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Fabrication of PVCL-co-PMMA Nanofibers with Tunable Volume Phase Transition Temperatures and Maintainable Shape for Anti-cancer Drug Release

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ABSTRACT

This work is to fabricate thermo responsive nanofibers of which the thermo response temperatures could be easily tuned, and of which the fibrous shapes could be maintained after the heating-cooling cycles in the aqueous solution. The nanofibers were further fabricated into nonwoven mat with the size -variable pores for temperature controlled release of model drug of Erlotinib. The thermo responsive nanofibers were electrospun from the copolymers of PMMA-co-PVCL (synthesized from MMA and PVCL, and had different LCSTs) by changing the solvents and the ratio of initiator/monomer. FT-IR and ^{1}H NMR were used for molecular structural characterization; UV-vis spectra were used for LCST measurement; SEM and metalloscope were used to determine the optimize electrospinning parameters and to observe the shape maintaining abilities of the nanofibers after the heating-cooling recycles. Then anti-cancer drug of Erlotinib was incorporated into PMMA/PVCL nanofibers (represent as 'model I'),or put in drug reservoir and covered with PMMA/PVCL electrospinning mat (represent as 'model II'). Uv-vis spectra were used to study the drug release behavior of each model. Results indicate that in model I, drug release was "switch on" below LCST, and "switch off" above LCST; in model II, drug release faster above LCST than that below LCST.

Keywords: PVCL-co-MMA, thermo responsive polymers, electrospinning, nanofibers, Erlotinib

1. Introduction

During the past decades, considerable interests have been attracted to utilizing stimuli responsive polymers as smart drug delivery systems (smart-DDS) [1, 2]. Thermo responsive polymers with lower critical solution temperature (LCST) close to human body are probably the predominantly studied stimuli responsive polymers and have been widely explored in oncology [3].The polymers are hydrophilic while below LCST, and are lipophilic while above LCST. With the heat inducing phase transition, the polymer would shrink quickly and then form dense skin layers in the interfaces, lead to an initially burst release and then a stopped release [4].

One of the most feasible methods to avoid burst release is to encapsulate drugs into nanofibers [5]. Electrospinning, as a unique and cost-effective way for the fabricating of high specific surface area nanostructures [6], has been proven an efficient approach to obtain drug delivery vehicles [7,8,9]. Electrospinning thermo responsive nanofibers tend to loss their shapes below LCST. Hydrophobic monomers have been used to enhance the longevity of the thermo responsive nanofibers [10]. As a widely used hydrophobic monomer, methyl methacrylate (MMA) could be copolymerized with PNIPAM, and the products have been studied in fabrication of thermo-responsive nanofibrous mats for bioseparation [11],nano particles for adsorption of human IgG[12] , and hybrid capsules for controlled drug release [13] etc.

Poly vinyl caprolactam (PVCL) is a promising thermo responsive polymer. Compared with the most studied PNIPAM, PVCL shows advantages in non-producing small toxic amide compounds under hydrolysis conditions [14,15,16]. Besides, PVCL is non-ionic, non-toxic and biocompatible, makes it an ideal material for bio applications [17]. Besides, PVCL exhibits the critical miscibility behavior of type□ Flory–Huggins (FH) [18], and its LCST values could be affected by the polymerization degree. Vinyl caprolactam consists of a six member cyclic amide group and the nitrogen within the amide group is connected directly to the vinyl group. This makes it a typical unconjugated monomer, and could undergo the polymerization radically [19]. Even though the low reactivity of VCL(vinyl caprolactam) makes the atom transfer radical polymerization [20] (ATRP) and the reversible addition fragmentation transfer polymerization [21] (RAFT) a difficult task, the low reactivity meanwhile enables the control of polymerization degrees by changing the solvent and the initiator/monomer ratio feasible. Up to now, studies on fabricating PVCL and PVCL-based composites in to nanostructure drug delivery system are relatively scarce [22].

