

MICROFLUIDIC SELF-ASSEMBLY OF TUMOR SPHEROIDS FOR STUDIES OF ANTICANCER DRUG ACTIONS AND INTERACTIONS

Liz Y. Wu^{*}, Dino Di Carlo^{*}, and Luke P. Lee

Biomolecular Nanotechnology Center, Berkeley Sensor and Actuator Center
Department of Bioengineering, University of California, Berkeley, CA 94720 USA

Abstract

Multicellular tumor spheroids may provide a better model than monolayer culture of *in vivo* tumors for drug assays. We present a novel microfluidic method for self-assembly of tumor spheroids for these studies. Our device traps cancer cells hydrodynamically and spheroid formation is enhanced by maintaining compact groups of the trapped cells due to continuous perfusion. A large amount of tumor spheroids with a narrow size distribution can be formed in the device to provide a good platform for anticancer drug assays.

Key words: Self-assembly of spheroids, drug discovery, tumor spheroids, MCTS

1. Introduction

Multicellular tumor spheroids (MCTS) have recently received a great deal of attention in cancer research and have been applied to the evaluation of anticancer drugs since the 3D multi-cellular aggregates simulate more accurately the tumor micro-environment *in vivo* by reproducing nutrient and signal gradients and removing the effect of unnatural adhesion to artificial surfaces or gels [1]. For example, the IC50 value of Taxol drug on MCF-7 cells spheroids was reported to be much higher than that of monolayer MCF-7 cells, and this information is valuable for clinical dosing. [2]

There are several techniques to generate MCTS, such as growth on non-adherent surfaces, in suspension [3], or by the hanging drop method [4], but most lack the ability to precisely control the number of cells in each spheroid or allow testing on the growth platform. Here we employed a purely hydrodynamic cell trapping method and precisely control the number of cells by the size of the traps (Fig. 1). We enhanced spheroid formation by maintaining compact groups of cells due to continuous perfusion (Fig. 2). After spheroid formation, drug assays may be performed immediately.

^{*} These authors contributed equally to this work

2. Experimental

The microfluidic trapping array used for self-assembly of tumor spheroids was micromolded in PDMS (Fig. 2f). The bonded PDMS chambers maintain a sterile culture environment. We generated a non-adherent trapping array by flowing BSA or PEG into the device. MCF-7 suspension with 10^6 cells/ml was then loaded into the device until each trap in the device was filled with cells (Fig. 3a). Continuous media perfusion was controlled by syringe pump after cell trapping. Drug injection can then be conducted using the same channels. Spheroid formation was then observed using time-lapse phase contrast microscopy.

3. Results and Discussion

MCTS formation in the culture array is shown in Fig. 3. We observed tumor cells adhering to its neighboring cells in the same trap gradually under the time lapse microscope. The time for MCTS formation is approximately 24 hrs. To further investigate the structure of the spheroids, we dyed the cell membranes with C16-fluorescein and the cell nuclei with Hoechst (Fig. 3e, 3f). The fluorescent probes indicated separate cells were adhering to form a combined structure. This was further supported when after 24 hours of culture we applied high pressure and dislodged the entire spheroids – not individual cells. In Figure 3g a well-formed spheroid that was pushed out from one of the microfluidic traps is shown. Approximately 15% of spheroids partially adhered to the glass substrate, even after we coated the surface with BSA or PEG, indicating that improved non-adherent surface coatings are required. Different size MCTS were also generated by adjusting the size of culture chamber (images not shown).

4. Conclusions

A new method to self-assemble arrays of 3D tumor spheroids was demonstrated. The microfluidic chip was also capable of continuous media perfusion and a sterile culture environment. With this platform, anti-tumor assays can be done immediately after the spheroid formation right in the growth platform.

Acknowledgements

Liz Y. Wu was supported by Taiwan Merit Scholarship TMS-094-2-A-008 and Intel Research Fund. Dino Di Carlo was supported by a Whitaker Foundation Graduate

Fellowship and GSK.

