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# Self-Assembling Peptide Amphiphile-Based Nanofiber Gel for Bioresponsive Cisplatin Delivery

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**Abstract:** The aim of this study is to develop a bioresponsive cisplatin (CDDP) delivery system with a self-assembling peptide amphiphile (PA) comprising a cell-adhesive matrix metalloproteinase-2 (MMP-2)-sensitive GTAGLIGQRGDS and a fatty acid. A biomimetic CDDP-PA gel was spontaneously formed upon incubating a mixture of CDDP and the PA for 5 h at 37 °C. CDDP-PA gel formation was confirmed by rheological analysis. The structure of self-assembled CDDP-PA nanofibers inside the gel was determined by transmission electron microscopy (TEM). Bioresponsive drug release from the biomimetic gel was demonstrated by in vitro MMP-2-triggered CDDP release. The MMP-2-sensitive CDDP release was dependent on the enzyme concentration in the medium. Enzymatic degradation of the CDDP-PA gel was confirmed by TEM images of the gel degraded in an MMP-2 containing medium. The MMP-2-triggered CDDP release as well as the presentation of RGDS in the gel would potentially provide a spatially and temporally controlled delivery system for targeted anticancer drug delivery.

**Keywords:** Cisplatin; matrix metalloproteinase-2; nanofiber; peptide amphiphile; targeted drug delivery

## Introduction

Peptide amphiphiles (PAs) composed of a hydrophilic peptide head and a hydrophobic tail have evolved as biofunctional molecules that self-assemble into highly ordered nanostructures under certain conditions such as high divalent ion concentration and pH change.[1−3] The self-assembly of PAs leads to the formation of nanofibers, which can then undergo physical cross-linking to form three-dimensional networks to provide gel structures.[4,5] The self-assembled PA nanofiber gels have been extensively studied as potential biomaterials for tissue engineering scaffolds

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because of their ability to mimic the important properties of the natural extracellular matrix (ECM) such as cell adhesion, proliferation, differentiation, and organization.[6−10] To facilitate the recognition of PA gels by cells via specific interactions, cell-adhesive moieties such as RGD (arginine-glycine-aspartic acid) have been incorporated into the hydrophilic peptide within PA.[7,8,11,12] The controlled biodegradation, another important characteristic of the ECM, has also been accomplished by incorporation of an enzyme-degradable peptide sequence into PA.[13−15]

Even though PA-based nanomaterials have been intensively suggested for a drug delivery application, only limited studies have been performed to validate their potential as biomimetic drug delivery vehicles. The main reason for this limitation could be found from the cumbersome synthesis of drug-incorporated PA nanostructures because of the variables and complexities related to drug incorporation and fine-tuning of the spatial arrangement of the peptides to elicit biomimeticity. PAs complexed with the magnetic resonance imaging (MRI) agent, Gd(III), have formed a nanofiber-networked gel, and in vitro MRI could be achieved with the PA−Gd(III) complex-based nanomaterial.[16,17] Additionally, hydrophobic model compounds, pyrene, and poorly water-soluble doxorubicin have been successfully incorporated into the hydrophobic cores of self-assembled PA nanofibers.[18,19] However, the incorporation efficiency of the model drugs into PA nanofiber matrices might not be enough to attain a therapeutic level, since the partition of drug into hydrophobic core of the nanofibers has been limited.

On the other hand, previous works have demonstrated that the PA with cell-adhesive matrix metalloproteinase-2 (MMP-2)-sensitive GTAGLIGQRGDS could mimic characteristic properties of the ECM, and thereby, has great potential for various biomedical applications.[13−15] The RGDS sequence in the PA is a well-known epitope for cell adhesion and specifically binds to upregulated $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins during tumor growth and metastasis.[20,21] It also has been established that MMP-2 is overexpressed in different kinds of invasive tumors and plays a critical role in tumor progression, angiogenesis, and metastasis.[22,23] Taken together, this PA synthesized from GTAGLIGQRGDS could be a desirable biomaterial for a targeted anticancer drug delivery system that releases a cytotoxic drug into the localized tumor region at clinically significant levels.

