Breast Cancer Tissue Bioreactor For Direct Interrogation And Observation Of Response To Antitumor Therapies

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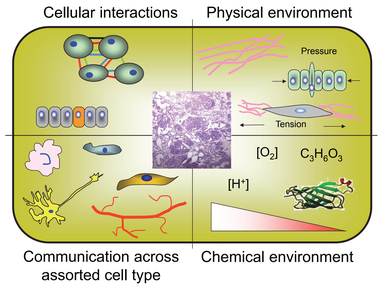
Abstract

Breast tumors are routinely biopsied to assess the pathological stage of a tumor. We hypothesize that under the proper conditions, this diseased tissue could be used to predicatively gauge therapeutic response. In vivo tumor behavior is modified by its own microenvironment, including cues received by structural information of surrounding extracellular matrix, and by the chemical nature of the surrounding microenvironment. The tumor microenvironment exists in a less oxygenated state than does normal tissue; the surrounding cellular matrix in the tumor tissue is maintained under higher structural matrix tension because of either structural differences or increased interstitial fluid pressure. These microenvironmental parameters regulate cellular behavior, including the tumor cells' responsiveness to antitumor therapy. Our goal is to develop and validate a microfabricated breast cancer tissue bioreactor (BCTB) that will provide a controlled artificial tumor tissue microenvironment to maintain intact small breast cancer biopsy samples ex vivo for testing the response of an individual patient's tumor to chemotherapy or molecular targeted therapy. **The objective of this project is to develop biomicroelectromechanical systems (BioMEMS) that will support the combination of the 3-D culture methodology with control over microenvironmental oxygen, acidity, and matrix rigidity to better approximate the in vivo tumor microenvironment ex vivo.**

Tumor microenvironment



To validate maintenance of long-term drug delivery in our tissue bioreactor, we observed the inhibition of morphogenic growth of human mammary epithelial cell lines—MCF-10A and their invasive variants, cultured under 3-D conditions inside the system. The MCF10A and their variants undergo a distinct morphogenic transformation that results in the formation of hollow mammospheres. This complex process requires alterations in a variety of cellular functions including the degradation of the extracellular matrix that is regulated by matrix proteinases. For our drug delivery testing and validation experiments, we have introduced proteinase inhibitors into the system and detected inhibition of both proteinase activity and cellular morphogenesis. Currently, we are extending functionality of the bioreactor to include controlled oxygen and pH. Once the base reactor system is validated, we will determine whether drug response of a tumor biopsy sample evaluated within the BCTB predicts in vivo responsiveness by using murine models of breast cancer. We predict that a close approximation of the tumor microenvironment ex vivo will maintain an intact tumor biopsy specimen under proper disease conditions and that the corresponding biopsies' response to antitumor therapy will directly correlate to the in vivo responsiveness of that same agent against the tumor in situ.

Objectives

- Develop Breast Cancer Tissue Bioreactor (BCTB) for long-term cell culture
- Demonstrate delivery of the "drug" agents by analyzing both activity inhibition (i.e proteinase) and changes to cellular morphogenesis
- To functionalize the Breast Cancer Tissue Bioreactor (BCTB) with controlled oxygen and pH in the surrounding microenvironment.

Cell Lines: **MCF-10A** and **MCF-10A pBabe** (vector control) cell lines were obtained from Joan Brugge (Harvard Medical School, Boston, MA). Ras-transformed **MCF-10 AT**, and the tumorigenic **MCF-10 CA 1a and MCF-10 CA 1d** variants were obtained from Dr. Fred Miller (Barbara Ann Karmanos Cancer Center, Wayne State University, Detroit, MI). These MCF-10A and cell line variants form varying degrees of invasive structures in 3D Matrigel dependent upon the degree of transformation and tumorigenecity of the cell line.

Cell Culture: Cells were maintained in "Growth Media" (DMEM/F12, 5% Horse Serum, 0.1 mg.ml insulin, 0.5 mg/ml hydrocortisone and 20 ng/ml EGF) unless specified otherwise. Cells were plated into 8-well chamber slides (2000 cells/chamber) and treated in the presence or absence of increasing concentrations of **Proteinase Inhibitor cocktail** [1X: 25 μM GM-6001(metallo-); 250 μM E-64 (cysteine); 100 μM Pepstatin A (aspartate); 2 μM Leupeptin (Cathepsin D); 2.2 μM Aprotinin (Serine)]. Cell viability was assessed using Live/Dead Assay (Molecular probes) according to manufacture's directions using 1μM of Calcein AM (live) and 2μM of Ethidium homodimer-1(dead). Fluorescence pictures were taken with Zeiss Axiovert 200M inverted microscope and with FITC and DAPI filters.

