Matrix Metalloprotease Triggered Delivery of Cancer Chemotherapeutics from Hydrogel Matrixes

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Glioblastoma multiforme (GBM) is a highly advanced and invasive brain tumor due to which current treatments cannot completely treat GBM or prevent recurrence. Therefore, adjunctive treatments are required. As part of the invasive and angiogenic nature of GBM, it has been well established that matrix metalloprotease-2 (MMP-2) and MMP-9 are overactive. To better treat GBM using chemotherapy, we have designed a hydrogel-based delivery system that can control the release of drugs based on the activity of MMPs. A model chemotherapeutic agent, cisplatin (CDDP), complexed to an MMP substrate (peptide-linker) was incorporated into poly(ethylene glycol) diacrylate hydrogel wafers having different poly(ethylene glycol) chain lengths ($M_n \approx 574$ and 4000). Hydrogel wafers were studied for physical characteristics and drug release in the presence and absence of MMPs. There was a substantial increase in CDDP release for the poly(ethylene glycol) 4000 hydrogel indicating that this chain length provides a mesh size that is sufficient to permit MMP activity within the hydrogel. CDDP bioactivity increased when the cell media was spiked with MMPs (0% cell survival) in case of the longer chain length as compared to in the absence of MMPs (approximately 50% cell survival). The results suggest that this system can be used for selective, local delivery of drugs where higher amounts of the drug are released in response to metastasis, angiogenesis, and invasion-promoting proteases. This strategy may prove to be a novel and effective method to overcome inadequacies in current controlled drug release systems.

INTRODUCTION

Brain tumors are especially difficult to treat due to the lack of chemotherapeutic agent blood-brain barrier (BBB) transport. Of all brain tumors, the most advanced stage of astrocytic brain tumor, glioblastoma multiforme (GBM) or World Health Organization Grade IV glioma, is highly proliferative and shows wide areas of necrosis surrounded by invasive tumor tissue and extensive neovascularization (*1*, *2*). The mean survival time for patients with GBM is one year due to the highly invasive nature of the disease (*1*) leading to recurrence even after treatment (*3*). Currently available chemotherapeutic agents, including cisplatin (CDDP), can only slightly prolong the mean survival time of patients (*4*). Therefore, better treatment strategies are necessary to effectively treat GBM and other forms of brain tumor. In an attempt to better treat brain tumors, local delivery of chemotherapeutics has been utilized (*5*). These local drug delivery systems release drugs at a constant rate that is dictated by hydrolysis of a polymeric carrier; drug delivery is not correlated with any biologic response. Over the last several decades, it has been recognized that the current paradigm of arbitrary and nonspecific delivery of chemotherapeutics can be improved by targeting the activation or release of the chemotherapeutic at a desired site via specific active mechanisms (*6*, *7*). With this in mind, systems that utilize tumor biology have been proposed and utilized to improve the outcome for brain tumor and other cancer patients (*8*).

Several aspects of tumor biology can be exploited to utilize tumor biology for delivery of chemotherapeutics, including tissue invasion, angiogenesis, and metastasis, all of which require the degradation of extracellular matrix (ECM) proteins. Many proteases are implicated in ECM degradation and matrix metalloproteases (MMPs) have been shown to actively participate in degradation of ECM and activation of invasion promoting growth factors (*9*). MMPs play an important and beneficial role in tissue remodeling, wound healing, and angiogenesis along with negative roles in tumor invasion and neovascularization (*10*, *11*). Unequivocal data confirms that the levels of MMP-2 and MMP-9 are observed to increase in correlation with tumor progression in human gliomas (*12*, *13*). Initial attempts to exploit MMPs as a chemotherapeutic target using synthetic MMP inhibitors (MMPis) prevented glioma invasion by inhibiting MMP activity. MMPis reduced glioma growth and invasion in vitro and in animal models (*14*, *15*) but have achieved disappointing results in Phase I, II, and III clinical trials for many reasons (*16*). Despite these disappointing clinical results with MMPis, MMPs may still be an excellent target for chemotherapeutic delivery if an alternate approach is taken: a prodruglike approach.

