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A fibrin encapsulated liposomes-in-chitosan matrix (FLCM) for delivering water-soluble drugs Influences of the surface properties of liposomes and the crosslinked fibrin network

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Abstract

A depot drug delivery system, fibrin encapsulated liposome-in-chitosan matrix (FLCM), has been developed to deliver a water-soluble drug which is configured by a porous chitosan matrix containing a bovine fibrin network encapsulated different surface properties of liposomes. Quinacrine (QR), a water-soluble, low-molecular weight fluorescent marker, is used as a model drug to evaluate the delivery characteristics of the system.

The SEM photographs show that the fibrin network adheres to the surfaces and pores of the chitosan matrix of a FLCM system. The QR release periods of the FLCM are sustained for about four times longer than those of QR encapsulated into the liposomes. However, the QR release periods and profiles of the FLCM are influenced by the surface properties of liposomes. The release of QR from FLCM is sustained for 9 days for neutral liposomes and only 5 days for PEG modified liposomes (PEG-liposome). After crosslinking the fibrin network of the FLCM with 0.5% of glutaldehyde, the release of QR is further sustained for 17 days with good linear profiles (e.g., 13 days) and with 50% of reduced burst release compared with those of without crosslinking, indicating that the stability of the fibrin network plays an important role on QR release of the system. More interestingly, the release periods and profiles of QR of the FLCM system are highly similar to those of Tirofiban, low-molecular weight of a water-soluble clinical cardiovascular drug, although the study has been done by human platelet poor plasma instead of bovine fibrinogen as a source of fibrin network. It suggests that the QR is a suitable model for investigating the drug delivery behaviors for water-soluble, low-molecular weight drugs of the FLCM. In conclusion, with QR as a model drug, FLCM with crosslinked fibrin network can effectively sustain the release of QR for 17 days but the release profiles are influenced by the surface properties of encapsulated liposomes. This study suggests that FLCM may have the potential as a depot drug delivery system for water-soluble drugs.

Keywords: Depot drug delivery system; Liposomes; Fibrin; Chitosan; Crosslinked

1. Introduction

Developing a delivery system to enhance the stability and prolong the biological activity of water-soluble drugs such as peptides for medical therapy is an important issue for controlled release. To achieve the objective, micro-encapsulation approaches with different techniques were tried and reported by many investigators (Blanco and Alonso, 1998; Cohen et al., 1991; Chung et al., 2002a; Tabata et al., 1993). For example, incorporation of those drugs into synthetic biodegradable polymers such as polyesters, and poly-anhydride microspheres has been widely used so that drug release is controlled by diffusion from degradation of polymer matrix (Chung et al., 2002a; Tabata et al., 1993). However, the disadvantages of the polymer-based technology are the loss of bioactivities of drugs during fabricating process like heat of sonication or organic solvents (Chung et al., 2002a; Manning et al., 1989; Sandor et al., 2002; Tabata et al., 1993). Another drug delivery system mainly used in blood stream injection for cancer therapy or gene delivery is small lipid based vesicles namely liposomes that are made

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from natural biodegradable, non-toxic lipid molecules which can entrap or bind different kinds of drugs into or on the lipid membrane (Allen et al., 1991; Moribe et al., 1999; Takeuchi et al., 2001). Beside used in blood stream by injection, liposomes encapsulated into a fibrin clot/network have been proposed for delivery different proteins (Sakiyama-Elbert and Hubbell, 2000; Meyenburg et al., 2000). Since the fibrin clot is a natural polymer with good biocompatibility and biodegradability, it has been considered as a matrix for drug delivery or tissue engineering (Meyenburg et al., 2000; Ye et al., 2000). However, the effects of a fibrin clot/network containing different surface properties of liposomes on the release of water-soluble, low molecular weight drugs such as Tirofiban, an anti-thrombosis drug, have not been studied.

Porous chitosan matrix is a potential biomaterial for developing a biodegradable and biocompatible depot delivery system with a high volume capacity of reservoir. Chitosan, an amino polysaccharide derived from chitin by de-acetylation, is a non-toxic, biodegradable and biocompatible biomaterial containing reactive amine and hydroxyl groups that are easily modified for applying in tissue engineering and drug delivery (Chatelet et al., 2001; Chung et al., 2002a,b). In addition, it is relatively simple to fabricate a porous chitosan matrix with different pore structures or sizes by a simple lyophilization technique and fix by crosslinked agents such as sodium citrate (Chung et al., 2002b; Shu et al., 2001).

