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Review article

Biomaterial-based *in vitro* 3D modeling of glioblastoma multiforme

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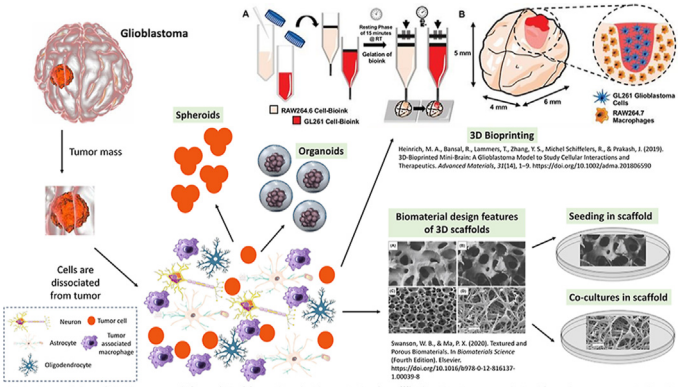
HIGHLIGHTS

- There is an urgent need to uncover major aberrant molecular pathways that could be targeted by new potential lead drug candidates in the treatment of glioblastoma multiforme (GBM).
- Lack of acceptable & reliable *in vitro* models which must guide the choice of *in vivo* GBM animal models hinders GBM therapy development.
- Development of 3D *in vitro* models could mimic the tumor microenvironment and the progression of the disease.
- Engineered biomaterials and technologies like 3D bioprinting could simulate GBM as closely as possible.
- 3D *in vitro* models will accelerate the preclinical testing and will aid in the development of an effective treatment for GBM

GRAPHICAL ABSTRACT



3D *in vitro* modeling of glioblastoma multiforme (GBM) (reproduced with permission from [111]). The GBM tissues are derived from the tumor mass isolated from the patients. Then different kinds of cells are dissociated from the tumor mass. These are: neurons, astrocytes, oligodendrocytes, tumor cells, tumor associated macrophages. *In-vitro* modeling is done in several ways: spheroid formation, organoid formation, formation of 3D scaffolds with the use of engineered biomaterials (reprinted with permission from [235]), 3D bioprinting etc. The bioprinting procedure and bioprinted mini brains are depicted using a schematic. A) Bioprinting procedure depicting the fabrication of cell-laden GelMA-gelatin bioinks and the subsequent two-step bioprinting of mini-brains. B) Close-up and cross-sectional view of the bioprinted mini-brains containing living cells (adapted with permission from [130]).

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ABSTRACT

Adult-onset brain cancers, such as glioblastomas, are particularly lethal. People with glioblastoma multiforme (GBM) do not anticipate living for more than 15 months if there is no cure. The results of conventional treatments over the past 20 years have been underwhelming. Tumor aggressiveness, location, and lack of systemic therapies that can penetrate the blood–brain barrier are all contributing factors. For GBM treatments that appear promising in preclinical studies, there is a considerable rate of failure in phase I and II clinical trials. Unfortunately, access becomes impossible due to the intricate architecture of tumors. *In vitro*, bioengineered cancer models are currently being used by researchers to study disease development, test novel therapies, and advance specialized medications. Many different techniques for creating *in vitro* systems have arisen over the past few decades due to developments in cellular and tissue engineering. Later-stage research may yield better results if *in vitro* models that resemble brain tissue and the blood–brain barrier are used. With the use of 3D preclinical models made available by biomaterials, researchers have discovered that it is possible to overcome these limitations. Innovative *in vitro* models for the treatment of GBM are possible using biomaterials and novel drug carriers. This review discusses the benefits and drawbacks of 3D *in vitro* glioblastoma modeling systems.

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Introduction

Glioblastoma multiforme (GBM), the most aggressive and common subtype of glioma, is a high-grade brain tumor comprising a heterogeneous group of gliomas. Surgery for diffuse gliomas is not an option because of the near impossibility of complete resection due to the considerable infiltration of the central nervous system (CNS) parenchyma that is present in all grades of glioma.[1] Patients with GBM have a life expectancy of fewer than 12 months without surgical intervention at the time of diagnosis, and the prognosis is poor even with surgery, radiation, and chemotherapy with the DNA-alkylating medication temozolomide.[2] Because GBM is a challenging and complex disease, extensive *in vivo* and *in vitro* studies have been conducted to develop a fundamental understanding of this condition.[3–5] Despite the potential of such research, *in vivo* models have several shortcomings. Among them are the limitations placed on the conditions that can be evaluated using a single animal,[3] the difficulty in obtaining reliable real-time data,[3,6] the high cost of experiments,[3] the inadequate translation of research to humans,[7] and the inability to accurately modify settings.[3] Owing to these limitations, it is challenging to employ *in vivo* models to determine causal links or isolate processes.[3,8] High-grade glioma treatment has not advanced much compared to that of other tumor types. This failure can be attributed to two main factors: first, the highly proliferative and infiltrative nature of GBM precludes surgical removal of the tumor and renders conventional therapeutic approaches nearly ineffective; second, the highly intra- and inter-heterogeneous nature of the tumor mass makes the identification of therapeutic targets very difficult.[9] Genome-wide molecular fingerprinting has revealed numerous potential genetic and epigenetic causes of glioma, revealing a wealth of new opportunities for drug discovery and more precise molecular classifications.[9,10] Identifying important aberrant molecular pathways that new prospective lead drugs might target atthe pharmacological treatment of GBM is urgently needed. It is anticipated that this fundamental knowledge will eventually lead to new therapies and improved patient outcomes. The lack of appropriate and trustworthy *in vitro* models, which should guide the choice of subsequent, more in-depth, and advanced *in vivo* GBM animal models, is a key problem hindering advances in GBM treatment [Table 1].[2,11,12]

Biomimetic models are essential for a deeper understanding of the relationship between tumor microenvironments (TMEs). Models that accurately represent the brain ME may yield more reliable results. To

Table 1
Cell culturing of glioma based on bio-scaffolds.

SL No.	Scaffold	Material	Cell type	Study type	Application	Disadvantage	References
1.	Microfluidic system	Cells cultured with alginate hydrogel tubes filled with circulating media	Cells from GBM patient resection	*In vitro*	More dynamic microenvironment, producing GSCs from the small initial cell population	N/A	68,189
2.	Matrigels	Gelatinous protein	E2, R10, G7 cell line	*In vitro* and mouse	Mimics brain ECM stiffness	Monoculture, does not provide structure equivalent to brain tumor ECM (E.g. high concentration of collagen and laminin	78,164
3.	Neurospheres	Cells are grown in suspension and transferred to geltrex-coated PEG and gelatin scaffold	GSC, human H9 ESC	*In vitro* and mouse	Allows cells to grow in 3D, ensures cells examined are tumorigenic, likely key target for anti-glaucoma therapy	N/A	32,73,263
4.	Spheroids	Multicellular cell aggregates grown in/on matrix made of polyethylene glycol/ polyvinyl alcohol/ polylactide-co-glycolide/ polycaprolactone	Cells from GBM patient resection	*In vitro*	Facilitates cell–cell and cell–matrix interaction, angiogenic, promotes stemness marker expression, secretion of cytokines, chemokines	Diffusion gradient with increased spheroid size	72,160
5.	Alvetex	Highly porous polystyrene scaffold embedded in Matrigel	E2, R10, G7 cell line	*In vitro* and mouse	Consistent structure, adaptable to existing cell format, compatible with current methods of analysis	Inadequate for cell maintenance or for experiments requiring cell suspensions in fluorescence activated cell sorting	32,85,264
6.	Electrospun scaffolds	Polycaprolactone, gelatin, hyaluronic acid	U251 cell line	*In vitro*	Thin scaffold limits intracranial pressure, contours resection cavity to provide stem cells with direct access to the brain	Low cell loading capacity, surface seeding that exposes cells to hostile resection microenvironment	191,265
7.	Microcapsule hydrogels	Alginate/collagen/fibrin/ chitosan/gelatin/ hyaluronic acid	Neural stem cell, GSC	*In vitro*	Long term persistence due to shielding from the immune system, potential universal stem cell line use	Stem cell immobilization that prevents utilization of tumor tropic migration, limited by tumoricidal agent diffusion	91,130,186
8.	Bulk hydrogels	2-hydroxyethyl methacrylate, agarose, or GelMA	GSC	*In vitro*	High loading capacity, embedded cells more shielded from hostile resection microenvironment	Increased intracranial pressure due to cavity filling, potentially toxic degradation of by-products from chemical cross-linkers	153,266,267

E2: Human estradiol; ECM: Extracellular matrix; G7: Cellosaurus cell line; GBM: Glioblastoma multiforme; GelMA: Gelatin-methacryloyl; GSC: Glioma stem cell; PEG: Poly(ethylene glycol); R10: Glycophorin A antibody; U251: ECACC general cell collection.

study GBM, *in vitro* cultures were used for the reasons described above. Unlike *in vivo* models, many culture factors can be included and controlled in *in vitro* systems.[3] This is significant because the makeup of the extracellular matrix (ECM), interactions between stromal cells, and tumor heterogeneity affect cell behavior.[4,13–15] Two- and three-dimensional (2D and 3D) *in vitro* cultures have been developed to study cell invasion, migration, and proliferation under various conditions, frequently including a tumor-specific circumstance, such as hypoxia.[3,4]

Standard 2D cell cultures involve layer-growing cells on a relatively stiff plastic substrate and maintaining their viability in a solution containing ECM proteins. Similar methods, such as laminin-coated plates or a layer of ECM mixture, are used to place cells directly on the surface and allow them to grow until they form a confluent monolayer. This platform is useful for studying cell morphology using various imaging techniques, antibody staining, and functional research because it makes use of readily available, specialized test kits from the market.[16,17] Current standard 2D cellular *in vitro* preclinical models are of low value and poorly informative because of several fundamental limitations that have a substantial impact on phenotypic, cell signaling, and medicinal responses. Inadequate cell density, gradients of medium components, unphysiological oxygen levels, disruption of the original spatial context, a lack of interactions with the ECM, and the presence of non-tumor cells in the GBM ME are the main causes of these biological consequences. It is essential to develop new *in vitro* models that are more accurate and practical to better understand the molecular biology and treatment of GBM. Considering the immune system components is crucial when evaluating GBM and, more broadly, brain models. The intricacy of the TME in GBM and the interactions between cancer cells and other immune system elements in the brain, and the comparatively scant understanding of these interactions, have slowed down advances in the treatment of GBM.[17] Advances in immunotherapy have considerably augmented the therapeutic toolbox for most solid tumors.