Cisplatin (*cis*-dichlorodiamineplatinum(II), CDDP) is one of the extensively used chemotherapeutic agents for the treatment of different cancers such as testicular cancer, ovarian cancer, bladder cancer, lymphoma, and glioma.[24] However, severe side effects including acute nephrotoxicity and chronic neurotoxicity have limited the clinical use of CDDP.[25,26] To reduce these side effects and enhance the anticancer activity, the tumor-specific accumulation and controlled release of CDDP at the site have been intended by drug carriers such as microparticles, hydrogels, and


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polymer complexes.[27−29] In these formulations, the controlled release of CDDP and retention of anticancer activity were achieved by the complex formation between CDDP and carboxylic acid groups in peptide sequence or polymers. A PA with cell-adhesive MMP-2-sensitive GTAGLIGQRGDS, as used in this study, bears carboxylic acid groups on aspartic acid (D) and at the C-terminal end of the peptide sequence. Hence, it is anticipated that the PA can self-assemble into nanofibers upon complexation between CDDP and carboxylic acid groups in PA and eventually form a nanofiber-networked gel. The complexation would account for the modulation of surface charge of PA nanofibers as accomplished by a pH change or adding divalent cations such as $Ca^{2+}$. The spontaneous gel formation between CDDP and PA would provide a simple mechanism to develop effective nano drug delivery systems from self-assembling PAs.

The aim of this study is to develop a bioresponsive CDDP delivery system based on spontaneous association of CDDP and PA into a gel upon a physical cross-linking mechanism. The bioresponsive CDDP delivery system is anticipated for spatially and temporally controlled drug release via RGDS-mediated cellular interaction and MMP-2-specific biodegradation after implantation at the tumor site. To simulate the tumor-specific degradation of PA gel and the triggered release of CDDP, an in vitro release study was performed in the presence of MMP-2.

## Experimental Section

Cisplatin (*cis*-dichlorodiamineplatinum(II); CDDP, $M_w$ = 300.05) and type IV collagenase (MMP-2) were obtained from Sigma (St. Louis, MO). Amino acid derivatives were purchased from Novabiochem (San Diego, CA). All other chemicals were of reagent grade and used without further purification.

**Synthesis of Peptide Amphiphile.** Peptide amphiphile (PA) was synthesized by a coupling reaction to acylate the N-terminus of peptide sequence with fatty acid as described elsewhere.[13,15] A 12-amino-acid peptide consisting of an MMP-2-sensitive sequence with a cell-adhesive sequence (GTAGLIGQRGDS) was synthesized using standard Fmoc chemistry on an Advanced Chemtech Apex 396 peptide synthesizer. The N-termini of the peptides were acylated repeatedly with 2 equiv of palmitic acid, 2 equiv of obenzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate (HBTU), and 4 equiv of diisopropylethylamine (DiEA) in dimethylformamide (DMF) for 12 h at room temperature. Cleavage and deprotection of PA were done with a mixture of trifluoroacetic acid (TFA), deionized (DI) water, triisopropylsilane (TIPS), and anisole in the ratio of 91:3:3:3 for 3 h at room temperature. The PA solution was precipitated in cold ether followed by centrifugation and dried under vacuum. The PA was dissolved in DI water at a concentration of 1 wt %, and the pH of the solution was adjusted to 10. The solution was centrifuged after adjusting the pH to 4, and the supernatant was removed. After repetition of this procedure, the recovered PA was dried under vacuum. The synthesized PA ($CH_3(CH_2)_{14}$CONH-GTAGLIGQRGDS-COOH) was characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. The calculated monoisotopic mass of PA was 1369.97.

**Preparation of CDDP-PA Gel.** CDDP-PA gels were prepared by mixing equal volumes of CDDP dispersion and 4% (w/v) PA solution at different molar ratios. The molar ratios of CDDP to PA (MR = [CDDP]/[PA]) were varied as MR = 1, 1.5, and 2, while the final concentration of PA was fixed as 2% (w/v). PA stock solutions were prepared in 50 mM phosphate buffer (pH 7.4) and adjusted to pH 7.4 by addition of 0.1 M NaOH. CDDP dispersions were prepared in DI water with different concentrations. To prepare 100 $\mu$L of CDDP-PA gel with MR = 2, 50 $\mu$L of 17.52 mg/mL CDDP dispersion ($2.92 \times 10^{-6}$ mol) was mixed with 50 $\mu$L of 4% (w/v) PA solution ($1.46 \times 10^{-6}$ mol). For the preparation of PA gel with MR = 1 and 1.5, 8.76 ($1.46 \times 10^{-6}$ mol) and 13.14 ($2.19 \times 10^{-6}$ mol) mg/mL CDDP dispersions, respectively, were used in the same condition. The CDDP-PA mixture was sonicated for 5 min to disperse CDDP homogenously and incubated at 37 °C until the gel was formed.

**Viscoelasticity and Gelation Behavior.** Viscoelasticity and gelation behaviors of the CDDP-PA gels were evaluated using an AR2000 rheometer (TA Instruments, U.K.). The CDDP-PA gels with different molar ratios were prepared as described above, and the corresponding rheometry analysis was performed after 5 h incubation. Dynamic oscillatory shear measurements were performed using an aluminum flat-plate circular disk with a 10 mm diameter. For each frequency sweep performance, preshear was applied for 5 s at 1 Hz, followed by a 5 s period to allow for an equilibrium state. The storage modulus ($G'$) and loss modulus ($G''$) were measured as a function of frequency in the range from 0.1 to 25 Hz at 25 °C.

