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Review article

Biocompatible copolymer formulations to treat glioblastoma multiforme

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ABSTRACT

The treatment for glioblastoma multiforme (GBM) has not changed for more than 20 years while the prognosis for the patients is still poor and most of them survive less than 1 year after diagnosis. The standard of care for GBM is comprised of surgical resection followed by radiotherapy and oral chemotherapy with temozolomide. The placement of carmustine wafers in the brain after tumour removal is added in cases of recurrent glioma. Significant research is underway to improve the GBM therapy outcome and patient quality of life. Biomaterials are in the front line of the research focus for new treatment options. Specially, biocompatible polymers have been proposed in hydrogel-based formulations aiming at injectable and localized therapies. These formulations can comprise many different pharmacological agents such as chemotherapeutic drugs, nanoparticles, cells, nucleic acids, and diagnostic agents. In this manuscript, we review the most recent formulations developed and tested both *in vitro* and *in vivo* using different types of hydrogels. Firstly, we describe three common types of thermo-responsive polymers addressing the advantages and drawbacks of their formulations. Then, we focus on formulations specifically developed for GBM treatment.

Statement of significance

Biomaterial formulations have been designed for the localized treatment of glioblastoma multiforme generating nanocomposite delivery systems aiming at better treatment outcomes. Hydrogels and polymers combined with drugs, nanoparticles, nucleic acids, immune cells or contrast agents originate formulations with different properties. The development of such formulations is constantly evolving and contributing to increase treatment options for glioblastoma patients in the future.

1. Advances in the treatment of GBM: from standard chemotherapy to carmustine wafers and liquid polymers

Malignant gliomas are tumours that originate in glial cells and represent the most common type of primary brain tumours. Among them, glioblastoma multiforme (GBM) is the most prominent and aggressive brain tumour in adults, classified as grade IV tumour by WHO [1] (World Health Organization) and presenting a poor patient survival prognosis of less than one year. This prognosis is not only due to the severity of the disease but also to the hurdles in the treatment imposed by the localization and biological characteristics of this type of tumour [2].

Systemic treatments of neurological disorders are very challenging due to the presence of the blood brain barrier (BBB) which impairs the penetration of drugs into the brain. One of the mechanisms responsible for the selective permeability of the BBB is the presence of protein transporters (also known as efflux pumps) that move several molecules, including hydrophobic drugs and chemotherapeutics, across the cell membranes and outside the brain in an ATP-dependent manner [3,4]. Therefore, both the permeability of the BBB and the existence of efflux pumps are responsible for the difficulty to achieve an effective concentration of drug in the brain [5]. Moreover, systemic chemotherapy imposes many severe side effects to patients. For this reason, the standard of care for GBM, composed of surgical resection followed by radiotherapy combined with oral chemotherapy with temozolomide (TMZ) [6], still needs to be refined to provide better outcomes associated with improvement of quality of life, with less systemic side effects. In

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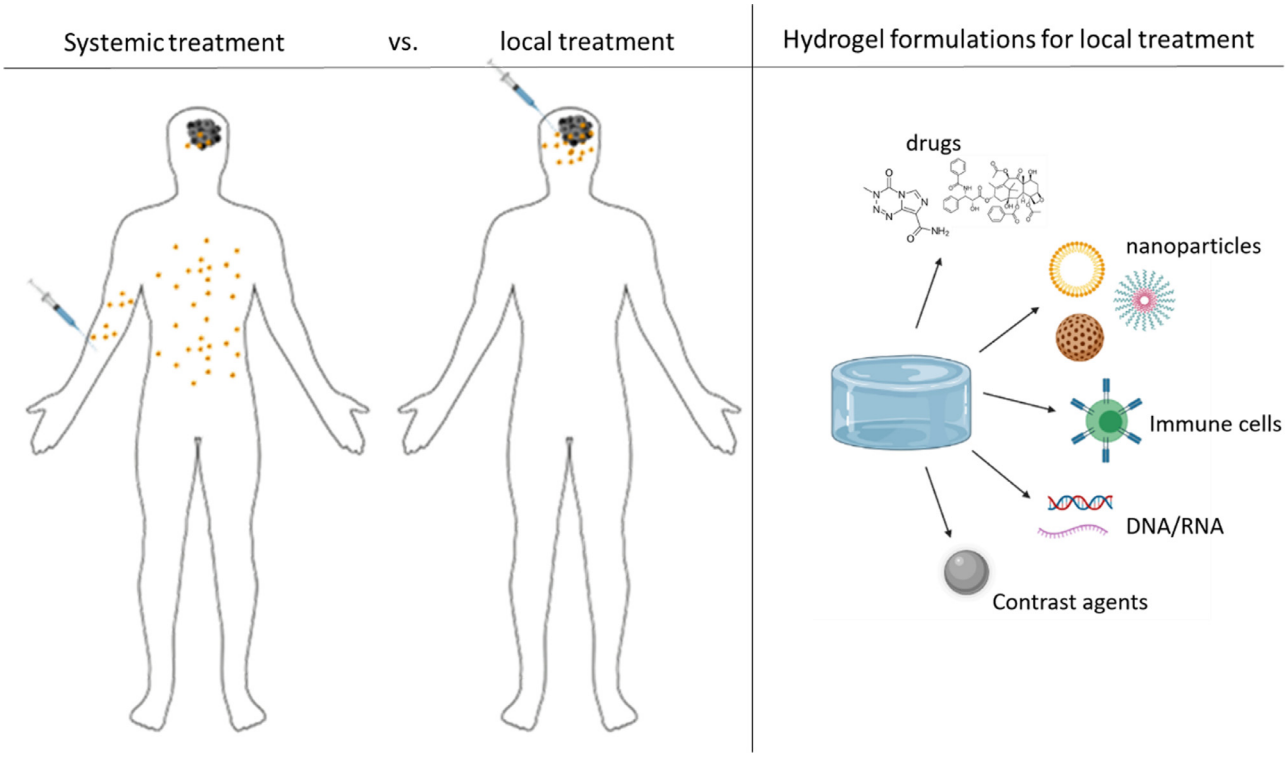

Fig. 1. Systemic *vs* local treatment of GBM.

addition, episodes of recurrence and resistance to the chemotherapeutic drug are very common [7]. Therefore, to avoid all these problems and increase treatment safety and efficacy, new drug delivery strategies have been proposed against GBM [8].

In 1996, the first localized treatment for GBM was approved with the commercial name Gliadel [9]. This drug delivery device consists of wafers of Polifeprosan 20 embedded with carmustine (bis-chloroethylnitrosourea, BCNU). Its development intended to improve the treatment outcome and decrease the side effects experienced by patients following systemic chemotherapy. Since then, in some cases the surgical resection of the tumour is followed by the local implantation of these wafers. However, the use of Gliadel presents some challenges related to the resection area, which is not effectively covered by the wafers, and the low amount of drug that can diffuse into the brain reaching the cancer cells [10,11]. For this reason, other local delivery approaches to the brain are under investigation to improve treatment outcomes (Fig. 1).

In addition to the likelihood to decrease side effects, local therapy seems convenient for GBM since most of the patients will undergo surgery as one of the first steps of their treatment. In this scenario, formulations consisting of copolymers forming hydrogels or polymeric nanoparticles (hydrogel nanoparticles, nanospheres, nanocapsules, microspheres and micelles), and nanocarriers including metal and inorganic nanoparticles, liposomes, polymeric micelles and microspheres are gaining more attention as possible alternatives in the development of a localized treatment [12]. It is important to highlight that, besides the use of hydrogels and nanocarriers, other approaches have been investigated in the field. They include methods to improve the delivery of drugs such as the convection enhanced delivery (CED) [13], and the intra-nasal delivery of formulations [14], as one example of strategy to overcome the BBB.

The incorporation of drugs into nanoparticles protects them from degradation while can also provide targeted and controlled release of drugs depending on the characteristics of the nanodelivery system. Moreover, the combination of nanoparticles into hydrogels adds the possibility to combine drugs and to control the delivery of not only drugs, but also nanoparticles from the hydrogels.

The use of hydrogels in healthcare devices has many technical advantages. Firstly, many of them allow the solubilisation of hydrophobic drugs. For instance, as demonstrated by Zentner et al., the incorporation of paclitaxel (PTX), a highly hydrophobic drug, in a biodegradable triblock copolymer significantly increased drug solubility and stability, providing a sustained release during approximately 6 weeks [15]. Secondly, encapsulation in hydrogels protects drugs from phagocytic cells and unfavourable environmental factors that can promote drug degradation. Thus, hydrogel formulations can increase the half-life of chemotherapeutic drugs while reducing the frequency of drug administration and improving patient compliance [16,17].

To obtain a suitable hydrogel formulation for drug delivery, some material characteristics are important, specifically viscoelasticity and swelling capacity, encapsulation stability and minimal toxicity. Other characteristics that may be tuned to improve the formulation include the response to stimulus, passive and active targeting, controlled and sustained drug release [18].