Thermo responsive drug delivery systems can be classified into the negative model and the positive model [23].For the negative model, drugs release relative faster at temperatures than that below LCST. In this model, mechanism may be attributed to that the swollen thermo responsive polymers could permeate more faster than the shrunken one. Jian Qian et al [24] fabricated semi-hollow spheres that consisting of low density cores, dense shells and separated cavities between cores and shells. The shell could be swollen below LCST, and

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the drug of doxorubicin thus penetrated faster than the dense shell. Ye-Zi You et al [25] fabricated PNIPAM-modified porous silica nanoparticles. The drug of fluorescein was encapsulated in the pores of silica nanoparticles. When PNIPAM was lipophilic, the pores were blocked, and when PNIPAM became hydrophilic, the polymer would swell and fluorescein could be released. The similar results could be seen in the works of Sebastian Berger [26] and the works of Bala Yerri Swamy [27]. And the negatively thermo responsive drug release systems should be triggered by the external thermal signals.

For positive thermo responsive drug release system, drugs release relative faster at temperature above LCST. In this model, mechanism may be attributed to that the thermo responsive polymers could block the passages of drug molecules at lower temperatures, thus would shrink and clear the passages above LCST. Xian-Zheng Zhang et al [28] used a dialysis bag to envelope PNIPAM hydrogel. The pores on the dialysis bag was blocked by the swollen PNIPAM that blow LCST, and generated many voids above LCST, thus led to a positive release; Todd Hoare [29]capsuled a drug reservoir with nanocomposite membranes that containing thermo responsive microgels. When the temperature was above LCST, the microgels shrunk and generated channels for drug release. Similar results could be seen in the work of Liu et al [30] and Chang et al [31]. As afflicted tissues are usually 1~2 centigrade higher than that normal tissues, the drug release of the positively thermosensitive system could be triggered by the fever at focus spontaneously, usually is preferable for targeting release [32].

In this work, PVCL with different LCSTs were synthesized via radical polymerization. PMMA was graft onto PVCL to improve the hydrophobicity of PVCL and help to keep the shape of PVCL nanostructure during heating-cooling circles. The PVCL-co-PMMA nanofibers were electrospun from copolymers of PMMA-co-PVCL, which were synthesized by coplymerization the monomer of MMA with PVCL. The LCSTs of PVCL could be modulated by changing the polarity of the solvents and the ratios of initiator/ monomer. Nanofibers of PMMA-co-PVCL and nonwoven mat composed of the nanofibers were used as drug delivery carriers. Anti cancer drug of Erlotinib was used as model drug. Erlotinib was incorporated into PMMA/PVCL nanofibers(as model I); or was put into drug reservoir and covered with PMMA/PVCL nonwoven mat(as model II).

2. Results and discussion

Table 1 listed the monomer conversions that corresponding to the different reaction conditions. All the reactions were carried out at the temperatures of about 70 ^{0}C. Exceptions were that tetrahydrofuran and methanol as solvents (the boil points were 65 ^{0}C and 67 ^{0}C, respectively).

Table 1. The monomer conversions of the polymerization of PVCLunder different reaction conditions a, b, c- with the weight ratio of AIBN and NVCL of 0.01,0.004 and 0.002, respectively. The concentration of NVCL is 1g/4mL.

C	5 \ 3 3119 \ 7 979 7
o	9. 0. .2 .2 0. .3 1. 7.
nv	6 3 5 6 8
er	
Ti	1 1 1 1212 1 1 122 2
m	2 2 2 2 2 4 4
e(	
Te	6 7 7 7070 7 7 676 6
m	5 0 0 0 0 7 7
pe	
ra	
Ini	0. 0. 0. 0. 0. 0. 0. 0. 0.
tia	0 0 0 0101 0 0 010 0 0
to	1 1 1 1 1 0 0
So	T D Di *is* C D Et M M M
lv	H Mox *o*- hl Mhaet et et
en	F S anpr or F n hahaha
t	O e o of ol n n n

As can be seen in table 1, the conversions of vinyl caprolactam were significantly affected by solvents and initiator/monomer ratio. Similar phenomenon could be seen in previous published literature [33, 34]. A conclusion could be drawn that conversions of NVCL were higher in polar aprotic solvents than in nonpolar solvents, and higher in small molecular solvents than in that larger ones.