**Determination of CDDP Concentration in CDDP-PA Gel.** The amount of CDDP loaded in CDDP-PA gels was determined by spectrophotometric *o*-phenylenediamine (OPDA) assay.[29] Briefly, about 5 mg of CDDP-PA gel was collected and subjected to five freeze−thaw cycles to break down the self-supporting gel. The gel solution was then diluted with 50 mM phosphate buffer (pH 7.4) (final volume = 200 $\mu$L), and the sample was mixed with 1 mL of 1.2 mg/mL OPDA dissolved in DMF. The reaction mixture was incubated in a 100 °C water bath for 10 min, and the absorbance of the solution was measured at 703 nm. The amount of CDDP was calculated using the calibration curve

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obtained from standard solutions of free CDDP. The calibration curves were linear with a correlation coefficient of 0.9991 over the concentration range of 5−200 $\mu$g/mL ($n$ = 3).

**Transmission Electron Microscopy (TEM).** The formation of nanofiber networks in the CDDP-PA gels was confirmed by TEM. After a CDDP-PA mixture was incubated at 37 °C for 5 h, 10 $\mu$L of solution sample was placed on a TEM grid for 1 min. The TEM grid sample was then negatively stained for 30 s with 2% (w/v) phosphotungstic acid (PTA), excess staining solution was removed, and the grid was air-dried prior to TEM imaging. To verify the enzymatic degradation of nanofiber networks, TEM imaging was performed after the incubation of CDDP-PA gel in MMP-2 solution (2 and 5 mg/mL in phosphate buffered saline (PBS) containing 0.5 mM $CaCl_2$) at 37 °C for 7 days.

**CDDP Release from CDDP-PA Gel.** The CDDP release from CDDP-PA gel by enzymatic degradation was studied using a Franz-diffusion cell at 37 °C. The CDDP-PA gel with MR = 1.5 was used for release study because this formulation showed a self-supporting property and a high loading efficiency of CDDP. About 50 mg of the CDDP-PA gel was applied to the upper donor chamber, and 500 $\mu$L of MMP-2 solution in PBS containing 0.5 mM $CaCl_2$ was added at the start of the release study. PBS (pH 7.4) containing 0.5 mM $CaCl_2$ was used as receptor fluid, and 250 $\mu$L of samples were collected for 24 h at the designated time points (0.25, 0.5, 1, 2, 4, 8, 12, and 24 h). A cellulose dialysis membrane with a molecular cutoff value of 6000−8000 was present between the donor and receptor chambers. To identify the enzyme concentration-dependent degradation of the CDDP-PA gel, different concentrations of MMP-2 solution (2 and 5 mg/mL) were added to the donor chamber. The release profiles in enzyme-free condition were also investigated for comparison with those from enzymatic degradation. Moreover, the triggered release of CDDP from CDDP-PA gel was studied by addition of MMP-2 solution (5 mg/mL) after 12 h incubation of CDDP-PA gel in PBS solution. The released CDDP was determined by spectrophotometric OPDA assay as previously described.

**Statistical Analysis.** Statistical analyses of data were performed using Student's $t$ test and ANOVA. The differences were considered significant for $p$ values of <0.05.

## Results

**Formation of Self-Supporting CDDP-PA Gel.** CDDP-PA gels were prepared by simple mixing of CDDP dispersion and PA solution. The synthesized PA ($CH_3(CH_2)_{14}$CONH-GTAGLIGQRGDS-COOH) composed of a hydrophobic palmitic acid and a cell-adhesive MMP-2-sensitive sequence was used for the preparation of CDDP-PA gel. After 5 h incubation at 37 °C, the CDDP-PA mixture formed a self-supporting gel at MR = 1.5 and 2. A self-supporting CDDP-PA gel resulted from the cross-linking of highly concentrated nanofibers (Figure 1A). It was proposed that the self-assembly of PAs into cylindrical nanofibers was induced by complexation of Pt in CDDP with the carboxylic group of