Many polymers are available to be used in local treatment formulations as hydrogels. Some examples of hydrogel formulations developed for GBM therapy include poly-NIPAM (poly-N-isopropylacrylamide) [19], alginates [20,21], chitosan [22] and PEI (polyethylenimine) [23]. Moreover, block copolymers derived from the combination of two or more polymers can also be used as hydrogel formulations. Copolymer design considering characteristics such as hydrophobic/hydrophilic ratio, block length and molecular weight, influences gel formation, release profiles, degradation and biocompatibility, which can be tuned based on hydrogel composition [24]. In addition, the sol-gel transition of hydrogels, for example, can be controlled by changing the block lengths, the polymers ratio and concentration [25]. Therefore, these design possibilities

make copolymer hydrogels interesting materials for the development of *in situ* delivery formulations.

2. Thermo-responsive block copolymers

Hydrogels can be classified according to their structure, mechanical properties, method of preparation and responsiveness to an external stimulus. The ones in the latter category are known as stimuli-sensitive hydrogels and they can be responsive to pH, light, redox environment, magnetic field, and temperature among others [26]. Specifically, thermo-responsive hydrogels are formed in response to changes in the local temperature and can be used to deliver drugs and/or diagnostic agents in a controlled and sustained manner.

The hydrogel chemical and physical properties influence the loading and delivery of compounds. Different mechanisms are responsible for the delivery of a drug from a hydrogel and the most important is passive diffusion. However, there are different models to predict release profiles from hydrogels, and these models are classified in three categories: (1) diffusion-controlled, (2) swelling-controlled, and (3) chemically controlled. Moreover, the structure and consequently the release characteristics of a hydrogel can be tuned by monomer composition, different degrees of crosslinking and the intensity of external stimuli [27]. Therefore, the possibility to build different formulations to achieve the desired delivery profile has stimulated an increased research in this area, especially for biomedical applications [28].

For drug delivery purposes, the use of *in situ* forming hydrogels is favourable since this type of gels offers a number of benefits; namely, they can be administered in liquid state in the absence of a trigger mechanism, and can rapidly undergo gelation after injection [29]. In this way, implantation by invasive surgical procedures is avoided. Furthermore, the incorporation of different types of drugs to the polymer solution can be achieved by simple physical mixing [30]. Thermosensitive hydrogels that can undergo an *in situ* gelation process at body temperature have received significant attention for the design of local depots for anticancer drugs due to their ease of preparation and administration. In this review, we summarize some types of block copolymers (Fig. 2) and different formulations that were built focused on the treatment of GBM.

2.1. PCLA-PEG-PCLA

Copolymers of PCLA-PEG-PCLA (poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(ε-caprolactone-co-lactide)) have been used as drug delivery systems in different biomedical formulations since they are biodegradable and biocompatible, and therefore suitable for *in vivo* use. This triblock copolymer has thermosensitive properties, which contributes to tuning the loading and release of small molecules. Besides that, the rheological properties of this hydrogel can be improved to make them more suitable for drug delivery applications.

Several studies investigated and compared the PCLA-PEG-PCLA properties after chemical modification on the polymer chain by different capping systems and through blending of the modified systems. Xun et al. synthetized a peptide functionalized hydrogel (KRGDKK- PCLA-PEG-PCLA- KRGDKK) that kept the thermosensitive properties while displaying improved rheological characteristics [31]. The peptide added to the structure contributed with hydrogen bonds to the formation of stronger gels at lower temperatures, and this characteristic is important to facilitate the use of the hydrogels for implantation. Another important improvement was the sustained and more prolonged release (over 1 month *vs.* 10 days) of doxorubicin (DOX), which was also attributed to the presence of the peptide moiety on the hydrogel structure.

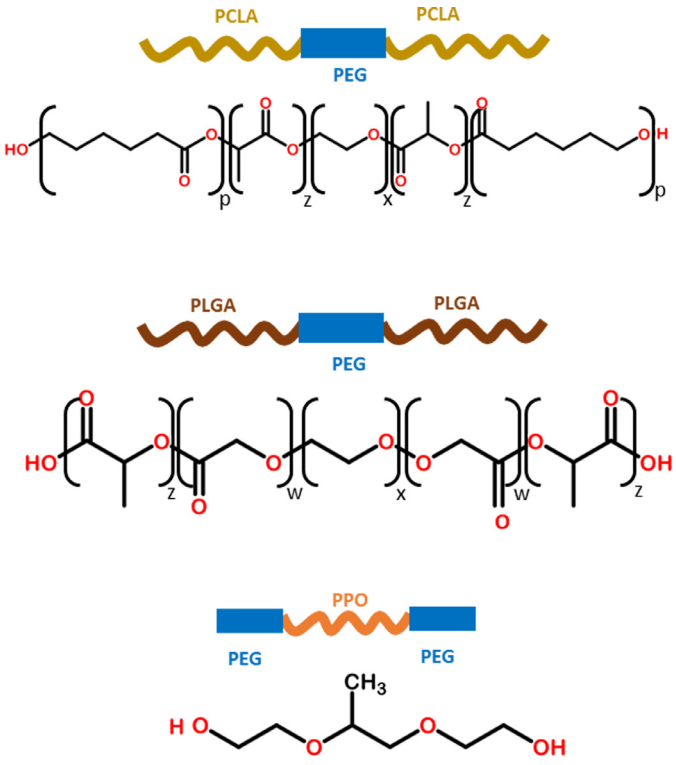

Fig. 2. Representative thermosensitive tri-block copolymers and their chemical structures. These polymers were used in different formulations for the local treatment of GBM due to their temperature sensitivity and favourable chemical characteristics to incorporate drugs and nanocomposites.

Petit et al. studied PCLA-PEG-PCLA aqueous solution of uncapped (hydroxyl-terminated), hexanoyl [32], acetyl and propionyl-capped [33] copolymers regarding the gelation and degradation behaviours. The degradation and sol-gel transition are very important characteristics for the material applicability and, in these studies, the possibility to modify and mix thermosensitive triblock copolymers to build the desired drug delivery formulation was shown. They demonstrated that the hydrogel degradation occurred through dissolution rather than hydrolysis over 280 days depending on the hydrogel composition, with the most hydrophobic and semi-crystalline copolymers having a slower dissolution. Moreover, the composition of the block copolymers provides the possibility to tune the temperature for sol-gel transition.

Besides the thermosensitive property, this copolymer can be functionalized to present other stimuli-responsive characteristics such as pH sensitivity [34,35]. Shim et al. were able to both tune the pH sensitivity and the biodegradability of PCLA-PEG-PCLA polymers by adding pH-sensitive sulfamethazine oligomers (SMOs) to either end of the block polymer. The constructed polymer undergoes sol-gel transition at physiological conditions (pH 7.4 and 37°C). This modified hydrogel was then tested for the ability to incorporate and deliver PTX to tumour-bearing mice through subcutaneous injections [36]. The effect of drug incorporation on the hydrogel properties was studied and it was shown that regardless of the PTX amount loaded into the hydrogel, the sustained drug release was maintained, although the gelation temperature shifted to lower temperatures in highly loaded gels (10 mg/ml PTX). These characteristics are important regarding the practical aspect of administration in a clinical setting.

Therefore, the formulation containing 5 mg/ml PTX that maintained the sustained drug release and the sol-gel transition similar to the unloaded gel was considered the most suitable for an injectable treatment. *In vivo* evaluation of the formulations in

