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Selenium-containing thermogel for controlled drug delivery by coordination competition†

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A novel coordination-responsive block copolymer composed of polyether and polyester with selenium at the center was designed and synthesized for controlled drug delivery. The aqueous system of the new copolymer was a free-flowing solution at ambient temperature and turned into a semi-solid thermogel at body temperature. We demonstrate that the selenium-containing copolymer is capable of coordinating with the antitumor drug cisplatin. The efficient coordination between them significantly increased the loading capacity of cisplatin in the selenium-containing thermogel matrix, and the presence of glutathione in the release medium triggered the cisplatin release by coordination competition in a sustained release manner.

Stimuli-responsive polymers show remarkable property changes in response to external stimuli such as temperature, pH, light, enzyme, and redox.[1–11] Among them, thermogelling polymers exhibiting a sol–gel transition in water with increasing temperature have attracted extensive attention as injectable biomaterials for drug delivery and tissue engineering *etc.* with advantages of (1) syringe injection with minimal invasiveness, (2) free of organic solvents during the fabrication of the implant, and (3) facile sterilization by filtration.[4,5,12–17] Such a system enables drugs or cells to be easily entrapped in the sol state, followed by injection into a target site forming an *in situ* hydrogel, acting as a sustained drug delivery depot or cell-growing matrix.[4–6,12–14,18–21] To date, biocompatible poly (ethylene glycol) (PEG) has been widely used as the hydrophilic segment of thermogelling polymers, while biodegradable polyester, polypeptide, poly(phosphazenes) and their modified products have been selected as the hydrophobic part.[19–24] Compared with other drug delivery systems, such as polymersomes and micelles,[7–9] the thermogelling systems are suitable as local carriers to deliver various drugs for a long time and the drug release profiles can be manipulated by polymer concentration, block composition, adding excipients, external stimuli, *etc.*[4,5,12,18,21,25] Nevertheless, it is still challenging to develop new ways to tune the drug release manner under physiological environments.

As an essential trace element for human beings and its unique redox-/coordination-responsive properties,[26] selenium has recently been introduced into polymers to construct new smart biomaterials.[7] In particular, Xu *et al.* synthesized a variety of selenium-containing block copolymers that could self-assemble into aggregates in water and thus developed a series of redox-responsive or coordination-responsive systems for controlled drug delivery.[7,27,28] However, multi-stimuli-responsive selenium-containing hydrogels are rather limited,[29] and no thermogelling system based on selenium-containing polymers has been reported so far.

This work reports the first selenium-containing thermogelling copolymer. It is known that the thermogelation of amphiphilic PEG/polyester copolymers originates from the delicate balance of hydrophilicity/hydrophobicity and ABA-typed polyester–PEG–polyester triblock copolymers suffer from a subtle end-group effect.[18,30–32] In this study, two novel thermogelling polymer–selenium conjugates were designed and synthesized *via* the introduction of a selenium compound, 3,3′-selenodipropionic acid (SePA), to the hydrophobic end of methoxyl poly(ethylene glycol)-*b*-poly(lactic acid-*co*-glycolic acid) (mPEG-PLGA) diblock copolymers, leading to the formation of BAB-typed PEG–polyester–PEG triblock copolymer, Bi(mPEG-PLGA)–Se. The basic idea in the present study is schematically illustrated in Scheme 1 and our route of design successfully avoids the potential end-group effect of thermogelling ABA-typed triblock copolymers. We found that the amphiphilic conjugates could self-assemble into micelles in aqueous medium and the concentrated aqueous solution exhibited a sol–gel transition with increasing temperature. To shed light on the potential in biomedical application, the Bi(mPEG-PLGA)–Se conjugate thermogel was further employed to

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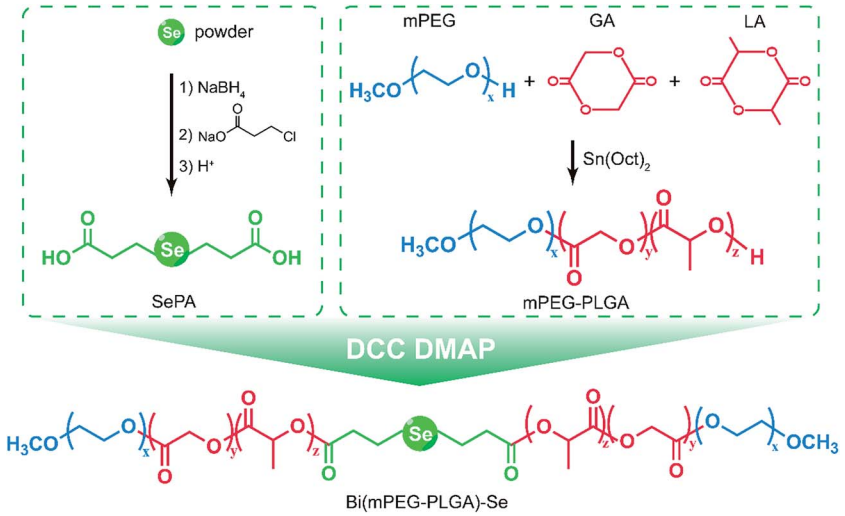

