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To cite this article: Xingliang Dai et al 2016 Biofabrication 8 045005

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3D bioprinted glioma stem cells for brain tumor model and applications of drug susceptibility

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Keywords: 3D bioprinting, glioma stem cells, brain tumor model, drug susceptibility

Abstract

Glioma is still difficult to treat because of its high malignancy, high recurrence rate, and high resistance to anticancer drugs. An alternative method for research of gliomagenesis and drug resistance is to use in vitro tumor model that closely mimics the in vivo tumor microenvironment. In this study, we established a 3D bioprinted glioma stem cell model, using modified porous gelatin/alginate/fibrinogen hydrogel that mimics the extracellular matrix. Glioma stem cells achieved a survival rate of 86.92%, and proliferated with high cellular activity immediately following bioprinting. During the in vitro culture period, the printed glioma stem cells not only maintained their inherent characteristics of cancer stem cells (Nestin), but also showed differentiation potential (glial fibrillary acidic protein and β-tubulin III). In order to verify the vascularization potential of glioma stem cells, tumor angiogenesis biomarker, vascular endothelial growth factor was detected by immunohistochemistry, and its expression increased from week one to three during the culture period. Drug-sensitivity results showed that 3D printed tumor model was more resistant to temozolomide than 2D monolayer model at TMZ concentrations of 400–1600 μg ml⁻¹. In summary, 3D bioprinted glioma model provides a novel alternative tool for studying gliomagenesis, glioma stem cell biology, drug resistance, and anticancer drug susceptibility in vitro.

1. Introduction

Despite recent improvements in the treatment of brain tumors, the prediction of clinical behavior, response to therapeutic drug, and treatment outcome of patients with glioma is still challenging [1]. Patients suffering from high grade glioma (GBM) have a very poor prognosis with a median survival time of approximately 15 months for a newly diagnosed GBM and 5–7 months for a recurrent GBM [2]. The main cause is considered to be glioma stem cells [3]. So, therapies targeting glioma stem cells are considered to be a promising way to treat brain tumors, and there is a demand of ideal tools that can be used for studying glioma stem cell biology and intrinsic resistance of glioma to chemotherapeutics for a better management of patients [4].

Over the past few decades, two-dimensional (2D) monolayer of various cancer cell lines, including glioma, has been the most widely used tumor model for the research of cancer development and progression, as well as for drug evaluations [5]. However, this method fails to recapitulate the three-dimensional (3D) environment and cells quickly lose their intrinsic characteristics and functions [6], such as cell–cell and cell–matrix interactions, spatiotemporal signaling, metabolic gradients, and mechanical restriction. It is also impossible for the 2D models to reflect the in vivo malignant progression, such as hypoxia/necrosis, stem cell niche characteristics, slow proliferation, and drug resistance of cancer cells in solid tumor [7, 8]. Different cell responses between 2D and 3D cancer models have been shown in protein and gene expression, protein gradient profiles, cell signaling, migration, morphology, proliferation, viability, organization, and drug response [5]. It may be the reason why most anticancer drugs, even targeted ones,
were proved effective in vitro, but exhibited low therapeutically efficacy in clinical trials. Additionally, drug test results obtained with animal model may not accurately predict the results in human patients because of cross-species differences [9]. Hence, there is a growing demand for innovative tumor models which can simulate real tumor biology, reflect in vitro the in vivo characteristics, and illustrate mechanisms of cancer development and progression. Such models can be used to develop truly effective drugs for tumors [10].

3D bioprinting is an ideal method for fabricating complex 3D biological structures by depositing bioinks, which are biomaterials mixed with cells, layer by layer from computer-aided designs. Based on the 3D printing techniques, bioprinting has shown distinct advantages on creating geometrically complex scaffolds containing viable cells, and it is also an effective method with low cost, high throughput, and precise reproducibility [5]. 3D bioprinting has offered a broad range of applications in tissue engineering, regenerative medicine, disease modeling, pharmaceutical research, and cancer research [10–15]. In terms of cancer research, in vitro tumor model can be fabricated via two bioprinting methods [5]. One method is the two-step biofabrication in which tumor cells are seeded on the preprinted 3D hydrogel scaffolds made of printable and biocompatible materials [16]. Another method is the one-step biofabrication in which tumor cells are encapsulated in hydrogels and printed directly [17, 18].

