ON-CHIP TRAPPING AND VIABILITY ASSESSMENT OF SUBMICROLITER PRIMARY TISSUES FOR PERSONALIZED TREATMENT OF OVARIAN CANCER

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ABSTRACT

Ovarian cancer appears in numerous forms with variable responses to the ever-growing panel of chemotherapeutic drugs available, underscoring the need for a personalized approach to select the best treatment for patients. Here, we report two designs of miniaturized platforms to trap live 3D primary tissue microsections and assay their viability through on-chip live-dead cell imaging. To achieve this goal, we combine microfluidics with a new technique to reproducibly cut tumor tissues down to submicroliter pellets using a vibratome followed by a biopsy punch. The described methods are also compatible with a wide range of tissue samples, both healthy and malignant.

KEYWORDS: Microfluidics, Tissue Viability, Cancer, Tumor, Personalized Therapy, Drug Testing

INTRODUCTION

Ovarian cancer is often asymptomatic with 63% of patients initially diagnosed at an advanced stage [1]. The wide range of tumor types and high intratumoral heterogeneity make this disease difficult to completely eradicate, which emphasizes the need for a method to determine the best treatment for each patient. Genomic personalized medicine applied to cancer is still of limited use due to the elevated number of genes implicated in the disease and the complexity of their expression which depends on multiple factors such as cell type and tumor micro-environment [2]. To circumvent these difficulties, empirical approaches to drug efficacy prediction have been developed, including the culture of primary precision-cut tumor slices that preserve cellular architecture and tumor-stroma interactions [3]. However, very few groups actually cultured cancerous tissue on chip [4, 5] and all types of primary tissue cultured to date in microfluidic devices were several millimeters in diameter [4-6], making them challenging to manipulate en masse in microchannels. Our group recently developed a microfluidic platform to trap and analyze the chemosensitivity of cancer spheroids [7]. Though they constitute a simple and promising 3D tissue model, spheroids currently lack the unique cell characteristics of primary tissue that are essential to personalized medicine. We hereby extend the application of our trapping devices to tissues of increased complexity by introducing a method that adapts vibratome cutting to the production of submicroliter pellets with high size reproducibility and varying viability. Combined with either of the two proposed microfluidic trapping mechanisms, it enables the parallel testing of multiple chemotherapies on individual tumor microsections produced from minimal amounts of material, such as those typically obtained from biopsies.

THEORY

Loading of primary tissue on chip, so far, has been achieved by inserting precision-cut slices [6] or biopsied tissue [4, 5] into large chambers, the dimensions of which were above one millimeter. Our technique combines two traditional instruments for soft tissue sectioning, namely a vibratome and a biopsy punch, to produce submicroliter tissue pellets compatible with microfluidic manipulation (Figure 1A). The pellets were loaded into two types of systems in which their viability was assessed: resistive trapping and gravitational trapping. As previously described for spheroid entrapment [7], the resistive trapping systems are composed of bottleneck-shaped center channels of inferior hydraulic resistance where tissues get immobilized and bypass channels for the circulation of additional samples to the following traps (Figure 1B). In the gravitational trapping system, the tissue pellets circulate in the upper layer and fall into microfluidic pits due to the gravitational forces acting upon them (Figure 1C). Since sedimentation velocity increases as the square of the particle radius at low Reynolds numbers, tissue pellets achieve much faster sedimentation than single cells. Live-dead cell confocal imaging of tissue pellets through the microfluidic chip provides an overall representation of viability to depths of about 40 μm inside individual samples while not appearing to affect image quality.

EXPERIMENTAL

To form the mouse xenografts, one million epithelial ovarian cancer cells (TOV112D) were mixed in Matrigel (BD Biosciences, Mississauga, Canada) and were subcutaneously injected into severely combined immunodeficient (SCID) female mice (Charles River Development, Burlington, USA). Xenografts, with volumes between 900 and 2200 mm^3, were harvested, cut to fragments of 1-3 mm in width by approximately 10 mm in length, and immediately placed in ice-cold Hank's buffered saline solution (HBSS). Ovarian tissue from consenting cancer patients was obtained post-surgery and resized tissue fragments were placed in ice-cold HBSS. Tissue fragments were then embedded in low melting point agarose (Invitrogen Corp., Grand Island, USA) supplemented with 10% fetal bovine serum (FBS) while the solution was still liquid at 37°C. Once the agarose solidified, the fragments were repositioned vertically for slicing (300 μm slices)

using a traditional vibratome (The Vibratome Company, St. Louis, USA) and further sectioned using 300 µm and 500 µm biopsy punches (Zivic Instruments, Pittsburgh, USA) into submicroliter disk-like pellets. The reported tissue pellet diameters represent the average of two perpendicular measurements per sample calculated from confocal microscope images.