NVCL was considered to be an inertia monomer, can only be initiated by free radical polymerization, and the reactivity of VCL was 20 times lower than MMA[35]. The polymerization route of PVCL-co-PMMA in our work was illustrated in Fig. 1.

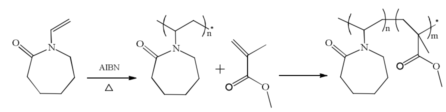


Fig 1. The free radical polymerization process of PVCL& PVCL-co-PMMA

2.1 FT-IR spectra of PVCL and PVCL-co-PMMA

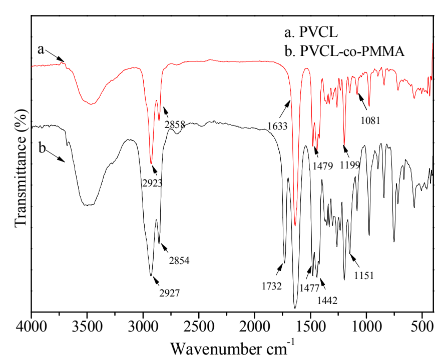


Fig 2. FT-IR spectrum of a.PVCL and b. PVCL-co-PMMA

FT-IR spectrum was used to determine the polymerization of PVCL and the copolymer. As represented in Fig.2(a), peaks at 1081 cm^{-1} and 1199 cm^{-1} were corresponded to the bending vibration of C-N, and the peak at 1479 cm^{-1} was corresponded to the stretching vibration of C-H. The peaks at 2923 cm^{-1} and 2858 cm^{-1} were corresponded to the C-H stretching vibration of methyl and the peak at 1633 cm^{-1} was corresponded to the C=O stretching vibration. Compared to NVCL monomer, peak at 3190 cm^{-1} which corresponding to the C-H stretching vibration of vinyl, peak at 1606 cm^{-1} which corresponding to the C=C stretching vibration and peak at 994 cm^{-1} which corresponding to the bending vibration of vinyl all vanished within the spectra of PVCL, indicating the polymerization of NVCL. In Fig.2(b),peaks at 1151 cm^{-1} and 1732 cm^{-1} were assigned to C=O stretching and $–O-CH_3$ stretching, respectively. Peaks at 2927 cm^{-1} and 2854 cm^{-1} were assigned to C-H stretching vibration of methyl, peaks at 1442 and 1479 cm^{-1} were assigned to the stretching vibration of C-H, indicating the copolymerization of PVCL and PMMA.

2.2 ^{1}H NMR spectra of PVCL and PVCL-co-PMMA

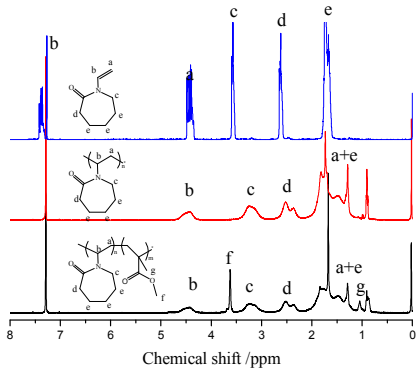


Fig 3. ^{1}HNMR of(a) VCL monomer, (b) PVCL and (c) PVCL-co-PMMAin $CDCl_3$ that measured at 400 MHz. (The inset was the resonance's assignments).