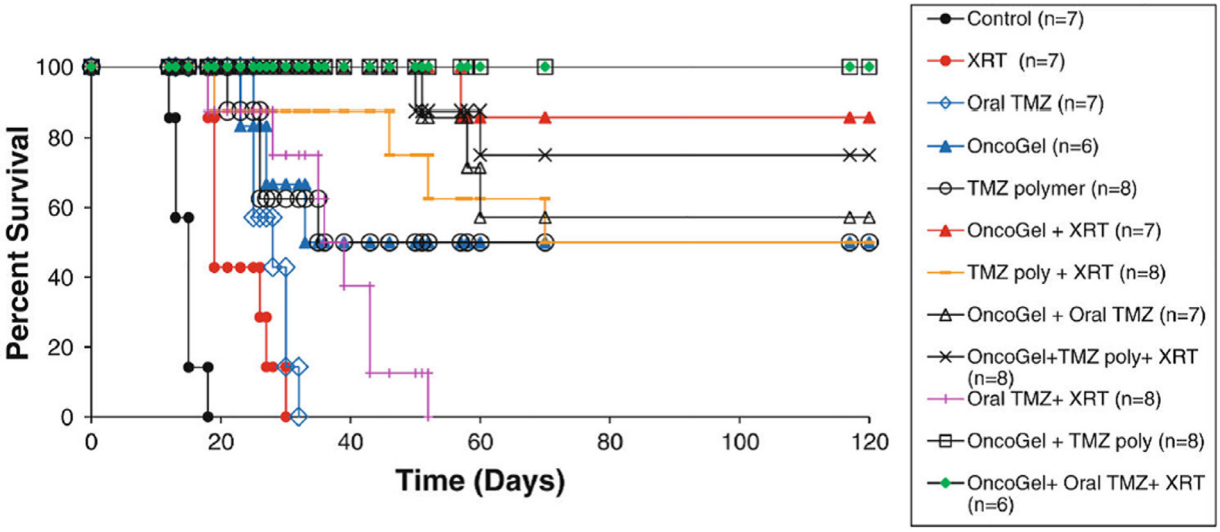

Fig. 3. Oncogel *vs* TMZ/radiation therapies. Efficacy of intracranial TMZ or oral TMZ in combination with OncoGel with or without radiation on Fischer-344 rats. Median survivals: Control- 15 days; Radiation Day 5- 19 days; Oral TMZ on Days 5-9- 28 days; OncoGel 6.3 on Day 0- 33 days; TMZ polymer Day 5 – 35 days; OncoGel 6.3 +radiation- 85% long term survivors; TMZ polymer +radiation- 70 days; OncoGel 6.3 +oral TMZ 57% long-term survivors; OncoGel 6.3+TMZpolymer +radiation – 75% long-term survivors; Oral TMZ +radiation – 35 days; OncoGel 6.3 + TMZ polymer and OncoGel 6.3 + oral TMZ + radiation – 100% long-term survivors. Reproduced with permission.[40] 2013, Springer Nature.

C57BL/6 male mice bearing tumours in the left flank showed a significant decrease in tumour volume as compared to the control group (saline – 17 cm3) and PTX hydrogel treated groups (smaller than 7 cm3) after 2 weeks of treatment. This result was confirmed by TUNEL analysis of apoptotic cells, which were very prominent in PTX hydrogel treatment but not in the control group.

2.2. PLGA-PEG-PLGA

Poly(D,L-lactic-co-glycolic acid)-(polyethylene glycol)-poly(D,L-lactic-co-glycolic acid) (PLGA-PEG-PLGA) polymers have been extensively studied as drug delivery systems for poorly water soluble drugs and drug combination therapy. In this class of polymers, the triblock thermosensitive copolymer named ReGel^{TM} is one of the most commonly used. This hydrogel has been studied as a potential drug delivery vehicle against various solid tumours such as breast cancer, oesophageal cancer, spinal cancer, peritoneal and ovarian cancer, and high-grade gliomas [37].

Regarding GBM, ReGel^{TM} was loaded with PTX, originating a commercial product named OncoGel^{TM}, which is in clinical trials [38]. OncoGel^{TM} has been studied as a monotherapy and as combination therapy with TMZ or radiation (Fig. 3). For instance, Tyler *et al.* treated mice intracranially implanted with 9L gliosarcoma cells with different OncoGel formulations or ReGel (with no drug, as negative control) to investigate the synergistic effects of OncoGel and radiation therapy in comparison with each therapy alone [39]. The results demonstrated that the group treated with OncoGel had extended survival periods compared with the group treated with ReGel. Moreover, the authors demonstrated that the group treated with both OncoGel and radiotherapy had the longest survival period (31 days), improving the results obtained with OncoGel (17 days) or radiotherapy alone (26 days). In another study with 9L gliosarcoma xenografts, Vellimana et al. treated mice with an individual therapy of OncoGel, TMZ (oral therapy or poly (1,3-bis-[p-carboxyphenoxy propane]-co-[sebacic anhydride]) local implant), radiotherapy or combinations of these therapies [40]. It was shown that the combination of OncoGel and TMZ was more effective than the individual treatments. Moreover, the addition of radiotherapy to the combination treatment with oral TMZ significantly improved the therapeutic outcome, as 100% of the animals were alive at the end of the study (120 days). Furthermore, a similar outcome was obtained for the group treated with OncoGel and an intracranial implant of TMZ.

Table 1
Physicochemical characteristics of some Pluronic copolymers [45–47].

Pluronic copolymers	Molecular weight	HLB[a]	CMC (M)[b]
F 127	12,600	22	2.8 × 10-6
P 85	4,600	16	6.5 × 10-5
P 103	4,950	9	6.0 × 10-6
P105	6,500	15	6.2 × 10-6
P 123	5,750	8	4.4 × 10-6

[a] HBL: hydrophilic-lipophilic balance.
[b] CMC: critical micelle concentration.

2.3. Pluronics

Linear Pluronic, also known as poloxamers, are non-ionic amphiphilic triblock copolymers. They are composed of poly(ethylene oxide) and poly(propylene oxide) ordered as an ABA triblock copolymer (PEO-PPO-PEO) with thermo-reversible properties in aqueous solutions. Pluronics can be used as hydrogels in injectable and topical formulations. For instance, a thermogel application of Pluronic copolymers include vaginal formulations to deliver amphotericin B locally taking advantage of the relative *in vivo* stability of this system [41]. They can also form and are most used as micellar systems for drug delivery [42].

Pluronic copolymers can self-assemble into polymeric micelles above their critical micellar temperature (CMT) forming nanodelivery systems. The polymeric micelles formed can incorporate hydrophobic drugs and this is directly dependent on the copolymer composition. The size and composition ratio between the hydrophilic (PEO) and the hydrophobic (PPO) chains influence the solubilisation of different types of drugs as well as their release characteristics [43]. Drug release is also controlled by polymer dissolution rate which can be tuned by the polymer concentration [44]. Some types of poloxamers and their physicochemical characteristics are summarized in Table 1 [45–47].

The encapsulation of drugs in the core of these polymers allows the delivery of hydrophobic drugs to tumour sites while the hydrophilic surface protects the drug from degradation and inactivation. Therefore, hydrophobic drugs, such as genistein, PTX

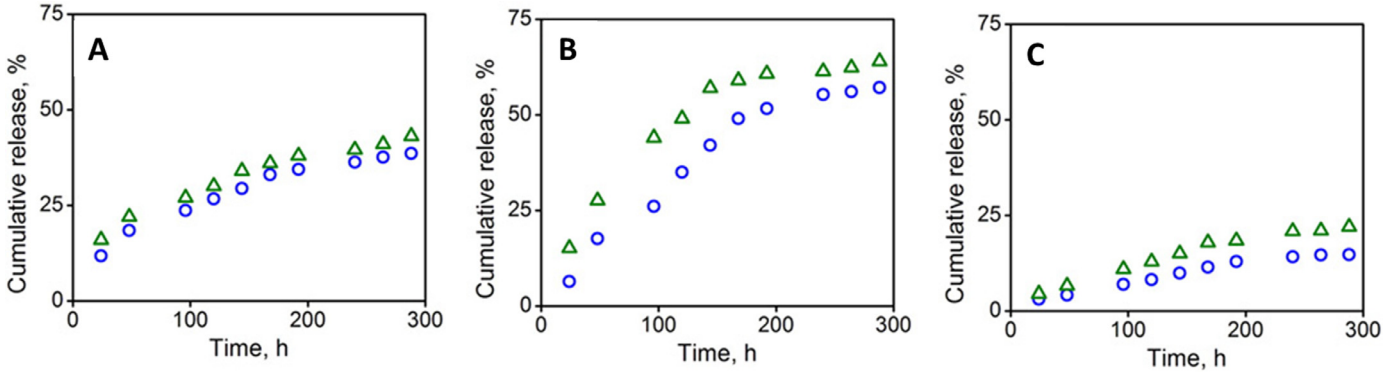

Fig. 4. Pluronic micelles release of hydrophobic drugs. Release profiles of (A) genistein, (B) paclitaxel, and (C) quercetin from 1% (○) P103 and (△) P123 at 37°C. Reproduced with permission.[48] 2017, Elsevier.