Understanding how certain microenvironmental features contribute to tumor formation *in vivo* is extremely challenging because of the extraordinary complexity of the TME. Building straightforward, reductionist systems that mimic specific microenvironmental components is becoming increasingly important to isolate the effects of these aspects while enabling a level of reproducibility and interpretability that is not feasible with *in vivo* systems.[4] The significant limitations of earlier modeling modalities can be overcome using advanced biofabrication processes to create 3D tissue models with high levels of flexibility, reproducibility, and scalability. Biofabrication techniques can be categorized based on whether cellular components are seeded onto constructs after device fabrication or are encapsulated in biomaterials during production. Compared to the cell-seeding method, the cell-encapsulating method offers greater control over the quantity and distribution of deposited cells and molecules, thus improving reproducibility.[18,19] Cells embedded in hydrogels experience ECM signals from all angles, similar to their normal states, whereas implanted cells only receive ECM cues from the hydrogel-contacting side. Although they are not frequently used for cell encapsulation, electrospinning, fused deposition modeling, and selective laser sintering can produce acellular scaffolds or devices with excellent resolution and throughput.[20] 3D bioprinting has become a tool for enhancing the study of cancer and tissue modeling because of its ability to accurately control tissue architecture and matrix properties while encapsulating live cells in biomaterials.[18,21,22] Modeling conditions with significant intratumoral and inter-patient heterogeneity, such as GBM, are well suited for 3D bioprinting because they allow for the creation of individualized and repeatable models. The focus of this review is to discuss the *in vitro* models of glioblastoma based on biomaterials. It discusses the models based on scaffolding, tissue engineering, bioprinting, microfluidic lab-on-a-chip regarding the ECM of the TME, and glioma tumor models. It focuses on recent advancements in the *in vitro* modeling field using natural or synthetic biomaterials for preclinical testing and analyzing the disease progression of GBM. It concludes with the limitations and future perspectives of the use of engineered biomaterials for the *in vitro* modeling of GBM. Figure 1 provides an overview of biomaterial-based *in vitro* 3D modeling of GBM. *In vitro* models that capture the complexity of the human brain are lacking in both neuro-oncology and neurology.[11,23] Therefore, different models that genuinely capture the complicated phenotypes of GBM are required.

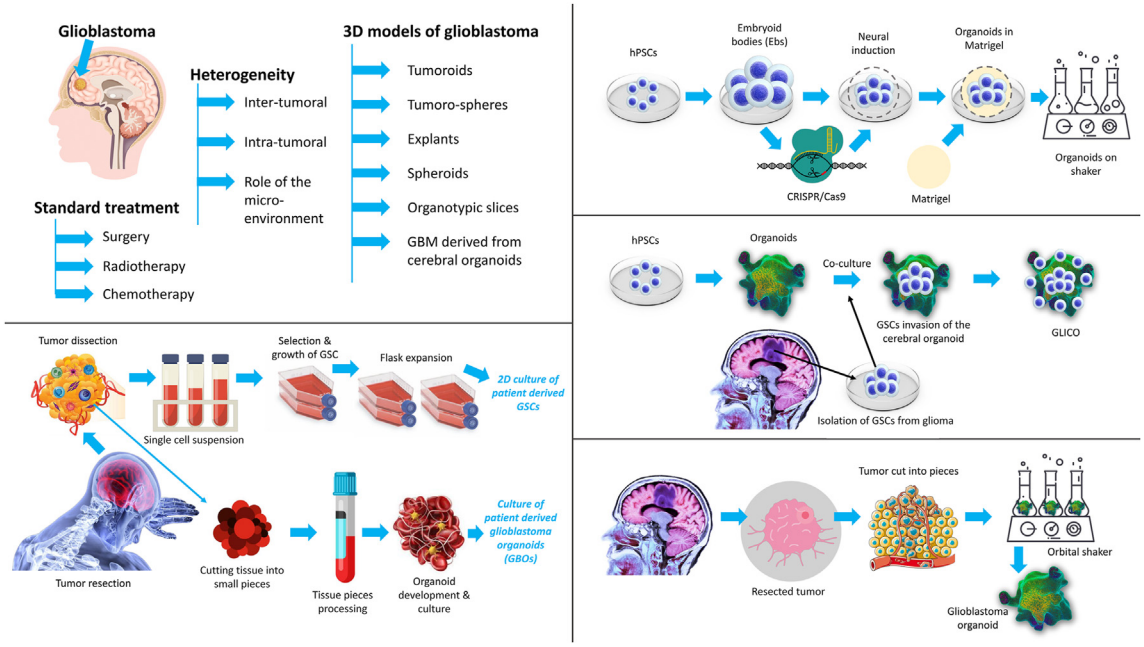

Figure 1. Overview of the biomaterial-based *in vitro* 3D modeling of glioblastoma multiforme (reproduced with permission).[44,215,259] The illustration on the left mentions the standard treatment options of glioblastoma, the tumor heterogeneity, and the 3D models of glioblastoma. It then focused on the culturing of the glioma stem cells (GSCs) obtained from the tumor dissection and growing them into 2D GSCs and glioblastoma organoids (GBOs). The right side of the illustration mentions the derivation of the organoids from hPSCs (human pluripotent stem cells). The middle right portion portrays the derivation of the GSCs from hPSCs and forming cerebral organoid glioma (GLICO). The bottom right part of the illustration of the resected glioma tumor and turning it into an organoid.

Developing, invading, and testing potential therapeutics for these models is essential for understanding gliomas.[24–26] The following sections provide an overview of 3D glioblastoma models with a specific focus on biomaterials and engineering.

Glioblastoma *in vitro* models

Tumor cells, healthy tissues, and ECM have been linked in 3D glioma cell culture systems. The ultimate objective is to create biomimetic platforms that can be used without previous experience.[27,28] The development of 3D culture methods has provided competitive alternatives to both 2D and animal models.[29–31] Scientists have already evaluated the current state of 3D tumor models grown *in vitro*. More advanced glioma models incorporate a wide variety of cell types, proteins found in the ECM, and soluble factor gradients.[32,33] Despite the rising prevalence of 3D TME models, incorporating macrophages and other immune cells is a more recent development.[34,35] Researchers have examined 3D models of the interaction between macrophages and cancer, namely glioma models, to gain further insight into tumor-associated macrophages (TAMs).[36–38] The next section discusses the 3D cell culture types of GBM.

Three-dimensional glioblastoma multiforme cultured cell types

In a model of GBM in which the tissues of the tumor explants were grown in Petri plates covered with collagen, both the TME and anatomy were maintained.[39,40] Jung et al. (2001) examined tumor infiltration into healthy tissues on an organotypic brain slice. The mentioned models have issues with tissue preservation and repeatability.[41,42] This enhanced the reliability of the GBM-specific 3D cell culture models. Both scaffold-free and scaffold-based cell culture environments are currently in use.[32,43,44] The ME in which a GBM tumor is embedded is highly intricate. In addition to microglia and astrocytes, mesenchymal stem cells (MSCs), TAMs, neurons, and perivascular cells are frequently observed in GBM TME samples.[45,46] Approximately 30–50% of GBM necrotic zone tumor tissues are M2 TAMs.[47] Creating an immunosuppressive milieu within the anti-inflammatory M2 phenotype facilitates tumor growth.[48,49] The CNS microglia are part of the immune system that is triggered in response to signals originating from tumors.[50] Through the upregulation of matrix metallopeptidase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), these immune components facilitate tumor invasion. Cancer cells can activate astrocytes through the use of efflux transporters and extracellular vesicles.[51] Tumor-associated reactive astrocytes create an immunosuppressive GBM ME by producing immunosuppressive cytokines, including transforming growth factor (TGF), which promotes the invasion of CD133+ glioma stem cells (GSCs).[52,53] Neurons have autocrine and paracrine effects on GBM in addition to glutamatergic synaptic connections. MSCs can promote tumor growth by releasing interleukin-6 and exosomes containing microRNA-1587.[54,55] Single-cell omics investigations have revealed many physiological stages in neoplastic cancer cells, notably stem-like GSCs, that promote tumor initiation, therapeutic resistance, and recurrence following therapy.[56]

There is no way to completely remove GBM tumors by surgery because the cells spread throughout the brain parenchyma. Adhesion molecules, including CD44, and receptors for hyaluronan-mediated motility (RHAMM), are highly expressed in GBM cells and aid in the ability of cells to attach to and migrate along the hyaluronic acid (HA)-rich ECM of the brain.[57,58] Neoplastic cells produce proteases to modify ECM pilocytic astrocytomas (PAs). Cancerous tissues may have adapted to the CNS, as GBM seldom spreads.[59]

