Materials and methods

Materials

PEG–PLGA microparticles were prepared by a two-stage solvent evaporation method. In the first stage, the polymer/monomer solution was prepared by mixing PEG–PLGA (1:1 w/w) and tetramethoxysilane (TMOS) with a stoichiometric ratio of 1:0.06 in decalin (10 mL). The mixture was heated at 120°C for 1 hour under vacuum to remove volatile solvents. In the second stage, the polymer/monomer solution was added dropwise to a mixture of methanol (100 mL) and water (25 mL) with constant stirring. The mixture was then dried in a vacuum oven at 40°C for 24 h. The resulting polymer/monomer mixture was then ground to a fine powder and sieved to obtain PEG–PLGA microparticles with a size of 50–100 μm.

Results

The drug release profiles from the drug-loaded PEG–PLGA microparticles are shown in Fig. 1. The release of fluorouracil from the drug-loaded PEG–PLGA microparticles was complete within 24 h, indicating that the microparticles were not suitable for the delivery of fluorouracil. In contrast, the release of capecitabine from the drug-loaded PEG–PLGA microparticles was slow and sustained for more than 10 days, indicating that the microparticles were suitable for the delivery of capecitabine.
biodegradable polymers such as PAAIO-Rh123 (Ke et al., 2010), CS-PEG (Park et al., 2010), PEG-PLA (Wang et al., 2012; Lu et al., 2011), PLGA–PEG (Chen et al., 2012; Yoo and Park, 2004), PEG-PCL (Guo et al., 2013) have been found to increase the cellular uptake and cell cytotoxicity of the formulated anticancer drugs via FA receptor mediated endocytosis. Such protocol was also used for the CAP's target releasing and site specific delivery (Wong et al., 2007).

However, poor drug loading efficiency of PLGA-based NPs as low as 1% for some drugs constitute a major problem for these NPs (Danhier et al., 2012). A silica shell along with the NPs presented a promising solving protocol for this. A silica shell could be specially confined at the interface between the core and corona of amphipathic block copolymer micelles (Tan et al., 2010; Yuan et al., 2007). By employing a microemulsion approach (Darbandi et al., 2005; Santra et al., 2005), silica-based core–shell NPs have been synthesized for bioanalytical applications and drug delivery (Tan et al., 2004). The uptake and release of various drugs and dyes from the resulting NPs was studied and the silica shells appeared to retard diffusional release rates as expected (Huo et al., 2006).

On the basis of these considerations and in continuum to our previous work (Ling et al., 2011), we developed FA biodegradable NPs with silica shell for CAP targeting delivery system. In this study, the amphipathic diblock copolymers PEG–PLGA with the FA and CAP end molecules were synthesized respectively. Then, these two copolymers were blended with CAP and TMOS to form self-assembled NPs in aqueous solution. To our knowledge, no related report existed dealing with NPs carriers of FA-PEG–PLGA/PEG–PLGA–CAP+CAP/TMOS to enhance CAP releasing. The synthesized polymer and the NPs were studied in the present manuscript. The CAP release behavior and release kinetics were also analyzed. These round morphology, stable drug-loaded NPs, formed by self-assembly and crosslinking of the silica shell, represent a new group of efficient drug carrier that appear to be suitable for controlled and targeted release in a wide range of drugs.

2. Materials and methods

2.1. Materials

PLGA (50:50, Mw: 10,000) was purchased from Daigang Biomaterials Inc. (Jinan, China). Alpha-hydroxy-omega-carboxy poly(ethylene glycol) (HO-PEG-COOH, Mw: 10,000) was purchased from Sigma Chemical Co. (USA). Capectabine (CAP, 99%) was purchased from Dalian Meilun Biology Technology Co. Ltd. (Dalian, China). FA, TMOS (98%), succinic anhydride, pyrene, dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were obtained from Sigma (St. Louis, MO).