Scheme 1 Synthetic procedures of the coupling agent SePA, diblock copolymer mPEG-PLGA and selenium-containing Bi(mPEG-PLGA)–Se conjugate.

encapsulate cisplatin, a famous anticancer drug. The high coordination efficiency between cisplatin and the selenium-containing conjugates led to a significant increase of drug loading capacity in the thermogel matrix. Finally, glutathione (GSH), a coordination competition agent, was tried to trigger the release of cisplatin from the conjugate thermogel depot.

NMR (^{1}H and ^{77}Se), GPC and ICP-OES measurements demonstrated the successful synthesis of Bi(mPEG-PLGA)–Se conjugates (Fig. S1 and S2†). Table 1 summarizes the molecular parameters of polymers investigated in this study. All the specimens obtained in this study exhibited a reversible sol–gel transition in water as the temperature increased. Different from the original mPEG-PLGA diblock copolymers (Polymer-1 and Polymer-2), the sol–gel transition temperatures of Bi(mPEG-PLGA)–Se conjugates (Conjugate-1 and Conjugate-2) shifted to a higher range after the coupling reactions, as shown in Table 1 and Fig. S3.† This feature is consistent with the previous publications elsewhere,[33,34] but differs from thermogelling polymer–platinum(IV) conjugates reported by us recently.[35] The cisplatin Pt(IV) analogue was covalently linked to the copolymer as a polymeric prodrug, and the introduction of Pt(IV) analogue resulted in the decrease in sol–gel transition temperature.[35] Also, the significant difference of the present study comes from that cisplatin was coordinated with the selenium-containing copolymer and then released by the addition of its coordination competitor GSH, as will be illustrated later. In this study, Conjugate-1 and its corresponding control polymer (Control-1, namely, mPEG–PLGA–mPEG) in Table 1 were used for subsequent experiments due to their proper sol–gel temperatures for biomedical application.

Amphiphilic PEG/polyester copolymers easily self-assemble into spherical micelles in water with hydrophilic PEG blocks as the micellar corona, while hydrophobic polyester segments as the core of micelles.[36,37] TEM observations clearly demonstrated that Conjugate-1 also formed similar spherical micelles in water and DLS measurement showed that the average hydrodynamic diameter of micelles was about 25 nm, as presented in Fig. 1A. Meanwhile, for the concentrated aqueous solution of Conjugate-1, an abrupt increase in both storage modulus G' and viscosity η as a function of temperature was observed by dynamic rheology analysis, as displayed in Fig. 1B. This result confirms the occurrence of a sol–gel transition. Our previous work has revealed that the formation of a percolated micelle network *via* micellar aggregations was responsible for the gelation mechanism and a possible driving force of aggregation came from the hydrophobic interaction.[31,35,38] Compared

Table 1 Parameters of polymers investigated in this study

Sample	M_n[a] (g mol^{-1})	M_n[b] (g mol^{-1})	$Đ_M$[b]	LA/GA[a] (mol mol^{-1})	Sol–gel transition temperature[c]
Polymer-1	550-1450	2410	1.48	2.9	23 °C
Conjugate-1	550-1700-223-1700-550	4390	1.34	2.9	33 °C
Control-1[d]	550-1760-170-1760-550	4360	1.24	2.9	31 °C
Polymer-2	750-1940	2610	1.42	3.0	35 °C
Conjugate-2	750-2110-223-2110-750	4690	1.29	3.0	47 °C

[a] The number average molecular weight (M_n) of mPEG was provided by Aldrich. M_n of each PLGA block and the molar ratio of LA/GA were calculated *via* ^{1}H NMR. [b] Determined by GPC. [c] Determined *via* the test tube inverting method. Polymer concentration was 25 wt%. [d] A control copolymer mPEG–PLGA–mPEG was obtained using HMDI as the coupling agent.

