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Improving matrix metalloproteinase-2 specific response of a hydrogel system using electrophoresis

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ABSTRACT

Matrix metalloproteinases (MMPs) overexpression plays a critical role in cancer invasion and metastasis. We utilized this key feature of tumor microenvironment to develop a disease-stimuli triggered drug delivery system. Poly(acrylic acid) hydrogels were synthesized by UV polymerization and pendant MMP-2 sensitive peptides (Gly-Pro-Leu-Gly-Val-Arg-Gly-Lys) conjugated throughout using EDC/sulfo-NHS chemistry. There were significantly more peptides released in the presence of MMP-2 compared with the control groups. The released peptide fragments were analyzed by HPLC and MALDI-MS and confirmed to be the expected fragments. In order to avoid nonspecific release of nonconjugated (*i.e.* unreacted) peptides, a novel method of electrophoretic washing was developed disrupting the strong electrostatic interactions between the peptides and the pendant groups of the hydrogel. After electrophoresis, the nonspecific peptide release in the absence of MMP-2 was minimized. This newly developed purification system significantly improved the control of release to be in response of the magnitude of the stimuli, *i.e.* MMP. Specifically, peptides were released proportionally to the concentration of MMP-2 present. Now that many of the design parameters have been examined, anticancer drugs will be conjugated to the MMP sensitive peptide linkers with the goal of implantation in a tumor void releasing anticancer reagent in response to elevated level of MMPs.

1. Introduction

In order to overcome the limitation in systemic drug administration, significant effort has been devoted toward developing site, organ or disease specific drug delivery system. Further, drug delivery systems that detect the signals sent out by diseased tissue and release the appropriate amount of drug in response to the signal magnitude, *i.e.* smart drug delivery, have great potential for therapeutic applications (Qiu and Park, 2001). Hydrogels are ideal carriers for therapeutic molecules due to limited inflammation and biologic acceptance due to the similar properties and water content compared to natural extracellular matrix (Peppas et al., 2000). However, most of hydrogel drug delivery systems encapsulate drug molecules by noncovalent bonds and diffusion of the entrapped drug during swelling or shrinking in response to non-specific, physicochemical stimuli (Huynh et al., 2009; Wu et al., 2011) or biochemical ligand binding (King et al., 2009; Miyata et al., 1999). Although drug release can be triggered through volumetric changes, it is challenging to reliably regulate the release profile based on the magnitude of these stimuli beyond subtle changes in diffusion rate.

To overcome the limitation of diffusive release, prodrug like approaches have been employed to selectively release therapeutic molecules in response to biologic signals (Tauro and Gemeinhart, 2005a,b; Tauro et al., 2008; Thornton et al., 2005; Ulijn, 2006; Ulijn et al., 2007; West and Hubbell, 1999). To accomplish the triggered release, chemical conjugation is generally needed either by cross-linking the system or by covalently attaching therapeutic molecules. Several general difficulties lie in conjugating drug molecules with hydrogel through covalent bonds: (1) selection of appropriate chemistry to conjugate drug molecule without affecting therapeutic efficacy, (2) selection of appropriate disease specific stimuli to trigger drug release, and (3) removal of uncoupled molecules after conjugation, without which will lead to non-specific release in the absence of stimuli.

Matrix metalloproteinases (MMPs) have long been associated with many different types and stages of cancer and MMP proteolysis controls homeostasis of the extracellular environment (Overall and Kleifeld, 2006). MMP-2 and MMP-9 can degrade the extracellular matrix (ECM), creating space for cells to migrate (Roy et al., 2006). MMP-2 activation potential is associated with

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metastatic progression in cancer and high level of expression of MMP-2 is present in the invasive and metastatic regions (Forsyth et al., 1998; Hu et al., 2003). MMPs as targets have become more accepted (Vartak and Gemeinhart, 2007) as further understanding of the mechanisms has advanced (Overall and Kleifeld, 2006). Previous research indicated the possibility of utilizing the elevated MMP-2 level to release anticancer reagent conjugated in the poly(ethylene glycol) diacrylate hydrogel through MMP-2 sensitive peptide linker, using hydrogel as an implantable catheter after glioma resection to prevent tumor recurrence (Tauro and Gemeinhart, 2005a,b; Tauro et al., 2008). In these systems, there was clear release upon stimulation by MMP; however, there was significant non-specific release in the absence of MMP. Ideally speaking, the anticancer drug will only be released in response to elevated MMP-2 level, achieving disease stimuli-triggered drug delivery.

