



Review article

Anticancer drug-loaded hydrogels as drug delivery systems for the local treatment of glioblastoma

C. Bastiancich^a, P. Danhier^b, V. Préat^{a,*,1}, F. Danhier^{a,*,1}^a Université catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Avenue Mounier, B1 73.12, 1200 Brussels, Belgium^b Université catholique de Louvain, Louvain Drug Research Institute, Biomedical Magnetic Resonance Unit, Avenue Mounier, B1 73.08, 1200 Brussels, Belgium

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ABSTRACT

Among central nervous system tumors, Glioblastoma (GBM) is the most common, aggressive and neurological destructive primary brain tumor in adults. Standard care therapy for GBM consists in surgical resection of the accessible tumor (without causing neurological damage) followed by chemoradiation. However, several obstacles limit the assessment of tumor response and the delivery of cytotoxic agents at the tumor site, leading to a lack of effectiveness of conventional treatments against GBM and fatal outcome. Despite the efforts of the scientific community to increase the long-term benefits of GBM therapy, at the moment GBM remains incurable. Among the strategies that have been adopted in the last two decades to find new and efficacious therapies for the treatment of GBM, the local delivery of chemotherapeutic drugs in the tumor resection cavity emerged.

In this review, our aim is to provide an overview on hydrogels loaded with anticancer drugs for the treatment of GBM recently used in preclinical and clinical studies, their advantages and major limitations for clinical translation. This review is divided in three parts: the first one describes the context of GBM and its current treatments, with a highlight on the role of local delivery in GBM treatment and the development of GBM resection murine models. Then, recent developments in the use of anticancer drug-loaded hydrogels for the treatment of GBM will be detailed. The final section will be focused on the limitations for *in vivo* studies, clinical translation and the clinical perspectives to the development of hydrogels.

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* Corresponding authors at: Université catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Avenue Mounier, 73, B1 73.12, 1200 Brussels, Belgium.

E-mail addresses: veronique.preat@uclouvain.be (V. Préat), fabienne.danhier@uclouvain.be (F. Danhier).

¹ These two authors equally contributed to the work.

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1. Introduction

1.1. Glioblastoma

Brain tumors only count 2% of the adult population affected by cancer. However, they are considered among the worst diseases as they have a direct impact on patient's life from a physical, psychological and neurological point of view [1]. Among brain tumors, Glioblastoma (GBM) is the most common and aggressive in adults, and also the most feared by patients, physicians and oncologists [2,3]. Indeed, GBM has been classified as grade IV astrocytoma as it is highly malignant and arises from astrocytes or supportive brain tissue [4]. Preventive measures, such as life style changes, early diagnosis and treatment unfortunately do not impede the development of the disease and do not improve its outcome, precluding the utility of screening for this tumor [1]. Based on the clinical history of the tumor, GBM can be divided into primary GBM (90%) or secondary GBM (10%): in the first case the tumor arises in an acute *de novo* manner without previous lower grade pathology or symptoms, while the secondary GBM derives from the progressive evolution and transformation of lower grade astrocytomas and normally affects younger patients. The two subtypes of GBM present different genetic profiles and can be identified by specific cell markers but are morphologically and clinically indistinguishable. Moreover, both have the same poor prognosis (median survival below 15 months) and remain incurable [5]. Signs and symptoms from GBM usually result from infiltration or compression of normal brain by tumor, edema, hemorrhage or increased intracranial pressure and include headaches, seizures, focal neurologic deficits and changes in mental

status [6]. Despite the low number of patients affected by this disease (the US and EU incidence is 3 in 10,000 persons) [7], in the last decades many researchers have focused their attention to find new efficacious treatment strategies to improve the quality of life of patients affected by GBM and their clinical outcome.

Several obstacles limit the assessment of tumor response and the delivery of cytotoxic agents leading to a lack of effectiveness of GBM treatments (Fig. 1): (i) the anatomical location of the tumor in the brain often impedes a complete surgical resection without damaging the neurological tissue and affects the cognitive functions of the patient. Moreover, the central nervous system (CNS) barriers (blood cerebrospinal fluid barrier; arachnoid barrier; blood-brain barrier, BBB; blood-tumor barrier) represent a challenge to the delivery of cytotoxic drugs at therapeutic concentrations at the tumor site. (ii) GBM is highly heterogeneous at all levels, from the tissue level to the molecular and genetic point of view to the cell type [2,8]. This heterogeneity, represented also within the same tumor, leads to high variability in tumor histopathology making the classification of these tumors very difficult and resulting in low predictability of tumor response to treatments [9]; (iii) the hallmark characteristics of GBM are uncontrolled cellular proliferation, propensity for necrosis and angiogenesis, resistance to apoptosis, high genomic instability, chemoresistance and fatal outcome [5]. GBM cells are able to extend their tendrils into the normal surrounding parenchyma infiltrating diffusely beyond the primary lesion in the early stages of tumor development (GBM is also known as “octopus tumor”) [10]. Many individual genes implicated in GBM cells migration and invasion have been identified and their presence has been correlated with poor patient survival [11]. It has also been shown that GBM invasion is

GBM: THE OCTOPUS TUMOR

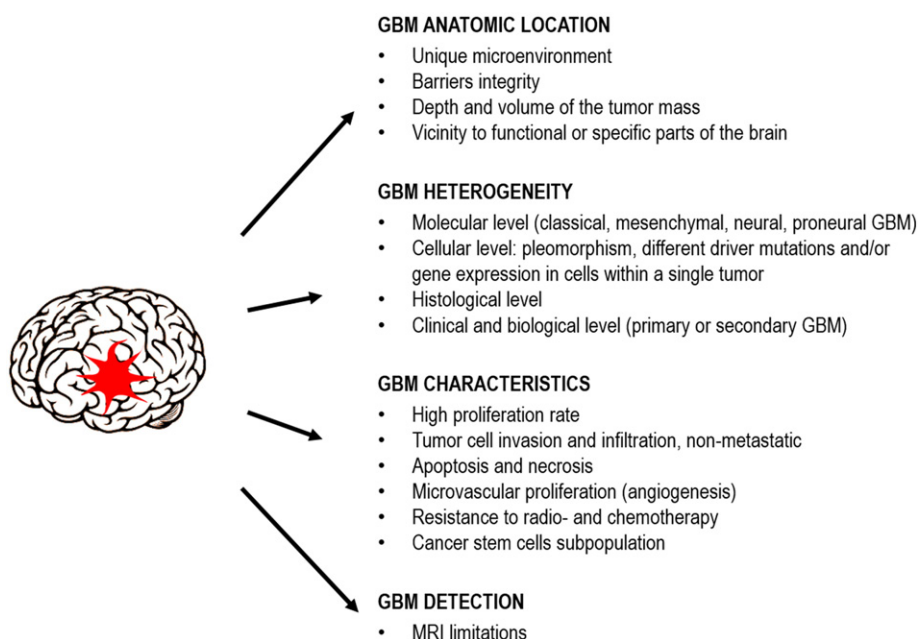


Fig. 1. Obstacles for effective treatment of GBM that contribute to its fatal outcome.

not random and occurs along white matter tracts, basement membranes of blood vessels, along the subependyma, adjacent to neurons and, sometimes, it reaches the contralateral hemisphere [5,11,12]. Moreover, the presence of a subpopulation of cancer stem cells able to act as “disease reservoirs” and self-renew themselves increases the ability of forming GBM recurrences [11,13]; (iv) A key role in the diagnosis and GBM progression evaluation is played by the Magnetic Resonance Imaging (MRI). Indeed, due to its high soft tissue contrast, MRI is the preferred method for the noninvasive detection of brain tumors. On standard gadolinium-enhanced T₁-weighted images, GBM appears as heterogeneous hyperintense signals at the tumor rim with the presence of a necrotic core [14]. Initial imaging exams aim to determine the location of the lesion for treatment/biopsy/resection planning, to evaluate mass effect on the brain, and characterizing tumor location, vascularity, mass effect, peritumoral edema, and proximity to areas of potential functional significance [15,16]. Advanced MRI imaging techniques provide additional information on the tumor such as cellularity, invasiveness, mitotic activity, angiogenesis, and necrosis [17]. MRI is also important for characterizing early recurrences but this task is often difficult as recurrences have similar radiologic features than treatment-associated changes [14,18]. However, conventional MRI of GBM suffers from important limitations. First, standard sequences hardly distinguish neoplastic from non-neoplastic tissues [19]. High-grade primary brain tumors, intracranial metastases, abscess, or inflammation induce BBB disturbances and appear as contrast-enhancing lesions on Gd-enhanced T₁-weighted images. Secondly, conventional MRI poorly differentiates low-grade from high-grade gliomas meaning that biopsies and histological studies are required to establish a definitive diagnosis [16,19]. Finally, GBM cells extents are not always detectable by modern neuroimaging meaning that MRI often fails to delineate the tumor margins [19,20]. Indeed, Yamahara et al. showed that invasive tumor cells can be found from 6 to 14 mm beyond the enhancing area in high-grade GBM [10].