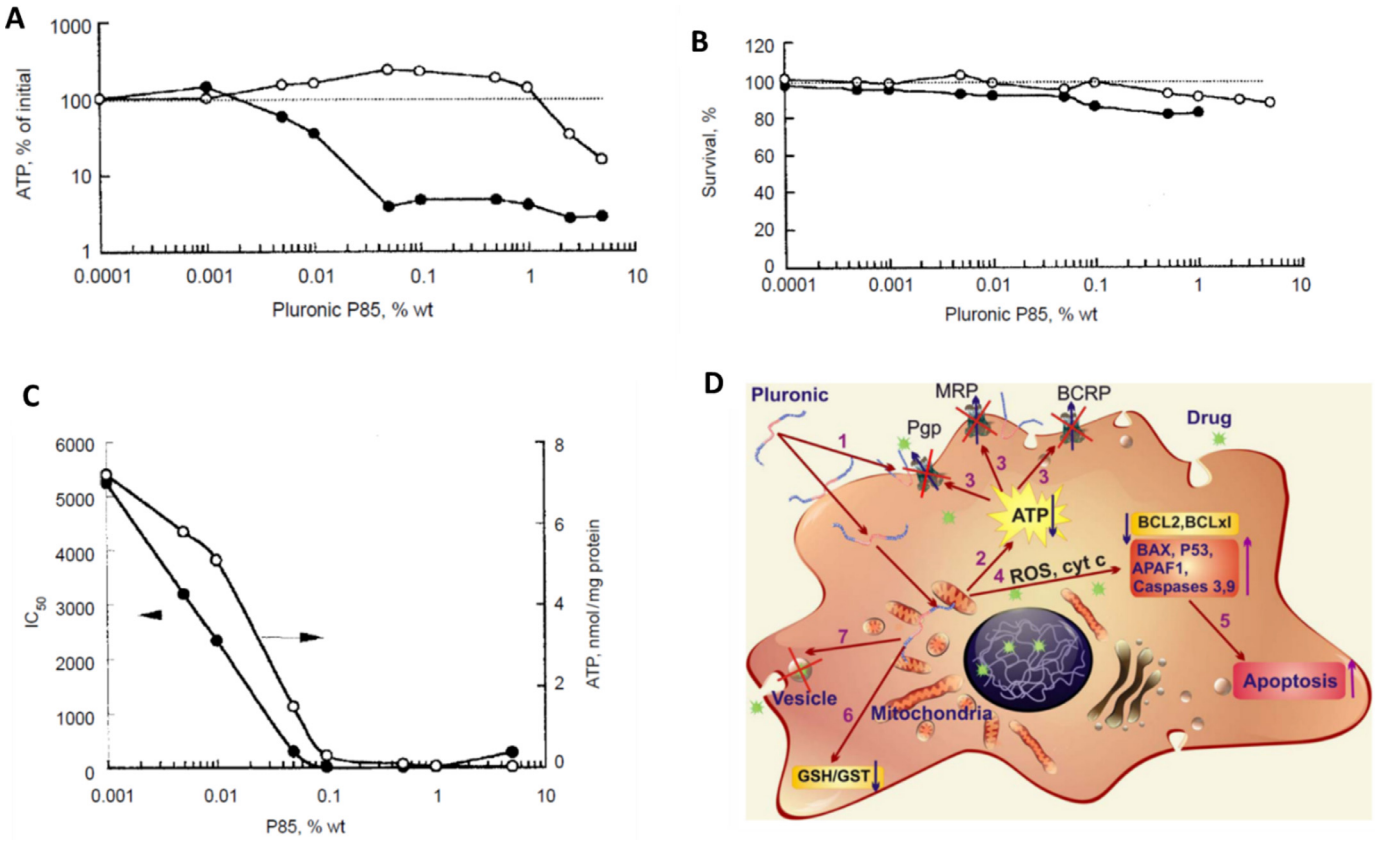

Fig. 5. Pluronic copolymers are non inert drug delivery vehicles. Effects of P85 on (A) intracellular ATP levels; and (B) cell survival in resistant MCF-7/ADR (filled circles) and sensitive MCF-7 (empty circles). (C) Effects of P85 on IC50 of doxorubicin (filled symbols) and ATP intracellular levels (empty symbols) in KBv cells. (D) Schematic on the Pluronic copolymer effects in MDR cells. Reproduced with permission.[50] 2008, Elsevier and [51]. 2001, Springer Nature (Creative Commons copyright).

and quercetin were already incorporated in different Pluronic micelles [48]. The solubility of these drugs was higher in moderately hydrophobic Pluronics P103 and P123, and was favoured by increased temperature and salt concentration. The release of the drugs from the micelles fits a first-order model equation. Therefore, the drugs are released in a sustained manner from the micelles mainly through diffusion (Fig. 4). Pluronics have also been applied as gene delivery systems and it has been proposed that both physical forms of the material, the micellar solution and the gel, can contribute differently to the gene transfection capability of the system [49].

Some studies have shown that Pluronic copolymers are not inert drug delivery vehicles (Fig. 5). The effect of Pluronic administration on several cell processes includes inhibition of efflux transport, activation of glutathione/glutathione S-transferase detox system and drug sequestration on vesicles, among others [50]. For example, Batrakova et al. showed that Pluronic P85 acts as a chemosensitizer agent in multi drug resistant (MDR) cancer cells making them more sensitive to DOX. Using Pgp expressing membranes, they demonstrated that the Pluronic effect is due to both ATP depletion and Pgp ATPase activity inhibition [51]. Importantly, the authors highlight that the drug resistance mechanisms in these cells are coupled with other factors, such as activation of glutathione/glutathione S-transferase detoxification system, that dramatically increases the energy requirements of these cells, also contributing to the Pluronic cytotoxic effects observed.

Besides the ability to take advantage of passive targeting due to their nanometric size, Pluronic polymeric micelles have been engineered as targeted drug delivery systems able to cross the BBB. Niu et al. designed Pluronic P105 micelles with two targeting

moieties, glucose and folic acid, and evaluated the dual-targeting system loaded with DOX (GF-DOX) in brain tumour models [52]. While glucose can increase the BBB penetration through the glucose receptor in the brain, folic acid can target its receptor present in glioma cells. Using an *in vitro* BBB model of murine brain microvascular endothelial cells (BMVECs), they observed a 5 times higher transportation of the glucose modified micelles loaded with DOX (GP-DOX) compared to free DOX, and a decrease in transport when an excess of glucose was present. Moreover, rat C6 glioma cells showed higher uptakes of folic acid modified micelles (FP-DOX) compared to non-modified micelles (P105-DOX) and GP-DOX. The dual-target micelles (GF-DOX) decreased C6 cell viability *in vitro* by more than 80% and, after an intravenous administration in C6 intracranial tumour model, it significantly decreased the tumour volume compared to mice treated with P105-DOX, GP-DOX and FP-DOX (approximately 7.5, 3.5 and 4 times smaller, respectively).

In another example of active targeting with Pluronics, Zhang et al. proposed a folate functionalization of Pluronic P123/F127 mixed micelles (FPF) [53]. The functionalized micelles loaded with paclitaxel (FPF-PTX) showed higher cell uptake of drug compared to the non-functionalized micelles, while also increased the blood circulation time compared to the free drug. Interestingly, the authors observed the co-localization of the uptaken micelles and mitochondria, which are important organelles for both cell metabolism and cell death. Therefore, the co-localization of Pluronic micelles with mitochondria highlights the dual effect of the targeted micelles interfering on cell metabolism and cell death induction through mitochondria sensing and protein release. Finally, in the *in vivo* efficacy test against MDR tumour bearing mice, FPF-PTX showed superior results regarding inhibition of tumour growth likely due to both active targeting and the chemosensitization effect of the Pluronic system. Additionally, Pellosi et al. demonstrated that Pluronics can be used as delivery systems of both drugs and photosensitizer molecules, which normally are very poorly soluble in water [54]. In this report, Pluronic P123/F127 micelles loaded with the isomers mixture of benzoporphyrin derivatives (BPDMA and BPDMB) showed photo-toxicity against HeLa and A549 cells.

3. Hydrogel formulations for localized treatment of GBM

For biomedical applications, hydrogels are being used to develop several formulations with enhanced physicochemical, mechanical and biological properties. These formulations can include not only drugs but also NPs, cells, nucleic acids and diagnostic agents (Table 2). For instance, the combination of two drug delivery platforms, hydrogel and NPs, may prevent the burst release of encapsulated drugs and extend the release period, thus decreasing potential adverse effects and increasing drug bioavailability [55]. Moreover, the incorporation of nanostructures into hydrogel matrices may alter the hydrogel physicochemical properties, providing tailored functionalities and an improved drug delivery efficiency of the composite [56]. A significant advantage of hydrogel formulations and, specifically, thermosensitive hydrogels, is the formation of a localized therapeutic depot that can be implanted after surgical resection of a tumour for prevention of metastasis or recurrence.

Recently, hydrogel research for GBM treatment has been focused on two main areas, hydrogel formulations for treatment and hydrogels for brain tumour cell culture [57]. In the latter approach, hydrogels are being used in the development of *in vitro* models to understand the blood brain barrier [58], to develop test platforms for different therapeutic modalities such as Photothermal Therapy [59] and to establish 3D models of tumours [60,61] using patient derived cells or established cell lines aiming to understand tumour development and drug resistance in a mimic tumour microenvironment.

Regarding the therapeutic application, hydrogels are mainly used as drug delivery systems. Specially, hydrogels are used in drug combination therapies such as the most recent development of an enzyme-responsive hydrogel loaded with TMZ and an MGMT inhibitor (O6-benzylamine) that sensitizes TMZ resistant cells after resection surgery *in vivo,* decreasing recurrence [62]. Besides combination therapies, drug penetration into the brain parenchyma is another concern and hydrogels are being designed to help solve this problem. Wang et al. designed a hydrogel based on a penetrating cyclic peptide covalently linked to two camptothecin drug molecules, which can also encapsulate other drugs for combined therapy [63]. The formulation improves penetration and antitumour effect *in vitro* on spheroids and *in vivo*. Other recent developments include a copolymer formulation that maintains high local concentrations of PTX *in vivo* [64] and a camptothecin-based self-assembling hydrogel [65]. These formulations, applied locally after tumour resection, showed effect on suppressing tumour recurrence and prolonging survival in mice GBM resection models.