By taking advantage of the biodegradable and biocompatible properties of fibrin encapsulated liposomes and chitosan, we developed the FLCM for sustained release of water-soluble, low-molecular weight drugs (e.g., Tirofiban) (Fig. 1). To characterize drug delivery properties of the FLCM system, the influence of different charges/properties of liposomes on encapsulation efficiency and the stability factors of FLCM such as the crosslinking fibrin network on the release of quinacrine (QR) were evaluated. Moreover, the release profiles of QR of FLCM system have been compared with those of Tirofiban of same system although the fibrin network for the comparison study was formed from human poor platelet plasma (PPP) instead of bovine fibrinogen. Based on the results, the sustained release of QR and the high similarity of the release profiles between QR and Tirofiban, FLCM may have a potential to be applied as a depot system for delivering water-soluble drugs.

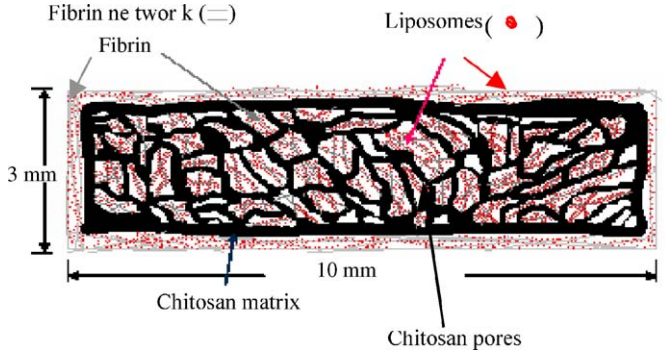

Fig. 1. The schematic diagram of a FLCM system containing fibrin network, liposomes and porous chitosan matrix.

2. Materials and methods

2.1. Preparation of QR loaded liposomes with different surface properties

Egg phosphatidylcholine (PC) of 90% purity, stearylamine (SA), dicetyl phosphate (DP), cholesterol, vitamin E and quinacrine were all purchased from Sigma Chemical Co., St. Louis, MO, USA. Di-stearoyl-phosphatidyl-ethanolamine-methoxy-poly(ethylene glycol) with molecular weight of 2000 Da for poly(ethylene glycol) (DSPE-PEG-2000) was purchased from Shearwater Corp. (Nippon Oil Corp., Japan). To prepare liposomes, PC, cholesterol and vitamin E were all dissolved in 20 ml of 95% alcohol with the molar ratio of 11:4:0.1. The mixture was dried with a rotary evaporator to form a lipid membrane film and further purged with nitrogen gas. To form SA, DP and DSPE-PEG-2000 contained liposomes, the molar ratios of PC, cholesterol, vitamin E and above-mentioned components (e.g., SA) were changed to 11:4:0.1:1, respectively, and the preparation methods were the same as the above-mentioned steps. The lipid film was hydrated by sodium citrate buffer followed by ultra-sonication (GT 50T, Cole-Palmer Co., IL, USA). After centrifugation, the liposome suspensions were filtered through 0.45 μm of polycarbonate membrane to form homogeneous particles. To prepare QR loaded liposomes, 60 μl QR at concentration of 50 mg/ml was added to 10 ml of liposome suspensions and then extruded three times through a polycarbonate membrane filter with 0.1 μm pore size at 42 °C followed by three times of freeze-and-thaw process (Meyenburg et al., 2000; Castile and Taylor, 1999). After the preparation process, the suspensions were dialyzed through a flow-type dialyzer with a cut off molecular weight of 10 kDa (Spectrum Laboratory Inc., USA) to remove un-encapsulated QR and also to concentrate the liposome suspension to a finite volume. Then a fresh medium was added in the concentrate liposome medium and dialysis processes were repeated two to three times till that the concentration of QR was extremely low (less than 1×10^{-5} M, detection limit of the calibration curve of QR by the fluorescent spectrophotometer). The concentrated QR loaded liposomes were ready for further testing.

2.2. Characterization of QR loaded liposomes and encapsulation efficiency

The sizes and surface charges of liposomes in saline were determined by a particle and zeta potential analyzer (Zeta 90 plus particle sizer, Brookhaven Instrument Corp., USA). To determine the encapsulation efficiency of QR loaded liposomes, the dialysate obtained from the above-mentioned flow-type dialyzer was first collected for measuring the concentration of QR by a fluorescent spectrophotometer (Hitachi F-4500, Kobe, Japan) and quantified. The excitation and emission wavelengths for measuring the concentration of QR in the medium were set at 420 and 500 nm, respectively. The encapsulation efficiency of QR into liposomes was determined by subtracting the quantities of OR in the dialysate from the initially loaded QR and then divided by initial loaded of QR. To double check the accuracy of

the encapsulation efficiency, the quantities of the initial loading of QR were frequently compared with the quantities for adding QR release from liposome suspensions with the QR collected in the dialysate.