1.2. Current Treatments

Standard of care therapy for GBM is represented by surgical resection of the accessible tumor (without causing neurological damage) followed by chemoradiation. This consists in radiotherapy and concomitant chemotherapy with Temozolomide (TMZ), carmustine (BCNU) or other cytotoxic agents (Fig. 2) [21]. Some clinical factors have been associated to better prognosis such as younger age, lack of motor and language deficit, mutations in biological markers (e.g. O⁶-methylguanine-

DNA methyltransferase (MGMT) promotor methylation, isocitrate dehydrogenase-1 mutations), increased extent of resection and minimal residual tumor volume, tumor location near neurogenic niches and not adjacent to the lateral ventricles [22–25]. However, despite the increase on GBM knowledge and therapeutic advance, only 8% of GBM treated patients reaches the long-term survival status of 2.5 years and very few survive over this period [26].

The early and distant dissemination of malignant cells renders GBM a surgically incurable neoplasm. Indeed, 35% of newly diagnosed GBM patients cannot be considered for surgery while the remaining ones can receive a complete or partial resection, depending on the extension and the location of the tumor [27,28]. There are several techniques that can be used to obtain a safe maximal tumor reduction, such as awake craniotomy, neuronavigation and image-guided surgery, intraoperative MRI, laser interstitial thermal therapy or fluorescence-guided surgery [16]. The selection of the safest and appropriate method depends on tumor location, characteristics and size, and the clinical and neurological conditions of the patient before the surgery [16]. Studies have shown that significantly longer survival times are observed in patients who undertake aggressive resection surgery (<98% of tumor volume resected) and also if recurrences often develop, surgery has a critical role in the management of patients [27,29]. Actually, it relieves symptoms resulting from mass effect, reduces the number of cells requiring treatment and often removes the hypoxic core of the tumor that is relatively resistant to radiation and inaccessible to chemotherapy [6]. Moreover, it allows an accurate diagnosis and provides adequate tissue for histological and molecular tumor characterization [6].

As the chemoradiation can have an impact on the wound healing process, GBM patients generally follow the standard treatment regimen once the surgical wound has healed (several weeks) [30]. This regimen consists in 6 weeks of external beam radiation 5 times a week (fractionated focal irradiation in daily fractions of 2 Gy for a total of 60 Gy) in the area around the original tumor plus oral TMZ daily (75 mg/m² of body-surface area per day). After the end of radiation, patients will receive TMZ daily (150 to 200 mg/m²) for 5 days every 4 weeks for six cycles [21].

After this “gold standard” therapy, most patients develop GBM recurrence within two years of their original diagnosis [31]. For patients progressing after prior chemotherapy, there is no established chemotherapy regimen available and patients are best treated within investigational clinical protocols [21]. Chemotherapy with procarbazine, lomustine, vincristine (PCV) or single agent nitrosourea (BCNU or lomustine) may achieve similar tumor control rates compared to TMZ

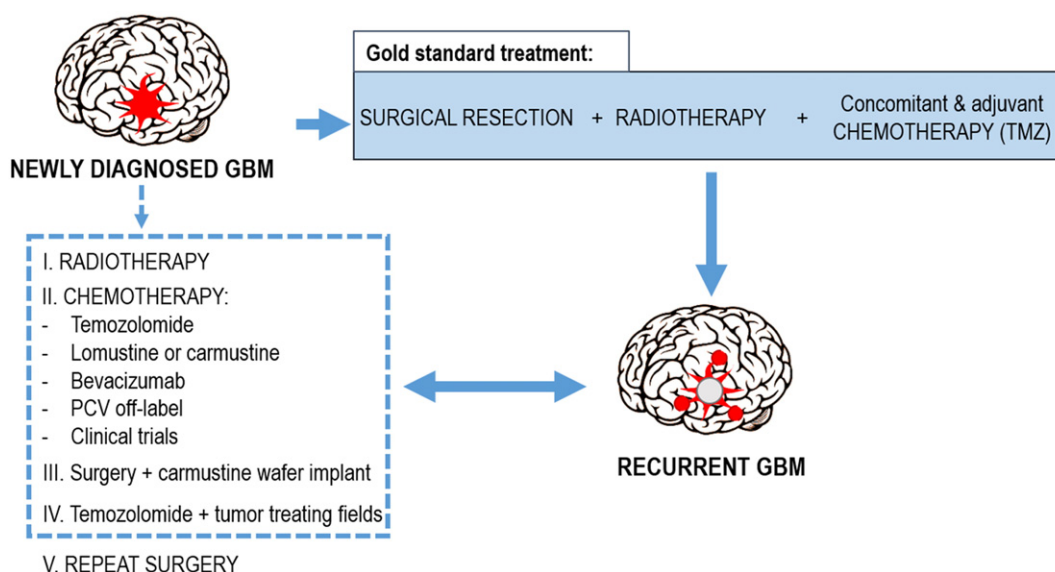


Fig. 2. Schematic representation of GBM treatment strategies. TMZ: temozolomide; PCV: procarbazine, lomustine, vincristine.

[21,32]. However, alkylating agents are subject by the same chemoresistance pathways as TMZ as their mechanism of action is similar, therefore their effectiveness is often limited [33]. Their chemoresistance is mediated by different mechanisms including DNA repair pathways, deregulation of apoptosis regulating genes or tumor cells overexpression of proteins such Galectin-1 or Epidermal Growth Factor Receptor [34,35].

In 2009 the US Food and Drug administration (FDA) approved the humanized monoclonal antibody bevacizumab for the first-line treatment of recurrent GBM patients. Its use, alone or in combination with irinotecan [36,37], has shown the improvement of the progression-free survival and maintenance of the quality of life and performance status in these patients. However, its impact on the overall survival time is controversial [38–40]. The application of a medical device on the patient's skull that deliver low-intensity, alternating electric fields – called tumor treating fields – is also approved by the FDA as adjuvant treatment for newly diagnosed and recurrent patients [41]. A second surgery could be considered, mostly if the recurrent tumor exerts an acute mass effect. Eventually, BCNU wafers can be implanted in the resection cavity (see Section 1.3). Re-irradiation is also considered, especially for small tumors [21]. Many clinical trials have terminated or are currently on-going using chemotherapeutical salvage approaches aiming at target different biological or molecular pathways (e.g. TMZ-resistance mechanisms inhibition, anti-angiogenic agents) [42].