Models devoid of a scaffold

Cultures can assemble into a spherical multicellular mass that closely resembles biochemical gradients when grown without the assistance of a scaffold.[60,61] By producing gradients in oxygen, nutrients, growth factors, and signaling, they can mimic the absorption of drugs into solid tumors.[62,63] Spherical models dominate the 3D modeling world. The interactions between cells were effectively mimicked. There are three cellular zones in spheroids: proliferative, necrotic, and quiescent zones.[61,64] Multicellular spheroids can mimic some aspects of solid tumors, including hypoxic centers and cell-to-cell communication. GBM cells can grow in many different types of media, such as hydrogels, plastic dishes, bioreactors, and hanging drops. The co-culture of stromal cells and preservation of cell–matrix linkages is made possible by hydrogel spheroids. Cell–matrix interactions are not taken into consideration by suspended spheroid models, despite the fact that they are easy, cheap, and quick to make.[65] When tumor cell lines are in an environment where they cannot stick together, they group together and form structures with many cells that look like spheres. Monocarboxylate transporters (MCTS) can be expressed in many different manners.[9,66] Organoids are multi-level, 3D constructions that reflect tissue variety more accurately. In this context, “self-renewing cells” refer to cells that can divide indefinitely and generate their own distinct tissue architectures.[54,67] The ability of cells to differentiate and organize is contingent on the presence of specific substances or ECM components in the growing media.[68–70] In organoid cultures, there is much less vascularization and growth of the duplicated organ.[71–73]

Scaffolding-supported models

Using biocompatible scaffolds that mimic the biochemical and mechanical characteristics of ECM, it is feasible to create *in vitro* models of the milieu in which GBMs are found. Cell invasion, microenvironmental interactions, and therapeutic outcomes can be studied within scaffolds.[46,74,75] Collagen, fibrinogen, HA, and basement membrane extracts are frequently used in the construction of biomaterial-based scaffolds.[76,77] Cells can transduce information and respond to scaffolds. Because these materials are found in mammals, infections, soluble substances, and protein concentrations may have an impact on the outcomes. Non-mammalian polymers, such as alginate and chitosan, can achieve this.[78–80]

Scaffolds come in various forms, including hydrogels, fibrous materials, and porous materials. Hydrogels, or water-absorbing microporous polymers, are materials with several practical applications. Scaffolds are formed when liquid precursors are cross-linked.[81,82] Therefore, cells may become encased in their membranes at an early stage. Marine hydrogels improve the accessibility of nutrients and development factors.[83–85] Hydrogels are frequently used for developing *in vitro* models. GBM cells penetrate the brain using a fibrous structure that looks like white matter pathways or blood vessels.[86,87] Polycaprolactone (PCL) and polydimethylsiloxane (PDMS) are examples of synthetic polymers used to prepare these structures. Seeded cells adhere to and migrate along the scaffold.[88–90]

Porous scaffolds in solids have pore networks. They support cellular processes that lead to the creation of 3D structures.[91,92] Cells proliferate and form spheroids as they attach to the scaffold. Scaffolding can be performed using several different techniques.[93–95] Techniques such as bioprinting, micromolding, gas foaming, and solvent casting/particulate leaching are examples of such procedures.[12,96,97]

3D bioprinting of tissues and organs employs polymers based on hydrogels (bioinks) [Table 2]. During the fabrication process, various layers and cells of the material are printed using images that have been digitally designed and segmented.[98,99] Similar biological, physiological, and biophysical features characterize the TME and may be seen in this arrangement of cells and ECM.[100–102] Accordingly, achieving the best possible results is of utmost importance. Several GBM bioprinted models have been constructed recently due to the promising potential of this technology for GBM research. For bioprinted models of GBM, other cell types, such as macrophages and astrocytomas, were introduced.[3,103] Different characteristics of glioma invasion are presented in the 2D and 3D models, as shown in Table 3.

Table 2
3D models of glioblastoma development with the use of different biomaterials.

SL No.	Biomaterial	3D model	Contributions	References
1.	Chitosan-alginate	Scaffolds	The use of a chitosan-alginate scaffold and Matrigel to evaluate the secretion of factors promoting tumor malignancy in a glioblastoma cell culture. Scaffolds were subsequently implanted into nude mice to evaluate tumor growth and blood vessel recruitment. The results show that chitosan alginate scaffolds promote the formation of a more malignant GBM phenotype than in monolayer or Matrigel culture solutions. This 3D model mimics the microenvironment of glioblastoma cells and may constitute an effective platform for the development of GBM treatments.	148,153
2.	Gelatin, alginate, fibrinogen	Bioprinting	The construction of a vascularized tumor by seeding spheroids into a bio-printed blood vessel layer. This study investigated blood vessel marker expression and tested drug efficacy. This bio-printed model mimics the tumor microenvironment and is useful for understanding tumor biology and for *in vitro* drug testing.	148,153
3.	Polyethylene glycol and hyaluronic acid	Hydrogel	An investigation into the effects of ECM stiffness on proliferation, dissemination, and gene expression of glioblastoma cells. Evaluation of matrices with two different grades of stiffness of GBM tissue. The results suggest that changes in ECM stiffness in tumors play a major role in modulating tumor progression.	11,12,152
4.	Methacrylated gelatin and gelatin	Bioprinting	The study of the interactions between GBM cells and glioblastoma-associated macrophages and evaluation of drugs aimed at inhibiting these interactions. The 3D models showed that glioblastoma-associated macrophages induce glioblastoma progression and invasion. Drugs inhibiting the interaction between tumors and macrophages reduce tumor growth.	149,151
5.	Hyaluronic acid and methacrylated gelatin	Hydrogel	Evaluation of hyaluronic acid of different molecular weights in methacrylated gelatin hydrogels. HA molecular weight impacts the migration of GBM cells. These results may be useful in research into new targeted therapies.	150,154,156
6.	Chitosan and hyaluronic acid	Scaffolds	Scaffold grown cells exhibited features of cancer stem cells, such as the expression of genes that mediate epithelial–mesenchymal transition. Furthermore, they presented an undifferentiated phenotype and displayed greater resistance to drugs than monolayer cell models. This model mimics tumor behavior and may be useful in basic research and preclinical studies.	155,164
7.	Collagen-hyaluronic acid	Hydrogel	Evaluation of combinations of hyaluronic acid with different types of collagens (type I/III and IV). The cell morphology was influenced by the type of collagen. The cell propagation and migration were dependent on the concentration of hyaluronic acid. The results suggest that GBM cells are sensitive to ECM mimetic biomaterials.	160,167
8.	Hyaluronic acid	Hydrogel	HA-CD44 and integrin-RGD interactions promote chemoresistance. The hydrogels with higher HA content protected glioblastoma cells against chemotherapy. Similar findings have been reported in a clinical trial setting where increased HA expression is positively correlated with tumor aggression.	163,166,268

CD44: Surface adhesion receptor; ECM: Extracellular matrix; GBM: Glioblastoma multiforme; HA: Hyaluronic acid; RGD: Arginyl-glycyl-aspartic acid.

Three-dimensional tumor microenvironment modeling

3D-cultured cells display a unique cytoskeletal architecture, gene expression, and metabolic activity in contrast to 2D cultures, which more closely resemble the conditions encountered in real organisms.[104–106] The TME mediates alterations in tumor morphology and bioactivity.[107–109] In 3D models, various cell types and materials are intimately entwined with tumor cells. The ECM may comprise up to 60% of the TME overall, according to certain studies. ECM compositional changes caused by tumor growth result in structural and mechanical/-physicochemical changes.[110–113] They have integrin-binding characteristics that promote tumor development and invasion.[114,115] Biophysical and pharmacological signals guide tumor cell growth and migration.[116–118] For cells to communicate while developing on a polystyrene surface and in touch with the cell medium, connections are required in 2D preparations.[119–121]

Limitations of current preclinical glioblastoma multiforme models

The current state of GBM culture and mouse models must be discussed before the development of novel culture systems to ascertain the therapeutic potential of candidate drugs more efficiently. Furthermore, we need to specify how their limited efficacy is due to the enormous complexity of brain tumor biology and the influence of the brain ME.

Status of preclinical glioblastoma multiforme modeling

The ideal system for the *in vitro* growth systems is patient-derived (PD) cells. Owing to the lack of resemblance to GBM tumor cells and the controversy surrounding their origins, human-immortalized glioma cells U87 (Uppsala 87) and U251 (permanent cell line) have lost favor.[122] These have been replaced with PD lines. These cells are created from surgically removed tumor tissue and transformed into cell lines that may be transmitted to and cultured as monolayers or in suspension in immunodeficient mice. Numerous cell lines have been produced at academic institutions and traded through partnerships in neuro-oncology research. Different settings were used to develop the cells *in vitro*. Media and additives have also been examined in recent studies.[123] The majority of laboratories utilize F12 (coagulation factor XII) or Dulbecco's modified Eagle's medium (DMEM) alone (Sigma–Aldrich, St Louis, Missouri, USA). Glucose, amino acids, minerals, and vitamins were also present in the medium. Currently, many organizations include growth factors in their medium, most frequently EGF and/or basic fibroblast growth factor (bFGF). In samples from human patients, these growth factors promote gene expression and proliferation.[124] N2 and B27 supplements are increasingly being used in GBM culture.