Fig 3(a) showed the ^{1}H NMR spectra of NVCL.The signalat 7.35 ppm was assigned to the proton of the –CH= group, and the signal at 4.39 corresponded to the two protons of the allyl group (CH_2=). The signal at 1.73 ppm was assigned to the $–CH_2–CH_2–CH_2–$ groups, and the signal at 2.61 ppm corresponded to the two protons of methylene group. Fig 3(b) showed the shift of the peak of proton (a) to 1.7 ppm and of the peak of proton (b) to 4.41 ppm. And the disappearance of the peak at 7.35 ppm indicated the consuming of the group of C=C. Fig 3(c) showed the ^{1}H NMR spectra of PVCL-co-PMMA.The signal at 3.75 was assigned to the $–O-CH_3$ of PMMA and the signal at 1.2 was assigned to the $–CH_3$ of PMMA. The results of ^{1}HNMR indicate that the the product possess function group of both PVCL and PMMA.

2.3 GPC detection of PVCL

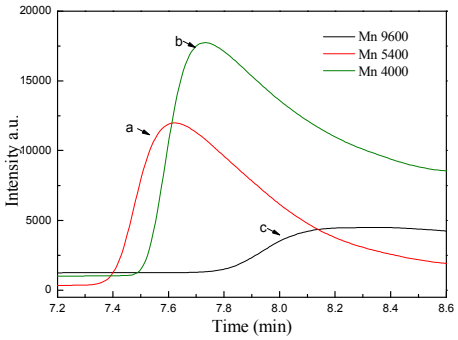


Fig 4. GPC of PVCL that synthesized with methanol as solvent and with different amounts of initiator. a- 1% of AIBN; b- 0.4% of AIBN; c- 0.2% of AIBN.

PVCL with different LCST values that at 33.0^{0}C, 36.2^{0}C and 37.2^{0}C were selected to determine the molecular weights and the molecular weight distributions by size exclusion chromatography detection. The products were synthesized with methanol as solvent and with different initiator/monomer weight ratios of AIBN/NVCL(0.4%, 0.2% and 1%). The concentration of NVCL was 4g/mL. Fig 4 gave the relations between the LCST values and the molecular weights. As can be seen, the molecular weights were 9600, 5400 and 4000 for the LCST value of 33.0^{0}C, 36.2^{0}C and 37.2^{0}C, respectively. And the polydispersity indexes were 1.34, 1.36 and 1.28, respectively.

2.4 Cloud point determination of PVCL

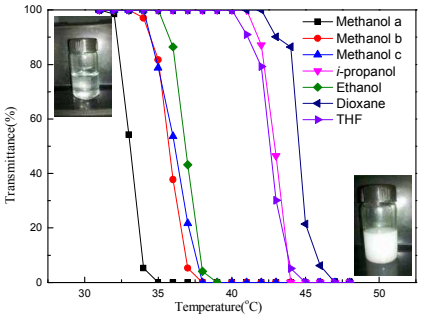


Fig 5. The cloud points of the synthesized PVCL within different solvents(The insets were the optical photos of the samples at different temperatures)

As a Flory-Huggins type Ⅰ polymer, the LCST values of PVCL were associated with its molecular weights [36]. As shown in Fig 5, vinyl caprolactam polymerized in stronger polarity solvents possess higher molecular weight; less initiator would

induce the lower polymerization degrees, show hint of coupling termination. As data showed in table 1, vinyl caprolactam polymerized in methanol exhibited higher conversions and larger molecular weights. Methanol was chosen as appropriate solvent for polymerization of vinyl caprolactam.

