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Extracellular Protease Activation of Chemotherapeutics from Hydrogel Matrices: A New Paradigm for Local Chemotherapy

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Abstract: A novel paradigm for local cancer chemotherapy, based upon local activation of chemotherapeutic molecules by soluble proteases, is presented. In the presence of matrix metalloproteases, a family of cancer-associated proteases, cisplatin is released from a hydrogel matrix in an active form. In the absence of matrix metalloproteases, cisplatin is released at a much lower rate. The mesh size of the polymer controls metalloprotease-based cisplatin release from the hydrogel matrix with approximate doubling of cisplatin release in the presence of matrix metalloprotease. Overall, this novel paradigm shows much potential for local chemotherapy where local chemotherapeutic release is in response to the invasive properties of the tumor.

Keywords: Prodrug; cancer; chemotherapy; hydrogel; drug activation; targeted delivery; drug delivery

Cancer chemotherapy is, in many cases, restricted by the pharmacologic toxicity of a chosen drug or combination of drugs. Despite the class of anticancer drug, a balance between activity and toxicity is needed to adequately treat tumors. Of the many methods of controlling toxicity, localized delivery of chemotherapeutics has emerged with the advent of novel biodegradable polymers.[1] Typically, hydrolytically degradable matrices have been applied to local cancer treatment with several prominent examples including Gliadel reaching the clinic for the treatment of brain tumors.[2] Most local biodegradable devices release drug in the local region of a tumor at a rate solely determined by the polymer chosen.

Alternative therapeutic approaches for treatment of cancer have also begun to emerge including locally placed, externally activated microfabricated drug delivery devices.[2] Prodrugs have also been widely utilized to allow local activation in the vicinity of a tumor. Prodrugs typically have a half-life similar to that of the parent drug and need frequent administration. Polymer therapeutics, or polymeric prodrugs, have been proposed to localize delivery of chemotherapeutic agents by active and passive targeting of tumors, thus increasing half-life and increasing local concentration. Polymer therapeutics are typically dependent upon cellular internalization.[3] Alternative activation strategies for polymer therapeutics, where the drug is released by extracellular proteases, have also been proposed but not as widely accepted.[4]

It is proposed that local drug delivery system placement will enhance currently available chemotherapeutic regimens; however, local release should be based upon active cancer biology. In this communication, initial data is presented in support of a novel paradigm for local chemotherapeutic delivery in the vicinity of active tumor cells (Figure 1). Current focus is centered on brain and ocular tumors due to the frequent surgical resection for these tumors;[2] however, this paradigm can be applied to any solid tumor mass. Based upon the knowledge that matrix metalloproteases (MMPs) are expressed and overactive in malignant glioma[5] and many other tumor types, the polymer therapeutic idea is expanded to a hydrogel matrix much like a biodegradable device. Hydrogel matrices have been chosen due to the ability of proteases to diffuse through the hydrogel matrix in a manner similar to the extracellular matrix surrounding cells.[6] Therefore, the hypothesis that release of chemotherapeutic from

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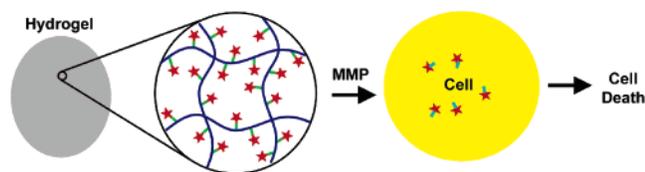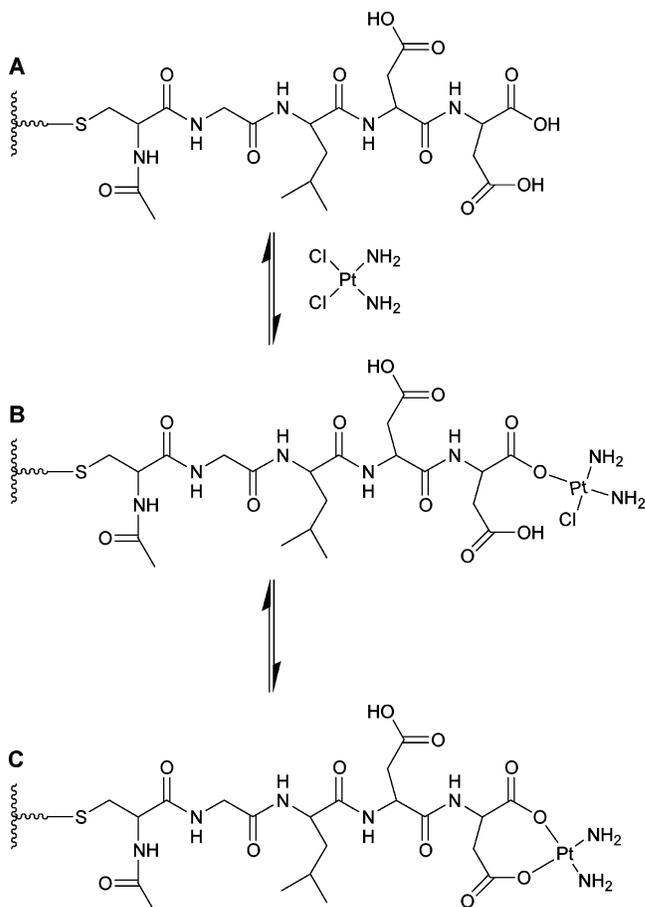
Figure 1. Schematic representation of the paradigm for local drug delivery proposed. In this, cisplatin (red stars) is complexed to peptides (light green lines) pendant on the backbone of a hydrogel matrix (dark blue lines). As matrix metalloproteases diffuse through the matrix, the peptides are cleaved, releasing cisplatin−peptide (aqua lines attached to orange stars) complexes. These complexes may dissociate or enter cells as a complex. Cisplatin is then able to cross-link DNA and have a therapeutic effect only when released from the hydrogel matrix.

