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ARTICLE TYPE

A supramolecular hydrogel for the delivery of bortezomib

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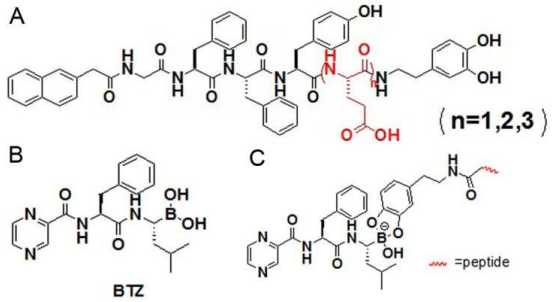
Here we reported on a supramolecular hydrogel for the delivery of an anti-cancer drug bortezomib.

Supramolecular hydrogels are formed by the self-assembly of low molecular weight hydrogelators.[1] They have showed big potential in cell culture,[2] regenerative medicine,[3] and materials preparation.[4] Recently, their application in controllable drug delivery attracts extensive research interests.[5] They can serve either as physical carriers[6] or as self-delivery systems[7, 8, 9] to deliver therapeutic agents. Among the above two ways to deliver therapeutic agents, the latter holds advantages because of the high and designable drug loading, sustained and long term release, and possible targeting effect. In order to produce supramolecular hydrogels or supramolecular nanofibers as self-delivery systems, therapeutic agents are generally conjugated with peptides through cleavable chemical bonds to yield self-assembling drug-peptide amphiphiles. These cleavable chemical bonds include ester bond,[10] disulfide bond,[11] and azo group.[12, 13] The resulting drug-peptide amphiphiles can self-assemble *via* non-covalent interactions into nanofibers, nanotubes, and so on. Such self-delivery systems can be administrated locally as hydrogels or used for intraveneous injection as diluted dispersions, and they have shown excellent capacities to inhibit tumor growth and inflammation.

The self-delivery system is especially beneficial to deliver hydrophobic therapeutic agents because of the following reasons: 1) the conjugation between hydrophobic therapeutic agents and hydrophilic/amphiphilic peptides can enhance the water solubility of therapeutic agents and in most cases, will result in self-assembling molecules;[7] 2) through choosing different peptides connected to the therapeutic agents, the drug loadings can be fixed and designed and targeting ability can also be achieved.[8] Until now, several hydrophobic therapeutic agents have been selected and developed into such self-delivery systems, including camptothecin/10-hydroxy camptothecin,[14, 15] taxol,[10] dexamethasone,[14, 16] olsalazine,[12] etc. In this study, we reported on another example of supramolecular hydrogel/nanofiber as a self-delivery system for bortezomib (BTZ).

BTZ is a proteasome inhibitor for cancer.[17] It has been approved by FDA for the treatment of multiple myeloma and shown big potential in the treatment of other cancers. However, the adverse pharmacokinetic effects of BTZ, including nonspecific binding to proteins, dose-limiting toxicities, and rapid clearance from blood, may result in its limited efficacy against many solid tumors. In order to improve the uptake of BTZ by solid tumors, Messersmith and co-workers have developed a polymer-BTZ conjugate *via* the catechol-boronic acid interaction, which can release the BTZ in acidic conditions.[18] Stimulated by their results and other self-assembling drug-peptide amphiphiles, we opt to develop a supramolecular hydrogel to locally deliver BTZ.



Scheme 1. Chemical structures of A) peptides of Nap-GFFYE-Cat, Nap-GFFYEE-Cat, and Nap-GFFYEEE-Cat when n was 1, 2, and 3, respectively; B) bortezomib (BTZ), and C) possible interaction between the peptide and BTZ.

