A new microfluidics-based 3D tissue culture method facilitates rapid and simultaneous viability measurements on multiple samples, offering the possibility of patient-specific assays.

With certain cancers, such as ovarian cancers, a significant number of patients are non-responsive to the standard chemotherapy treatment. These patients, therefore, experience the negative side effects of chemotherapy without clinical benefits. With the ever-increasing number of available anti-cancer drug alternatives to chemotherapy, there is tremendous pressure on clinicians to make the right treatment choice. The biomarker approach—a statistical method that associates drug-response rates with specific patient characteristics—is effective for predicting which patients will respond best to a given treatment. However, only very specific cancer subtypes have associated biomarkers (i.e., the BRCA mutation for breast cancer). There is a crucial need, therefore, for a complementary predictive method that is applicable to virtually all types of cancers.

Clusters of cells, known as spheroids, are the most popular 3D tissue model in cancer research. These samples, which have standard diameters of around 400μm, are relatively easy to culture and they represent patient tumors better than traditional 2D cell cultures. The spheroids are often formed and cultured in miniaturized fluidic systems—or microfluidic chips—in which biological assays are performed. However, spheroids have been of little use in personalized therapy because they are formed using generic cell lines that do not reproduce the specificities of a patient’s tumor. A promising alternative would be to directly test therapies on small amounts of cancer tissue from patients, but this approach has had limited success in the past because of challenges associated with culturing patient tissue outside the human body and with developing detection methods to measure drug response in 3D tissue.

In response to these issues, we have proposed a novel approach for testing many drugs simultaneously on tissue from a specific patient (see Figure 1). We first developed a technique to cut biopsied tumor tissue down to the size of spheroid-sized samples. These individual samples are then introduced into a microsystem, in which different treatment options can be tested and their effects measured using various detection systems. Inset shows a top-view image of a sample trapped inside a well. The sample is labeled with fluorescent probes marking cells that are viable (green) and dead (red), and is imaged using confocal fluorescence microscopy. The results of the test may help medical specialists choose the most effective treatment for each patient.

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microfluidic chip. Typically, these probes have broad emission spectra. This means that we can detect only a limited number of the probes using traditional instruments, i.e., without overlapping signals. To quantify the overall viability of the microtissues, we used fluorescent probes that targeted viable and dead cells. We detected these probes with confocal microscopy and flow cytometry. We thus showed that microtissues—formed from mouse xenograft tumors and used as a cancer tissue model—remained alive for more than a week when cultured under non-treated conditions within our microsystems (see Figure 2).

![Figure 2](image1.jpg)

**Figure 2.** Viability results of the micro-dissected tumor tissue samples produced from mouse xenografts (formed using the OV90 ovarian cancer cell line) and cultured in a microfluidic chip. (A) Confocal microscopy results representing the relative area of live cells, over the total area of both viable and dead cells. The live and dead cells are labeled with CellTracker Green™ (green) and with propidium iodide (red), respectively. Representative confocal images are shown with their respective computed viability scores. Scale bars indicate 100μm. (B) Flow cytometry results of the samples dissociated into single cells and categorized by the instrument into three cell groups: late apoptotic/dead (7-aminoactinomycin D labeling), early apoptotic (annexin V labeling), and viable (absence of labeling). Error bars show the standard error of the mean, from at least three independent experiments.

We used these results to spearhead tests on human tumor tissue that was obtained from consenting patients who had undergone surgery at the University of Montreal’s hospital. Results from our preliminary chemoresponse tests seem promising. Ongoing improvements to our detection system, however, will increase the statistical significance of our results through greater multiplexing of fluorophores, allowing multiple measurements to be taken from a single microtissue at multiple time points.

To enhance the imaging speed and increase our ability to multiplex probes, we are developing a fluorescence spectroscopic imaging system that collects the fluorescence emitted by the samples at all wavelengths and forms a complete emission spectrum at each pixel of an image (i.e., a hyperspectral

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data cube). This custom-built widefield spectroscopic imaging system is based on the use of a liquid crystal tunable filter and has transmittance and fluorescence capabilities.\(^4\) As a proof of concept, we measured the fluorescence of spheroids that have two fluorescent markers. Our results showed that we can easily separate their contribution to the overall fluorescence, over the whole field of view of 1cm\(^2\) (see Figure 3). Since our imaging system is widefield and quantitative, the fluorescence intensity for each marker can be correlated to a number of marked cells, allowing us to measure cell viability of many samples over a wide area.

In summary, we have validated that microtissues remain viable within specifically designed microsystems, and have shown that it is possible to obtain rapid and simultaneous viability measurements on multiple samples. To take full advantage of the enhanced capabilities of our spectroscopic imaging system, we are currently optimizing the imaging intervals and determining the assortment of fluorescent probes that can be used to most accurately detect how anti-cancer agents affect the tissue. Our work will have a significant impact on patient survival and quality of life by providing medical teams with a new tool to predict patient responses to anti-cancer drugs.

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References