3D Matrigel Culture/ Mammosphere Formation. Cell chambers were coated with 3D gel Matrigel. Cells were resuspended in Matrigel ($3x10^5$ cells/ml) and plated into 8-well cell chamber (~70 μl/well) or bioreactor cell chamber (~30 μl/well) and allowed to gel for 1 hr at 37°C. Cells were maintained with Growth Media by either media changes every 3 days (8-well chamber) or continuously in bioreactor.

BCTB supports long-term organotypic culture

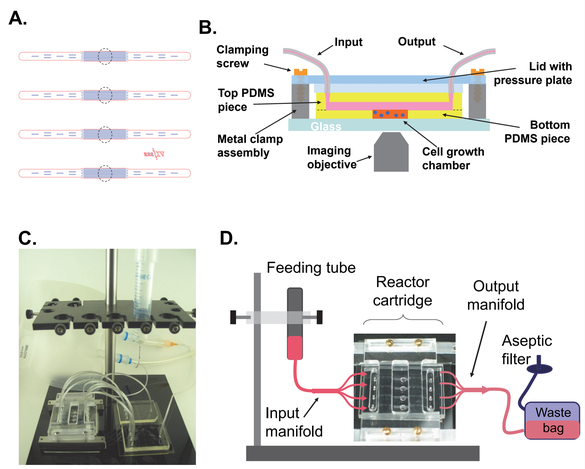


Figure 1. **Principle design of the Breast Cancer Tissue Bioreactor (BCTB).** A) AutoCAD drawing of the lithographic mask for BCTB microfluidic channels. Dotted circles indicate cell culture chambers; B) Block-diagram of the typical layout for the clamped Breast Cancer TB indicating major components; C) A photo of the fully assembled Breast Cancer TB prototype with the gravity driven feeding system, passively regulated flow through the input/output manifolds, and a "Labtainer™" from HyClone as a waste containment system; D) A photo of the clamped Breast Cancer TB cartridge with a diagram illustrating set up for long term culture.

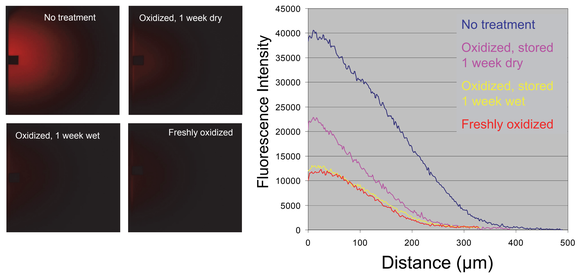


Figure 2. **Partition of small molecules into PDMS as a function of surface properties and storage conditions.** (A) Cross-sectional view of the rhodamine B diffusing into bulk PDMS observed after 24 h incubation period. (B) Intensity profiles corresponding to fluorescence images in (A). Note: Absence of any surface treatment can result in a high degree of adsorption of small molecules to the surface, which can lead to unpredictable changes in local chemokine concentrations. This potentially can have a pronounced effect during long-term culture or when total volumes of handled media is on the order of several μL or nL. This also may affect the actual concentration of "drugs" delivered into BCTB for treatments.

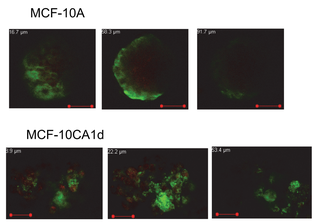


Figure 3. **Selected confocal slices of MCF cell variants cultured within the Breaset Cancer TB.** Mammmospheres formed by MCF-10A and MCF-10CA1d cells labeled with Alexa 488 phalloidin (Green) and with SYTO-63 (Red).

Inhibition of mammosphere formation in BCTB

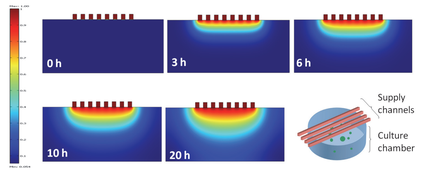


Figure 4. **Diffusion model of drug delivery into the BCTB filled with collagen.** Supply channels are 100 by 100 μm, culture chamber is 1 by 3 mm and a large molecule "drug" with a diffusion coefficient D = 2.75e-12 m^2/s (~2 MDa fitc-labeled dextran through collagen gels). Lower Left Panel: 3-D view of the Cell culture chamber used in the initial modeling efforts with only 4 channels pictured. Actual devices have 8 supply channels.

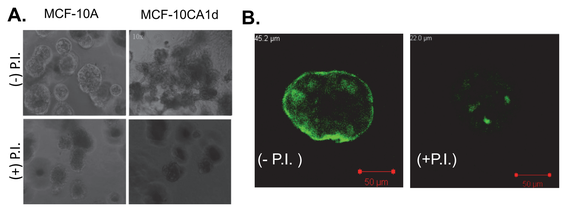


Figure 5. **Proteinase Inhibitor Delivery into BCTB.** (A) Matrix Proteinase Inhibitor (PI) treatments of organotypic culture of MCF10A cell variants shows a reduction in mammosphere size. (B) Inhibition of proteinase activity was confirmed by confocal microscopy of MCF-10A cell variants that were grown for 5 days within TTB and then overlaid with matrix containing fluorogenic protease activity probe (25 μg/ml DQ-gelatin in Hydrogel). Following 48 hr, protease activity was detected by an increase in fluorescence due to proteolysis of DQ-gelatin (Ex/Em 495/515 nm; Molecular Probes). Images representative of one plane of a z-stack (40x).