References

[1] Faute MAD et al. Clin Exp Metastasis 19: 161-168, 2002.
 [2] Nicholson KM et al. European Journal of Cancer Vol. 33, PP. 1291-1298, 1997
 [3] Kunz-Schughart et al., Kreutz M, Knuechel R. 1998. Int J Exp Pathol 79:1-23, 1998
 [4] Jens MK et al. Biotechnology and bioengineering 83:173-180, 2003

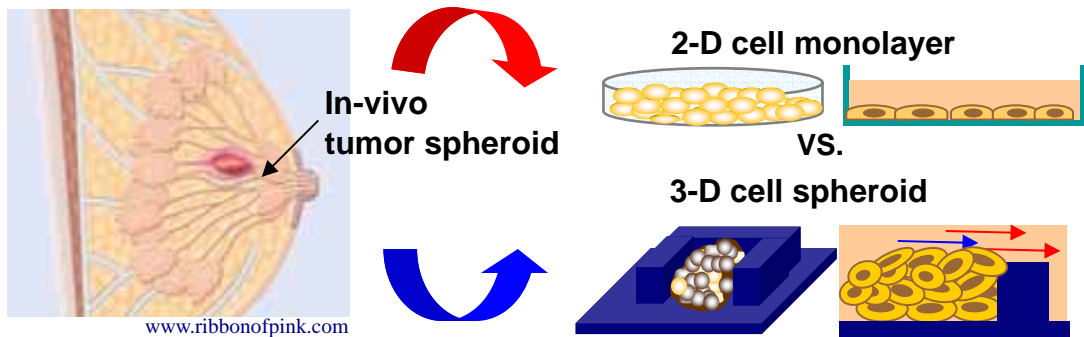


Figure 1. Formation of tumor spheroid. 3D tumor spheroids formed by trap geometry with additional perfusion control provides a better in-vivo like platform for anticancer drug analysis

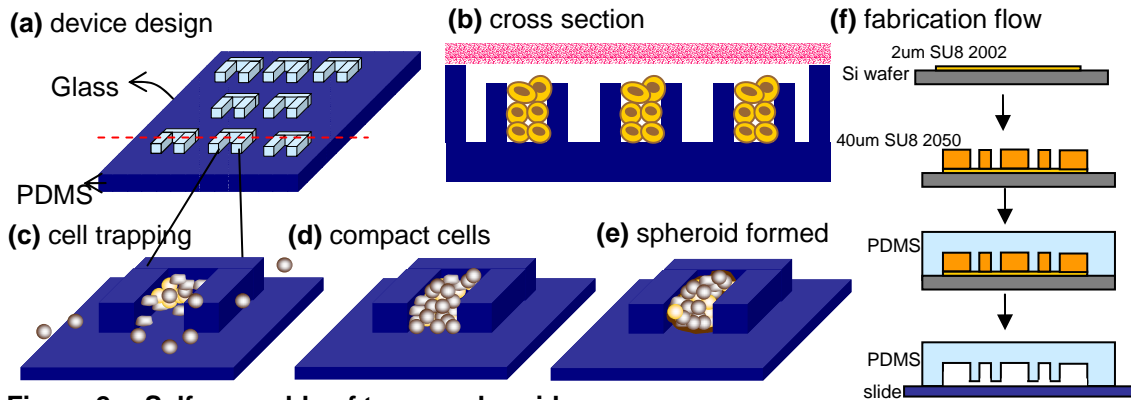


Figure 2. Self-assembly of tumor spheroid.

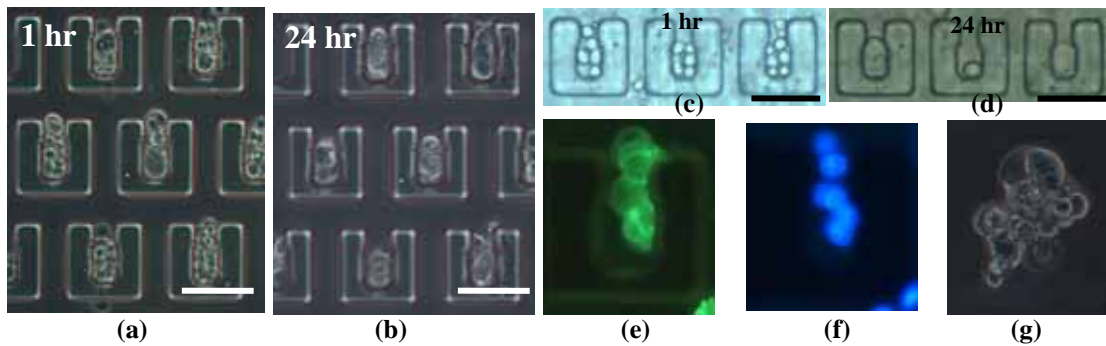


Figure 3. Spheroid culture results phase contrast image of the cells (a)1hr after loading (b) spheroids formed in the trap array after 36 hrs of culture (c)dark field image of cells after loading (d) spheroids formation (e) image of C16-dyed cell membranes (d) image of Hoechst dyed cell nucleus (e) suspended spheroid taken out from the trap (Scale bar =100 μm)