2.2. Synthesis of polymeric carriers

2.2.1. Synthesis of conjugated di-block copolymers (PEG–PLGA)

HO-PEG-COOH, DCC and DMAP (molar ratio = 1:2:0.01) were dissolved in CH$_2$Cl$_2$ (20 ml) for 12 h at room temperature under nitrogen. After the precipitate being filtered, drop the solution into ice-cold diethyl ether to form the end carboxyl activated PEG sediment and then dried under vacuum. Dissolve the precipitate (1 g) and PLGA (0.175 g) in CH$_2$Cl$_2$ (10 ml), after 24 h stirring at room temperature under nitrogen, the target product PEG–PLGA was precipitated by dropping the solution into ice-cold diethyl ether.

2.2.2. Synthesis of capectabine-conjugated copolymer (PEG–PLGA–CAP)

A mixture of PEG–PLGA, DCC, DMAP and pyridine with a molar ratio of 1:2:0:1.0:0.01 were dissolved in CH$_2$Cl$_2$ (5 ml) and stirred at room temperature for 12 h under nitrogen. 22 mg CAP were added into the solution for three days reacting in an ice bath under nitrogen (molar ratio of activated PEG–PLGA/CAP = 1/1.2). Filtration, dialysis against deionized water with the dialysis bags (Spectra/Por 7, MWCO 3500) and freeze dry were performed to get the final PEG–PLGA–CAP.

2.2.3. Synthesis of folate-conjugated diblock copolymer (FA-PEG–PLGA)

FA (0.23 g, 5.2 × 10$^{-4}$ mol) was reacted with DCC (0.15 g, 7.3 × 10$^{-4}$ mol) and NHS (0.06 g, 5.2 × 10$^{-4}$ mol) in anhydrous DMSO (15 mL) solution overnight under nitrogen at room temperature. After the byproduct DCU was filtered, the activated FA solution was added 5 ml DMSO solution of PEG–PLGA (10 g, 5 × 10$^{-4}$ mol) and DMAP (12.2 mg, 1 × 10$^{-4}$ mol) following with another 24 h reaction under nitrogen atmosphere. The product was centrifuged, filtered, purified by dialyzing against DMSO for 24 h to remove excess DCC and dialyzing against water to remove DMSO. The final FA-PLGA–PEG product was obtained after freeze dry.

2.3. Characterization of the conjugates

The chemical structures of the synthesized FA-PEG–PLGA, PEG–PLGA-CAP conjugate were characterized by $^1$H NMR (Bruker, 500 MHz) using tetramethylsilane as an internal standard and DMSO-d$_6$ as the solvent. The number average molecular weight (Mn) and the weight average molecular weight (Mw) of hybrid copolymer were estimated by GPC on Viscotek Max VE 2001 Solvent/Sample Module system equipped with a Viscotek TDA 305 Triple detector array, a Malvern CLM 3021 T6000 M column using THF as eluent at a flow rate of 1 ml/min at 40 °C. The instrument was calibrated vs. PS standards (19,000 g/mol) and data was analyzed using OmniSec 4.6.0 software.

2.4. Formulation of CAP drug-loaded NPs

TMOS (2 ml) was added into 10 ml transparent yellow CH$_2$Cl$_2$ solution of FA-PEG–PLGA (0.080 g), PEG–PLGA-CAP (1.5 g) and CAP (0.23 g) and stirred at 200 rpm for 10 min. Then, they were dropped into 100 ml PVA aqueous solution (1%wt.) to spontaneously form the silica cross-linked NPs (SSCL NPs) with stirring speed of 600 rpm. The solution was kept stirring for over a night to evaporate the organic phase. The NPs were collected by centrifugation at 10,000 rpm for 10 min at room temperature and washed twice using distilled water. The non-silica cross-linked NPs (NSSCL NPs) were prepared with the similar method but without the addition of TMOS.

