Agarose multi-wells for tumor spheroid formation and anti-cancer drug test

Yadong Tang a, Jianmiao Liu a and Yong Chen a,c,d*

a Ecole Normale Supérieure-PSL Research University, Département de Chimie, Sorbonne Universités - UPMC Univ Paris 06, CNRS UMR 8640 PASTEUR, 24, rue Lhomond, 75005 Paris, France
b Sino-France Laboratory for Drug Screening, Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Huazhong University Of Science And Technology, Wuhan, China
c Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
d Institute for Interdisciplinary Research, Jianghan University, Wuhan, China

E-mail: yong.chen@ens.fr

Keywords: Agarose, Tumor spheroids, Drug assay

Spheroid culture has recently received a great deal of attention in stem cell and cancer studies [1,2]. Unlike 2D monolayer culture, spheroid culture can more accurately mimic the in vivo microenvironments and more easily avoid unnatural adhesion of cells to material surfaces. In anti-cancer research, for example, one is particularly interested in capturing the spheroid complexity of solid tumors [2,3]. Previously, various methods such as hanging drop, suspension in spinning flask and hydrogel patterning have been used for the formation of large size spheroids but few of them are easy and reliable for systematic studies [2,4]. Here, we propose an assay form based on agarose multi-well patterning which allow easy forming millimetre size tumor spheroids and easy evaluation of anticancer drug effect.

Figure 1 shows schematic process flow of agarose well array fabrication. A flat PDMS film with a thickness of 2 mm was made at 1:10 mixture ratio. Then, an array of holes was created on the PDMS layer with a biopsy punch of 2 mm diameter and a computer numeric controlled milling machine. After exposed in trimethylchlorosilane (TMCS) vapour for 1 mm, the PDMS layer was used as master to cast a second layer of PDMS. After curing at 80°C for 2 h, the PDMS mold with pillars was peeled off. A 5% agarose in DI water solution was mixed at 120°C for 5 min and then immediately casted on the PDMS pillar mold. After cooling down the system to room temperature and waiting for 10 min, the agarose layer was gently removed from the PDMS mold, resulting in a multi-well device for spheroid culture.

To study the tumor spheroids formation in agarose multi-wells, U87 cells were seeded after the device was equilibrated with culture medium for 15 min. After 24 h, tumor spheroids could form spontaneously in each well. Figure 2 shows microphotographs of tumor spheroid formation. As expected, these tumor cells aggregated first and then formed spheroids by compaction. Afterwards, the spheroids grew until day 10 due to cell proliferation. After 10 days, the size of the tumor spheroid was unchanged and further increase of the cell seed density had no effect on the final size of the spheroid which was always about 1.4 mm. This limitation in size is due to the fact that when the size of the spheroid becomes too larger, the cells in the center of the spheroid will get less and less nutrient due to decreased diffusion and the fact that the spheroid is a self-organized entity in which the proliferation of all cells is under regulation due to mutual interaction.

To study the anticancer drug effect, Combretastatin A-4 (CA4) was added in the medium at day 4. As expect, the spheroid stopped to grow and some of the cells were detached from the spheroid (Fig.3). The live/dead cell staining images showed that in the culture medium with the drug, many cells dead at day 7 whereas the most of cells were alive in the medium without drug.

In conclusion, agarose multi-wells are reliable for the formation of millimeter size tumor spheroids and anti-cancer drug studies.