Hydrogels are porous materials with the advantages of high permeability of nutrients and metabolism wastes. They can be easily fabricated to customized shapes and printed at micron precision at larger dimensions [19]. Alginate hydrogel is a popular biologically inert material that is widely used in 3D bioprinting, especially in extrusion-based printing [20]. Gelatin is a mixture of peptide sequences derived from partially hydrolyzed collagen, and has been used widely in extrusion bioprinting because its melting temperature typically lies between 30 °C and 35 °C [21]. The combination of alginate and gelatin is advantageous because of the chemical similarity to the extracellular matrix (ECM) [22, 23], as alginate hydrogels covalently crosslinked with gelatin resulted in the highest degree of cell adhesion, spreading, migration, and proliferation, and had a fast degradation rate [24]. Fibrinogen, a natural polymer, is a promising material for applications in biomedical engineering because of its biocompatibility and biodegradability. It has been reported as an ideal material for tissue regeneration, healing, and regulating cell differentiation [25–27]. Similar material composition has been used for cervical tumor matrix, and showed Hela cells in 3D environment had a higher proliferation rate and formed cellular spheroids [18]. Consequently, gelatin/alginate/fibrinogen (GAF) hydrogel was used as the matrix of 3D bioprinted brain tumor in this study.

In this study, first we enhanced the stability of the GAF hydrogel system by adding the crosslinker, transglutaminase (TG) to crosslink gelatin, the main component of the system. TG is a monomeric protein containing active sites, which can catalyze intramolecular and intermolecular covalent cross-linking of protein polypeptides via acyl transfer reaction. In addition, TG is a non-toxic transferase, which exists widely and naturally in the human body [28]. Then, glioma stem cell-laden GAF was printed directly by 3D bioprinter, the growth characteristics, stemness, and differentiation potentials of the cells were verified. Finally, we evaluated the sensitivity of cells in the 3D printed GAF against the chemotherapeutic drug TMZ, and compared it to that at 2D culture condition.

2. Materials and methods

2.1. Cell culture

Glioma stem cell line, SU3 was previously established and characterized in our lab [29, 30]. Human glioma cell line, U87 was purchased from ATCC [31]. Differentiated cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, NY, USA) containing 10% FBS (Gibco). Glioma stem cell lines were cultured in DMEM/F12 containing 20 ng/ml basic fibroblast growth factor (bFGF), 20 ng ml−1 epidermal growth factor (EGF), B27 supplement (50X), 2 mM L-glutamine, MEM vitamin solution and 100 mM sodium pyruvate (100X) (all from Gibco, Frederick, MD, USA).

2.2. GAF hydrogel scaffolds

Sodium alginate was purchased from WaKo (WaKo Pure Chemical Industries, Ltd, Osaka, Japan). Gelatin, fibrinogen, and thrombin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Transglutaminase (TG) was bought from Ruibio (BoMei, HeFei, China). GAF hydrogel scaffolds were printed by a 3D multi-nozzle bioprinter (Tissform III) based on a previously reported method [32]. Briefly, sodium alginate, gelatin, and fibrinogen were sterilized by gamma ray and dissolved into phosphate buffered saline (PBS) without calcium and magnesium ions at the concentration of 4%, 20% and 4% (all w/v), respectively. For printing, 20% gelatin, 4% sodium alginate, and 4% fibrinogen were mixed at a volumetric ratio of 2:1:1 at 37 °C. In order to enhance the strength and stability of the scaffold, 1% TG (w/v) was added uniformly to the hydrogel system before printing by mixing with the GAF solution. Porous grid scaffolds with pre-designed circular or square shape were printed under the optimized parameters. Specifically, the nozzle insulation temperature and printing chamber temperature were controlled at 25 °C and 4 °C respectively, to retain scaffold shape. The nozzle diameter was 0.26 mm. Extrusion speed was adjusted to best fit the nozzle scanning speed. After printing,
grid scaffolds were first immersed into 3% (w/v) calcium chloride solution for cross-linking sodium alginate, and then in 20 U ml\(^{-1}\) thrombin for cross-linking fibrinogen, resulting cell-free scaffolds.

