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# FROM EMULSION TO SINGLE-PHASE MICROFLUIDICS: AN INTEGRATED APPROACH TO CULTURE AND PERFUSION OF MULTICELLULAR SPHEROIDS

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## ABSTRACT

This study presents a novel microfluidic approach for developing large scale screening assays of anticancer compounds on 3D multicellular spheroids. We have developed a microfluidic device with associated protocols that combine the high-throughput characteristics of droplet microfluidics for spheroid formation and aggregation with those of single-phase microfluidics for substance exchange, long term culture and drug perfusion.

**KEYWORDS:** Droplet microfluidics, spheroids, cancer, 3D cell culture

## INTRODUCTION

In the past decade, there has been an increase in the identification of potential anticancer drugs. However, less than 10% are approved after clinical trials[1]. One of the major issues is the unsuitability of the in vitro models used in preclinical settings. The use of 3D cell culture models, such as those based on multicellular tumour spheroids, is expected to provide a more accurate reflection of the tumour microenvironment in vivo. Currently used spheroid formation techniques suffer from several limitations, such as being labour intensive and low throughput. Emulsion based technologies can overcome such disadvantages by offering increased mechanical stability and high throughput methodologies through miniaturisation via droplet microfluidics. However, a drawback of the technology for cell culture is compartmentalization and we have previously shown the related challenges and detrimental effects involved in medium exchange and delivery of compounds[2]. Therefore, microfluidic solutions must provide access to the encapsulated cellular environment and typically these are based on double emulsions and gel particles[3], [4], conditions that increase the complexity of the microfluidic setup.

## EXPERIMENTAL

In this study, we present a microfluidic device based on a passive channel network[5], [6] that uses first droplet microfluidics to create and position spheroids in predefined locations and, subsequently, induces destabilization of the emulsion interface without compromising the cell culture conditions, transitioning from segmented flows to single-phase flows (Figure 1). It was discovered that the properties of cell culture medium caused coalescence of the emulsion interface without reducing the surfactant concentration. This mechanism was exploited to coalesce droplets containing spheroids, thus allowing for substance exchange (Figure 1C).

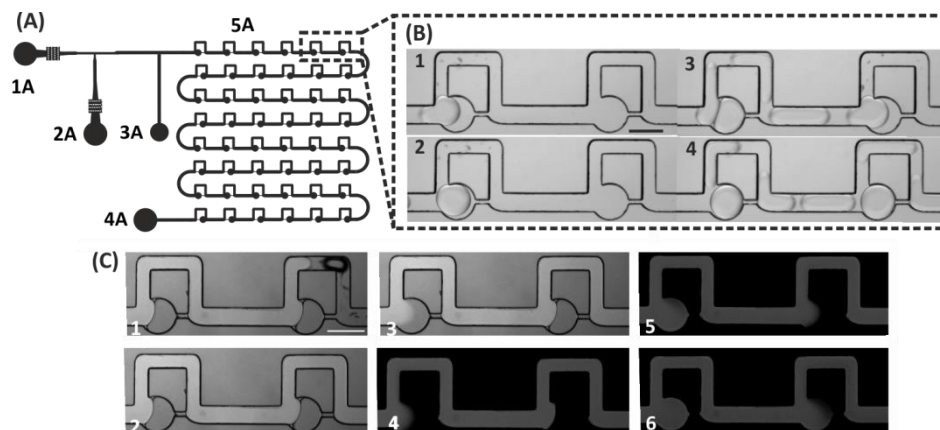


Figure 1: (A) Design of microfluidic device with oil inlet (1A); aqueous phase inlet (2A); air bypass channel (3A); outlet (4A) and chamber array (5A). (B) Brightfield images showing the trapping of

droplets (1) and the bypassing of a droplet to fill the next trap (2 to 4). (C) Brightfield and fluorescent images showing the coalescence of a long plug containing calcein with trapped droplets only containing medium. The scale bars are 400  $\mu\text{m}$ .

## RESULTS AND DISCUSSION

We used UVW glioma cells to initially create spheroids within droplets, taking advantage of the non-adherent interface features created by the PEG-fluorosurfactants<sup>2</sup>. The oil-medium interface was controllably coalesced to allow for long term culture, as well as medium and compound perfusion such as drugs and viability stains. Spheroids (64 per device) were cultured for up to 17 days with no detrimental effects and their viability confirmed using live/dead staining (Figure 2). Furthermore, proof-of-concept treatment of spheroids with anticancer compounds was carried out using cisplatin, producing a significant reduction in spheroid growth. Preliminary experiments also showed that it was possible to use the device for the formation of alginate beads for the culture of spheroids.

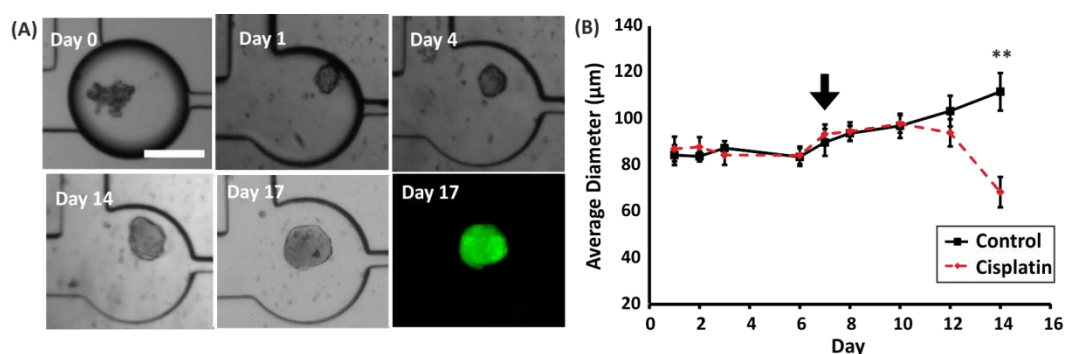


Figure 2: (A) Time-lapse of bright-field images of a spheroid within a trapping site terminating with a fluorescent image showing spheroid staining by fluorescein diacetate (green) and propidium iodide (red) on day 17. (B) Growth curve of average spheroid diameter ( $\mu\text{m}$ )  $\pm$  standard error of the mean (error bars) over 14 days for a control and treated (50  $\mu\text{M}$  of cisplatin) spheroid populations. \*\* indicates a p value of less than 0.01. The scale bar is 200  $\mu\text{m}$ .

## CONCLUSION

Overall, we have developed a microfluidic system for the long term culture of multicellular spheroids formed within aqueous droplets and alginate beads which is suitable for chemotherapy treatment. In the future, this device has the potential to be used for the screening of spheroids formed from biopsy samples for development of personalised combination therapies.

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