the hydrogel matrix will be similar to activation mechanisms at play in the extracellular matrix during the natural biologic progression of cancer[7] was investigated.

We have produced poly(ethylene glycol) diacrylate (PEG-DA) hydrogels with varying molecular weight between cross-links containing pendant MMP sensitive peptides. Cisplatin, as a model chemotherapeutic agent, was either entrapped in hydrogels (without peptide) or complexed to MMP-sensitive peptides within hydrogels.[8] Cisplatin was present in the hydrogel therapeutic as free cisplatin (Scheme 1A) and as complexed cisplatin (Scheme 1B,1C). The complex may have one (Scheme 1B) or two (Scheme 1C) chlorine ions replaced with carboxylic acid groups.[9] According to this scheme, cisplatin is released from hydrogels without peptides as free, native cisplatin. When peptides are incorporated and no proteases are active, cisplatin is still released as free, native cisplatin. However, when peptides are incorporated and MMPs are active, cisplatin is released as free, native cisplatin and cisplatin−peptide complex. It has previously been reported that the peptide and MMP-cleaved peptide fragment are not toxic and that the peptide−cisplatin complex is active (toxic).[10]

Cisplatin entrapped within the hydrogel matrix was released to a much greater extent than cisplatin complexed within the hydrogels in the presence or absence of MMPs regardless of PEGDA molecular weight (Figure 2). MMPs

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Scheme 1. Cisplatin−Peptide Complexation Scheme Used in This Study[a]

[a] The peptide is attached to the hydrogel through poly(ethylene glycol) acrylate spacers (wavy lines). It should be noted that cisplatin complexes with any of the three carboxylic acids in the di-aspartic acid containing peptide. Cisplatin may also complex with carboxylic acids in two adjacent peptides in the same polymer chain or in adjacent chains.

were able to release cisplatin depending upon PEGDA molecular weight used to make hydrogels. This was because the molecular weight of PEGDA determines the mesh size, or pore size, of the hydrogel.[11] When low molecular weight PEGDA comprised the hydrogel matrix, hydrogels had a mesh size of 21 ± 4 nm. Cisplatin was released from low molecular weight PEGDA hydrogels with MMP and without MMP to the same extent (Figure 2a) due to the hydrogels having a mesh size smaller than or similar to the size of MMPs. However, when higher molecular weight PEGDAs comprised the hydrogel matrix, hydrogels had a mesh size of 79 ± 7 nm. In this case, cisplatin was released to a greater extent from hydrogels when MMPs were present (Figure 2b) due to the larger mesh size allowing MMPs to freely diffuse in the matrix.

Since cisplatin is highly reactive with many chemical groups,[12] release of a platinum compound is not sufficient

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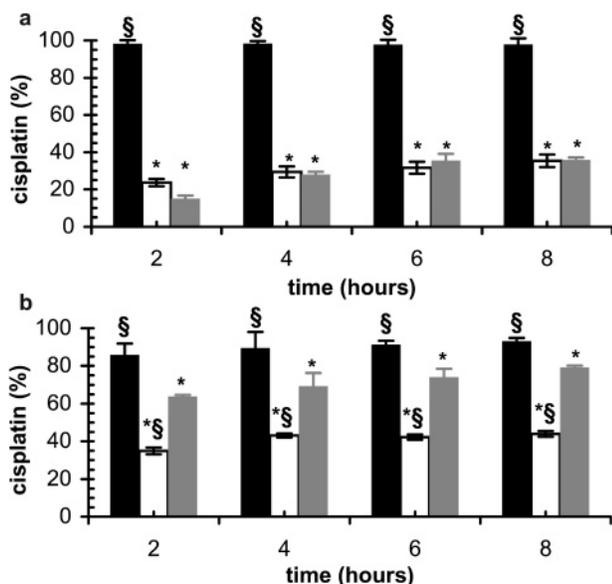
Figure 2. Comparison of cisplatin released from poly-(ethylene glycol) diacrylate hydrogels at several time points. Cisplatin release from short-chain poly(ethylene glycol) diacrylate hydrogels (panel a; PEG $M_n \sim 574$ g/mol) and long-chain poly(ethylene glycol) diacrylate hydrogels (panel b; PEG $M_n \sim 4000$ g/mol). In each panel, cisplatin release is presented in the absence of complexing peptide (black bars), in the presence of complexing peptide with (gray bars) and without (white bars) MMP-9. Data is reported as mean and standard deviation of a minimum of three independent measurements. Statistical significance ($p < 0.05$) is specified by ∗ indicating statistical significant difference from cisplatin entrapped in PEGDA (black bars) and § indicating statistically significant difference from cisplatin release with MMP present (gray bars) at a given time point.