### 2.3. 3D bioprinting of glioma stem cells

For bioprinting glioma stem cells, SU3 cells (5 \times 10^5) were mixed into 0.25 ml of solution containing 4% fibrinogen and 4% TG, then added into 0.5 ml of 20% gelatin solution and 0.25 ml of 4% sodium alginate solutions, achieving final concentrations of 1%, 10% and 1% for fibrinogen, gelatin, and sodium alginate, respectively. The most suitable printing parameters, such as 25 °C incubation temperature, 4 °C printing chamber temperature, and 0.26 mm nozzle diameter, were used to make appropriate cell-laden constructs. After crosslinking, 2 ml fresh medium was added into each well and the structures were maintained in culture condition at 37 °C with 5% CO2, and medium were replaced every 2 d. The schematic of the printing process and crosslinking of hydrogel components is shown in figure 1.

### 2.4. Scanning electron microscopy (SEM) analysis

Cell-free scaffolds and cell-laden constructs were fixed with 4% glutaraldehyde for 4 h at room temperature. The samples were then dehydrated in a series of ethanol solutions (30%, 50%, 75%, 90%, 95%, 100%, 100%), by soaking the samples in each solution for 30 min. Subsequently, the samples were critical point dried. For all samples, cross sections were observed after brittle fracture using liquid nitrogen, and the surfaces were also photographed by SEM: constructs were sputter coated with platinum, and imaged with the ULTRA 55 Scanning Electron Microscope (ZEISS, Germany).

### 2.5. Live/dead assay

Cell survival was assessed by fluorescent live/dead viability assay kit (KeyGEN BioTECH, Nanjing, China) according to the manufacturer’s instructions. 3D cell-laden constructs were immersed in PBS solutions containing 8 μM propidium iodide (PI) and 2 μM Calcein-AM to stain dead (red) and live cells (green). These constructs were incubated for 15 min in dark at room temperature, and washed with PBS. Images were acquired from fluorescent microscope. Live/dead cells were counted in 5 random sights of magnifying at 200x for each sample (n = 3).

### 2.6. Cell proliferation/cytotoxicity analyses

Cell proliferation and scaffold cytotoxicity was evaluated by the Alamar Blue Kit (MAIBIO, Shanghai, China) according to the manufacturer’s instruction. 3D cell-laden constructs were washed with PBS, then 1 ml Alamar Blue working solution (100 μl of Alamar Blue diluted with 900 μl fresh medium) was added to each well of 24-well plates, and incubated at 37 °C for 1 h. The solutions were transferred to a 96-well plate to read the optical density at wavelengths of 570 nm and 630 nm (BioTek ELX800, VT, USA). After staining, the constructs were washed with PBS to remove the remaining Alamar Blue, and fresh medium was added. For each sample, this procedure was repeated every other day for 21 d. Another cell cytotoxicity assay was performed using CCK-8 kit (Dojindo, Japan) following the instruction. Briefly, mouse embryonic fibroblasts, L929 (4 \times 10^5/well) were seeded in 96-well plates for 24 h, cell-free scaffolds were added into the well and incubated for 24 h. Then, detection reagent (90 μl medium + 10 μl CCK-8) was added to each well for 2 h before detecting the optical density at 450 nm exciting light.

### 2.7. Histology and immunohistochemistry

For paraffin embedding, printed cell-laden constructs were fixed in 4% paraformaldehyde for 24 h, and embedded in paraffin following the routine approach. Then, samples were cut with a microtome (Leica, Germany) to obtain 2 μm thick sections, and stained with haematoxylin and eosin (HE) following the protocol. Based on previously reported methods [33], immunohistochemical analyses were performed using the primary antibody for nestin, GFAP, β-tubulin III, Ki67 and VEGF (all from BOSTER, Wuhan, China). Secondary antibodies and other related reagents were

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**Figure 1.** The schematic of the printing process and crosslinking of hydrogel components of the in vitro brain tumor model.
all purchased from Beyotime Biotechnology (Shanghai, China).