2.3. Preparing porous chitosan matrix

Chitosan was purchased from Primex Ingredients ASA, Norway (batch #: TM732, Mw. of 200–700 kDa) with 96% of deacetylation, and with the characteristic viscosity for 1% chitosan in 1% acetic acid solution of 57 mPa S. To prepare the chitosan solution, 1.5 g of chitosan was dissolved in 100 ml of 1% acetic acid solution and then filtered through a 0.45 μm polycarbonate membrane. The solution was poured into glass disks and then solidified in the frozen state at liquid carbon dioxide for 24–48 h followed by lyophilizing in a freeze-dryer to form a porous chitosan film (Chung et al., 2002b; Shu et al., 2001; Madihally and Matthew, 1999). The morphology and pore size of the chitosan film were taken by SEM and then analyzed by a computer image software. The mean pore size of chitosan film was about 200 μm with 85% of porosity and fixed by sodium citrate and dried at room temperature for further applications. Chitosan matrix of 1 cm^2 of area with 3 mm thickness was cut for preparing FLCM.

2.4. Preparing FLCM with or without crosslinked fibrin network by 0.5% of glutaldehyde

Bovine thrombin (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in sodium citrate buffer to prepare thrombin solution at a concentration of 50 unit/ml. To prepare the FLCM system without fibrin cross-linkage, 1.0 ml of QR loaded with different types of liposomes were mixed with 1.0 ml of 7.0 mg/ml bovine fibrinogen (Sigma Chemical Co., St. Louis, MO, USA) solution containing with 0.11% sodium citrate, 0.038% NaCl and 0.15% $CaCl_2$ and pH at 6.4 (Meyenburg et al., 2000). After 2 ml of QR loaded liposomes/fibrinogen gently mixed and injected to porous chitosan films, 0.1 ml of thrombin solution was also injected to initiate fibrin clot and waited for being stabilized. To prepare FLCM with crosslinked fibrin network, the FLCM was further dipped into 0.5% of glutaldehyde solution for 5 min and then washed with distilled water. The FLCM with a capacity of 7 ml solutions/cm^3 of matrix was further tested to characterize the delivery properties of water-soluble drugs (Fig. 1).

2.5. In vitro QR release of FLCM with or without fibrin crosslinking

To study the release of QR of the FLCM system, it was placed within an osmosis membrane with a cut off molecular weight of 300 kDa (Spectrum medical industries Inc., USA), suspended in vials containing 20 ml of saline solution containing 0.1 wt.% of sodium azide as dissolution medium (Chung et al., 2002a,b,c,d). The system was shaken at 60 rpm at 25 °C. The dissolution medium of 0.5 ml was periodically drawn out and the concentration of QR was examined by a spectrophotometer (Hitachi F-4500, Kobe, Japan). Same volumes of fresh medium were also replaced to the dissolution medium. The cumulative QR concentrations in the dissolution medium for different times were collected and measured by the spectrophotometer (Chung et al., 2002a). The release profiles of QR from fibrin crosslinked FLCM was studied with the same conditions as that of non-crosslinked one.

2.6. Preparing platelet poor plasma configured FLCM with Tirofiban loaded liposomes, and the release of Tirofiban of the FLCM

For testing whether QR was a suitable model drug for the system, the similarity between the release profiles of the QR and Tirofiban were compared. Moreover, to construct a FLCM with high biocompatible to human subjects, the platelet poor plasma of normal subjects instead of bovine fibrinogen solution was induced by thrombin to form the fibrin network of FLCM to encapsulate QR and Tirofiban loaded liposomes.

The procedures for preparing Tirofiban loaded liposomes were the same as those for preparing QR loaded ones except that 500 μg of Tirofiban was loaded into 10 ml of liposome suspensions. The encapsulation efficiency and in-vitro release of Tirofiban were determined with the same protocols as those for QR except that the concentration of Tirofiban was determined by HPLC technique (Bergquist et al., 2001). In general, the samples were injected into an HPLC (Jasco PU-1580, Kobe, Japan) equipped with a C_{18} column (BETASIL, 4.6 mm × 25 cm i.d., CA, USA) at 40 °C. For this analysis, 10% acetonitrile dissolved in 90% 10 mM of potassium phosphate at a pH of 2.3 was used as the mobile phase with 0.8 ml/min flow rate, and the quantity of Tirofiban was detected by absorption intensity of wavelength at 227 nm that was analyzed with built-in standard software of the HPLC (Bergquist et al., 2001).

To prepare the PPP of normal subjects, 5 ml of fresh blood suspension mixed with 3.8% of sodium citrate ($n = 3$) was centrifuged at 1500 × g for 10 min for separating blood cells and plasma as in our earlier study (Yang, 2003). After the upper level plasma suspension was separated, its platelet number was counted by a platelet particle counter (SYSMEX PDA-500, TOA Medical Electronic, Kobe, Japan). The number of platelets less than 1.0×10^4 μl^{-1} was defined as PPP for this study. To prepare FLCM with PPP as a source of fibrin network, the conditions were the same as those for the FLCM configured by bovine fibrinogen except that 1.0 ml PPP instead of 1.0 ml of bovine fibrinogen solution.