To achieve a prodruglike approach, an understanding of MMPs proteolytic character is needed. Each MMP has different specificities for the constituents of the ECM; however, MMP-2 and MMP-9 show a great amount of overlap in their substrate specificities. Substrates common to both gelatinases include gelatins, collagens IV and V, elastin, and aggrecans (*17*). By use of these overlapping specificities, it is possible to design peptide sub-

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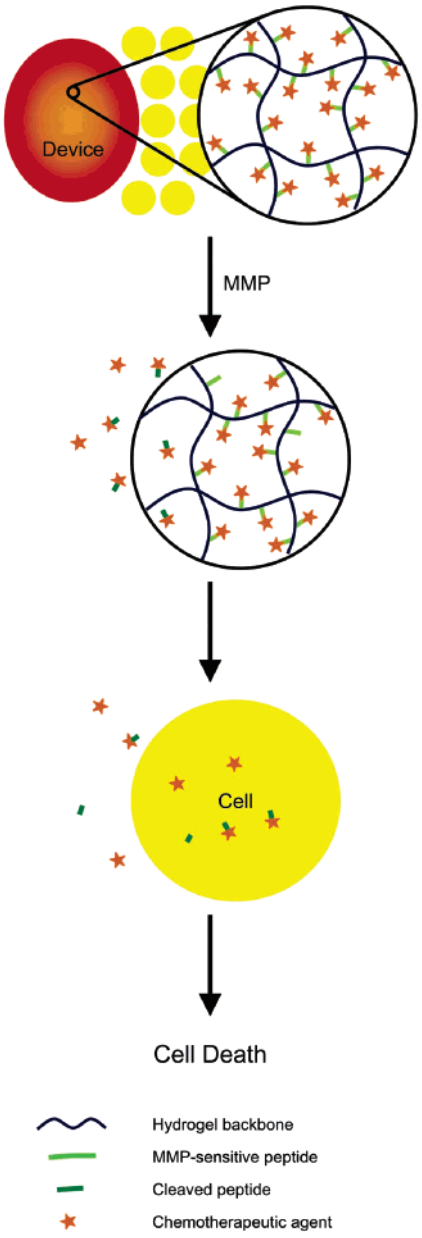

Figure 1. Schematic representation of the drug activation mechanism proposed. In this, CDDP (orange star) is complexed to peptides (light-green lines) pendant on the backbone of a hydrogel matrix (blue lines) of a hydrogel device (red circle) placed near tumor cells (yellow circles). As matrix metalloproteases diffuse through the matrix, the peptides are cleaved releasing CDDP−peptide (dark-green lines) complexes. These complexes may dissociate or enter cells as a complex. CDDP is then able to have the therapeutic effect only when released from the hydrogel matrix. Once released by MMP cleavage (or nonspecific means), the chemotherapeutic agent can enter a cell and have the therapeutic effect (cell death).

strates for releasing drug when MMPs are active similar to a traditional prodrug. In this hydrogel-based prodrug system, peptide substrates act as MMP degradable spacers binding the chemotherapeutic agent to a hydrogel matrix; therefore, the prodrug is retained at the site of implantation by the hydrogel matrix. Our rationale for this design was to use a peptide−drug complex that will release an active form of the drug from the matrix when the peptide is cleaved by MMPs (Figure 1).

Hydrogels were chosen as matrixes due to the inherent biocompatibility of the cross-linked polymeric network (*18*). Hydrogel biocompatibility can be attributed to their hydrophilicity and ability to imbibe large amounts of biological fluids (*19*, *20*). Also, hydrogels closely resemble natural living ECM (*21*) creating a perfect environment for a matrix to mimic MMPs natural activity. In addition, poly(ethylene glycol) diacrylate (PEGDA) hydrogels of different PEG chain lengths are known to allow protein diffusion through the matrix if appropriate mesh size is attained (*22*). Hydrogels of this type have even been produced to allow degradation via MMPs thereby releasing PEG chains and natural products and allowing for aid in regeneration (*23*).