Despite the efforts of the scientific community in order to increase the long-term benefits of GBM therapy, at the moment this tumor remains incurable. In many cases the clinical end point for GBM is to stabilize the disease, to slightly extend the life of the patients and to improve their quality of life in the latest phases of the disease [43]. The improvement of the treatment to reach an actual cure and a long-term survival is limited by several obstacles. The location of the tumor in the brain does not always allow a complete resection of all tumor cells, firstly because of their invasiveness and secondly because broadening the resection area could lead to neurological deficit (motor, sensory, cognitive) and loss of functional brain tissue. On the other side, increasing the perimeter or intensity of the radiation could lead to harmful and unacceptable side effects. For what concerns the chemotherapy, the presence of the BBB, the intrinsic and acquired chemoresistance of GBM cells and the formation of recurrences close to the resection borders limit the achievement of effective treatments. New, specific and more effective drugs and/or multi-drug synergistic approaches that allow to target different tumorigenic pathways need to be discovered in order to reach the goal of eradicating GBM. Also, more efficient drug delivery strategies able to achieve the drug release at optimal concentrations over a sustained period of time and able to suppress tumor growth need to be used against GBM. In this sense, the use of nanomedicines and/or local delivery strategies could be promising approaches for the treatment of GBM.

1.3. Local delivery

Brain is a soft tissue characterized by a unique microenvironment maintained by internal and external mechanisms of defense (skull and vertebral column, meninges, cerebrospinal fluid (CSF), CNS barriers) [2]. Many strategies have been developed to circumvent the CNS barriers and reach therapeutic concentrations of chemotherapeutic drugs in brain tumors. Among them, small lipophilic drugs have been used to passively pass the BBB, while active compounds have been modified or incorporated into nanocarriers in order to reach the brain parenchyma by passive targeting or active targeting of the BBB endothelial cells [44]. Others have tried to modify the BBB permeability or used focused ultrasounds to transiently open the BBB for drug delivery [45].

Among the strategies that have been adopted in the last two decades, there is the local delivery of chemotherapeutic drugs in the tumor resection cavity. Local drug delivery, using implantable or injectable systems with sustained drug release characteristics, aims at

preventing the growth of cancer cells that cannot be resected by surgery [46]. GBM cells are highly infiltrative throughout the brain but they do not disseminate *via* the lymphatic system meaning that they are unable to metastases outside the CNS [5]. In >90% of cases the formation of recurrences appears in the resection margins or within several cm of the resection cavity [47]. For these reasons the use of local delivery strategies that increase the drug concentrations at the tumor site avoiding systemic side effects without interacting and/or interfering with the CNS barriers and without modifying the drug chemical structure and pharmacological properties is a promising strategy for the treatment of GBM.

Direct injection of chemotherapeutics into the tumor resection cavity, surrounding brain parenchyma and/or into the ventricle *via* repeated needle-based injection or catheter implants connected to a reservoir was the earliest strategy used for GBM local drug delivery. This method is simple and can be easily repeated, large volumes of drugs can be injected with minimal systemic toxicity and can be adapted for continued delivery of chemotherapeutics [25]. However, the depth of distribution of the drug from the injection site is often very limited (<3 mm) and repeated surgeries are needed, leading to an increased risk of local side effects (e.g. intracranial hemorrhage, infections). Another approach that has been widely studied for the local treatment of GBM is the convection-enhanced delivery. This consists in direct continuous infusion of an agent in the brain parenchyma using a micro-catheter connected to a pumping device. This device is able to create a pressure gradient that allows the drug to distribute further in the brain tissue compared to the method previously described (2–3 cm) [25,48]. However, its reservoir needs to be continually refilled and the drug distribution depends on the infusion parameters (volume, rate and duration of infusion), the device design and the drug characteristics. Moreover, neurotoxicity can be induced by the infusate backflow in the catheter or by the leakage of the therapeutic agent out of the brain parenchyma into the cerebrospinal fluid [25,49,50].

Another approach is the craniotomy-based drug delivery. This consists in the use of drug-impregnated gels, nanoparticles or polymeric-based delivery systems (such as films, disks, rods or wafers) that can be implanted or injected in the resection cavity and are able to guarantee a sustained release of the drug in the surrounding brain tissue by degradation (if biodegradable) or diffusion (if non-biodegradable) [51].

The most-successful drug delivery implant, and the only one approved by the FDA for the treatment of newly-diagnosed and recurrent GBM, is the Gliadel® wafer. This is a biodegradable co-polymer formed of 1,3-bis-(p-carboxyphenoxy)propane (pCPP) and sebacic acid (SA) in a 20:80 ratio (polifeprosan 20) impregnated with the chemotherapeutic drug BCNU [52]. Polifeprosan 20 is able to protect BCNU from degradation and release it over time. The recommended dose of BCNU is 61.6 mg, represented by 8 wafers (7.7 mg BCNU each) that are implanted intracranially to fill the resection cavity. Prolonged overall survival was observed with Gliadel® compared to placebo-treated patients, and low systemic toxicities were observed (gastrointestinal disorders, asthenia, fever and depression). On the other side, serious local side effects include seizures, intracranial hypertension, meningitis, cerebral edema, impaired neurosurgical wound healing, wafer migration [53–55]. Gliadel® wafers release the drug in approximately three weeks, but *in vivo* studies in mammalian models showed that the majority of the drug release takes place in the first 5–7 days [53]. For what concerns the drug penetration depth, in different animal models, high concentrations of drug were observed adjacent to the polifeprosan 20 implants (3–6 mm from the polymer/tissue interface during the first 7 days, 2–3 mm for the next two weeks) while low drug concentrations were observed in distant regions of the brain [56–58].

Since only one third of GBM patients are responsive to alkylating agents [59] and Gliadel® wafers show some inconvenients (poor drug diffusion and fast drug release, one-drug system, implant dislodgement, big resection cavity size needed), several groups tried to improve the efficacy of polymer-mediated implants for the controlled release of other

Table 1

Examples of polymeric implants developed for intracranial implantation and tested for the local treatment of GBM in preclinical and clinical studies.

Local delivery system	Drug	Clinical stage	Reference
pCPP:SA wafer	BCNU	FDA approved	[54]
	PTX	Preclinical	[61]
	Mitoxantrone	Preclinical	[60]
	DOX	Preclinical	[63]
	Camptothecin	Preclinical	[62]
	Minocycline	Preclinical	[64]
6-Carboxylcellulose plates	Riluzole + memantine	Preclinical	[65]
	Cisplatin	Pilot study	[69]
Open cell polylactic acid solution	Cisplatin	Preclinical	[84]
Caprolactone-glycolide polymer beads	Rapamycin	Preclinical	[66]
Drug-PLGA implant	DOX	Preclinical	[67]
Liquid crystalline cubic phases	PTX + carboplatin	Pilot study	[68]
EVAc polymer	Camptothecin	Preclinical	[70]
PLGA wafer	BCNU	Preclinical	[80]
PLGA microassemblies implants	PTX	Preclinical	[71,72,77,79]
	PTX + etanidazole	Preclinical	[78]
	5-FU	Phase II	[81]
	Carboplatin	Preclinical	[85]
	BCNU	Preclinical	[85]
Nanofiber membranes	BCNU + irinotecan + cisplatin	Preclinical	[83]

Abbreviations: pCPP:SA: 1,3-bis-(p-carboxyphenoxy)propane and sebacic acid; EVAc: ethylene-vinyl acetate co-polymer; PLGA: poly(lactic-co-glycolic acid); BCNU: carmustine; PTX: paclitaxel; DOX: doxorubicin.

