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REVIEW

Engineered hydrogels for brain tumor culture and therapy

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Abstract

Brain tumors' severity ranges from benign to highly aggressive and invasive. Bioengineering tools can assist in understanding the pathophysiology of these tumors from outside the body and facilitate development of suitable antitumoral treatments. Here, we first describe the physiology and cellular composition of brain tumors. Then, we discuss the development of three-dimensional tissue models utilizing brain tumor cells. In particular, we highlight the role of hydrogels in providing a biomimetic support for the cells to grow into defined structures. Microscale technologies, such as electrospinning and bioprinting, and advanced cellular models aim to mimic the extracellular matrix and natural cellular localization in engineered tumor tissues. Lastly, we review current applications and prospects of hydrogels for therapeutic purposes, such as drug delivery and co-administration with other therapies. Through further development, hydrogels can serve as a reliable option for in vitro modeling and treatment of brain tumors for translational medicine.

Keywords Brain tumor · Bioengineering · Cancer cells · Drug delivery · Hydrogel

Introduction

Our understanding of composition, pathogenesis, and available treatments for brain tumors has significantly increased in recent years. Tumors of the nervous system are heterogeneous depending on the original cell type, and contain distinct genomic and epigenomic changes affecting cellular mechanisms [1]. Primary intracranial tumors occur in 10 per 100,000 adults, which increases with age [2]. Preferred treatment for brain tumors is individualized based upon the tumor's metastatic potential, patient's age and sex, and his/her physical conditions [3]. Most brain tumors are treated with a combination of surgery, chemotherapy, and radiotherapy. During the surgery, the tumor is physically removed; however, resection of significant tumor mass may cause a midline shift or herniation [4]. Radiotherapy uses high intensity rays to destroy tumor tissues, and chemotherapy utilizes drugs to kill tumor cells [5–7].

Traditional brain tumor treatments have limitations including off-target toxicity and the need for frequent administration of the therapeutic agent. To circumvent these hindrances, biomaterials can be used to encapsulate drugs in order to localize their impacts, reduce their side effects, and provide additional benefits, such as controlled and prolonged drug release [8–12]. For nearly 20 years, carmustine-loaded

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copolymers known as "Gliadel wafers" have served as the only clinically approved chemotherapy-secreting biomaterial for brain cancer treatment [13–15]. These polymeric scaffolds and other developing biomaterials have served as successful vehicles for drug delivery to tumor tissues.

There is a great need to develop new biomaterial-based platforms for brain tumor therapy. Next-generation drug delivery systems need to engage more with surrounding tissues in order to better manipulate the local environment and improve the therapeutic outcome. While cells are the basic unit of the human body, the extracellular matrix (ECM) provides a framework for tissues and plays a role in the modulation of tissue development and disease state. Biomaterials are being developed to mimic several features of the ECM, specifically its composition and functions [16–20]. Among these biomaterials, hydrogels are able to recapitulate the organization of the tumor microenvironment and offer biocompatible vehicles for cancer therapies [16, 21, 22]. Different gel structures have been developed at the micro- and nanoscale to properly encapsulate or culture individual cells and other biological components in order to provide advantages in therapies and tissue modeling [23–27]. Hydrogels are commonly synthesized from engineered macromolecules or obtained from natural sources, such as polypeptides and polysaccharides, which have inherent roles within natural tissues [27]. To expand their properties, natural hydrogels have been augmented with functional groups to achieve the same functionality as synthetic biomaterials, such as greater elasticity and electrical conductivity [28–31].

In this review, we discuss the key features of central nervous system (CNS)-related tumors to better understand their physiological differences compared to normal tissues. Also, we describe current in vitro models of these tumors including those derived from cancer cells and scaffolds, particularly hydrogels. We also provide insights into tumor pathology to help further development of more effective treatments. Hydrogels engineered with microscale technologies are also described to highlight the recent efforts on developing functional platforms to culture and treat brain tumors. Lastly, challenges and future perspectives in this research area are discussed to help design next-generation bioengineering platforms.

Native microenvironment of brain tumors

The tumor microenvironment is composed of the ECM, blood vessels, stromal cells, cancer cells, and signaling factors that promote cancer tissue formation and function [32]. The ECM contains primarily hyaluronic acid (HA), a polysaccharide commonly found in brain tissue, as well as several proteins [33]. HA plays a large role in supporting tissues, separating heterogeneous tissues from one another, and regulating intercellular communication. A brain tumor is graded on a scale of 1–4, which indicates how abnormal the tumor cells and tissue look and their likelihood to grow further [34]. Meanwhile, a brain tumor can be categorized as either primary or metastatic, where it spreads from a different primary tumor in the body. Brain metastases commonly develop from lung cancer, breast cancer, melanoma, and gastrointestinal cancers [35]. Primary tumors can initiate from a particular cell type in the CNS including astrocytes, meningothelial cells, ependymal cells, oligodendrocytes, Schwann cells, neuroectoderm, or any position along the spine (chordoma) [36, 37]. Brain cancers include glioblastoma multiforme (GBM, a form of astrocytoma) [34], medulloblastoma (from the neuroectoderm) [38], and ependymoma (from ependymal cells) [39]. The most common CNS tumors in children ages 0–14 are pilocytic astrocytoma, malignant glioma, and embryonal tumors, whereas meningioma, glioblastoma, and pituitary tumors are the most common in adults (Fig. 1a) [37].

Cancer cells from malignant tumors can enter the circulatory system in early stages before being diagnosed and circulate through the blood until finding other niches to grow [40]. The existence of cancer stem cells (CSCs) greatly supports cancer metastasis in different parts of the body, including the brain. Metastasis occurs in several steps and only a small proportion of cancer cells can overcome obstacles, infiltrate other tissues and organs, and survive in them (Fig. 1b). CSCs can continually self-renew and differentiate into more proliferative cells that populate tumors [41]. Several factors of the tumor microenvironment promote cancer progression in other tissues or organs. As cancer cells migrate into new tissues, their enzymes degrade surrounding proteins, allowing the cells to invade the tissues without hindrance. Tumor cells have shown to upregulate ECM-remodeling enzymes, such as serine and cysteine proteases and metalloproteinases, which target the ECM components, such as collagen [42]. In gliomas, collagen and lysyl oxidase, a collagen cross-linking enzyme, are upregulated to promote tumor cell proliferation and migration [43, 44]. Mammoto et al. [44] demonstrated that disruption of collagen structure in a tumor tissue via inhibition of lysyl oxidase reduced expression of vascular endothelial growth factor (VEGF) and, as a result, suppressed angiogenesis. The lysyl oxidase inhibition was also shown to reduce tumor size and tumor cell proliferation, as well as metalloproteinase expression in brain tumors, indicating that collagen plays a significant role in GBM metastasis. Meanwhile, tumor-associated macrophages secrete transforming growth factor (TGF)-β and matrix metalloproteinase-9 to remodel the ECM. TGF-β can stimulate glioma stem cells (GSCs) to transdifferentiate into pericytes, which are supportive cells to the endothelium, and can replace normal brain pericytes [45].

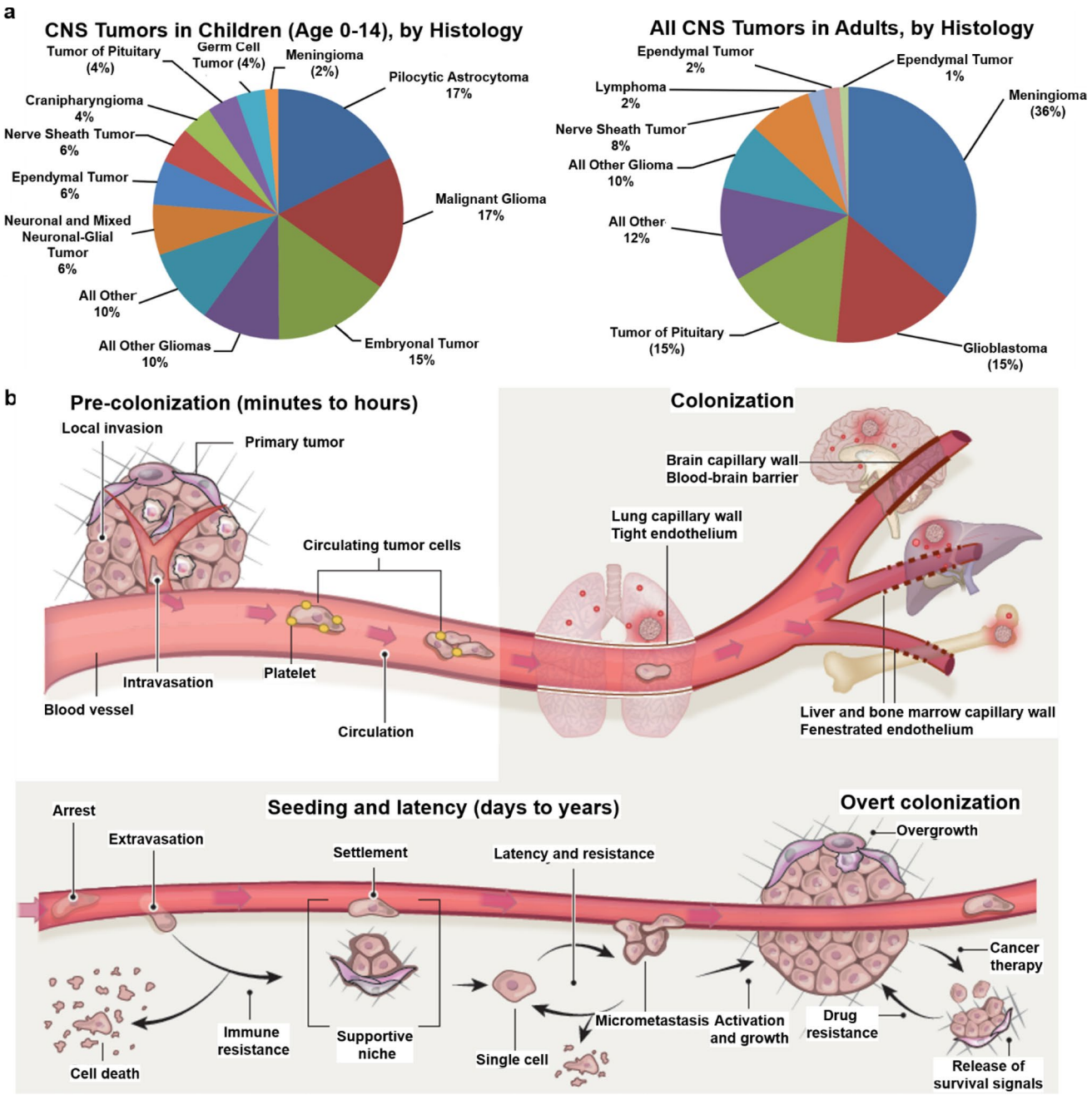


Fig. 1 **a** Types of CNS tumors and incidence by age group among children (ages 0–14) and adults (Adapted from McNeill et al. [37]). **b** Multiple steps of metastatic colonization including pre-colonization phase, which occurs in minutes to hours, followed by colonization phase, which occurs in years. Pre-colonization steps include intravasation of cancer cells into the tumor vasculature, entry into the circulatory system, and extravasation into the parenchyma of target tissues or organs (Adapted from Massague et al. [40])

This cancer vasculogenesis contributes greatly to patient mortality [46]. The brain contains highly vascularized regions, which support the continual survival and function of cells. In gliomas, microvascular proliferation occurs widely depending on the severity of the cancer and involves astrocytes, macrophages, microglia, stromal cells, and endothelial cells [47]. Blood vessels and accompanying endothelial cells are shown to surround stem cell-like cancer cells and promote the survival of self-renewing cells [48]. Endothelial cells line the interwalls of capillaries, thin blood vessels in the brain and other bodily regions. Compared to the rest of the body, endothelial cells in the brain form tighter and more complex junctions through claudins and occludins [49]. Certain GSCs are also shown to differentiate into endothelial

cells, promoting the formation of denser vascular networks [50].