The described study utilizes a PEGDA-based hydrogel to locally target one of the major characteristics of GBM and utilizes this characteristic to selectively deliver a model chemotherapeutic agent, CDDP. Physical properties and in vitro activity of the system have been studied to confirm that MMPs can be used to activate a chemotherapeutic agent from a synthetic hydrogel matrix. With the application of this technology, the problems of recurrence and invasion are expected to be mitigated as the drug will be activated as a tumor attempts to metastasize or recur locally. Localized delivery minimizes systemic toxicity and targets activation of the drug to the site of malignancy. The ability of a delivery system to release the drug when activated by MMPs may help in the development of a successful strategy for treatment of GBM and should be explored further in relation to GBM and other solid tumors.

EXPERIMENTAL PROCEDURES

The MMP-sensitive peptide substrate, CGLDD, and the expected cleaved fragment, LDD (*24*), were synthesized by the University of Illinois at Chicago Research Resources Center with the N-terminal amine blocked by acylation. Peptide structures were confirmed by liquid chromatography mass spectrometry. MMP-2 and MMP-9 were purchased from Calbiochem and activated as suggested by the manufactured using 4-(hydroxymercury)-benzoic acid (Acros Organics, NJ). Short-chain PEGDA (M_n = 700, PEG(574)DA) macromer was purchased from Sigma Aldrich Chemical company (Milwaukee, WI). Long-chain PEGDA (M_n = 4126, PEG(4000)DA) macromer was purchased from Polysciences, Inc. (Warrington, PA). All remaining chemicals were purchased from Fisher Scientific (Fairlawn, NJ) and used without further purification unless specified.

Preparation and Characterization of Hydrogels. MMP-sensitive peptide substrate (2 mM) was dissolved in water and mixed with PEG(574)DA (100 mM) or PEG-(4000)DA (15.9 mM) and stirred overnight. Michael addition of the acrylate group to the sulfhydryl group on cysteine (*25*) was confirmed using Ellman's reagent (Pierce, Milwaukee, WI), thereby quantifying reduction in free sulfhydryl groups (*26*). PEGDA−peptide solutions (or peptide-free PEGDA at the concentrations listed above) were polymerized without further purification between glass slides to form sheets of hydrogels using ammonium persulfate (20 mM) and N,N,N,N-tetramethylethylenediamine (51.6 mM) as the initiator pair. Hydrogels were cut into 6 mm diameter wafers using a biopsy punch, washed repeatedly in deionized water to remove unpolymerized monomers, and finally allowed to dry in a vacuum oven. CDDP was aquated at pH 7.4 using silver nitrate (*27*) and reacted overnight with hydrogels. Hydrogels containing the peptide were washed to remove excess CDDP. CDDP was entrapped in hydrogels without peptide by using the same procedure as described without washing. CDDP entrapment and complexation in hydrogels was determined by spectropho-

tometeric *o*-phenylenediamine (OPDA) assay (*28*). The mesh size (ξ) of hydrogels was determined to compare with MMP penetration (*29*).

Cell Culture and MMP Activity. A malignant glioma cell line (U-87 MG, ATTC #HTB-14) was grown and maintained in Eagles minimum essential media with 10% fetal bovine serum, 1% penicillin/streptomycin, sodium pyruvate (110 mg/L), L-glutamine (292 mg/L), and nonessential amino acids. Cells were cultured at 37 °C in 5% CO_2. To confirm MMP expression and activity in U-87 MG cells, cells were subcultured under standard conditions for 24 h, then supplemented media was replaced with serum-free media and cultured for an additional 24 or 48 h. The serum-free media was removed and analyzed for presence for MMPs by gelatin zymography (*30*). Cell-treated media (10 μL) was loaded on Ready Gel zymography gels (Bio-Rad Laboratories, Hercules, Ca) and electrophoresed at 100 V. The gels were then washed, incubated, stained, and analyzed for presence of MMPs.