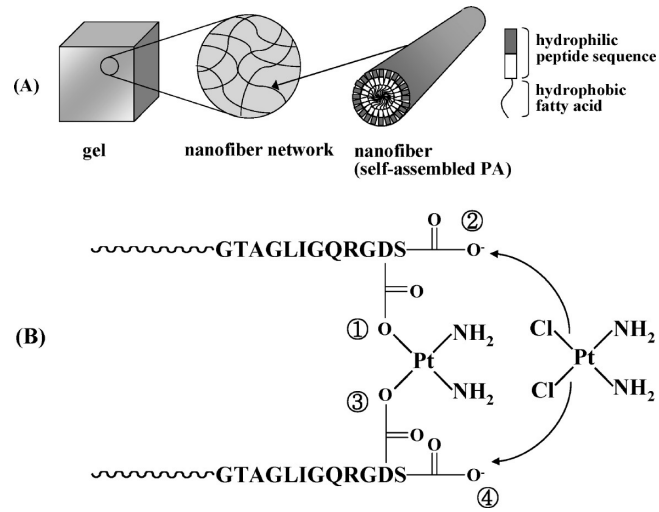


***Figure 1.*** (A) Schematic diagram of a nanofiber-networked gel. (B) Presumed mechanism of the interactions between CDDP and PA. Pt can interact with the carboxylic group of aspartic acid (①,③) or C-terminal end (②, ④). In addition to the complexation mechanism shown (①-Pt-③, ②-Pt-④), ②-Pt-③ and ①-Pt-④ are possible mechanisms between PA and CDDP.

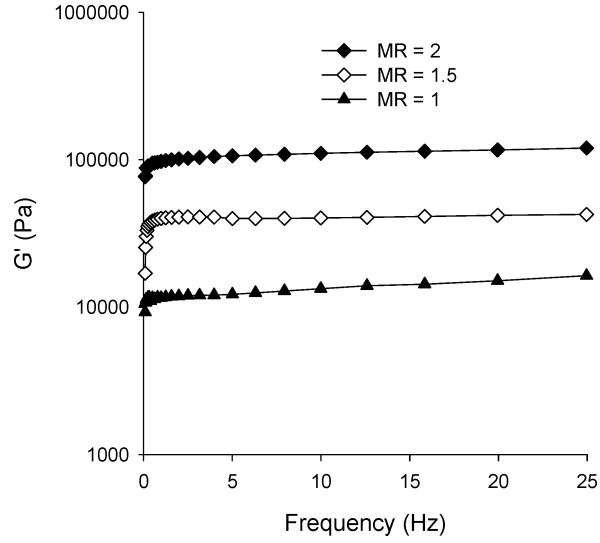


***Figure 2.*** Storage modulus ($G'$) of nanofiber-networked gels with different molar ratios (MR = [CDDP]/[PA]).

aspartic acid or C-terminal end in PA (Figure 1B). In the case of MR = 1, the mixed solution became viscous but did not form a self-supporting gel after incubation at 37 °C for 24 h.

**Rheometric Analysis of CDDP-PA Gel.** To investigate the mechanical properties of the CDDP-PA gels, the viscoelasticity and gelation behaviors were determined by rheometry. The storage modulus ($G'$) with respect to frequency was dependent on the amount of CDDP in CDDP-PA gels. As MR ([CDDP]/[PA]) increased, the storage modulus of CDDP-PA gels increased (Figure 2). The storage moduli at 2 Hz of CDDP-PA gels with MR = 1.5 and 2 were 3.4- and 8.5-fold higher than that with MR = 1, respectively. The loss modulus ($G''$) of CDDP-PA gels also

***Table 1.*** Ratio of Storage Modulus to Loss Modulus ($G'/G''$) and Loading Efficiency of CDDP-PA Gels at Different Molar Ratios

| molar ratio ([CDDP]/[PA]) | $G'/G''$ [a] | self-supporting gel formation | loading efficiency (%) [b] |
|---|---|---|---|
| 2 | 14.18 | yes | 87.1 ± 3.2 |
| 1.5 | 4.97 | yes | 98.7 ± 5.8 |
| 1 | 3.42 | no | |

[a] Ratio of storage modulus ($G'$) to loss modulus ($G''$) at 2 Hz. [b] Results are expressed as the mean ± SD ($n = 3$).

increased as a function of MR, but the difference between MR = 1.5 and MR = 2 was not large (data not shown). The ratio of storage modulus to loss modulus ($G'/G''$) was used to characterize gel structure, and $G'/G'' = 1$ is regarded as a critical point of gelation. At 2 Hz, the ratios of storage modulus to loss modulus ($G'/G''$) of all CDDP-PA gels exceeded 1 (Table 1). Even though CDDP-PA gel with MR = 1 showed the relatively high value of $G'/G''$ (= 3.42), it did not show a self-supporting property. It was observed that $G'/G''$ values of CDDP-PA gels with MR = 1.5 and 2 were 4.97 and 14.18, respectively, and these two formulations formed the self-supporting gels.