2.5 Influence of polymer concentrations on fibers morphology

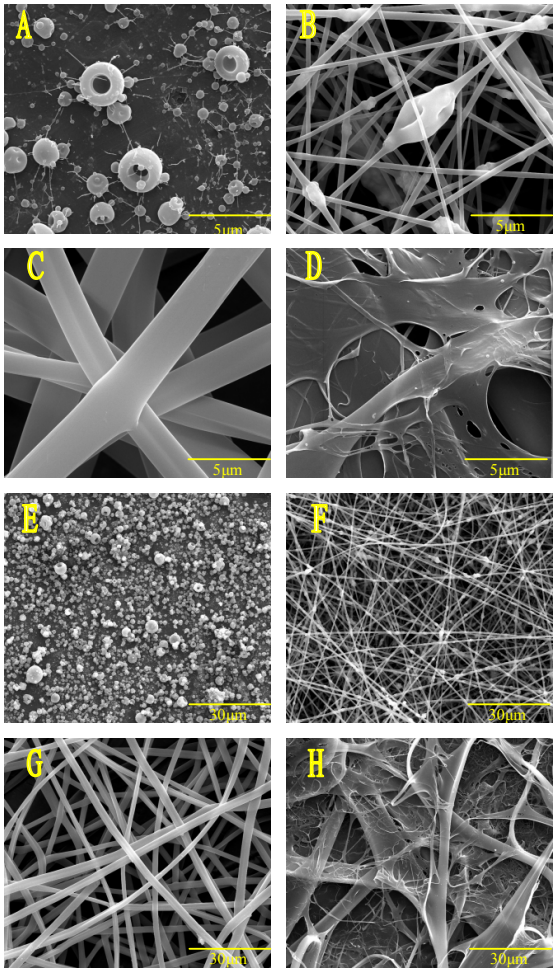


Fig 6. Morphology of nanofibers electrospinning from PVCL-co-PMMA, with PMMA to PVCL of 10% in weights, at different concentration with DMF as solvent; A,E 0.1 g/mL; B,F 0.2g /mL; C,G 0.4 g/mL; D,H 0.6g/mL.

The effect of electrospinning parameters on the morphology of the electrospinning nanofibers and nonwoven mats was investigated. As shown in Fig 6, PVCL-co-PMMA solution drips with concentration of 1.0 g/mL were attracted to the receptor without the formation of fibers. When the concentration of the electrospinning solution increased to 0.2 g/mL, beads on string morphology were formed. Increased the concentration to 0.4 g/mL, the smooth fibers were formed without beads onto. Increased the concentration further to 0.6 g/mL, the fibers increased the diameters, and in ribbon like belts. Conclusion can be drawn from Fig 6 that, with the increase of the concentration of the polymer solution, the diameters of the fibers increased.

2.6 Influence of PMMA content on fiber Stability

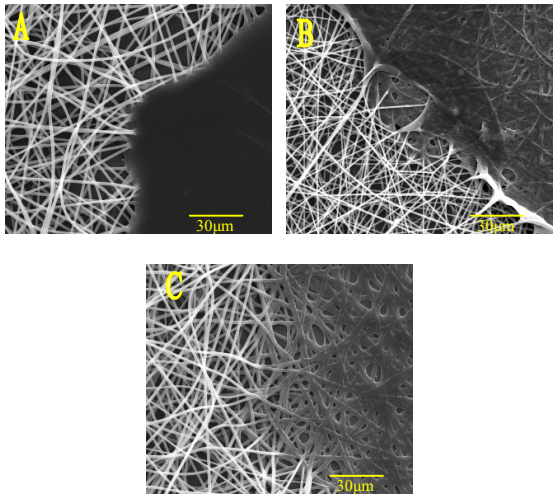


Fig 7. Morphology of nowoven mats with partically dripping of a drop of water; A pure PVCL;B PVCL-co-PMMA, with PMMA to PVCL of 10%; C PVCL-co-PMMA, with PMMA to PVCL of 20%.

Electrospun PVCL nanofibers could not keep the original shapes in aqueous solution, while below its LCST value. And the same phenomenon appeared for the electrospun copolymer of PVCL with the crosslinker of BMA in weight of 2%. This might caused by the entropic-driven elasticity and which led to the random coil conformation, and further resulted in the destroying of the fibrous morphology. Increasing the amount of MBA, the polymer could not dissolve within the solvent anymore; and thus handicapped the electrospinning. Thus, biocompatible hydrophobic PMMA segment would be employed to further improve the stability of the fibers. As shown in Fig 7 (B), PVCL mats with 2% MBA in weight and 10% MMA in weight could keep the fibrous morphology. As shown in Fig 7(C), PVCL electrospinning mats with 2% MBA and 20% MMA in weights mainly maintained the porous morphology.