to suggest that this type of delivery mechanism could be used for cancer chemotherapy. Therefore, in vitro activity of cisplatin released from hydrogels was examined. Hydrogels without cisplatin exhibited no toxicity toward glioma cells (Figure 3) or any other cells tested by our laboratory. PEGDA-entrapped cisplatin (with no complexation) exhibited toxicity similar to that of cisplatin added to the solution. However, when cisplatin was complexed with peptides, the activity was reduced for all PEGDA chain lengths due to cisplatin retention in the hydrogel matrix. High molecular weight PEGDA exhibited greater cisplatin activity in this model due to the ability of MMP to diffuse into the hydrogel matrix and activate the drug while MMPs could not enter the lower molecular weight PEGDA hydrogel. This was further confirmed upon the addition of MMP to the culture media. MMP was able to fully recover cisplatin activity to that of cisplatin entrapped in the hydrogel for the large molecular weight PEGDA hydrogels but not for the low molecular weight PEGDA hydrogels. The mesh size difference between the two hydrogels was able to control the
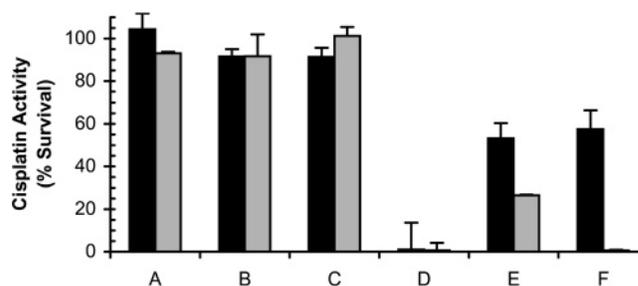
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Figure 3. Comparison of in vitro cisplatin activity following release from poly(ethylene glycol) diacrylate hydrogels. Cell survival is presented for short-chain poly(ethylene glycol) diacrylate hydrogels (black bars; PEG $M_n \sim 574$ g/mol) and long-chain poly(ethylene glycol) diacrylate hydrogels (gray bars; PEG $M_n \sim 4000$ g/mol). (A) PEGDA hydrogels. (B) PEGDA hydrogels with pendant peptide. (C) PEGDA hydrogels with pendant peptide and added MMP-9. (D) Cisplatin-entrapped, but not complexed, PEGDA hydrogels. (E) Cisplatin-complexed PEGDA hydrogels. (F) Cisplatin-complexed PEGDA hydrogels with added MMP-9. Data is reported as mean and standard deviation of a minimum of three independent measurements.

activation of cisplatin when complexed to MMP-sensitive peptides.

This in vitro data is confirmation that MMPs and other extracellular proteases can be utilized to deliver active chemotherapeutic agents to a tumor dependent primarily on the biology expressed by the tumor cells. These results even further suggest that cellular MMPs influence cisplatin release from the devices; activity of cisplatin is greater for large molecular weight PEGDA than small molecular weight PEGDA (Figure 3) despite a similar cisplatin release rate with no supplemental MMP (Figure 2). This is true because most cells release MMPs when in culture and MMPs are released by the cells used.[10] This work can be expanded to other extracellular proteases, and similar results have been observed with both gelatinases, MMP-2 and MMP-9. Not only can these hydrogels be implanted but this type of device could also be formed by locally injecting a prepolymer solution and utilizing a photopolymerization mechanism.[13] Therefore, this type of drug delivery system could be readily adapted to many situations beyond surgical implantation.

In summary, this study suggests that extracellular protease activation of chemotherapeutic agents from hydrogel matrices can be utilized for cancer treatment. To the knowledge of the authors, this is the first report of the use of hydrogel matrices for locally retaining a chemotherapeutic as a hydrogel-prodrug that is activated by naturally occurring extracellular proteases. This is also the first report of control of proteolytic drug release from a hydrogel matrix mediated by the molecular weight between cross-links of the polymer. Although these results are compelling, animal experiments

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are needed to determine if the hydrogel matrices can, in fact, reduce tumor size. A current research focus is development of a stronger complexation (or conjugation) mechanism that will retain the drug until it is released by the proteases and more selectively deliver chemotherapeutic agents from hydrogel using this novel drug delivery paradigm.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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