The methods and protocols to determine the release of Tirofiban and QR of FLCM with the PPP as a source of fibrin network were the same as those of the FLCM with bovine fibrinogen.

2.7. Studying the hydrolysis of fibrin network of FLCM with or without fibrin crosslinked

To study the influence of hydrolysis of fibrin network of FLCM on the release of QR, the concentration of fibrin products and chitosan dissociated into the dissolution medium was mea-

sured for FLCM with or without crosslinking fibrin network after it was immersed into saline solution by using the same conditions as QR release study. The hydrolysis of fibrin network and dissociation of chitosan into dissolution medium for OR loaded liposomes of the FLCM were periodically collected and measured by the fluorescent spectrophotometer with excitation wavelengths at 280 and 340 nm, and emission wavelengths at 340 and 500 nm, respectively (Yang, 2003).

All of the calculations were performed using the Sigmastat statistical software (Jandel Science Corp., San Rafael, CA, USA). Statistical significance corresponded to a confidence level of at least 95%. Data are presented as mean ± S.D., having been measured at least in triplicate.

3. Results and discussion

This study is to develop a depot delivery system configured by non-toxic, biocompatible, biodegradable biomaterials with a high capacity of reservoir that can sustain the release of water-soluble drugs. Moreover, no toxic organic solvents or ultra-sonication was applied during the preparation QR or Tirofiban loaded FLCM that assured the stability of loaded proteins or drugs that could avoid the disadvantages of drugs delivery by polyesters with emulsion technique (Chung et al., 2002a; Sandor et al., 2002; Tabata et al., 1993). For illustration, the schematic diagram for the FLCM is shown in Fig. 1.

3.1. Characterizing QR encapsulated into different surface properties of liposomes

Since surface properties of liposomes might affect the release properties of FLCM system, the characteristics of QR encapsulated by positive or negative charge, PEG shielded and neutral liposomes (e.g., SA-, DP-, PEG-liposome and liposome, respectively) were first characterized. The surface charges, particle sizes and encapsulation efficiency of those liposomes are shown in Table 1. The surface charges of the liposomes are consistent with the formula of the liposome compositions. As expected, DP-liposomes have much negative charges compared to other liposomes. In addition, DP-liposomes have the largest particle size because the added DP molecules into lipid bi-layer would increase the glass transition temperature, and resulted in increased rigid of lipid membrane to cause large size during freeze-and-thaw fabricating process (Felgner and Ringold, 1989). In contrast, PEG-liposome has the lowest particle size since the shielded PEG on the surface of lipid bi-layer enhanced the stability of liposome (Allen et al., 1991; Takeuchi et al., 2001) and reduced the fusion of liposome during freeze-and-thaw process. DP-liposomes show the highest en-capsulation efficiency (e.g., 75.0 ± 0.2%) while PEG-liposomes show the lowest (e.g., 47.0 ± 0.5%) that corresponds to the size of liposomes (Table 1) which is consistent with the report by Chapman et al. (1990). However, the burst release (e.g., 80% within 12 h) with a short release period (e.g., 100% release within 24–36 h) of QR from all of the prepared liposomes are observed in this study which are weakly dependent on the sizes, encapsulation efficiencies and surface charges (data not shown). It has been reported that the release characteristics of drug from liposomes is influenced by the molecular weights and hydrophobic properties of the drugs (Chapman et al., 1990). For instance, in the same conditions the release period of trypsin (e.g., about 96 h) is about three times of that of QR (e.g., 24–36 h) (data not shown) which may be attributed to high molecular weight of trypsin (e.g., 23 kDa) compared to that of QR (e.g., 428 Da). Besides the molecular weights of loaded drugs, the compositions of lipid bi-layer such as DSPC instead of egg PC and cholesterol contents also affect drug release behaviors of liposomes. Since this study concentrates on investigating the effects of surface properties of liposomes such as surface charges affecting their interactions with fibrin networks, and stability of the networks on drug release behaviors, the effects of different lipid compositions on the liposome stability and release phenomena shall be considered in a future study.

For comparisons, liposomes–chitosan system without fibrin network was also evaluated. In general, high burst release of QR from the system were observed for all tested liposomes. The releases of QR were sustained from 24 (e.g., SA-liposomes) –36 h (e.g., DP-liposomes) to 72–96 h (data not shown), respectively. Therefore, chitosan matrix could sustain the release periods although they are dependent on the types of liposomes loaded in the matrix. The sustained QR release by chitosan matrix could be resulted from increasing the diffusion path of QR from the inner side of liposome cores to dissolution medium.