chemotherapeutic drugs in the GBM resection cavity (Table 1). For example, polyanhydride polymers (pCPP:SA at different ratios) wafers were loaded with paclitaxel (PTX), mitoxantrone, camptothecin, doxorubicin (DOX), minocycline and, more recently, riluzole and memantine. They were safely and effectively delivered intracranially in animal models and their efficacy has been tested in different GBM models [60–65]. Rapamycin was incorporated into biodegradable caprolactone-glycolide polymer beads and tested *in vivo* in combination with radiotherapy for the local treatment of GBM [66]. Manome et al. developed and tested an implantable drug-conjugated device of DOX-PLGA [67] while Von Eckardstein et al. developed a PTX/carboplatin liquid crystalline cubic phases and implanted it in the surgical resection cavity of GBM patients in a pilot study that showed its feasibility and safety. Another pilot study has been realized on GBM patients using 6-carboxylcellulose plates loaded with cisplatin, however the clinical benefit of these two last approaches remain to be examined in further clinical studies [68,69]. A controlled-release ethylene-vinyl acetate co-polymer (EVAc) has been loaded with camptothecin and showed a controlled release over at least 21 days. Due to its low solubility, the efficacy of camptothecin in a GBM model was greatly enhanced by intracerebral implantation using this polymeric delivery system [70]. On the other side, a wide range of active compounds (both lipophilic and hydrophilic) has been incorporated in degradable polymeric microspheres and extensively studied for the local treatment of GBM. In particular, promising preclinical studies were obtained using microspheres encapsulated with PTX, 5-Fluorouracil (5-FU), Carboplatin or BCNU following direct intracranial injection (peritumorally, intratumorally or in the tumor resection cavity) or pressed into disks or wafers for implantation in the resection cavity [71–76]. PTX has also been loaded in PLGA microfiber disks, sheets, foams or wafers with different form and geometry alone or in combination with Etanidazole, obtaining good pharmacokinetic and diffusion profiles and promising preliminary efficacy results *in vivo* [77–80]. The 5-FU encapsulated PLGA microspheres injected locally with concomitant radiotherapy reached good results in phase I and II clinical trials. However, the median overall survival between groups

was not sufficiently different as to show a significant result [81,82]. Other chemotherapeutic polymer-based systems have also been developed to be injected as solutions peritumorally or in the resection cavity or to be placed on top of the cerebral cortex [80,83–87].

Many interesting reviews have been recently published related to many different aspects of GBM management, challenges and future options [25,27,44,45,73,88–91]. Here, we will focus on a relatively novel approach that, we believe, holds great potential: the hydrogels, conceived as chemotherapeutic drugs reservoirs and delivery platforms for the local treatment of GBM.

1.4. *In vivo* rodent models for GBM

Different GBM rodent models have been developed to evaluate the *in vivo* efficacy of new treatment options, depending on the histologic markers and growth characteristics requested from the model (ex. invasiveness). Indeed, allotopic as well as orthotopic GBM using both xenograft and non-immunogenic models are well described in the literature. The complete description of different GBM rodent models (ex. U251, U87MG, GL261, C6, 9L, CNS-1 glioma models) is beyond the scope of this paper but for more details about the biology of the different cell lines used, an excellent review can be found here [92]. To have a more clinically-relevant model, some authors have also developed and validated tumor resection models of orthotopic GBM in rodents. For example, Akbar et al. were the first ones to perform a C6-green fluorescent protein (GFP) glioma complete resection after craniotomy in rats. They used a dissecting fluorescence microscope able to detect the tumor and guide a suction tip that allows a precise microsurgical resection of the tumor. Thrombin-soaked gel foam pledgets were used to obtain hemostasis whereas a collagen matrix device was used to repair the dura window. To reconstruct the cranial window, impermeable sheet of titanium meshes were fitted in the skull and adhered with super glue as shown in Fig. 3 [93]. This method was also reproduced in nude rats by Denbo et al. and modified by Kauer et al. to develop an efficient GBM subtotal resection model in nude mice [94,95]. More recently, Sweeney et al. validated an imageable surgical GBM resection model in rats. They used an operating microscope to develop a macroscopic surgical plane along the brain/tumor interface. They removed the tumor with a microdissector and achieved hemostasis using hand-held electrocautery pen with a fine needle tip. Finally, they repaired the cranial defect with a sterile circular glass microscope coverslips fixed with cyanoacrylate glue. This modified cranial window technique allows, simultaneously, the separation of the resection cavity from the overlying tissues and the post-operative bioluminescence imaging (BLI) to follow-up the tumor recurrences formation [96]. Another group isolated and removed the GBM tumor by inserting an aspirator in the brain at specific coordinates for 5 s. This technique, which is easier to perform, seems less effective. Indeed, no difference was observed in the survival of untreated rats compared with those treated with the surgical resection (18 days *versus* 18.5 days) meaning that the tumor was not completely and/or efficiently resected [97].

2. Hydrogels for the treatment of GBM

2.1. Hydrogels

Hydrogels are three-dimensional (3D) polymeric and hydrophilic networks able to imbibe large amounts of water or biological fluid without the dissolution of the polymer due to their hydrophilic but crosslinked structure. Hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media [98]. Hydrogels are used for numerous applications in the medical and pharmaceutical field, for example as membranes for biosensors, materials for contact lenses or artificial skin, linings for artificial hearts. Moreover, they are used for 3D cell culture and as drug delivery devices [98–100].

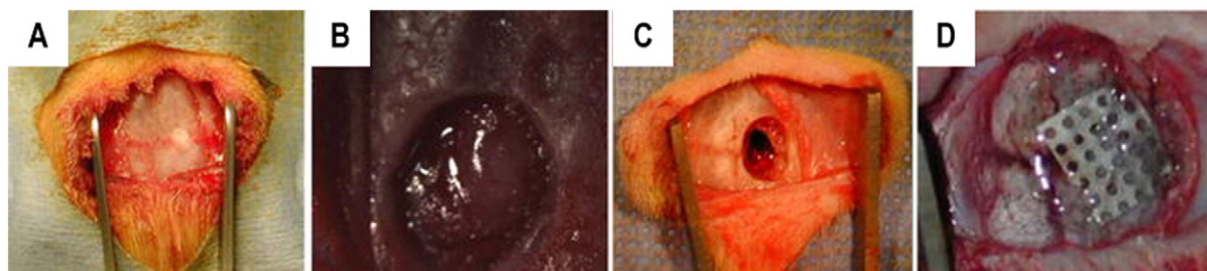


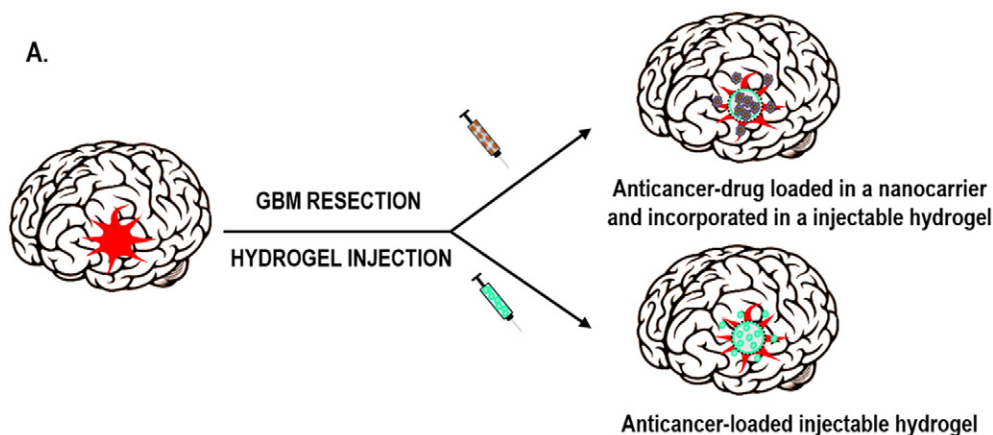
Fig. 3. Intracranial resection glioma model. (A) Burr hole at the time of tumor inoculation shown lateral to bregma and closed with bone wax after stereotactic implantation of C6-GFP glioma cells. (B) Plain color photograph of craniotomy at the time of resection immediately surrounding the burr hole demonstrated in (A). (C) Tumor resected through dural window after craniotomy leaving behind the resection cavity and intact margins of dura. (D) DUREPAIR® processed collagen covering the dural defect and TiMesh® covering the cranial defect. Adapted with permission from [93].