The two different polydimethylsiloxane (PDMS) channel configurations and trapping systems – resistive and gravitational trapping – were fabricated using traditional soft lithography techniques. Tumor pellets were cultured inside the microfluidic chips for up to 72 hours in media supplemented with 10% FBS. Dual fluorescent staining of the tissue pellets was performed at different time points using CellTracker™ Green (CTG) (Invitrogen Corp., Grand Island, USA) labeling the cytoplasm of viable cells and propidium iodide (PI) (Sigma-Aldrich Corp., St. Louis, USA) labeling the nucleus of apoptotic or necrotic cells. Finally, the tumor pellets were imaged on-chip in 10 µm-spaced optical slices using an inverted confocal microscope (Leica Microsystems GmbH, Wetslar, Germany) and maximum projections of the z-sections were computed.

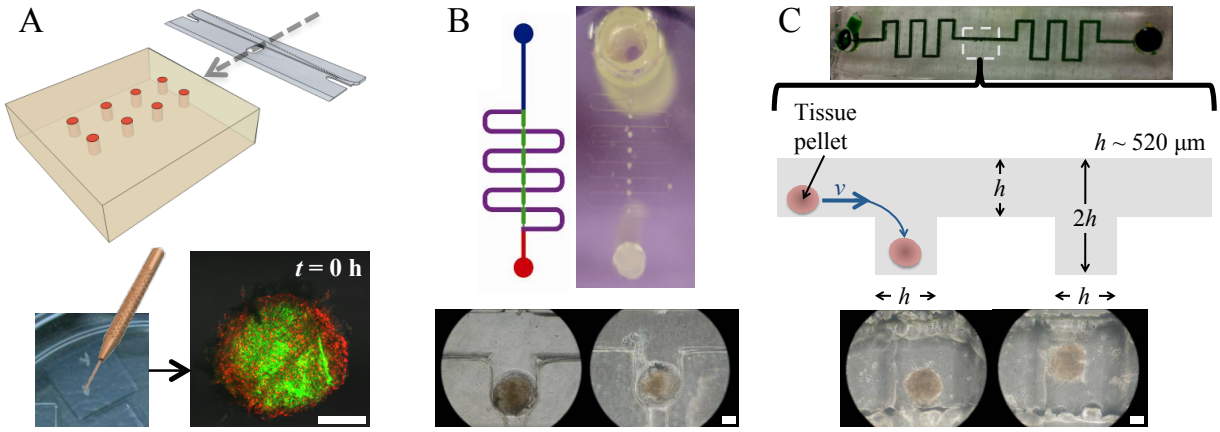

Figure 1: Tissue sectioning and loading into microfluidic trapping devices. A) Schematic of tissue sectioning using a vibratome (top) and a biopsy punch (bottom, left); confocal z-stack projection of a viable human ovarian tissue pellet labeled with CTG and PI (bottom, right). B) Resistive trapping with bottleneck-shaped center traps marked in green and bypass channels marked in violet (top, left); image of system with trapped pellets (top, right); pellets imaged in resistive traps using a light microscope (bottom). C) Image of a gravitational trapping system filled with colored solution (top); side view schematic of gravitational trapping where samples sediment into microfluidic pits (center); pellets imaged in gravitational traps (bottom). Scale bars = 100 µm.

