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Disulfide-crosslinked Poly(*L*-glutamic acid) Grafted Mesoporous Silica Nanoparticles and Their Potential Application in Drug Delivery

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Abstract Poly(*L*-glutamic acid)(PLGA) was grafted onto the surface of mesoporous silica nanoparticles(MSN) *via* the ring opening polymerization of *γ*-benzyl-*L*-glutamate *N*-carboxyanhydride(BLG-NCA) and its subsequent deprotection of benzyl groups. The PLGA chains were cross-linked with cystamine, and thus forming a type of redox responsive drug delivery system(MSN-*c*PLGA). The structures were characterized by Fourier transform infrared spectrometry(FTIR), transmission electron microscopy(TEM) and energy disperse spectrometry(EDS), demonstrating that disulfide groups existed on the surfaces of MSN-*c*PLGA particles. The thermal gravimetric analysis(TGA) results show that the PLGA mass fraction is about 33.4% in the MSN-*c*PLGA hybrid. The *in vitro* drug release experiments showed that the MSN-*c*PLGA hybrid can realize the controlled release of model drugs(5-fluorouracil) in response to redox environment. Even 0.1 mmol/L dithiothreitol(DTT) can accelerate the drug release speed, and a concentration of 10.0 mmol/L DTT is higher enough to trigger the open of cross-linked PLGA network so as to realize rapid release of drugs. All the results demonstrate that the cross-linked PLGA chains on the surface of MSN could act as efficient gatekeepers to control the on-off of the pores, showing potential application in drug delivery system.

Keywords Mesoporous silica nanoparticle; Redox responsive; Drug delivery; Surface modification; Hybrid

1 Introduction

In recent years, mesoporous silica nanopartcles(MSN) have received much attention due to their ordered mesopores, high surface areas, easily functionalized surface, large pore volume and excellent biocompatibility[1,2]. The tunable porous channel can be used to absorb or load drug molecules to achieve a large drug loading efficiency. These unique advantages make MSN an ideal carrier for drug controlled release systems[3]. However, in order to prevent premature drug release from MSN and achieve drug controlled release, various types of materials, such as smart valves based on host-guest systems[4—6], DNA based molecular or peptide sequences[7,8], stimuli-responsive polymers[9,10] and nanoparticles[11] have been anchored on the surface of MSN to act as gatekeepers, which thus endow MSN with the intelligent properties so that controlled drug release behaviors can be obtained *via* the external changes. For example, Yang's group[12,13] has reviewed the MSN surface immobilized with biocompatible molecues and supramolecular swithches, and also introduced that various external stimuli, such as pH, enzyme, light, temperature and redox can trigger the release of model drugs.

It has been reported that the concentration of reducing glutathione(GSH) is approximately 2—10 mmol/L in the cytosol and cell nucleus, while the concentration of GSH in the extracellular fluids and circulation system is about 2—20 μmol/L[14]. The redox-responsive drug delivery systems which use disulfide linkage as the gatekeeper have aroused much attention[15] due to this kind of drug controlled delivery system realizing intracellular drug release to enhance the cancer therapy. For example, Cai *et al.*[16] reported the fabrication of nano reservoirs based on MSN that are end-capped with collagen, which demonstrates great potential for both cell-specific targeting and redox-responsive controlled drug release. Feng *et al.*[17] synthesized a cross-linked poly(*N*-acryloxysuccinimide) network on the surface of MSN to cap the nano-pores. The uncapping can be easily achieved *via* the addition of dithiothreitol(DTT) to induce the cleavage of disulfide bonds. Zhang *et al.*[18] synthesized a *β*-cyclodextrin(*β*-CD) anchored on the surface of mesoporous silica nanoparticles *via* disulfide linking for glutathione-induced drug release.