**Loading Efficiency of CDDP in PA Gel.** The loading efficiency (%) of CDDP was calculated by the following equation.

$$\text{loading efficiency (\%)} = \frac{\text{mass of CDDP in PA gel}}{\text{mass of CDDP added in formulation}} \times 100$$

The loading efficiencies of CDDP in PA gels with MR = 1.5 and 2 were 98.7% and 87.1%, respectively (Table 1). From the results, the PA gel with MR = 1.5 achieved a complete CDDP loading. In the case of CDDP-PA gel with MR = 2, the precipitate of CDDP was found at the bottom of the tube during the formation of the self-supporting gel. It was assumed that the precipitated CDDP caused an incomplete loading of CDDP in the PA gel. However, the concentration of CDDP in PA gel with MR = 2 was 7.63 mg/g, which is 3 times higher than the water solubility of CDDP (2.53 mg/g). The loading amount of CDDP in PA gel with MR = 1.5 was 6.49 mg/g.

**TEM Image of CDDP-PA Gel.** The self-assembly of the PA into nanofibers and the nanofiber networks in the PA gel were observed by TEM (Figure 3). The nanofibers were identically formed in both formulations by addition of CDDP into PA solution. TEM images exhibited that the CDDP-PA gels were composed of nanofibers of 8−10 nm in diameter and several micrometers in length. Moreover, the physical cross-linking of the self-assembled nanofibers was pronounced in the PA gel, and these structures coincided with the proposed mechanism of gel formation (Figure 1A). The only difference between the two formulations was the density of cross-linking in nanofiber networks. The CDDP-PA gel with MR = 2 showed more close-packed structures compared to that with MR = 1.5. The densely cross-linked structures of nanofibers in CDDP-PA gel with MR = 2 were in accordance with its higher storage modulus implying greater gel strength.

**In Vitro Release of CDDP from CDDP-PA Gel.** In order to determine the CDDP release from CDDP-PA gel by the enzymatic degradation of the MMP-2-sensitive sequence, in vitro release studies were performed in the presence of MMP-2. As shown in Figure 4, the release of CDDP from CDDP-PA gel was concentration-dependent. In enzyme-free PBS, 45% of CDDP was released from CDDP-PA gel within 24 h. This unexpected high release of CDDP in enzyme-free conditions can be attributed to the initial burst release. More than 28% of CDDP was released within 4 h, and then 17% of CDDP was slowly released over 20 h. The CDDP release rate was markedly increased by the addition of MMP-2 solution in release media. At 2 and 5 mg/mL MMP-2 solution, the amounts of released CDDP for 24 h were 72% and 85%, respectively. Although the release profile at an early stage in MMP-2 solution was influenced by the initial burst release, the enzymatic degradation of the nanofiber-networked gel was the main mechanism of release. Compared with 28% of CDDP release in enzyme-free conditions for 4 h, 50% and 67% of CDDP release in MMP-2 solution (2 and 5 mg/mL) at the same time interval showed that the MMP-2-sensitive sequence in PA gel was susceptible to enzymatic cleavage. The CDDP-PA gel underwent concentration-dependent enzymatic degradation in that all points from the release profiles for 5 mg/mL collagenase are significantly different from those for 2 mg/mL collagenase ($p < 0.05$).

The triggered release of CDDP from CDDP-PA gel was observed by the addition of 5 mg/mL MMP-2 solution (Figure 5). Until 12 h, the amount of released CDDP was only 46%, which was similar to that of previous studies in PBS. Upon addition of MMP-2, the amount of released CDDP gradually increased and reached up to 85% within next 12 h. These results demonstrated that CDDP could be released from the CDDP-PA gel by enzymatic cleavage of the MMP-2-sensitive sequence.

**TEM Image after Enzymatic Degradation of CDDP-PA Gel.** The enzymatic degradation of nanofiber networks by proteolytic activity of MMP-2 was characterized by TEM imaging after the incubation of CDDP-PA gel with different concentrations of MMP-2 (2 and 5 mg/mL) at 37 °C for 7 days. There was no noticeable change in the physical appearance of CDDP-PA gel after 7 days of incubation in MMP-2 solution. It is assumed that the cleavage at the MMP-2-sensitive sequence in PA did not cause an immediate breakdown of the nanofiber-networked gel. The slight decrease in the volume of CDDP-PA gel was observed after 7 days, but the change was too small to be measured. From the in vitro release study, however, it was evident that the specific peptide sequence was cleaved by the enzyme and the nanostructure of the CDDP-PA gel changed. From the TEM images, the structural change in nanofiber-networked gel after enzymatic degradation was verified (Figure 6). The self-assembled nanofibers in the enzyme solution were degraded into shorter fragments, typically observed as several