Michael-type addition for incorporation of cysteine-containing peptides at the same time as hydrogel polymerization allowed peptides dimerization through disulfide bond formation and other side reactions (Salinas and Anseth, 2008), which led to low drug loading efficiency and non-specific release. To overcome this shortcoming, in this study we first made poly(acrylic acid) hydrogels with UV polymerization and incorporated lysine-containing MMP-2 sensitive peptides by reacting the ε-amine group of lysine with pendant carboxyl groups in hydrogel. This method eliminated free radical side reactions with peptides or drug molecules. To minimize non-specific release when MMP-2 was not present, free peptides were removed by a novel electrophoresis process. The MMP-2 triggered specific release was significantly increased in the presence of MMP-2. Moreover, this drug delivery system was sensitive to the amount of stimuli in the environment, specifically MMP-2 concentration. Higher concentration released significantly more peptide from the hydrogel than the lower concentration. In this study, we optimized a disease stimuli-triggered hydrogel drug delivery system that can adjust the amount of drug released based on the magnitude of stimuli.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) diacrylate (PEGDA, MW 3400 g/mol; Laysan Bio, AL), acrylic acid (99%), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) all from Sigma–Aldrich, USA. Human active recombinant MMP-2 was purchased from Calbiochem, EMD, San Diego, CA. Irgacure 2959 was obtained from Ciba Specialty Chemicals Corp. (Tarrytown, NY). MMP-2 sensitive peptides (methoxycoumarin(mca)-GPLGVRGK) were synthesized by UIC research supply center. HPLC grade acetonitrile, trifluoroacetic acid (TFA), and all the other chemicals were purchased from Fisher Scientific without further purification (Fairlawn, NJ).

2.2. Synthesis of poly(acrylic acid) hydrogel

The crosslinker, PEGDA, was dissolved in water and mixed with acrylic acid (25%, w/v) at the molar ratio of 1:9. The photoinitiator (Irgacure 2959) was used at 1% weight of the total monomer. To initiate the reaction, the mixture was polymerized under 345 nm UV light at an intensity of 2.7 mW/cm^2. The polymerization took place in a mold composed of two glass slides separated by Teflon spacer. The hydrogel film was cut into discs with a biopsy punch (6 mm diameter), followed by washing in deionized water for 24 h to remove the unreacted monomers and initiators.

2.3. Conjugation of MMP-2 sensitive peptide with hydrogel

Aqueous carbodiimide (EDC) coupling was used for conjugating MMP-2 sensitive peptides with the carboxyl groups in hydrogel. To optimize the conjugation conditions, the pH value of the reaction medium and the molar feed ratio between acrylic acid and peptide were varied. Briefly, hydrogels were submerged in MES buffer (at pH 4.7, 5.7, and 6.7), EDC and sulfo-NHS solutions were sequentially added into the MES buffer achieving final concentration of 72 and 36 mM, respectively. Varying amounts of MMP-2 sensitive peptide were added for acrylic acid to peptide molar ratios of 50, 10 and 6 (final concentration 36, 7.2 and 4.3 mM). The reaction volume was maintained at 500 μL across all conditions. The mixture was covered with foil and allowed to react overnight on the shaker. To remove the nonconjugated peptides from the hydrogel, hydrogels were washed with tris buffered saline (TBS/Zn: 10 mM Ca^{2+}, 0.05 mM Zn^{2+} and 0.05% Brij-35; pH 7.6) by changing the buffer every 24 h until equilibrium. To determine total loaded peptide, the hydrogels were then hydrolyzed with 1 mL NaOH (1 N). The peptide content was determined, after titration to neutral pH with 1 N HCl, by measuring the fluorescence intensity at an emission and excitation wavelength of 320 and 410 nm (Molecular Devices SpectraMax Gemini XS, CA) and compared with a standard curve.

2.4. Investigation of the interaction between nonconjugated peptides and hydrogel

Aqueous solutions with varying pH value (pH 2.5, 5, and 7.5), ionic strength (20, 200, and 1000 mM NaCl solution), and polarity index (7.6, 7.7, 8.0 and 10.2; derived from 50% mixtures of water and methanol, ethanol, and acetonitrile, and 100% water) were used to extract the nonconjugated peptides from the hydrogel. The polarity indices were calculated according to Snyder polarity index (Braithwaite et al., 1985). The fluorescence intensity in the extraction solution was determined as previously described with appropriate standard curves in the specific solvent conditions of the experiment.

