

Short Note

Not peer-reviewed version

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Posted Date: 30 December 2025

doi: 10.20944/preprints202512.2627.v1

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Short Note

Commentary of Paper Describing a Microfluidic Cancer-on-Chip Platform Predicts Drug Response Using Organotypic Tumour Slice Culture

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Abstract

This preprint describes contemporary research on using microfluidic platform to culture organ-like tumour slice for the purpose of drug screening and for predicting drug response. The original paper is at: A Microfluidic Cancer-on-Chip Platform Predicts Drug Response Using Organotypic Tumour Slice Culture, *Cancer Research*, Vol. 82, Issue. 3, pp. 510-520, Year: 2022.

Keywords: tumour slice; microfluidic chip; drug screening; drug response; organotypic

Subject areas: biochemistry; cell biology; biomedical engineering; biotechnology; medicine

Microfluidics applications in biological research have seen important advances in our fundamental understanding of microflow in biological mimicry systems. One area that has made important contributions to drug screening and disease modelling is microfluidic organ on a chip (OoC) system. Manuscript that is reviewed in this commentary solves an important challenge in the OoC field that may help made conclusions that we derived from OoC drug screening studies for cancer patients more physiologically relevant and personalized.

Typically, researchers using OoC systems to model cancer cells behaviour and screen for drugs useful for treating particular cancer would first use primary cancer cells extracted from a solid tumour biopsy to seed the microfluidic channels of the OoC.¹ With infusion of nutrients from microfluidic flow, the cells would grow to confluency in the microchannels. Depending on the cancer, additional growth factors may be needed in the growth medium to maintain the cancer cells' phenotype.² Upon achieving confluency, drugs are added to the growth medium to screen for their potency against the patient's derived cancer cells.

Examining the aforementioned system would reveal that a typical OoC cancer drug screening chip could only support a limited number of cancer cells. From a physiological perspective, this setup may not mimic the tumour environment in a patient such as inability to fully recapitulate the metabolism, growth behaviour, and gene expression pattern of cancer cells in a malignant solid tumour. Thus, experiment data derived from the common OoC chip may be biased towards low efficacy drugs that consequently leads to poor patients' outcome.

The approach taken by the authors (Chakrabarty et al.) in this manuscript is to use microflow system to support a tumour slice obtained from the patient instead of growing tissue-like constructs from primary tumour cells in microchannels. Using an innovative microfluidics cancer on chip (CoC) design, the authors published early results of their endeavour to develop a microfluidics system that could mimic the growth and gene expression profile of a tumour slice. Such engineered systems would bring forth new insights into the physiology of tumour cells in a solid tumour (basic science research) as well as better inform oncologists of suitable drug treatments with better efficacy for each patient (medical research). A key feature of the authors' chip is the ability to provide nutrients to both the top and bottom surfaces of the tumour slice as they are in contact with microfluidic flow, and is the primary reason why large multilayer tissue slice could be maintained for up to 14 days on the

chip. As it is impossible to compare the CoC chip's performance with a patient derived xenograft (PDX) on a mouse using a time-course design, the authors settled on an alternative ex vivo culture of similar tumour slices from the same patient in tissue culture plates on a rotary shaker that ensures good mixing and diffusion of oxygen and removal of carbon dioxide from growth medium. The ex vivo tumour slice culture system serves as a good comparison of the authors' CoC system.

In the project, the authors first assessed the performance of the cancer on chip (CoC) method compared to ex vivo tumour slice culture for cisplatin sensitive and resistant tumour PDX using staining for cell nuclei (proxy for cell number), S-phase cells (proxy for cell proliferation), and apoptotic cells. The same approach is extended to another class of drug, apalutamide for PC82 and prostate tumour PDX. Next, the authors undertook a long-duration (14 days) culture of CoC with breast PDX to check for reproducibility of Ki-67, cell nuclei numbers, S-phase cells, and apoptotic cell between CoC and ex vivo tumour slice culture. Finally, gene expression analysis was conducted via a RT-qPCR panel and RNA-seq to assess for concordance in gene expression pattern for CoC and ex vivo culture relative to Day 0 PDX.