RESULTS AND DISCUSSION

Our tissue cutting technique typically enabled the formation of 10 to 20 individual tissue pellets from a single 10 mm long fragment, thus allowing for multiple tests to be run in parallel. Although the heterogeneity of tissue consistency sometimes impeded slicing, the technique produced pellets of human ovarian tissue from the limited number of available human cases (Figures 1A and 2B) and pellets of mouse xenograft tissue (Figure 2A). The latter were preferred as an initial tissue model since higher control on cellular composition, tumor formation and tissue extraction was achieved. The disk-like tissue pellets had mean diameters of 348 ± 57 µm (average ± standard deviation, N=74) and 225 ± 27 µm (N=12) when micro-dissected using the 500 µm and 300 µm biopsy punches respectively. The pellets were loaded into resistive and gravitational trapping systems with success rates above 80% (number of trapped pellets / number of micro-dissected pellets). With each trap usually capturing only one sample, the number of loaded pellets per device was solely limited by the number of designed traps: 6 to 8 traps for the resistive trapping systems and 2 traps for the gravitational trapping systems. The gravitational trapping offered superior sample stability compared to resistive trapping with trapped pellets withstanding flow rates up to 800 µL/min (average velocities in channel of ~5 cm/s) and resisting slight perturbations of the devices during manipulation.

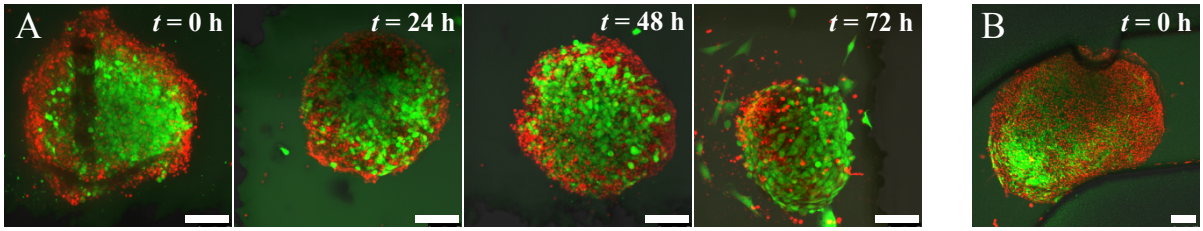

Figure 2: Representative confocal z-stack projections of tissue pellets of intermediate viability labeled with CTG (green) and PI (red) at different time points. A) Mouse xenograft tissue pellets loaded in gravitational trapping systems. B) Human ovarian tissue pellet imaged in the bypass channel of a resistive trapping system. Scale bars =100 µm.

Tumor pellet viability was variable between samples treated similarly within a same experiment. While some samples remained viable for up to 72 hours inside the microfluidic devices (Figure 2A), as was also shown with liver precision-cut slices by van Midwoud et al. [6], others lost viability after only 24 hours (Figure 3C). We observed reduced viability in the ring-shaped region on the periphery of many pellets (Figures 2A and 3B), possibly due to the stress exerted by the biopsy punch on the cells adjacent to the sectioned surfaces. This effect, however, wasn't noted for certain highly viable pellets (Figure 3A) and couldn't explain the mortality distribution of other samples (Figure 3C). We suspect that the variable mortality may be due to tumor pellets being extracted from regions affected by hypoxia and nutrients deprivation to different degrees. Although better control on viability is desirable, close to half of the observed xenograft samples at 24 hours had retained high to intermediate viability, which might prove sufficient to measure the early response of tissues to chemotherapeutics.

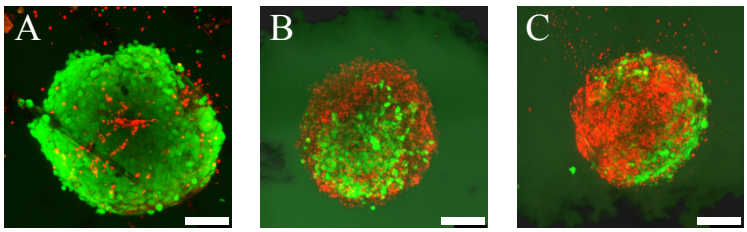

Figure 3: Representative confocal z-stack maximum projections of mouse xenograft tissue pellets displaying high (A), intermediate (B) and low viability (C) after 24 hours within the gravitational trapping systems. Scale bars =100 μm.

CONCLUSION

The microfluidic approach described here can readily be adapted to biopsied tissue of different cancer types and was designed with maximum simplicity in mind to eliminate the need for user specialization. The viability of some of the tested primary tumors, up to 72 hours, is sufficient to allow incubation or perfusion of samples with drugs. The number of traps per system could easily be scaled up to accommodate a larger number of samples so that statistically relevant data on drug efficacy can be extracted prior to treatment, even from a limited proportion of viable pellets. This demonstrates the potential of our method as a direct, empirical way to test chemosensitivity on chip that could directly complement current methods to predict the outcome of a therapy, such as biomarkers and histopathology.

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