2.5. Removal of the nonconjugated peptides with electrophoresis

Three 8.5 mm × 8.5 mm wells were formed on 3% agarose gel formed in citric acid-Na_2HPO_4 buffer (pH 2.6). After polymerization and peptide conjugation, solid hydrogels were cut into squares (8.5 mm × 8.5 mm) using a blade to fit the wells formed in the agarose. Hydrogel squares placed in the wells were electrophoresed for 1, 2 and 3 h in citric acid-Na_2HPO_4 buffer (pH 2.6) in BIO-RAD mini horizontal submarine electrophoresis unit. Following electrophoresis, the solid hydrogels were easily recovered. To demonstrate the fluorescence intensity change from electrophoresis, hydrogels were cut with a 6 mm biopsy punch and placed in black 96-well plates. Similarly, the agarose gels were cut with 6 mm biopsy pouch and placed in black 96-well plates. Fluorescence intensity was measured in each sample with fluorometer as previously described (Fig. 4A). Otherwise, the hydrogel squares were taken out of the loading wells for further experiment after removing the nonconjugated peptides from the hydrogel with electrophoresis.

2.6. MMP-2 triggered release

Electrophoresed hydrogels were equilibrated in TBS/Zn buffer for 24 h before the release experiment was initiated. Hydrogels were placed in TBS/Zn buffer in the presence or absence of MMP-2 at concentrations of 9 and 27 nM. Fluorescence intensity of the releasate was measured and all media replaced with new buffer at predetermined time. At each time point, MMP-2 was also added.

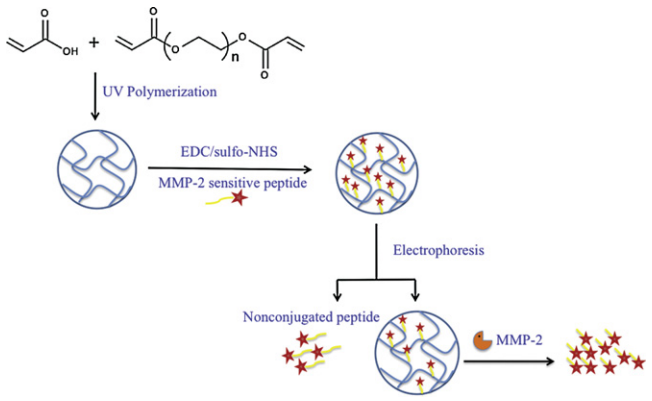


Fig. 1. Schematic illustration of the formation, optimization, and characterization of the MMP-sensitive drug delivery system.

The cumulative peptide-released amount was calculated based upon the peptide in the release media.

2.7. Identification of the released peptides

MMP-2 sensitive peptides were digested in TBS/Zn buffer by MMP-2 for 0.5 h. Both the full-length peptides and MMP-2 digested peptides were analyzed by high performance liquid chromatography (HPLC) on a Waters Symmetry® C18 column (4.6 mm × 150 mm, 5 μm) with a Waters 717 autosampler, and 474 fluorescence detector (Waters, Milford, MA); chromatographic conditions were a linear gradient of 90% water with 0.1% (w/v) TFA and 10% acetonitrile with 0.1% (w/v) TFA inverting over 30 min at a flow rate of 1 mL/min. Peptides were detected at excitation and emission wavelengths of 320 and 410 nm, respectively. The HPLC peaks were fractionated for matrix assisted laser desorption/ionization (MALDI) mass spectrometry (MS).

2.8. Statistical analysis

At least three independent replicates of each experiment were performed. Analysis of variance (ANOVA) was used to test significant difference among groups. Post hoc analysis was performed using Tukey's test when p-value was less than 0.05. GraphPad Prism v.4.0 was used for all the statistical analyses (GraphPad Software, CA).

3. Results and discussion

3.1. Optimization of peptide conjugated amount

MMP-2 sensitive peptides were conjugated to the pendant carboxyl groups brought about by acrylic acid monomers after hydrogel formation (Fig. 1). In order to exploit the conjugation potential of hydrogel for peptides, different hydrogel formulations were examined by increasing the acrylic acid amount while maintaining the carbon–carbon double bond concentration (molar) constant. The acrylic acid and PEGDA molar ratio of 9–1 was used for the remaining experiments where the hydrogels had appropriate tactile stability and the acrylic acid amount was maximal.