Poly(*L*-glutamic acid)(PLGA) is one of the widely studied synthetic polypeptides. Owing to its biodegradability, biocompatibility and non-toxic features, PLGA has become a

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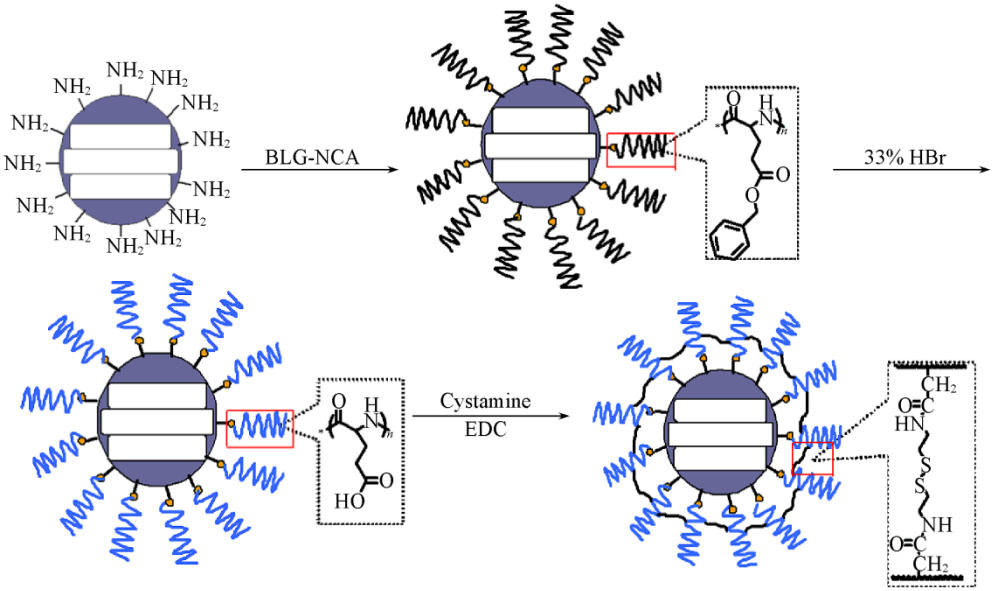
promising material in drug delivery[19]. Herein, a novel reduction-responsive controlled drug delivery system based on MSN grfated with cystamine cross-linked poly(*L*-glutamic acid) (MSN-*c*PLGA) was prepared. As shown in Scheme 1, MSN was firstly functionalized with amino groups, and then PLGA chains were grafted on the surface of MSN *via* the ring opening polymerization of *γ*-benzyl-*L*-glutamate *N*-carboxyanhydride (BLG-NCA) and deprotection of benzyl groups. Then the PLGA layers were cross-linked by reactions between the carboxyl groups and cystamine. The surface grafted PLGA chains act as gatekeepers to control the drug release. The *in vitro* release of the drug from MSN-*c*PLGA was a typical redox-responsive, implying that the MSN-*c*PLGA may have potential application in intracellular drug delivery system.



Scheme 1 Preparation route of MSN-*c*PLGA

EDC: 1-Ethyl-3-(3-dimethylamino-propyl) carbondiimide.

2 Materials and Method

2.1 Materials and Instruments

Triphosgene, *L*-glutamic acid 5-benzylester, dithiothreitol (DTT) and 5-fluorouracil(5-Fu) were purchased from Aladdin Reagent Company(Shanghai, China). 3-Aminopropyltriethoxysilane(APS) was purchased from Alfa Aesar. Tetraethyl orthosilicate(TEOS) was purchased from Sinopharm Chemical Reagent Co., Ltd. *N*-Cetyltri-methylammonium bromide (CTAB), 1,3,5-trimethylbenzene(TMS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide(EDC) and cystamine dihydrochloride were purchased from Shanghai Chemical Reagent Co., Ltd.(China). Fourier transform infrared(FTIR) spectrophotometer, Shimadzu IR prestige-21, Japan; field-emission transmission electron microscope(TEM), JEOL JEM-2100, Japan; thermal gravimetric analyzer(TGA), Netzsch STA409PC, Germany; accelerated surface area and porosity system, Micromeritics ASAP2020C, USA; Zetasizer, MALVERN NANO ZS90; Ultraviolet-visible(UV-Vis) spectrophotometer, Perkin-Elmer Lambda 750, USA.

2.2 Preparation of BLG-NCA

BLG-NCA was synthesized according to the reported approach[20]. Briefly, *L*-glutamic acid 5-benzylester(10.0 g, 42 mmol) and triphosgene(5.0 g, 20.1 mmol) were suspended in anhydrous tetrahydrofuran(THF, 100 mL) and then heated to 60 °C under nitrogen atmosphere. The mixture was stirred for a certain reaction time until the suspension became transparent. Then the solution was poured into petroleum ether(800 mL), and a white precipitate was obtained. The white product was collected and recrystallized three times from a mixture of anhydrous ethyl acetate/petroleum ether.

