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ARTICLE TYPE

Controlling the width of nanosheets by peptide length in peptoid-peptide biohybrid hydrogels

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Through changing the peptide length, the width of self-assembled nanosheets can be controlled in peptoid-peptide biohybrid hydrogels.

In the late 1980s, peptoids were exploited as synthetically convenient, peptidomimetic molecules for combinatorial drug discovery and therapeutics.[1] The side chains of peptoids connect to the nitrogen of amides while those of peptides link the α-carbons.[2] Such structural change in peptoids renders their resistance to degradation by proteases. Due to the chemical similarity to peptides, better stability in biological systems, and bioactivity, peptoids have gained extensive research interests.[3] Recent research interest about peptoid moves to the development of self-assembling peptoids and the study of their functions.[4] For example, Zuckermann and co-workers found that peptoids with a special secondary structure of Sigma-stand could self-assemble into nanosheets, which could serve as 2D templates for mineral growth.[5] Furthermore, they discovered that peptoids with more than 12 residues could form more stable nanosheets than shorter peptoids.[6] Meanwhile, the longer peptoids formed nanosheets with flatter structure and higher thickness. Compared with the strategy to control the thickness of self-assembled nanosheets, the one to control the width of nanosheets of peptoids remains as a challenge.

Molecular hydrogels of peptides are promising biomaterials[7] for drug delivery,[8] cancer cells inhibition,[9] regenerative medicine,[10] and detection of important analytes.[11] However, the stability of peptide-based hydrogels in biological system is needed to be improved because peptides are easily degraded by digestion enzymes. We recently reported several molecular hydrogelators of peptoid–peptide conjugates with superior stability against enzyme digestion of proteinase K and good biocompatibility to different cells.[12] In the study, we also found that a peptoid-peptide biohybrid of F'F'F'F'GRGD could self-assemble into nanosheets. We opted to test whether we could change the self-assembling behavior and gelation property of F'F'F'F'GRGD by variation of the number of glycine (G) between the peptoid F'F'F'F' and the peptide RGD. We finally found in this study that the mechanical property of resulting gels and more importantly, the width of self-assembled nanosheets formed by these peptoid-peptide biohybrids (F'$_4$G$_n$RGD, n = 0-3) could be controlled by the variation of the number of G.

Similar to our previous strategy to prepare peptoid-peptide biohybrids and peptides,[12,13] we prepared the designed compounds using standard solid phase peptide synthesis and then used reverse phase high performance liquid chromatography (HPLC) to purify the compounds. After the successful synthesis of our designed compounds, we tested their gelation ability by the invert-tube method. As shown in Fig. 1, through a heating–cooling process, the four compounds we designed could form gels at a concentration of 1.0 wt% in phosphate buffer saline (PBS) buffer solutions (pH=7.4, G$_0$gel, G$_1$gel, G$_2$gel, and G$_3$gel for F'$_4$RGD, F'$_4$GRGD, F'$_4$GGRGD, and F'$_4$GGGRGD, respectively). The minimum gelation concentration of four compounds was similar (around 0.4-0.5 wt%), and the resulting four gels showed similar appearance with slightly opaque property.

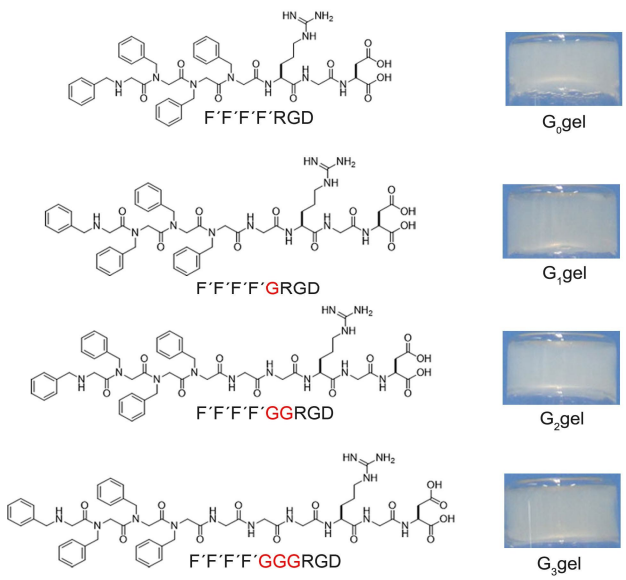

Fig. 1. Chemical structures of molecular hydrogelators of peptoid-peptide conjugates and optical images of their corresponding gels containing 1.0 wt% of compounds in PBS buffer solutions (pH=7.4)