We firstly designed and synthesized three peptide conjugates shown in Scheme 1A. They were based on the efficient gelator of Nap-GFFY and possessed the boronate binding moiety of catechol (Cat). The number of glutamic acid (E) was used to adjust the amphiphilicity of the peptides. We imaged that one of these peptides might chelate with BTZ to yield a self-assembling hydrogelator that would form supramolecular hydrogels for locally deliver BTZ. The synthesis of these peptide derivatives were described in Scheme S-1. After obtaining the designed compounds, we firstly tested the gelation ability of the three peptide derivatives in phosphate buffer saline solution (PBS, pH = 7.4) by the heating-cooling process. The Nap-GFFYE-Cat could not form homogeneous solution even upon heating to near 100 ^{o}C and therefore was unable to form hydrogels by the heating-cooling process. The Nap-GFFYEEE-Cat was well soluble and also failed to form hydrogels in PBS solution at the concentration up to 3 wt% (30 mg/mL). The compound of Nap-GFFYEE-Cat could form hydrogels when its concentration was higher than 0.5 wt% (gel I, Fig. 1A inset). These observations indicated that the number of E and the

amphiphilicity of the conjugate were essential for the gelation ability of the conjugates.

We then added BTZ to the gel I to test whether its addition would disrupt the gel I or not. The results indicated that the mixture of Nap-GFFYEE-Cat (0.5 wt%) and 1 equiv. of BTZ could also form hydrogels by the heating-cooling process (gel II, Fig. 1B inset), suggesting that the BTZ would not disrupt the gel. The mixture of Nap-GFFY (0.5 wt%) and 1 equiv. of BTZ in PBS solution could not form a homogeneous solution even upon heating to near 100°C, suggesting that the catechol-boronate interaction was crucial to improve the solubility of BTZ and the formation of mix-component hydrogels.

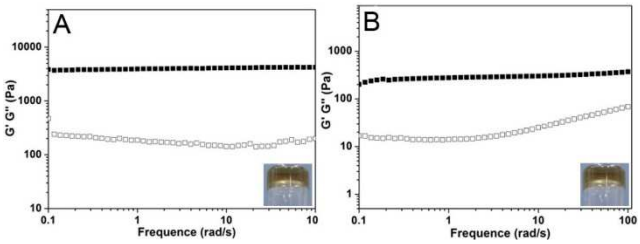


Fig. 1. A) Optical image and dynamic frequency sweep of the gel I formed by PBS solution of Nap-GFFYEE-Cat (0.5 wt%), B) Optical image and dynamic frequency sweep of the gel II formed by PBS solution of Nap-GFFYEE-Cat (0.5 wt%) and 1 equiv. of bortezomib (filled symbols: G' and empty symbols: G'').

We characterized the mechanical properties of both hydrogels by the rheometer. The hot PBS solution of gel I or gel II was transferred to the rheometer, and the gel would form after cooling back to room temperature between the two parallel plates of the rheometer. After incubation for two hours at room temperature to allow the formation of a stable hydrogel, the dynamic frequency sweep was then performed at the strain of 1% and at 37 °C. As shown in Fig. 1, the elasticity value (G′) of the gels was at least one order of magnitude bigger than their corresponding viscocity value (G″), indicating the formation of true gels.[19] The addition of BTZ decreased the mechanical property of resulting gels (Fig. S-9 and Fig. S-10). The G' value of gel I was about 13 times bigger than that of gel II, suggesting that the addition of BTZ slightly disrupted the molecular packing between peptides.

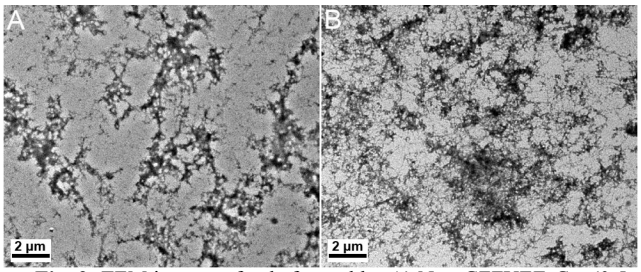


Fig. 2. TEM images of gels formed by A) Nap-GFFYEE-Cat (0.5 wt%), B) Nap-GFFYEE-Cat (0.5 wt%) with 1 equiv. of BTZ.

The morphologies of the self-assembled structures in the hydrogels were characterized by the transmission electron microscopy (TEM). As shown in Fig. 2, we observed networks of nanofibers in both hydrogels. However, the density and morphology of fibers were quite different. Gel I (Fig. 2A) exhibited networks of fibers with the width of around 20-50 nm. The length of nanofibers was longer than several microns and entangled with each to form networks for hydrogel formation. Compared with gel I, gel II showed a totally different morphology of nanosturctures in which we observed a network of very short nanofibers. Similar results were observed in scanning electron microscopy (SEM) images of both gels (Fig. S-12). We observed short fibers in gel II, while relative longer fibers in gel I. These observations were consistent with the mechanical properties of the two gels, that is gel II with weaker mechanical strength possessed a network of much shorter fibers.