3.2. Sustained release of QR from FLCM for different surface properties of liposomes

To observe fibrin network within the pore structured chitosan matrix, SEM micrographs were taken (Fig. 2a and b). The fibrin network adhered to the surface of chitosan is also observed. The adherence of fibrin to chitosan surface to form FLCM system could be due to some interactions between the negative charges of carboxyl groups of amino acids such as glutamate of fibrin and the positive charges of amine groups of chitosan polymers.

Table 1
Particle size, encapsulation efficiency of QR and zeta potential of different surface properties of liposomes ($n = 3$)

Properties	Liposomes	PEG-liposomes	DP-liposomes	SA-liposomes
Particle sizes (nm)	287.1 ± 10.6*	187.8 ± 9.2*	455.8 ± 13.7*	315.6 ± 4.9*
Encapsulation efficiency (%)	63.0 ± 0.5#	47.0 ± 0.5#	74.9 ± 0.1#	71.6 ± 0.3#
Zeta potential (mV)	−3.1 ± 0.4	−1.7 ± 0.3	−41.4 ± 1.5	12.8 ± 0.4

* The differences in particle sizes between PEG-liposomes and others, $P < 0.005$.
# The differences in encapsulation efficiency of QR between PEG-liposomes and others, $P < 0.001$.

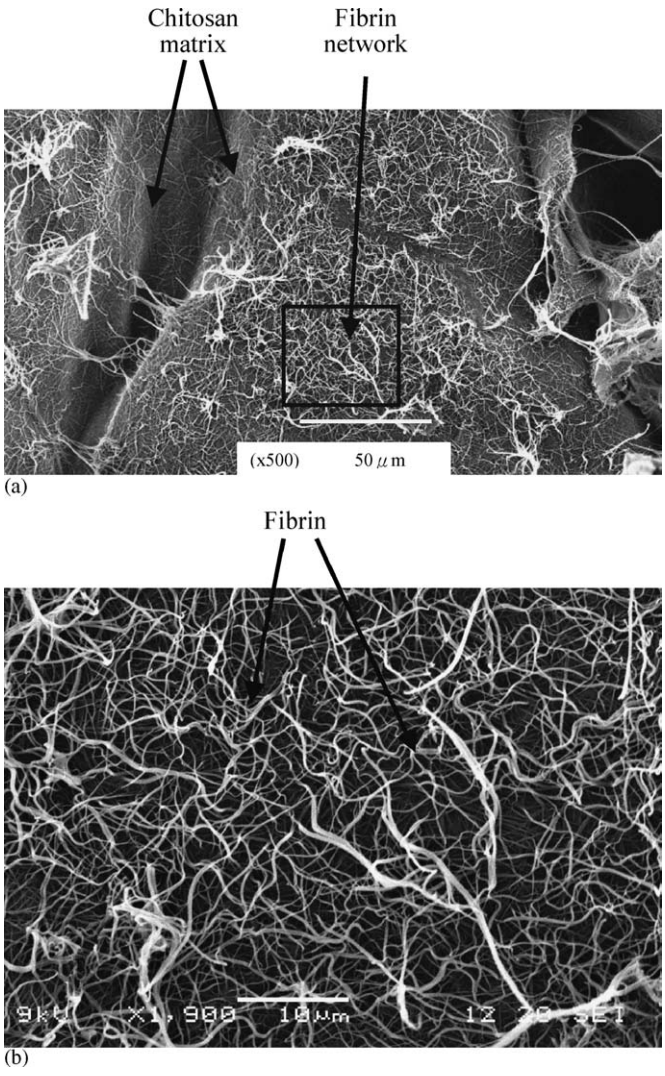

Fig. 2. The SEM micrograph shows the adhesion of fibrin network on the surface and pores of chitosan matrix of a FLCM system (a). The SEM micrograph shows fibrin network of a FLCM system (b).

Although the micrographs do not show the encapsulated liposomes within the fibrin network, liposomes might still keep in an intact state according to another report (Meyenburg et al., 2000).

Fig. 3 shows the cumulative release curves of QR for FLCM system containing different surface properties of liposomes. Although the burst release of QR from FLCM for tested liposomes are observed, they are much reduced compared with those of liposomes and liposomes–chitosan system. Notably, the burst release of QR of the FLCM is influenced by the surface properties of liposomes. For example, the burst release of QR for PEG-liposomes (e.g., 12 h) has the highest (68%) while DP-liposome has the lowest (42%) (Fig. 3). Moreover, the differences between the burst release of QR of PEG-liposomes and that of SA-liposome, and DP-liposome are significant, $P < 0.02$ and 0.001, respectively. Moreover, the cumulative release of QR of FLCM are effectively sustained to 120–168 h that are influenced by the surface properties of liposomes (Fig. 3). For instance, the release period of QR of PEG-liposomes containing FLCM is the shortest (e.g., 120 h) and that of the neutral liposomes is the longest (e.g., 168 h). Moreover, the burst and sustained release of QR from DP-liposomes are similar to neutral ones even though the former has a higher QR encapsulation efficiency than the latter (Table 1). In general, the sustained release of QR from FLCM is about four and two times longer than that from liposomes and liposomes–chitosan system, respectively.