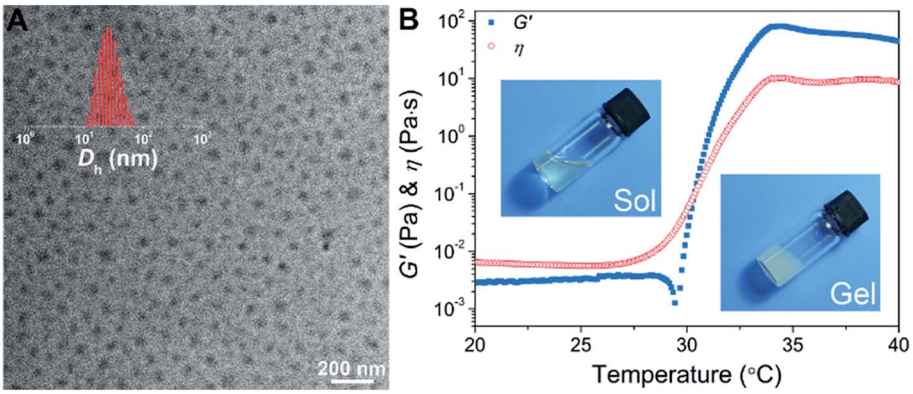

Fig. 1 (A) TEM image showing Conjugate-1 micelles. The conjugate concentration was 0.5 wt%. The inset shows the corresponding distribution of micellar size (intensity-weighted) measured by DLS. (B) Storage modulus G' and viscosity η of the Conjugate-1 aqueous solution (25 wt%) as a function of temperature. The inset photographs show the conjugate aqueous solution (25 wt%) exhibiting sol and opaque gel states at 20 and 37 °C, respectively.

with the original PEG–polyester diblock copolymers, the increased sol–gel transition temperature of PEG–polyester–PEG triblock copolymers was attributed to its weak capacity of micellar aggregation as a function of temperature.[33,34]

It is acknowledged that selenium as a ligand is capable of interacting with a variety of metals.[27,28,39–41] For example, Xu *et al.* revealed that polymer–selenium conjugate micelles could coordinate with $PtCl_2$ or cisplatin to form coordination complexes.[27,28] Herein, a similar experiment was performed to verify the ability of Conjugate-1 to coordinate with cisplatin. The coordination process was monitored as a function of time using ICP-OES following the addition of excess cisplatin powder (the drug amount added was significantly greater than the solubility of cisplatin in water at 25 °C (2.5 mg mL^{-1})[42]) into the Conjugate-1 aqueous solution (25 wt%, a much higher polymer concentration than the general micelle system) with constant magnetic stirring. As shown in Fig. 2A, the Pt/Se molar ratio was 1 : 16 after 6 h of coordination and then gradually increased as the coordination time prolonged. The stoichiometric ratio reached an almost constant value (1 : 2) after 1 week coordination. Correspondingly, the cisplatin coordinating amount in the 25 wt% Conjugate-1 solution increased from 1.1 mg mL^{-1} after 6 h of coordination to approximately 8.0 mg mL^{-1} 7 d later, as presented in Fig. S4.† Consequently, considering the solubility of cisplatin itself in water (2.5 mg mL^{-1} at 25 °C),[42] the drug loading amount of cisplatin in the 25 wt% conjugate solution could exceed 10 mg mL^{-1}.

Next, a simple, yet visualized experiment was performed to further affirm the existence of coordination between Conjugate-1 and cisplatin. As shown in Fig. S5A,† restricted by the solubility of drug in water, the Control-1 system containing 8 mg mL^{-1} cisplatin maintained a turbid state even after 4 d stirring. In contrast, for the Conjugate-1 aqueous solution containing the same amount drug after stirring for the same duration, the system changed gradually from a yellow, turbid solution to a brown, transparent state (Fig. S5B†), reflecting the increased solubility of drug by coordination.

Additionally, XPS was employed to examine the influence of coordination on the binding energies of Se and Pt (Fig. 2B). The 3d binding energy of Se shifted upwards from 54.7 to 55.3 eV after coordinating with cisplatin for 4 d. The positive shift of binding energy means the decrease of shielding effect, namely representing a lower electron density in the vicinity of the Se atom. This feature indicates that Se atom is acting as an electron donor. In contrast, Pt atom plays the role of electron acceptor since the 4f binding energy of Pt decreased from 76.0 and 72.7 to 75.2 and 71.8 eV, respectively. These changes of binding energy further confirm the occurrence of coordination between Se and Pt. XPS was also used to analyze the surface atomic concentration of the examined sample and the results showed that the atomic concentrations of Pt and Se were 0.14% and 0.27% respectively, namely with the ratio close to 1 : 2. This finding is in agreement with the ICP-OES results.