2.5. Characterization of the CAP-loaded NPs

2.5.1. Particle size, zeta potential measurement and morphology

The sizes of the NPs (diameter, nm) and size distribution of the drug-loaded NPs were measured using a zeta sizer (Nano ZS, Malvern Instrument, UK) at 25 °C. The samples were diluted to ensure free diffusion and unhindered Brownian motion of the particles. The sizes results were the mean value of five test runs. The morphology of the NPs was confirmed using a scanning electron microscopy (SEM, Nano, FEI, American).

2.5.2. Nanoparticle yield, drug loading and encapsulation efficiency

The yield of nanoparticles (NPs) was obtained according to Eq. (1). To measure the drug loading amount, a pre-determined weights of the freeze-dried drug-loaded NPs were dissolved in DMSO and then underwent centrifugation at the speed of over 8000 rpm. The supernatant CAP concentration was determined using a UV–vis
spectrometer at wavelength of 303.6 nm, by reference to a calibration curve of CAP in DMSO at the same wavelength, which is

\[ C = 19.55 \times (\text{Abs}) – 1.10, \text{R}^2 = 0.9977. \]

The encapsulation efficiency (EE) and drug loadings (DL) were calculated using the following equation:

- **Nanoparticle yield** = \( \frac{\text{Weight of the nanoparticles}}{\text{Total weight of drug and copolymer}} \times 100\% \) (1)

- **Drug loading** = \( \frac{\text{Weight of encapsulated drug}}{\text{Weight of the nanoparticles}} \times 100\% \) (2)

- **Encapsulation efficiency** = \( \frac{\text{Weight of encapsulated drug}}{\text{Total weight of drug}} \times 100\% \) (3)

### 2.5.3. Determination of critical micelle concentration (CMC) of FA-PEG–PLGA–PEG–CAP aggregation

Pyrene solution (6.0 x 10^{-4} M) in acetonitrile was added to PBS to attain a pyrene concentration of 12 x 10^{-7} M. The solution was then distilled under vacuum at 60 °C for 1 h to remove acetone from the solution. Different amounts of copolymers were added to acetonitrile free pyrene solution to make mixed solution with the concentration ranging from 10^{-7} to 10^{-1} mg/mL. The solution was incubated at 60 °C for 1 h, then at room temperature overnight with mild stirring so that the pyrene could equilibrate between the NPs and the aqueous phase completely. The change of the intensity ratio (I_{378}/I_{384}) of the pyrene with polymer content was chosen to plot excitation spectra from 360 to 410 nm and emission wavelength 390 nm. The CMC was determined by taking a mid-point of the copolymer concentration at which the relative excitation fluorescence intensity ratio I_{378}/I_{384} nm shift abruptly.

### 2.5.4. Stability study

Stability evaluation was supported by size variation with the time experiment and zeta potential measurement. Size variation experiment was carried out as follows: the SSCL NPs (50 mg) and NSSSL NPs (50 mg) were suspended in phosphate-buffered saline (PBS, pH 7.4) under stirring (110 rpm) at 37 °C, respectively. The NPs sizes were measured and compared at the first 3 days to examine the stability of the NPs.

Zeta potential (surface charge) of NPs was measured using a zeta sizer (Nano ZS, Malvern, Instrument, UK) at 25 °C. The samples were diluted to ensure free diffusion and unhindered Brownian motion of the particles. The zeta potential was the mean value of three test runs.

### 2.6. In vitro drug release

The SSCL NPs and NSSCL NPs (50 mg) were suspended in 10 ml PBS (pH = 7.4), respectively, and placed in dialysis bag with a molecular weight cut-off of 3500 Da. The dialysis bag was suspended in 100 ml of PBS at 37 °C and stirred at 110 rpm; 5 ml PBS was taken out and replaced by another 5 ml fresh PBS periodically. The release rate of both SSCL NPs and NSSCL NPs was compared by a UV–vis spectrometer at the wavelength of 303.6 nm.

### 3. Results and discussion


The principle in synthesis of FA-PEG–PLGA and PEG–PLGA-CAP is the esterification reaction. We synthesized HOOC-PLGA–PEG–OH by direct conjugation of HOOC-PLGA–OH with activated HO-PEG-COOH. PEG–PLGA-CAP and FA-PEG–PLGA were synthesized as shown in Fig. 1, respectively.

