DEVELOPMENT OF A DROPLET MICROFLUIDIC ASSAY FOR RADIOTHERAPY TREATMENT OF MULTICELLULAR SPHEROIDS

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ABSTRACT

In this study, we report the development of a droplet microfluidic assay for the formation and radiotherapy treatment of multicellular spheroids. Glioma cells formed multicellular spheroids following their encapsulation within biocompatible medium-in-fluorinated oil (M/O) droplets with a non-adherent surfactant interface. Our studies identified that the ratio of volume-of-medium to number-of-cells and the refreshment of medium are important parameters for healthy spheroid growth within M/O droplets. In addition, a protocol was validated to extract radiotherapy dose responses from the spheroids based on their size variation via brightfield microscopy.

KEYWORDS: Cancer, spheroids, radiotherapy, droplet microfluidics, 3D cell culture

INTRODUCTION

One major disadvantage with current *in vitro* technologies for testing anti-cancer therapies is the use of 2D cell culture models which provide a poor reflection of the behaviour of a treatment *in vivo.* These do not take into consideration the influence of cell to cell contact and the three-dimensional (3D) topology of tissues, contributing to the poor efficacy of the treatment [1]. A 3D cell culture model that provides a more accurate representation of the tumour tissue is a multicellular spheroid which is defined as a stable spherical cluster of cells. This 3D model is obtained by preventing adhesion of cells to a surface, thus inducing their aggregation and clustering [2]. Although currently used methods are effective, they suffer from several drawbacks in that they are labour intensive, produce spheroids of different sizes and they are low throughput [1]. In recent years, the development of droplet microfluidics techniques has shown the potential to solve these issues, also allowing smaller sample volumes of cells to be used and offering a means for automation [3]. However, the main disadvantage of emulsion based technologies for cell culture is their compartmentalized nature that does not allow a straightforward exchange of cell nutrients. Hence, our experiments have investigated first which parameters were required to achieve long-term culture of spheroids within M/O droplets off-chip before carrying out our experiments within a microfluidic device. Finally, the multicellular spheroids formed within droplets were exposed to X-radiations and their effects were quantified.

EXPERIMENTAL

UVW glioma cells were encapsulated into PEG-PFPE surfactant-stabilised M/O droplets within polydimethylsiloxane (PDMS) wells (Figure 1) and microfluidic devices (Figure 2), with the oil-surfactant interface providing the non-adherent substrate [3][4]. Spheroids formed from a suspension of cells encapsulated in M/O droplets in PDMS wells were investigated for the effect on spheroid formation and growth due to (i) cell concentration, (ii) frequency of refreshment of medium and (iii) ratio of volume of medium to cell number per droplet. The microfluidic device design used was based on a modified version of the one developed by Schmitz *et al.*[5] and contains a chamber array with over 2000 sites to allow for long term culture of multicellular spheroids within M/O droplets. Multicellular spheroids within M/O droplets were exposed to X-radiation and cultured for up to 28 days to investigate radio-therapeutic effects on their proliferation.

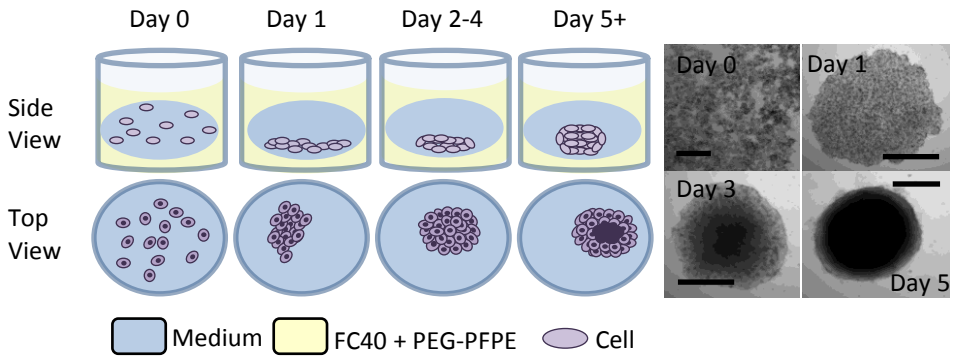

Figure 1: (Left) Schematic diagram showing the principle of formation of multicellular spheroids within M/O droplets. From a cell suspension, 3D aggregates formed within 5 days. (Right) Brightfield images of spheroid formation in 100 µL M/O droplets (scale bar = 300 µm).

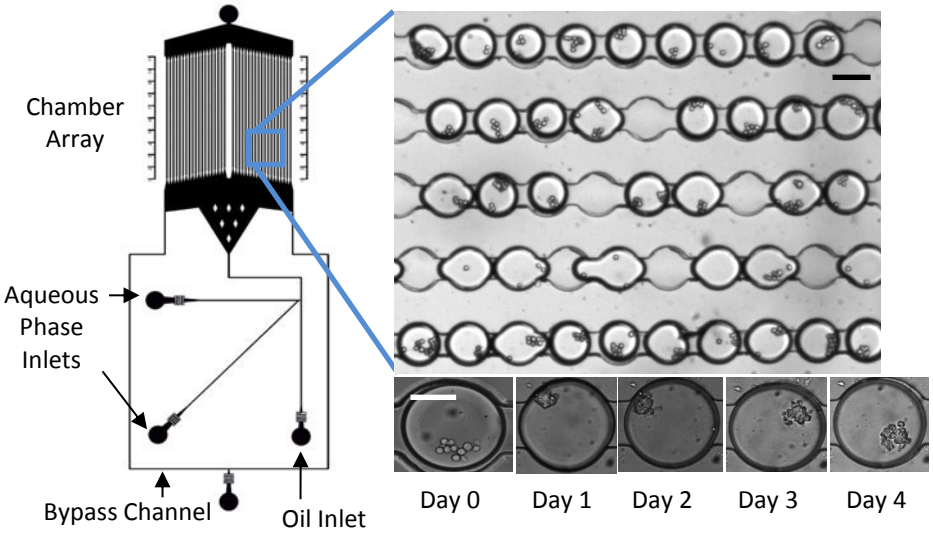

Figure 2: (Left) Diagram of the microfluidic device used for spheroid formation within droplets and their on-chip storage. (Right) Brightfield image of M/O droplets containing spheroids stored within the chamber array (top right image scale bar = 230 µm and bottom left scale bar = 115 µm).