N2 mostly consists of putrescine, a diamine created from amino acids (Thermo Fisher, Waltham, Massachusetts, USA). Linoleic acid, corticosterone, and progesterone were all components of B27 (Thermo Fisher). We found that the medium composition can influence the phenotype of GBM cells, restricting the applicability of the present models. The 3D culture of lung and head and neck cancer cells embedded in laminin-rich ECM promotes radiation resistance relative to 2D culture by condensing chromatin and repairing DNA double-strand breaks.[125–127] Monolayer-grown cells differ phenotypically and respond in a variety of ways to cytotoxic treatments than do patients.[125,128]

The morphology, phenotype, and gene expression of colorectal cancer cell lines grown under laminin-rich ECM 3D conditions changed, and they developed resistance to epidermal growth factor receptor(EGFR)

Table 3
Aspects of glioma invasion in 2D and 3D models.

Model	Mechanism	Significance	Reference
Two-dimensional model			
Scratch assays: the monolayer culture on glass/plastic slides	Cell motility	To define the effect of several ECM components and soluble factors on glioma cell motility	61,230
Transwell migration	Cell invasion and cell motility, which depends on the chemotactic gradient	To define whether the factors will be able to favor or inhibit the invasion of GBM	51,269
	Insert coating	To assess the role of ECM components on cellular invasion	86,267
	Pore size	The glioma cells require myosin II only when migrating through the 3 μm diameter of the pore	270
			164,190
Three-dimensional model			
Migration of Transwell (modified)	Endothelial assay: to determine the effect of endothelial cells on glioma invasion	Perivascular invasion due to bradykinin	78,271,272
	Brain slice invasion assay: invasion of brain tissue slices	Effects on glioma cell motility, soluble factors, ECM components	272,273
Spheroids	Multicellular tumor spheroids (MTS)	The effects of motogenic substances and irradiation on migration upon adhesion on plastic substances	72,274
	Organotypic multicellular spheroids (OMS)	The role of different ECM components on cell migration from patient-derived spheroids	36,51
Ex vivo tumor sections	The tumor slices of PDGF-driven rat gliomas: glioma migration in living brain tissue through extracellular species in the sub-micrometer range	When invading the extracellular spaces, glioma cells squeeze through pores smaller than their nuclear diameter, and this process requires myosin II	268,275
	The tumor slices of brains xenotransplanted with human tumor cells and perivascular invasion of glioma cells	Perivascular glioma cells disrupt both astrocyte vascular coupling and the blood–brain barrier	154,276
Engineered models			
Two-dimensional	Stiffness: substrate with controlled elastic modulus	Motility is induced in glioma cells due to increased ECM stiffness	10,103
	Physical topography and confinement of cells	Increased motility is seen in substrates with aligned nanofibers and in cells cultured in micron-sized channels	44,277,278
	ECM composition and chemotactic gradients	Function of ECM components and chemotactic gradient on tumor cell motility	85,166
Three-dimensional	Stiffness	Cell motility is inversely related to stiffness	264,279
	ECM composition	The role of different ECM components to construct 3D hydrogels	191,265
	Migration along constrained paths	Parenchyma invasion mechanisms	91,163,231
		Perivascular invasion: currently available 3D models have not been tested with glioma cells	33,61
	Interstitial flow	The pro- and anti-migratory effects of interstitial flow	96,227
	3D cell–cell interaction	Tumor cells co-cultured with astrocytes show anti-apoptotic effects	9
		Tumor cells co-cultured with microglial cells show pro-migratory effects	81,156,215

GBM: Glioblastoma multiforme; PDGF: Platelet-derived growth factor.

inhibitors.[128,129] EGFR and VEGF inhibitors differentially radiosensitized cells in 2D and 3D GBM systems.[130] These findings may help explain why conventional 2D cell culture systems frequently underpredict treatment efficacy,[131,132] which results in an overuse of animal testing.

Intrinsic tumor factors

Every GBM tumor demonstrates a distinct genetic makeup. Studies have shown that GBM is a highly adaptable tumor type. Any attempt to simulate this cancer in a dish must consider many factors while initiating culture and selecting culture parameters. Based on the genetic characteristics, four tumor subtypes were discovered in a landmark study involving 400 patient samples. The groups were identified by mutations in receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR).[133] The four tumor subtypes have been reduced to three in subsequent research: classical, proneural, and mesenchymal, but their therapeutic utility is debatable. Recent findings from single-cell RNA sequencing have demonstrated that all three types of cancer are present in many patient cases.[134] With evidence of mosaic amplification of multiple receptor tyrosine kinases[135] and subpopulations inside tumors expressing several kinase variants,[136] intratumoral diversity includes crucial growth drivers. These cells are similar to GSCs. Owing to their capacity to self-renew, produce a variety of cell types, and express crucial stem cell genes.[137,138] *In vitro* GBM models are complicated by genetic variation. It should be noted that each PD cell line represents one of several genetic variations present in GBM. The Mayo Clinic created a panel of PD GBM lines, among which there were significant differences in the well-known GBM mutations *EGFR*, *PTEN* (phosphatase and tensin homolog), *p53*, and platelet-derived growth factor receptor A (*PDGFRA*).[139] Although these changes persisted in mouse intracranial xenografts, employing PD cell lines in studies introduces bias. Similar varieties of genetically and phenotypically varied cell lines have been developed by a team at Cambridge University.[140] It is crucial to use various cell lines in every inquiry to prevent skewed results. These panels will also shed light on potential predictive biomarkers, as the success of cell lines with particular mutations suggests that they may be useful in a subset of patient populations. Selection is necessary to generate a cell line from a patient tumor. Since 1929, very few brain tumor samples have been developed *in vitro*. As they only represent a portion of the tumor, specific tumor clones that persist and flourish in culture will skew the assay results. For preclinical studies and animal models, laboratories must use several cell lines of various subtypes to account for inter-tumoral variability. As a result, the amount of time and money required to develop GBM treatment has increased. The inability to cultivate GBM cells may be attributed to intratumoral variability. Evidence shows that several tumor subpopulations play a specific role in the maintenance and growth of tumors. Bidirectional communication between differentiated GBM cells and GSCs promotes GBM development.[141] There is a significant risk of losing tumor subpopulations and subpopulation interactions that are essential for tumor activity when cells are chosen from patient samples. Current cell culture techniques promote uniformity, limiting their utility. The ability

to sustain diverse cell mixtures has been improved with new *in vitro* techniques.

Because GBM cells are flexible, the development of representative tumor cultures is difficult. GBM cells are unstable and are in a state of dynamic equilibrium.[142] There are many different phenotypes of brain tumors due to GBM cellular plasticity. The expression of GBM genes and their functional state, including the development of the GSC state, can be affected by acidity, hypoxia, chemotherapy, and radiation stress.[143,144] There have been more recent activators of cellular plasticity. Extracellular vesicles from GBM cells that have died transmit splicing factors to neighboring cells that are still alive, altering their transcription and promoting an aggressive character.[145]

The development of *in vitro* brain tumor models is affected by the sensitivity of GBM cells to their environment because even minute changes in culture conditions can have a significant impact on the phenotype. Fetal bovine serum (FBS), a common additive in cell culture media, modifies the phenotype of GBM cells generated by patients. The serum decreased GSC subpopulations and promoted the expansion of cells with distinct genetic and functional properties.[124] The growth factor-containing serum-free cultures used today were inspired by this study. Small changes in growth factor concentration can affect how cells behave and how sensitive they are to medication.[123] Passaging cells induce cellular plasticity, changing the expression and phenotype of PD GBM cells, including their sensitivity to the medicine.[146] Uneven treatment sensitivity is caused by these mechanisms, which have been shown to cause mouse-specific changes in the tumor phenotype and gene expression.[147] Laboratories and research teams must maintain consistency because GBM cells adapt to their environments.

Tissue engineering-inspired glioblastoma multiforme models

Inspired by tissue engineering, biomaterial platforms are now being exploited as synthetic models of cell activity to better understand the mechanisms underlying tumor formation.[148] Hydrogels and electrospun fibers, which are both types of biomaterials that are frequently used in tissue engineering, are currently being used as two of the most helpful *in vitro* disease models which are currently being used.[149,150] These two types have several desirable properties, including the ability to be tweaked chemically and mechanically and the incorporation of a wide range of cell-responsive signals (including adhesion molecules and growth factors) into the material.[12,151,152] glycosaminoglycan (GAG) and proteoglycans (PGs) are naturally found in the extracellular environment of the brain; therefore, cross-linked polymeric biomaterials termed hydrogels can duplicate the structural and mechanical features of brain tissue.[153,154] To facilitate the spread of GBM in living organisms, electrospun strands are fashioned to resemble fibrous components (e.g., white matter and blood arteries).[11,155] These materials, whether synthetic or derived from nature, are designed to mimic the TME in three dimensions.[156–158]