Glioblastomas promote angiogenesis to transport nutrients and oxygen to the cancerous tissue. This transport process requires expression of particular genes in vasculature and peritumoral tissues, like nestin, a neural stem cell marker, and CD105, a tumor neoangiogenesis marker [51]. These markers colocalize in microvascular tissue of peritumoral regions. Sica et al. [51] observed that patients with CD105-positive tumors had decreased survival times and higher risk of death, indicating that characteristics of peritumoral vasculature influences GBM patient outcomes. Some cancer treatments, such as bevacizumab, inhibit blood vessel formation by blocking VEGF signaling, which highlights the effects of hypoxia in cancerous regions [50].

Stromal cells make up an important structure known as the perivascular niche (PVN), which maintains and disseminates the region's resident stem cells (including CSCs). These cell types include cancer-associated fibroblasts, astrocytes, pericytes, neurovascular endothelial cells, macrophages, adipocytes, and leukocytes. Stromal cells are recruited to tumor sites and assist with growth, migration, and metastasis of tumor cells. To do so, they secrete soluble cues, such as growth factors, cytokines, chemokines, and other signaling entities like exosomes (Fig. 2a) [52]. Tumor cells, in turn, interact with stromal cells to modulate their microenvironment. For example, endothelial cells in the PVN secrete signals to migrating glioma cells to attract them, which leads to the adhesion and invasion of glioma cells into the surrounding tissue as shown in Fig. 2b [52]. Meanwhile, astrocytes reduce cell death among GBM cells through the formation of gap junctions via the gap junction alpha-1 protein, also known as connexin 43 (Cx43) [53]. Cx43 facilitates communication between these cells, generating a protective effect [53].

Astrocytes are connected with neurons and blood vessels, becoming an integral component of the blood–brain barrier (BBB). The BBB prevents the introduction of pathogens into the CNS. Immune cells that induce a response to pathogens are present in low numbers in the BBB compared to the rest of the body [54]. Even though the brain is considered immune-privileged, Louveau et al. [55] discussed that it still contains a lymphatic system whereby antigens and other solutes drain into cervical lymph nodes. Lymphatics typically surround blood vessels and meninges, and drain along dural sinuses to the cervical lymph nodes [55]. Despite this evidence, physicians still argue that the CNS does not contain a lymphatic system, and tumor cells travel through blood vessels.

In view of the discussed brain tumor biology, treatment options should be further developed and optimized to target key facets of tumor cell growth and migration. Tissue constructs obtained from patient cells and biomaterials can also recapitulate the complex biology of the CNS during cancer therapy.

In vitro cell culture models

For many years, two-dimensional (2D) cell culture systems dominated biomedical research due to their cost-effectiveness and ease of handling. In the realm of neurobiology, new therapies were often developed using 2D cellular models and evaluated using changes in axon and dendrite growth, neuronal cell survival, and synapse formation [57]. Through the introduction of three-dimensional (3D) cellular models, researchers were able to develop more physiologically accurate structures for cell-based studies (Fig. 3a) [58]. Examples of such cellular models are organoids and spheroids, which are non-scaffold-based cultures containing a single or multiple cell type(s) [59]. These constructs aim to mimic homogeneous and heterogeneous cell–cell interactions in the native tissues. The complex cellular architecture of the nervous system demands these constructs that are highly organized and amenable to manipulation. For example, cerebral vasculature allows the transport of nutrients for neural cell viability and requires both neural and vascular/endothelial components for tissue modeling [60]. Scaffolds have also been developed containing a cellular and microenvironmental architecture mimicking the ECM. These scaffolds are derived from either natural or synthetic biomaterials with relatively defined and tunable properties, including architecture, stiffness, pore size, and ligand density [61]. 3D culture platforms have also been used to study tumor cells in a controlled microenvironment.

Spheroids

Spheroids can be created using a number of techniques, including growth in a bioreactor [62], microwell-plates (e.g., AggreWell from STEMCELL technologies) [63], and the hanging-drop method [64]. As early as 1990, tumor tissues derived from distinct patient samples, including glioblastoma, astrocytoma, and ependymoma, have been shown to form spheroids when cultured in flasks for 4–20 days [65]. Such structures showed invasive properties reflective of malignancy of the biopsied/resected cancer when implanted into animal models [42, 66–68].

Spheroids can also be incorporated into microfluidic devices to evaluate the effects of different compounds and growth conditions on cancer cells. For example, Ko et al. [69] designed a microfluidic culture platform to develop vascularized cancer spheroids (Sphero-IMPACT) in a 96-well plate (Fig. 3b). The platform consisted of a tapered hole in the center of a rail to pattern GBM cells into a large spheroid. The group investigated angiogenesis,

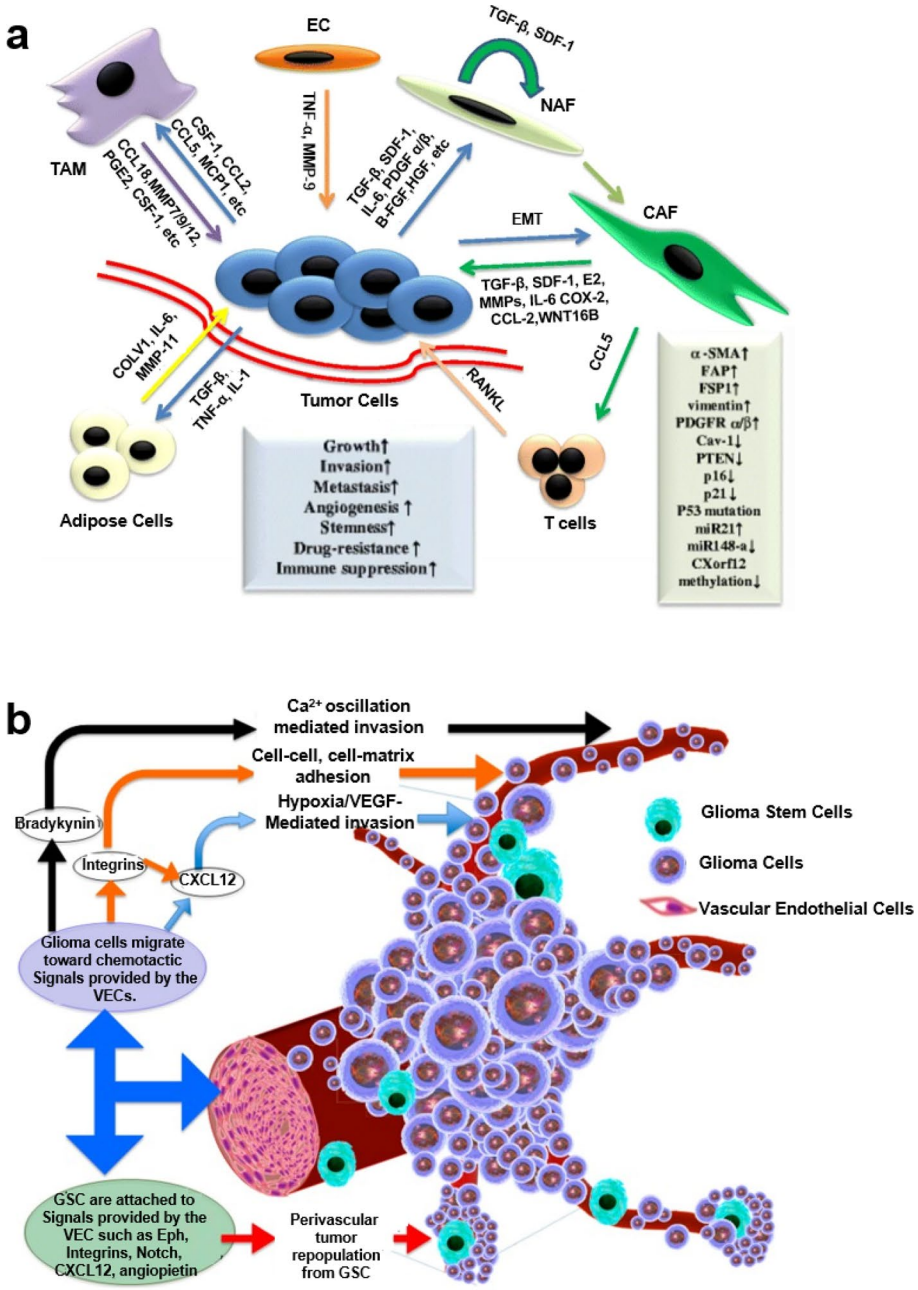
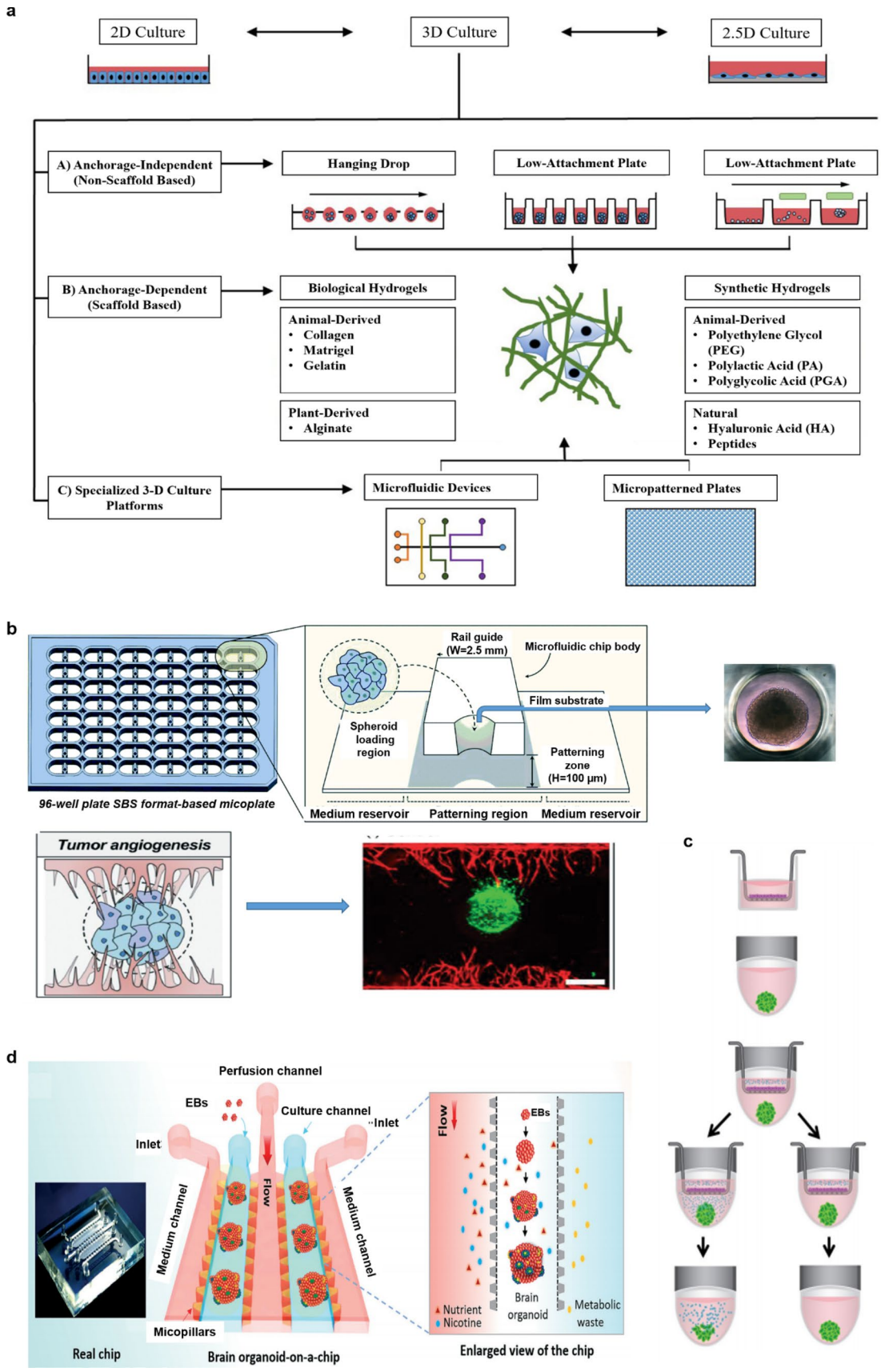