CDDP Release. CDDP release from the hydrogels was determined at 37 °C in a buffer containing Tris−HCl (50 mM), NaCl (0.2 M), $CaCl_2 \cdot 2H_2O$ (10 mM), Brij-35 (0.05%), and $ZnSO_4 \cdot 7H_2O$ (50 μM) in the presence and absence of MMPs (at a final concentration of 10 nM). Released CDDP was determined using the spectrophotometeric OPDA assay (*28*). CDDP release profiles were compared to CDDP release profiles for hydrogels with entrapped CDDP; no peptide was present in the hydrogel for entrapped CDDP studies, which act as a diffusional release control.

Peptide Toxicity and CDDP Activity. To determine the activity of released CDDP, the cleaved peptide fragment LDD was complexed with CDDP using the same procedure as described earlier for hydrogels. Cytotoxicity of the MMP cleaved fragment, LDD, and LDD−CDDP complex was determined by incubating U-87 MG cells with varying concentrations of these peptides in supplemented serum free media for 24 h.

To determine the activity of the hydrogel−CDDP complex devices, hydrogels were incubated with cells in supplemented serum-free media for 24 h. For specific samples, MMP (10 nM) was added to media to supplement the MMPs expressed by U-87 MG cells (*31*) and thus accentuate the MMP effect on CDDP release. Activity of the hydrogel−CDDP complex devices was also studied in the absence of added MMPs. Cell viability with respect to the untreated control was calculated using a modified MTT assay (Cell Titer 96 Aqueous One Solution Cell Proliferation Assay; Promega; Madison, WI) (*32*).

RESULTS

Preparation of Hydrogels. Peptide−PEGDA conjugation was nearly complete after overnight incubation at room temperature as determined by quantitative analysis of free sulfhydryl groups. There was 74.1 ± 3.2% efficiency for conjugation with the PEG(574)DA and 76.1 ± 1% efficiency for conjugation with the PEG(4000)-DA, and the reaction did not proceed further following extended incubation under these conditions. Since this reaction mixture was not further purified prior to polymerization, further incorporation of the peptide is expected by further Michael reaction or chain-transfer reactions with the peptide (*33*). In similar studies with fluorescent-labeled peptide, no peptide was released from the hydrogel without addition of MMPs (data not shown).

PEGDA and PEGDA−peptide hydrogels reached the critical gelation point within 5 min of initiation, but the reaction was continued at 37 °C for a minimum of 1 h to increase the extent of polymerization. PEG(574)DA hydrogels had a mesh size (21±4 nm) that was smaller than PEG(4000)DA hydrogels (79±7 nm). This was expected since PEG(4000)DA hydrogels have significantly longer PEG chains; this increased mesh size allows for substantially higher water content. This larger mesh size was expected to allow MMP-2 and MMP-9 to more freely diffuse in the PEG(4000)DA hydrogel than the PEG(574)-DA hydrogel.

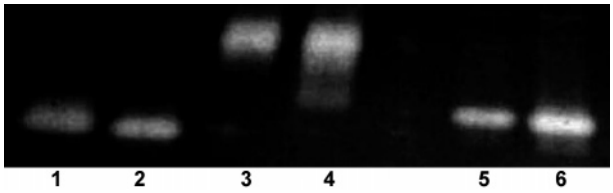

Figure 2. Detection of MMPs by gelatin zymography. Lane 1, Pro-MMP-2; Lane 2, active MMP-2; Lane 3, Pro-MMP-9; Lane 4, active MMP-9; Lane 5, 24 h U-87 MG expression; Lane 6, 48 h U-87 MG expression.