TheGPC trace indicated the Mn of the FA-PLGA-PLGA and PEG–PLGA-CAP were 19,280 g/mol and 18,092 g/mol, and the Mw of 20,396 g/mol and 20,230 g/mol, respectively. The structures were characterized and verified with 1H NMR (Fig. 2).

The 1H NMR spectra of PEG–PLGA, FA–PEG–PLGA and PEG–PLGA–CAP were shown in Fig. 2. The inserted spectrum was the higher magnification of the region between 7 and 8.5 ppm. The peak at 2.50 ppm corresponded to DMSO. All three spectrum exhibited similar peaks of the main chain PEG–PLGA marked 1–5, while the FA–PEG–PLGA (middle) showed a typical peak of 6–9 ppm, which represented the aromatic protons and pteridine proton. The spectrum of PEG–PLGA–CAP (top) exhibited two peaks marked as 10, 11, which were the characteristic of methylene protons near the F atom in the CAP. The peaks marked as 12 and 13 referred to the imino proton and one hydroxyl protons on CAP molecule and the peak marked as 14 is the other imino proton. Besides, it also exhibited two relatively obvious peaks marked as 15 and 16, which were the characteristic of methyl and methylene protons of amyl hydrde in CAP. These peaks were relatively much weaker than the signals of FA and CAP due to the small molecular weight of FA (441 Da) and CAP (359 Da) compared to that of PEG–PLGA (20,000 Da).

#### 3.2. Preparation of CAP-loaded SSCL system and control system NPs

The NPs were prepared by using the emulsion–solvent evaporation method with some modification. It was found that when PVA was 1.0% (w/v), the NPs would not easily aggregated, the viscosity of the emulsion was moderate and the particle size was smallest (Chen et al., 2012). So we here used PVA aqueous solution (1.0%, w/v) as an emulsifier agent for the preparation of NPs.

In this study, we consider that the FA-PEG–PLGA–PEG–PLGA-CAP mixture have only 5 mol% FA-PEG–PLGA content to form either SSCL NPs or NSSCL NPs to guarantee NPs stability, as the presence of too many FA molecules on the surface of polymeric micelles has a detrimental effect on the stability. This is confirmed by a study that the appearance of micelles prepared from H40–PLA–b-MPEG–FA copolymer having 5 mol% PEG–FA did not change notably over two weeks (Prabaharan et al., 2009).

Upon injecting the mixed organic solution dropwise into an surfactant solution, polymeric NPs were formed as PLGA segments with terminally conjugated CAP were buried in the core, and PEG segments with terminally conjugated FA were oriented outside toward aqueous medium (Liu et al., 2007). The free CAP was physically entrapped in the core with the O/W method. This process was simultaneously accompanied by the hydrolysis reaction of TMOS due to its hydrophobic properties. The hydrolysis reaction was limited to occur at the interface region between the hydrophilic and hydrophobic domain, which resulted in the formation of a thin silica shell between PEG and PLGA (Kim et al., 2010). This process was schematically shown in Fig. 3.

#### 3.3. Characterization of formulated NPs

##### 3.3.1. Size and morphology of the drug-loaded NPs

Particle size distribution of SSCL NPs was shown as Fig. 4(A). The average diameter of SSCL NPs was around 203 ± 0.47 nm, with a polydispersity index of 0.391. SEM micrographs shown as Fig. 4(B) revealed that the NPs had a spherical morphology and smooth surface.
3.3.2. NPs yield, drug loading, encapsulation efficiency of SSCL NPs and NSSCL NPs

The NPs yield, drug loading, and encapsulation efficiency of SSCL NPs and NSSCL NPs has been recognized by UV–visible spectrophotometer. The results were shown in Table 1.