3.1. Hydrogels loaded with drugs

One of the earliest studies to investigate the potential of *in situ* forming thermo responsive hydrogels as local depots to deliver a chemotherapeutic drug is the research work by Arai et al. [66] They incorporated DOX in a thermo responsive hydrogel composed of PEG and poly-N-isopropylamide, and the anticancer effect of the formulation was tested in T98 and U87 GBM cell lines as well as in a mice model with a subcutaneous U87 xenograft. The hydrogel triggered significant apoptosis on both cell lines *in vitro* and decreased the tumour weight when locally injected in mice.

In an attempt to develop a novel local drug delivery system, Akbar et al. designed a hydrogel system composed of PLGA with different common plasticizers, to locally deliver TMZ to tumour cells following tumour resection surgery [67]. In the study, U87 cells were implanted in mice, and after 35 days the tumours were surgically removed followed by the hydrogel injection in the resected area. There were no significant differences in adverse effects between control groups and groups treated with TMZ-loaded hydrogels. However, in terms of treatment efficacy, the tumour weight in groups injected with hydrogels decreased up to about 95%.

Another innovative system, based on a lipid nanocapsule hydrogel composed of a triglyceride core surrounded by a shell containing two surfactants (Span 80 and Kolliphor HS15) and the chemotherapeutic agent lauroyl-Gemcitabine (GemC12), was developed to be locally delivered in the treatment of brain tumours [68,69]. The intratumoral injection of the hydrogel formulation was well tolerated in the GBM *in vivo* models used (nude mice and NMRI mice). Furthermore, a significant increase in the median survival of groups treated with the hydrogel (62 days) compared to control groups (no treatment – 35.5 days) was observed as well as a lower rate of tumour recurrence. The group of Chen et al. reported on a different phospholipid based gel system aimed to deliver PTX [70]. The formulation, liquid at room temperature, turns into a gel upon injection into the tumour due to diffusion of the ethanol that is included in the formulation. PTX was delivered in a sustained manner, and the gel was well tolerated and significantly increased the median survival of C6 tumour bearing mice compared to mice receiving no treatment (26.5 days *versus* 15.5 days) or local injections of free drug (18 days).

Erkoc et al. proposed a dual therapy for GBM through the combination of a degradable hydrogel loaded with free TRAIL (tumour necrosis factor α-related apoptosis-inducing ligand) and a TRAIL sensitizer drug, quinacrine [71]. The hydrogel was composed of PEG particles that were sensitive to matrix-metalloproteinases, se-

Table 2
Hydrogel formulations tested for GBM treatment in *in vitro* and *in vivo* models.

Polymer composition	Main features	Drugs/small molecules/genetic material	*In vitro* tested cell line	*In vivo* model	Reference
PCLA-PEG-PCLA end capped with the pH-sensitive sulfamethazine oligomers (SMOs)	The formulations showed a significant decrease in tumour volume *in vivo* comparing to the control group (no treatment)	Paclitaxel	–	C57BL/6 male mice bearing tumours in the left flank	[36]
Oncogel (PLGA -PEG -PLGA)	*In vivo* treatment with OncoGel and radiotherapy increased the survival period (31 days) compared to both treatments alone (OncoGel – 17 days and radiotherapy – 26 days)	Paclitaxel	9L gliosarcoma cells	Female Fischer-344 rats intracranially implanted with 9L gliosarcoma cells	[39]
Oncogel (PLGA -PEG -PLGA)	The combination of OncoGel and TMZ was more effective than the individual treatments.	Paclitaxel/TMZ	–	Female Fischer-344 rats implanted with 9L gliosarcoma cells	[40]
Pluronic P85	Pluronic P85 causes ATP depletion and Pgp ATPase inhibition on cells acting as a chemosensitizer.	Doxorubicin	Multi drug resistant cancer cells (MDR cells)	-	[51]
Pluronic P105	The Pluronic polymeric micelles were modified with two target molecules, glucose and folic acid. Intravenously administered formulation significantly decreased the tumour volume in mice.	Doxorubicin	Murine brain microvascular endothelial cells (BMVECs) and rat C6 glioma cells	Male Institute of Cancer Research (ICR) mice implanted with C6 cells	[52]
Pluronic P123/F127 micelles mixture	The Pluronic micelles were functionalized with folate. The formulation inhibited tumour growth *in vivo*.	Paclitaxel	KBv and KB cells and A-549 cells	Mice subcutaneously implanted with KBv cells on the flank	[53]
PEG and poly-N-isopropylamide	The hydrogel triggered significant apoptosis *in vitro* and decreased the tumour weight when locally injected in mice.	Doxorubicin	T98 and U87MG GBM cell lines	Male BALB/c nude mice model with a subcutaneous U87 grafted tumour	[66]
PLGA with different common plasticizers	The tumour weight in groups injected with drug-loaded hydrogels decreased up to about 95%.	Temozolomide	C6 and U87MG GBM cell lines	Adult PRKDC CB-17 mice model with subcutaneous U-87 cell-grafted tumour and adult Wistar rats with C6 cells intracranial tumour	[67]
Triglyceride core surrounded by a shell containing two surfactants (Span 80 and Kolliphor HS15)	The formulation promoted a significant increase in the median survival of groups treated with the hydrogel (62 days) compared to control groups (no treatment – 35.5 days) and a lower rate of tumour recurrence.	Lauroyl-Gemcitabine (GemC12)	U251, T98G and U87MG glioma cells	Nude mice and NMRI mice	[68, 69]
Phospholipid based gel system	The gel system significantly increased the median survival of C6 tumour bearing mice compared to mice receiving no treatment (26.5 days versus 15.5 days) or local injections of free drug (18 days).	Paclitaxel	-	Male Balb/c mice intracranially implanted with C6 cells	[70]
PEG hydrogel	The PEG particles are surface functionalized with RGDS peptide and it is sensitive to MMP2. The formulation showed synergistic effect between the components and induced apoptosis gene expression *in vitro*.	Quinacrine-loaded particles and free TRAIL	U87MG cells	-	[71]
Alginate gel matrix	The gel matrix entrapped PLGA microspheres had a sustained release of drug (over 60 days) and reduced tumour volume more effectively than Taxol.	Paclitaxel	C6 cells	BALB/c nude male mice subcutaneously injected with C6 cells	[20]

(*continued on next page*)

Table 2 (*continued*)