2.8. Dose-response assay
To test the chemosensitivity, U87 and SU3 cells were biprinted in cell-laden structures, grew on 2D surfaces for 24 h, and treated with TMZ solutions at various concentrations for 48 h before replaced with fresh medium. Drug concentrations at 0, 100, 200, 400, 800 and 1600 μg ml⁻¹ were tested for each trial (n = 3). Cell viability was examined at 48 h after treatment via the Alamar blue assay as described above and reported as optical density value of viable cells relative to an untreated control.

2.9. Statistical analysis
Each experiment was performed in three replicas, and results are presented as the mean ± SD where applicable. Comparison between any two groups was evaluated using the Student’s t-tests. P < 0.05 was considered to indicate a statistically significant result. The data was analyzed and presented by GraphPad Prism 5.0 software.

3. Results

3.1. Characterization of GAF scaffolds
First, the advantages of adding the crosslinking agent were determined. In order to enhance the mechanical strength and structural stability, TG was added to crosslink gelatin before printing. As shown in figure 2, GAF scaffolds can be well printed and preserved their original shapes after freeze dried, and can be crosslinked with TG without additional cytotoxicity (figures 2(A) and (B)). The cell cytotoxicity of the TG was evaluated by comparing to that of carbodiimide (EDC) and CaCl₂ solutions, respectively, and it was observed that even the concentration of TG was increased to 6% (w/v), it was still almost non-toxic, as cells maintained 97.18% of the relative growth rate compared to blank control (figure 2(B)). GAF scaffolds with TG maintained their morphologies, structures, and water-retention property after incubation in PBS for 15 d at 37 °C (figure 2(B)), while the GAF scaffolds without TG collapsed under the same condition (figure 2(B)). The equilibrium swelling ratio of GAF scaffolds was calculated using the following formula: SW = [(W_e – W_d)]/W_d × 100\%, where W_e is the weight of swelling-equilibrium gel in the distilled water, and W_d is the weight of the dried gel. Equilibrium swelling ratio of the GAF scaffolds cross-linked by TG was (518.18 ± 60.58)%, with no difference to that of GAF scaffolds cross-linked by CaCl₂ (501.85 ± 62.31)% (figure 2(B)). The morphologic features of GAF scaffolds were determined subsequently. In the interior of the scaffolds, porous structures were shown on the SEM images, with uniform diameter (2–4 μm) of the porous structure (figure 2(C)).

3.2. 3D bioprinted cell-laden GAF hydrogels
We have previously reported that SU3 cells grew into spheres and sustained their stemness in stem cell medium, and their potential of differentiation appeared when FBS was added (figures 3(A) and (B)) [30]. Here, SU3 cells were encapsulated into GAF hydrogels for 3D bioprinting. First, 3D cell-laden porous scaffolds were fabricated under the preset printing parameters (figure 3(C)). Then, growth characteristics of 3D bioprinted SU3 cells were compared to these at 2D culture condition (figure 3(D)). For 2D condition, cells proliferated faster at the beginning, as the logarithmic multiplication phase and the plateau phase appeared earlier than these at the 3D condition. After that, a lot of cells detached from the substrate surface, and showed a declined proliferation. On the other hand, 3D bioprinted SU3 cells grew slowly in the first two weeks, with subsequently increasing cellular activities. To assess the effect of bioprinting process on cell survival, live/dead assay was performed. As shown in figures 3(E)–(G), most cells remained viable (green) and only a small amount of dead cells (red) were observed. Live/dead cell ratio was quantified to be 86.92%, which was comparable to that of cells mixed into GAF hydrogel system without bioprinting process (figure 3(H)).

When cultured in vitro for the first week, cells scattered within the scaffold without obvious proliferation. Then cell proliferation rate accelerated slowly over time. After three weeks, cells grew into spheroids and pushed the surrounding hydrogels aside to occupy a larger space within the scaffolds. This growth process is extremely similar to the in vivo tumor growth (figures 4(A)–(C)). SEM showed the spheroids bulged out over the scaffolds surface, as well as the trace of cell division and movement (figures 4(D)–(F)).