CAP was loaded into the NPs by an emulsion–solvent evaporation method. The drug loading into the SSCL NPs was 7.9% with an encapsulation efficiency of 69.2%, while the drug loading amount and encapsulation efficiency of NSSCL NPs are respectively 6.8% and 58.8%. This comparison confirms that the SSCL NPs are more effective for encapsulating the hydrophobic drug and have higher encapsulation efficiency, which may be due to the establishment of the strong interactions both between silica shell with drug functional groups and with hydrophilic chains, as there are one hydroxyl groups and two imino group in one CAP molecule, which could act as H-bonding sites with our H-bonding hydrolyzed TMOS (Fig. 5).

### Table 1

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Drug/copolymer ratio in feed (w/w)</th>
<th>Yield (%)</th>
<th>Drug loading (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSCL</td>
<td>1/10</td>
<td>83</td>
<td>7.9</td>
<td>69.2</td>
</tr>
<tr>
<td>NSSCL</td>
<td>1/10</td>
<td>72</td>
<td>6.8</td>
<td>58.8</td>
</tr>
</tbody>
</table>

* NPs dissolve in distilled water at a concentration of 0.6 mg/mL followed by 10 min sonication.

3.3.3. Critical micelle concentration (CMC) study

The formation of polymeric micelles is thermodynamically favorable only above CMC of the amphiphilic molecules. So we use pyrene as an extrinsic probe to determine the CMC of FA-PEG–PLGA/PEG–PLGA-CAP. Still, the FA-PEG–PLGA takes 5 mol% of the copolymer blends, as the PEG–PLGA-CAP content increases,
FA-PEG–PLGA content increases accordingly. The results were shown as Fig. 6(A) and (B).

In the excitation spectra, although the apparent pyrene concentration remains unchanged, the fluorescence intensity increased with increasing polymer concentration. A definite shift from 378 to 383 nm was observed as FA-PEG–PLGA/PEG–PLGA-CAP concentration increased, indicating the formation of aggregations. The intensity ratio \( (I_{378}/I_{383}) \) of the pyrene excitation spectra versus the logarithm of copolymer concentration was shown as Fig. 6(B), which indicated that the CMC of FA-PEG–PLGA/PEG–PLGA-CAP was approximately 1.2 \( \mu \)g/ml in PBS, as estimated from the cross over point of the intensity ratio \( (I_{378}/I_{383}) \). The low CMC value indicates a strong tendency of FA-PEG–PLGA/PEG–PLGA-CAP block copolymer toward NPs formation in aqueous media at a lower copolymer concentration and also suggests the NPs may have a good stability characteristic in blood stream (Mehdizadeh et al., 2013; Yoo and Park, 2001).

3.3.4. Stability study

For further use of the NPs, stability is significant for long-term storage, transportation and so on. Stability study was carried out by putting the NPs in PBS (pH = 7.4) at 37 °C for 4 days, the size were measured respectively and the results were shown as Fig. 7, there was no significant change in the average diameter of NPs during the study.

These results were also confirmed by the zeta potential analysis. Zeta potential is to measure the surface charge of colloidal dispersions, it has been usually used to predict and control dispersion stability. Electrostatic repulsion between particles with the same electric charge prevents the aggregation of these NPs. Here,
the zeta potential value of NSSCL NPs was $-27.54 \pm 0.40$ mv, which showed very similar zeta potentials of SSCL NPs ($-28.43 \pm 2.55$ mv), for they had the same group on the shell of the NPs. A slightly more negative potential of SSCL NPs may be due to the smaller size of the SSCL NPs than SSCL NPs, which rendered less charge density. These results also supported the deposition of silica within these NPs. From the stable analysis, these two NPs systems both had no problem with regard to stability (Song et al., 2011).

3.4. In vitro drug release

Considering that the controlled and continuous drug release is essential for the success of pharmaceuticals, we further examined the drug release profiles of SSCL NPs and SSCL NPs which were formed with different formulations, the mass ratios of polymer complex/drug is 5/1, 10/1 and 20/1 respectively. The in vitro release profiles had been obtained at pH 7.4 by using a membrane dialysis method, the results were shown as Fig. 8.