Hydrogels also emerged as excellent candidates for controlled release, bioadhesive and/or targeted drug delivery as they are able to encapsulate biomacromolecules including proteins and DNA as well as hydrophilic or hydrophobic drugs [101]. Hydrogel-based drug delivery systems can be used for oral, rectal, ocular, epidermal, and subcutaneous applications [98]. A key point in the success of hydrogels development is the *in situ* gelation. This can be achieved by UV polymerization, introducing non-reversible covalent bonds, or *via* self-assembly by either reversible interactions or non-reversible chemical reactions. The gelation can also be time-dependent or be triggered by specific stimulus (e.g. pH, temperature, light, etc.) [102].

In the field of GBM, hydrogels have been used i) as mimicking platforms in 3D *in vitro* tumor microenvironment models to study the tumor cells biology, motility, migration and angiogenesis behavior [2, 103,104]; ii) as tools for preclinical screening to grow *ex vivo* cultures

of GBM and assess their sensitivity to radiation and drugs [105] iii) as anticancer drug delivery systems for the treatment of GBM.

In this review, we will focus on the description of anticancer drug-loaded hydrogels for the treatment of GBM. These systems are directly administered in the brain after a craniotomy *via* intracerebral implantation or intracerebroventricular injection. They can be administered intratumorally or in the surgical resection cavity [44]. In some cases, the drug is directly loaded in the hydrogel matrix while some authors have incorporated anticancer-loaded nanomedicines into the hydrogels, in order to prolong the sustained release of the drug (Fig. 4A). Even if the administration of hydrogels in the GBM resection cavity is very little described in the literature, this route of administration seems very promising due to its clinical relevance. An optimal anticancer-loaded hydrogel for the treatment of GBM should have the characteristics reported in Fig. 4B.



B. IDEAL DRUG-LOADED HYDROGEL FOR THE TREATMENT OF GBM

- Sterile
- Injectable and implantable *in situ*
- Rheological properties close to brain modulus and able to mechanically mime CNS microenvironment
- Adhesion to resection cavity border
- Sustained drug release over one month with initial controlled burst effect
- Biocompatible, non-immunogenic and biodegradable within 6 to 12 months
- Do not allow cell infiltration

Fig. 4. (A) Schematic representation of the use of anticancer-loaded hydrogels for the treatment of GBM; (B) Optimal characteristics that a hydrogel should possess to be efficiently used as treatment for GBM.

Table 2

Non exhaustive list of anticancer-loaded hydrogels developed for intracranial implantation and tested for the local treatment of GBM.

Hydrogel matrix	Active agent	Type of study	Reference
PLGA:plasticizers (40:60)	TMZ	C6 rat glioma resection model	[93]
ReGel™	PTX	Phase 1/2 dose escalation study	[106,107]
PLGA/PEG microparticles	Trichostatin A, etoposide and methotrexate	<i>In vitro</i> studies	[108]
PEG-DMA and water (75:25)	TMZ	U87MG subcutaneous GBM xenograft model	[109]
Lipid nanocapsules	GemC ₁₂	U87MG subcutaneous GBM xenograft model	[110]
Poly(organophosphazene) hydrogel	Irinotecan	U87MG orthotopic GBM xenograft model	[111]
Mebiol™Gel	Free or encapsulated DOX	U87MG subcutaneous GBM xenograft model	[112,113]
	Camptothecin-loaded PLGA microspheres	C6 rat glioma orthotopic and resection models	[97,114]
Polyvinyl alcohol hydrogel with sulfonate groups and 0.6% alginate solution	DOX	9L rat orthotopic glioma model	[115]
Alginate	PTX-loaded PLGA microspheres	U87MG-luc2 orthotopic GBM xenograft model	[116,117]
Vesicular phospholipid gels	Cytarabine	U87MG subcutaneous GBM xenograft model	[118]
Chitosan/β-glycerophosphate hydrogel	Ellagic acid	<i>In vitro</i> studies	[119]
PEG-g-Chitosan hydrogel	T lymphocytes	<i>In vitro</i> studies	[120]
PEG diacrylate-based hydrogel	Peptide-cisplatin prodrug	<i>In vitro</i> studies	[121]
PEG-MMA / PEG-DMA hydrogel	PTX-loaded iron oxide nanoparticles	<i>In vitro</i> studies	[122]
Monomethoxy PEG-PLGA nanocomposite hydrogel	PTX and TMZ	<i>In vitro</i> studies	[123]

PLGA: poly(lactide-co-glycolide); PEG: polyethylene glycol; PEG-DMA: polyethylene glycol dimethacrylate; PEG-MMA: polyethylene glycol methacrylate; TMZ: temozolomide; PTX: paclitaxel; DOX: doxorubicin; GemC₁₂: Lauroyl-gemcitabine.

Hereunder is presented a non-exhaustive examples list of recent developments in the use of hydrogels for the delivery of *anti*-cancer drugs in the treatment of glioblastoma (Table 2).

2.2. PLGA-based hydrogels

Hydrophobic polymeric networks can be constructed with poly(lactic acid) PLA or poly(lactide-co-glycolide) PLGA. PLGA is one of the most successfully used biodegradable polymers because its hydrolysis leads to the formation of lactic acid and glycolic acid. These two monomers are endogenous and easily metabolized by the body via the Krebs cycle, therefore minimal systemic toxicity is associated with the use of PLGA for drug delivery or biomaterial applications. PLGA is approved by the FDA and European Medicine Agency (EMA) in various parenteral drug delivery systems in humans and the polymers are commercially available with different molecular weights and copolymers composition. Nevertheless, PLGA-based hydrogels have limited water absorption capabilities (<5–10 wt.%) [124,125].

A biodegradable gel matrix for the delivery of temozolomide (TMZ) constituted by PLGA:plasticizers (40:60) was developed by Akbar et al. The plasticizers were acetyl triethyl citrate (ATEC) and triethyl citrate (TEC) (30:30). As aforementioned, to test their drug delivery system in a clinically relevant model, this group developed a surgical resection model for intracranial C6-GFP glioma in rats. A significant reduction of tumor load was observed in the 30% TMZ group compared to blank control (94% reduction in tumor load) [93].

OncoGel™ was tested as adjuvant to radiation therapy in an intracranial 9L GBM model, alone or in combination with temozolomide and radiation therapy by Tyler et al. [106,107]. OncoGel™ is a non-Cremophor® EL based formulation of Paclitaxel (PTX) in ReGel™, designed for the local delivery of PTX for the treatment of solid tumors. ReGel™ is a copolymer of PLGA and polyethylene glycol (PEG) and it is an environmentally-sensitive controlled release delivery system. Indeed, ReGel™ is a low viscous solution at temperatures between 2 and 15 °C and become a viscous, water insoluble biodegradable controlled-release gel at body temperature. Its biocompatibility has been extensively demonstrated using different preclinical settings (three animal species, various tissues and administration pathways). OncoGel™ can be injected in the proximity of the tumor (e.g. *via* intralesional injection or placement into the tumor cavity) and offers a controlled release of PTX during 6 weeks maintaining high local concentrations. OncoGel™ has been evaluated in three completed clinical studies in superficially

accessible solid tumors and in combination with radiotherapy in esophageal cancer [126]. An interventional study started in 2007 in order to evaluate the safety and tolerability of this system in the GBM tumor resection cavity (Phase 1/2 dose escalation study of locally-administered OncoGel™ in subjects with recurrent glioma) but it has been terminated for sponsor business decision.

A novel thermosensitive formulation of chemotherapeutic drug-loaded PLGA/PEG microparticles able to form matrices that mold around the resection cavity walls was developed by Rahman et al. These microparticles have the consistency of a free-flowing powder at room temperature but they create a paste when mixed with a saline-based carrier solution. Although the formed matrices cannot be really defined as hydrogels, we believe that this system can be taken into account in this section due to its physico-chemical properties and use. Indeed, the formulation can be injected or pasted at room temperature until it gradually solidifies into a solid, porous matrix at body temperature. The *in vitro* release kinetics of different drugs (Trichostatin A, etoposide and methotrexate) suggest that they could gradually release the active ingredients over time. Moreover, the matrices properties are not affected by irradiation meaning that they could be used in a combination regimen and no *in vitro* cytotoxicity was observed with drug-free matrices [108].