Synthetic biomaterials

Research into GBM cell activity has been made possible with the help of synthetic biomaterials. Hydrogels based on poly(methylphenyl) siloxane (silicone rubber) and poly(acrylamide) have been used to investigate glioma cell movement.[101] These findings indicate that substrate stiffness is related to migratory processes. The significant influence of the mechanical environment on migration control was demonstrated with poly(acrylamide)-based hydrogels, where migration declined significantly when stiffness (E) (E × 0.8 kPa (kilo Pascal)) was in a manner comparable to that of brain tissue. To achieve the primary goal of these tests, which was to alter the mechanical properties, synthetic materials were used due to their adaptability.[159,160] The topography of GBMs was also studied using electrospun poly(ε-caprolactone) (PCL) fibers. GBM cells migrated more quickly on oriented PCL fibers than on random PCL fibers.[161,162] This special topographic susceptibility of materials influenced by tissue engineering was demonstrated in a second study, in which glioblastoma tumor progression was susceptible to modest quantities of signal transducer and activator of transcription 3 (STAT-3) inhibitors in cultivated brain segments and on oriented nanofibers, but not in TCPS41. Cell translocation was not affected by STAT-3 inhibition in a transwell migration experiment, unlike in nanofibers and brain slices.[163,164] Cell mobility is connected to STAT-3 signaling, a recognized regulator of cellular proliferation *in vivo*. At the intermediate modulus (*8 MPa) of the matched PCL nanofibers, the GBM migration speed peaked. Atomic force microscopy (AFM) has rarely been used to study white matter pathways.[165] This process results in the production of nanofibers with properties comparable to those mentioned in a previous publication. Research on electrospun nanofibers may have a greater physiological impact if additional studies are conducted to investigate how cancer affects the mechanical modulus underlying the white matter tracts.[165]

Synthetic materials allow users to control their properties, but they cannot reproduce the complex and time-varying chemistry of the *in vivo* milieu. This chemistry encompasses both chemical and mechanical changes.[166,167] *In vitro* cell migration is analogous to *in vivo* migration; however, there is no 3D ECM structure to test cell migration. This 2.5-dimensional environment connects the 3D and 2D environments. Without an ECM-like barrier, proteases and glycans cannot be studied for their role in tumor cell migration.[168,169] It is anticipated that the number of studies utilizing these materials as 3D tissue analogs will dramatically increase over the next several years due to the development of novel biomaterial combinations.

Synthetic polymers

Synthetic biomaterials, such as polyethylene glycol (PEG), polyurethane (PU), and poly(N-isopropylacrylamide) (PNIPAAm), have been used in GBM research. PNIPAAm and its composite materials are thermoresponsive hydrogels that exhibit good printability in bioprinters utilizing extrusion. PNIPAAm implanted with gold nanorods can be printed using multiphoton lithography to achieve nanoscale resolution and dynamic post-printing modulations.[170] Primary GSCs grown in a PNIPAAm-PEG matrix retained their stemness for an extended period of time and were simple to extract and re-encapsulate by altering the temperature of the hydrogel.[89] Hydrogels have the capacity to proliferate GSCs in the vast quantities necessary for screening. PEG is a popular biomaterial for 3D tissue modeling owing to its high biocompatibility, inert biochemical properties, and adaptable mechanical properties.[171] PEG and its derivatives are amenable to the addition of bioactive components to enhance biomimicry and printability as bioinks.[172–174] The effects of stiffness on the advancement of GBM have been investigated using PEG hydrogels coupled with a predetermined concentration of HA and functionalized with arginyl-glycyl-aspartic acid (RGD) peptides and MMP degradation cross-linkers.[175] GBM cells cultured in a stiff PEG hydrogel (26 kPa) produced denser tumor spheroids than those cultured in a soft structure (1 kPa). Owing to its biocompatibility and photopolymerisability, photo-polymerizable poly(ethylene) glycol diacrylate (PEGDA), a PEG derivative, has been widely used in 3D bioprinting.[176] Glioblastoma cells were co-cultured with endothelial cells in PEGDA-based microwells for *in vitro* glioblastoma cell culture for high-throughput drug screening.[177,178] PU hydrogels are biodegradable and thermo-responsive. Through the use of 3D bioprinting, brain stem cells have exhibited excellent growth and differentiation capabilities when embedded in water-based PU hydrogels.[179]

Self-assembled peptides

Superabsorbent polymer (SAP)-based hydrogels are complexed to produce nanofibrous sheets that resemble biological ECM structures by the physical or chemical interaction of the peptides.[154,180] Peptides are amino acid chains with inherent biological properties. Fibrous SAP

hydrogels are viable bioinks for extrusion-based bioprinting owing to their adaptable mechanical properties and stimuli-responsive gelatinization processes (such as enzymatic activation).[181,182] Fluorescent SAP hydrogels exhibit exceptional mechanical stability and a low rate of solution deterioration during extrusion-based proof-of-concept printing.[183] Owing to its injectability and ability to conform to irregular shapes, SAP is a fantastic option for CNS regeneration, including blood–brain barrier (BBB) repair or repair of damaged brain tissue after GBM resection. After injection into the brain of a zebrafish brain injury model, a peptide RADA (RADA16 is a 16-amino acid Type I-SAP containing repeated R [positively charged arginine], A [hydrophobic alanine], and D [negatively charged aspartic acid] amino acid residues) 16-SVVYGLR (osteopontin-derived synthetic peptide)-forming hydrogel with a stiffness ranging from 0.326 to 5.336 kPa promotes both angiogenesis and neurogenesis.[184]

Natural biomaterials

Natural scaffold materials can be used in conjunction with 3D tumor cell migration models. Matrigel and collagen tests were routinely performed. Experiments were conducted to examine radial cell migration away from the tumor center, using both tumor tissue spheroids and cell seeding on the surface of the gel.[160] These experiments are useful for understanding cell movement in 3D environments, but they lack physicochemical parameters, such as stiffness and ligand density, making tumor cell properties difficult to discern. To isolate the effects of certain parts on 3D cell behaviors, researchers have constructed complicated biomaterial models comprising multicomponent and customizable systems.[149,185,186] The migration of GBM cells in 3D hybrid collagen-agarose hydrogels is inversely related to matrix rigidity. Amoeba-like migration occurred during the mesenchymal transition. This study also examined the effects of pore size on GBM migration in 3D collagen gel networks. When the pore diameters ranged from 5 to 12 mm, there was no correlation between the pore size and invasion distance.[46,157] There was an increase in the secretion of tumor-promoting chemicals from human glioblastoma cell lines cultured in 3D compared to 2D cultures. Researchers have used hydrogel biomaterials that contain HA to mimic the real world.[35,187] These biomaterials were used in 2.5D and 3D cultures to analyze GBM cell movement. In 2.5D culture, GBM cell motility was strongly influenced by the stiffness of the gel and ligand density (0–5 mg/mL RGD).[166] Lower-density HA cultures allowed migration, while higher-density 3D HA hydrogel cultures prevented migration. In collagen gels, chondroitin sulfate acts as a mobility inhibitor for GBM. In the model with regularized white matter topography, a HA "shell" on a PCL "core" nanofiber halted GBM migration. The factors that promote GBM migration in HA-based hydrogel systems are kappa-elastin, stromal cell-derived factor-1a, and bFGF.[160] The types of cells that invaded the HA hydrogels varied. Although HA has several beneficial properties, one of which is the promotion of cell migration, HA alone is not sufficient. More resources are required to achieve a motion similar to that observed *in vivo*. This may be due to chemical variables and the thick structure of the HA hydrogel, which has few open pores to facilitate migration (HA hydrogels do not promote cell attachment on their surfaces).[11,149] There are several benefits of using biomaterials sourced from nature. Tumor cell responses to contrasting stimuli (such as chemistry or stiffness) can be studied using adaptable systems.[148,188] Although it is possible to mimic *in vivo* conditions with synthetic biomaterials, natural biomaterials produce signaling responses that affect cell function. It is possible that only the mechanical and topographical features of natural systems are observable; however, variations in composition can influence the experimental results. Synthesized peptide- and protein-based ECMs can incorporate native architectural elements with the physiologically appropriate stiffness and can be used to study GBM behavior.[189–191] By maintaining appropriate gradients, including flow, microfluidic devices can provide time-sensitive migration resolution in both 2D and 3D culture microenvironments. 3D biomaterials are more reproducible than animal tissue models.[192,193] Compared with brain slice models and direct confrontational testing, these offer fewer disadvantages in terms of cost, duration, and ease of application.[42,46] To completely characterize the activity of GBM tumor cells, it may be necessary to combine hydrogel, brain segment, and/or fiber-based tests. High-throughput screening using 3D cell culture models can help determine their potential effects on animal models.[68,77] Research into intracellular signaling cascades and migratory regulatory circuits using present and future physiological biomaterial systems may lead to the discovery of novel therapeutic targets.[9,159] The next section provides an overview of GBM modeling through 3D bioprinting. Figure 2 shows the stem cell-derived GBM organoids, *in vitro* models, and 3D cell cultures. Table 4 lists the advantages and disadvantages of different glioblastoma models.

Hyaluronic acid

d-Glucuronic acid and N-acetyl-D-glucosamine alternate to form a negatively charged linear polysaccharide known as HA, which is produced in the plasma membranes of glial and neuronal cells.[194] HA-based hydrogels are the most pertinent matrix materials for mimicking brain tissue due to the dominance of HA in the brain and GBM stroma and its crucial function in controlling various physiological and pathological processes. HA hydrogels imitate the stroma of the brain and GBM with nanoporous structures and a range of elastic moduli.[195] To construct 3D GBM models, HA was combined with type I collagen,[196] gelatin methacrylate (GelMA),[197] chitosan,[159] laminin, fibrin,[198] and PEG.[175] It is important to consider the size-dependent regulatory actions of HA when building models. HA larger than 1000 kDa is required to model healthy brain tissues.