After the printed cell-laden scaffold were cultured for 7 d, as shown in the pathological sections, individual cells scattered in the hydrogel (figures 5(A) and (B)). During the culture period, natural gelatin degraded gradually via hydrolysis in the culturing medium, as well as by the secreted enzymes of tumor cells to provide space for expanding tumor cells with increased proliferation activity (figures 5(C) and (D)). As the ECM of tumor cell deposited, GAF hydrogel system simulated the characteristics of in vivo tumor stroma and provided an alternative platform for tumor biology research.

3.3. Stemness and differentiation potential of bioprinted glioma stem cells
In order to verify the stemness and differentiation capability, 3D bioprinted SU3 cells were cultured in vitro in stem cell medium and complete medium, respectively. Nestin was the most recognized...
biomarkers of glioma stem cells, and it was chosen for immuno-histochemical staining. Regardless of the culturing conditions (2D or 3D), SU3 cells had high expression level of nestin when cultured in stem cell medium. Unlike those cultured in the 2D condition, high level of nestin expression was observed in 3D printed SU3 cells even after FBS was added for 3 weeks, indicating a better capability of the 3D printed SU3 cells to maintain their stemness.

For differentiation potential, GFAP and \( \beta \)-tubulin III were chosen for staining. After culturing for 3 weeks, expression of GFAP and \( \beta \)-tubulin III increased significantly for SU3 cells cultured in 3D condition with the addition of FBS. The expression level of Ki67, a marker for cell proliferation activity was also evaluated, and no obvious difference was observed between groups (figure 6).

In addition, the expression of VEGF, one of the main biomarkers for malignant brain tumor progression and tumor angiogenesis was measured (figure 7). Over time, expression of VEGF increased gradually. It is known that tumor angiogenesis is a necessary condition of tumor progression \textit{in vivo}. Herein, over-expression of VEGF on 3D bioprinted glioma stem cells in the hydrogels was observed, indicating that GAF mimicked the \textit{in vivo} tumor stroma microenvironment, and glioma stem cells tend to transdifferentiate to tumor endothelial cell when necessary, similar to the \textit{in vivo} condition of tumor progression and characteristics of cancer stem cell biology.

3.4. Drug resistance

Chemotherapy drug resistance assay of 3D bioprinted glioma cells U87 and glioma stem cells SU3 were performed and compared to that of 2D monolayer cells using the most common type of brain tumor chemotherapy drugs TMZ. For both cell lines, the 3D bioprinted cells were more resistant to drug compared to those in 2D culture condition (figures 8(A) and (B)). After treated for 48 h at the drug concentration of 1600 \( \mu \)g ml\(^{-1}\), a large number of apoptotic U87 cells were observed regardless if they were cultured in 2D or 3D conditions (figures 8(C) and (D)); while the
situation of SU3 cells were better than U87 cells with a small number of live cells (figures 8(E) and (F)). Relative growth rates of SU3 in 3D and 2D environments were (107.20 ± 4.94)% and (72.73 ± 3.38)%; and that of U87 were (87.85 ± 4.57)% and (39.07 ± 3.57)% respectively (figure 8(G)). It can be seen that (1) 3D bioprinted glioma tumor model was more resistant to TMZ than 2D condition, no matter if glioma cell lines or glioma stem cell lines were used; (2) Glioma stem cells were more resistant to chemotherapy drugs, regardless if they were in 3D or 2D culturing conditions. These findings match with what have been reported in literature [34–36].

4. Discussion

Because of ethical and safety constraints, direct clinical studies of tumorigenesis and antineoplastic agents are
not allowed. Therefore, in vitro preclinical models that mimic the physiological environments of tumors are in need [37]. As described above, 2D culture and animal xenografts are not ideal methods. On the other hand, 3D tumor models are expected to mimic the human tumor tissues in vitro [38].