Fig. 2 **a** Mechanism of interaction of tumor cells with stromal cells in tumor microenvironment promoting tumor growth, invasion, and metastasis (Adapted from Mao et al. [52]). **b** The glioma perivascular niche. Vascular endothelial cells provide chemotactic signals to migrating glioma cells in order to attract them to blood vessels. GSCs may also migrate to the site and differentiate to multiple cell types due to signals in the PVN (Adapted from Diksin et al. [56])

tumor cell migration, and invasion using the spheroids. Sphero-IMPACT was also applied to conduct cell-laden patterning of human umbilical vein endothelial cells (HUVECs) and lung fibroblasts, resulting in the generation of sprouting cell patterns. The latter approach was used to study the effect of anti-cancer drugs on spheroids. Another study grew patient-derived stem cell-like populations in 1536-well plates, producing spheroids positive for GSC biomarkers, such as Nestin, Sox2, and Sox9 [70]. When expanded, they were able to recapitulate the self-renewal and cellular heterogeneity observed in the primary tumor after differentiation [70].

Designed wells may also contain a tapered bottom, which promotes cell aggregate formation in the well (Fig. 3c). Such plates have been developed for generating spheroids of uniform size and composition [63]. Sherman et al. [71] studied the delivery of anti-cancer drugs to 3D glioma tumor spheroids with an in vitro model of BBB that combined permeable tissue culture plates and 96-well plates (Fig. 3c). The geometry of round-bottom 96-well plates enabled them to generate a single multicellular tumor spheroid in each well. Madin-Darby canine kidney epithelial cells over-expressing the human multi-drug resistance (MDCKII/MDR1) gene were seeded on the permeable inserts, generating


a
2D Culture
3D Culture
2.5D Culture
A) Anchorage-Independent (Non-Scaffold Based)
Hanging Drop
Low-Attachment Plate
Low-Attachment Plate
B) Anchorage-Dependent (Scaffold Based)
Biological Hydrogels
Animal-Derived
• Collagen
• Matrigel
• Gelatin
Plant-Derived
• Alginate
Synthetic Hydrogels
Animal-Derived
• Polyethylene Glycol (PEG)
• Polylactic Acid (PA)
• Polyglycolic Acid (PGA)
Natural
• Hyaluronic Acid (HA)
• Peptides
C) Specialized 3-D Culture Platforms
Microfluidic Devices
Micropatterned Plates
b
96-well plate SBS format-based micoplate
Rail guide (W=2.5 mm)
Microfluidic chip body
Film substrate
Spheroid loading region
Patterning zone (H=100 μm)
Medium reservoir
Patterning region
Medium reservoir
Tumor angiogenesis
c
d
Perfusion channel
EBs
Culture channel
Inlet
Inlet
Flow
Medium channel
Medium channel
Micopillars
Real chip
Brain organoid-on-a-chip
Flow
EBs
Nutrient
Nicotine
Brain organoid
Metabolic waste
Enlarged view of the chip

◂**Fig. 3** **a** In vitro brain tumor models. Models consist of 2D and 3D platforms, subdivided into: (1) anchorage independent, which do not utilize scaffolds; [79] anchorage dependent, (2) integrating scaffolds for culture; and (3) specialized devices. Non-scaffold methods to generate 3D masses use hanging-drop method, low-attachment plates, or magnetic levitation, while scaffold-based methods include hydrogels. Specialized platforms include microfluidic devices and micropatterned plates (Adapted from Langhans et al. [58]). **b** Incorporation of brain glioblastoma spheroids within a microfluidic 3D culture platform of 96-well plates containing a tapered hole in the center of a rail for brain glioblastoma spheroids to generate angiogenic sprouting patterns (Adapted from Ko et al. [69]). **c** A 3D model generated by combination of transwell 96-well-plates and 96-well spheroid microplates to simulate penetration of anti-cancer drugs through the BBB (Adapted from Sherman et al. [71]). **d** Generation of brain organoids on a brain organoid-on-a-chip device to investigate the effect of nicotine on early brain development (Adapted from Wang et al. [74])

monolayers, which simulate the BBB. After creating the glioma-BBB system, the effect of anti-cancer drugs on the spheroids was investigated.

Compared to other structures, spheroids typically grow cells to a mature cell fate [59]. If stem cells are aggregated into a spheroid, they can be induced to differentiate and secrete ECM factors. The small diameter of spheroids also allows them to be transferred between plates when analysis is required [69]. However, the interior and core of the spheroid is often difficult to image without sectioning and with traditional light microscopy [64]. Spheroids can also be applied to a number of systems to better study neurological disorders (Table 1).

Organoids

Organoids are grown from stem cells or early multipotent progenitor cells. Upon exposure to biological cues, these cells differentiate into more mature cell types that perform specific functions. Linkous et al. [72] demonstrated that GSCs grown within cerebral organoids are highly proliferative and form more invasive tumors. Such cancer cells formed intercellular tracks from microtubes that facilitated cell migration. In general, glioma cells have a tendency to aggregate and form larger structures over time in culture compared to normal cells.

Advances in bioengineering tools including miniaturized spinning bioreactors, lab-on-a-chip devices, and microfluidic systems have improved the development of organoids in shape and function [58]. In addition, the introduction of specific neurotrophic factors during culture has allowed researchers to generate brain region-specific organoids, including those of the forebrain, midbrain, and hypothalamus [62], thalamus [73], hindbrain and preplate [74], dorsal cortex, prefrontal cortex, hippocampus, ventral forebrain, and choroid plexus [75]. Wang et al. [74] proposed a lab-on-a-chip device for the generation of brain organoids through neural differentiation of stem cell aggregates as embryonic bodies (EBs) in a 3D microenvironment (Fig. 3d). They investigated the effect of nicotine exposure on fetal brain development, which showed abnormal neural differentiation when exposed to nicotine. Cancers of neural cell types can be investigated as complete organoids or in the presence of non-cancerous cells to study the effects of cell–cell communication on the behavior of cancer cells. When placed within brain tissue, brain cancer cells tend to be highly invasive and migrate along vasculature, white matter tracts, parenchyma, and fibrous ECM [17, 76, 77]. In this regard, organoids can be used to demonstrate inherent characteristics of malignant cells and serve as models for cancer treatment.

Compared to spheroids, organoids have distinct disadvantages that should direct their use in research (Table 1). For example, as they are growing, organoids need specialized culture conditions including low-attachment plates or bioreactors that allow for high oxygen exchange [58]. With cells differentiating into specific brain regions, the time for culture prior to experimentation is often high. Studies also use multiple growth media, which can be expensive [57].

Scaffold-based models

Models of cellular systems should mimic both cell–cell and cell–matrix interactions. While spheroids and organoids properly recapitulate cell–cell interactions, scaffolds allow control of cell–matrix conditions and interactions [20]. Cells can be embedded within or on the surface of scaffolds, which in turn can be tuned to optimize cell growth conditions. Scaffolds are intended to recapitulate the cellular microenvironment, which provides mechanical cues to cells for adherence and proliferation [20]. They can be composed of natural polymers, plant-based or animal-based, or synthetic polymers, which can provide different benefits for cell culture (Fig. 3a) [58]. Combinations of these polymers or use of stimuli-sensitive polymers have been reported to modulate the scaffold response to temperature, pH, light, and ions [78].