Reduced molecular weight HA affects the growth and migration of GBM cells. Invasiveness was increased by lower molecular weight HAs (10 and 60 kDa) by stabilizing the poro-elastic properties of HA-GelMA hydrogels (500 kDa). The elastic modulus of HA-GelMA hydrogels was similar across all groups and unaffected by the molecular weight of HA, which was approximately 3 kPa.[197] The growth and vascular formation of human embryonic neural progenitor cells (NPCs) are facilitated by HA, laminin, and fibrin scaffolds. Chemical changes to produce HA derivatives have been reviewed for 3D modeling and bioprinting.[195] The carboxylate group of the D-glucuronic acid moiety, the N-acetyl-D-glucosamine moiety, and the hydroxyl groups of both moieties were the focus of the modifications. The HA hydrogels were prepared by radical polymerization. In the presence of photo-initiators, such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), HA functionalized with glycidyl methacrylate (GMHA) or methacrylic anhydride on the N-acetyl-D-glucosamine C-6 hydroxyl group can be photopolymerized to create a hydrogel.[195] GMHA and methacrylated HA (MeHA) are promising bioinks for light-assisted liver tissues. GBM models were bioprinted using the GMHA-based hydrogel combination.[22,199] To encourage cell adhesion to the 3D matrix, RGD peptides have also been functionalized into MeHA.[200] Additionally, HA-based hydrogels can be produced via condensation and addition processes. The ideal bioink for extrusion or inkjet bioprinting is HA thiol derivatives, which crosslink via disulfide bond formation in the air without initiators. Biocompatible hydrogels were produced through the addition and condensation of HA modified with aldehydes, dihydrazides, and haloacetate.

Collagen

Collagen is a widespread ECM component. Although the brain lacks type I fibrillar collagen, the vascular basement membrane is rich in types IV and V. Collagen-derived biomaterials can be used to model the BBB. Various GBM studies have used collagen biomaterials owing to their well-studied gelation mechanism (both pH- and temperature-based), the quantity of cell-binding sites, and variable mechanical properties. GBM cells develop diverse 3D morphologies in type IV and type I/III collagen matrices.[15] Tissue modeling uses collagen, HA, agarose, and synthetic

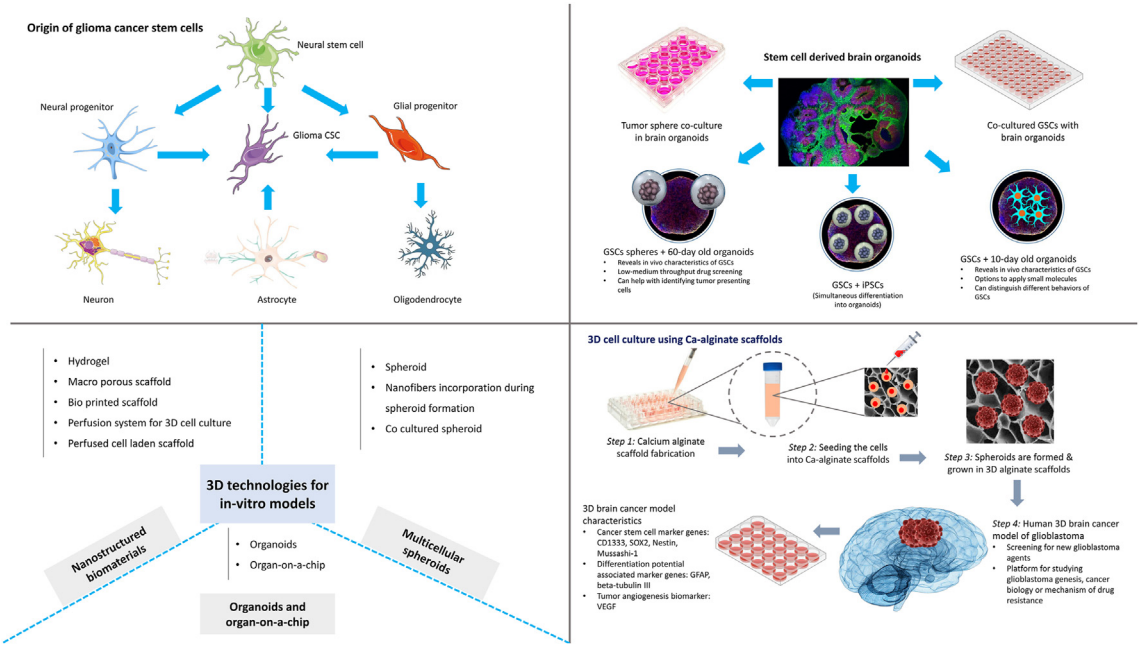

Figure 2. Overview of the 3D technologies for *in vitro* modeling of glioblastoma multiforme and the origin of glioma stem cells (reproduced with permission).[220,228,260–262] The upper left part of the illustration shows the origin of the glioma stem cells. The bottom left portion shows the examples of the 3D technologies for the in-vitro models. The upper right portion shows the examples of stem cells derived brain organoid techniques. The bottom right portion shows the method of 3D cell culture using calcium alginate scaffolds. GSCs: Glioma stem cells; iPSCs: Induced pluripotent stem cells; VEGF: Vascular endothelial growth factor; GFAP: Glial fibrillary acidic protein.

materials. Only type IV collagen supported GBM cell proliferation in the hybrid matrix with HA.[201] Pure collagen solutions gel and thicken slowly. Adding riboflavin or increasing the collagen concentration enhances bioprinting accuracy.[202] The addition of riboflavin to collagen bioinks improves printability. Bioprinting with collagen-based inks is employed in tissue engineering applications, such as heart regeneration and liver modeling.[203] The hydrogel elastic modulus can be customized between 0.9 and 3.6 kPa, which is ideal for brain tissues.[202]

Gelatin

Gelatin was used as the collagen hydrolysate. Gelatin and its derivatives are frequently used in 3D tissue modeling because of their bioactive characteristics, which include MMP digestion sites and integrin-binding RGD sequences. Angiogenesis and ECM remodeling were boosted when PVN and GBM cells were co-cultured in a 3D gelatin matrix. Gelatin-based bioinks are widely used due to their favorable rheological and thermal characteristics. Hepatocytes are maintained alive and functional in 3D-printed tissue for two months by encapsulating the cells in a gelatin hydrogel.[204] Gelatin can be used with synthetic materials, such as PU, to increase the window and resolution of bioprinting. MSCs thrived in the gelatin-PU matrix. GelMA, a gelatin derivative, has been employed in 3D bioprinting. The GelMA bioink is photopolymerizable in the presence of photo-initiators when exposed to UV light by modifying lysine and hydroxyl groups with methacrylamide and methacrylate side groups. GelMA offers adaptable 3D matrices while preserving the biological properties of the gelatin. GelMA can be used as a fundamental matrix for studies of functional ECMs, such as HA, that are associated with the brain. Tumor growth is influenced by the presence of soluble or immobilized HA in a gelatin-based matrix.[205] The biphasic

Table 4
Advantages and disadvantages of different glioblastoma models.

SL No.	Model	Advantages	Disadvantages	References
1.	Bio-printed chip systems	Ability to build 3D microstructures of various cell patterns in microfluidic devices	Critical choice of supporting scaffold composition and bioink printability	213,280
2.	Organotypic slice cultures	Useful to study infiltration processes	Mouse brain slices are required	61,189
3.	Organoids	Suitable to study the niche microenvironment	Organoid composition may vary between different experiments	77,230
4.	hiPSCs	Can be produced in the lab via genetic manipulation	Genetic manipulation may not reflect the genotype of GSC from human samples	93,269
5.	Microtubes	Opportunity to study intercellular communication and niche formation	Critical effects of cell spatial organization and structural marker identification	60
6.	Glioma cell lines grown in 3D	Enhanced invasiveness, increased integrin expression, expression of stemness markers	Not well-characterized middle ground between cell lines and GSC	86,214
7.	2D-3D cultures	Share features of GBM, such as resistance to therapeutic treatments, high invasiveness etc.	Must be isolated from fresh human samples and require extensive characterization	68,95
8.	Glioblastoma stem cells (GSC)	Grown as adherent cells or neurospheres	Spheres environment could limit stem cell divisions	62,66
9.	Glioma cell lines grown in 2D	Suitable for high throughput drug screening and is commercially available	Variations in genotype and phenotype, and does not closely resemble GBM	90,209

GBM: Glioblastoma multiforme; GSC: Glioblastoma stem cells; hiPSC: Human induced pluripotent stem cell.

maxima for angiogenic and hypoxia markers were between 0.3% and 0.5% HA.[206] Gradients of HA, crosslinking, and GBM cell density can be produced using GelMA-based hydrogels.[206] Local MMP2 expression has an inverse relationship with cell density, whereas tumor cell proliferation and proangiogenic expression are correlated with local crosslinking density and tumor cell density. GelMA and PEGDA were employed to create a cardiac patch for myocardial infarction.