Polymer composition	Main features	Drugs/small molecules/genetic material	*In vitro* tested cell line	*In vivo* model	Reference
PLGA (Poly Lactic-co-Glycolic Acid)	PLGA nanofiber discs promoted a deeper penetration of drug into the tumour, inhibiting tumour growth *in vivo* more effectively compared to PLGA microspheres.	Paclitaxel	-	BALB/c nude mice intracranially implanted with U87 MG-luc2	[21]
Polyethylene glycol (PEG) and poly-N-isopropylacrylamide	PLGA microspheres were mixed with the thermosensitive hydrogel and the formulation significantly increased the survival period of animals compared with both the hydrogel (placebo) and drug-loaded hydrogel without the microspheres.	Camptothecin and vincristine	-	Mice models bearing C6 glioma tumours (Male Sprague–Dawley rats)	[72],[73]
Poly(N-isopropylamide-co-n-butylmethacrylate) (poly(NIPAAm-co-BMA) and PEG	The formulation containing polymeric microspheres or liposomes loaded with drug had a more sustained release compared to free drug into the gel, and inhibited tumour growth.	Doxorubicin	U87MG, LN229 and G55 cells	Nude mice subcutaneously implanted with U87MG cells	[75]
PEG-dipalmitoyl-phosphatidyl-ethanolamine (m-PEG-DPPE)	Calcium phosphate nanoparticles (NPs) were included in the formulation and provided a sustained drug delivery and significantly increased the survival rate of rats.	Paclitaxel and temozolomide	C6 cells	SPF male Wistar rats bearing C6 gliomas (after resection)	[76]
Poly(ethylene glycol) dimethacrylate and Lucirin-TPO, a photoiniator	Drug loaded PEG-PCLA micelles were incorporated into the hydrogel formulation, which induced higher extent of apoptosis *in vivo* compared to mice treated with systemic TMZ.	Temozolomide	-	Female athymic nude subcutaneously injected with U87MG cells	[77]
Poly(ethylene glycol)-g-chitosan hydrogels	T-lymphocyte cells infiltrated better and retained their cytotoxic activity against U87 cells compared to Matrigel.		U87MG	-	[22]
Nanogel of cholesteryl pullulan (CHP)	The nanogel formulation with a peptide antigen triggered an immune response against syngeneic tumours in mice. The nanogel was already tested in clinical trials.	Peptide antigen	-	Female BALB/c mice subcutaneously injected with the vaccine	[80]
Cationic polymer composed of RGD peptide and Polyethylenimine (PEI) (RGD-PEG-SS-PEI)	The intravenous administration in mice bearing U87 tumours showed an efficient targeting to the brain after analysis of reporter gene systems.	pDNA complexes	U87MG cells	Nude mice intracranially implanted with U87MG cells	[83]
Dexamethasone-conjugated-polyethylenimine (PEI-Dexa)	The combination between the polymer carrier and the gene vector was more effective in reducing the tumour volume compared to the polymer alone (three times less effective) or combined with a non-specific plasmid sequence (twice less effective).	Plasmid carrier for nestin intron 2 (NI2) and erythropoietin enhancer	C6 and U87MG GBM cell lines	Subcutaneous (Balb/cSlc nude mice) and intracranial (male Sprague−Dawley rats) models of GBM	[84]
mPEG-PEI polymers	The formulation promoted higher transfection both *in vitro* (almost 2-fold targeting enhancement in U87 cells) and *in vivo* in nude mice bearing U87 tumours.	Target peptide sequence (retro-inverso CendR peptide (D(RPPREGR))	U87MG cells	Male BALB/c nude mice intracranially implanted with U87MG cells	[23]
Poly(organophosphazene) hydrogel	The hydrogel formulations containing cobalt ferrite nanoparticles generated higher inhibition effects on tumour growth (up to 48% decrease) compared with the chemotherapeutic drug only, and suitable MRI contrast-enhancing effects.	7-ethyl-10-hydroxycamptothecin (SN-38)	NIH3T3 mouse embryo fibroblast cells and U87MG cells	6-week-old female BALB/c-nu mice (U87 ectopic xenograft model and orthotopic brain tumour model)	[85]
Carboxymethyl cellulose (CMC)-grafted poly(N-isopropylacrylamide co-methacrylic acid)	The formulation includes albumin nanoparticles loaded with paclitaxel. The theranostic formulation increases the average *in vivo* survival compared to the control group from 18 to 63 days (MBR-641 models) and from 27 to 69 days (U87 models).	gadolinium/epirucibin /paclitaxel	MBR 614 human brain tumour cell	C57BL/6 and Nu/Nu mice subcutaneously implanted with MBR-614 or U87MG cells	[19]

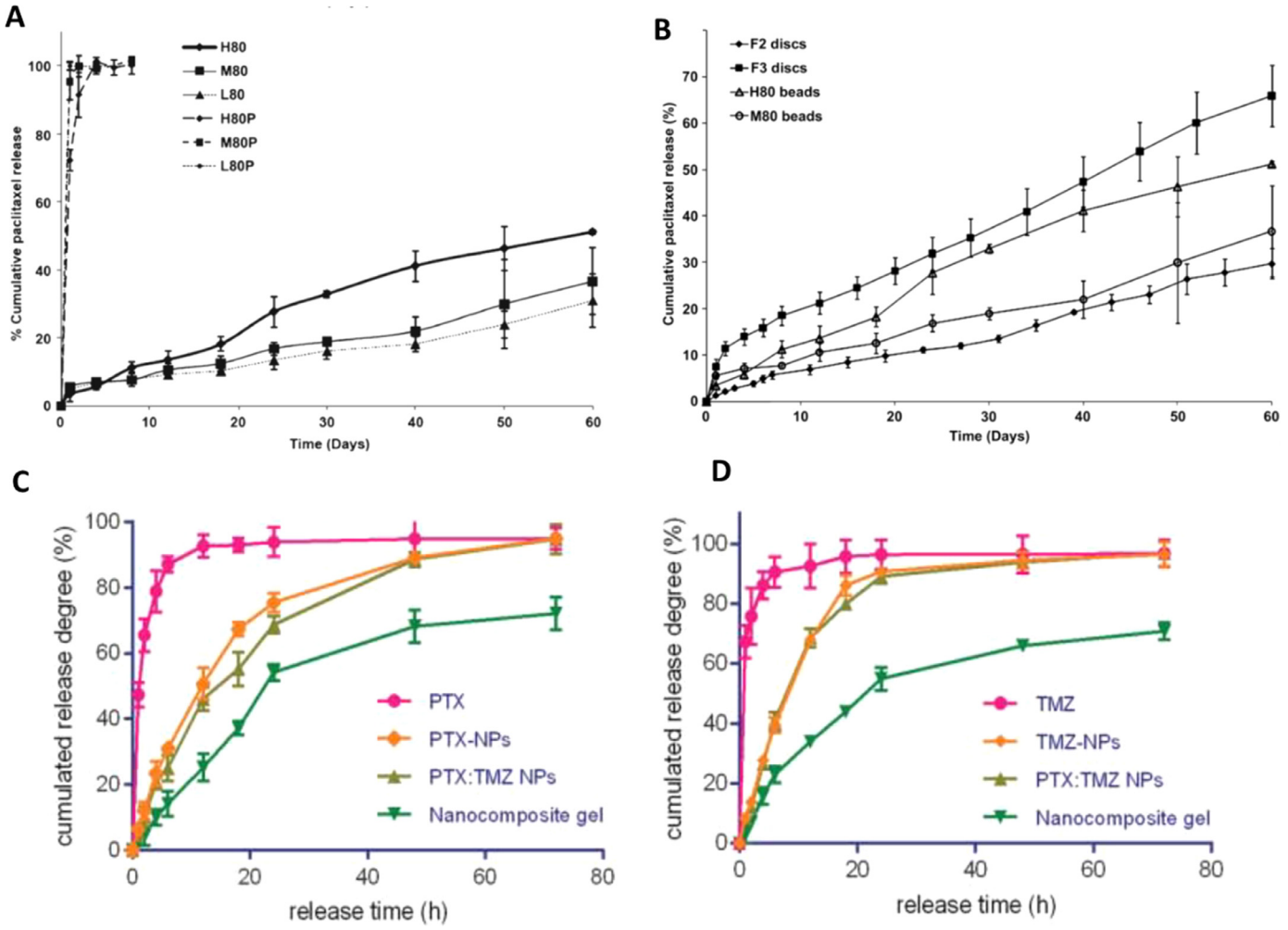

Fig. 6. Nanoparticles loaded into hydrogels provide a more sustained release of drugs over time. (A) *In vitro* release of paclitaxel from different formulations of the alginate beads. 80% (w/w) microsphere-loaded alginate beads and paclitaxel-loaded beads; the prefixes H, M and L refer to the extent of crosslinking in the beads (high, medium and low). H80P, M80P, L80P refer to paclitaxel-loaded beads with equivalent amount of paclitaxel as compared to its microsphere loaded beads. Reproduced with permission.[20] 2009, Springer Nature. (B) *in vitro* release of paclitaxel from 9.1% paclitaxel-loaded F3 discs, 9.1% paclitaxel-loaded F2 discs, H80 and M80 beads [From previous Ref. [20] and Reproduced with permission.[21] 2010, Elsevier. (C) Cumulative release profiles of PTX from PTX NPs, PTX:TMZ NPs and nanocomposite gel (D) Cumulative release profiles of TMZ from TMZ NPs PTX:TMZ NPs and nanocomposite gel. Reproduced with permission.[76] 2017, Taylor & Francis.

creted by the tumour cells, and were functionalized with targeting RGDS peptides. The authors proved the *in vitro* synergistic effect between the quinacrine-loaded PEG particles and TRAIL, also showing that the treatment induces apoptosis specific gene expression in U87 cells.

3.2. Hydrogels loaded with drug-loaded micro/nanoparticles

The properties and applicability of drug-loaded hydrogel formulations can be improved by the combination with different types of particles. The addition of a particulate component opens the possibility to combine different chemotherapeutic agents in the same formulation, and allows the exploitation of additional ways to control and target the drug release (Fig. 6). For instance, Ranganath et al. developed an implant formed by an alginate gel matrix entrapping PTX-loaded PLGA microspheres [20]. The incorporation of microspheres in a gel matrix resulted in formulations with a highly sustained *in vitro* release profile of PTX of more than 60 days at a near-constant rate and with a minimum initial burst. Moreover, when implanted subcutaneously in mice, it reduced the tumour volume more effectively than Taxol, demonstrating its potential as a local chemotherapy for glioma treatment.

The same group compared these PTX-loaded PLGA microspheres entrapped in alginate hydrogel matrices with PTX-loaded PLGA nanofiber discs [21]. At this time, the formulations were intracranially implanted in BALB/c nude mice with glioblastoma xenografts (U87 MG-luc2). They observed that the nanofiber discs formulations had a higher release rate *in vitro* and provided deeper penetration of drug in the tumour, inhibiting tumour growth *in vivo* more effectively compared to the formulations developed previously. This result was attributed to a higher drug concentration at the implant surface. In a typical post-surgical chemotherapy regimen, the initial tumour growth inhibition will be critical to slow down the rate of glioma recurrence, thereby inhibiting migration and invasion into healthy brain tissue. Thus, the authors concluded that these implants could improve the treatment outcome for recurrent GBM.

