Colloids and Surfaces B: Biointerfaces 172 (2018) 665–673

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The comparison genomics analysis with glioblastoma multiforme (GBM) cells under 3D and 2D cell culture conditions

Liang Ma[a,b,*], Bin Zhang[a,b,*], Changchun Zhou[c], Yuting Li[a,b], Binjie Li[a,b], Mengfei Yu[d], Yichen Luo[a,b], Lei Gao[a,b], Duo Zhang[e], Qian Xue[a,b], Qingchong Qiu[f], Biaoyang Lin[f], Jun Zou[a,b], Huayong Yang[a,b]

[a] State Key Lab of Fluid Power and Mechatronic Systems, Zhejiang University, Hangzhou, 310058, People's Republic of China
[b] School of Mechanical Engineering, Zhejiang University, Hangzhou, 310058, People's Republic of China
[c] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, 610064, People's Republic of China
[d] The Affiliated Stomatologic Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, People's Republic of China
[e] Department of Engineering, University of Cambridge, Trumpington Street, Cambridge, CB2 1PZ, United Kingdom
[f] Zhejiang California International NanoSystems Institute, Zhejiang University, Hangzhou, 310029, People's Republic of China

ARTICLE INFO

Keywords:
GBM
3D tumor model
DNA microarray

ABSTRACT

GBM, the most common and aggressive malignant primary brain tumors which needs new research approach to reveal the underline molecular mechanism of tumor progression. The 3D *in vitro* tumor model can be a simple and effective way to study tumor characteristics with ability to replicate of the tumor milieu. In the current study, we adopted the DNA microarray to analyze the gene expression of GBM tumor cells cultured under 2D cell culture flasks and 3D PLA porous scaffolds for 4,7 and 14 days. For 14 day old cultures, 8117 and 3060 genes expression were upregulated and downregulated respectively. Further KEGG pathway analysis revealed, the upregulated genes were mainly enriched/involved in PPAR and PI3K-Akt signaling pathways whereas the downregulated genes were mainly contributed in metabolism, ECM related and TGF-beta pathways. Thus, our approach of establishing 3D *in vitro* tumor model provides realistic results and proves itself a powerful tool for understanding the inner nature of GBM and can be considered as potential platform for drug screening.

1. Introduction

Glioblastoma multiforme (GBM), originating from glial or glial-precursor cells in the central nervous system. GBM is one of the most common and aggressive malignant primary brain tumors which accounts 52% of all functional tissue brain tumor cases and affects over 20,000 individuals in the United States, annually [1]. Various efforts have been made to improve the treatment of this disease including neuro-surgery, neuro-imaging, radiation therapy as well as chemotherapy. However, the results are still dismal with median patient's survival time, along with optimal care around 14 months, and 5 year survival rate as low as 5%. A unique feature of these aggressive tumors is their infiltration ability to diffusively invade normal brain tissue. Given such highly infiltrative characteristic, GBM is easy to reoccur both locally and distantly within the brain [2,3].

To understand this infiltrative nature and reveal the mechanism of tumor progression, it is essential to develop an effective *in vitro* tumor model. The ideal *ex vivo* tumor model requires reproducible *in vivo* like microenvironment with extracellular matrix (ECM) for tumor cells to grow, form three dimensional architectures and represent their invasive behavior. The ECM has been recognized playing an important role in tumorigenesis [4,5] and tumor cell invasion [6–8]. However, existing 2D tumor models grow cells on the flat surface of polystyrene dishes, which cannot replicate *in vivo* cellular behavior. Thus, limits the predictive effect of such 2D *in vitro* models. Commonly used animal models such as rodents or rabbits, usually shows ethical problems and the results gained cannot simulate with the human body. In order to gain realistic, reliable and appropriate cellular response of GBM tumor cells, establishing highly reproducible and tunable microenvironments are in need.

Many 3D *in vitro* GBM models have been established using ECM-based scaffolds [9–11]. However, these models lack in comparative studies between 2D and 3D platforms at gene level. Thus, relationship between actual cellular response and dimensionality remained

* Corresponding authors at: State Key Lab of Fluid Power and Mechatronic Systems, School of Mechanical Engineering, Zhejiang University, Hangzhou, 310058, People's Republic of China.
E-mail addresses: liangma@zju.edu.cn (L. Ma), zbzju@zju.edu.cn (B. Zhang).

https://doi.org/10.1016/j.colsurfb.2018.09.034
Received 25 April 2018; Received in revised form 27 July 2018; Accepted 14 September 2018
Available online 15 September 2018

unanswered. Hereby we report a comparative study about genomewide analysis of GBM cells between 2D and 3D culture conditions. In this study we observed, GBM cells grown in 2D and 3D platforms not only had different in their morphologies but also had dramatic difference with their gene expressions. Different gene expression would result in variation of signaling pathways and finally lead to changes in cellular behavior. From such observations, we highlighted the importance of cellular dimension in GBM cell culture to show impact on genetic profile. Thus, 3D models can be a powerful tool to understand the realistic cellular behavior under different culture conditions.

2. Materials and methods

2.1. Fabrication of porous scaffolds

Porous scaffolds for 3D cell culture were fabricated with solvent-free solid state foaming method as described in the previous work [12]. Briefly, biodegradable polylactic acid (WMI, Taiwan) samples were put into a pressure chamber for 7 days gas saturation with CO_2 at 2 MPa, then desorption for 0.5 h at room temperature in air, followed by foamed at 100℃ for 30 s with hot bath, the pore size and porosity can be easily controlled by adjusting the foaming parameters. Foamed samples were then treated with 20 kHZ power ultrasound to break the pore walls in the PLA foam. Fabricated PLA scaffolds were cut into disc-shaped of diameter 13 mm. Fig. 1 shows the saturation process, the shape of foamed samples and the microstructure of fabricated porous PLA scaffold. The average pore size was 250–300 μm with the average porosity 70%.