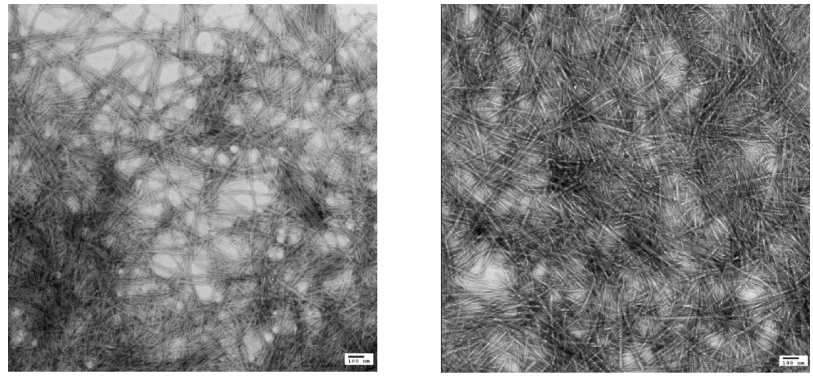


***Figure 3.*** TEM images of nanofiber-networked CDDP-PA gels (scale bar, 100 nm): (A) MR = 1.5 and (B) MR = 2 (MR = [CDDP]/[PA]).

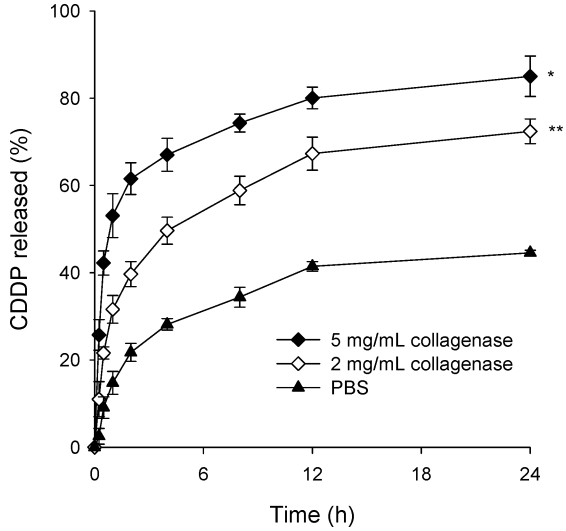


***Figure 4.*** Release profiles of CDDP from CDDP-PA gels at different concentrations of type IV collagenase (MMP-2) solution. The release study was performed with Franz diffusion cells at 37 °C, and the enzyme solution was prepared in PBS containing 0.5 mM $CaCl_2$. (*) All points are significantly different from those of 2 mg/mL collagenase ($p < 0.05$). (**) All points are significantly different from those of PBS ($p < 0.05$). The results are expressed as the mean ± SD ($n = 3$).

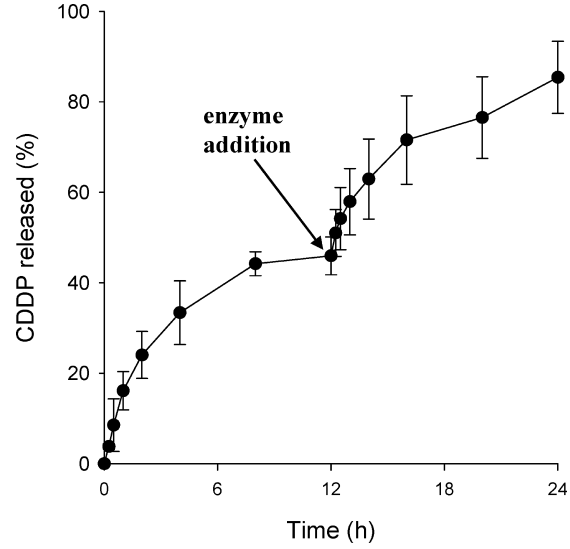


***Figure 5.*** MMP-2-triggered CDDP release from CDDP-PA gels. The replacement of release media from MMP-2-free PBS to a medium containing MMP-2 at a concentration of 5 mg/mL of type IV collagenase (MMP-2) solution induced rapid CDDP release. The release test was performed with Franz diffusion cells at 37 °C, and PBS was replaced with enzyme solution after 12 h. The results are expressed as the mean ± SD ($n = 3$).

hundred nanometers or less. On the other hand, the gel in PBS solution showed no structural changes even after incubation for 7 days at 37 °C.

