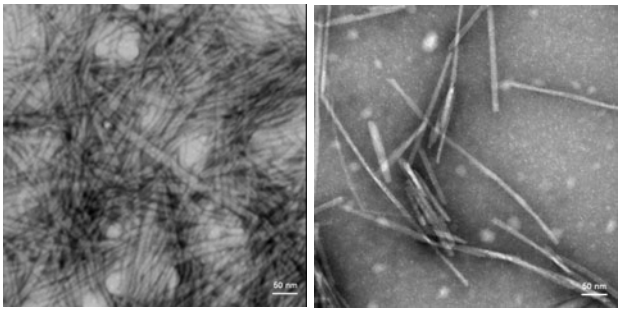
Self-assembling Peptide Amphiphile-based Nanofiber Gel for Bioresponsive Cisplatin Delivery

Seongbong Jo[1], Jin-Ki Kim[1], Joel Anderson[2], Ho-Wook Jun[2], Michael A. Repka[1]
[1] Department of Pharmaceutics, School of Pharmacy, The University of Mississippi,
[2] Department of Biomedical Engineering, University of Alabama at Birmingham

Statement of Purpose: Peptide amphiphiles (PAs) composed of a hydrophilic biomimetic peptide sequence and a hydrophobic alkyl chain have been extensively studied as biomaterials which mimic extracellular matrices [1-3]. Although in situ formed PA gels have been intensively examined for cellular engineering, their applications for drug delivery have been limited thus far. The aim of this study is to develop a bioresponsive cisplatin (CDDP) delivery system with a biomimetic peptide amphiphile (PA). The self-assembling PA comprised of RGDS, MMP-2-sensitive GTAGLIGQ and a fatty acid has been investigated for spontaneous gel formation via complexation with CDDP. MMP-sensitive CDDP release from the PA gel has been examined to simulate tumor-responsive CDDP release in vitro.

Methods: A peptide consisting of RGDS and MMP-sensitive GTAGLIGQ was synthesized by standard Fmoc-chemistry. PA was successfully synthesized by a coupling reaction to acylate the N-terminus of the peptide with palmitic acid. CDDP/PA gels were prepared by mixing equal volumes of a CDDP dispersion and a 4% (w/v) PA solution at different molar ratios. The molar ratios of CDDP to PA (MR = [CDDP]/[PA]) were 1, 1.5 and 2, while the final concentration of PA was adjusted to be 2% (w/v). The amounts of CDDP incorporated in CDDP/PA gel were determined by spectrophotometric *o*-phenylene-diamine (OPDA) assay. Gelation behavior of the CDDP/PA and viscoelasticity of the resulting gel were determined by rheometry. The ratio of storage modulus to loss modulus (G′/G″) of the PA gel with different MR was also determined by a rheometer. The structures of CDDP/PA gels were confirmed by transmission electron microscopy (TEM). The CDDP/PA gel at an MR of 1.5 was used for release study due to favorable mechanical property and CDDP loading. MMP-2-sensitive CDDP release were examined by in vitro CDDP release in Franz diffusion cells using PBS and release media containing MMP-2 at concentrations of 2 and 5 mg/mL. Additionally, an alternating challenge of MMP-2 at a concentration of 5 mg/mL after 12 h-CDDP release in PBS was performed to observe the CDDP release triggered by MMP-2. The structural changes in the degraded gel were also observed by TEM.

Results: Self-supporting CDDP/PA gels were formed at MRs of 1.5 and 2 after 5 h incubation of CDDP/PA mixture at 37°C. The percentages of CDDP loading in PA gels at MRs 1.5 and 2 were 98.7% and 87.1%, respectively. Rheometric analysis has also demonstrated gel formation in a mixture of CDDP and the PA. At a dynamic oscillatory shear frequency of 2 Hz, the ratio of storage modulus to loss modulus (G′/G″) for all CDDP/PA gels exceeded 1. As MR increased, the storage modulus of CDDP-PA gel increased. Storage moduli of the CDDP-PA gels with MR = 1.5 and 2 were 3.4 and 8.5-fold greater than that with MR = 1, respectively. TEM images exhibited that the CDDP/PA gels were composed of the nanofibers of 8-10 nm in diameter and several micrometers in length. In addition, physical crosslinking of the self-assembled nanofibers was pronounced in the PA gel (Fig. 1A).



(A) (B)

Figure 1. TEM images of nanofiber-networked CDDP-PA gels with MR = 1.5 at preparation (A) and after enzymatic degradation (B).

CDDP release from the CDDP/PA gel was dependent on MMP-2 concentration (Fig. 2A). In PBS, only 45% of incorporated CDDP was released within 24 h. However, the amounts of released CDDP in 24 h increased to 72% and 85% at MMP-2 concentrations of 2 and 5 mg/mL, respectively. Fig. 2B exhibits MMP-2-triggered CDDP release from the PA nanofiber gel. Moreover, the MMP-2-sensitive gel degradation resulted in structural changes of CDDP-PA gels as shown in Fig. 1B.

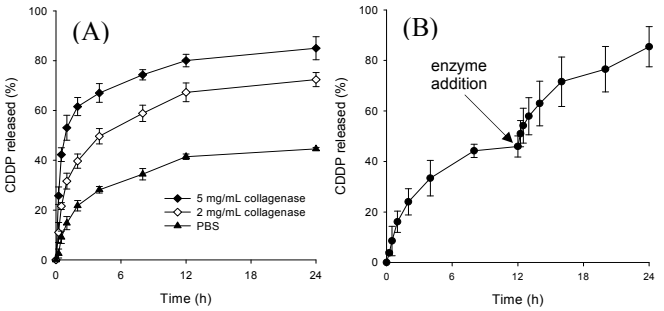


Figure 2. In vitro CDDP release from CDDP/PA nanofiber gels at the different MMP-2 concentrations (A) and triggered CDDP release upon an alternating challenge of MMP-2 at concentration of 5 mg/mL after 12 h pretreatment of PBS (B).

Conclusions: CDDP-loaded nanofiber gels were prepared from a PA with RGDS, MMP-2-sensitive GTAGLIGQ and palmitic acid. The CDDP delivery system based on the self-assembling PA nanofibers may find applications as a bioresponsive drug delivery system for cancers.

References

1. Hosseinkhani H. Biomaterials 2006;27;4079-4086.
2. Sargeant TD. Biomaterials 2008;29;161-171.
3. Jun HW. J Biomater Sci Polym Ed. 2008;19; 665-676.