2.2. Cell cultures

For 2D cell cultures, GBM cell line U87 was purchased from American Type Culture Collection (ATCC, Manassas, VA) and maintained in T25-flasks with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (1:1) culture medium with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY). The T25-flasks were placed in an incubator (Thermo, Boston, MA) maintained at 37℃ and 5% CO_2.The culture medium was replaced every 2–3 days.

For 3D cell cultures, PLA scaffolds were sterilized by 70% ethanol for 1 h followed by 30 min UV irradiation. Sterilized PLA scaffolds were then placed in 24-well cell culture plate with complete cell culture medium for several days prior cell seeding. Approximately, 10^5 cells were taken in 100 μl culture medium and were seeded on each scaffold, after which scaffolds were incubated for 6 h. Later, cultures were topped with 1 ml culture media and media was renewed once in every 2–3 days. Live/Dead cytotoxic assay was performed to evaluate the cell viability.

2.3. Gene expression analysis

The Affymertrix gene chips (Primeview Array) were used for mRNA expression profiling. Experimental protocols for gene chips were based on the manufacture's technical instruction. Following is a brief description.

At days 4, 7 and14, total RNA of GBM cells was isolated with Trizol followed by manufacturer's instruction (Invitrogen, San Diego, CA). For 2D cell cultures, regular isolation protocol was adopted. For 3D cell cultures, the scaffolds containing cells were cut into small pieces by scissors, then 1 ml Trizol was added to the mixture. Total RNA was purified by Qiagen RNeasy mini kit (Qiagen, Velencia, CA). Purified RNA was analyzed on a ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). The quality and quantity of total RNA were verified using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Three independent RNA samples at each time point with 260/280 ratios between 1.9 and 2.0 and the RIN (RNA integrity number) was greater than 8.5 were pooled for subsequent analysis. Single and double stranded cDNA was synthesized from total RNA samples using SuperScript II (Invitrogen, CA, USA). High quality total RNA (250 ng) was used as the starting material. The genechip 3′ IVT expression kit was used for the first-strand, second-strand cDNA Synthesis, and *in vitro* transcription to synthesize labeled cRNA. The cRNA was then purified and fragmented for hybridization analysis. 12.5 mg aliquants of the fragmented cRNA were hybridized with the Primeview array (Affymetrix, Santa Clara, CA) in hybridization cocktail (0.5 mg/ml cRNA, 50 pM control oligonucleotide B2, 1.5 pM bioB, 5 pM bioC, 25 pM bioD, 100 pM cre, 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, 100 mM MES, 1 M Na^+, 20 mM EDTA, 0.01% Tween 20, 10%DMSO). Hybridization was allowed to proceed overnight (16 h) at 45 °C, 60 rpm, followed by washing and staining with the Affymetrix hybridization kit (Affymetrix, Santa Clara, CA). Hybridization assay procedures including preparation of solutions were carried out as described in the Affymetrix GeneChip Expression Analysis Technical Manual. The distribution of fluorescent material on the array was obtained using 7G3000 GeneChip Scanner (Affymetrix, Santa Clara, CA). Microarray Suite (MAS) version 5.0 and Affymetrix Genechip Command Console (AGCC) supplied by Affymetrix was used for gene expression analysis.

2.4. Microarray data analysis

Chip data were presented as intensity of the probes of each sample in raw format files(.chp). The data normalization, statistical analysis fold change and clustering were performed with GeneSpring 11.5 (Aglient Technologies, Foster City, CA) software. Signal values < 0.01 were set to 0.01, the gene expression filtering cut off was set to 20%. Fold change > 2 was selected as differentially expressed genes and only those genes with more than 2-fold change were used for Gene

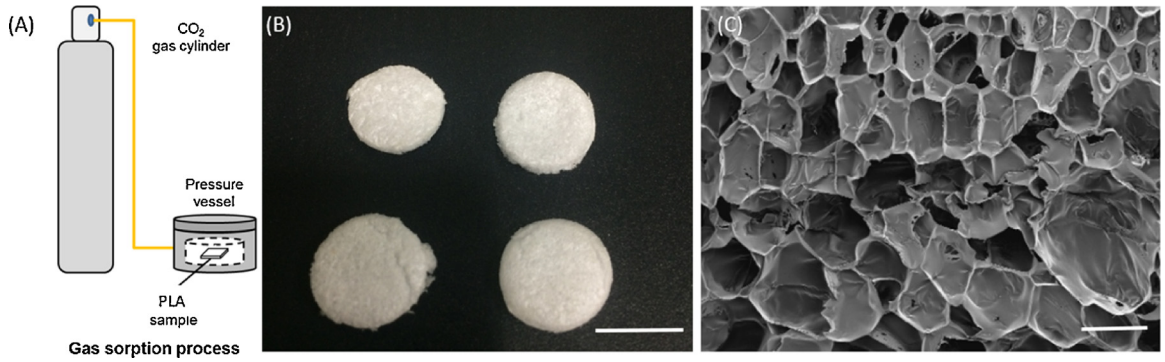

Fig. 1. PLA scaffolds fabrication. (A)PLA scaffold fabricated by solvent-free solid-state-foaming approach.(B)Disk-shaped PLA scaffolds. Scale bar: 10 mm. (C) Cross section of scanning electron microscopic (SEM) image of a PLA scaffold. Scale bar: 500 μm.