2.3 Preparation of Amino Group Functionalized MSN(MSN-APS)

Firstly, MSN was prepared based on the following procedure[21]. Typically, 0.8 g of CTAB in 5.6 mL of TMS was dissolved in 300 mL of deionized water, heated to 80 °C, and then 2.8 mL of NaOH aqueous solution(2 mol/L) was added to the mixture. 30 minutes' later, 5.6 mL of TEOS was dropped into the mixture and the reaction continued for another 2 h. In the end, the white precipitate was collected by centrifugation, washed with ethanol and deionized water separately, and then lyophilized. The obtained product was denoted as MSN-CTAB. Secondly, MSN-APS was prepared by the following procedure. MSN-CTAB(100 mg) was dispersed in 40 mL of ethanol containing 200 μL of APS, then the suspension was heated to 80 °C and refluxed for 6 h. The raw product(MSN-CTAB-APS) was collected by centrifugation, washed with ethanol several times and lyophilized. In order to remove the template CTAB, MSN-CTAB-APS was added to an ethanol solution of ammonium nitrate(NH_4NO_3/C_2H_5OH, 10 mg/mL) and refluxed for 6 h at 80 °C. In the end, the final product(MSN-APS) was collected by centrifugation and lyophilized.

2.4 Preparation of Poly(*γ*-benzyl-*L*-glutamate) Grafted MSN(MSN-PBLG)

MSN-APS(100 mg) and BLG-NCA(400 mg) were put

into a flame-dried polymerization bottle, and the air in the bottle was replaced with water-free nitrogen. Then anhydrous DMF(10 mL) was injected into the bottle. The mixture was firstly dispersed by ultrasonic treatment and then stirred at 37 °C for 3 d. Then the raw product was collected by centrifugation and washed with DMF and deionized water several times, respectively. In the end, a white product(MSN-PBLG) was obtained by lyophilization.

2.5 Preparation of MSN-PLGA

The protecting group in MSN-PBLG was removed according to the reported method[22]. MSN-PBLG(100 mg) was dispersed in trifluoroacetic acid(10 mL) in the ice bath and then HBr(1 mL, 33% in acetic acid) was added to the mixture. The dispersion was stirred in the ice bath for 2 h. The reaction mixture was poured into 40 mL of ice-cold dry ether, followed by centrifugation, and washed with deionized water several times. The product(MSN-PLGA) was lyophilized.

2.6 Drug Loading and Release

To evaluate the drug loading and release properties, 5-fluorouracil(5-Fu) was chosen as a model drug. 5-Fu was dissolved in 8 mL of PBS solution(pH=7.4) at a concentration of 1 mg/mL. MSN-PLGA(10 mg) was ultrasonically dispersed in the above solution and stirred at room temperature for 24 h. Then EDC(140 mg) and cystamin(20 mg) were added to the suspension. The mixture was stirred for another 12 h, and then the drug loaded MSN-*c*PLGA was collected by centrifugation, washed with PBS solution several times and lyophilized.

5-Fu loading efficiency(LE) on MSN-*c*PLGA was quantified by UV-Vis spectroscopy, where LE was calculated according to Eq.(1).

$$\mathrm{LE}(\%)=\frac{\text{Mass of 5-Fu in MSN}}{\text{Mass of total 5-Fu added}}\times 100\% \quad (1)$$

The cumulative amounts of 5-Fu released from MSN-*c*PLGA at different time intervals were calculated according to Eq.(2).

$$\text{Cumulative release}(\%)=\frac{m(\text{5-Fu released from MSN})}{m(\text{5-Fu initially loaded in MSN})}\times 100\% \quad (2)$$

The release of 5-Fu from MSN-*c*PLGA(2.0 mg) was carried out in 20 mL of PBS solution at different DTT concentrations. Release profiles were obtained by plotting the cumulative release of 5-Fu in solution as a function of time.