3D bioprinting has recently emerged as an extension of 3D material printing, using biocompatible or cellular components to build structures in an additive, layer-by-layer method for encapsulation and culturing of cells [39]. The in vitro 3D cellular environments simulate the complexity of the in vivo environment and the natural ECM. The 3D constructs have proven to promote cell viability, stemness, differentiation, migration, and cell–cell interactions that are not observed in 2D culture systems [40–42]. In addition, bimolecular gradients play an important role in chemotaxis and cancer metastasis [43], cells grew in hydrogel formed spheroids under non-adherent conditions, forming a gradient of nutrients and oxygen, which can more closely simulate nutrient concentration of the tumor in human body.

Compared to 2D system, 3D bioprinted tumor models recapitulate the in vivo tumor microenvironment more closely, at least in spatial dimension, ECM, and tumor-stroma interactions. Therefore, it is expected the 3D bioprinted tumor model would lead to results more similar to an in vivo tumor compared to 2D culture systems would. Comparing to seeding tumor cells on scaffolds or mixing tumor cells directly into hydrogel, 3D bioprinted tumor model has other advantages: (1) size and shape of the model can be designed before printing; (2) size of hydrogel filaments can be controlled based on the printer’s nozzle and relative matching parameters; (3) several different types of cells can be accurately patterned at the same time; (4) bimolecular gradients can be fabricated in multilevel: on one hand, internal and external bimolecular gradients can be presented on the bioprinted tumor-like tissue; on the other hand, internal and external biomolecules can also be presented in gradient on the hydrogel filaments.

3D bioprinting provides a huge space of application on cancer research. By precise patterning, various types of cell-laden biomaterials that mimic the in vivo tumor microenvironment can be used to study tumorigenesis, tumor heterogeneity, invasion and metastasis, and sensitivity to antineoplastic drugs [5, 44]. Demirci and colleagues reported a bioprinted human ovarian cancer model for the first time, which provided a tool for studying multiple unknown regulatory feedback mechanisms between tumor and stromal cells, and high-throughput drug screening [45]. Recently, Sun and colleagues reported that the bioprinted HeLa cells formed cervical tumor models: in the GAF composite hydrogel system, HeLa cells migrated and aggregated within hydrogel filaments with high viability. Even more importantly, 3D bioprinted cervical tumors were more resistant to chemotherapeutics of paclitaxel [18]. For brain tumors, chemotherapy drug resistance is also a huge challenge to overcome, and brain tumor stem cells are considered to be the main cause of drug resistance [46–48]. Research on brain tumor stem cell biology is the key solution to drug resistance, as well as tumor recurrence.
Figure 6. Immunohistochemistry staining (from top to bottom: nestin, GFAP, β-tubulin III, Ki67) of 3D printed and 2D cultured SU3. Scale bars: 50 μm.

Figure 7. Immunohistochemistry of 3D bioprinted SU3: VEGF expression at 7, 14, and 21 d in culture. GBM tissue (G), adjacent normal tissue (H) and SU3 xenograft tumor tissue (I) are used as positive control (PC). Scale bars: 50 μm.
In this study, first the biocompatibility of printed GAF hydrogel scaffolds was tested; results showed that TG can maintain morphological structure of the scaffold without additional cytotoxicity, or changing its water retention capacity, which is consistent with previous report \[49\]. Cell viability was detected immediately after bioprinting, and a high survival rate of 86.92% was observed, which was comparable to 90.4% survival rate for cells without printing process, similar to the results reported previously, without TG \[14\].

The proliferation potential of 3D bioprinted glioma stem cells was tested and compared to that of 2D cultured cells during prolonged in vitro culture. Results showed that cells on 2D substrate grew rapidly and faster than in 3D hydrogel at the beginning. However, after nine days, 2D monolayer cells became apoptotic and fell off from the substrate, with their activities dropped sharply. On the other hand, 3D printed cells grew slower at the first several days, but growth rate increased gradually, which lasted to 21 days in vitro, as observed in both electron microscopy and pathological section images. Therefore, 3D bioprinting is an ideal method for long-term in vitro cell culture with high cell viability.