The EDC and sulfo-NHS chemistry has optimal efficiency in the pH range of 6–7 (Hermanson, 1996). Expanding upon this knowledge, we designed a 3^2 orthogonal experiment to further optimize the reaction efficiency (Fig. 2). Higher pH and higher peptide feed ratio were significantly correlated with increased peptide conjugation. Based upon the information learned in this optimization experiment, the peptide conjugation reaction conditions were set at pH 6.7 and an acrylic acid to peptide molar ratio of 10:1. This

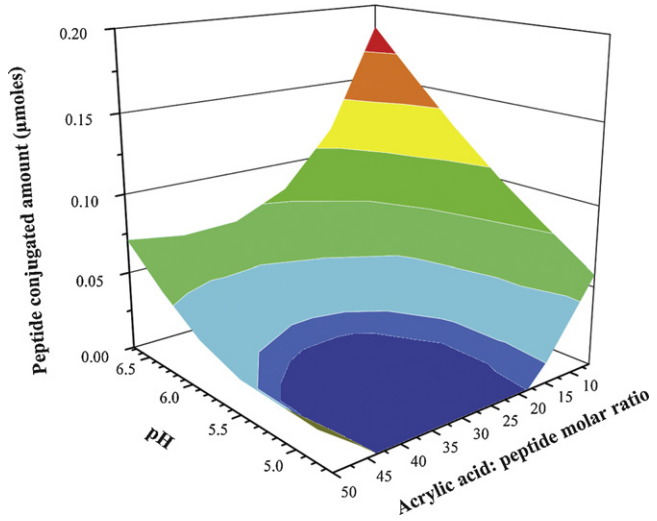


Fig. 2. Response surface plot of MMP-2 sensitive peptide conjugation amount as a function of pH and acrylic acid to peptide feed ratio derived from three independent samples examined at each condition. The optimal conditions are predicted by the red regions ranging to the least optimal conditions in the regions of blue following the color spectrum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

optimal condition is bounded by the original 3^2 orthogonal design and further optimization may be possible.

3.2. Investigation of the interaction between peptide and hydrogel

In order to take advantage of the upregulated expression and activation of MMP-2 in cancer, it is crucial that the non-specific release in the absence of MMP-2 is minimal. However, MMP-2 sensitive peptides were resistant to washing with TBS/Zn buffer, *i.e.* it took about a week to reach equilibrium. This fact intrigued us, and we sought to understand the forces that held free peptides within the hydrogels. We hypothesized that the primary interactions between unreacted peptides and the hydrogel pendant groups to be ionic in nature. As an alternative hypothesis, the hydrophobic portion of the peptide sequence (mca-GPLGV) could cause local peptide aggregation and/or interactions with the hydrophobic poly(ethylene) backbone of the hydrogel. Hydrophobicity was reported to have a major influence on the interaction between a kininogen-derived antimicrobial peptide (GKHKNKGKKNGKHNGWK) and poly(acrylic acid) microgels (Bysell et al., 2010). Based on these analyses, we designed protein extraction conditions with solvents of different pH, ionic strength, and polarity directly testing the primary and alternative hypothesis. Low pH and high ionic strength were most effective in removing peptides from the hydrogel (Fig. 3).

Low pH and high ionic strength conditions disrupted the interactions between peptides and hydrogel, suggesting the interaction is electrostatic attraction. At each of the pHs examined, MMP-2 sensitive peptide carries two positive charges resulting from protonation of guanidinium group and amine group in arginine and lysine, respectively. The pK_a of pendant carboxyl groups in the hydrogel is approximately 5 (Elliott et al., 2004), and thus, these groups are expected to be protonated, *i.e.* uncharged, at pH 2.6, which minimized the electrostatic attraction between peptides and hydrogel. At the higher pHs, there is expected to be significant, if not complete, deprotonation of the pendant groups.

Higher ionic strength (Fig. 3B) had a similar effect as low pH (Fig. 3A) due to electrostatic shielding effect (Israelachvili, 1991). The charge on the groups do not change in this instance, but