This 1 : 2 coordination ratio of Pt/Se is interpreted as follows. Pt–N bonds of cisplatin are rather stable,[41] therefore, only the two chloride ions of cisplatin can be substituted by Se atoms in the Bi(mPEG-PLGA)–Se conjugates. Different from O atom, Se ([Ar] $3d^{10}4s^24p^4$) hardly adopts the sp^3 hybridization.[43] This could also be deduced from the different valence angles between H_2O (104.5°) and H_2Se (91°), which suggests little contribution from interaction with the 4s orbital of Se.[43] Consequently, Se atom in the conjugate can only donate one lone pair of electrons from 4p orbital during the coordination with cisplatin. As a result, the possible maximum value of the Pt/Se coordination ratio is 1 : 2, as schematically displayed in Fig. 2C. In fact, this coordination ratio is also in consistency with the stoichiometry of coordination between other selenides and Pt.[28,39,40]

We subsequently examined the effects of coordination between cisplatin and Conjugate-1 on the micellar behavior and thermogelling properties. As revealed in Fig. 3A, the spherical morphology of micelles remained but the size of polymeric micelles increased from 25 nm before coordination (Fig. 1A) to 32 nm after 4 d of coordination. This finding indicates that the coordination between them did not significantly affect the

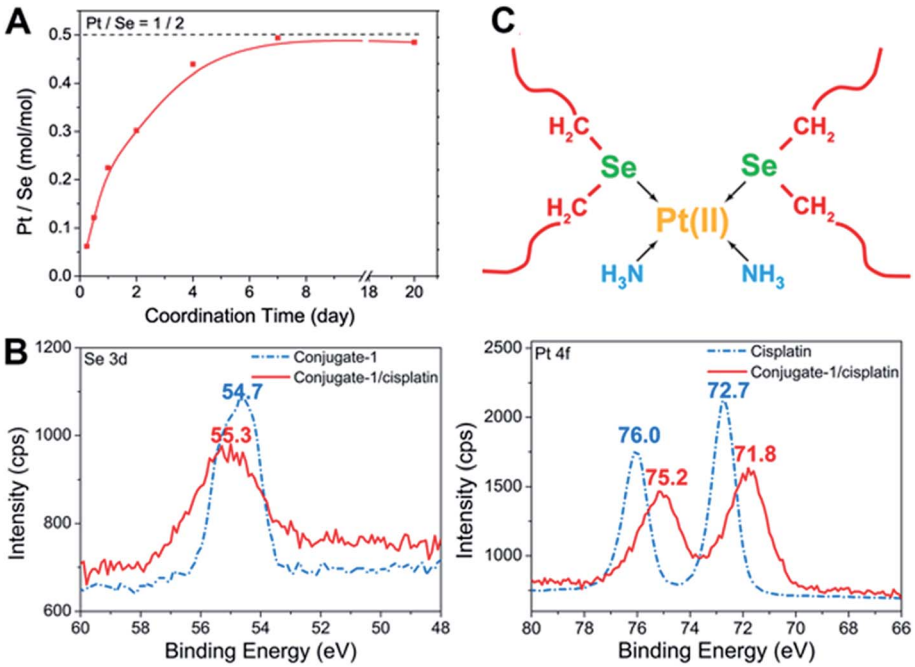

Fig. 2 (A) Pt/Se coordination ratio in the Conjugate-1 aqueous solution (25 wt%) as a function of coordinating time. Excess drugs (>100 mg mL^{-1}) were added. 0.1 mL sample was taken out at the predetermined time points and diluted with 15 mL water, followed by dialyzing against deionized water for 24 h to remove uncoordinated cisplatin in the conjugate solution before measurement *via* ICP-OES. (B) XPS analysis of the selenium and platinum binding energies before and after coordination. (C) Schematic representation of the coordination between cisplatin and polymer–selenium conjugate. The electron configuration for Pt(II) is [Xe] $4f^{14}5d^{8}$ with a dsp^{2} hybridization producing a square planar structure.