In vitro release of BTZ from gels was also monitored in PBS solutions. We fixed the peptide concentration to be 0.5 wt% and using different equivalents of BTZ to form gels. A 0.5 mL of PBS was added to the formed gels, 0.45 mL of the upper PBS solution was taken out at desired intervals, and a fresh 0.45 mL of PBS solution was added back to the gel. The accumulating release profile of BTZ from gels was then determined by the LC-MS. As shown in Fig. 3, the gels released BTZ at a mean rate of 12.5, 22.9, 41.4, and 67.1 μg mL^{-1} per hour for gels containing 0.125, 0.25, 0.5, and 1 equiv. of BTZ, respectively. There was no burst release phenomenon being observed in the 12 h experimental period. The accumulating release percentage of BTZ was about 68.6, 62.7, 56.7, and 45.9% for gels containing 0.125, 0.25, 0.5, and 1 equiv. of BTZ, respectively during the 12h experimental period (Fig. S-11). These observations implied that our peptide hydrogels could be used to sustainably release of BTZ for cancer therapy.

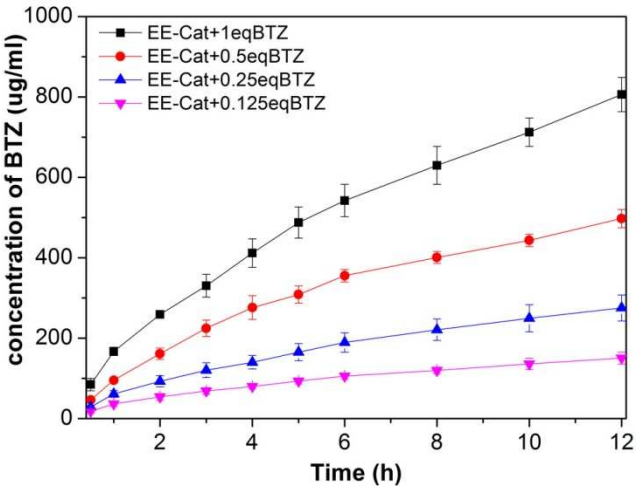


Fig. 3. Release profile of BTZ from hydrogels to PBS buffer solution at 37 °C.

To evaluate the inhibition capacity of the peptide-drug conjugates to cancer cells, we performed the MTT assay to obtain their IC_{50} values to HeLa, HepG2, and NIH 3T3 cells. Compared with free BTZ, peptide-BTZ conjugate exhibited slightly decreased cytotoxicity to both cancer cells (HeLa and HepG2) and normal cell of 3T3 cells (Table 1). Our recent study indicated that drug-peptide conjugate in nanofiber form

would change the cellular distribution of the drug molecule, thus decreasing the inhibition capacity of anti-cancer drugs to cells.[20] These observations indicated the distribution of drug-peptide nanostructures in cells was crucial to the inhibition capacity of drug molecules to cells. Though the drug-peptide conjugate exhibited decreased inhibition capacity to cells, the hydrogels of this conjugate might be applied for topical administration in near tumor spaces to inhibit tumor growth.

Table 1. IC_{50} value (nM) of BTZ and the peptide-BTZ conjugate to different cells.

Compound	HeLa	HepG2	3T3
BTZ	32.2	38.7	101.1
Peptide-BTZ	82.7	106.5	162.5

In summary, a catechol containing peptide derivative was designed and synthesized that could form a conjugate with the anti-cancer drug of BTZ through the complex interaction between boronic acid and catechol moieties. The conjugate could also form supramolecular hydrogels and this is the first example of supramolecular hydrogelator of BTZ. The hydrogel could sustainably release BTZ through boronate ester hydrolysis, which suggested its potential for topical administration to release BTZ and inhibit tumor growth. Our study provides useful information to design nanomaterials to deliver the anti-cancer drug bortezomib.

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Notes and references

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