A GRAVITY-DRIVEN MULTI MICRO PHYSIOLOGICAL SYSTEM TO STUDY TISSUE RESPONSES TO CANCER THERAPEUTICS

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Statement of Purpose: Multi Micro Physiological Systems (MMPS) are in vitro models to study systemic drug responses in preclinical studies. However, many MMPS are complex, require tedious assembly, and are incompatible with high throughput drug testing. To address this, we developed a multi-well MMPS that is similar to a standard 96-well plate. Our system has three main tissue compartments that represent liver, tumor, and bone marrow. We selected liver and bone marrow because they are both common sites of off-target toxicity from cancer therapeutics and because the liver is a major site of drug metabolism. Our system is facile, utilizes 3D extracellular matrix-embedded cells, captures tissue toxicities, and is compatible with automated liquid handling. This model will facilitate evaluating effects of cancer therapeutics on both tumor and normal tissues in preclinical studies.

METHODS: The device was designed in SolidWorks. The distance between the wells matched that of a standard 96-well plate. The wells were connected via circular microchannels (d=800 µm) that reside higher than the well surface. Fluid flow among different wells was driven by hydrostatic pressure difference between two end reservoirs and tilting of the device. Fluid flow and mass transport in the device were simulated using COMSOL Multiphysics. The device consisted of a 3D printed block of PMMA attached to a thin clear film at the bottom. Three different polymer films were used to seal the bottom of the block and enable microscopy: a plate sealer with adhesive, a PMMA film, and a polycarbonate (PC) film. Each fabricated plate had 16 MMPSs and each MMPS had five linearly connected wells. Out of the five wells, the outer two were used as media reservoirs and the inner three were used as tissue compartments for human cell lines HEPG2/C3A, MDA-MB-231, and HS-5 to represent liver, tumor, and bone marrow stroma, respectively. Spheroids of cancer cells were formed prior to placing them in the device using our established aqueous two-phase system (ATPS). Tumor spheroids were embedded in a 4 mg/ml rat tail type I collagen hydrogel. Bone marrow and liver cells were embedded as single cells in collagen. Before confocal microscopy of cultures, cells in all the wells were stained with Calcein AM (ThermoFisher). A chemotherapy drug, Fluorouracil (5FU; 100 µM) and its prodrug, Tegafur (200 µM), were used to test drug responses of different cells over 4 days of culture. Tegafur was mixed with uracil at a 1:4 molar ratio, also known as a UFT cocktail, to prevent 5FU break down by hepatic cells. Each experiment had 4 replicates. Viability of cells were measured using Prestoblue (ThermoFisher).

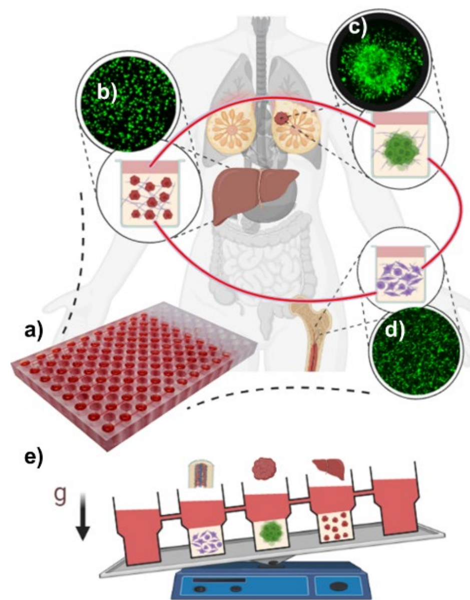

Figure 1. **Concept of the fabricated MMPS. a) the fabricated device, b-d) z-project images of HEPG2 (CAAM), MDA-MB-231 (GFP), and HS5 (CAAM) cells, e) gravity driven flow maintained by cyclical tilting of the device. Created in Biorender.com**

RESULTS: Fluid flow in the MMPS was simulated using an estimated pressure function. The simulation showed that metabolites are transported across different compartments by the cyclical advective flow. For example, a hypothetical compound ($D=10^{-9}$ m^2/s; 200 µM; 12.5 µl) introduced in one of the reservoirs was evenly distributed among the compartments in about 20 minutes. Next, we found that viability of cells on the PC and PMMA films was similar to that in standard culture plates. In contrast, the plate sealer showed markedly high cytotoxicity. Cancer cells invaded from the spheroid into the collagen matrix, and single-cell cultures of normal cells showed a uniform distribution and proliferation in collagen. Then, we measured the toxicity of 5FU and UFT to cells in all compartments. Viability of cells dropped to 50.4%, 22.6%, and 23.9%, respectively, for HEPG2/C3A, MDA-MB-231, and HS-5 cells when exposed to 5FU and 86.6%, 67.3%, and 87.8% when exposed to UFT.

Our design avoids the complexities of tubing and absorptions of lipophilic compounds associated with silicone. We aim to use our system to further investigate cancer therapeutics and provide a tool that can be used in preclinical studies.

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