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A new microfluidic workflow to study the effects of spatiotemporal gradients in tumor microenvironments

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Globally almost 10M people die from cancer each year. While a large body of research exists on cancer-specific genetics, the role of the tumor microenvironment (TME) in driving cancer development, metastasis, and recurrence remains vastly unexplored. The TME is highly complex in vivo and can only be studied in an observational setting. To understand causality of environmental effects, in vitro methods are necessary.

Currently, one of the main challenges for in vitro analysis is to measure how cells respond to long-term gradients of environmental states, beyond imaging experiments. Specifically, gene expression analysis of cells with precise spatiotemporal gradient information is necessary for an in-depth understanding of these processes.

To address this challenge, we aimed to design a microfluidic device that enables analysis of how multifactorial microenvironmental gradients impact cancer cell biology and behavior in pH mimicking that in the TME. To do so, we developed a device with a workflow that can create a gradient over a large area ($cm^2$), containing in situ sensors, and with a reversible sealing to later perform spatial transcriptomics.

Our microfluidic device consists of a PDMS chip in which channels are molded following the design in (a). This chip is sealed onto a glass slide on which both cells and spheroids have been cultured in biologically relevant hydrogels (b). Next, pH gradients and temporal pH oscillations are applied on the cells (c), following a prior

successful characterization of the gradients using fluorescent sensor beads or pH-responsive dyes (d). This device allows us to perform live cell imaging. It can be subsequently opened (e) for immunofluorescence analysis (e.g., live/dead staining, (f)). The ultimate goal is to apply spatial transcriptomics to characterize the gene expression response to each TME condition imposed by the device (g); to that end, spatially resolved RNA extraction and capture has been optimized.

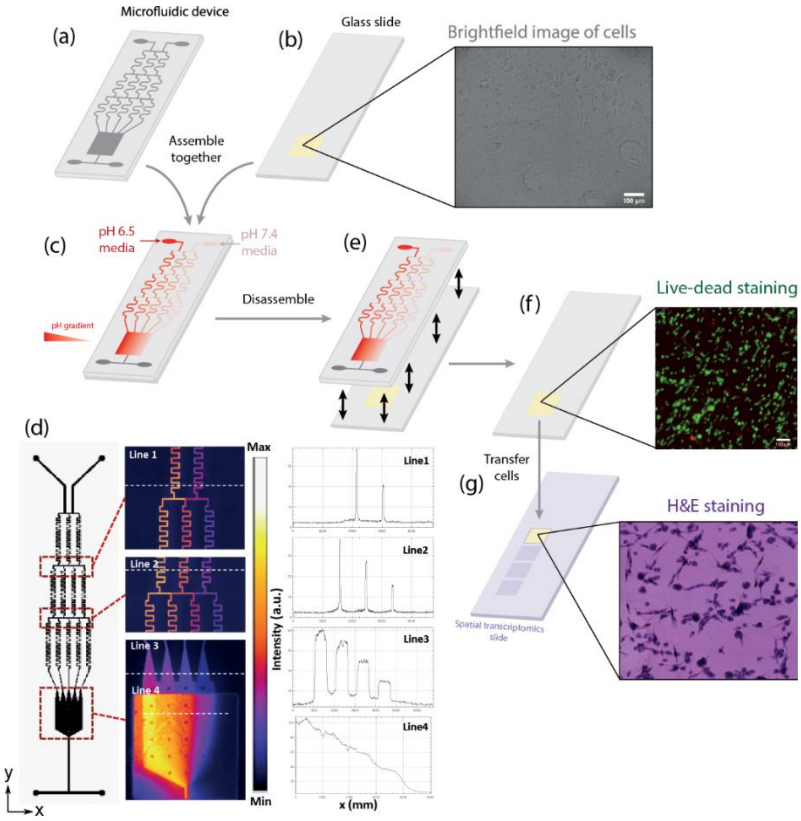


Figure: (a) schematic view of the PDMS chip with the pH gradient design; (b) growth of cells and spheroids in hydrogels on glass slides; (c) pH gradient applied on cells or spheroids in hydrogel, using the microfluidic device; (d) characterization of pH gradients and temporal pH oscillations obtained in microfluidics; (e) microfluidic chip disassembled from the glass slide; (f) live dead staining using Calcein AM (3 µM) and Propidium Iodide (3 µM); (g) H&E staining of the cells to optimize for RNA extraction and spatial transcriptomics

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