2.7 Thermo response of the electrospun materials

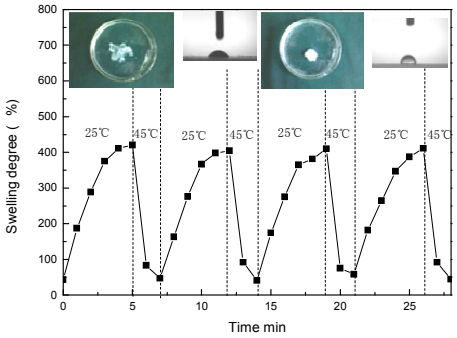


Fig 8. Thermo response of PVCL-co-PMMA with PMMA to PVCL of 10% and BMA of 2% in weights, in the heating and cooling circles between 25^{0}C to 45^{0}C for 4 times. Insets were a. photo of the gel at 25^{0}C, and contact angle of the polymer that dehydrated; b. photo of the gel at 45^{0}C, and contact angle of the polymer that hydrated.

Thermo response behaviors of the obtained PVCL-co-PMMA were tested by heating the gel to 45^{0}C and cooling down to 25^{0}C for 4 times. Fig 8 reflected that the gel contracted and discharged the absorbed solvents while heating to 45^{0}C; and the gel swelled and absorbed the solvent while cooling down to 25^{0}C, reversibly. So the synthesized copolymer and the further fabricated electrospun materials could both release the loaded drugs switchably with the changing of the temperatures. This would be illustrated further by the detection results of the fluorescence microscope images of the drug loaded nanofibers with and without the contact of water.

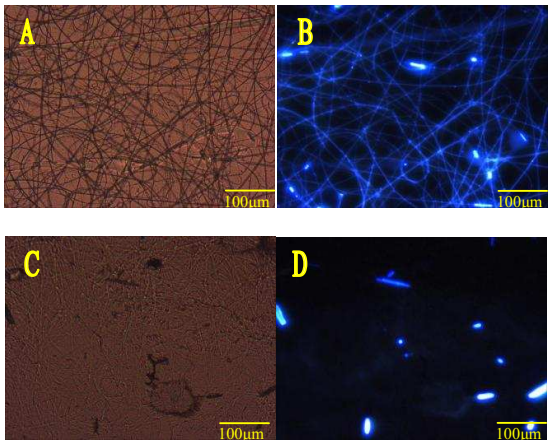
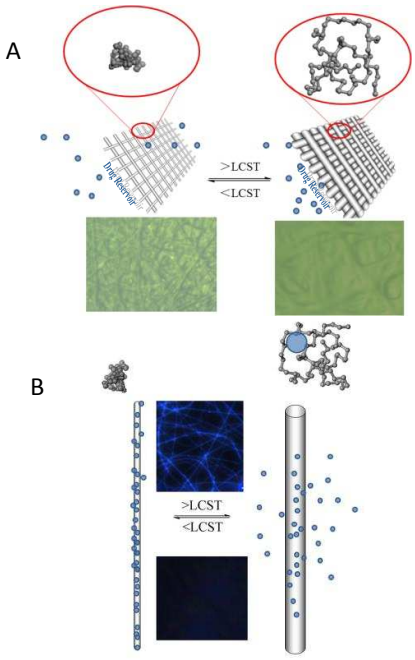


Fig 9. Fluorescence microscope images of (A) PVCL-PMMA nanofibers loaded with Erlotinib; (B) area corresponding to (A) under 365 nm UV radiation; (C) PVCL-PMMA nanofibers after dipped in water for 40 min; (D) area corresponding to C under 365 nm UV radiation.

Fig 9. reflects the existence and the release behavior of Erlotinib. Under the radiation of ultra violet at 365 nm, compared to the bright blue fluorescence of Erlotinib, PMMA-co-PVCL exhibited neglectablefluorescence. After dripping a drop of water on PVCL-co-PMMA nanofibers, as shown in Fig 9 (B) (with the same area that corresponding to (A)), the sample could maintain its original fibrous shape, and the fluorescent areas would not be restricted on the fibers anymore, indicating the release of the drug from the electrospun nanofibers.