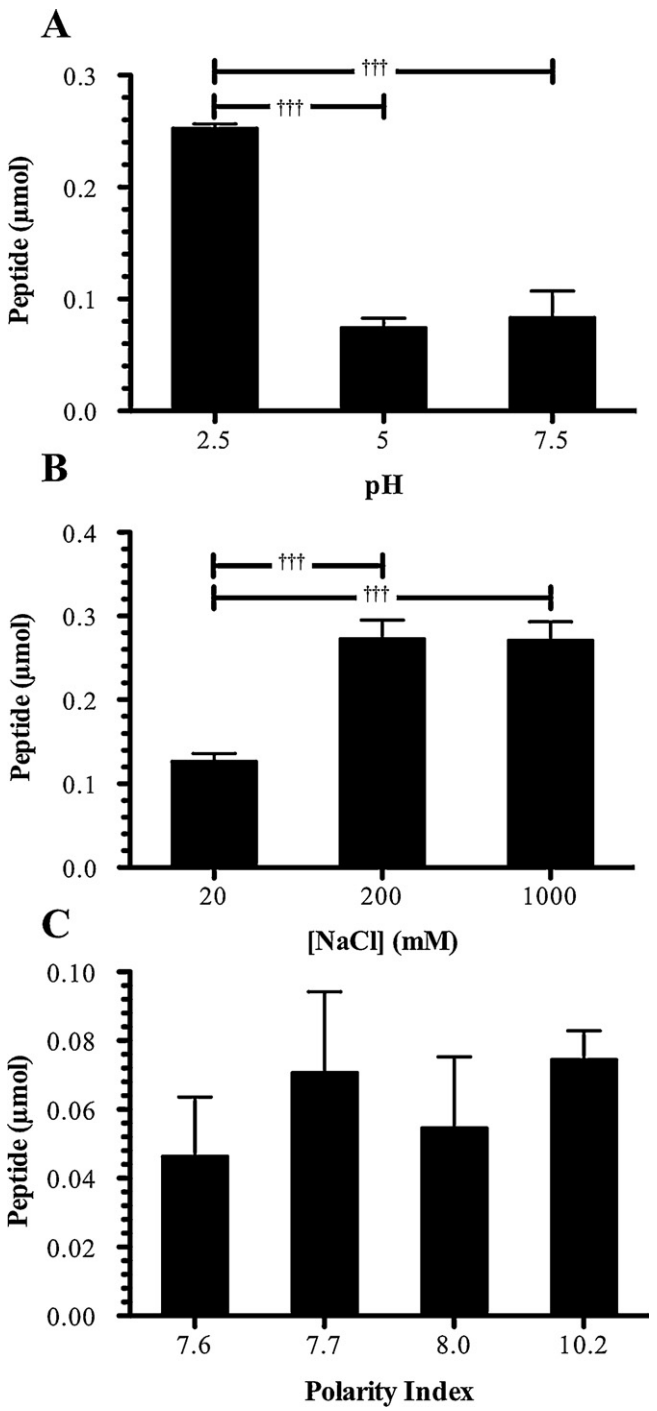


Fig. 3. Peptide extraction capacities under various conditions. Peptide was extracted using aqueous solvents at varying pH (A), different ionic strength (B), and different polarity (C) where greater extraction (Peptide-μmol) indicates more thorough removal of unreacted peptides. Data represent the mean plus or minus (±) the standard deviation of three independent samples where ††† indicates statistical difference between the groups at $p < 0.001$.

a greater number of interacting counterions are present which displace the peptides from the pendant carboxylate ions. Similar observation were made when antimicrobial peptides, $(Ala\text{-}His\text{-}His\text{-}Ala\text{-}His\text{-}Ala)_4$ and $(Ala\text{-}His\text{-}His\text{-}His\text{-}Ala\text{-}Ala\text{-}His\text{-}Ala)_3$, were interacted with poly(acrylic acid) microgels (Bysell et al., 2009). Although both of these observations suggest ionic interactions are present, they do not exclude the alternate hypothesis where significant hydrophobic interactions take place.

Solvents of different polarity did not show significant difference in extracting peptides from the hydrogels (Fig. 3C). Although not conclusive, it would be expected that there would be more, not less extraction with lower polarity index if the interactions within the hydrogels were significantly hydrophobic in nature. Only aqueous mixtures were used to maintain the swollen nature of the hydrogel. There were subtle changes in the swollen state in pure solvents (*i.e.* ethanol, methanol, and acetonitrile), but pure solvents resulted in a significant dehydration making interpretation of results much more difficult. However, this restricted the range of polarity that was possible. Also complicating the interpretation, methoxycoumarin (mca) is not sensitive to environment pH or ionic strength; however, mca is sensitive to environmental polarity. Standard curves made in solvents of different polarity were used to assure that the fluorescence of the observed signal was not the reason for the observations. Based upon the lack of clear solvent effect, it was suggested that the predominant force for the stable interactions was ionic in nature. This also explains why in our prior experiments extensive washes with neutral solutions did not adequately remove unreacted peptides (Tauro and Gemeinhart, 2005a,b; Tauro et al., 2008).