Design of BCTB for Oxygen Control and Sensing

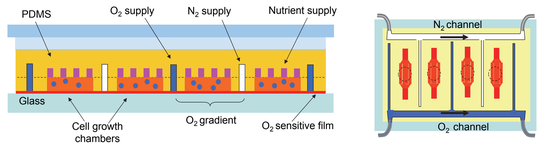


Figure 6. : **Oxygen Gradient Formation**. A) A scheme for O_2 delivery inside BCTB. Source and sink channels contain proper mixture of O_2 and N_2 to achieve target (normoxic / hypoxic) conditions.

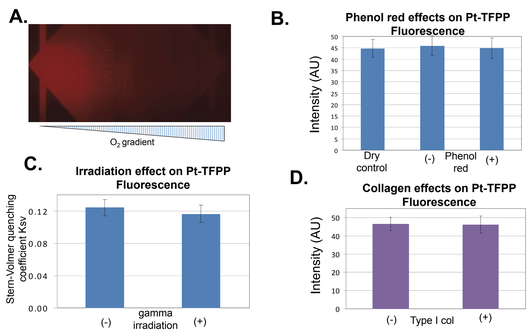


Figure 7. **Oxygen film and its biocompatibility**. A. Oxygen gradient visualized by Pt-TFPP in PS in a flow-through chamber (Ex=540nm, Em=650nm); B) Effects of phenol red auto-fluorescence on observed O_2 film fluorescence; C) Effects of sterilization with gamma-irradiation on Pt-TFPP in PS film to O_2 changes; D) Effects of Type I collagen auto-fluorescence on observed O_2 film fluorescence.

Thin films for optical assessment pH levels within the BCTB cell chamber

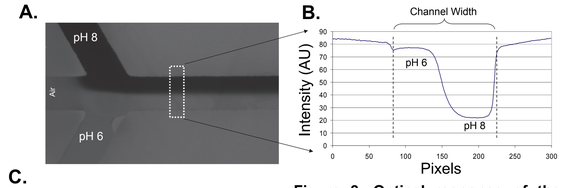
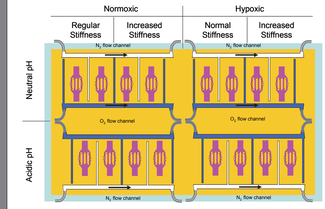


Figure 8. **Optical response of the SNARF – Nafion pH sensitive film in presence of different pH calibration buffers.** A) Observed changes in fluorescence of the pH film in response to pH 6 and pH 8 buffers flowing through the 100 μm wide microfluidic channel (Ex = 488 nm, Em = 575 / 40 nm). B) Line profile of the observed fluorescence signal across microfluidic channel as two buffers flow on top of pH sensitive film (40 pixel average along channel length). C) Measured emission spectra of the pH sensitive film in: absence of fluid contact (native fluorescence); in presence of buffers with pH 6 and 8; and auto-fluorescence of the Nafion film without SNARF dye.

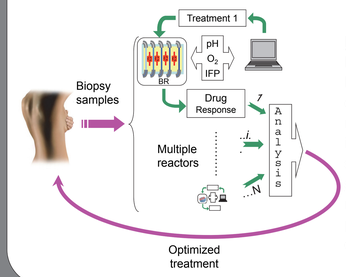
Conclusions

- BCTB Modular design: each reactor can be maintained under different flow (nutrient exchange) profile or connected to a single supply source via manifolds
- System allows for efficient delivery of a drug treatment (as confirmed by reduction of mammosphere formation by inhibiting proteinase activity)
- Mathematical model for diffusion inside cell chamber has been developed
- Oxygen and pH sensitive film for imaging of O_2 and pH distributions within the BCTB has been developed. O_2 film is compatible with standard cell culture. Its performance is unaffected by auto-fluorescence of collagen, phenol red, and gamma ray sterilization.
- Proper pH and oxygenation conditions within BCTB can be established by controlling pH of the supplied media and via oxygen diffusion through bulk PDMS.

Future Directions



Fully developed BCTBs to test a tumor biopsy's response to anti-tumor therapy under proper physiological conditions against the *in vivo* responsiveness of the tumor *in situ* to that same agent.



Personalized Treatment: A Breast Cancer Tissue Bioreactor will provide the controlled artificial tumor tissue microenvironment to maintain intact small breast cancer biopsy samples *ex vivo* under tumor specific conditions for testing the response of an individual patient's tumor to chemotherapy or molecular targeted therapies.

Acknowledgements

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