For the function of 3D bioprinted cells, stem cell characteristics and differentiation potential have been verified. Without FBS, high expressing level of nestin was observed, and when FBS was added, the expression of differentiation markers GFAP and \(\beta\)-tubulin III increased. Cell viability was evaluated by Ki-67. It is interesting to see that cells printed within the hydrogel filaments showed spontaneous, high expression of VEGF, which is one of the most important markers of tumor angiogenesis \[50\]. Tumor vascularization via endothelial differentiation of glioblastoma stem cells have been reported earlier with 2D culture and mouse model \[51–53\]. 3D bioprinted cancer stem cell model showed enormous potential for studying tumor angiogenesis mechanisms, as well as relevant targeted therapies.

Most of the current drug evaluation methods are based on 2D monolayer cells. These screening methods, however, often fail to get accurate and reliable results, thus failure in predicting chemotherapy tolerance. For the same drug, tumor cells would show different responses in 2D and 3D models. In addition, due to the cross-species differences, the drug test results obtained from animal model cannot accurately reflect what would be observed in human body, as more than 95% of the drugs that are effective in

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**Figure 8.** Resistance of 3D bioprinted glioma tumor model to high drug concentration: (A): TMZ-susceptibility of U87 at 3D and 2D; (B): TMZ-susceptibility of SU3 at 3D and 2D; (C) and (D): TMZ treated U87 in 3D and 2D at 1600 \(\mu\)g ml\(^{-1}\) for 48 h; (E) and (F): TMZ treated SU3 in 3D and 2D at 1600 \(\mu\)g ml\(^{-1}\) for 48 h; (G): relative growth rate of SU3 and U87 in 3D and 2D treated with TMZ at the concentration of 1600 \(\mu\)g ml\(^{-1}\) for 48 h. Scale bars: (C)–(F) 50 \(\mu\)m. "***" \(p < 0.01\), "**" \(p < 0.05\).
animals are not as effective in human. Ultimately, most of the drugs fail at preclinical trial [9, 54, 55]. Therefore, 2D culture and animal models are not ideal for tumorigenesis and antitumor drug researches. To create 3D cell models, encapsulate cells in hydrogel by direct mixing is not ideal since it is limited by inadequate diffusion of nutrients and drugs into the thick hydrogel constructs. 3D bioprinting is an ideal method for drug evaluation because of its unique advantages described above. In this study, we find that compared to 2D model, regardless of the cell line used (glioma cell line U87 or glioma stem cell line SU3) 3D printed glioma cells showed resistance to TMZ at high concentrations between 400 and 1600 μg ml−1, especially 800–1600 μg ml−1. 3D bioprinted tumor model provides an excellent platform for drug evaluation and discovery, showing huge potential on precursor drug screening and preclinical study. Furthermore, with this technology, tumors in patients can be replicated in vitro, and individualized tumor can be tested for pre-treatments of anti-tumor drug. Eventually, individualized accurate treatment of cancer will be realized [56–58].

5. Conclusions

In this study, 3D bioprinted glioma stem cell model offered a novel platform, mimicking the brain tumor microenvironment with high cell viability and inherent characteristics, for long term in vitro culture, not only expressing its biomarkers, but also revealing potential functions when needed. In addition, 3D bioprinted brain tumor model showed more resistance to chemotherapeutics (TMZ) compared to 2D model, in which glioma stem cell model was more resistant to chemotherapy drugs than differentiated cell model. 3D bioprinted brain tumor model brings broad implications for glioma stem cell biology and anticancer drugs susceptibility.

Acknowledgments

This study was financially supported by the following programs: 1. Program of Medical Innovation Team and Leading Talent of Jiangsu Province, China (No. LJ201150), 2. Science and Technology Plan Projects of Jiangsu Province, China (No. BL2012048), 3. The National High Technology Research and Development Program of China (863 Program, No. 2015AA020303), 4. Guangdong Innovative Research Team Program, China (No. 20115055), 5. China Shenzhen Peacock Plan Project (No. KQTD201209), 6. Clinical cutting-edge technology, social development projects in Jiangsu Province (BE2016668).

Financial conflicts of interest

None.

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