As expected, entrapped CDDP content was found to be much higher than complexed CDDP content since washing removed CDDP that was not complexed to the hydrogel. PEG(574)DA entrapped 22.1 ± 0.9 μg (0.317 μg/mm^3), and 8.6 ± 0.1 μg (0.122 μg/mm^3) was retained as CDDP−hydrogel complexes. Also, the CDDP content was found to be higher in the PEG(4000)DA hydrogels than in the PEG(574)DA hydrogels. PEG(4000)DA hydrogels entrapped 55.7 ± 6.1 μg (0.43 μg/mm^3) while 20.5 ± 1.4 μg (0.157 μg/mm^3) was retained as peptide−CDDP complexes.

MMP Activity. U-87 MG cells express MMP-2 when cultured in media for 24 and 48 h (Figure 2). Pro-MMP-2 increased as cells are incubated for extended periods. Active MMP-2 was detected at 48 h. MMP-9 was not expressed at detectable levels in the cells for up to 48 h. However, this cell line may be used as an in vitro model since the expression and functions of MMP-2 and MMP-9 are known to be heterogeneous in clinical GBM presentation (*9*, *12*) and MMP-2 and MMP-9 have overlapping substrate specificities.

CDDP Release. CDDP was complexed with the carboxyl groups of aspartic acids in the substrate peptide (Figure 3) (*27*). We have previously shown that CDDP complexation delays the release of CDDP from hydrogel particles (*34*). This was confirmed for peptide complexation in this study. CDDP entrapped in PEGDA hydrogels with no carboxylic content was released nearly instantaneously in a burst profile (Figure 4). Peptide complexed CDDP exhibited a slowed release over time even without MMPs present indicating that the complexation was taking place but was not sufficient for complete retention of the drug. However, with the addition of MMPs, there was a marked increase in the amount of CDDP released from the PEG(4000)DA hydrogels but not the PEG(574)DA hydrogels. This suggests that the peptide CGLDD was cleaved from the PEG-(4000)DA hydrogels in a manner dependent upon MMPs if the MMPs are able to enter the hydrogel matrix.

Peptide Toxicity and CDDP Activity. As anticipated, the expected cleaved fragment, LDD, showed no toxicity when incubated with U-87 MG cells. However, the complex, LDD−CDDP, had activity (EC_{50} = 234.7 μM) approximately an order of magnitude below CDDP (EC_{50} = 41.5 μM) (Figure 5). Complexed CDDP retained activity with some loss due to complexation. PEGDA hydrogels and PEGDA−peptide hydrogels without CDDP

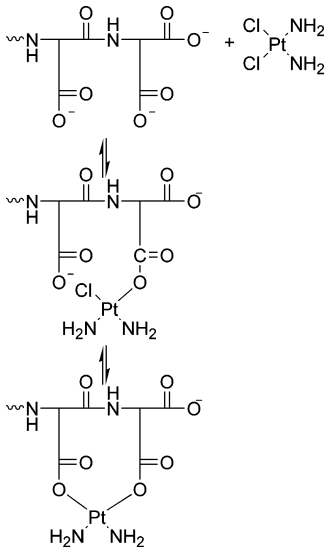

Figure 3. Generalized complexation chemistry of CDDP with carboxylic acid groups, which was utilized in this study. Platinum can interact with aspartic acid side groups as shown or with the carboxyl-terminal group in the peptide. The complexation is dynamic, so both interactions are expected.