In terms of overall experiment design, the authors used a cell biological combined brightfield and fluorescence microscopy approach to image different biomarkers and cell state in the tumour slice. Quantification of the expression level of specific biomarkers or cells in different cell states (i.e., proliferating or apoptotic) is through automated artificial intelligence accounting for image pixel with particular wavelength of light, and aggregating light intensities of these pixels. Such an approach provides good estimates of expression level of protein biomarkers, but would require careful calibration for size and shape in the case of extracting cell number information from the aggregated light intensity measured.

Fluorescence detection methods have gained prominence in many areas of cell biology and biomedical engineering research. In essence, implementation of fluorescence detection technique requires a recognition module and fluorophore module. Traditionally, these fluorescence biomolecules need to be conjugated and introduced into cells through various means. But, recently, there is an alternative method to implement fluorescence detection in both live and dead (fixed) cells. In this approach, the recognition module is separated from the fluorescence (fluorophore) module, and both modules are separately introduced and "conjugated" together in the cell through biorthogonal click chemistry. This approach ameliorated the challenges of introducing fluorophore conjugated antibodies or aptamers into cells as they may not transit smoothly through the ion channels or porins on the cell envelope. Fluorescence biomolecules, EdU and TUNEL, chosen by the manuscript's authors uses the latter approach. Although both reagents are commercially available, utilisation of click chemistry may have as-yet unknown biological effects on cells or the phenomenon we sought to observe. Fortunately, the authors did not seek to perform time-course live cell imaging with their chosen fluorescence biomolecules, and the quantitative imaging approach is robust given the molecular specificity of the recognition module on both fluorescence biomolecules.

Gene expression analysis is one major approach that many researchers sought to compare the biological effects of drug treatment or to assess how closely engineered cell culture system mimic ex vivo culture. Two major techniques, OncoSignal RT-qPCR and RNA-seq, were employed by the authors. Specifically, OncoSignal uses a set of pre-defined genes to assess activity of specific oncogenic pathways, while RNA-seq is an unbiased profiling of expression level of all genes. Uses of two complementary approaches enriches the discussion.

Summary of data presented: Across cisplatin sensitive and resistant breast tumour slices, the CoC chip culture resembles the PDX Day 0 in morphology and staining pattern for DAPI, EdU (cell proliferation) and TUNEL (apoptosis). In the case of tumour slices that have undergone drug treatment, fluorescence microscopy images showed consistent response of the tumour slice across ex vivo and CoC culture. For example, cell nuclei decreased, which is accompanied by drastic decline in cell proliferation and significant increase in cell apoptosis. Quantification numbers of the cell proliferation (S-phase cells) and apoptosis cells revealed consistent response to drug treatment in

both ex vivo culture and tumour slice CoC systems, i.e., decline in proliferating cells, and increase in apoptotic cells with drug treatment.

Similar approach was used for apalutamide treatment of PC82 and prostate cancer tumour slice. Specifically, Day 7 CoC tumour slice resembles Day 0 PDX in morphology (H&E stain), cell numbers (DAPI stain), and relative abundance of proliferating cells (EdU) and apoptotic cells (TUNEL), thereby, showing utility of the CoC system. Treatment with apalutamide in PDX CoC changed cell morphology, reduced number of proliferating cells, and increased numbers of apoptotic cells. Quantification of fluorescence signals showed that prostate CoC cultures is able to recapitulate androgen receptor (AR) expression in ex vivo cultures at Day 7. Automated quantification of S-phase and apoptotic cells again showed a treatment response and CoC tumour slice could recapitulate the responses from ex vivo tumour slice culture.