2.3. Photo-polymerizable hydrogels

Photopolymerization is a technique that uses light (visible or ultraviolet; UV) to initiate and propagate a polymerization reaction to form a linear or crosslinked polymer structure. The use of photopolymerization has thus been proposed for the production of biomaterial-based polymer networks for specific biomedical applications (e.g. drug delivery) [127]. In particular, photopolymerized polymer networks can be used in tissue engineering due to their capacity to entrap a wide range of substances and cells [128].

To our knowledge, at the moment, just one paper described a photopolymerizable hydrogel for the delivery of TMZ as a possible local treatment for GBM. This injectable hydrogel consisted in a mixture of PEG dimethacrylate (PEG-DMA) and water (75:25), while 0.5% of Lucirin-TPO® was used as photoinitiator. When this solution was irradiated with a light at 400 nm during 15 s, the hydrogel was rapidly formed (<2 min) and presented a viscous modulus (≈ 10 kPa). The TMZ *in vitro* release kinetics was characterized by a linear burst release of 45% of TMZ during the first 24 h, followed by a logarithmic release of 20%

over the first week. An *in vivo* short-term tolerability study showed that the unloaded hydrogel did not induce apoptosis in mice brains nor increased microglial activation. The anti-tumor efficacy of this hydrogel was evaluated in nude mice on a subcutaneous human GBM model, which showed that the tumor growth of mice treated with the photopolymerized TMZ hydrogel significantly decreased compared to the controls [109].

2.4. Nanomedicine-based hydrogels: lipid nanocapsules

Lipid nanocapsules (LNC) are nanocarriers composed of an oily core of triglycerides surrounded by a shell of surfactants [129]. They have been extensively studied as drug delivery systems for the treatment in GBM thanks to their easy and cost-effective preparation procedure, long-term stability, biocompatibility and high encapsulation rate with different drugs [130]. They have been tested against GBM *via* different administration routes such as subcutaneous, intravenous, and local delivery (e.g. CED). Recently, a specific and innovative hydrogel uniquely formed of LNC and GemC₁₂ has been developed and its use has been suggested as local treatment for GBM (Fig. 5A) [131]. This formulation fulfills with the definition of the hydrogel excepted that no constituent is polymeric. Indeed, the formation of the hydrogel is due to the location of the GemC₁₂ at the oil-water interface of the LNC, which allows the formation of H-bond cross-linkings between the drug moieties and the immobilization of the water phase. Compared to the previous cited hydrogels, the advantage of this system is that the degradation of the gel corresponds to the release of the GemC₁₂-LNC, as no other components (synthetic or natural polymers, gelling agents, external stimuli) are present in the formulation, reducing the risk of side effects [110]. *In vivo*, this system showed to be well tolerated in mice brain in the short-term (one week) and to reduce the tumor growth in a subcutaneous human GBM model compared to the free drug (Fig. 5B) [110].

2.5. Theranostic hydrogels

Hydrogels constitute excellent candidates for theranostic applications. Indeed, the combination of treatments within an imaging platform, could allow to (i) assess noninvasively the biodistribution and target site accumulation of the drug, (ii) control the drug release, (iii) enhance the therapeutic efficacy *via* triggered drug release and (iv) predict the therapeutic response [132]. Extensive research attempts to

monitor the drug delivery to brain tumors using MRI. For this reason, hydrogels containing MRI contrast agents have been developed to monitor the drug response or to improve the tumor delineation before surgical resection [133]. For instance, Kim et al. designed an injectable 'MRI-monitored long-term therapeutic hydrogel' for brain tumors (MLTH) [111,134]. Authors synthesized a thermosensitive/magnetic poly(organophosphazene) hydrogel containing both an anticancer drug (the active metabolite of irinotecan SN-38) and a hydrophobic CoFe₂O₃ magnetic core. Using the MLTH, authors succeeded in delivering SN-38 to rodent U87MG brain tumors. MRI experiments at 7 Tesla allowed distinguishing MLTH-treated and non-treated areas of brain tumor regions. Moreover, the *in vivo* long-term inhibition tendency of tumor growth demonstrated the potential of the MLTH system as MRI-monitored therapeutic agent [111].

Another example of theranostic hydrogel is the pH/temperature sensitive magnetic nanogel containing contrast agents for MR and fluorescence imaging. This nanogel, developed by Jian et al., is intended for systemic use but has the ability to accumulate in the rat brain acidic tumor microenvironment [135]. Indeed, superparamagnetic iron oxide (SPIO) nanoparticles loaded poly(N-isopropylacrilamide-co-acrylic acid) nanogels were conjugated with Cy5.5-lactoferrin for targeting *in vivo* rat C6 glioma tumors. The grafted Cy5.5 fluorophore allowed fluorescence imaging, SPIO allowed the MR detection of nanoparticle accumulation in brain tumors, while the lactoferrin is a ligand of low-density lipoprotein receptor-related protein 1 (LRP-1), which is overexpressed in GBM [136].

2.6. Other types of anticancer drug-loaded hydrogels

Some groups used the thermoreversible gelation polymer (TPG) as a novel drug delivery system for the local treatment of GBM. TPG hydrogel (Mebiol™Gel), which is a gel at body temperature but a solution at room temperature, is composed of polyethylene glycol (PEG) conjugated with the thermoresponsive polymer poly-N-isopropylamide. TPG is biocompatible, non-cytotoxic and completely pathogen-free. Arai et al. evaluated the antitumor activity of TPG loaded with free or encapsulated DOX (in PLGA microspheres or liposomes) in a subcutaneous human GBM xenograft model showing a significant inhibition in tumor growth when the drug is encapsulated [112,113]. Ozeki et al. developed a Camptothecin (CPT)-loaded PLGA microspheres-containing TPG hydrogel and evaluated its therapeutic efficacy (comparison of survival) in a

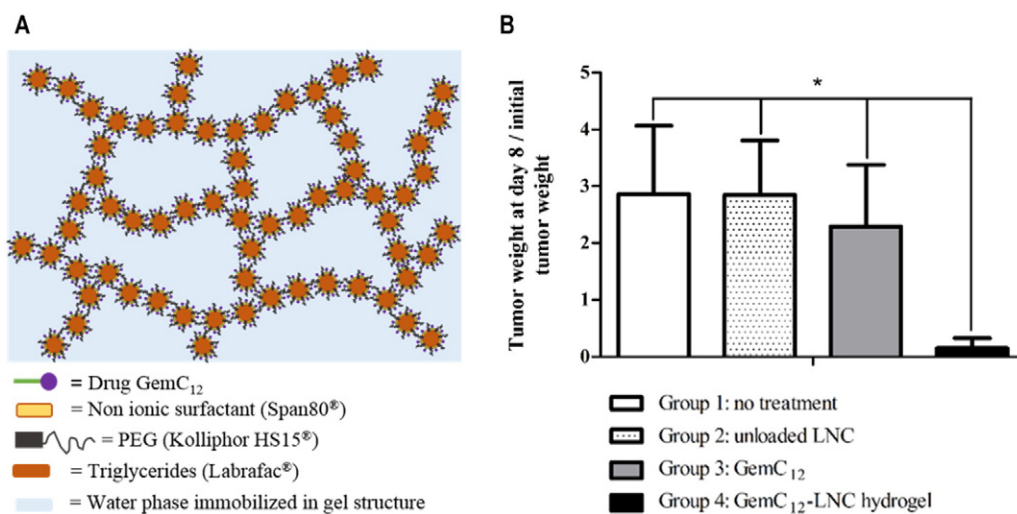


Fig. 5. Lauroyl-gemcitabine loaded lipid nanocapsule hydrogel for the local treatment of Glioblastoma. (A) GemC₁₂-LNC hydrogel schematic representation; (B) Short-term anti-tumor efficacy of GemC₁₂-LNC hydrogel in a subcutaneous GBM model. The dose administered was 19.5 mg of GemC₁₂ per kilogram of body weight. Results are expressed as the tumor weight at day 8/initial tumor weight ratio $\pm$ SEM, * $p < 0.05$. Adapted with permission from [110].