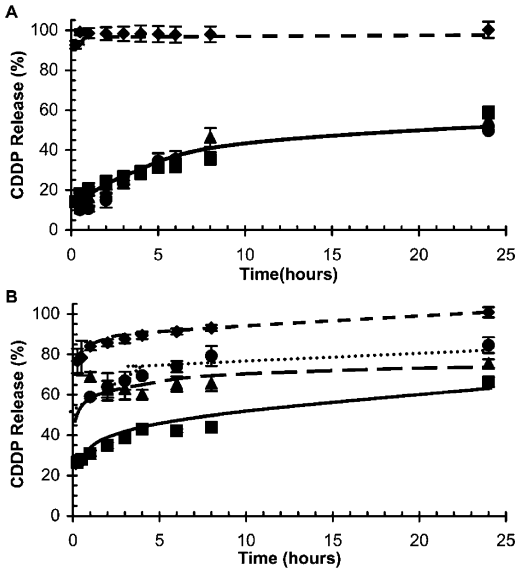

Figure 4. (a). CDDP release from PEG(574)DA hydrogels where (◆) CDDP was entrapped with no complexation, (■) CDDP was complexed to the hydrogel, (●) CDDP was complexed to the hydrogel with MMP-9 added to the release media (10 nM), and (▲) CDDP was complexed to the hydrogel with MMP-2 added to the release media (10 nM). (b) CDDP release from PEG(4000)DA hydrogels where (◆) CDDP was entrapped with no complexation, (■) CDDP was complexed to the hydrogel, (●) CDDP was complexed to the hydrogel with MMP-9 added to the release media (10 nM), and (▲) CDDP was complexed to the hydrogel with MMP-2 added to the release media (10 nM).

were not cytotoxic in the presence or absence of MMP-2 or MMP-9 (Figure 6). CDDP entrapped in PEGDA hydrogels exhibited similar activity to the amount of CDDP that was loaded into the hydrogel. Upon complexation, PEGDA−peptide−CDDP hydrogels exhibited activity at a level well below the amount of CDDP loaded. Activity of CDDP released from PEG(574)DA peptide−CDDP complex hydrogels was not influenced by MMP addition. CDDP released from PEG(4000)DA peptide−CDDP complex hydrogels showed higher activity than CDDP released from PEG(574)DA hydrogels with and without the addition of MMPs. Also, with the addition

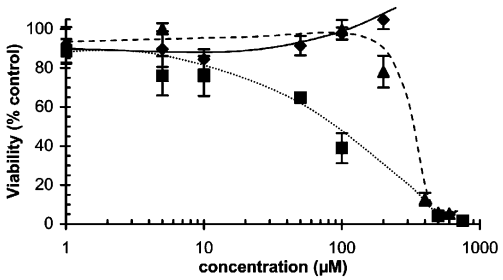

Figure 5. Cytotoxic activity of the (◆) cleaved fragment, LDD, the (▲) expected released peptide−CDDP complex, LDD−CDDP, and (■) CDDP as determined by modified MTT assay using U-87 MG malignant glioma cells.

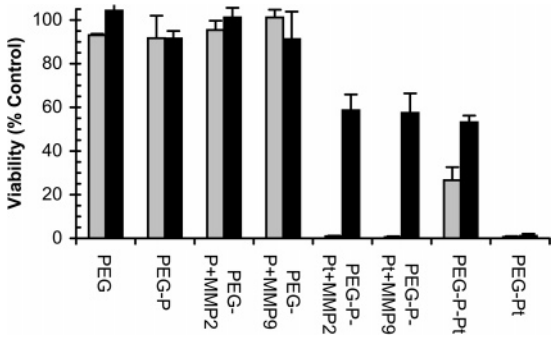

Figure 6. Activity of CDDP released from (dark bars) PEG(574)DA and (light bars) PEG(4000)DA hydrogels. Hydrogels without CDDP or peptides (PEG), hydrogels with peptide incorporated but with CDDP in the absence (PEG-P), and presence of MMP-2 (PEG-P + MMP2) or MMP-9 (PEG-P + MMP9), hydrogels with entrapped CDDP but without peptide for complexation (PEG-Pt), and hydrogels with peptide−CDDP complex in the absence (PEG-P−Pt) and presence of MMP-2 (PEG-P−Pt + MMP2) or MMP-9 (PEG-P−Pt + MMP9).

of MMP-2 or MMP-9 to PEG(4000)DA peptide−CDDP complex hydrogels, higher CDDP activity was observed than in the absence of MMPs. This was not seen with the PEG(574)DA hydrogels. The increase in CDDP activity supports and reinforces the data obtained by CDDP release suggesting that more CDDP was released in the presence of MMPs.