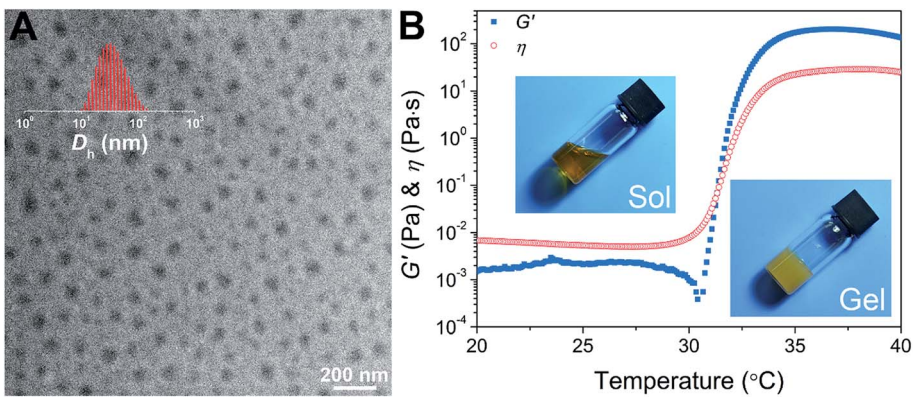

Fig. 3 (A) TEM image showing micelles of Conjugate-1/cisplatin. The conjugate concentration was 0.5 wt% and the drug concentration was 0.16 mg mL^{-1}. The inset shows the corresponding distribution of micellar size (intensity-weighted) measured by DLS. (B) Storage modulus G' and viscosity η of the Conjugate-1/cisplatin aqueous solution as a function of temperature. The conjugate concentration was 25 wt% and the drug concentration was 8 mg mL^{-1}. The inset photographs show the conjugate aqueous solution (25 wt%) exhibiting sol and opaque gel states at 20 and 37 °C, respectively.

micellar morphology of Conjugate-1. On the other hand, the concentrated Conjugate-1 aqueous solution maintained the thermo-reversible sol–gel transition and formed a semi-solid gel at body temperature after the coordination with cisplatin, as illustrated in the insets of Fig. 3B. Meanwhile, the coordination of cisplatin with Conjugate-1 brought a yellow color compared with the colorless conjugate aqueous solution alone. The dynamic rheology analysis in Fig. 3B further revealed that the introduction of cisplatin by coordination resulted in the significant increase in the maximum G' value of conjugate/water system (200 *versus* 80 Pa).

To demonstrate that the coordination between selenium of Bi(mPEG-PLGA)–Se conjugate and platinum of cisplatin plays a vital role in the cisplatin release profile, Control-1 was employed as the control carrier and *in vitro* release experiments were performed. In the case of cisplatin-loaded Control-1 gel formulation, more than 80% drug was released into the PBS media (pH 7.4) after 36 h, as shown in Fig. 4A. In contrast, for

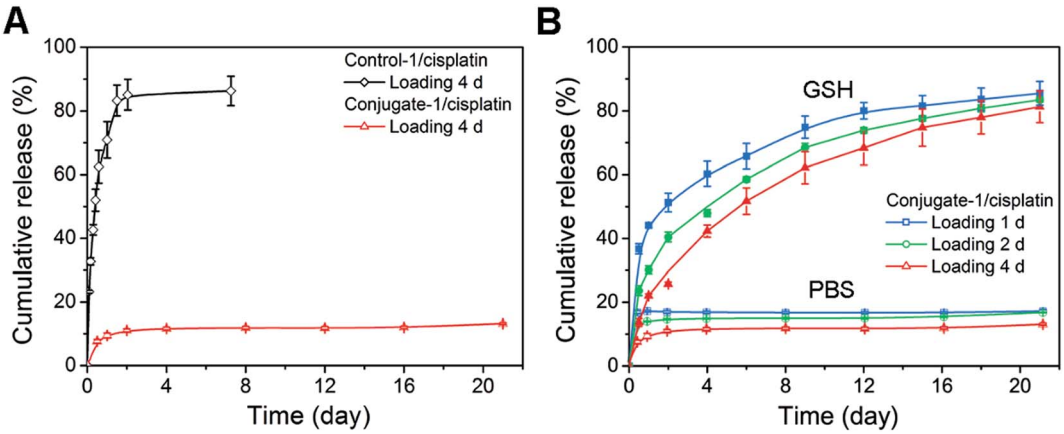

Fig. 4 (A) *In vitro* release profiles of cisplatin from Control-1 and Conjugate-1 thermogels in PBS. (B) *In vitro* release profiles of cispatin from Conjugate-1 thermogels in PBS with or without 10 mM GSH. "Loading 4 d" means the coordination time between the carrier and cisplatin before the *in vitro* release examination. Conjugate-1 concentration was 25 wt%, Control-1 concentration was 25 wt%, and the cisplatin loading amount was 8 mg mL^{-1}. The results are presented as mean ± standard deviation ($n = 3$). Lines are just for guidance of eyes.

the Conjugate-1/cisplatin system, a very low amount of cisplatin (only 13.2%) was released up to 3 weeks, indicating that once being coordinated with selenium, only the uncoordinated cisplatin could be released into the media through diffusion while the coordinated parts were anchored stably within the conjugate thermogel.