3 Results and Discussion

3.1 Characterization of MSN-PLGA

The preparation process of MSN-PLGA is illustrated in Scheme. 1. MSN was prepared *via* the sol-gel method. Amino groups were connected on the surface of MSN by the reaction between MSN and APS. Since the meso-pores of MSN were filled with CTAB, the reaction between APS and MSN only took place on the exterior surface of MSN. CTAB was removed by refluxing in an ethanol solution of ammonium nitrate. Poly(γ-benzyl-*L*-glutamate)(PBLG) was grafted on the surface of MSN *via* the ring-opening polymerization of BLG-NCA initiated by MSN-APS, and the benzyl groups on PBLG chains were deprotected, resulting in the formation of MSN-PLGA.

The infrared absorption spectra of MSN, MSN-APS and MSN-PLGA are shown in Fig.1. Different peaks at 1639, 796, 960 and 1084 cm^{-1} on all the curves are attributed to the stretching vibrations of O—H, the flexible vibrations of Si—O, the stretching vibrations of Si—OH and Si—O—Si, respectively[23]. As shown in Fig.1 curve *b*, a new peak at 1547 cm^{-1} appears, which is attributed to the flexible vibrations of N—H bonds, identifying that amino groups have been connected on the surface of MSN. Compared with Fig.1 curve *b*, Fig.1 curve *c* shows some peaks at 1737, 1658, 740 and 698 cm^{-1}, and these peaks are characteristic peaks of PBLG chains on the surface of MSN, which are attributed to the vibrations of carbonyl ester in the side chain of PBLG, amide I and benzene ring, respectively, demonstrating PBLG has been successfully grafted on the surface of MSN. After the reaction between MSN-PBLG and HBr in acetic acid, benzyl groups were deprotected from PBLG chains, and thus the peaks at 740 and 698 cm^{-1} disappeared(Fig.1 curce *d*), demonstrating that MSN-PLGA was prepared. At the same time, the peak intensity at 1737 cm^{-1} decreased, indicating the successful deprotection of benzyl groups.

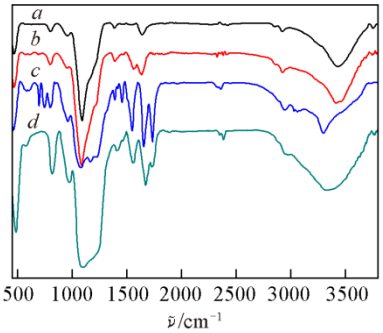


Fig.1 FTIR spectra of MSN(*a*), MSN-APS(*b*), MSN-PBLG(*c*) and MSN-PLGA(*d*)

The TGA curves shown in Fig.2 were used to evaluate the graft amount of PLGA on the surface of MSN. After the temperature increased to 800 °C, the mass loss of MSN and MSN-APS were 8.69% and 12.7%, respectively. Moreover, after ring-opening polymerization of BLG-NCA, the mass loss increased to 53.2%, indicating that the mass of PBLG was about 40.5%. After deprotection of the benzyl groups, the mass

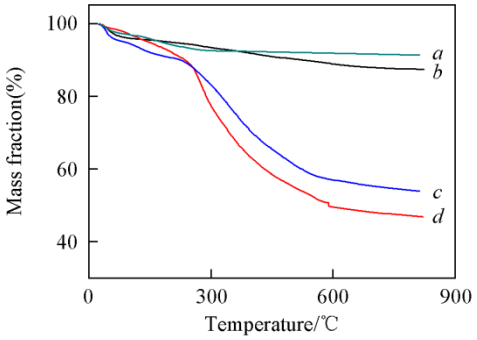


Fig.2 TGA curves of MSN(*a*), MSN-APS(*b*), MSN-PLGA(*c*) and MSN-PBLG(*d*)

loss of MSN-PLGA decreased to 46.1%, indicating that the mass of PLGA layer was about 33.4%.

TEM images of MSN-APS and MSN-*c*PLGA are shown in Fig.3. The synthesized MSN-APS was uniform nanospheres with regular array channels[Fig.3(A) and (B)]. From Fig.3(C) and (D), it can be seen that the edge of MSN was sealed by a thin layer of polymer, which was composed of cross-linked PLGA. The diameters of MSN-APS and MSN-*c*PLGA were determined by measuring the size of more than ten particles from the TEM image, and the corresponding results were given in the form of mean value±standard deviation. The diameters of MSN-APS was about (106±13) nm, while the diameter of MSN-*c*PLGA nanoparticles increased to (124±9) nm due to the polymer layers on its surface.