DISCUSSION

PEGDA hydrogels have been widely accepted in many biomedical applications over the last several years (*35*). Hydrogels prepared using PEG(574)DA and PEG(4000)-DA have adequate physical characteristics for implantation and manipulation prior to placement. The current formulations are biodegradable, but the time course for biodegradation is quite slow. This would cause accumulation of PEGDA hydrogel at the site of implantation. To overcome this problem, PEGDA hydrogels could be made to biodegrade at a faster rate by several modifications (*36*). PEGDA hydrogels can be made biodegradable by incorporating a biodegradable cross linker or by utilizing biodegradable copolymers (*37*−*40*). The extent and rate or degradation would be controlled by the specific degradation mechanism used. Future generations of this type of system should integrate such biodegradable nature. However, it was necessary to confirm the possibility of drug activation prior to adding a potentially confounding variable such as biodegradation. Once proteolytic release of anticancer agents is understood, appropriate degradation mechanisms can be incorporated into the system that allow full control of release while limiting accumulation of the hydrogel at the site of implantation. It should be noted, however, that PEGDA

hydrogels are well tolerated in the body even when left for significant periods.

PEGDA hydrogels are hydrophilic, biocompatible, nontoxic, and exhibit variable mesh size depending upon PEG macromer length. Large PEG chain lengths allow proteins to diffuse throughout the matrix while smaller PEG chain lengths can be used to control the accessibility of the matrix to specific proteins (*22*). This work has shown that adequate PEGDA chain length can be attained to allow diffusion of MMPs throughout the matrix, thus releasing a chemotherapeutic agent from the matrix via MMP cleavage of peptide substrates. Hydrogels containing peptide−CDDP complex provided a level of control for the release of CDDP to the tumor cells. We did not show complete control over delivery of CDDP; the CDDP−peptide complex allows nonspecific release of approximately 50% of bound CDDP in the first day regardless of chain length. Increasing CDDP binding efficiency with a hydrogel by increasing the carboxylic acid content has been shown to slow the nonspecific CDDP release from hydrogel particles (*34*). Alternate platinum chemistries are also possible if platinum-based drugs are desired to be delivered. Our group has successfully bound platinum to amine groups of hydrogel and showed limited release over a week or longer (*41*). Utilization of covalent coupling of alternate drugs could also alleviate the problem of nonspecific release (*8*). Alternate chemistries should allow a more specific release of chemotherapeutics from the device. A perfectly designed system would allow release of the chemotherapeutic agent in a MMP dose-dependent manner. Most importantly, when no MMP is present, no chemotherapeutic should be released. As the concentration of MMP increases, the amount of chemotherapeutic should increase. This would ensure minimal toxicity due to the chemotherapeutic and maximum release with protease activity. Despite the setback of nonspecific release, we were able to confirm that MMP-specific release of CDDP is possible from a hydrogel matrix.