Considering that the efficiency of Se/Pt coordination was dependent on the coordination time, the effects of coordination time on the cisplatin release profiles were evaluated as well. Fig. 4B revealed that cisplatin hardly released in PBS medium except the initial burst after the indicated coordination procedures. The initial burst amount of cisplatin depended on the coordination time. It is obvious that the longer coordination time produced the more coordination between cisplatin and Bi(mPEG-PLGA)–Se conjugate. As a result, the less free cisplatin was left, leading to the lower burst amount.

Compared with the coordination with selenium, platinum has a stronger coordinating ability with other ligands, such as GSH, DTT, spermine, *etc.*[27,44,45] Hence, we hypothesized that the release of cisplatin from the selenium-containing thermogel depot might be triggered by competitive ligands. To verify this hypothesis, the most prevalent intracellular thiol species GSH was selected as the competitive ligand to trigger the cisplatin release. As shown in Fig. 4B, the existence of GSH in the release medium greatly promoted the cisplatin release in a sustained and complete release manner. Different from less than 20% cisplatin released in PBS media after 3 weeks, more than 80% loaded drugs were released with the treatment of GSH. This enhanced release profile was attributed to the thiol groups of GSH which possess a stronger competing coordinating ability with the platinum of cisplatin.

Interestingly, we found that the coordination time could be well utilized to adjust the release profile of cisplatin, which is vital for the potential application. When the coordination proceeded for 1 d, a remarkable burst with about 36.7% drug released was detected within the initial 12 h, followed by

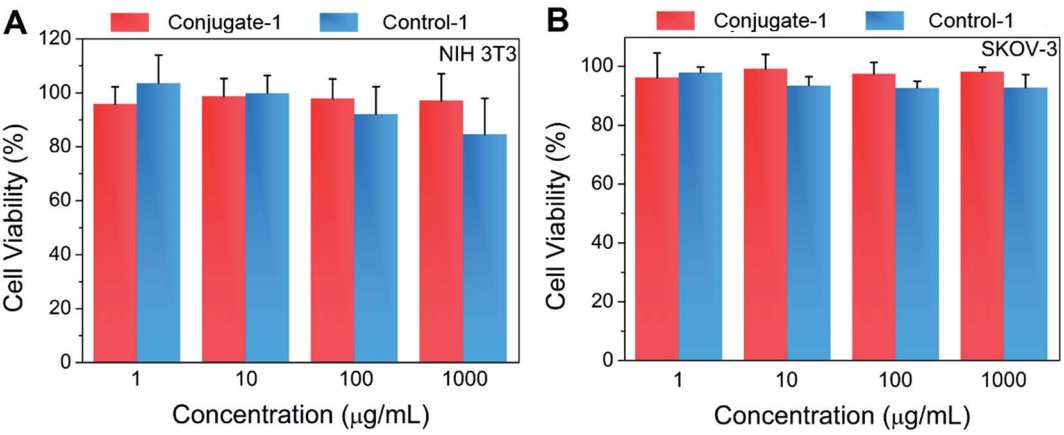

Fig. 5 *In vitro* cytocompatibility of Bi(mPEG-PLGA)–Se conjugate (Conjugate-1) and mPEG–PLGA–mPEG polymer (Control-1) with varied concentrations against NIH 3T3 (a normal cell line) (A) and SKOV-3 (an ovarian cancer cell line) (B) cells after incubating for 24 h. The results are presented as mean ± standard deviation ($n = 6$). The cell viability of culture medium as the blank control was defined as 100%.

a sustained release lasting for 3 weeks. As the coordination time increased to 2 and 4 d, the burst release was well inhibited with only 23.7% and 13.7% cisplatin released within the initial 12 h. Furthermore, all samples reached a similar cumulative cisplatin release amount regardless of the initial drug release profiles. Thus, a coordination responsive polymer–selenium conjugate hydrogel with facile-tuned cisplatin release behavior was achieved *via* simply adjusting the coordination time. Some other questions such as the effects of different GSH concentrations on the release profiles are still open and thus further studies are called for.

Finally, the cytocompatibility of Conjugate-1 was evaluated using a cell counting kit-8 (CCK-8) assay. After 24 h of incubation, the viabilities of two different cell lines remained over 95% in the Conjugate-1 solutions with various concentrations, as shown in Fig. 5. This finding confirms that the Bi(mPEG-PLGA)–Se conjugate is biocompatible and suitable for the potential biomedical applications.

