance to apoptosis, and multi-drug resistance.[2] For decades, Multicellular Tumor Spheroids (MCTS) have been used to model solid tumors and emulate key aspects of tumor biology such as hypoxia.[3] However, challenges with MCTS formation and reproducibility, inadequate biomechanical cues provided to cells, and uncontrolled oxygen depletion among other limitations led to non-physiological tumor cell responses.[4] As a result, predicting clinical responses to anticancer drugs with MCTS remain a major challenge in cancer treatment.[5] To model solid tumors more accurately, our team has recently developed an innovative approach using cryogel scaffolds[6] inducing rapid oxygen depletion while enabling cell-rearrangement into spherical or ellipsoidal cellular aggregates embedded within a 3D polymer network. Our main objectives were: (1) to engineer hypoxia-inducing cryogels (HIC) to induce cellular hypoxia; (2) to provide a biophysical support enabling tumor cell attachment, proliferation, and remodeling, and (3) to evaluate acquired resistance of hypoxic B16-F10 melanoma cells to an anticancer drug. **Methods:** HIC were fabricated via a cryogelation process[6] using methacrylated hyaluronic acid (HAGM). RGD peptide, catalase, and glucose oxidase (GO) were incorporated into the gel to promote cell attachment and viability while depleting oxygen. To image the polymer network by confocal microscopy, rhodamine-labelled HAGM was utilized (yellow). Oxygen depletion was monitored using needle type oxygen microprobes in the presence or absence of glucose. B16-F10 cell viability was evaluated by confocal imaging. Cells were stained with DAPI (blue), a fixable dead cell stain (red), and FITC-phalloidin (green). Similarly, cellular hypoxia was monitored using a fluorescent hypoxyprobe (magenta). Finally, AlamarBlue assay was performed to evaluate drug resistance of B16-F10 cells to various concentrations of doxorubicin (DOX), a common chemotherapy drug.

Results: Fig. 1A shows that 0.1% HIC induce a controlled and sustained depletion of oxygen leading to hypoxia (~1% O_2) up to 48h. Hypoxia induction is dependent on both GO and glucose contents. Fig. 1B shows that unlike regular cryogels (control), HIC promoted cell remodeling into MCTS-like structure with high cell viability (95 ± 2%), while inducing a substantial level of cellular hypoxia (94 ± 3%) after 24h as depicted in Fig. 1C. Finally, HIC significantly induced melanoma resistance to DOX. With our highest DOX concentration (2µM), 100% and 77% of drug-resistant B16-F10 cells in HIC remained alive after 24h and 48h treatment, respectively. In comparison, all B16-F10 cells died within 48h in our control condition (blank cryogels).

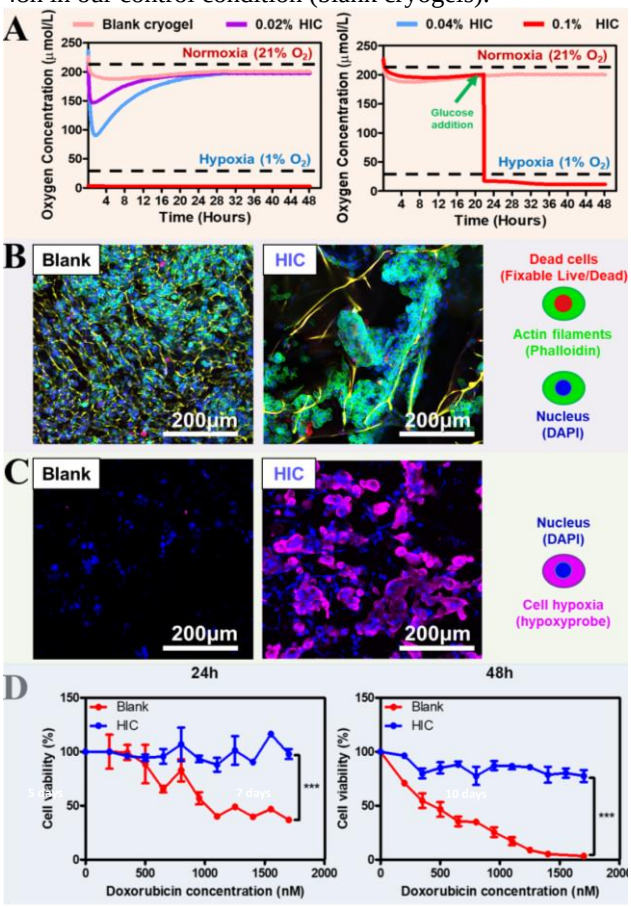

Figure 1. A HIC-supported tumor model. A) Oxygen depletion as a function of GO (left) or glucose (right) concentration. B-C) Confocal imaging of B16-F10 cells seeded within blank cryogels or HIC showing cell viability (B) or cellular hypoxia (C) after 24h incubation. D) B16-F10 resistance to DOX after 24h (left) or 48h (right) treatment.

Conclusions: We have engineered a new class of macroporous and biomimetic materials, namely HIC. HIC are capable of inducing local hypoxia while promoting tumor cell remodeling, leading to anticancer drug resistance. This suggests that tumor-laden HIC may mimic key aspects of the native tumor microenvironment, making these advanced cellularized scaffolds a promising platform for drug screening in 3D and potentially improving the efficiency of cancer drug discovery.

References: 1. Vaupel, P. Cancer Metastasis Rev. 2007;26,225-239, **2**. Wilson, WR. Nat Rev Cancer. 2011;11(6):393–410, **3**. Leek, R. Adv Exp Med Biol. 2016;899:167–96, **4.** Yamada, K. M. Cell. 2007;130,601–610 (2007), **5.** Seruga, B. Nat Rev Clin Oncol. 2011 Jan;8(1):12-23, **6.** Bencherif, S. A. PNAS 2012;109,19590–19595.