C6 rat glioma model and in a resection model of this tumor [97,114]. The treatment with CPT/PLGA/TGP formulation exhibited significant survival compared with the untreated rats (26 vs 18 days respectively). Similar therapeutic effects were observed in the groups treated with CPT/PLGA/TGP alone and surgical tumor resection plus CPT/PLGA/TGP, but some long-term survivors (>60 days) were observed in this last group, meaning that the combination therapy could be a good strategy for this hydrogel [97].

In another study, DOX eluting beads (CM-BC1) have been evaluated for their safety and efficacy in a 9L glioma model. The bead microspheres were produced from a polyvinyl alcohol (PVA) hydrogel modified with sulfonate groups and mixed with 0.6% alginate solution. This system shows a controlled loading and delivery of DOX. The beads with a low dose of drug (1 mg/ml) showed to be well tolerated *in vivo* long term studies (6 months). *In vivo* efficacy studies of the beads administered alone or in combination with radiotherapy (3×6 Gy whole-brain irradiation) gave significant results compared to the untreated animals in terms of survival (44, 54 vs 26 days respectively). Interestingly, this system could be loaded with other therapeutic agents such as irinotecan, topotecan and mitoxantrone [115].

Alginate has been used to entrap PLGA-PTX microspheres in a solid hydrogel matrix in order to avoid initial burst effect and control the drug release from the microcarriers. This hydrogel has been designed and characterized, tested *in vitro* for its release pharmacokinetics and cytotoxicity and *in vivo* in a subcutaneous tumor study showing promising results. Moreover, using an intracranial human GBM xenograft model this hydrogel showed to significantly inhibit tumor growth and the drug penetrates up to 5 mm from the implant site until 42 days post implantation [116,117].

Vesicular phospholipid gels were loaded with cytarabine and characterized as local delivery depots for GBM treatment [118,137]. This gel has showed high stability to autoclave, responding to one of the main requisites of hydrogels for brain cancer use: the sterility [137]. Then, it has shown to be able to release the drug *in vivo* for at least 28 days with a good drug bio-distribution profile and penetration depth after intracerebral injection. Moreover, the efficacy of this system has been tested in a human subcutaneous GBM model showing a good tumor-suppression compared to the free drug [118].

Another study developed a body temperature gelling chitosan/ β -glycerophosphate hydrogel loaded with ellagic acid. Its biocompatibility and *anti*-tumor effect was tested *in vitro* on GBM cell lines (U87MG and C6 cells) to suggest its use as GBM treatment option [119]. A thermoreversible PEG-g-Chitosan hydrogel could serve as depot for the delivery of T lymphocytes for localized GBM immunotherapy, as suggested by Tsao et al. When implanted intratumorally, or in the resection cavity, the released T cells could come into contact with GBM cells and selectively kill them [120]. Another approach for the local treatment of GBM is the use of a PEG diacrylate-based hydrogel complexed with a peptide-cisplatin prodrug. Here, the linking peptide can be selectively cleaved by the matrix metalloproteases (MMPs), which are highly expressed in GBM cells, releasing the active drug from the hydrogel in a controlled manner. When administered locally, this system is able to deliver a higher dose of the drug selectively to the most invasive portion of the tumor, which is where the MMPs are located [121]. Alternatively, PEG methyl ether methacrylate (PEG-MMA)/PEG dimethacrylate (PEG-DMA) magnetic hydrogel containing iron oxide nanoparticles loaded with PTX was synthesized and tested *in vitro* on M059 K GBM cells as a proof of concept for its use as hyperthermia local treatment [122]. Xu et al. developed a PTX and TMZ-loaded polymer monomethoxy PEG-PLGA nanocomposite under the form of a thermosensitive gel. This gel presents optimal gelation and rheological properties for a local application in the brain and possesses much higher growth-inhibiting effect and apoptosis-inducing rate in U87 and C6 cells compared to the controls [123]. However, the *in vivo* tolerability, biocompatibility and *anti*-tumor efficacy studies using established GBM animal models still need to be performed for these last systems. Moreover, a

tunable diblock copolypeptide hydrogel and has been developed for the delivery of hydrophobic compounds and studied for local application in restricted sites of the CNS. Also if its application has not been tested specifically for the local treatment of GBM (nor *in vitro* or *in vivo*), its ability to incorporate TMZ could suggest its use for this purpose [138].

3. Clinical perspectives and opinion

The last few years have witnessed an extraordinary expansion in drug delivery research for cancer therapy. Indeed, when conventional chemotherapeutic agents fail to show clinical relevance in the treatment of tumors, due to their toxicity or chemoresistance, the research on local delivery emerges as a good alternative. The rationale of the use of the local delivery strategies in the treatment of GBM has been highlighted by the FDA approval of Gliadel® and subsequently, as illustrated in this review, by the development of many hydrogels for this purpose. However, the clinical application of Gliadel® showed poor and controversial advantages compared to the standard chemotherapy (no significant increase in the median survival time of patients, many adverse effects) and no other local treatments have been approved since its entrance in the market. We consider that we need to understand why this very promising strategy did not work as expected and why the number of clinical trials performed on hydrogel-based drug delivery systems for the treatment of GBM is so limited (Fig. 6). Hereunder, we list the main factors that could allow the development of an efficient hydrogel for the local treatment of GBM.

3.1. The anti-cancer drug

It is possible that one of the reasons of the fail of the Gliadel® wafers consists in the choice of the drug. Although carmustine has shown to be effective on GBM cells, the first line treatment of GBM is TMZ. Efforts should may be focused on the local delivery of TMZ and how to avoid its chemoresistance. Nevertheless, the particular physico-chemical properties of TMZ lead researchers to focus on other molecules which are not always in full compliance with clinical recommendations. Interestingly, as alternatives to TMZ, some researchers developed systems for the delivery of anticancer drugs showing radiosensitizing properties such as Gemcitabine, PTX, 5-FU etc. As the combination of local chemotherapy with systemic chemotherapy and/or radiation may improve the therapeutic efficacy by targeting complementary cancer-based cellular mechanisms, the rationale of the use of multiple chemotherapeutic drugs with different mechanisms of action and/or radiosensitizing agents is high.

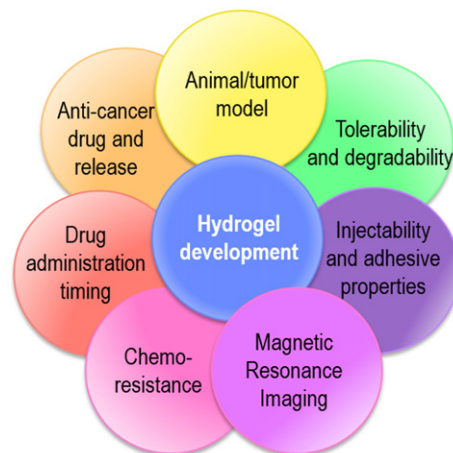


Fig. 6. Schematic representation of the main issues to overcome for the development of anticancer drug-loaded hydrogels for the treatment of GBM.

3.2. The sustained release of the drug

In the development of many drug delivery systems, the sustained release of the drug is a major concern. Indeed, when working with these systems for a CNS application it is important to take into account all the parameters that might influence the drug release kinetics, diffusion rate and penetration depth. These depend, for example, on: (i) the polymer and/or nanocarrier characteristics (type and nature, concentration, molecular weight or size, organic/water ratio, presence of stabilizers); (ii) type of technique used for the preparation and morphology of the resulting inner and external structure; (iii) drug characteristics (chemical structure, concentration, and type of interaction with the polymer); (iv) tolerability and biocompatibility to the brain tissue; (v) brain tissue conditions post-surgery, as they can influence the drug diffusion behavior (CSF flow, BBB leakage, enhanced interstitial fluid convection). Some computational simulations have been proposed to increase the effectiveness of drug-loaded gels by predicting factors that can influence the drug release rate and its delivery to the tumor (e.g. [139]).

