Microfluidic Tissue Processing Platform for Single Cell Analysis and Primary Cell Isolation

[1]Jeremy Lombardo, [1]Marzieh Aliaghaei, and [1]Jered Haun

[1]Deparment of Biomedical Engineering, University of California, Irvine

Introduction: Tissues are highly complex ecosystems of different cell types. To capture this heterogeneity, high throughput single cell analysis methods are increasingly being employed. These methods, however, require that tissues first be dissociated into cellular suspensions, and this process represents a bottleneck hindering these efforts. Conventional protocols are inefficient, relying on manual, time-consuming, and variable steps. Advances in microfabricated technologies, however, hold exciting potential to carry out these procedures on-chip in a high throughput, repeatable, and automated fashion. Here, we present a microfluidic platform consisting of 3 different tissue-processing technologies that significantly improves the breakdown of diverse tissue types into high quality cell suspensions that are ready for downstream single cell analysis. First, we integrated our Dissociation Device, which uses a network of branching channels to break down aggregates into single cells and small clusters, with our Filter Device, which eliminates remaining aggregates and dissociates small cell clusters into single cells. Next, we created a new Minced Digestion Device concept for working with minced tissue (Fig. 1B), and which can integrate with the combined device in Fig. 1A.

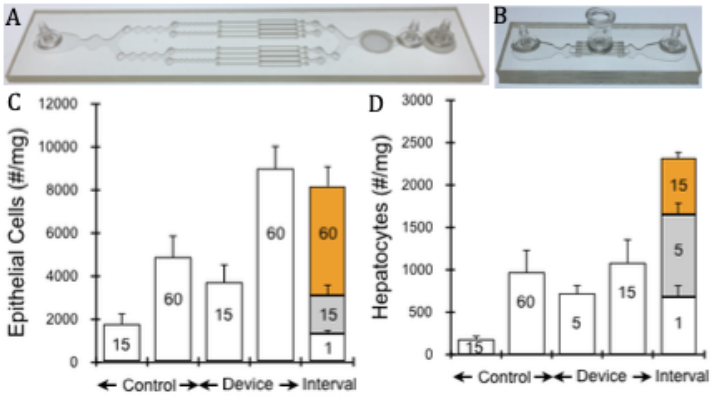

Figure 1. A) Integrated Dissociation and Filter Device. **B)** Minced Digestion Device. **C&D)** Mouse (C) mammary tumor and (D) liver tissues were minced, digested for the indicated times (in minutes), and passed through the dissociation/filter device.

Materials and Methods: Microfluidic devices were fabricated by Aline, Inc. using a commercial laminate microfabrication process. Briefly, fluidic channels were laser-machined into hard plastic layers, which were then pressure laminated. Devices were tested using murine mammary tumor, liver, kidney, and heart tissues. Samples were first minced with a scalpel to ~1 mm^3 pieces and then processed with the Digestion Device for different period of time. This was followed by a single pass through the dissociation/filter unit. These tests also utilized a novel concept, interval processing, in which sample is recovered from the devices at periodic intervals to minimize overprocessing and maximize cell. Control samples were digested with collagenase, with repeated pipetting/vortexing to mechanically disrupt aggregates, and filtering with a cell strainer as with standard manual protocols. Cell counts were obtained using flow cytometry to identify live and dead epithelial cells (tumor & kidney), hepatocytes (liver), or cardiomyocytes (heart), along with endothelial cells and leukocytes. Single cell RNA sequencing (scRNA-seq) was also performed on kidney and tumor samples to comprehensively assess cellular stress responses.

Results: For murine tumor tissue, 15 and 60 min device digestions generated ~2-fold more epithelial cells than their respective controls (Fig. 1C). Interval recovery did not affect recovery for tumors. Similar results were seen with murine kidney (data not shown). Liver is known to be a softer, so shorter 5 and 15 min static device digestions and 1, 5, and 15 min interval digestions were chosen. The 15 min static condition produced more hepatocytes than a 60 min control. Interval collections greatly increased hepatocyte yield, with the sum of the intervals yielding >2-fold more cells than the 60 min control, with 4-fold shorter in processing time (Fig. 4D). Similar results were seen with heart tissue (data not shown). Preliminary scRNA-seq results with kidney also suggested that device processing can produce more tubule and endothelial cells, which are hard to liberate. Moreover, stress responses can also be minimized using digestion times. Viability after all device treatments remained comparable to controls conditions (data not shown).

Conclusions: To improve single cell analysis of tissues, we have designed and fabricated a suite of microfluidic devices that function on different size scales to improve the quality of single cell suspensions obtained from tissues. Our experimental results demonstrate that integrating these device increases single cell recovery from diverse tissues without compromising cell viability. Notably, the interval operation may establish a new paradigm for tissue dissociation that can reduce stress responses and better preserve softer and more fragile tissues. In future work, we envision incorporating cell sorting and analysis capabilities on-chip to achieve fully point-of-care single cell diagnostic platforms.