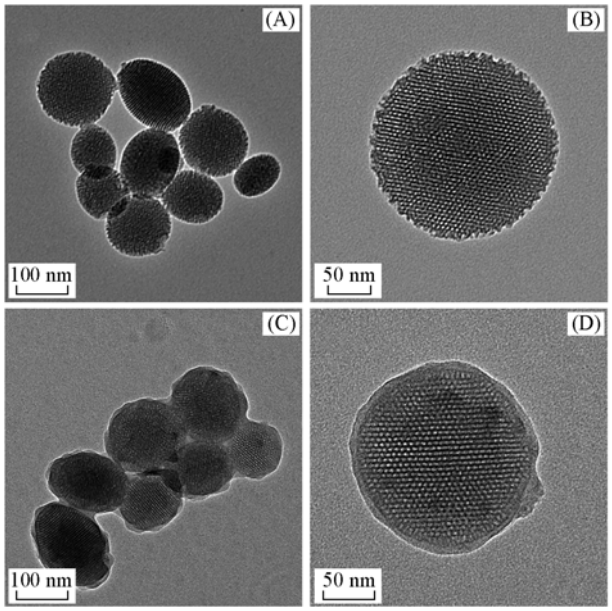


Fig.3 TEM imges of MSN(A, B) and MSN-*c*PLGA(C, D)
(B) and (D) are the magnified images of (A) and (C), respectively.

In order to further confirm the successful crosslink of PLGA chains on the surface of MSN, energy disperse spectrum was used to determine the existence of sulfur elements. As shown in Fig.4, the appearance of a new peak assigned to S element derived from disulfide groups confirms that the reaction between cystamine and the carboxyl group of PLGA side chain has completed.

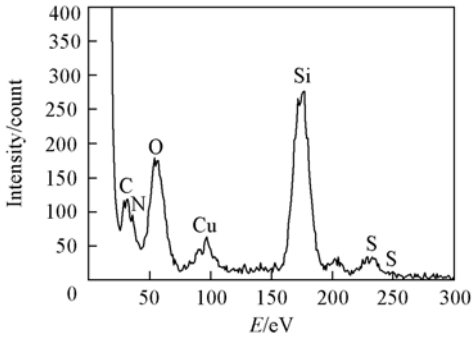


Fig.4 Energy dispersive spectrum of MSN-*c*PLGA

Fig.5(A) shows the nitrogen adsorption-desorption isotherms of MSN, MSN-APS and MSN-*c*PLGA. The BET isotherms of MSN and MSN-APS displayed typical IV isotherm with a hysteresis loop which was the typical characteristic of mesoporous materials. The pore size distributions of MSN, MSN-APS and MSN-*c*PLGA calculated *via* Barrett-Joyner-Halenda(BJH) method are shown in Fig.5(B). The BET surface areas of MSN and MSN-APS were about 823 and 748 m^2/g, respectively, while the surface areas of MSN-*c*PLGA descreased to 79 m^2/g, indicating the surface of MSN was closely capped by PLGA chains.

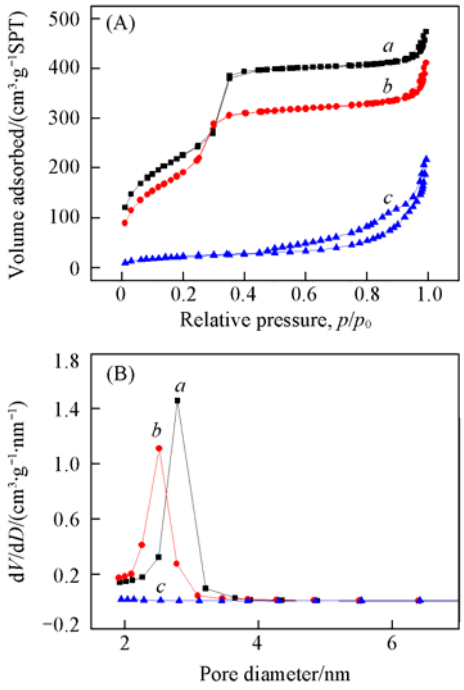


Fig.5 Nitrogen adsorption-desorption isotherms(A) and pore size distribution curves(B) of MSN(*a*), MSN-APS(*b*) and MSN-*c*PLGA(*c*)

The zeta potential changes can also identify the changes of MSN structure. The zeta potential of MSN was –23 mV. After MSN was treated with APS, amino groups appeared on the surface of MSN, and thus the zeta potential of MSN-APS increased to –1.76 mV. After MSN was grafted with PLGA, the zeta potential of MSN-PLGA was decreased to –43.6 mV, indicating the existence of a large amount of carboxyl groups. The zeta potential of MSN-*c*PLGA was only –29.3 mV, indicating that the amidation reaction between cystamine and carboxyl groups of PLGA was successful and the amount of carboxyl groups on PLGA chains was decreased.