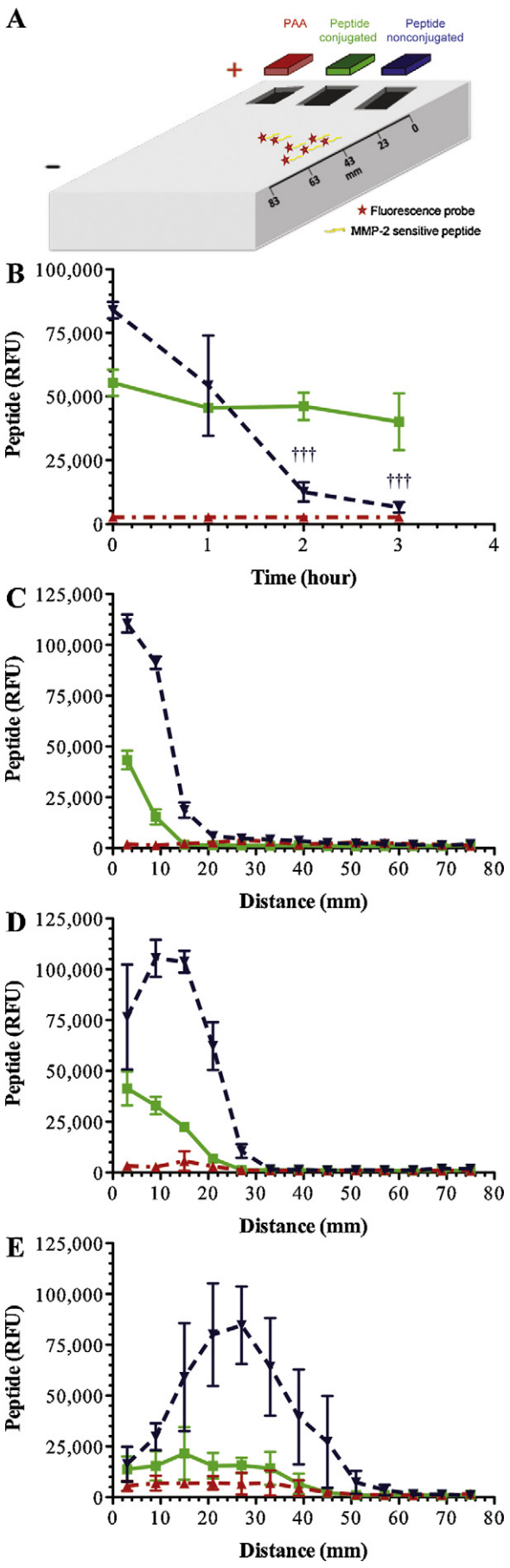
3.3. Removal of nonconjugated peptides with electrophoresis

Based on our understanding of the interaction between MMP-2 sensitive peptides and the pendant groups within hydrogels, we hypothesized that the non-covalently retained peptides could be removed by electrophoresis. Hydrogel (without peptide) and hydrogel with non-covalently incorporated peptide were run as control groups together with peptide conjugated hydrogels to confirm the conditions necessary to drive the peptides from a hydrogel (Fig. 4A). Using this method, the amount of fluorescence within the nonconjugated peptide-containing hydrogels (Fig. 4B) decreased to background within 3 h while the conjugated peptide-containing hydrogels decreased to about 73% of the initial value in 3 h. Clear migration of the peptide into the agarose was measurable (Fig. 4C–E).

This demonstrates that electrophoresis was sufficient to disrupt the interactions between peptides and the hydrogel, which also supports electrostatic interactions between the peptides and the pendant groups within the hydrogel. The peptides migrated toward the negative electrode as time increased (Fig. 4C–E), and the area under the curve, *i.e.* total peptide, from the conjugated peptide-containing hydrogels was much smaller than the nonconjugated peptide-containing hydrogels. There was also a delay in terms of peptides migrating, which could be due to electroendosmosis (Kirby, 2010). Even though peptides were conjugated within the hydrogel matrix, the charged groups still have the tendency to move toward the negative electrode, which causes more anions to flood to the positive electrode, composing a resistant force to retard migration of the nonconjugated peptides. This effect is less pronounced when the peptides are free to migrate.

The utilization of electrophoresis on macroscopic hydrogels could cause inhomogeneous distribution of unbound peptides. Unbound peptides would reside in the hydrogel with an increasing gradient toward the negative electrode. Due to chemical binding to the crosslinked hydrogel network in our system, the ability of bound peptides is restricted. If sufficient time in electrophoresis, all unbound peptides should be removed and few inhomogeneities should be present.

The method of removing nonconjugated peptides with electrophoresis could also be applied to any hydrogel drug delivery system that releases drug molecules by degradation. The non-negligible amount of noncovalently attached drug molecules in the hydrogel limit the specificity of the release obtained and stands in the way of transition from bench to bedside (Brandl et al., 2010). The



difficulty of removing nonconjugated peptides after reaction also challenges researchers working with other drug delivery system such as dendrimers (Hong et al., 2006), micelles (Bae et al., 2003), and nanoparticles (Bae et al., 2010), which are usually overcome by time-consuming dialysis. It is foreseeable that the electrophoresis washing method developed here could also be applied to those systems with minimal adaptation.