Hydrogels for applications in brain tumor studies

Hydrogels have received significant attention for applications in biotechnology and medicine. They are often referred to as water-saturated polymeric gels [18] and have been employed as implants or injectable biomaterials loaded with therapeutic agents for brain tumor therapy [5, 10, 80, 81]. Scaffolds are also used for 3D modeling of brain tissues toward in vitro studies [19, 29, 32, 33] as they are capable of mimicking the brain ECM. Such structures provide distinct biophysical cues that direct cell growth and allow cells to form biomimetic tissues. Hydrogels can be derived from a

Table 1 In vitro cell culture systems for modeling brain tumors

In vitro cell culture models	Advantages	Disadvantages	Applications
Spheroids	Recapitulate self-renewal and cellular heterogeneity	Limited culture time	Drug screening
	Allow for mature cell differentiation	Required transfer to new plate for analysis	
	Secrete ECM	Difficult to image interior regions	
	Highly controllable		Understanding neurodevelopment and pathology
	Inexpensive to make		
	Easy to handle		
Organoids	Resemble organ structures and mimic functions	Need specialized tissue culture conditions (low-attachment plates, high oxygen exchange)	Drug screening
	Can induce whole-brain and region-specific differentiation	Require time to differentiate into defined subregions	
	Mini-bioreactors allow their scaled-up production	Need multiple growth media (cost)	
		Optimization is needed	Finding suitable treatment for specific cancer subtypes
			Understanding neurodevelopment and pathology
Scaffold-based models	Able to control the cell–matrix interactions	Scaffold materials should recapitulate biological and mechanical properties	Modulate the scaffold response to temperature, pH, light, and ions
	Optimize cellular microenvironment and growth conditions	Scaffolds should be tested on multiple cell types as in the human body	
	Provide controlled mechanical cues to cells		

number of polymers, such as chitosan, alginate, HA, collagen, agarose, and gelatin, or be chemically synthesized [78].

Origin of hydrogels

Hydrogels are composed of synthetic, natural, and semi-synthetic/hybrid materials [17]. Natural polymers can be found in biological tissues, while synthetic polymers are composed of non-biological materials. Semi-synthetic materials, on the other hand, are composed of a combination of natural and synthetic polymers. When researchers generate hydrogels from both natural and synthetic substances, they can specify the most beneficial properties of both and synthesize stable and biocompatible options.

Natural hydrogels

Commonly used natural biomaterials, including collagen, fibrin, Matrigel®, HA, and many others, have inherent roles within tissues in the body [27]. These natural biopolymers have been shown to improve cancer cell viability, proliferation, and differentiation, and replicate phenotypes and processes observed in vivo [58]. Hydrogels derived from these polymers are being used to study the assembly of CSCs into 3D tumorspheres, as well as their morphology, survival, and distinct pathways they undergo. Cancer cells, for example, often break down ECM components, migrate, and invade other matrix regions depending on the degree of malignancy of the cancer [82].

Per their derivation from biological tissues, natural polymers are highly biocompatible and interact with cells to maintain physiological functions (Table 2). They can be used to generate structures with various mechanical properties and provide different levels of support to cells cultured in 2D and 3D formats. Varying the protein concentrations and cross-linking conditions of natural polymers changes gel stiffness which, in turn, affects biological processes. The degradation rate of these hydrogels must also be taken into account when discussing their desired application, as it affects their mechanical strength and swelling rate. Fluctuations result in changes to cell behavior, such as the migration and spreading of cancer cells. Natural hydrogels' inherent mechanical strength is also affected if they are too soft. However, Chen et al. [19] showed that GBM cells invaded quicker in softer hydrogels and slower in hydrogels containing greater HA. Most natural hydrogels contain many hydroxyl and primary amine groups which improve the structures' hydrophilicity, but can be targeted by proteases

Table 2 Hydrogel scaffolds in brain tumor culture and therapy

Hydrogels	Advantages	Disadvantages	Applications
Natural hydrogels	Does not require synthetic modifications	Immunogenic to a certain extent	Assembly of CSCs for tumorigenesis
	Generate structures with low stiffness	Fast degradation	Study brain cancer invasion and ECM degradation
	Low toxicity byproducts	Structural weakness	
		Low mechanical strength	
		Batch-to-batch variation	
		Animal derived materials may pass biological contaminations	
Synthetic hydrogels	Can vary cross-linking density	Can include toxic substances	In situ devices
	Provide unique topography		Cell/regenerative medicine
	Highly tunable, with the ability to respond to external stimuli		Long-term, controlled drug delivery
	Capability of being electrically conductive		
	Precisely controlled with scalable production		
	Can minimize risk of biological pathogens or contaminants		
Hybrid hydrogels	Maximize benefits of natural hydrogels modified with synthetic polymers		In vitro brain tumor studies
			Regenerative medicine
			Drug and gene delivery platforms

[83]. In this way, stiffer hydrogels promote cell aggregation, as in tumors, and invasion, while upregulating the secretion of matrix-remodeling enzymes [26].

Natural hydrogels are also immunogenic to a certain extent. Thus, grafts carrying therapeutic agents, such as chemotherapies or radioactive isotopes, could lead to rejection of implanted biomaterials due to incompatibility or transferal of viruses and diseases from host tissues [25]. Optimal biomaterials must be chosen to achieve desired functions, reduce side effects, and provide required mechanical strength.

Synthetic hydrogels

Similar to natural hydrogels, synthetics must first and foremost should have high biocompatibility. The Food and Drug Administration (FDA) has approved many synthetic devices made of polyethylene glycol (PEG) [84], polyacrylic acid (PAA) [85], and poly (hydroxyethyl methacrylate) (P-HEMA) [86], which are now being produced in commercial scale [25].

Synthetic polymers can provide unique topography to direct cellular fate. They can be specially functionalized with cell adhesion groups and growth factors, as well as chemically and physically modified with bioactive molecules to enhance growth. As GBM cells are highly invasive, the porosity and stiffness of the scaffold are particularly important. Different processes that can induce cross-linking of hydrogels include chemical cross-linking, thermal annealing, supramolecular self-assembly, ionic gelation, and electrostatic interactions [87]. Varying the cross-linking density and polymer concentration can tune physical properties of a synthetic scaffold, such as its architecture, stiffness, pore size, diffusion of soluble factors, and ligand density [61]. As the result, the hydrogel controls cell degradation and invasion of surrounding tissue [28]. A synthetic hydrogel is able to act as a substrate with a strong similarity to soft living tissue through characteristics such as elasticity, softness, and high-water retention. Synthetic hydrogels can also be customized for a variety of biomedical applications by adjusting and controlling the physical cross-linking network, pore size, and density of the cross-linked network [22, 88, 89].

Electrically conductive hydrogels can be utilized among in situ devices to monitor cell growth, as impedance of these conductive polymer networks is affected by the encapsulation of cells within them [90]. Inal et al. discussed that biologically derived functional groups can be added to conductive synthetic polymers to enhance their biocompatibility; however, it also reduces their electrical monitoring ability (Table 2). Thus, the beneficial mechanical properties of synthetic materials may be sacrificed when attempting to create electrically sensitive and hydrogel-infused models to monitor neural activity.

Hybrid hydrogels

Semi-synthetic or hybrid hydrogels are comprised of natural hydrogels chemically modified with synthetic chemical groups or blended with synthetic polymers. Natural hydrogels have their own inherent chemical and physical characteristics with certain disadvantages, such as structural weakness, rapid degradation, and source variability [91]; however, the addition of synthetic polymers can adjust these properties prior to the hydrogel's application in tissue regeneration. Hybrid hydrogels contain bioactive cues and highly tunable properties which drive their popularity in bioengineering [28]. HA is a common polysaccharide in the brain ECM and is used in hydrogel systems to model and treat a variety of cancers. In order to improve its functionality, an HA hydrogel was augmented with RGD ligands, which increased glioma cell spreading and actin fiber assembly with greater ligand density [29]. Such additions allow researchers to improve upon problems in the hydrogel material's mechanics such as elasticity. Employing hybrid polymers as scaffolds improves the quality of brain tumor studies in vitro to better understand cellular adhesion and invasion characteristics. Unfortunately, hydrogels with a synthetic polymer origin may still remain immunogenic and incompatible with the body following transplantation (Table 2).

Hydrogel properties

Hydrogels are widely used for brain tumor culture and therapy [5, 10, 29, 92]. The brain tumor itself is a dynamic and multifaceted system with various biochemical and mechanical indications that cause GBM cell transition and growth. Likewise, GBM cell growth is significantly correlated with the density and stiffness of the hydrogel scaffold in which it is encapsulated [32, 92]. Depending on different applications and environmental conditions, hydrogels can have variations in their biophysical properties, including permeability, swelling rate, stiffness, absorption capacity, and surface properties. As they are permeable, hydrogels can facilitate the transfer of substances such as proteins, fluids, and even cells through invasion [22, 93, 94]. Therefore, the characteristics of hydrogels associated with permeability is crucial for organ transplantation and drug delivery. Hydrogels can be modified for these purposes by adjusting the cross-linking density during synthesis or introducing additional monomers, hydrophilic or hydrophobic, into the polymer network.

Aside from permeability, the swelling and absorption capacities are principal aspects of hydrogels in relation to their biomedical applications since the swelling behavior influences surface properties, mechanical properties of hydrogels. Oh et al. [95] found that poly(ethylene glycol diacrylate) (PEGDA)-alginate hydrogels had a greater swelling ratio, pore diameter, and porosity than PEGDA hydrogels. These properties resulted in the formation of larger tumorspheres due to the greater voids in the hybrid hydrogel. Meanwhile, the cross-linking density, composition, temperature, and ionic strength of the hydrogel influence the hydrogel's swelling ratio and wettability. From a biotechnology point of view, dense and microporous hydrogels are suitable substrates for tissue engineering, drug delivery, and controlled release of drugs since their microstructure can help carry a wide range of substances. Further, greater cancer cell infiltration and migration are reported in softer and more porous hydrogel networks [96, 97].

Hydrogel stiffness

Stiffness, the resistance to deformation, is one of the essential mechanical factors acting on cells and hydrogel behavior. It is measured as Young's elastic modulus (E) by applying force using a stress–strain relationship diagram [98]. Measurement of Young's modulus determined that stiff hydrogels provide more resistance than soft hydrogels [99]. In general, glioblastoma cell differentiation and proliferation increase with the stiffness of hydrogels, as the stiffness of the hydrogel substrate directly affects cell growth and migration [19, 100]. These examples raise the importance of Young's modulus and the effects of stiffness on the properties of hydrogels and their suitability for cell culture.

Recent research from many laboratories indicated that hydrogel stiffness has a broad impact on cell function. Hydrogel stiffness tunes the degree of cell adhesion and the size of focal adhesions, and it could also regulate stiffness of cultured tumor cells [101–103]. Cell migration, passage, motility, and alignment are also associated with substrate stiffness [32, 88, 101]. Multiple cell types show a morphological dependence on matrix stiffness [44, 104–106]. Therefore, remodeling the hydrogel stiffness to degrees above and below physiological levels leads to changes in the cell populations suitable for growth on the gel surface.