We characterized the self-assembled nanostructures in four gels using transmission electron microscopy (TEM). Fig. 2 showed the results, and the four peptoid–peptide biohybrids self-assembled into nanosheets in hydrogels (1.0 wt%). However, it could be clearly seen that the width of nanosheets was significantly different. We randomly chose 50 fibres in each sample to determine the width of the nanosheets. The average width of nanosheets in G$_0$gel was the largest, which was about

277 ± 51 nm. The width of nanosheets in G_1gel, G_2gel, and G_3gel was 251 ± 32, 195 ± 20, and 162 ± 16 nm, respectively. These observations suggested that the width of nanosheets decreased along with the increase of number of G. This is the first example of control over the width of self-assembled nanosheets in peptoid-peptide hydrogels, and it provided a versatile strategy to manipulate the property and probably the function of such nanomaterials.

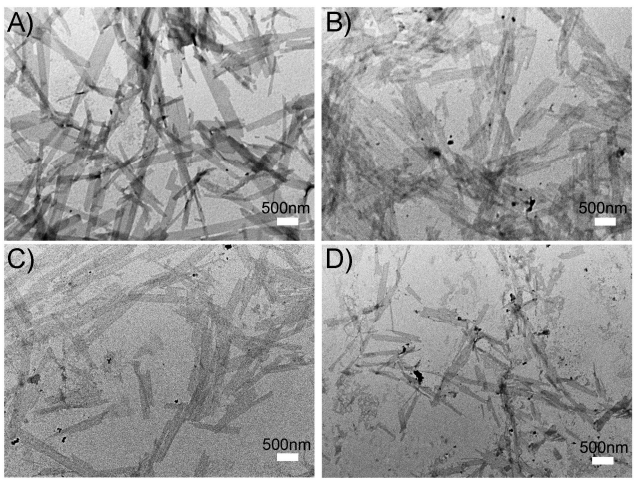

Fig. 2. The transmission electron microscopy (TEM) images of A) G_0gel, B) G_1gel, C) G_2gel, and D) G_3gel.

We then characterized the mechanical properties of the hydrogels by a rheometer. The hot solutions of compounds were transferred to parallel plates in the rheometer, and gels would form after cooling back to room temperature. We performed dynamic strain sweep and dynamic frequency sweep successively when the gels were stable at room temperature (20-25 ^{0}C). As shown in Fig. 3, at the frequency range of 0.1–100 rad/s, the gels exhibited weak frequency dependence, suggesting high elasticity of the hydrogels. The loss modulus (G'') of the gels were at least one magnitude lower than their storage modulus (G'), indicating the formation of true gels.[13] Meanwhile, the G' value of the gels

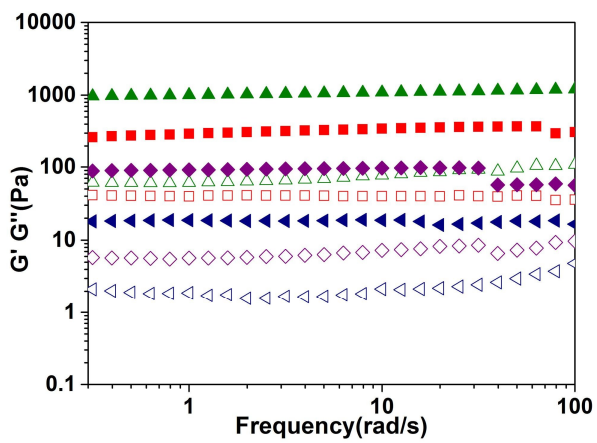

Fig. 3. Dynamic frequency sweep at the strain of 1% of four hydrogels at 1.0 wt% (Triangles: G_0gel, squares: G_1gel, rhombuses: G_2gel, and left triangles: G_3gel, filled symbols: G' and open symbols: G'')

decreased with the incremental number of glycine. For example, the G' value was about 1,000, 300, 100, and 20 Pa at the frequency of 1 rad/s for G_0gel, G_1gel, G_2gel, and G_3gel, respectively. The results, in combine with those obtained by TEM observations, suggested that nanosheets with larger width could form mechanically stronger hydrogels.

