PATTERNED MULTICELLULAR SPHEROIDS IN 3D MATRIX FOR TUMOR INVASION AND VASCULOGENIC MIMICRY IN GLIOMA CELLS
Xu Zhang, Jingyun Ma, Jianhua Qin*
Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China.

ABSTRACT
Multicellular spheroid is an appropriate in vitro system for simulation of 3-D tumor micro-milieu that can be used for evaluating and predicting tumor response to therapeutic agents. Herein, we presented a novel and straightforward approach to generate patterned multicellular spheroids in collagen matrix for the investigation of tumor invasion and vasculogenic mimicry in glioma cells. The results demonstrated that the interior of collagen matrix was hypoxic condition, which would enhance U87 invasion and vasculogenic mimicry, whereas the invasion and vasculogenic mimicry of U87 could be inhibited by resveratrol.

KEYWORDS: glioma multicellular spheroid, 3-D pattern, tumor invasion, vasculogenic mimicry.

INTRODUCTION
Multicellular spheroid is a useful platform for simulation of 3-D tumor micro-milieu that can be used for evaluating and predicting tumor response to therapeutic agents. The existing techniques to generate cell spheroids mainly included hanging droplets method, non-adhesive 96-well plate method and direct-written methods, which had been used in drugs evaluation widely [1-3]. As compared to these techniques, our approach exhibited obvious advantages to produce patterned multicellular spheroids within 3D matrix in terms of simple operation, low cost and high throughput format. In particular, it can not only facilitates the investigation of tumor invasion in real-time within 3-D microenvironment, but also enables the exploration of angiogenesis in tumor progress and development.

EXPERIMENTAL
The proposed approach is simply based on non-adhesive PDMS substrate replicated from SU-8 mold with array micro-well concave structure. After the initial formation of multicellular spheroids in micro-well concave array, the patterned multicellular spheroids embedded in 3D matrix are produced by dropping double layers of collagen solution onto the spheroids followed by collagen solidification (Fig. 1). Based on this approach, the size, distance, position and the pattern of cell spheroids could be controlled flexibly by changing the design of SU8 mold as shown in Fig.2.

RESULTS AND DISCUSSION

![Fig. 1: Schematic diagram of the method to generate well-patterned cell spheroid array in collagen matrix.](image)

![Fig. 2: Generation of patterned cell spheroid array in collagen matrix with controllable size, distance and position.](image)
In this work, the patterned multicellular spheroids were applied for the investigation of glioma cells invasion and vasculogenic mimicry (VM), a new alternative mechanism in vascularization in aggressive tumors. As shown in Fig. 3 (a-c), U87 cell spheroids demonstrated obvious invasive behaviors in 3-D collagen matrix. The quantitative invasive distance and area were both time-dependant. In particular, the invasive U87 cells exhibited an enhanced acquisition of mesenchymal trait with the increased expressions of vimentin as compared to that in 2D culture. We also found that the hypoxia-relevant genes were obviously up-regulated within collagen matrix in U87 cell spheroids compared to that cell spheroids cultured alone (Fig. 3d).

**Fig.3**: a-c. Quantitative evaluation of glioma cell spheroid invasion in collagen matrix during 12h; d. The expression of hypoxia-relative genes of U87 cell spheroids.

The results indicated the creation of a hypoxic condition by collagen matrix (thickness 800-1000 μm), which can closely mimic the in vivo realistic tumor environment. In addition to cell invasion, we also explore the formation of vasculogenic mimicry in glioma U87 cells after long term culture and evaluate the effect of resveratrol on cell invasion and VM formation. As shown in Fig. 4 -5, three dimensional in vitro cultured stimulated vasculogenic mimicry and re-capitulates key features of high grade brain tumours. Resveratrol can significantly inhibit the invasive behavior and VM formation in U87 cells.

**Fig.4**: Vasculogenic mimicry formation within patterned U87 cell spheroids.
CONCLUSION
We present a flexible and high-throughput approach to generate patterned cell spheroids in hypoxia collagen matrix. The proposed approach can facilitate the quantitative study of cell migration and network formation in both tumor cells and neural cells et al, providing a useful platform for the study of cancer biology and regenerative medicine.

ACKNOWLEDGEMENTS
This research was supported by the National Nature science Foundation of China (O. 91227123, O. 11161160552), Key Project in National Science & Technology Pillar Program in the Twelfth Five-year Plan Period of China (O. 2012BAK02B00, O. 2012BAK02B03).

REFERENCES

CONTACT
X. Zhang, tel: +86-411-8437-9059; zhangxu@dicp.ac.cn
*J.H. Qin, tel: +86-411-8437-9650; jhqin@dicp.ac.cn