Various cell types react differently to varying degrees of substrate stiffness. GBM cells obtain different invasive behaviors and proliferation rates with fluctuations in stiffness [17]. Moreover, hydrogel stiffness can control differentiation of tumor progenitor cells into GBM cells and their proliferation, and contribute to the branching and invasiveness of tumors [107]. GBM tumor cell proliferation and invasion is less dependent on matrix stiffness and shows greater metabolism when spreading on surfaces with greater stiffness [107]. Thus, the stiffness of the hydrogel scaffold can provide insight into designing culture platforms for GBM.

The brain ECM also has biophysical effects on GBM invasion. Naturally derived hydrogels such as Matrigel, collagen, and laminin have been used to construct an ECM-mimicking microenvironment for investigating GBM invasion [32, 88]. In Matrigel, growing glioma spheroids exerted compressive

forces, while invading cells exerted traction forces on the ECM [32]. Increasing collagen I concentration, and consequently the matrix stiffness, facilitated glioma spheroid invasion because the increased fiber density allowed for more cell-ECM adhesions and traction. However, it resulted in reduced spheroid growth, as the dense matrix inhibited the proliferation of cells. Additionally, enhanced glioma spheroid invasion was observed on collagen matrices with more porous network architectures [97]. Different parameters such as porosity, concentration, and cross-linking density affect stiffness, which is shown to be a major factor in cell migration. Migration plays a direct effect on cell growth by promoting multiple cellular processes.

Bioconjugation of hydrogels with therapeutic agents to expand functionality

Hydrogels have different biochemical properties depending on the ligands, growth factors, regulatory sites, and therapeutics that are incorporated into their structure. These properties induce various pathways that can support or inhibit cellular function. For example, peptides conjugated to a hydrogel can promote cell adhesion and proliferation. In this way, hydrogels can be used in different modularities.

Incorporation of therapeutic and diagnostic agents including anti-cancer drugs, imaging agents, and nanoparticles within hydrogels expands their usability as functional implants for brain cancer therapy [5, 12, 81, 108, 109]. Entrapment of drug-loaded nanoparticles, for example, in hydrogels results in local delivery of drugs to specific sites in a sustained release manner for efficient glioma chemotherapy. For this purpose, poly(lactic-co-glycolic acid) (PLGA) nanoparticles are incorporated into hydrogels derived from substances such as alginate. Alginate is found to be a valuable choice due to its biocompatibility, biodegradability, and ability to encapsulate drugs for sustained release [12]. Incorporation of PLGA can help encapsulation of lipophilic drugs like PTX which would normally have difficulty solubilizing in body fluids. Polymeric scaffolds can be inserted into the resected tumor cavity following surgery to prevent recurrence of the cancer, or they can be injected prior to the surgery [81]. Unique characteristics of certain nanoparticles, such as fluorescent or magnetic properties, suggests that nanoparticle-loaded hydrogels can assist in fluorescence studies or magnetic resonance imaging (MRI), in addition to drug delivery [109, 110].

Microscale technologies to engineer hydrogels in brain cancer culture

In this section, we discuss a few microscale technologies featuring hydrogels. Microgels can be engineered by a variety of methods, including micropatterning, electrospinning, microfluidics, and bioprinting. A combination of microscale technologies and hydrogel precursors defines the ultimate chemical, physical, and mechanical behaviors of hydrogels. Major advantages of microscale structures include the small experimental scale, minimized experimental times, cost-effectiveness, and physical reduction of the experimental platform from the benchtop scale to the microscale. Microscale technologies also permit researchers to independently control numerous experimental factors (e.g., number, size, shape, and density of cells) in cell-laden hydrogels, resulting in precise control of cells for encapsulation in natural or synthetic hydrogels. Table 3 highlights the advantages of these methodologies and hindrances to their applications in brain tumor engineering.

Micropatterning

Micropatterning is used to print specific cell and tissue architectures at different scales and complexities. It involves utilizing cell attachment, shape, and spreading on the controlled spatial area of the culture surface. The general steps of micropatterning on these surfaces involve: (1) generation of adhesiveness, (2) seeding of cells onto newly adhesive surfaces, and (3) washing unattached cells to generate specified patterns. ECM proteins and polypeptides used in patterning mediate attachment through anchorage-dependent cellular mechanisms [113].

The size of patterns affects properties of attached cells. When patterns at the subcellular to single-cell levels (≈ 10 μm) are made, cell spreading is hindered, which causes cells to rearrange their cytoskeleton to fit into the constrained space [114]. At the multicellular scale, micropatterning is used to form microscale islands, which causes cells to appear as sheets with specific cell shape, polarization, and signaling [115]. These "microsheets" can be engineered to represent specific shapes that define networks for cell types, like neural networks for synaptic signaling and neural polarization, or cardiac structures to mimic the cellular architecture of the heart [113]. Micropatterning can also recapitulate the linear-like invasive routes of aggressive cancers within the CNS, similar to the paths of important blood vessels. Monzo et al. [111] created micropatterned linear tracks coated with laminin to mimic vasculature and showed that when seeded onto these surfaces, GBM cells adhered and microtubules became polarized (Fig. 4a). Other ECM components including fibronectin, laminin, and Matrigel also promote cellular elongation to mimic natural structures like blood vessels (Fig. 5a).

In order to generate these patterns, different methods can be used to create adhesive surfaces to direct the culture of cells. Photolithography uses a silicon wafer coated with photoresist, which is baked, hardened, and exposed to UV light, revealing a designed pattern on the wafer [113].

Table 3 Applications of microscale technologies in brain tumor engineering

Microscale technologies	Advantages	Disadvantages	Applications
Micropatterning	Ability to make complex geometries	Requires clean room facilities	Study of cell behavior in controlled population or cell size and/or geometry
	Patterns are stable for days	Limited to 2D surfaces	
	Microscale patterns are feasible		
Electrospinning	Can create aligned fibrous meshes	Can only create fibers	Wound dressing materials
	Can be used with biological polymers such as collagen	Jet instability	Skin regeneration
	Fibers with diameter sizes of nanometer to few microns	Toxic solvents	
	High aspect ratio		
	Enhanced mechanical properties		
	Continuous process		
Microfluidics	Complex geometry (in microsheets)	Requires clean room facilities	Microfluidic chips
	Cells can be seeded along with protein patterning	Costs associated with photolithography	Preclinical testing of drugs on living cells
		Multistep and requires substantial optimization	Structure-based drug discovery
			Pharmacological profiling and toxicity testing
Bioprinting	Offers high and precise resolution	Large-scale construction increases the complexity	Create entire living organs
		Incapable of printing complicated tissues	Newly developed drugs can be tested out on manufactured cellular organs
	Rapid manufacturing of tissues and organs	Difficulty keeping cells alive	Imprinting cells directly onto human body
		Implanted organ can be rejected as body may not accept them as functional tissues	

Soft lithography, on the other hand, generates an elastomeric stamp from materials such as poly(dimethylsiloxane) (PDMS), with features of the topography of a previously patterned wafer [106]. For microcontact printing, the PDMS-based stamp is plasma-treated and coated with a protein solution, such as collagen or fibronectin, which can then be transferred onto surfaces for cell culture [113]. Microfluidic-assisted patterning uses channels made from photolithography to transfer protein solutions in a desired pattern. In contrast, methods utilizing microstencils require individuals to seed cells onto a PDMS elastomeric sheet with holes in defined geometries and then remove the PDMS to leave only the cells. Among studies using microcontact printing, Endler et al. [116] tested the effects of hydrogel surface chemistry on tumoral astrocyte growth. Cells were sparsely plated, adhered, and, upon growth, delineated regions that were clearly adhesive or repulsive to cells.

Nanoparticles can also be used to pattern the surface of a biocompatible material. Guo et al. [117] patterned the surface of an agarose hydrogel with a magnetic nanoparticle suspension and used a magnetostatic field to direct the pattern. Cells were able to attach, elongate, and, in some cases, even form aggregates.

Electrospinning

Electrospinning utilizes an electrical field to generate fibers of a desired size, orientation, and material depending on the parameters used. The basic setup uses a high-voltage system, a spinner, and a collector. A polymer solution is ejected from a pipettor, while voltage is being applied to a needle tip as well as the grounded fiber collector [118]. Repulsive forces from induction are broken at a critical voltage, and the polymer solution is deposited onto the collector. Variables of the solution such as concentration, conductivity, viscosity, molecular weight, solvent volatility, and molecular structure affect the efficacy of the process and the resulting fiber morphology [119]. Meanwhile, the voltage, flow rate, and distance of the pipette with polymer solution from the collector impact the solution's ability to collect on and adhere to the collector. Several conditions of the electrospinning setup and even the room's environmental conditions