RESULTS AND DISCUSSION

To determine the optimal conditions required for spheroid formation, it was observed from our off-chip experiments that the ratio of droplet volume to number of cells was fundamental to control spheroid health and to prevent break up (for UVW cells a ratio of 100 cells per µL was required). Furthermore, to ensure the proliferative growth of spheroids for up to 28 days and to prevent spheroids remaining within a dormant state the refreshment of medium was essential. As shown in Figure 3, when medium was refreshed every 2 days, it was observed that cells seeded at a lower concentration formed spheroids quicker and increased in diameter at faster rate than cells seeded at a higher concentration. In addition, from our radiotherapy studies, we observed a significant decrease in spheroid proliferation following a treatment with radiation (tested at 4 Gy and 8 Gy) (Figure 3). In the on-chip experiments, similar results were obtained by scaling down the volumes and spheroid size, thus increasing the throughput of experiments (approximately 1000 droplets containing spheroids per microfluidic device). Further modification of the microfluidic device design is however required to allow for refreshment of medium.

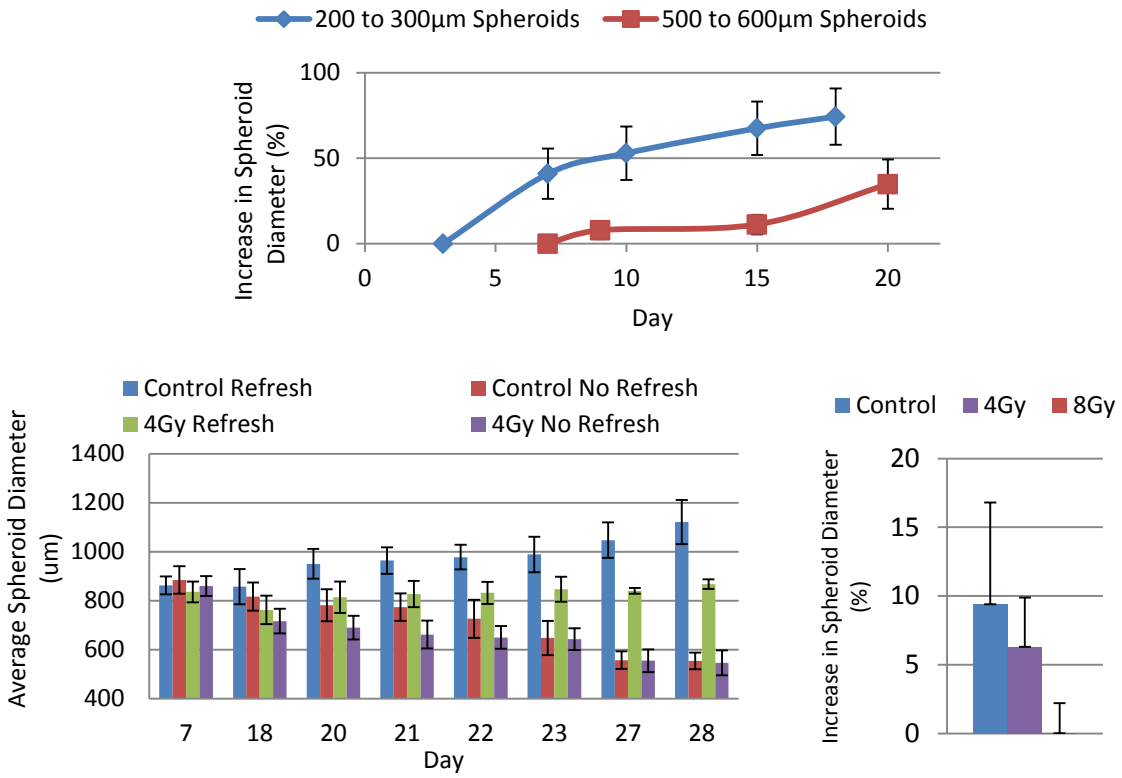

Figure 3: (Top) Representative line graph of day at which spheroid formation occurred and percentage increase in spheroid diameter over days for spheroids initially at 200-300 µm and 500-600 µm in diameter. (Bottom Left) Representative bar chart of off-chip spheroid growth over 28 days which were formed within M/O droplets. Media was refreshed every two days from day 15 and spheroids were irradiated on day 7 at 4Gy. (Bottom Right) Representative bar chart of spheroid increase in diameter (%) 10 days after exposure with 4 Gy and 8 Gy, respectively. Initial size was 400-500 µm in diameter.

CONCLUSION

Overall, we have identified the conditions required for developing a droplet-microfluidics device for long term culture of multicellular spheroids for radiotherapy studies. This miniaturized approach has therefore the potential to be used with biopsy samples, thus providing a microfluidic platform for developing stratified cancer treatments.

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