## Discussion

For successful anticancer therapy, chemotherapeutic agents should be efficiently delivered to cancer cells. Targeted anticancer drug delivery has been accomplished by intelligent drug delivery system (DDS). The intelligent DDS frequently represents targeting ligands specifically directed to the binding sites on cancer cells and releases the incorporated drug in response to the tumor-induced environmental conditions such as pH changes and enzymes.[30−32] In the previous studies, PAs with similar chemical structures to the PA synthesized for this study have been applied for cell encapsulation.[13,15] The self-assembly of the PA into nanofibers resulted in a biomimetic nanostructured gel which presented cell-adhesive RGDS ligands on its surface and underwent cell-mediated degradation in the presence of MMP-2. These characteristics of the PA gel mimic the

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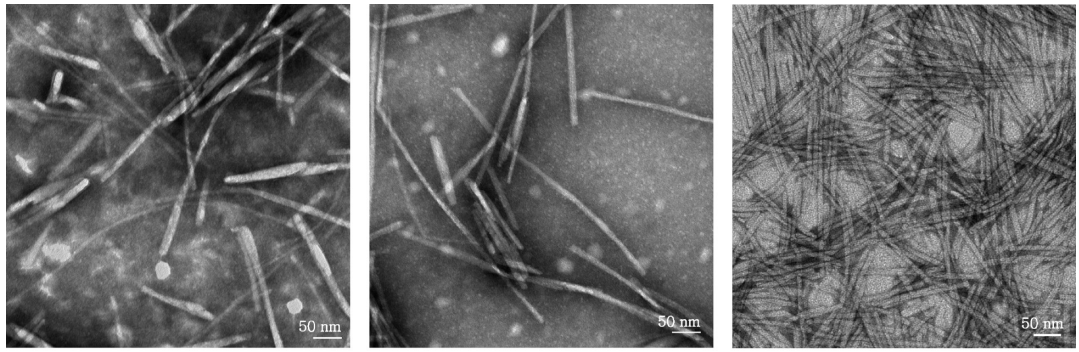


***Figure 6.*** TEM images of nanofiber networks after degradation in different concentrations of type IV collagenase (MMP-2) solution or PBS (scale bar, 50 nm): (A) 2 mg/mL, (B) 5 mg/mL, and (C) PBS (control). CDDP-PA gels were prepared at MR = 1.5 (MR = [CDDP]/[PA]) and incubated in different concentrations of enzyme solution or PBS for 7 days.

natural ECM, thus providing a biomimetic scaffold for tissue engineering applications. In addition, the characteristics of the PA offer a potential for the development of a targeted anticancer drug delivery vehicle.

A CDDP-PA gel formed via physical cross-linking of self-assembled PA nanofibers is able to function as a reservoir for anticancer drug. Furthermore, the MMP-2-sensitive peptides in the PA would be substrates for the protease produced by cancer cells. The gel was designed to release CDDP upon cancer cell-specific matrix degradation. After enzymatic degradation, the integrin-binding RGDS located at the periphery of nanofibers would guide the released CDDP-peptide complex to integrin receptors overexpressed in cancer cells.

Self-supporting CDDP-PA gels were successfully formulated by the presumable complexation of Pt with carboxylic groups in the PA. In general, the self-assembly of PAs into nanofibers might be induced by the minimization of repulsive anionic charges in their structure. Addition of divalent ion or pH modulation has been effective for the self-assembly of PAs into nanofibers. Formation of nanofibers from PAs could also be weakly initiated by cell culture media and physiological fluid.[3,33] However, the formation of PA nanofibers and their subsequent transformation into self-supporting gel by a drug-induced mechanism has never been investigated thus far. CDDP-induced gelation of the PA required 5 h incubation at 37 °C after CDDP addition, which was a slower process than calcium-mediated gelation. The slow complexation between CDDP and carboxylic acids has been pronounced in previous literature.[27−29,34,35] The complete complexation usually requires overnight to 72 h, since the substitution of chlorine with carboxylic groups on Pt in CDDP is not a prompt reaction. The relatively rapid gelation observed in our study might be due to the high concentration of CDDP and PA solution, mechanical agitation for good mixing, and an ordered arrangement of PA for easy interaction with CDDP. These factors might synergistically contribute to the effective interactions between CDDP and carboxylic groups in the PA.