3.4. MMP-2 triggered peptide release from hydrogel

The MMP-2 activation from the hydrogel drug delivery system was demonstrated in the presence or absence of MMP-2. MMP-2 concentrations used are within those accepted for demonstrating MMP-2 responsive mechanism *in vitro* (Chau and Langer, 2003; Chau et al., 2004; Sun et al., 2011; Tauro and Gemeinhart, 2005a,b; Terada et al., 2006). It should be noted, however, that the quantitation of MMP is typically complicated by the measurement of both active MMPs and latent MMPs including those bound to native inhibitors (Zucker et al., 1993, 1999). Also, MMPs tend to be locally sequestered at the site of invasion causing significant inhomogeneities in concentration and activity (Moscatelli and Rifkin, 1988).

There was significant MMP-2 sensitive peptide fragment released after 2-h incubation with 27 nM MMP-2 (Fig. 5). Nonspecific release was minimal when MMP-2 was not added which implies minimal nonspecific effect (toxicity or therapy) would be present when the tumor is in a dormant or non-invasive state. In addition, hydrogels release significantly more peptides at higher MMP-2 concentration (27 nM) than at lower concentration (9 nM). The MMP-2 responsive hydrogel drug delivery system is not only responsive to the environment, but adjusts the amount of drug molecules released according to the magnitude of triggering signal.

Peptide therapeutics have drawn substantial attention due to their wide involvement in various biological processes. The technology advancement for production of peptides in large-scale by solid-phase and recombinant DNA technologies has promoted research on peptides as potential drug candidates (Wurm, 2004). However, design of delivery systems for peptides remains challenging due to their sensitivity to degradation during long circulation and lack of accumulating at the disease site (Lu et al., 2006). These shortcomings could have been overcome by incorporating those peptides into implantable devices such as the system demonstrated here a localized smart drug delivery system. For example, a p53-based peptide which inhibits the E3 ubiquitin ligase HDM2 and leads to a therapeutic elevation in p53 levels and overcomes HDMX-mediated cancer resistance *in vitro* and *in vivo* but is limited by degradation and local accumulation (Bernal et al., 2010). This peptide inhibitor could be linked with MMP sensitive peptide linker and conjugated with the hydrogel for localized activity. Cell toxic and lytic peptides could be another class of therapeutic candidates for local delivery (Kawamoto et al., 2011; Yates et al., 2011) particularly due to their global side-effects that could be controlled with a

Fig. 4. Removal of nonconjugated peptides with electrophoresis. (A) Schematic illustration of the electrophoresis apparatus and regions measured. The agarose gel was cut consecutively from the loading well to the edge of the gel tray after electrophoresis. (B) The fluorescence intensity of hydrogel (red; ▲), peptide (nonconjugated) in hydrogel (blue; ▼), and conjugated peptide hydrogel (green; ■) before and after electrophoresis at varying times where ††† indicates statistical significant ($p < 0.001$) compared with the pre-electrophoresis condition. (C–E) Peptide distribution into the agarose gels downstream of the hydrogel (red; ▲), peptide (nonconjugated) in hydrogel (blue; ▼), and conjugated peptide hydrogel (green; ■) after electrophoresis for 1 h (C), 2 h (D), and 3 h (E). Data represent the mean plus or minus (±) the standard error of the mean of three independent samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

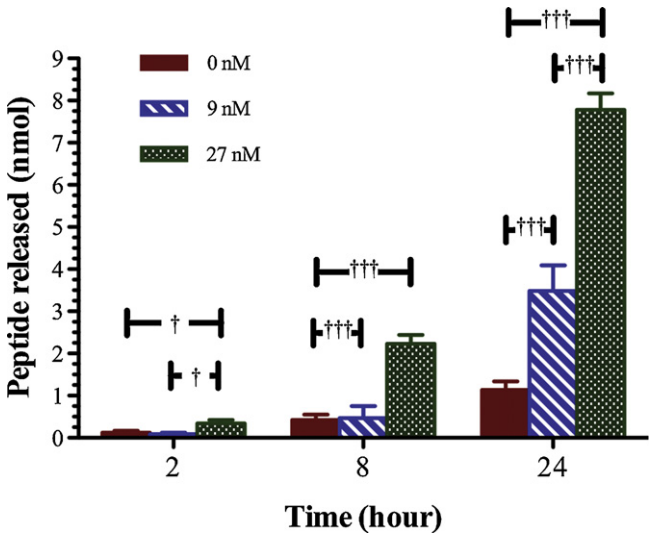


Fig. 5. Effect of MMP-2 concentrations on peptide release from hydrogel. Peptide release over time from hydrogels incubated in 0 nM (red, solid bars), 9 nM (blue, diagonally crossed bars), and 27 nM (green, dotted bars) MMP-2. Data represent the mean plus or minus (±) the standard deviation of three independent samples where †, and ††† indicate statistical difference between the groups at $p < 0.05$, and 0.001, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