3.3. The drug administration timing

As radiation has an impact on the wound healing process, GBM patients' needs to wait for several weeks after the surgical resection before starting the TMZ + radiation regimen [30]. Many studies have suggested that a longer time between surgery and radiotherapy correlates with an increase in local recurrences (as the residual tumor cells have time to proliferate). For this reason, the application of a hydrogel directly in the resection cavity after surgery reduces the risk of recurrences formation in this period of time. Considering this, the expected release kinetics from hydrogels is around one month, in order to allow the resection wound to heal but killing the residual infiltrative GBM cells at the same time.

3.4. The induction of chemoresistance

In cancer treatment, sustained release of drugs means that cancer cells may be exposed to suboptimal doses of drugs for long periods of time. Is it possible, in that case, to induce a drug resistance of cancer cells? It is true that, *in vitro*, periodic exposure of GBM cells to escalating doses of drugs such as TMZ produces a chemoresistant phenotypes [140]. However, we believe that *in vivo*, in the specific case of drug

delivery systems inserted in a tumor resection cavity, the answer to this question could be likely “no” for two reasons: (i) as the majority of the tumor cells has been resected with the surgery, the maintenance of lethal drug concentrations in the resection perimeter in the period immediately after the surgical resection (24–48 h) due to the burst effect will allow to kill rapidly the remaining cancer cells and prevent local tumor recurrence. After this, not only the healthy cells at the resection border but also in other parts of the brain would be subject to a low-concentration long-term exposure. If the drug is selective against the tumor cells, no local side effects should be observed and no chemoresistance should be induced, as the tumor cells do not remain in constant contact with the drug for long periods of time; (ii) the hydrogel protects the brain tissue (and so the healthy cells) from the direct contact with the drug, which is slowly released by the system. When the drug is released and penetrates in the brain tissue, it comes in contact with the residual infiltrating cells and kills them, avoiding the development of multifocal gliomas.

3.5. Magnetic resonance imaging

Magnetic resonance imaging play a central role in the detection, diagnosis and even in the development of new theranostic agents for GBM. Indeed, it allows to simultaneously measure and localize the primary tumor or its recurrences and evaluate its response to the treatments.

3.6. The tolerability

Even if the benefit-/risk balance is less important for this fatal type of cancers, the tolerability of hydrogels is a major concern in the development of drug delivery systems. Studies of brain tolerability of different systems have shown that an accurate and methodic work need to be performed in this sense to guarantee the safety of new products [141]. First of all, the drug delivery system and the drug inside should be sterilized. Then, the inflammatory reaction produced both by the mechanical trauma (GBM resection, implantation of the system, increase of intracranial pressure due to injection of the hydrogel and swelling) and the brain tissue contact with the delivery system should be analyzed in the short and long-term (acute and chronic tissue response).

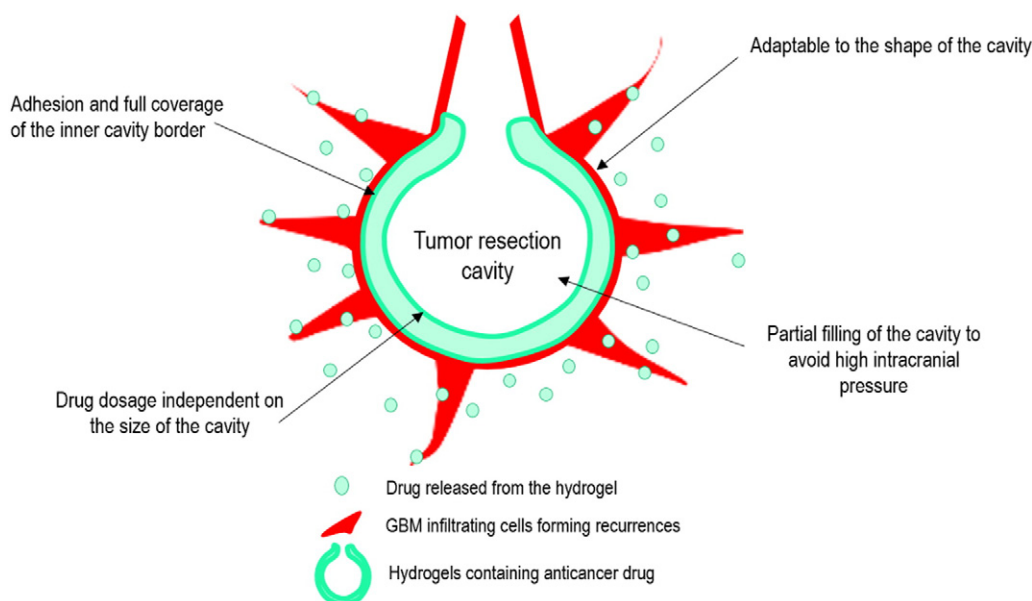


Fig. 7. Schematic representation of the clinical issues related to the development of injectable and adhesive hydrogels for the treatment of GBM.

3.7. The degradability

Systems delivered in the resection cavity should be degradable in order to avoid the repetition of brain surgeries. The degradation time limit acceptable for these systems should be between 6 months and one year.

3.8. The injectability and adhesion properties

One of the limitations of Gliadel® is that the wafers drug loading is limited and their structure is rigid. Therefore, to have an optimal BCNU dosage, the cavity needed to be tall enough to host 8 wafers, and needed to be carefully placed inside the cavity. However, as their size and shape are not adapted to the anatomy of the resection cavity and their structure is rigid, they often suffered from dislodgement. To overcome these limitations, hydrogels are a very good alternative. Indeed, they can be loaded with a sufficient amount of drug, injected in the cavity and adapt to its shape. If the gel has a good adherence profile, it will stick to the cavity borders, increasing its contact surface (Fig. 7). However, with injectable hydrogels there will always be parts of the cavity that will not benefit from the direct contact with the system. Probably, an appropriate device able to enter in the resection cavity and, by rotation, spray the gel all around the cavity borders could be an optimal option.

3.9. Animal/tumor models

Rodent models of GBM have been available for decades, however, very few new therapies have successfully translated into the clinic. Some of them are better to evaluate the *anti*-tumor efficacy of the new drugs while others are more appropriate for drug penetration and biotolerability studies. An ideal model should recapitulate the key histopathological, genetic and imaging features encountered in GBM's aggressive growth as well as being a reproducible and reliable model [92]. Researchers should take into account different parameters: (i) The size/species of the animal. For sure, the evaluation of the efficacy of a hydrogel in a resection cavity is easier in a rat model compared to a mouse one; (ii) Human GBM models are of course closer to the clinic situation. Nevertheless, this kind of models needs the use of athymic nude mice or rats. These models are then not adequate for the study of the tumor-immune microenvironment. (iii) The ability of the invasion of diffusion of GBM cells from the tumor mass to the brain parenchyma differs depending on the tumor GBM model. However, the brain and cavity sizes intra-species, the amount of drug that can be implanted as well as the differences in tumor growth pathways and timings make us wonder if we really have models strong enough to predict the effects of hydrogels in humans. In conclusion, the only certain thing is that new therapies should be validated on different GBM models showing different characteristics.

4. Conclusions

In conclusion, there is still a lot of research to do in this field in order to obtain a local administered system both efficacious and well tolerated, but we believe that hydrogels loaded with one or multiple chemotherapeutic drugs offer a drug delivery platform with many advantages for the treatment of GBM. In particular, they would allow (i) to fill the gap time between the GBM resection and the administration of chemotherapy and/or radiotherapy; (ii) to sustainably release the drug over a long period of time; (iii) to allow the diffusion from the resection cavity borders to the brain parenchyma in order to kill the infiltrating GBM cells responsible of recurrences.

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