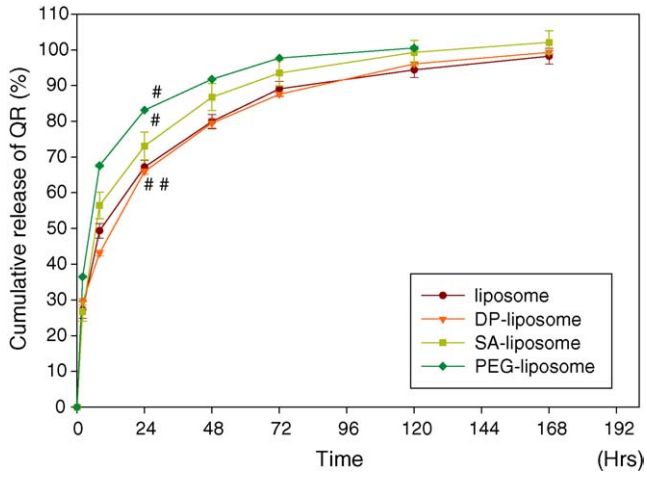

Fig. 3. The release profiles of QR from a FLCM system are effectively sustained for all tested liposomes although they are influenced by the surface properties of liposomes ($n = 3$) (# and ##: the differences between the burst release of QR of PEG-liposomes and that of SA-liposome, and DP-liposome are significant, $P < 0.02$ and 0.001, respectively).

The reduced effect on sustained release of QR for FLCM containing PEG-liposomes may have resulted from the shielded PEG on the surface of liposomes that hinder the interactions between PEG-liposome and fibrin network. However, high burst release of the PEG-liposome with low QR encapsulation may play a role on a short release period (Fig. 3). In contrast, a little lower burst release and release profiles of QR of FLCM containing DP-liposomes than SA ones may be due to some interactions between the surface charges of DP-liposomes and the fibrin network/or chitosan matrix. Significantly, the neutral liposomes can effectively sustain QR release although the dominant factors require further investigation.

In vitro release behaviors of different proteins (e.g., horseradish peroxidase (HRP) and NGF) from a fibrin network have been showed much difference (Meyenburg et al., 2000; Sakiyama-Elbert and Hubbell, 2000). For instance, in studying the release behavior of HRP from a fibrin network, only 1% or less of HRP was released from the network containing HRP loaded liposomes in 12 days of period while 100% of HRP was released from the same network without liposomes in 6 days. In contrast, the release of NGF from a fibrin network was very short (e.g., 100% <1 day). It did not reduce burst release of NGF (e.g., about 75% release on the first day) from the network after heparin modification (Sakiyama-Elbert and Hubbell, 2000). The release behaviors of QR of FLCM are in-between the above-mentioned ones. Therefore, the effects of the properties of loaded drug on influencing the release behaviors of a FLCM need to be further investigated.

3.3. Influence of crosslinked fibrin network on the release of QR of FLCM

To enhance the stability of the fibrin network of the FLCM such as the resistance of hydrolysis of the network, using coagulation factor XIII (Ichinose et al., 1983; Meyenburg et al., 2000; Schense and Hubbell, 1999) or low concentration of glutaldehyde to crosslink fibrin polymers to stabilize the network were proposed (Ho et al., 1994). Here, using 0.5% of glutaldehyde solution to crosslink the fibrin network of the system was applied, and the results of QR release from the system with crosslinked fibrin (crosslinked FLCM) were investigated and reported (Fig. 4). The release of QR of the crosslinked FLCM of three surface properties of liposomes are all sustained to 17 days with a long period of linear release (e.g., 1–13 days) although the release profiles of QR are influenced by the properties of liposomes (Fig. 4). For instance, the release of QR of crosslinked FLCM of neutral liposomes is higher than that of DP- or SA-liposomes. However, the roles of surface properties of liposomes on the release of QR from FLCM are not clear (Fig. 4). Notably, the QR release periods of the crosslinked FLCM do sustain about three times longer than those without crosslinked ones (e.g., Figs. 4 and 3, respectively). In addition, the system of crosslinked FLCM could effectively reduce 50% burst effects of QR for all of tested liposomes compared to non-crosslinked ones (e.g., Figs. 4 and 3, respectively). The results indicate that stabilizing the fibrin network by crosslinking the network would highly affect the release behaviors of QR of the FLCM.