3.2 Drug Loading and *In vitro* Release of 5-Fu

The loading efficiency(LE) of MSN-PLGA analyzed by UV-Vis spectroscopy was about 14.8%. To evaluate the drug release behavior of 5-Fu@MSN-*c*PLGA in cancer cells and normal human cells, the *in vitro* drug release experiment was carried out in PBS solutions of pH=7.4 with different concentrations of DTT at 37 °C. As shown in Fig.6, after 24 h, the total amount of released drug was only about 7.1% without the addition of DTT, suggesting that most of the drug was sealed in the channels of MSN by the cross-linked PLGA shell. With the increase of DTT content, the release speed of drug accelerated a lot(Fig.6 curve *b*, *c* and *d*). This is because the cross-linked

network of PLGA was dissociated under the action of DTT, and thus PLGA chains were spread into PBS solution, which opened the gate of MSN pores to make the drug released quickly. The fast release of 5-Fu at a concentration of 10 mmol/L DTT reached 44.5% at 1 h and 80.4% at 24 h, which was due to the cleavage of cross-linked PLGA networks in the DTT solution. To further investigate the redox response of disulfide bonds to external stimulus, DTT was added to MSN-*c*PLGA having been immersed in a PBS solution for 6 h. As is shown in Fig.7, only 6.8% of 5-Fu was released after the first 6 h(without DTT), while 62.2% of 5-Fu was released at a subsequent 18 h interval at the addition of DTT. The results suggest that drug delivery from MSN-*c*PLGA can be mediated by the disulfide reducing agent, thus resulting in redox-responsive controlled drug delivery. On the contrary, if the PLGA chains had not been crosslinked with the disulfide bonding, the MSN-PLGA would not have the redox responsive properties, and would only have had the pH responsive properties. As reported by Yang's group, the MSN-PLGA can be used to realize the controlled release of anticancer drug in a pH responsive manner[24].

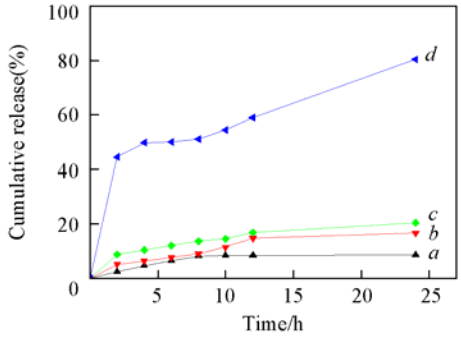


Fig.6 Cumulative release of 5-Fu from MSN-*c*PLGA nanoparticles at different concentration of DTT

Concentration of DTT/(mmol·L^{-1}): *a*. 0; *b*. 0.1; *c*. 1.0; *d*. 10.0.

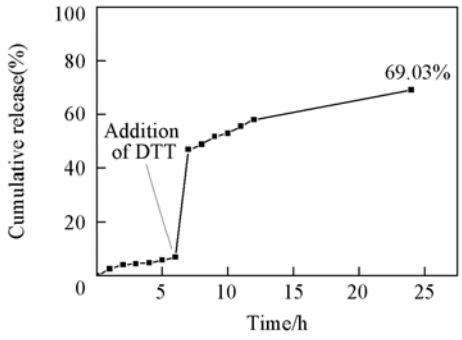


Fig.7 Accelerate release of 5-Fu from MSN-*c*PLGA by addition of DTT solution after incubation for 6 h

4 Conclusions

Disulfide crosslinked poly(*L*-glutamic)(PLGA) was successfully grafted onto the surface of MSN, and the new hybrid MSN-*c*PLGA with about 33.4%(mass fraction) PLGA was obtained. The *in vitro* drug loading and release experiments showed that the drug loading amount of MSN-*c*PLGA was about 14.8%. Owing to the existence of DTT, about 80.4% of drugs released from the MSN-cPLGA system, while only 7.1% of the drug released from the MSN-*c*PLGA without the addition of DTT, demonstrating that the MSN-*c*PLGA hybrid can be a promising drug delivery system in biomedical fields.