Using a similar approach, Ozeki et al. designed a system in which PLGA microspheres loaded with camptothecin were mixed with a thermosensitive polymer composed of PEG and poly-*N*-isopropylacrylamide [72]. The *in vivo* assessment using rat models bearing C6 glioma tumours showed that the formulation significantly increased the survival period of animals compared with both the hydrogel (placebo) and drug-loaded hydrogel without the microspheres. It was shown that the formulation with microspheres had higher retention times up to 14 days, which could contribute to enhance therapeutic outcomes. They also showed

that the microsphere-hydrogel composites may act as local depot after tumour resection surgery and different chemotherapeutic drugs can be incorporated into the gel, for example, camptothecin and vincristine, improving the survival period of the *in vivo* model (Male Sprague–Dawley rats bearing C6 intracranial tumours) [73,74].

Another example of hydrogel nano/micro-composites was developed by Arai et al. combining polymeric microspheres or liposomes loaded with DOX with a thermo-reversible gelation polymer (TGP), composed of poly(*N*-isopropylamide-*co*-*n*-butylmethacrylate) (poly(NIPAAm-co-BMA) and PEG [75]. *In vitro* results using U87MG, LN229 and G55 cells confirmed that TGP alone is non-toxic to glioma cell lines and that the DOX released from the TGP, liposomes and spheres retains its biological effect. In subsequent *in vivo* studies, the antitumour effect was evaluated in subcutaneous human glioma xenografts in nude mice. The free DOX entrapped in TGP was released faster than from the TGP combined with DOX-loaded spheres (2.5x times) or liposomes (4.3x times), which presented a more sustained drug release up to 30 days and, therefore, inhibited tumour growth up to 32 and 38 days, respectively.

More recently, Ding et al. developed an injectable thermo responsive hydrogel based on PEG-dipalmitoyl-phosphatidyl-ethanolamine (m-PEG-DPPE) and calcium phosphate NPs that provided a sustained and local delivery of both PTX and TMZ [76]. The formulation was able to inhibit C6 cell proliferation *in vitro* and significantly increased the survival rate of rats bearing C6 gliomas, which were injected with the hydrogel formulation after tumour resection.

Photopolymerisation methods have also been used to design hydrogel-nanostructure composites for the treatment of GBM. In the research by Fourniols et al., PEG Poly(ε-caprolactone-co-trimethylene carbonate) micelles loaded with TMZ were added to a polymer solution containing poly(ethylene glycol) dimethacrylate and Lucirin-TPO as photoiniator [77]. The formulation was locally injected in female athymic nude mice and UV light was applied to induce the polymerization reaction, forming the local gel depot at the tumour site. The *in vivo* results showed that the tumours from mice treated with the hydrogel-nanostructure formulation were significantly lighter, and a higher extent of apoptosis was observed compared to mice treated with systemic TMZ.

Taken together, these results confirm the advantages of a localized and combined strategy. On one hand, local administration can ensure the therapeutic dose maintenance at the tumour site. On the other hand, the NPs ensure a sustained and prolonged release of drugs to remaining or recurrent tumour cells. Therefore, this combination holds significant promise in the treatment of GBM.

3.3. Hydrogels for cancer immunotherapy

Immunotherapy is a modern and attractive approach in cancer treatment that attempts to stimulate the patient immune system to specifically reject and destroy tumours with minimal harm to healthy tissues. Thermosensitive hydrogels are not only able to carry small molecules and micro or nanoparticles but have also been reported to act as depots for immune cells-based therapy. Tsao et al. designed a thermosensitive poly(ethylene glycol)-g-chitosan hydrogel that was able to support the penetration of T-lymphocyte cells [22]. The hydrogel showed better compatibility for the infiltration and release of T-lymphocyte cells when compared to Matrigel, likely due to its bigger pore size (0.5−1μm *vs* 0.1-0.5 μm pore size distribution). Moreover, the cells retained their cytotoxic activity against U87 glioblastoma cell line. Although *in vivo* studies were not carried out, these results may lead to the development of a novel localized immunotherapy for glioblastoma or other CNS disorders.

Another immunotherapeutic approach studied for cancer treatment is the development of vaccines. Cancer vaccines aim to stimulate the immune system to act against cancer cells. However, some improvements are needed to get the desired immunogenicity for the formulations [78,79]. Muraoka et al. developed a cancer vaccine using a nanogel of cholesteryl pullulan [80]. The nanogel loaded with a peptide antigen was selectively internalized by macrophages in lymph nodes of female BALB/c mice subcutaneously injected with the vaccine. In addition, they showed that the macrophages could present the antigen to T cells, triggering the immune response against syngeneic tumours transplanted into the mice. Importantly, this nanogel system was already tested in clinical trials that confirm its safety and efficacy.

The above mentioned research works introduce new developments regarding immunotherapy and cell therapy against cancer. They show that hydrogels can be used both as cell supporting systems *in vitro* and as cell delivery vectors. The biocompatibility of these materials would allow their incorporation into new treatments for GBM and other tumour types.

3.4. Hydrogels loaded with DNA/RNA

Different strategies to deliver gene sequences have been developed and evaluated in the past years [81]. Polymers have been presented as a feasible strategy to increase the transfection efficiency of gene therapies, exploring stimuli-responsive and targeting mechanisms. For this purpose, oligonucleotides have been loaded or attached to hydrogel NPs using a range of approaches. For instance, Ma et al. attached siRNA to hydrogel NPs via disulphide bonds to improve the systemic and controlled delivery of gene therapy [82]. In this design, the siRNA release is responsive to reductive conditions. The developed material was tested *in vitro* using luciferase-expressing HeLa cells, and the inhibition of luciferase expression was observed upon treatment with the siRNA conjugated hydrogel NPs. *In vivo* tests were conducted with C57BL/6 mice, showing the efficiency of transfection (at both mRNA and protein level) through gene silencing of the Coagulation factor VII (FVII) produced by hepatocytes. Exploring the same principle with disulphide linkages, Lei et al. developed a non-viral gene delivery vector aimed to treat GBM [83]. A cationic polymer composed of RGD-PEG linked to polyethyleneimine (PEI) through a disulphide bond (RGD-PEG-SS-PEI) was synthesized. The PEG moiety was used to both decrease the polymer toxicity and increase the transfection efficiency. The superior performance of the reducible targeted gene vector was confirmed *in vitro* in U87 cells, and *in vivo* after intravenous administration in nude mice bearing U87 tumours. The analysis of reporter gene systems in the mice organs after polymer administration showed an efficient targeting to the brain.

Alternative stimuli-responsive strategies that take advantage of pathological characteristics of the tumour, such as hypoxic conditions, have been reported. Using dexamethasone-conjugated PEI as plasmid carrier, Kim et al. developed a specific therapy for GBM that combines two different regulatory elements, the nestin intron 2 (NI2), which has increased gene expression in glioblastoma, and the erythropoietin enhancer, which has increased expression under hypoxia, to deliver a suicide gene to the tumour [84]. They confirmed the specificity of their constructs and the cytotoxicity promoted by the delivered gene in C6 and U87 GBM cell lines. Moreover, *in vivo* subcutaneous and intracranial models of GBM showed response to this gene delivery therapy. Indeed, the study showed that the combination between the polymer carrier and the gene vector was more effective in reducing the tumour volume compared to the polymer alone (three times less effective) or combined with a non-specific plasmid sequence (twice less effective).