When glioma cells attempt to grow and invade the surrounding tissue or even in culture, MMPs are expressed (Figure 2). This led to increased CDDP release when MMPs were present (Figure 4). The fact that PEG(574)DA hydrogels do not allow increased release of CDDP upon MMP addition while PEG(4000)DA hydrogels do allow accelerated release of CDDP confirms our hypothesis that adequate mesh size can be obtained. Thus, this system will deliver a higher dose of the drug selectively to the most invasive portion of the tumor. This was also confirmed by examining the activity of the devices (Figure 6). Cells are not influenced by any hydrogels that do not possess CDDP, and when no CDDP complexation exists, cells are not able to survive. Upon peptide−CDDP complexation within PEG(574)DA hydrogels, intermediate CDDP activity was observed after 24 h with no increase upon MMP addition. When peptide−CDDP is complexed within PEG(4000)DA hydrogels, CDDP was substantially more active. Without MMPs, fewer cells survive when treated with PEG(4000)-DA hydrogels than when treated with equivalent PEG(574)DA hydrogels. This is due to MMP diffusion into the PEG(4000)DA hydrogels and subsequent increased release of CDDP. CDDP activity was increased by addition of MMPs as expected for a Michaelis−Menten-type kinetics ($V_{max} = k_{cat} \cdot [E]_{tot}$). Further studies are needed to better characterize the exact kinetics of release under these circumstances, and we hope to report these results in the future. The end result is not in question, increased CDDP was released when MMPs are added to the system. This type of delivery should greatly increase the efficacy of drug delivery systems.

Local delivery and controlled activation of the drug reduces unnecessary exposure to other organs. Since the drug was released in high doses at the tumor site it may have better efficacy. Problems with currently utilized routes of chemotherapeutic agent delivery, such as inability to cross the BBB and systemic toxicity, would be averted. Since the conjugation of the peptide within PEGDA has a high yield, loading of the drug can be maximized by increasing the concentration of the peptide. The peptide used for the study is a substrate for MMP-2 and MMP-9 with efforts underway to design substrates specific for these and other enzymes. Having an array of substrates would allow for tailoring a device to a specific patient or for a particular tumor type. Not only is peptide choice important for specificity and kinetic control of cleavage but activity of the released drug is also influenced by peptide modification.

Minimization of loss of activity of the drug due to complexation has to be considered in designing peptides; our current example indicated a reduction in activity from CDDP (Figure 5). By selective control over peptides, more active CDDP (or other drug) analogue can be released from the hydrogel matrix. Our data showed that peptide substrates for MMP-2 and MMP-9 can be cleaved by these enzymes within hydrogels thus releasing an active form of CDDP.

Other groups have shown activity of drugs when covalently attached to MMP substrates (*42*) or released from MMP-sensitive micelles (*31*). We have focused on localized delivery due to questions that exists concerning the heterogeneity of tumor vascular permeability in different tumor types and proteases present within the blood. In our system, the activity of the complexed drug toward the glioma cell line was retained (although slightly attenuated) after cleavage. We have not overlooked the fact that transport of materials away from the device may hinder the area of effectiveness, but we also believe that polymer therapeutics that rely on vascular permeability have potential liabilities that can be addressed using local device placement. We hope to report in vivo results with this system soon. From these studies, hydrogel-based matrixes may prove to be excellent carriers for chemotherapeutics when local delivery is desired particularly when a prodruglike approach is used.

CONCLUSION

Current treatments have not improved the poor prognosis of GBM to a significant degree. It is necessary to develop drug delivery systems that reduce the problems of toxicity, recurrence, and invasion in this disease. By utilization of the biology of GBM, we have proposed a novel method of utilizing the overactivity of MMP-2 and MMP-9 in GBM to activate a prodrug from a hydrogel matrix. When CDDP is entrapped within PEGDA hydrogels, CDDP is released within a short period (greater than 95% within an hour). CDDP can be retained in PEGDA hydrogels by complexation with aspartate-containing peptides (approximately 50% release in 24 h). When MMPs are present, CDDP is released at an accelerated rate when adequate mesh size of the hydrogel matrix is present. PEG(4000)DA hydrogels have sufficient mesh size for MMP diffusion but not PEG(574)-DA hydrogels. Thus, PEG(4000)DA hydrogels containing drug−peptide prodrugs have shown great promise for locally controlling the release of chemotherapeutics. Active drug was cleaved from the hydrogel in the pres-

ence of MMPs and the cleaved drug retained activity toward glioma cells. This system warrants future research with MMP substrates and drugs and should be further explored.

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