Next, the authors tested 14-day culture of breast PDX with CoC chip. Brightfield H&E staining data and fluorescence microscopy data reveals that Day 7 CoC culture resembles Day 7 ex vivo tumour slice culture in staining pattern for H&E stain, Ki-67 (cell proliferation), DAPI (cell nuclei proxy for cell number), EdU (S-phase cells) and TUNEL (apoptotic cells). Results indicate that Day 7 ex vivo tumour slice culture remain viable and resembles the Day 7 CoC culture. However, there is lack of resemblances in the Day 14 ex vivo and CoC tumour slice culture. Specifically, there is reduced proliferation (Ki-67 and S-phase cells), and more apoptotic cells in Day 14 ex vivo culture compared to Day 14 CoC culture. Results suggest that microfluidic flow could support long-term culture of tumour slice in CoC chip.

Quantitative image analysis for 10 field of views of each tumour slice reveals that CoC Day 7 culture resembles ex vivo tumour slice Day 7 culture and PDX Day 0 culture in cell proliferation (S-phase cells) and apoptotic cells profile. Results concur with qualitative images from microscopy analysis. The same quantitative image analysis was performed for Day 14 CoC and ex vivo cultures. Again, quantitative results concur with qualitative images from microscopy. Overall, Day 14 CoC culture more closely resembles Day 0 PDX culture than Day 14 ex vivo cultures. Specifically, Day 14 CoC culture has higher cell proliferation and less apoptosis compared to Day 14 ex vivo tumour slice culture. Phenomenon could be due to sufficient transport of nutrients brought forth by microfluid flow in CoC culture. Relatively broad spread in quantitative values could be due to spatial heterogeneity in the tumour slice.

The authors sought confirmatory support of the high abundance of growth phase cells in their CoC culture by employing staining for the nuclear protein Geminin, which is a marker for cell proliferation and cell lineage commitment. Fluorescence microscopy data reveals that Day 7 CoC culture resembles closely to Day 0 PDX in both cell nuclei (DAPI) and Geminin (red nuclei) staining. On the other hand, there is less concordance between the DAPI and Geminin staining pattern of ex vivo Day 7 tumour slice culture and Day 7 CoC culture as the ex vivo culture reveals smaller nuclei. Next, the authors attempt to quantify the expression level of Geminin per nucleus. Results reveal that there is higher expression of Geminin in Day 7 CoC cultures compared to Day 7 ex vivo culture. Highest number of Geminin expressing nuclei and nucleus with highest Geminin concentration are in Day 0 PDX. Plotting the ratio of Geminin expressing cells to total number of cells reveal that CoC Day 7 culture has higher number of Geminin+ cells compared to ex vivo Day 7 tumour slice culture; indicating CoC Day 7 culture may be more differentiated and viable.

Finally, gene expression analysis was employed to assess the phenotype of breast CoC and PDX culture. Seven pathways (MAPK, Notch, PI3K, TGFb, Hedgehog, AR, ER) were assessed via the OncoSignal RT-qPCR approach. Notch has the highest pathway activity level. Across all pathways, Day 7 and Day 14 CoC cultures show similar pathway activity level compared to their corresponding ex vivo tumour slice culture and Day 0 PDX, indicating that except for Notch, these pathways may not be transcriptionally connected to the effects of microfluidic culture. RNA-seq heatmap reveals higher expression of interferon (IFN) genes in ex vivo Day 7 culture compared to CoC Day 7 cultures, suggesting that microfluidic flow may reduce IFN signalling. This may come about due to removal

of antigens that bind cell surface receptors connected to interferon signalling through microfluidic perfusion flow in CoC Day 7 culture.

Future directions for the research may investigate how metastatic cancer cells leave the top and bottom surface of the tumour slice through optical imaging at the top, and profiling for circulating cancer cells in the bottom microfluidic flow. In addition, the CoC chip can be used to understand how different microflow velocity and shear stress affects growth, apoptosis, and gene expression pattern of the cancer cells in the tumour slice. One limitation for the current study is the lack of a tumour microenvironment in the CoC system. Perhaps, growing a monolayer of endothelial cells underneath the tumour slice may help.

Funding: No funding was used in this work.

Conflicts of interest: The author declares no conflicts of interest.

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