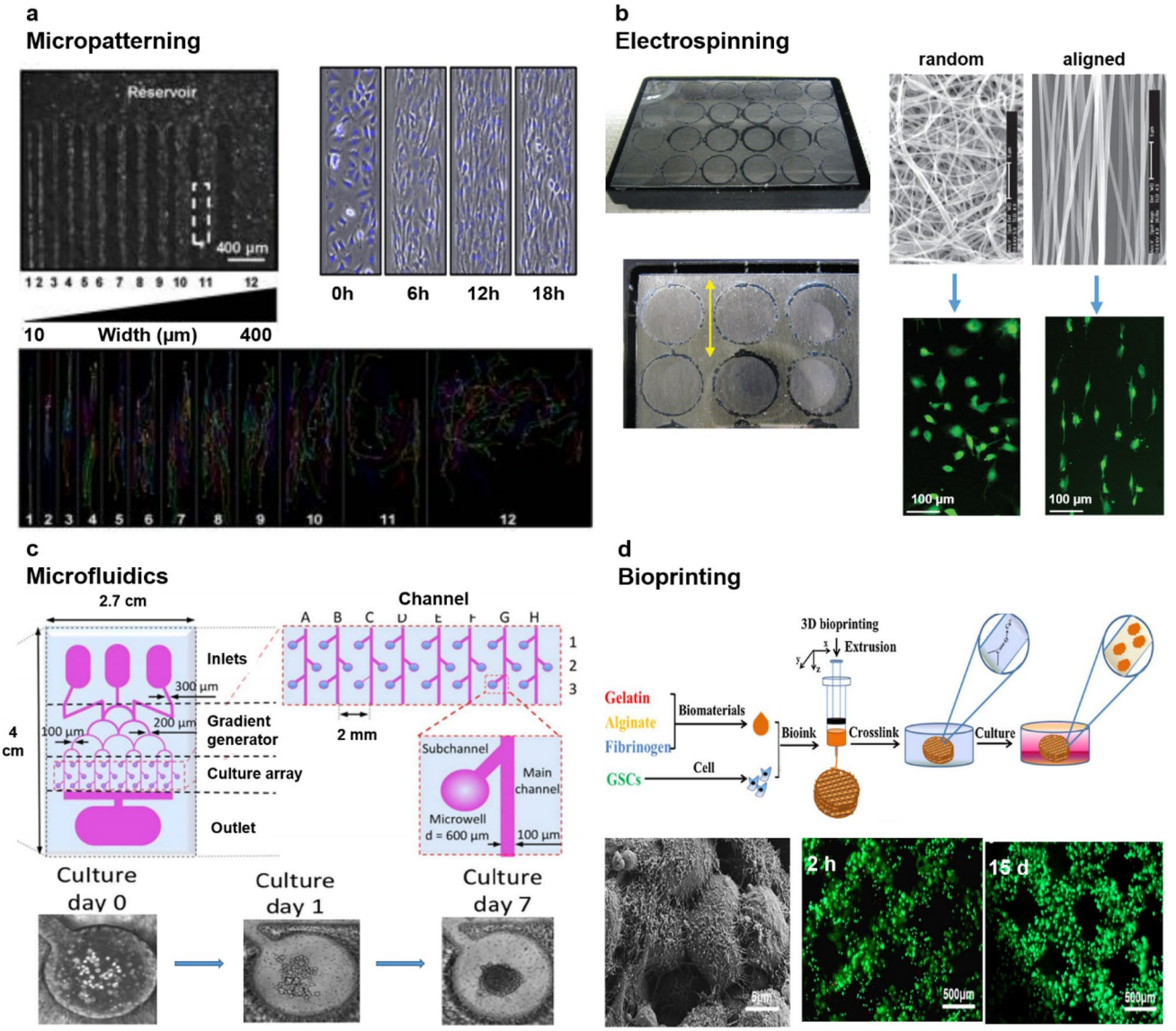


Fig. 4 Microscale procedures for directing cellular growth. **a** Microchannels patterned with laminin cause glioma cell adhesion and alignment as cell density increases (Adapted from Monzo et al. [111]). **b** Aligned PCL nanofibers promote directional contact guidance to glioma cells seeded on their surface, while randomly oriented fibers produce non-specific cell populations on their surface (Adapted from Agudelo-Garcia et al. [76]). **c** Microfluidic devices are compartmentalized and consist of wells which allow for GBM cell aggregation as the flow of media provides nutrients as cells proliferate (Adapted from Fan et al. [21]). **d** Bioprinting extrudes of alginate/gelatin/fibrin gel which encapsulates cells and causes them to grow in distinct arrays (Adapted from Wang et al. [112])

like temperature and relative humidity affect the generation of uniform nanofibers of a desired diameter and must thus be monitored.

The fibrous structure of nanofibers can direct the proliferation of cells. Similar nanotopography in the body, such as those along the white matter in the brain and blood vessels, can allow GBM tumor cells to migrate from their primary site and create secondary growths [120]. These tumor cells exhibit lower cytoskeletal stiffness, cell traction stress, and focal adhesion while downregulating proliferative genes and upregulating migratory genes, compared to normal astrocytes. Such factors allow for an increased potential for migration along aligned surfaces. Electrospinning can generate coated surfaces from polycaprolactone (PCL) and other polymeric nanofibers that act as substrates for growing cells (Fig. 4b). Glioma cells cultured on nanofiber scaffolds show elongated morphology, reduced migratory potential under myosin II inhibition, and STAT3 inhibition in neurospheres and tumor explants [76]. Electrospinning and surface patterning create fibrous structures with nanotopography that influences gene expression and signaling pathways to facilitate cellular invasion (Fig. 5b). Nanofiber scaffolds

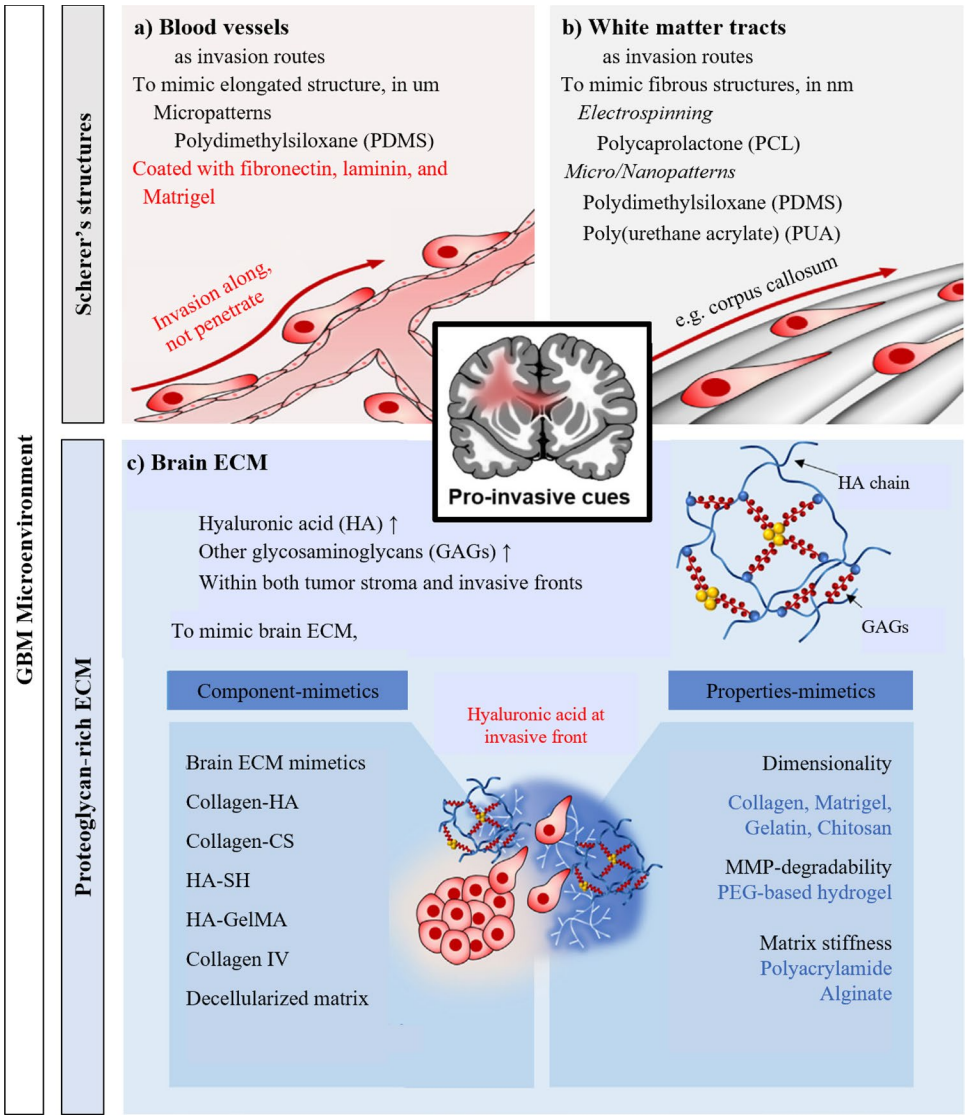


Fig. 5 Physiological structures in GBM microenvironment promote cancer invasion: **a** White matter tracts facilitating communication between brain regions serve as invasion routes for GBM cells. Tracts recapitulated on nanofiber-coated and pattered surfaces allow for cell adhesion and migration. **b** Blood vessels are invasion routes for migrating GBM cells. PDMS devices micropatterned with ECM proteins mimic elongated structures of blood vessels. **c** Brain ECM composed of high HA and other glycosaminoglycans in tumor stroma and invasive fronts. Polymers are able to mimic components and properties of brain ECM (Adapted from Cha et al. [17])

can also be used to carry cells to the tumor site. Bagó et al. [121] implanted a poly(L-lactic acid) electrospun scaffold containing mesenchymal stem cells (MSCs) into the tumor resection cavity. This approach induced the release of the TRAIL antitumor protein from the MSCs, which reduced GBM xenograft volume and inhibited recurrence.

Electrospun scaffolds are beneficial for drug treatments as they can provide sustained release of different therapeutic agents. Nanofibers have been utilized to locally deliver substances such as paclitaxel (PTX), temozolomide (TMZ), carmustine, rapamycin, mycophenolic acid, daunorubicin, *Bis*-chloroethylnitrosourea, irinotecan, cisplatin, combretastatin, SN-38, MMP-2 mRNA, TRAIL, and curcumin locally to brain tissue [122]. Local administration reduces the possibility of drug exposure leading to unwanted side effects in non-cancerous regions.

Specific 3D scaffolds can also be constructed to provide a microenvironment that supports cell proliferation. Rao et al. [123] combined different polymers, including HA, collagen, and Matrigel, to modulate the mechanical properties and form core–shell nanofibers while using mechanically robust PCL in the core. Under this structure, HA inhibited GBM cell migration, while other materials did not significantly affect cell properties. As a result, hybrid nanofibers allow researchers to promote the benefits of multiple materials, such as biocompatibility and mechanical integrity, to allow greater cellular engagement.

Microfluidics

Microfluidics provides an opportunity to model the physicochemical environment of tissues and cells. Microfluidic devices are typically composed of polymers, such as PDMS or poly(methyl methacrylate) (PMMA), to provide perfusable microchannels. Fluid flow in the channels generates shear stress, similar to blood in vasculature. This

vasculature-mimicking platform is shown to affect the growth of tissues in vitro. Depending on the purpose of the study, cells can populate the interior of the device to test the effects of mechanical forces or biochemical substrates on living cells. Researchers also alter the basic structure of these devices and create interfaces with either hydrogels or ECM-coated surfaces to reproduce physiological conditions. These devices hope to recapitulate oncologic processes, such as tumor growth, blood vessel expansion, epithelial-to-mesenchymal transition (EMT), and tissue invasion and metastasis [23].

Due to the unique pathobiology of the nervous system, microfluidic devices are being used in innovative ways to model the brain, spinal cord, and their interfaces with the rest of the body via the BBB. Brown et al. [124] designed a device with vascular and brain compartments, separated by a porous membrane. Between these compartments, the device developed a functioning neurovascular unit composed of endothelial cells, astrocytes, and pericytes that support molecular diffusion and hold a transepithelial electrical resistance. Such BBB devices have been adapted to simulate the processes of metastasis, such as intravasation and extravasation, when brain cancer cells migrate through the neurovascular barrier [125]. Xu et al. [126] tested the therapeutic effects of various agents in a glioma-BBB microfluidic device. They demonstrated that TMZ induced the highest level of cell death as it is lipophilic and able to traverse the BBB, while other compounds performed poorly. Similar devices recapitulate the permeability of tumors with vasculature for future studies of drug interaction with tumors and cancer cells [127].