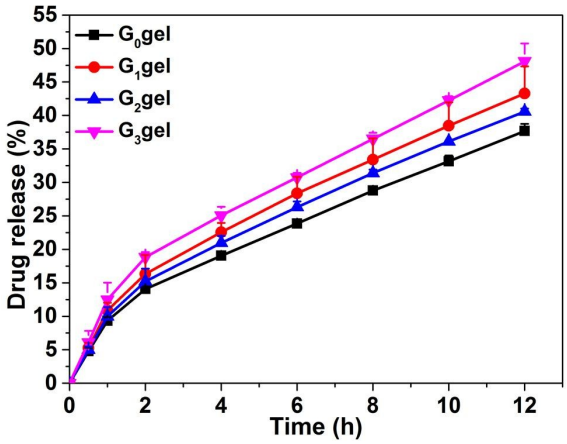

Fig. 4. Release profile of doxorubicin from G_0gel, G_1gel, G_2gel, and G_3gel, respectively.

Since the nanostructure in gels and mechanical property of gels were different, we then tested whether the release profile of a drug would be different from these gels. We chose the doxorubicin as the drug and monitored its release behavior from the gels at 37 ^{0}C. Peptoid–peptide biohybrid hydrogels (1 wt%) in PBS solution were formed by the heating–cooling process containing the same amount of doxorubicin (0.05 wt%). We added 250 μL of PBS (containing 0.5 % (v/v) Tween20) on the top of gels, and took out 200 μL of upper solution for measurement at each designed time point. At the same time, 200 μL of fresh PBS was added back to the gel. We measured the absorbance of doxorubicin at the wavelength of 490 nm to determine the accumulative amount of doxorubicin released from the gels. As shown in Fig. 4, doxorubicin gradually released from four gels during the 12h time period. However, the release speed was slightly different from these gels. It could be obviously seen that the release of doxorubicin was slower from mechanically stronger gels, and finally there was about 35.0, 38.0, 42.5, and 47.5% of doxorubicin released from G_0gel, G_1gel, G_2gel, and G_3gel, respectively during the 12h experimental period. Our previous study had shown that the peptoid-peptide biohybrids possessed better stabilities against enzyme digestion than peptides.[12] We obtained similar observations in this study, and F'F'F'F'RGD was much stable than FFFFRGD when treated with proteinase K (Fig. S-13). The controllable drug release behavior from our gel and better stability of peptoid-peptide biohybrids suggested the promise of the gels for sustained release of drugs.

In summary, we demonstrated in this study that, by simply changing the length of peptides, the width of self-assembled nanosheets and mechanical property of hydrogels could be manipulated. The hydrogels reported in this study had potential

for controlled drug release due to their good stability and controllable mechanical property. Our study provides a versatile method to control the property of self-assembling peptoid-peptide biohybrids, which will be useful to construct functional biomaterials based on peptoids.

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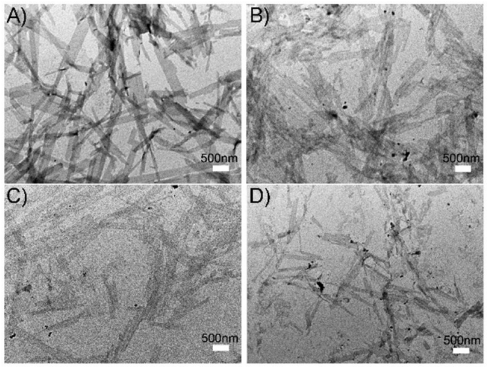

The width of self-assembling nanosheets could be controlled by the variation of peptide length.