Glioblastoma multiforme modeling through three-dimensional bioprinting

Researchers are creating patient-specific cancer models to better capture the complexities of the disease.[207,208] The 3D-bioprinted GBM model used extrusion and a segmented cancer-stroma concentric ring architecture, according to Yi et al.[102,185,209] The bioprinted GBM-on-a-chip reproduces all aspects of the ECM and microvessels (MVs) that normally surround malignant tumors. To obtain GBM cells prior to chemoradiation, the tumor tissue was removed.[210,211] The tolerance to radiation and temozolomide (TMZ) displayed by this model was successfully replicated. Patients with GBM who have become resistant to traditional first-line therapy may benefit from this approach by accessing more effective medication combinations.[3,212,213] Tumor progression, invasion, and angiogenesis are aided by glioma-associated macrophages (GAMs). Preclinical studies have shown that inhibition of cancer cells and macrophages can significantly decrease the progression of GBM.[191,214] Researchers have used 3D bioprinting with extrusion to create a miniature brain model with macrophages and GBM cells.[103,215,216] The relationship between these two factors may affect macrophages and cancer cells. This is a two-stage bioprinting process. This 3D mini-brain was initially made from a macrophage bioink (RAW264.7).[11,23,24] By excluding other parts of the TME, the model isolates interactions between macrophages and GBM cells. There was an increase in GAM-specific and matrix remodeling markers after macrophages interacted with GBM cells. In particular, prostate cancer cells or GBMs migrated.[28,148,217]

To better treat patients with glioma, models are required because of the stem cell nature of the tumor. Dai et al. constructed a 3D GSC model using extrusion and bioink for their study.[33,34,186] However, bioprinted GSCs have the potential to differentiate and develop blood vessels.[35,218,219] From weeks one to three, there was an increase in VEGF expression in GSCs. Regarding TMZ resistance, this model performed better than the 2D monolayer model.[36,220] Three weeks later, GSCs bioprinted in 3D outgrew their counterparts that had been cultured in 2D. It is possible that 3D bioprinting maintains cells in culture for a longer period.[37,38,221] Understanding the biology of GSCs will improve drug resistance and potential for anticancer treatment.[39,40,166] Cancer ecosystem models, including cells, the ECM, and anatomic heterogeneity, can be developed for individual patients using 3D bioprinting.[190,222,223] The following section provides an overview regarding the influence of the 3D culture ME and its dependence on drug responsiveness.

Biomaterials for three-dimensional bioprinting

Bioink, which can be made from either synthetic or natural biocompatible polymers, allows for the recreation of *in vivo* conditions through 3D printing.[149] Bioinks that allow for good strength, viscosity, and 3D tissue growth have been challenging to develop.[68,189] A new study concentrated on bioink formulation and 3D bioprinting processes in an effort to improve structural precision, cell viability, and production time. Biomaterials encase cells and offer an optimum environment for 3D bioprinting.[150] The viability and fidelity of 3D models printed with bioinks depend on their viscosity, shear thinning, and thixotropy. If the bioink is not thick enough, it will affect the print quality and may even prevent printing.[51,224] It is possible for the nozzle tip to become clogged when using a bioink with such high viscosity. Bioink deposition is enhanced by shear-thinning materials with thixotropic biomaterials, which change viscosity upon shear stress and can deform 3D-printed cells.[225,226] During bioprinting, cells are protected from damage and stress by hydrogels, alginate, and gelatin. Biopolymers, including collagen, fibrin, gelatin, and alginate, are preferred for bioprinting because of their thermo- and stimuli-response.[12,51] Cellular motility, proliferation, and differentiation are regulated by bioactive groups. Natural substances have both benefits and drawbacks. However, collagen does not have the necessary mechanical stability and viscosity for 3D printing despite its capacity to promote cell proliferation and adhesion in the ME.[227,228] As a collagen by-product, the reversible temperature-dependent gelation of gelatin makes it an ideal material for 3D bioprinting. The cell-binding domains and matrix metalloproteinase identification sequences in this protein are beneficial for cell health.[229] Gelatin, similar to collagen, is brittle and sticky. Alginate, a seaweed polymer with low toxicity, lacks cell-binding features, despite its biocompatibility. Fibrin is a naturally occurring polymer that promotes cell adhesion and proliferation; however, the degradation rate was reportedly too high to support a sustained cell culture.[230] GelMA, also known as gelatin methacryloyl, is a bioink that is both flexible and biocompatible. Due to its quick sol–gel transition at ambient temperature, SLA bioprinting is challenging to achieve with GelMA.[47,164] The mechanical stability and printing resolution of GelMA can be modified for rapid printing. The improved biocompatibility and resolution of GelMA make it a useful bioink for a wide range of biofabrication uses.[231] Synthetic polymers with excellent mechanical properties can be used in 3D printing and tissue engineering at low prices. They do not contain any active ingredients, and hence, they have no biological effects. PEG can be easily altered and is safe to use in biological systems.[167] PEG lacks cell-adhesion domains and is stable at physiological temperatures, light levels, and pH. A third biocompatible, biodegradable, and stiff bioink polymer is polycaprolactone (PCL). In its liquid state, PCL is hazardous to cells and cannot be used for cell encapsulation; however, its low melting point of 63 °C makes it a good material for 3D bioprinting.[232] Owing to its hydrophobic composition, it prevents cells from sticking together and multiplying. Similar to PEG derivatives, the PVA (polyvinyl alcohol) characteristics can be easily altered. PVA has low affinity for cells and is biodegradable, biocompatible, thermostable, and water-soluble.[188,233] PVA must be functionalized so that its chemical and mechanical properties can be controlled for use in cell culture platforms.[234] Polyurethane (PU) is a synthetic bioprinting substance that is biodegradable, aqueous, and has low toxicity. Light, heat, and pH can affect hydrogels made from polyurethane. Biomaterials for 3D printing may have varying qualities depending on their intended use.[100,192]

Glioblastoma multiforme organoids

The best *in vitro* model for studying GBM is GSC, despite issues with cell population heterogeneity and marker identification. There were no physiological or reciprocal interactions between GSC and vascular, non-tumor, or other cells in this model. In cancers, both vascular and non-tumor cells interact with GSC. By releasing soluble chemicals and altering the ECM, non-tumor and vascular cells promote the formation of GSCs. GSCs also produce angiogenic factors that affect the differentiation and tumorigenicity of nearby vascular pericytes.[235] Several research groups have provided organoid models. Organoids resemble the *in vivo* tissue architecture, cell proliferation, self-organization, and differentiation, making them an effective *in vitro* model.[236] Neural organoids were initially described in a study that created brain tissue from hiPSCs.[237] Organoids have recently attracted the attention of researchers as 3D models for brain physiology and physiopathology, such as brain cancer.[238–241] Other researchers have used this ground-breaking method to reproduce primary human GBM *ex vivo* for high-throughput drug screening in *in vitro* GBM models. To create GLICO (cerebral organoid glioma), researchers reverse-engineered GSCs and human embryonic stem cells (hESCs).[242] In this model, GSC tumors invade organoids and develop within the organoid, creating a web of microtubes that infiltrate healthy host tissue. This final advantage is crucial because it considers the most recent research on GBM tumor cells and healthy brain tissue. In

a related study, long-term 3D organoid culture was performed using GBM tissues.[243] This tumor organoid model has rapidly proliferating cells encircling the hypoxic core of GSC and non-stem cells. Orthotopic transplantation of these organoids resulted in tumors that were more invasive and had better histology than PD neurosphere cultures, lending credence to this theory. To create an organoid system for invasive altered cells, other authors used CRISPR/Cas9 genome editing.[244] The promise of the platform as a tool for deciphering GBM molecular pathways is highlighted by the development of invasive tumors when organoid-derived presumptive tumor cells are implanted into human cerebral organoids. The inter- and intratumoral heterogeneity of GBM has been mimicked in recent research using a new organoid.[245] The authors bio-banked PD glioblastoma organoids (GBOs), which closely mirror the genetic features of their parental tumors *in vitro* and can generate tumoral masses in *in vivo* animal models, using histological, transcriptomic, genomic, and single-cell studies. GBOs develop in EGF/bFGF-free media without ECM components while maintaining the original cell–cell interactions. Patient-specific GBM treatment regimens were improved using this novel method. This method has limitations because brain organoids lack cancer-causing cell types despite having positive outcomes. GBM mainly invades brain regions through blood vessels, and mesenchymal progenitors or endothelial cells may be of assistance. Non-GBM cells may be present in the organoids.[244] It requires specialized knowledge and months before morphological and functional studies can be conducted to create a brain organoid. Further research is required to standardize the culture conditions and medium composition. The proportion of various cell types needed at different stages of 3D model building varies by organoid type.

3D models showing the effects of drug therapy on GBM cells

3D GBM cultures are more resistant to pharmacological therapy than monolayer cultures and more closely imitate the chemotherapeutic response of patients with GBM. Han et al. created a microfluidic technique to examine resistance to GBM treatment. A total of 488 microchambers and two microchannels were included in the CDRA chip for antiparallel drug and medium delivery.[246] The purpose was to create a drug concentration gradient and monitor the development of drug-resistant cells. In 3D cell cultures, there are numerous pathways of drug resistance. The microfluidic technique used by Ayuso et al. revealed that TMZ had a minor effect on U-251 cell survival. The DNA replication-dependent activity of TMZ is inhibited when GBM cell proliferation decreases in the 3D hydrogel.[247] The vincristine sensitivity in the microfluidic method for single-cell separation created by Pang et al. was influenced by the biomechanical characteristics of GBM cells.[248] Chemotherapy failed to kill the tumor cells that were smaller or more deformed.

In 3D cell cultures, several gene clusters showed enhanced expression, which contributed to drug resistance.[249] These include genes related to apoptosis resistance (*ESR1*, *RARG*, *ERBB4*, *MET*), anti-apoptosis (*BCL2*, *B2M*), oxidative stress resistance (*NFKB* family members, *PPAR*, *SOD*, and *HIF1A*), DNA repair (*MGMT*, *XPC*, *TOP2B*, and *BRCA2*), DNA replication arrest (*MGMT*, *XPC*, *TOP2B*), drug detoxification, and drug efflux *ABCC5*, *ABCC3*, and *MVP* (*CDKN13* and *CCND1*).