Hydrogels such as gelatin methacryloyl (GelMA) and PEGDA are now being used in the development of these devices more commonly due to their permeability and ability to encapsulate cells and biomolecules. Lee et al. [22] tested the migration of U87MG glioblastoma cells through a GelMA hydrogel barrier treated with VEGF. Cells migrated toward the GelMA-coated microchannel over 5-day experiment. Meanwhile, Fan et al. designed a high-throughput 3D brain cancer chip composed of a photo-polymerizable PEGDA hydrogel containing microwells to create spheroids. They tested the effects of drug combinations of pitavastatin and irinotecan, finding that dual therapy had the most significant effect on the cell viability (Fig. 4c) [21]. Polymeric networks within hydrogels recapitulate components and properties of the brain ECM and remain susceptible to cancer cell invasion (Fig. 5c). Hydrogels are being used more commonly in bioengineering to control the release of drugs and growth factors or to encapsulate cells.

Bioprinting

Bioprinting is driven by the hypothesis that precise cell arrangement can signal physiological cues to generate functional tissues. It offers a revolutionary approach for the fabrication of neural tissues in a precise and controllable manner [59]. 3D bioprinting of tumor models has been used more commonly compared to 2D cellular monolayer models. Studies have shown varying cellular responses between 2D and 3D models, specifically changes in protein and gene expression, proliferation, viability, and drug response [16]. The resulting differences between these models, as well as the lack of ECM-mimicking properties of 2D models, have driven the development of more 3D models that are capable of mimicking the ECM of cancerous tumor cells [112]. The 3D bioprinting of hydrogel scaffolds integrated with GSCs accurately showed the vascularization potential of GSCs and expression of tumor angiogenesis (Fig. 4d) [112]. 3D bioprinted tumor models recapitulate the in vivo tumor microenvironment more closely, in terms of spatial dimension, ECM characteristics, and tumor–stroma interactions. Therefore, the 3D bioprinted tumor model can be expected to lead to formation of a microenvironment more similar to that produced by an in vivo tumor compared to those from 2D culture systems.

Bioprinting offers numerous advantages that are key to the design of scaffolds to mimic the ECM of tumors. These include versatility of scaffold fabrication (i.e., size, dimension, material), ability to use different types of cells that can be accurately patterned simultaneously, and bimolecular gradients that can be fabricated in multilayers. A recent study done by Dai et al. [16] showed that cells grown on 3D bioprinted scaffolds had a higher rate of survival than those typically grown in 2D models. Their prolonged growth would allow for better analysis of the mature tumor microenvironment.

For cancer research, bioprinting is utilized in one of two ways: (1) one-step biofabrication, in which tumor cells are encapsulated in hydrogels and printed directly into structures for drug treatment, or (2) seeding tumor cells onto preprinted scaffolds [128]. In a study by Lee et al., glioma/vascular cell–cell interactions were observed which led to better understanding of GBM cells' invasiveness [129]. Patient-derived GBM cells were seeded onto a collagen matrix to create a vascular channel that more accurately modeled GBM cells' ability to migrate through the body using micro-vessels. Bioprinting allowed direct observation of the patient cells' response and activity. In addition, the collagen matrix was printed with a uniform distribution of cells. The GBM cells were then loaded at varying concentrations to determine the ideal scaffold composition for future work in tumor-vascular models.

3D printed models that mimic the brain tumor microenvironment with high cell viability and inherent characteristics are needed to study potential functions of corresponding native tissues. Interestingly, in a study by Dai et al., a 3D bioprinted brain tumor model constructed from GSCs showed more resistance to chemotherapeutics (TMZ) compared to a standard 2D cell model [16]. This study highlights the difference in drug-eluting properties of typical 2D cancer cell models and its inaccuracies compared to the results obtained from 3D models.

Therapeutic hydrogels for brain tumor treatment

Survival time of patients with different types of brain tumors under current treatment modalities decreases with age and malignancy; however, mutations in genes and epigenetic regions can affect a patient's response to therapy [130]. Hydrogels can improve upon these various methods due to their injectability, mechanical strength, and ability to envelop other substances. These substances can include drugs, growth factors, and even whole cells depending on the application of the hydrogel. As a result, if properly applied in the nervous system, the hydrogel has the potential to treat an infected or injured region that is not easily accessible by common therapies.

Hydrogels for drug delivery to tumor cells

Physical and chemical properties of hydrogels control their release of encapsulated drugs. Drugs are infused in the hydrogel and are often enclosed within a cross-linked network which prevents these molecules from undergoing metabolic processes in the body [131]. As shown in Fig. 6a, hydrogels must be delivered to the desired site, which can occur locally by needle injection or systemically by intravenous delivery [8, 132]. The anti-cancer drugs loaded in different nanostructures integrated within a macroscopic non-injectable hydrogel can be locally delivered to brain tumors via implantation, while hydrogels with shear thinning properties can be injected via syringe [133–135]. Additionally, hydrogel nanoparticles functionalized with targeting agents can be administered intravenously to deliver loaded drugs to the specified brain tumors [93].

Drug delivery challenges

Delivery of a hydrogel containing therapeutic agents can occur either locally or systemically, such that it will circulate through the body to the intended site. Both processes have multiple hurdles which must be overcome before the treatment should be released. A drug must retain its bioactivity during encapsulation which is dependent on how it interacts with the drug carrier. In local delivery, the drug will be required to act on a tumor site quickly or slowly after administration. To do so, release needs to occur in frequent bursts or in a sustained manner, respectively. The hydrogel's physicochemical properties determine how it will release its cargo and react with the environment, so the properties must be studied well prior to use in the clinic.

For systemic delivery, drugs intended for entry into the CNS are released into the bloodstream, meaning that they must overcome the BBB. The BBB regulates the levels of nutrients, ions, and macromolecules, balances the pH, and keeps out harmful substances from the CNS. Typically, only small, lipophilic molecules are able to passively diffuse across the BBB, while active transport is difficult. This severely limits the drugs that can enter the CNS. As a result, drug properties must be modified or drug carriers must be engineered to improve their bioavailability.

Organized neurovascular units facilitate the aforementioned BBB functions with the assistance of pericytes [49]. The composition of the BBB and signaling that occurs between these cells should be understood well to improve upon current treatment options for brain tumors. Microvascular endothelial cells form tight junctions consisting of claudins and occludins with selective transmembrane proteins to regulate transport, while pericytes lead blood vessel formation and remain anchored to endothelial cells to maintain their differentiated state [136]. Astrocytes extend their "endfeet" and lie in unique positions between the endothelium and neurons, which indicates a role in mediating signaling between the different cell types. They limit permeability of the BBB through secretion of Sonic hedgehog (Shh), and regulate Wnt and angiotensin I and II signaling, which affects tight junction formation [137]. In addition, astrocytes' production of apolipoprotein E is thought to act on pericytes which regulate tight junctions. Researchers are assembling nano- and microscale gels that are highly permeable and biocompatible to transport drugs across the BBB to increase availability of the therapeutic agents to tumor cells [93].

In vitro studies

Among their numerous applications, the porosity of hydrogels makes them uniquely qualified to carry different molecules and allow for controlled release. Hydrogels loaded with nanostructures or nanocarriers produced from hydrogel substrates, called "nanogels," generally perform this controlled release [8]. Drugs that have been used with these hydrogels are typically hydrophilic, but the solubility of the drugs in media is still poor and needs to be improved through medicinal chemistry [138].

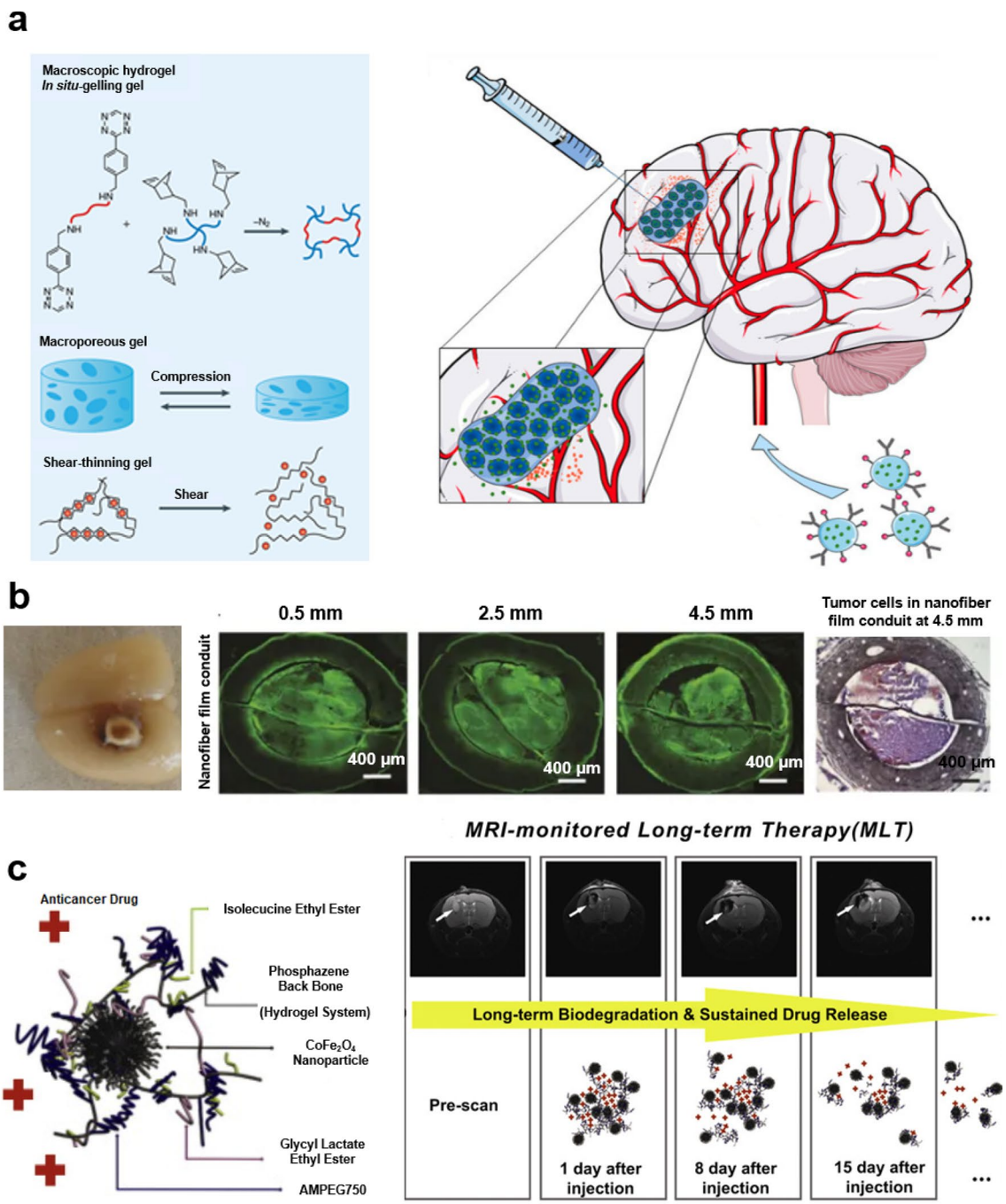


Fig. 6 **a** Hydrogel composition and routes of administration of hydrogels for glioblastoma treatment. Macroscopic hydrogels containing therapeutic agents with various chemical compositions were locally implanted or injected in the tumor site, while hydrogel nanoparticles were intravenously administered ((Adapted from Li et al. [132] and Basso et al. [8]). **b** Insertion of a PCL/polyurethane carrier conduit containing aligned PCL nanofiber films, and cyclopamine (anticancer)-conjugated collagen hydrogel serving as an apoptotic tumor sink in the brain. Migration of tumor cells along the aligned films throughout the cross section of conduit was observed at different distances from the interface of the tumor in the brain (Adapted from Jain et al. [141]. **c** Stereotactical injection of drug-loaded magnetic hydrogel, and simultaneous magnetic resonance images of treated region over time by degradation of hydrogel and sustained release of drug (Adapted from Kim et al. [144])