References

[1] Lu J., Liong M., Li Z. X., Zink J. I., Tamanoi F., *Small*, **2010**, *6*(16), 1794
[2] Meng H. A., Xue M., Xia T. A., Zhao Y. L., Tamanoi F., Stoddart J. F., Zink J. I., Nel A. E., *J. Am. Chem. Soc.*, **2010**, *132*, 12690
[3] He Q. J., Shi J. L., *J. Mater. Chem.*, **2011**, *21*(16), 5845
[4] Yang Y. W., Sun Y. L., Song N., *Acc. Chem. Res.*, **2014**, *47*, 1950
[5] Sun Y. L., Yang Y. W., Wu W., Zhang S. X. A., *Chem. J. Chinese Universities*, **2012**, *33*(8), 1635
[6] Li Q. L., Sun Y. F., Sun Y. L., Wen J. J., Zhou Y., Bing Q. M., Isaacs L. D., Jin Y. H., Gao H., Yang Y. W., *Chem. Mater.*, **2014**, *26*, 6418
[7] Schlossbauer A., Warncke S., Gramlich P. M. E., Kecht J., Manetto A., Carell T., Bein T., *Angew. Chem. Int. Ed.*, **2010**, *49*(28), 4734
[8] Coll C., Mondragon L., Martinez-Manez R., Sancenon F., Marcos M. D., Soto J., Amoros P., Perez-Paya E., *Angew. Chem. Int. Ed.*, **2011**, *50*(9), 2138
[9] Li Q. L., Wang L. Z., Qiu X. L., Sun Y. L., Wang P. X., Liu Y., Li F., Qi A. D., Gao H., Yang Y. W., *Polym. Chem.*, **2014**, *5*, 3389
[10] Sun Y. F., Sun Y. L., Wang L. Z., Ma J. B., Yang Y. W., Gao H., *Micropor. Mesopor. Mat.*, **2014**, *185*, 245
[11] Liu R., Zhang Y., Zhao X., Agarwal A., Mueller L. J., Feng P. Y., *J. Am. Chem. Soc.*, **2010**, *132*(5), 1500
[12] Yang Y. W., *Med. Chem. Commun.*, **2011**, *2*, 1033
[13] Song N., Yang Y. W., *Chem. Soc. Rev.*, **2015**, *44*, 3474
[14] Deng C., Jiang Y. J., Cheng R., Meng F. H., Zhong Z. Y., *Nano Today*, **2012**, *7*, 467
[15] Huo M., Yuan J. Y., Tao L., Wei Y., *Polym. Chem.*, **2014**, *5*, 1519
[16] Luo Z., Cai K., Hu Y., Zhao L., Liu P., Duan L., Yang W., *Angew. Chem. Int. Ed.*, **2011**, *l50*, 640
[17] Liu R., Zhao X., Wu T., Feng P. Y., *J. Am. Chem. Soc.*, **2008**, *130*(44), 14418
[18] Zhang J., Yuan Z. F., Wang Y., Chen W. H., Luo G. F., Cheng S. X., Zhuo R. X., Zhang X. Z., *J. Am. Chem. Soc.*, **2013**, *135*(13), 5068
[19] Yang Y. N., Cai J., Zhuang X. L., Guo Z. P., Jing X. B., Chen X. S., *Polymer*, **2010**, *51*(12), 2676
[20] Wei J. C., Liu A. X., Chen L., Zhang P. B., Chen X. S., Jing X. B., *Macromol. Biosci.*, **2009**, *9*(7), 631
[21] Slowing I. I., Trewyn B. G., Giri S., Lin V. S. Y., *Adv. Funct. Mater.*, **2007**, *17*(8), 1225
[22] Kar M., Pauline M., Sharma K., Kumaraswamy G., Sen G. S., *Langmuir*, **2011**, *27*(19), 12124
[23] Chang J. S., Kong Z. L., Hwang D. F., Chang K. L. B., *Chem. Mater.*, **2006**, *18*(3), 702
[24] Zheng J., Tian X. J., Sun Y. F., Lu D., Yang W. L., *Int. J. Pharmaceut.*, **2013**, *450*, 296