There was a relatively rapid release of drug within the first 30 h of all formulations. From the release profiles, the average release rate of SSCL NPs was slower than that of NSSCL NPs as a silica shell formed between PEG and PLGA in SSCL NPs posing a matrix barrier for drug releasing. These two NPs were able to sustained release CAP for more than 200 h with a rapid release of 15–28% in the first 15 h. The initial burst is due to the surface bound drugs dislodging themselves from the NPs surface when contacted with the release medium. The cumulative release of CAP from NPs was fastest when the polymer:drug ratio was 5:1, which may be due to the dense drug aggregate in the outer space or lodge on the surface of NPs where release is faster. However, the release was slower when the copolymer:drug ratio was 10:1 than 20:1 (76% vs. 80% cumulative CAP release at 200 h of NSSCL NPs, respectively; Fig. 8). In SSCL NPs, almost 54% of the drug was released in 160 h when the copolymer:drug ratio was 20:1. In contrast, when the polymer:drug ratio was 10:1, 54% of the drug was released till 200 h. The difference between the release profiles may be due to the hydrophobic interaction between the lipophilic CAP agent and the polymers (Miao et al., 2010). An increase in the amount of hydrophobic CAP within the NPs may enhance the interaction between CAP and the hydrophilic PLGA segment leading to decreased drug release (Allen et al., 2000).

The results from the in vitro release studies showed that these drug-loaded NPs have potential targeting capability as well as control effect on the rate of drug release. The above drug release experiments also suggested that the optimal formulation used for further drug release mechanism studies was polymer/drug ratio of 10:1.

3.5. Mechanism of drug release

In order to study the release kinetics in the mainstay, we further examined the drug release profiles of the formulation with 10:1 polymer/drug ratio, the release profile was shown as Fig. 9.

Two models (Fig. 10) were created according to core–shell structure to depict the release profile for CAP in these two NPs release systems. Here to ignore the surface adsorption of drugs, and from the drug release profile, it is thought to follow two processes:

From 30 h to 70 h, the release is relatively placid. The release medium permeate into NPs, unconjugated drugs (in the zone 1, drugs are considered uniformly distributed in the core of the system) dissolve and diffuse. Besides, conjugated drugs dislodge with the hydrolysis of the ester bond between the drug and copolymer.

![Fig. 4. Size (A) and morphology (B) of the drug-loaded NPs.](image)

![Fig. 5. The schematic presentation of an internal nanoparticle structure of hydrogen bond formed between the PEG and hydrolyzed TMOS.](image)
Fig. 6. The determination of the CMC (B) from the fluorescence intensity ratio $I_{378}/I_{383}$ from excitation spectra (A) vs. log concentration of the FA-PEG-PLGA/PEG-PLGA-CAP (pH 7.4).

Fig. 7. Size variation with days.

In this process, hydrophilic domain 3 take effect mostly on the drug releasing from the matrix is mainly based on a diffusion mechanism. We call this internal drug dissolution and diffusion process.

From 70 h to 180 h, the release rate is relatively higher. The drugs diffuse from the polymers matrix with the NPs swelling and polymers degradation. During this process, hydrophobic domain 2 take effect mostly on the drug release. With the release medium keeps penetrating into the domain of the release system, the hydrogen bond between the PEG and silica shell breakage; the shell collapse and drugs can diffuse easier depending on PEG–PLGA.
degradation and hydrolysis of ester bond. Due to denser drug packing, the disintegration process is slow, thereby leading to a relatively near-zero order release (Ranganath et al., 2009).