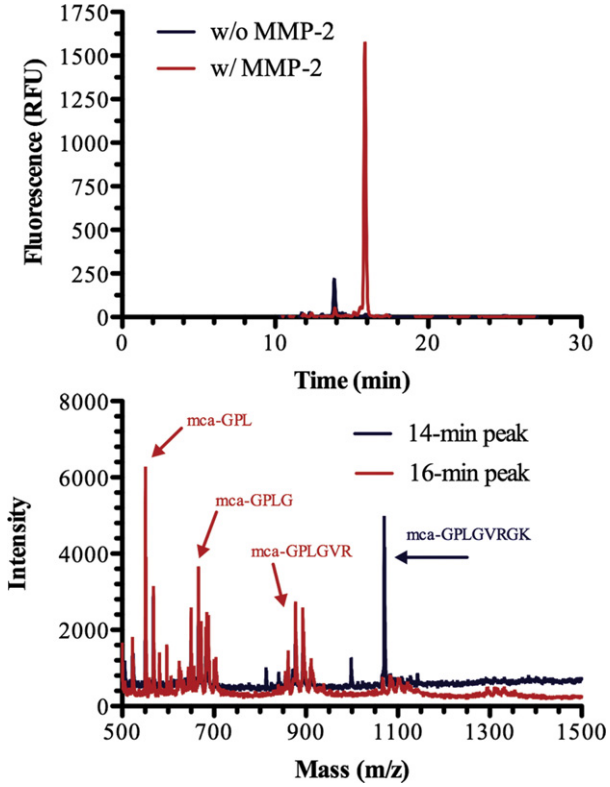


Fig. 6. Identification of peptide release fragments with HPLC and MS. (A) HPLC chromatograms of the cleaved peptide releasate following addition of MMP-2 (red) and nonspecific peptide released without addition of MMP-2 (blue). (B) MS spectrograms of the fractions corresponding to the 14 min HPLC peak of MMP-2 free sample (blue) and the 16 min peak of MMP-2 supplemented group (red). The expected fragments are marked with the one-letter peptide abbreviations and mca for methoxycoumarin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

local activation system. These cell toxic peptides are currently being tested in our further studies of this system. We also are examining cell-binding control and outside-in integrin targeting with these systems (Vartak and Gemeinhart, 2009).

3.5. Identification of peptide release fragments with HPLC and MS

In order to confirm that the increased peptide release in response to MMP-2 was due to MMP-2 cleavage, the identity of the released fragments was examined. The HPLC elution time of the full peptide sequence (14 min) and cleaved peptide fragment (16 min) was confirmed by running the MMP-2 digested peptides and full peptide sequences (data not shown). The releasate following MMP-2 addition had a prominent peak at 16 min (Fig. 6A) and a much less prominent peak at 14 min. When no MMP-2 was added to the incubation media, the releasate had one measurable peak at 14 min. From this, it was suggested that the 14 min peak was the full length peptide while the 16 min peak was indeed the cleaved fragment, but further confirmation was desired.

Mass spectrometry (Fig. 6B) of the fractionated HPLC peaks confirmed that the full peptide sequence predominated in the 14 min fraction. When MMP was added, fraction collected from the 16 min peak was composed of only cleaved peptide fragments. Several peptides were released, all of which can be explained by MMP-2 cleavage. No full length peptides were detected in this fraction. From this, it was clear that the electrophoretic wash method did limit the non-specific release of nonconjugated peptide, and that the fragments released in the presence of MMP were the expected fragments.

4. Conclusion

In the present study, we optimized a MMP-2 responsive drug delivery system. The hydrogel system responds to the magnitude of activating signal, in this case MMP-2. The interaction between nonconjugated MMP-2 sensitive peptides and hydrogel was investigated and the result suggests that the interaction was mainly driven by electrostatic interactions. A unique method of removing nonconjugated peptides with electrophoresis was developed and could be applied to other drug delivery system. The synthesis chemistry is simple, efficient and safe, suggesting the potential for utilization in delivering small molecule drugs or peptides for treatment of proteolytic disease such as cancer, rheumatic arthritis and wound healing.

Acknowledgments

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