In vitro models are used to avoid the use of animals or verify the efficacy of a therapy prior to clinical trials. These methods have developed significantly in recent years and given way to more sophisticated platforms. For example, cellular models have evolved from 2D monolayer culture to 3D culture systems, such as spheroids and organoids. Especially

among 3D cultures, models have become more physiologically accurate as they incorporate multiple cell types or microstructures seen in the body. In doing so, the effects of abnormal genotypes and phenotypes are better represented.

Nanogels loaded with macromolecules can cross boundaries such as the BBB and release their cargos at a particular site. However, computational studies found that the surface area-to-volume ratio is important to enhance diffusion of the drug or drug-loaded nanogels as brain tissue prevents the formation of an optimal concentration gradient for the drug [139]. In vitro studies must effectively control the dosage of therapeutics during administration in order to facilitate proper diffusion and uptake by cells. Drug release from a hydrogel has several advantages over direct administration when tested in vitro. Formulations of hydrogels into microspheres, nanogels, and injectable gels allow for sustained release at constant rates [8, 12, 94, 140]. When tested in cell cultures, hydrogels with single and multiple drug loading combinations induced greater cytotoxicity compared to drug administration in soluble form. Delivery of other anti-cancer drugs, such as camptothecin (CPT), has been studied using PEG-based hybrid hydrogels. These hydrogels encapsulated CPT-loaded silica nanoparticles which were bonded to a photo-triggerable chemical adaptor to provide stimuli-responsive release. Photo-irradiation induced the controlled release of CPT, causing significantly lower glioblastoma cell viability compared to controls [108]. Multiple drugs and drug combinations have shown such efficacy in these experiments.

Nanostructures can also utilize the properties of GBM cells to lure them outside primary tumor sites. Researchers have implanted aligned PCL nanofibrous films to guide migratory GBM cells outside the cortex and into a collagen hydrogel loaded with the drug cyclopamine (Fig. 6b) [141]. Following this procedure, tumor volume significantly decreased as the hydrogel killed GBM cells extracortically.

In vivo studies

In vivo studies elucidate on mechanisms that cannot be easily modeled in vitro due to their complex multicellular and microenvironmental interactions. The BBB and cerebrovascular structures are integrated with the brain in vivo which is difficult to recapitulate in vitro with physiological accuracy. The brain is also connected to many other bodily systems, which determine drug and nutrient absorption, circulation throughout the body, metabolism, and excretion of waste.

Animal studies utilize post-resection treatment whereby a hydrogel is injected intracerebrally or intracerebroventricularly after a brain tumor is removed. The hydrogel may be loaded with an anti-cancer drug to allow its sustained release. Administration in this route allows the material to be inserted into the target site without significant hindrance as the BBB presents a physicochemical barrier to substances. Puente et al. [5] injected a TMZ and ^{131}I-loaded chitosan hydrogel into the surgical cavity in the mouse brain post-resection. The hydrogel's semisolid nature presents a material that is neither too stiff nor releases TMZ too quickly. As a result, it effectively distributed the drug, improving the survival of the treated animal subjects. Hydrogels containing chemotherapy-loaded particles improve survival compared to untreated groups due to sustained drug release [11, 81, 142]. These hydrogels can also encapsulate radioactive isotopes to assist in concurrent radiotherapy [5].

Postoperative administration of hydrogels allows for the prevention of brain tumor recurrence, as nano- or microstructures loaded with drugs limit toxicity outside the target site where the hydrogel is injected [143]. Certain formulations of hydrogels can also be monitored in real-time through imaging of, for example, incorporated iron oxide magnetic nanoparticles (Fig. 6c) [144]. These theranostic hydrogels have significant benefits when considered in vivo for both treatment and tracking of patient condition.

Clinical studies

Although animal and cell models provide indications of the safety and efficacy of treatments on humans, a human clinical trial is still needed to determine the actual and most accurate responses. Ultimately, the physiology of animals has inherent differences when compared to that of humans, and current 2D and 3D cell models cannot fully recapitulate the complex organ structures seen in the human body. Human bodies have multiple organs and associated cells that process drugs, circulate metabolites, and eventually excrete the end products. In the end, all of these mechanisms must be considered before a treatment can be deemed safe for use outside the lab.

When used clinically, an important consideration is the site of administration of the hydrogel. Studies have shown that injecting gels following tumor resection prevents recurrence because leftover cancerous cells cannot repopulate the area [145]. The Gliadel wafer, a biodegradable copolymer loaded with carmustine, has long been approved for use clinically and shown positive results. Implantation of the Gliadel wafer in resection cavities has reduced risk of recurrence and improved survival in patients [14, 15, 146]. Torres et al. [139] created a PTX-loaded hydrogel implant that released the drug slower than the Gliadel wafer, and hypothesized that a hydrophobic drug like TMZ would be more efficient. Therapies can also be injected intratumorally which have shown low rates of edema and non-toxic levels of chemotherapy in other body regions [9]. Results from phase II clinical studies have shown that microspheres loaded with chemotherapeutic drugs were tolerated well in patients, without adverse symptoms, though the difference

in median survival time was not statistically significant [147, 148].

Complications from surgery may also require hydrogels as a form of sealant. During surgery, particularly when operating on the brain and spinal cord, blood leakage and leakage of cerebrospinal fluid (CSF) can occur, which must be addressed immediately. DuraSeal, for example, uses a PEG hydrogel to treat dural bleeds that occur during cranial surgery [149]. Boogaarts et al. [150] tested a different PEG sealant against CSF leakage and found excellent postsurgical wound healing, with few infections and adverse effects. A systematic review of dural sealants showed that while many did not reduce the number of CSF leaks, incisional leaks, or pseudomeningocele formation, dural sealants did reduce secondary infections at the site of the initial surgery [151].

Hydrogels combined with other therapies

The current standard-of-care for brain cancer consists of radiotherapy with adjuvant chemotherapy, most commonly TMZ [6]. To increase anti-cancer effects, TMZ can be supplemented with multiple drugs, including, but not limited to, curcumin [152], capecitabine [153–155], and O6-benzylguanine [156]. However, the lack of specificity toward the cancer cells leaves uncertainty for the patient's overall health. Thus, methods are being researched to improve the localization of new and old treatments such that healthy bodily areas are not harmed.

For several years, bioengineered particles have been used to mediate the release of drugs to specific tissues and provide controlled release. As described above, this method is now being combined with hydrogels to further improve drug release. Specifically, micro- and nanoparticles can be formed from hydrogels, and used to carry chemotherapeutics. These structures can fill the area surrounding the tumor or around the cavity formed following tumor resection [81, 157].

Hydrogels are also being applied to carry radiosensitive compounds. In addition to encapsulating chemotherapeutic agents, the hydrogel can be applied to image tumor structures with loaded radioactive isotopes, such as iodine-131 and cobalt ferrite nanoparticles, over time [5, 110, 144]. Each study showed that these magnetic hydrogels caused low cytotoxicity in vivo and provided long-term visualization of the tumor site. Physicians can monitor drug release and accumulation at the target site, and, if the condition of the tumor site worsens, they can supplement treatment.

Challenges and future perspectives

The current state of brain tumor modeling is promising as new materials and culture conditions are developed continuously. Our understanding of cancer biology has also improved with different brain tumors categorized by brain region, cell type, severity, and metastatic potential. Spheroids, organoids, and scaffolded cellular models have different benefits over one another. The processes used to develop them from cells and polymers allow for diversity in the model and the appropriate time of use.

The treatment of brain tumors can involve a range of therapies with hydrogels as one of the primary drug carriers used to deliver them. Hydrogels supplement the benefits of treatment methods as they provide physicochemical cues to proliferating cells. They are also highly adaptable depending on the need of the material. The ability of hydrogels to form particles, films, highly porous structures, or simply encapsulate other structures makes their applications in bioengineering very desirable. When hydrogels encapsulate other compounds, the sustained release that it provides allows the hydrogel to work alongside and bolster current treatments, such as chemotherapy or radiotherapy. This mode of delivery is shown to prevent recurrence of cancer and reduce toxicity, edema, and other side effects. They can also allow for better imaging if not used for treatment.

Methods reviewed here for overcoming challenges to drug delivery directly to the brain, such as the BBB, provide helpful insights for the further development of hydrogel systems in the clinical realm. The number of studies shows that significant thought has been given into the optimized formulations of hydrogels, modes of administration, methods for evaluation, and minimization of side effects.

Conclusion

Brain tumors can be categorized in a number of ways, such as primary cell type and metastatic potential, which determines the patient's available treatment options. Hydrogels have been used to recapitulate the brain tumor microenvironment in vitro and test potential therapies prior to use clinically. They have proven to be an effective medium to deliver treatments or physical blockage to the target regions of model systems. Thus, to make the most efficacious use of hydrogels, researchers should utilize hydrogels to encapsulate drugs and other compounds, but also postsurgically as a sealant or secondary therapy in the tumoral cavity to prevent recurrence of the disorder. Their biological and physicochemical properties can also be altered to match the properties of the tumor ECM, which should make it more biocompatible and reduce the likelihood of any immunologic rejection. The current applications of hydrogels in the study and treatment of brain tumors have made great success at both the experimental and clinical levels and show potential for future studies.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

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