Further, we studied and compared the effect of the silica shell toward these two stages. According to the profile (Fig. 9), there are periods of steady-state of release in the two processes. Illustrated as Fig. 10, we can monitor a straight line to approximately represent for the steady-state of each process. The straight lines all have contained more than 90% of the data points and can be expressed by the following liner equation:

\[ \frac{M_t}{M_\infty} \times 100(\%) = Kt \]  

(4)

where \( \frac{M_t}{M_\infty} \) is the cumulative released fraction; \( K \) is the slope of the straight line of the curve, which means the release rate in the steady-state. The value of \( K \) for different stages and different NPs systems are listed in Table 2. It reflects how much the release rate varied from stage 1 to stage 2. The \( K \) value ratios of the same stage in two systems (\( K1/K1 \) and \( K2/K2 \)) are also calculated to measure the effect of the silica shell to these two stages.

The \( K \) values calculated from the profile of cumulative release (Fig. 11) are shown in Table 2, the two drug release systems both have smaller \( K1 \), which means lower release rates in the first stage; The \( K2/K1 \) value of both NPs demonstrate the average release rate of second stage is more than 2.2 times faster than the first stage. Besides, the transition time of SSCl NPs from the 1st stage to the 2nd stage is also longer than NSSCl NPs. The \( K/K1 \) value measures the effect of the silica shell to two release stages. With 1.963041 and 1.78697 calculated, the first stage release is relatively affected more by the additives than the latter stage.

This phenomenon can be explained by different kinetics. The copolymer PEG–PLGA has a high molecular weight (over 20,000 Da) and is amphiphatic. So it is easy to form open pores when continually contact with PBS. Without another barrier, the drugs may release mainly through these pores and this is the first stage release of NSSCl NPs system. It can be called diffusion-controlled release stage though further studies are needed to substantiate this conclusion.

In SSCl NPs, the pores of NPs are separated by non-pores layers (domain 4), since there is no interconnected channels, the degradation of polymers and the erosion of silica shell has not generally begin or not obvious, the influence caused by this additional non-porous layer is much stronger in this stage. The drug release from this stage is probably diffusion-controlled, the similar transition time also indicate that drug release from these matrices is dominated by a similarly diffusion-mediated mechanism.

While in the second stage, with the hydrolysis of ester bond between PEG and PLGA chain, and with the polymer molecular become small, the release matrix shows a more porous structure which lead to higher rate of release. And during this period, the hydrogen bond collapses, also accompanied with silica shell erosion, the release rate is increased gradually. Of course, the rate of TMOS system is still lower for its additional barrier effect. The drug release from this stage can be concluded as degradation–erosion–diffusion-mediated controlled kinetics.

4. Conclusion

In this study, we chemically conjugated the CAP to the copolymers to make PEG–PLGA–CAP conjugates for the first time and also synthesized and characterized FA-PEG–PLGA. The blends of PEG–PLGA and PEG–PLGA–CAP were used to prepare NPs for targeted and controlled drug release, these NPs could contain a large amount of CAP by chemically conjugation and physically contraction in the inner core, which increased targeting capacity. Besides, the addition of TMOS which formed a shell in this system could cause a barrier affect on both the two release stages. Our successful NPs preparation and characterization results imply that the FA-PEG–PLGA/PEG–PLGA–CAP/TMOS NPs system could have high potential to be used for targeted and controlled drug delivery.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2013.12.047.

References


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Table 2

<table>
<thead>
<tr>
<th>Release system</th>
<th>Slope of the 1st stage ( K1 )</th>
<th>Slope of the 2nd stage ( K2 )</th>
<th>( K2/K1 )</th>
<th>Transition time* (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control system</td>
<td>0.199975</td>
<td>0.443362</td>
<td>2.217</td>
<td>60</td>
</tr>
<tr>
<td>TMOS system</td>
<td>0.101879</td>
<td>0.248108</td>
<td>2.435</td>
<td>80</td>
</tr>
<tr>
<td>( K/K1 )</td>
<td>1.963041</td>
<td>1.78697</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\( K/K1 \) is the ratio of the same stage of two drug release NPs systems.

Transition time* is the time from the 1st stage to the 2nd stage.

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Fig. 11. Release kinetics of CAP from SSCl NPs and NSSCl NPs systems based on the two stage release, showing that the linear slope changed with TMOS added in.


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