Conclusions

In summary, a novel coordination-responsive selenium-containing thermogel was successfully fabricated for the controlled release of cisplatin. The Bi(mPEG-PLGA)–Se conjugates were designed and synthesized by covalent linking of selenium ligand to the hydrophobic ends of two mPEG–PLGA polymer chains. The conjugate/water system was a free-flowing sol at low temperature and spontaneously transformed into a semi-solid thermogel as the temperature increased. The conjugate could effectively coordinate with cisplatin in a time-dependent manner and the loading amount of drug significantly increased to 8 mg mL^{-1} after 4 d of coordination. The corresponding thermogel formulation exhibited a sustained release manner of cisplatin up to 3 weeks triggered by the competitive ligand. Meanwhile, the initial burst of drug, a common problem in drug delivery system, could be well controlled simply by adjusting the time of coordination process without affecting the late complete release. The current work not only broadens the strategy of tuning drug release using thermogelling systems, but also stimulates the design of other coordination-responsive systems for biomedical applications.

Acknowledgements

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References

1 H. Lin, W. Xiao, S. Y. Qin, S. X. Cheng and X. Z. Zhang, *Polym. Chem.*, 2014, **5**, 4437–4440.
2 H. B. Wang, G. Y. Liu, H. Q. Gao and Y. B. Wang, *Polym. Chem.*, 2015, **6**, 4715–4718.
3 F. Li, J. L. He, M. Z. Zhang and P. H. Ni, *Polym. Chem.*, 2015, **6**, 5009–5014.
4 H. J. Moon, D. Y. Ko, M. H. Park, M. K. Joo and B. Jeong, *Chem. Soc. Rev.*, 2012, **41**, 4860–4883.
5 L. Yu and J. D. Ding, *Chem. Soc. Rev.*, 2008, **37**, 1473–1481.
6 S. Guragain, B. P. Bastakoti, V. Malgras, K. Nakashima and Y. Yamauchi, *Chem.–Eur. J.*, 2015, **21**, 13164–13174.
7 H. P. Xu, W. Cao and X. Zhang, *Acc. Chem. Res.*, 2013, **46**, 1647–1658.
8 H. Wei, L. R. Volpatti, D. L. Sellers, D. O. Maris, I. W. Andrews, A. S. Hemphill, L. W. Chan, D. S. H. Chu, P. J. Horner and S. H. Pun, *Angew. Chem., Int. Ed.*, 2013, **52**, 5377–5381.
9 P. P. Chen, M. Qiu, C. Deng, F. H. Meng, J. Zhang, R. Cheng and Z. Y. Zhong, *Biomacromolecules*, 2015, **16**, 1322–1330.
10 N. Miyamoto, K. Shimasaki, K. Yamamoto, M. Shintate, Y. Kamachi, B. P. Bastakoti, N. Suzuki, R. Motokawa and Y. Yamauchi, *Chem.–Eur. J.*, 2014, **20**, 14955–14958.
11 N. Miyamoto, M. Shintate, S. Ikeda, Y. Hoshida, Y. Yamauchi, R. Motokawa and M. Annaka, *Chem. Commun.*, 2013, **49**, 1082–1084.
12 T. Vermonden, R. Censi and W. E. Hennink, *Chem. Rev.*, 2012, **112**, 2853–2888.
13 M. H. Park, Y. Yu, H. J. Moon, D. Y. Ko, H. S. Kim, H. Lee, K. H. Ryu and B. Jeong, *Adv. Healthcare Mater.*, 2014, **3**, 1782–1791.
14 P. Z. Elias, G. W. Liu, H. Wei, M. C. Jensen, P. J. Horner and S. H. Pun, *J. Controlled Release*, 2015, **208**, 76–84.
15 K. W. Lei, W. J. Shen, L. P. Cao, L. Yu and J. D. Ding, *Chem. Commun.*, 2015, **51**, 6080–6083.
16 B. G. Yang, C. Y. Wang, Y. B. Zhang, L. Ye, Y. F. Qian, Y. Shu, J. M. Wang, J. J. Li and F. L. Yao, *Polym. Chem.*, 2015, **6**, 3431–3442.
17 X. Zhao, O. Coutelier, H. H. Nguyen, C. Delmas, M. Destarac and J. D. Marty, *Polym. Chem.*, 2015, **6**, 5233–5243.
18 A. Petit, M. Sandker, B. Muller, R. Meyboom, P. van Midwoud, P. Bruin, E. M. Redout, M. Versluijs-Helder, C. H. A. van der Lest, S. J. Buwalda, L. G. J. de Leede, T. Vermonden, R. J. Kok, H. Weinans and W. E. Hennink, *Biomaterials*, 2014, **35**, 7919–7928.
19 E. J. Kye, S. J. Kim, M. H. Park, H. J. Moon, K. H. Ryu and B. Jeong, *Biomacromolecules*, 2014, **15**, 2180–2187.
20 J. S. Kwon, S. W. Kim, D. Y. Kwon, S. H. Park, A. R. Son, J. H. Kim and M. S. Kim, *Biomaterials*, 2014, **35**, 5337–5346.
21 M. R. Park, B. B. Seo and S. C. Song, *Biomaterials*, 2013, **34**, 1327–1336.
22 Z. B. Li, Z. X. Zhang, K. L. Liu, X. P. Ni and J. Li, *Biomacromolecules*, 2012, **13**, 3977–3989.
23 G. Chang, T. Ci, L. Yu and J. D. Ding, *J. Controlled Release*, 2011, **156**, 21–27.
24 T. Y. Ci, L. Chen, L. Yu and J. D. Ding, *Sci. Rep.*, 2014, **4**, 5473.
25 K. Li, L. Yu, X. J. Liu, C. Chen, Q. H. Chen and J. D. Ding, *Biomaterials*, 2013, **34**, 2834–2842.
26 R. Boyd, *Nat. Chem.*, 2011, **3**, 570.
27 W. Cao, Y. Li, Y. Yi, S. B. Ji, L. W. Zeng, Z. W. Sun and H. P. Xu, *Chem. Sci.*, 2012, **3**, 3403–3408.