2.8 Temperature controlled release of Erlotinib



Scheme 1. Illustration of (A)reversible swell- deswell of the thermo responsive electrospinning mat valve and its drug release;(B) reversible swell of the drug loaded thermo responsive fiber and its drug release

As demonstrated by Fig.10 (A) and (B), the drug release of the fibers showed switchable on-off characters while changing the temperatures to below or above the LCST values of PVCL. This could be explained by the confirmation changes of the electrospun polymers. The polymer chains would be swollen and the produced pores of the chain would be big enough for Erlotinib molecule to permeate, while the temperature was set under the LCST values. While the temperature was set above the LCST values, the fibers of the polymer contracted and became less hydrophilic, thus the permeation of the drugs would be restricted.

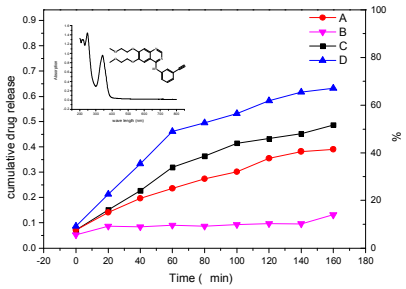


Fig 10. (A) drug release of Erlotinib loaded nanofiber at 25^{0}C;(B) drug release of Erlotinib loaded nanofiber at 39^{0}C; (C) drug release of Erlotinib wrapped electrospinning valve at 25^{0}C; (D) drug release of Erlotinib wrapped electrospinning valve at 39^{0}C. Insets were Ultra violet absorptionspectrum and structural formula of Erlotinib.

The Cumulative drug release (*c*) was linearly dependent to time (*t*), so it had the relation of -d*c*/d*t*=*kc* (where *k* referred to the rate constant), and *c*=0.0256*t*+0.11989 (the correlation coefficent of *R*=0.92,), indicating the release kinetics of the drug loaded fiber followed an approximate Zero order function at 25^{0}C. And the drug scarcely released at 39^{0}C.

As to Erlotinib wrapped PVCL electrospinning materials, nanofibers swelled at temperature that below LCST and deswelled that above it. The sizes of the pores on the electrospinning matswould shrink and expand accordingly. Erlotinib particles could be of acid soluble and of slow dissolution behavior at neutral pH values. Thus the drug particles would penetrate the pores when the nanofibers were in shrinking state, and while would be trapped when the nanofibers were in expanding state.

As reflected by Fig.10 (C) and (D), drug released at a faster rate at temperature that above the LCST values than that below one, indicating the positive relation of releases to temperatures. And although the drug particles were in trapped state at the “off” status, the nanofibers were still in more hydrophilic state while below the LCST values, and thus the gels were penetratable for drug molecules, so the drug still could be released.

3. Experimental Section

3.1 Materials

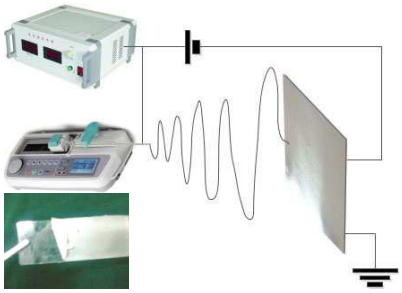
N-vinyl caprolactam (NVCL) was purchased from Sigma Co. Ltd.; N,N'-methylene diacrylamide (MBA) was purchased from A Damas Beta; 2,2'-Azobis (2-methylpropionitrile) (AIBN) , Methyl methacrylate (MMA) and solvents were purchased from Sinopharm chemical reagent Co. Ltd.; Erlotinib was supplied by courtesy of Sinopharm Co., Ltd. AIBN in analytical gradewas recrystallized with methanol, and other reagents were used as received.