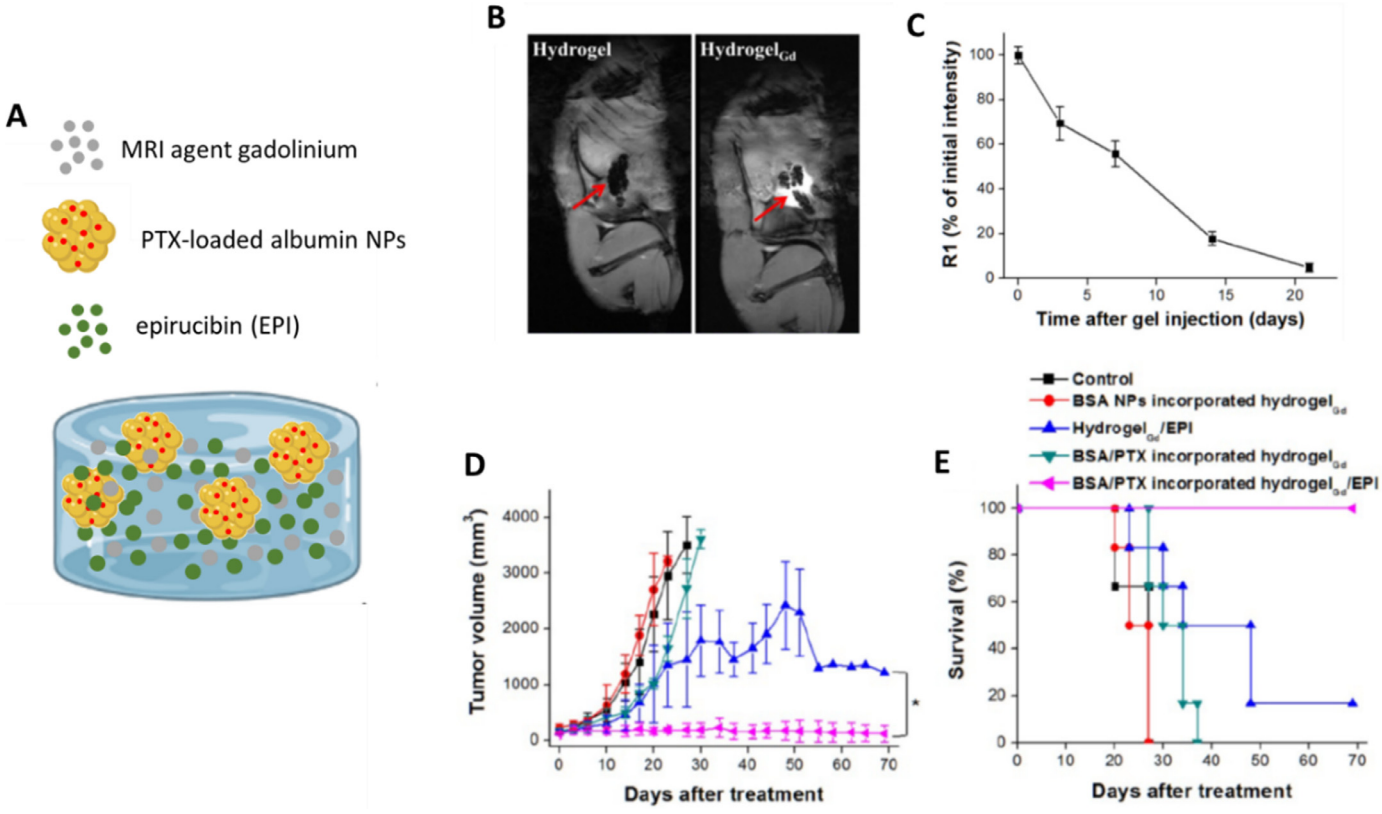

Fig. 7. Theranostic hydrogels. (A) Schematic illustration of the theranostic formulation proposed by Lin et al. [19] 2017, Ivyspring International Publisher (Creative Commons copyright). (B) MRI contrast-enhanced T1 images of (left) hydrogel and (right) hydrogelGd implanted in tumor-bearing mice. (C) The reduction of MR T1 intensity of BSA NPs-incorporated hydrogelGd in tumour site. (D) Tumour growth curves of mice bearing U87 tumours after surgical operation then treatment with BSA NPs incorporated hydrogelGd or hydrogelGd/EPI or BSA/PTX NPs incorporated hydrogelGd or BSA/PTX NPs incorporated hydrogelGd/EPI implantation. (E) Survival curves of mice bearing U87 tumours after different treatments.

A polymer based on mPEG-PEI was reported by Wang et al. to deliver gene sequences to GBM tumour cells using a retro-inverso CendR targeting peptide (D(RPPREGR)), known to increase cell penetration through binding the neuropilin-1 receptor [23]. Enhanced cell uptake and tumour spheroid penetration of the fluorescent peptide FITC-D(CRPPREGR) were achieved, as well as higher transfection using the gene delivery system both *in vitro* (almost 2-fold targeting enhancement in U87 cells) and *in vivo* in nude mice bearing U87 tumours.

In summary, polymer based formulations are showing promising results as gene delivery carrier systems for GBM therapy, and the use of polymers as non-viral delivery vectors is a very promising area of research to be expanded.

3.5. Theranostic hydrogels

The combination of therapeutic and diagnostic approaches, known as “theranostics”, has also been reported in the field of hydrogels. In this case, the polymer matrix contains both a chemotherapeutic and a contrast agent that allows the treatment monitoring in real time. As an example of nanotheranostic formulation, the “MRI-monitor long term therapeutic hydrogel” (MLTH) system consists of a thermosensitive poly(organophosphazene) hydrogel, cobalt ferrite NPs and the chemotherapeutic drug 7-ethyl-10-hydroxycamptothecin (SN-38) [85]. MLTH systems with different amounts of SN-38 were tested in U87 ectopic xenograft mice models to determine the MRI-enhancing effect and anticancer efficacy of the formulations. The hydrogel formulations generated higher inhibition effects on tumour growth (up to 48% decrease) compared with the chemotherapeutic drug only. Moreover, the cobalt-ferrite NPs in the composite had suitable MRI contrast-enhancing effects to distinguish between the untreated and treated areas in the brain. The MLTH system has been presented as an alternative approach to treat malignant brain tumours without any surgical resection.

Other formulations propose benefits following tumour resection and claim to impair tumour recurrence. This is the case of the recently reported theranostic hydrogel formulation with rapid gelation ability that is composed of carboxymethyl cellulose-grafted poly(*N*-isopropylacrylamide co-methacrylic acid) and the MRI agent gadolinium, loaded with a free drug, epirucibin (EPI), and PTX-loaded albumin NPs [19]. *In vivo* studies with mice bearing gliosarcoma tumours of MBR-614 or U87 cells showed that the theranostic formulation increases the average survival compared to the control group from 18 to 63 days (MBR-641 models) and from 27 to 69 days (U87 models). *In vivo* MRI of the group that received local administration of the hydrogel formulation showed a bright contrast at the site of implantation, and the signal intensity gradually decreased in 21 days, corresponding to the degradation and clearance of the hydrogel depot. The theranostic ability of this type of hydrogels could significantly improve treatment monitoring in the clinical setting (Fig. 7).

4. Conclusion

The treatment of GBM is a great medical challenge due to both the disease aggressiveness and tumour location. The research for new chemotherapeutic molecules as well as new forms of treatment administration are of high importance for current and future patients. Indeed, local administration of therapy is a promising strategy to improve therapeutic outcomes.

Several examples were covered in this manuscript and highlighted the importance and effectiveness of having a sustained release of drugs, for example through the combination of hydrogel and nanocarriers which includes hydrogel nanoparticles, nanospheres, nanocapsules, microspheres, micelles, metal and inorganic nanoparticles, liposomes and polymeric micelles. It has been proven that a sustained and increased exposure of the tumour to the treatment can lead to significant enhancement on survival time. On one hand, drugs loaded into nanoparticles and/or incorporated into hydrogels are protected from degradation and can have their solubility increased. On the other hand, the improvement of treatment outcomes will be accompanied with an expected decrease in side effects when a local administration is used.

Moreover, the tunability of hydrogel systems makes them very versatile materials regarding the mode of administration (injectable or implantable) and the formulation composition. The hydrogel characteristics, specially the rheological properties, can be modified according to the desired formulation. Besides drugs, incorporation of nucleic acid and contrast agents are also being explored to use hydrogels as non-viral gene delivery systems and to monitor treatment efficacy.

Despite all the above, clinical translation of hydrogel formulations to treat GBM is still poor and to date there are no clinical trials on GBM specifically evaluating hydrogel formulations. Some aspects of this approach that need to be further evaluated are: (1) the optimization of formulations to achieve a synchronised release of components due to the need of a specific ratio between drugs to achieve additive or synergistic effects, and (2) the analysis of the safety to use specific formulations directly into the human brain. Hence, to translate the use of *in situ* hydrogel formulation treatment into clinical settings, these possible drawbacks need to be addressed further in future works.

Remarkably, hydrogels are being used in GBM research to develop 3D cultures of GBM cells in order to compare drug cytotoxicity with monolayer cultures and perform drug screenings. It is known that cell spheroids usually show greater resistance compared to monolayer cultures. For this reason, they are models that better reflect the clinical manifestation of cancer. Examples of these studies include a chitosan-PEG hydrogel crosslinked with genipin, developed to form GBM cell spheroids in which the combination of TMZ and BCNU was tested showing higher resistance and shedding light into the possible reasons for the emergence of the resistance behaviour [86]. Another example is the development of a brain cancer chip using photo-polymerizable poly(ethylene) glycol diacrylate (PEGDA) hydrogel in which 3D GBM spheroids are formed and can be treated with different drug combinations for drug screening [87]. Thus, these platforms based on hydrogels can help to understand and circumvent drug resistance issues.

These studies are expected to help translate the use of new hydrogel formulations to the clinic while they improve our understanding of the disease. Therefore, hydrogels hold great promise as part of formulations aimed to the local delivery to GBM.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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