28 L. Zeng, Y. Li, T. Li, W. Cao, Y. Yi, W. Geng, Z. Sun and H. P. Xu, *Chem.–Asian J.*, 2014, **9**, 2295–2302.
29 W. Cao, X. L. Zhang, X. M. Miao, Z. M. Yang and H. P. Xu, *Angew. Chem., Int. Ed.*, 2013, **52**, 6233–6237.
30 L. Yu, H. Zhang and J. D. Ding, *Angew. Chem., Int. Ed.*, 2006, **45**, 2232–2235.
31 L. Yu, Z. Zhang, H. Zhang and J. D. Ding, *Biomacromolecules*, 2009, **10**, 1547–1553.
32 C. Chen, L. Chen, L. P. Cao, W. J. Shen, L. Yu and J. D. Ding, *RSC Adv.*, 2014, **4**, 8789–8798.
33 K. W. Kwon, M. J. Park, Y. H. Bae, H. D. Kim and K. Char, *Polymer*, 2002, **43**, 3353–3358.
34 K. T. Peng, C. F. Chen, I. M. Chu, Y. M. Li, W. H. Hsu, R. W. Hsu and P. J. Chang, *Biomaterials*, 2010, **31**, 5227–5236.
35 W. J. Shen, J. B. Luan, L. P. Cao, J. Sun, L. Yu and J. D. Ding, *Biomacromolecules*, 2015, **16**, 105–115.
36 B. Jeong, Y. H. Bae and S. W. Kim, *Macromolecules*, 1999, **32**, 7064–7069.
37 L. Yu, Z. Zhang and J. D. Ding, *Biomacromolecules*, 2011, **12**, 1290–1297.
38 T. Li, T. Y. Ci, L. Chen, L. Yu and J. D. Ding, *Polym. Chem.*, 2014, **5**, 979–991.
39 D. G. Sar, M. Montes-Bayon, E. B. Gonzalez, L. M. S. Zapico and A. Sanz-Medel, *Chem. Res. Toxicol.*, 2011, **24**, 896–904.
40 Q. Liu, J. Y. Zhang, X. K. Ke, Y. H. Mei, L. G. Zhu and Z. J. Guo, *J. Chem. Soc., Dalton Trans.*, 2001, 911–916.
41 G. S. Baldew, J. G. J. Mol, F. J. J. Dekanter, B. Vanbaar, J. J. M. Degoeij and N. P. E. Vermeulen, *Biochem. Pharmacol.*, 1991, **41**, 1429–1437.
42 S. Budavari, *The Merck Index*, Merck, Rahway, USA, 12th edn, 1996.
43 W. Kutzelnigg, *Angew. Chem., Int. Ed.*, 1984, **23**, 272–295.
44 W. Cao, L. Wang and H. P. Xu, *Chem. Commun.*, 2015, **51**, 5520–5522.
45 W. Cao, Y. W. Gu, M. Meineck, T. Y. Li and H. P. Xu, *J. Am. Chem. Soc.*, 2014, **136**, 5132–5137.