A microdroplet incubator to establish 3D organoids cultures from oesophageal adenocarcinoma.

Team : Gianmarco Contino (Rebecca Fitzgerald Lab, MRC-CU, Cambridge University) provides the biological expertize and reagents to design and optimize the incubator. **Ziyi Yu** (Chris Abell Lab, Department of Chemistry, University of Cambridge) manufactures the incubator and participates to its optimization. They contribute to the design of the incubator.

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Summary

We propose to build a self-partitioned microdroplet array to grow 3d organoid cultures from oesophageal cancer biopsies. Organoids faithfully recapitulate the biology of the primary tumour and are an ideal model to test chemo-sensitivity for personalized therapies and interrogate the role of specific mutations. Currently the use of organoids is limited by the lack of standardization, poor replicability and the high cost of culture reagents. A microdroplet incubator can overcome these problems by allowing the growth of multiple organoids from the same patients on a single scaffold and microdose them with different compounds including drugs and gene editing reagents.

Proposal

Over the last 40 years, the incidence of oesophageal adenocarcinoma (EA) has increased more than 6-fold in western countries. The overall age-adjusted incidence in the United States is 2.7 cases per 100,000—a figure that reaches 6 and 9.4 per 100,000 among American and British white men respectively. Overall 5-year survival is approximately 20% and about half of patients die within a year of diagnosis. However, less than 50% of patients diagnosed early enough for curative treatment (surgery and neoadjuvant chemo or chemoradiotherapy) survive for 5 years.

Currently, the lack of suitable in vitro and in vivo experimental models of oesophageal adenocarcinoma is the major bottleneck to understanding the biology and testing novel therapies. There are less than 15 commercial cell lines that do not faithfully represent the genomic heterogeneity of oesophageal adenocarcinoma and whose results are difficult to translate to clinical applications.

We have established a method to grow 3D cultures directly from patient's biopsies. This model system called organoid is a 3D cell culture established from patient's biopsies. Organoids faithfully recapitulate the biology of the primary tumour and therefore represent an ideal model to analyse the effect of novel mutations and predict the response to therapies.

To become an effective tool, culture of organoids should be cost-effective, reproducible and allow analysis of several different experimental conditions. Currently, successful organoid culture is operator-dependent and requires high quantities of expensive 3D matrices (i.e. matrigel) and custom made media. **There is a huge need of culture scaffolds that allow high throughput organoid growth, minimize the use of reagents and can be used for direct in-vitro analysis of multiple experimental conditions.**

Aim of the project:

We aim **to build a self-partitioned microdroplet array for 3D organoid culture** from a single cell solution of oesophageal adenocarcinoma with the following characteristics:

- Each cell is embedded in a separate droplet of matrigel
- Each droplet is hosted in an isolated compartment that allows selective exposure to specific reagents
- Each compartment will require a few microliter of media
- The device can be analysed using in vivo microscopy.

Specific Aims

Aim 1 Design and produce a microdroplet device with multiple compartment able to host organoid containing matrigel droplets.

A hydrophilic–hydrophobic patterned microfluidic device will be fabricated by laser surface treatment. Due to the extreme difference in wettability between hydrophobic and hydrophilic areas and the phenomenon of discontinuous de-wetting, matrigel solutions applied onto such surfaces spontaneously form an array of separated microdroplets. Each of these droplets can serve as a microreservoir, resulting a single cell could be

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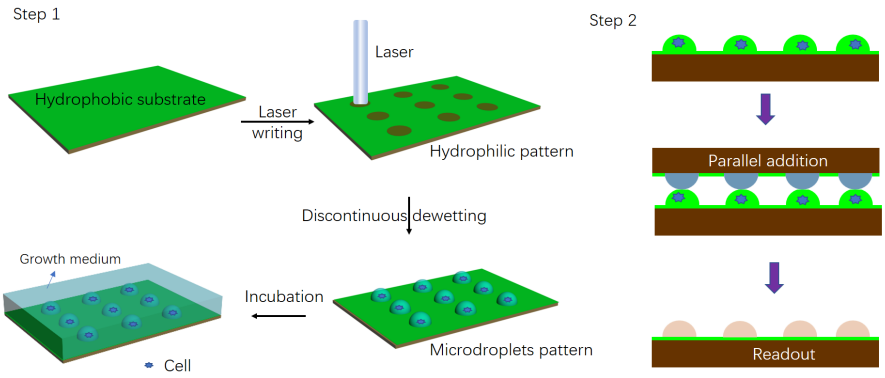

Aim 2. Test and optimize the device with an oesophageal adenocarcinoma cell line

We will test the device using an oesophageal adenocarcinoma cell line (MFD1) derived from a patient. We will prepare a single cell solution and embed each cells in droplets of matrigel with a microfluidic technology already developed in the Abell lab. In alternative we will prepare a low concentration solution of MFD cells and plate them on the microfluidic device. We will optimize the dilution to ensure the maximum number of compartment will contain a single cell. In order to ensure that the growth of organoids is clonal (i.e. derive from a single cell), we will utilize a fluorescent membrane marker whose intensity will be progressively diluted at each mitosis. In contrast, cell cluster will appear as a homogeneous staining of cell clusters. Cells will be grown in an organoid specific media until spheroids are formed.

Subsequently, we will dose each droplet with media containing increasing concentration of cisplatin and analyse cell viability/growth with time lapse microscopy.

Future developments

Upon optimization of design and culture conditions we will start testing single cell solutions of patient biopsies. There is potential to develop further this device for use to other cell types. This will include cells derived from biopsies of a common premalignant condition associated to development of esophageal adenocarcinoma called Barrett's oesophagus as well as other cancer types. We have an interest in develop this model system to perform functional assays, Crispr gene editing and single cell sequencing.

Estimate the components and budget that you need to complete the project

1) Microfluidic device fabrication: 3-month Makespace membership (120 GBP)
2) A4 mask for photolithography (105 GBP)
3) Chemicals including TRICHLORO(1H,1H,2H,2H-PERFLUOROOCTYL) etc. (240 GBP)
4) Matrigel (250 GBP)