The expression of genes related to detoxification and multidrug resistance, such as *CYP3A4* and *ABCB1*, did not differ significantly between the 3D scaffold and 2D cultures. As a potential mechanism of alkylating chemical resistance in a 3D environment, Lv et al. found increased expression of MGMT but no changes in the expression of key ABC transporters (ABCB1, ABCC1, ABCC2, ABCC4, and ABCG2).[186] According to a previous study, drug-resistant GBM cell lines prepared in three dimensions express higher levels of ABCG2 than usual.[249,250] According to Florczyk et al., the 3D cell line with the greatest resistance to alkylating chemicals showed increased ABCB1 expression.[159] The Cancer Drug Resistance Accelerator chip determined that an increase in drug efflux activity was the main driver of DOX resistance in U87 cells.[246] The authors extracted resistant U87 cells from the chip and performed exome and transcriptome sequencing. Numerous altered genes (*CHD1* and *FLNA*) associated with DOX resistance were identified, and genes with variable expression patterns related to the immune response, DOX metabolism, and NFB signaling. In addition to DOX and resveratrol, resistance to apoptosis results in drug resistance to alkylating agents in 3D cell cultures.[249,251] Kim et al. (2011) showed that apoptosis inducers, DOX, and resveratrol, boosted the production of survivin and Bcl2 in 3D cells.

Medication combinations can reverse 3D drug resistance. In 3D GSC, Fernandez-Fuente et al. revealed that PD98059 and LY294002 could overcome sunitinib resistance.[145] Simvastatin, an inhibitor of mevalonate synthase, stimulates TMZ-induced apoptosis by reducing autophagic flux.[145] In a recent study, scientists used TMZ and coenzyme Q10 in a collagen hydrogel 3D microfluidic system to sensitize TMZ-resistant RC6 cells.[145]

Traditional cell culture system limitations

In the past, 2D monolayer cell cultures were grown on glass or plastic substrates to investigate the function of ECM elements in the pathophysiology of malignancies. This is achieved by adsorbing the protein or PG of interest from the solution and then functionalizing the surface with it. The above section describes the procedure for functionalizing a surface with the desired protein or PG. Observing the effects of soluble signaling mediated by the ECM component can be accomplished by adding the molecule to the medium in which the cells are grown. This might be carried out to determine whether the molecule has the desired effect. This straightforward technique has made it possible to study the behavior of tumor cells in culture. This research has generated a wealth of knowledge regarding the function of several ECM molecules in the initiation and development of malignancies. In addition, it typically provides a framework for subsequent studies using models that are more appropriate for the physiological environment. For example, this technique was employed by Berens and colleagues in several significant investigations to define the function of ECM elements, such as fibronectin, vitronectin, and HA, in enhancing the adherence and migration of astrocytoma cells.[252] These investigations were significant because they established that ECM elements play a role in promoting astrocytoma cell adherence and migration. These researchers found that the presence of these elements increased the ability of astrocytoma cells to move and adhere to the surfaces. Matrix metalloproteases (MMPs) were initially discovered to play a role in the invasion of gliomas using monolayer culture methods.[253] Basic monolayer cultures have several limitations in the analysis of complicated cell-ECM interactions, and despite their effectiveness in fundamental functional studies, these cultures display a number of severe downsides. It is well known that cells grown in 3D behave slightly differently than those grown on flat 2D substrates. The fact that cells grown in 3D cultures have a larger area to move freely explains this behavioral difference. Significant variations in ECM protein presentation, organization, and polarity in a 3D matrix lead to simultaneous changes in the structure and makeup of cell-ECM adhesions and subsequent signaling events.[254,255] Conventional substrates made of plastic or glass are much stiffer than the brain, a tissue whose typical stiffness varies from 100 to 5000 Pa[256] with a stiffness greater than 3 GPa. The fact that most substrates are constructed of glass or plastic may explain the variance in stiffness. It is impossible to adequately reproduce the key components of a 3D ME of a tumor in 2D culture. These characteristics include gradients in cytokine concentration, increased interstitial fluid pressure, and hypoxia.[257,258] Flat monolayer cultures do not lend themselves to studying the impact of matrix remodeling or cell–cell interactions due to the nature of the culture. This is the final and crucial factor to consider. To address these issues, field researchers have begun to create microenvironments. In both 2D and 3D cultures, these microenvironments can provide precise control over the material composition, stiffness, and architecture.

Discussion

Using 3D models, scientists have extensively studied glioblastoma tumor cells for over a decade. Synthetic biomaterials enable *in vitro* models to be used to improve glioblastoma research. Research organizations are reducing the frequency of unsuccessful animal trials by relying on *in vitro* glioma models to predict the effectiveness of treatment protocols. In the long run, it would be fantastic if preclinical drug testing did not include animals. Costs would be lower, and testing on living organisms would no longer raise any moral dilemmas. Preclinical testing and medication development using 3D glioblastoma cell culture models require careful consideration of several factors. Creating 3D glioma models that resemble real GBM tissues in an injured brain is the most challenging task. This description can be used to explain many cancer-related events, such as metastasis and the dynamic TME. Researchers can move from straightforward spheroids to more accurate tumor models. Some engage in ECM interactions, while others do not. The observed tumor was three-dimensionally flat and biochemically uniform, despite being malignant. Medical researchers are currently interested in laboratory-grown organoids, which mimic benign tumors in appearance, but act differently and are more challenging to maintain over time. There may be fewer risks by controlling the structural complexity of the tumor tissue using 3D bioprinting. Fluid flow is crucial in physics and mechanics, although it is not necessary for bioprinted culture. Soon, a dynamic ME will be crucial for the deployment of organoids and bioprinted cultures. This is now possible owing to microfluidic technology and perfusion bioreactors. Microfluidic devices are currently the industrial standard for 3D cell culture. However, much work remains to be done to create microfluidic 3D glioblastoma cell cultures. Studies on biomaterials and tissue engineering typically focus on material choice and how to improve the 3D biomaterial properties of GBM cell culture scaffolds. These scaffolds may be more stable if the composition of their ECM more closely mimics that of GBMs (such as being high in HA). The scaffold must retain its original chemical and mechanical properties while GBM cells are present and interact with the cells. The results of chemosensitivity experiments can be affected by modifications to the biomaterials and pharmaceutical choices. Because there are significant differences across medicines in terms of permeability and absorption rates, there are various challenges in treating GBM that can only be overcome using 3D microfluidic systems. Research has focused on the migration, interaction, and mobility of GBM cells in relation to those of other brain cells. Creating 3D composite platforms for *in vitro* glioblastoma cell cultivation is expensive and time-consuming. It is anticipated that 3D glioblastoma cell cultures will be used more frequently as technology develops. Therefore, they may be used for high-throughput and high-resolution screening, which may increase their economic viability. It is crucial to monitor molecular and phenotypic changes in 3D glioblastoma cell platforms. Endpoint studies, time-lapse fluorescence, and confocal imaging are the most popular techniques. 3D cell cultures must be monitored for biomolecule transit using state-of-the-art methods, such as magnetic resonance imaging (MRI) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI). This information has multiplied as a direct result of the use of 3D culture techniques in cancer cell research. This volume of data is expected to grow as high-throughput 3D screening tools have become accessible. Machine learning applications in 3D cancer cell cultures have advanced quickly. Consequently, 3D cell cultures fetch higher prices on the market. We predict that tumors will increasingly resemble those of the original patients as 3D GBM cell cultures are replicated. Mathematical analysis can help scientists overcome reductionist barriers. Mathematical models based on *in vitro* data may be able to predict whether GBM develops therapeutic resistance. Drug screening and personalization can be facilitated using 3D glioblastoma cell cultures. Researchers will soon have a better knowledge of how well GBM responds to treatment and the best ways to overcome chemoresistance, thanks to developments in imaging technologies, artificial intelligence, and mathematical models. They can help us learn more about the development and operation of the BBB in patients with GBM. Medication concentrations in patients are considerably increased using iPSC-based BBB models. Since then, other elements have been added, such as flow-induced shear stress and a fully functional ECM. These improved BBB models were used to examine drug permeability and toxicity to identify potential GBM treatments. The TME and response to targeted therapy can be precisely simulated *in vitro* using technologies with synthetic biomaterials, such as 3D bioprinting and microfluidic glioma-on-a-chip models.

Conclusion

The current status of biomaterial- and scaffold-based 3D *in vitro* models of GBM are discussed in this review. The biology of glioblastoma tumors was simulated using this model. Simple GBMs are easy to understand. Physicians can better understand individual differences and the consequences of their treatment by visualizing patients in three dimensions. Translational studies are not as complex because they last longer. However, problems remain, even with GBM models. No one has proven a link between a variety of human malignancies, tumors, and healthy tissue microenvironments. The glioblastoma-on-a-chip was bioprinted using endothelial cells. This clearly illustrates the intratumoral hypoxia gradient and TME. Organoids have a structure superior than that for 2D cells. However, bioprinting is a complicated and expensive process. Endothelial cells that have been removed from the patient can be used to co-cultivate organoids. This approach examines how brain organoids function in their natural surroundings. Tumoroids have not been co-cultured with endothelial cells. Reports have suggested that immune cells and inflammation can facilitate communication between GBM cells and their environment. GBOs have been used in immunotherapy, and it is essential to assess CAR-T therapy. Immune systems and GBM co-culture may provide some benefits. Potential therapeutic strategies for reducing recurrence and improving prognosis may be identified as a result of recent research. GB tumoroids can be studied using state-of-the-art tools, such as single-cell sequencing, microfluidics, organ-on-a-chip, and four-dimensional real-time imaging. GBOs are being studied by an increasing number of researchers as possible treatment options.

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Ethics statement

None.

Data availability statement

The datasets used in this study are available from the corresponding author upon reasonable request.

Conflict of interest

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