The hydrophobic tail of PA aggregated into nanofibers while the interactions of CDDP with carboxylic acid could neutralize the negative charge in the PA. The synthesized PA formed cylindrical micelle structures because the hydrophilic peptide headgroup is bulkier than the narrow single chain hydrophobic tail. The alkyl chain in the PA, $CH_3(CH_2)_{14}CONH-$, provides a hydrophobic driving force for self-assembly. Moreover, glycine (G), alanine (A), and leucine (L) residues in the PA can reinforce hydrogen bonding between peptides to stabilize cylindrical nanofiber structures and to prevent the formation of spherical micelles, which are also typically observed in the self-assembly of amphiphilic molecules.[5,11,36] Once nanofibers were formed, free CDDP or CDDP interacting with one carboxylic group could complex with carboxylic groups on the adjacent nanofibers. These interactions result in physical cross-linkings between nanofibers and thus finally form a reinforced nanofiber gel. TEM images of CDDP-PA gels exhibited the cross-linked-nanofiber networks, which were similar to those of calcium-induced PA nanofiber gels. The gels exhibited greater values of storage moduli ($G'$) than calcium-induced PA gels, indicating that more stable gels were formed. Greater $G'$ also suggested strong interactions between CDDP and carboxylic groups of the PA favorable for the reinforcement of nanofiber networks. CDDP-PA mixtures at MR below 1 did not result in a self-supporting gel although its

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$G'/G''$ ratio was greater than 1. In this text, self-supporting gels are defined as those that remain attached to the bottom of the vial for at least 30 s with little or no observable viscous flow.[3]

In this study, GTAGLIGQ was incorporated into the PA as an MMP-2-specific peptide of which the cleavage site is expected to be between glycine (G) and leucine (L).[37,38] The incorporation of the MMP-2-specific sequence in the nanofiber resulted in proteolytic degradation of the network, enabling the bioresponsive release of CDDP−peptide complexes. The OPDA assay used in this study is ideal for the determination of CDDP because OPDA specifically reacts with the free amine group in CDDP and avoids any presumable analytical error associated with carboxylic groups in the peptide sequence. The CDDP release from CDDP-PA gel showed an initial burst release as 28% of CDDP was released within 4 h in PBS. The burst release of CDDP during the early stage can be explained by CDDP entrapped in the gel structure but not complexed with the PA. CDDP entrapped as a free molecule in the gel was quickly diffused into the release media, and consequently, the initial burst release was observed. However, the increased CDDP release in the presence of MMP-2 was also observed following the initial burst release. The increase in the CDDP release was dependent on MMP-2 concentration. Additionally, triggered CDDP release upon a medium change from MMP-2-free PBS to MMP-2-containing medium indicated that CDDP release from the nanofiber gel could be controlled by signals from biological changes. TEM images clearly evidence the degradation and structural change of CDDP-PA gels by MMP-2. It was found that the structure of CDDP-PA gel was changed into a sparse network of shortened nanofibers after enzymatic degradation. The structural change of CDDP-PA gel after enzymatic degradation was similar to that of calcium-induced PA gels incubated in the presence of collagenase.[13] Calcium-induced PA gels with an MMP-2-sensitive sequence showed substantial weight loss as well as defects in the nanofiber packing after enzymatic degradation.

To be cytotoxic to cancer cells, CDDP incorporated in the nanofiber-networked gel should be released by the substitution of two carboxylic acid ligands with $H_2O$ molecules. The aqueous complexes of CDDP can directly interact with DNA and express anticancer activity by interfering with cell division.[39] During the process, the released CDDP is still holding the intergrin-binding RGDS with a presumable structure of LIGQRGDS-CDDP-LIGQRGDS. The estimated molecular weight of CDDP/peptide adducts will be less than 2000, which is favorable for receptor-mediated uptake by cancer cells. The current CDDP-based cancer chemotherapies are predominantly mediated by intravenous infusions or intraperitoneal injection. The repeated high doses of CDDP via current regimens cause systemic nephrotoxicity and neurotoxicity. The bioresponsive CDDP-PA delivery system developed from the biomimetic PA would reduce the toxicity of CDDP via spatially and temporally controlled CDDP release.

## Conclusion

A bioresponsive CDDP delivery system was developed from PA with integrin-binding and MMP-2-sensitive peptide by CDDP-induced self-association of PA nanofibers. Self-assembly of the PA into nanofibers of 8−10 nm in diameter and physical association of the nanofibers in the PA gel were observed by TEM images. The amounts of CDDP loaded in the PA gel were approximately 3- and 2.5-fold greater than the aqueous solubility of CDDP at MR = 2 and 1.5, respectively. CDDP release from the PA gel was triggered by the cleavage of the MMP-2-sensitive sequence in the PA and was dependent on the concentration of the enzyme. The biomimetic PA composed of MMP-2-sensitive and integrin binding peptide provides great potential for the development of a bioresponsive anticancer drug delivery system.

## Abbreviations Used

CD, circular dichroism; CDDP, cisplatin (*cis*-dichlorodiamineplatinum(II)); ECM, extracellular matrix; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MMP-2, matrix metalloproteinase-2; MRI, magnetic resonance image; OPDA, *o*-phenylenediamine; PA, peptide amphiphile; PBS, phosphate buffered saline; Pt, platinum; TEM, transmission electron microscopy.

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