3.2 Synthesis of PVCL and cross linked PVCL-co-MMA

PVCL was synthesized via free radical polymerization by placing monomer of NVCL in three neck flask, with volume concentration of 1 g/4 mL. Initiator of AIBN was then added with the quality percentage of varying from 0.2% to 1%. The obtained PVCL-co-PMMA was dissolved in methanol and filtrated to remove insoluble compound; and then dissolved in tetrahydrofuran and precipitated by n-hexane for several times to remove other impurities.

In synthesis of PVCL-co-PMMA, 2% crosslinker of MBA and varied amounts of MMA were added, when the reaction time of the synthesis of PVCL reached 12 h. The amount of MMA was a variant in proportion to PVCL that ranging from 5% to 30%. After 5 h, the copolymer was collected and purified by chloroform and then precipitated by n-hexane for several times.

3.3 Electrospinning the thermo responsive nanofibers and the electrospinning mats



Scheme 2. Illustration of the electrospinning equipment for the nanofibers and mats

The copolymer in amount of 0.2 g, 0.4 g, 0.6 g, 0.8 g was dissolved in 1 mL N,N-dimethyl-Formamide. Ultrasonic equipment was used to assistant the dissolution. Then the homogeneous solution was loaded into a syringe that equipment with stainless steel needle and connected to the positive pole of the supplier. And an aluminum foil was connected to the negative pole of the supplier as a receptor. The electric field was provided by a high voltage power supplier. Glass slides were adhered above the receptor foil, to make the exfoliation of electrospinning mats easier. The electrospinning equipment for the nanofibers and mats was illustrated in Scheme 2.

For the drug loaded fibers, drug was directly dissolved into the electrospinning solution. For the fibrous wrapper, the drug was fixed in drug reservoir, and the reservoir was covered with fibrous wrapper.

3.4 Instruments and measurement

Structural information was investigated with FT-IR (Specturum one, Perkin-Elmer, USA) and ^{1}H NMR (ADVANCE III, Bruker, 400MHz,GER). Molecular Weight was measured by GPC with RID detector (HP Agilent 1100, USA). LCST values and the cloud points were determined by the transmittance changes of the solution by ultraviolet-visible spectrometer (760CRT, INEASA, CN), (0.02 g PVCL was dissolved into 10 mL ultra pure water, and then the temperature was increased with the rate of 1^{0}C per 5 minutes). Morphology was investigated by Scanning Electron Microscope (Helios Nanolab600i, FEI, USA) and metallographic microscope (XJP-6A, COIC, CN). Distribution of drug was observed by fluorescence microscope (IBE2003, COIC, CN).Two types of thermo controlled release systems were fabricated, i.e. Erlotinib directly dissolved in electrospinning solution to fabricate fibers that carrying the drug; and Erlotinib wrapped within PVCL electrospinning mats. The ratios of drug to polymer were both 1:5. The drug release system was soaked in 50mL beaker filled with 0.1mol/L PBS (pH=6.5) and the beaker were put into a shaker equipped with thermostat. Samples of PBS were taken at constant time interval. The release amount of the anti-cancer drugs was detected by HPLC equipped with PDA detector (E2695-2998 Waters, USA).

4. Conclusions

PVCL-co-PMMA with different LCST values were synthesized via free radical polymerization. Thermo responsive nanofibers that based on PVCL-co-PMMA were fabricated via electrospinning for temperature controlled drug release of Erlotinib. Relative higher molecular weights and narrow weight distributions in higher conversions of PVCL were polymerized in methanol. PMMA chain segment could improve the stability of the thermo responsive nanofibers in aqueous solutions. With the introduction of PMMA in mass ratio of 20%, the nanofiber maitained its fibrous shape after the reversible swell-deswell cycles. Furthermore, a switchable release of Erlotinib for drug loaded PVCL-co-PMMA nanofibers could be achieved for 4 cycles with the changing of the temperatures. The drug incorporated into the PVCL-co-PMMA nanofibers show switchable drug release behavior, and the drug release velocity at temperature below LCST follows zero order kinetics. And the drug wrapped by PVCL-co-PMMA nanofibers could exhibite the positive release behavior that relating to the temperatures.

5. Acknowledgements

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