

# ON-CHIP FORMATION OF 3D SPHEROIDS FOR PATIENT-DERIVED TISSUE SCREENING

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## ABSTRACT

Three-dimensional (3D) multicellular spheroids have been identified as a suitable model of solid tumours for drug screening and cancer research [1]. However, creating and monitoring a large number of spheroids using conventional methods remains labour-intensive. This work reports a novel double-layer microfluidic device for the generation and culture of 3D multicellular spheroids and its use with biopsy samples. The system enables drug concentration-response curves to be obtained from a limited amount of sample material by applying a drug concentration gradient using an integrated passive microchannel network.

## KEYWORDS

Spheroid, tumour, drug-screening

## INTRODUCTION

The majority of drug screening assays currently carried out in research institutes and industry employs two-dimensional (2D) models mainly based on cell lines, which do not mimic the tumour microenvironment found in *in vivo* tumours. 3D multicellular spheroids have been identified as a more physiologically relevant model, yet their routine use in drug screening remains laborious. A particular challenge is the screening of tumour biopsies which, due to the small number of cells contained in biopsies, imposes limitations on the number of compounds and concentrations to be tested.

## EXPERIMENTAL

The device was fabricated using polydimethylsiloxane (PDMS) and consists of a bottom layer, which contains microwells for spheroid generation and culture, and a top layer with microchannels and inlets that enable cell seeding, medium exchange and drug application. By dispensing pre-determined volumes of drug solutions and medium into opposing inlet wells, a hydrostatic pressure gradient is created across the microwell chamber, which results in the formation of a stable drug concentration gradient for up to 10 hours.

## RESULTS AND DISCUSSION

As initial validation, UVW cells, a high-grade glioma cell line, were used to form hundreds of spheroids per device (50-150  $\mu\text{m}$  in diameter), achieving viable culture conditions for up to 21 days [2]. UVW spheroids were exposed to a range of cisplatin concentrations on day 5 of culture and monitored for a further 16 days. Furthermore, a range of spheroid sizes can be generated in the device, which allows the evaluation of spheroid size-dependent effects of drugs on morphology and viability. Spheroid viability was assessed with fluorescence microscopy by staining with propidium iodide (PI) and fluorescein diacetate (FDA) (Figure 1). Using in-house developed software, areas and perimeters of all spheroids were determined from bright-field images. This allowed the application of a parametric analysis based on the spheroid 'shape factor' to quantify the extent of disaggregation after drug treatment (Figure 2) [3].

## CONCLUSION

This microfluidic design differs from existing solutions [4, 5] as it does not require active flow control for either cell injection or the creation of a drug concentration gradient. Work is currently ongoing using cell suspensions from human prostate cancer biopsies to screen patient-derived spheroids using the proposed microfluidic system. Overall, this device presents an invaluable tool for personalised medicine and cancer research when only a small amount of patient material is available.

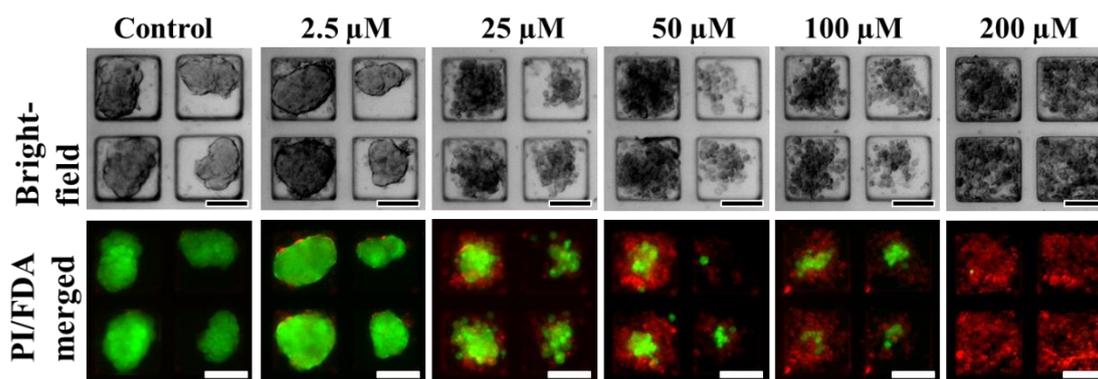


Figure 1: Representative images within the array of viable cells, stained green (FDA), and dead cells, stained red (PI), 3 days after cisplatin administration. Scale bars are 100  $\mu\text{m}$ .

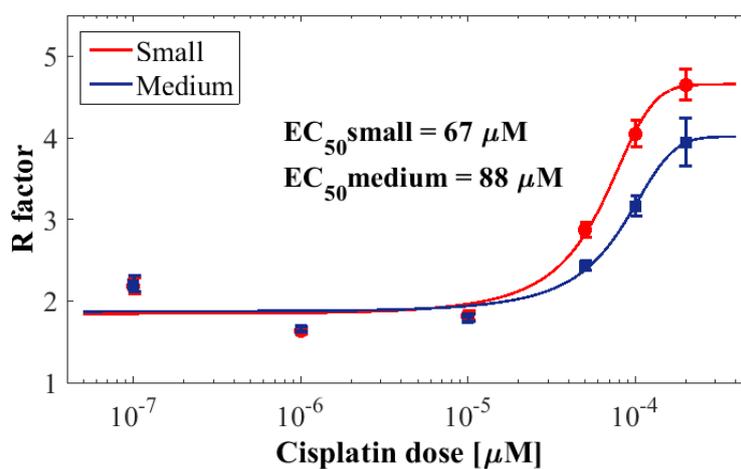


Figure 2: Dose response curves (data points and interpolation) quantifying spheroid disaggregation (shape factor) after treatment with a range of cisplatin concentrations for small (<75 $\mu\text{m}$  diameter) and medium-sized (<105 $\mu\text{m}$  diameter) spheroids and respective EC50s (drug concentration that produces a half-maximal response).

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