To further analyze whether the release of QR of FLCM is influenced by the stability/hydrolysis of the fibrin network, the concentrations of fibrin products for FLCM containing DP-liposomes with or without crosslinked, and the chitosan polymers from the disintegration of chitosan polymers/matrix were quantified for up to 7 days in saline solution (Table 2). Table 2 shows that fast hydrolysis of fibrin network after 2 days of lag time for FLCM without crosslinking while that for crosslinked

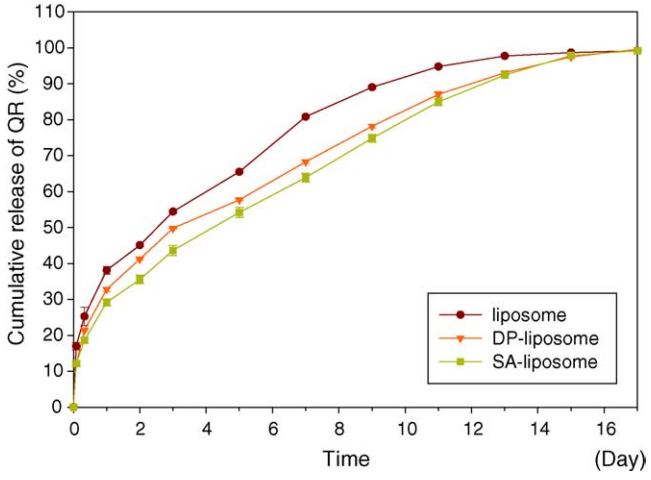

Fig. 4. This figure shows the release profiles of QR from a crosslinked FLCM system. The release periods of QR from the system are sustained to 17 days with 50% of reduced initial burst for all tested liposomes while the release profiles are influenced by the surface properties of liposomes.

Table 2
The percentages of hydrolyzed fibrin products and chitosan of a FLCM system to saline solution are measured

Time (h)	Fibrin products (%)	Chitosan (%)
0	0	0
8	0	0
24	0	0
48	3.19 ± 0.1	0.20 ± 0.1
72	16.66 ± 0.2	0.44 ± 0.1
96	41.63 ± 0.5	0.69 ± 0.3
120	62.19 ± 1.1	0.72 ± 0.1
144	79.98 ± 0.7	0.83 ± 0.2
168	86.08 ± 1.8	0.88 ± 0.1

The percentages of hydrolyzed fibrin products of a glutaldehyde crosslinked FLCM are very low till the end of tests (less than 1%) ($n = 3$).

one is very low till the end of the test. Moreover, chitosan matrix is relatively intact since the concentrations of measured chitosan are very low. The results of the hydrolysis of fibrin network would highly affect the release of QR for the non-crosslinked FLCM. It also suggests that the reduced hydrolysis of crosslinked fibrin network plays an important role on the sustained release of QR of crosslinked FLCM while the surface properties of liposomes play a minor role on QR release.

3.4. Comparing the release of Tirofiban and QR of FLCM configured by platelet poor plasma

To study whether QR is a suitable model drug for water-soluble drugs or not, the release profiles of QR and Tirofiban from FLCM system have been compared although human poor platelet plasma instead of bovine albumin was used for fibrin network to be more compatible to human subjects. Tirofiban is a clinical anti-thrombosis drug, which blocks the surface adhesive molecules (e.g., glycoprotein IIb/IIIa) of platelets and results in inhibiting the aggregations of platelets, and currently used by slow injection for therapy for the patients of unstable angina or myocardial infarction (PRISM-PLUS Investigators, 1998). Notably, Tirofiban is a low molecular weight (e.g., Mw. of 495 Da) and water-soluble drug which is one of the possible drugs for delivery by the FLCM system.

The particle sizes and encapsulation efficiency for Tirofiban loaded neutral, DP and SA-liposomes are 264.1 ± 6.6, 286.6 ± 8.3 and 310.0 ± 8.1 nm, and 41.7 ± 1.2%, 52.0 ± 1.4% and 46.5 ± 1.0%, respectively. Moreover, the total encapsulated Tirofiban within liposomes are 250–300 μg. Fig. 5a–c shows the cumulative release curves of QR and Tirofiban for FLCM containing the above three types of liposomes that have a good similarity to each other. Moreover, the less burst and a longer sustained release of Tirofiban (e.g., 11 days for DP-liposomes; Fig. 5b) for PPP forming FLCM than bovine fibrinogen are observed while those for QR are also similar but less effective (9 days for SA-liposomes; Fig. 5c). In addition, the burst release and release profiles for Tirofiban are also influenced by the surface properties of liposomes (e.g., Fig. 5a–c). Those results may be due to the fibrin network stabilized by the coagulation factor XIII of the PPP (Ichinose et al., 1983; Meyenburg

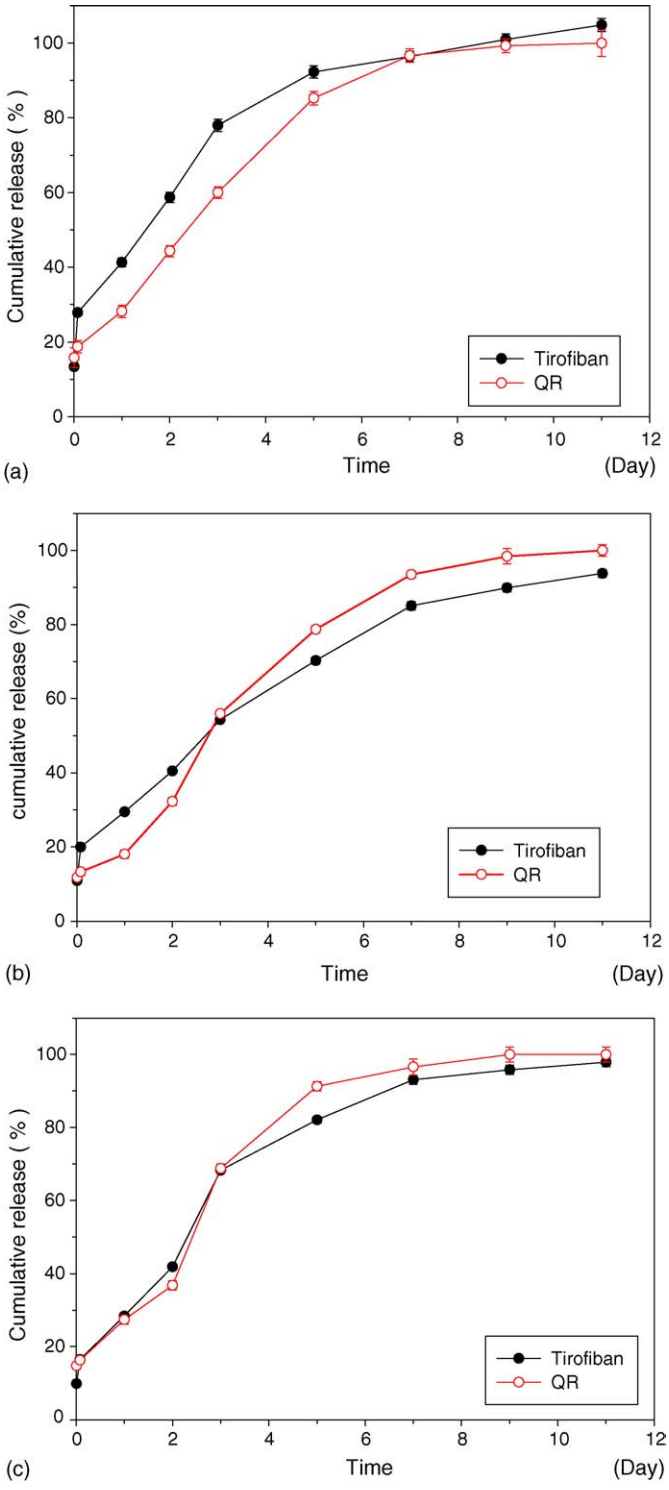

Fig. 5. The release profiles of Tirofiban and QR from a FLCM system with fibrin encapsulated different liposomes which are neutral liposomes (a), DP-liposomes (b) and SA-liposomes (c), respectively (note: PPP used as a source of the fibrin network).

et al., 2000; Schense and Hubbell, 1999). In another study, the released Tirofiban solution of 11 days shows effective inhibition of the aggregations of platelets induced by an aggregation reagent (e.g., ADP; data not shown) (Yang, 2003). The comparisons of the release profiles between Tirofiban and QR study (e.g., Fig. 5a–c) indicate that the results of QR may be a suitable model for studying the release behaviors of water-soluble and low-molecular weight clinical drugs for FLCM.

4. Conclusion

A FLCM system has been developed which can sustain the release of water-soluble model drugs such as QR in 5–7 days that are influenced by the surface properties of liposomes. Moreover, by crosslinking fibrin network of the FLCM with 0.5% glutaldehyde, the released QR can be further sustained to 17 days with 50% of reduced initial burst, suggesting that the stability of the network plays an important role on the release. By comparing the release behaviors between Tirofiban and QR from a FLCM system, they are highly similar to each other, indicating that the results of QR may be a suitable model for understanding the release behaviors of water-soluble drugs of the FLCM system.

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