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Streptokinase Loading in Liposomes for Vascular Targeted Nanomedicine Applications: Encapsulation Efficiency and Effects of Processing

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ABSTRACT: Vascular diseases leading to thrombo-occlusion are the leading cause of morbidity and mortality worldwide. Revascularization and restoration of antegrade blood flow is critical for tissue survival and patient health. In this aspect, systemic administration of thrombolytics (e.g., streptokinase) to dissolve occlusive thrombi is a clinically established strategy. However, this strategy typically necessitates the administration of large doses, leading to a high incidence of hemorrhagic complications due to systemic side effects. To minimize this risk, liposomes specifically targeted to the site of thrombo-occlusion have been bioengineered by exploiting ligand–receptor relationships pertinent to thrombus-associated cell phenotypes. This study focuses on encapsulating streptokinase within these liposomes, specifically regarding the effect of liposome processing conditions on streptokinase encapsulation and activity. Theoretical calculations of encapsulation capacity agreed well with that reported in the literature. The experimental encapsulation efficiency values are $45.9 \pm 34.0\%$ ($n = 9 \pm$ SD) and $21.6 \pm 30.0\%$ ($n = 6 \pm$ SD), using two different methods. The liposome processing conditions are found to decrease streptokinase activity; however, over 30% remain active after processing, maintaining

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enough activity to be therapeutic especially when protected inside a vehicle targeted to the site of thrombo-occlusion. The insight gained from the research reported here would enable refining the design and the processing conditions of liposomal formulations of fibrinolytics to yield reduced variability in encapsulation efficiency and streptokinase activity. The design of a thrombus-targeted 'stealth' liposome reported earlier and the current findings of fibrinolytics' encapsulation and activity in such liposomes can be efficiently integrated to develop an efficient strategy for vascular nanomedicine.

KEY WORDS: streptokinase, liposome, encapsulation efficiency, processing, vascular nanomedicine.

INTRODUCTION

Vascular diseases, including coronary artery disease, peripheral artery disease, and stroke, plague over 80 million American adults and are the leading causes of morbidity and mortality in the United States [1]. Nearly seven million vascular procedures are undertaken in the United States per year [2] at an estimated cost of $450 billion [3]. Irrespective of etiology, the major clinical event in vascular diseases is thrombo-occlusion and reduction of antegrade blood flow to critical organs and tissues. Rapid revascularization by therapeutic, angioplastic, or surgical intervention is of crucial importance to prevent tissue and organ damage [4,5]. The angioplastic (with or without stenting) and surgical (e.g., bypass surgery) strategies are currently widely practiced in the clinic for patients with acute occlusion. At the same time, with or without angioplastic/surgical procedures, systemic and/or oral administration of therapeutic agents (fibrinolytics, anti-coagulants, anti-inflammatory, anti-platelet, and anti-proliferatives) remains a major treatment modality for occlusive vascular diseases, globally [5].

The systemic and oral delivery of these agents, although currently well-established in clinical regimen [4–11], put patients at significant risk of side effects. For example, the current strategy of intravenous bolus administration of fibrinolytics often leads to hemorrhagic complications in patients [12–15]. This is because, to overcome the short plasma half-life of fibrinolytics and to ensure that an optimum concentration of the drug reaches the clot site, a large systemic dose is administered. The nonspecific uptake of a large fraction of the systemic drug dosage leads to activation of soluble plasminogen, which in effect causes breakdown of soluble fibrinogen and coagulation factors, resulting in adverse side effects, such as cerebral hemorrhage [12–15]. This was the case with using streptokinase (SK) [16–19] and urokinase as fibrinolytics in the United States [20–22], and though these

fibrinolytics are hardly used in the United States anymore, they still have significant usage in other countries, putting patients at risk.

The way to reduce such risks while maintaining therapeutic efficacy at the clot site is to develop methods of 'targeting' or 'localizing' optimum concentrations of drug to areas of occlusive thrombi. Various strategies of reducing systemic uptake while maintaining clot site-localized optimum drug concentration have been applied in pre-clinical and/or clinical settings, including using intra-arterial prolonged infusion of drug with specialized catheters [23–30], using drug-eluting stents (DES) in cases of angioplasty with stenting [31–34], using drugs with genetically engineered higher affinity for clot-specific biomolecules (e.g., tissue plasminogen activator (tPA) with higher affinity for clot-associated plasminogen [13,20]), formulation of the drug in degradable polymeric nanoparticles deployed via specialized intra-luminal catheters [35–38], and many strategies specifically with SK [39–45]. The DES and trans-catheter infusion approaches have demonstrated significant clinical success, while systemic delivery of tPA still suffers from limitations of short half-life, nonspecific uptake, and unwanted side effects. Considering the fact that a significant number of patients do not undergo invasive interventional procedures like catheterization and stenting, a clinical need still exists for pharmacotherapy strategies that can enhance the plasma half-life of vascular drugs, can reduce rapid drug washout, can ensure clot site-selective drug delivery, and can, in effect, prevent nonspecific uptake and systemic hemorrhagic side effects.

To this end, the authors are developing liposomes which can be surface-modified by ligands that have binding specificity toward cellular phenotypes involved in clot formation and stabilization. A schematic representation of the final liposome design is shown in Figure 1. Many articles have highlighted the potential of liposomes as a drug delivery vehicle [46–51], and a few preliminary investigations have evaluated different liposome formulations for vascular applications [52–58]. Encapsulating drugs or diagnostic probes within such thrombus-targeted liposomes will provide an effective strategy for maintaining long half-life of the encapsulants, affecting thrombus-selective delivery and action, and minimizing systemic uptake and harmful side effects. Based upon this rationale, success has already been demonstrated in developing liposomes that specifically bind activated platelets, which are a major component of primary thrombus [54,59]. As a next step toward the goal of a vascular drug-loaded thrombus-targeted liposome, studies on encapsulating the fibrinolytic SK within these liposomes are reported here, specifically the effect of liposome processing conditions on SK encapsulation and activity.

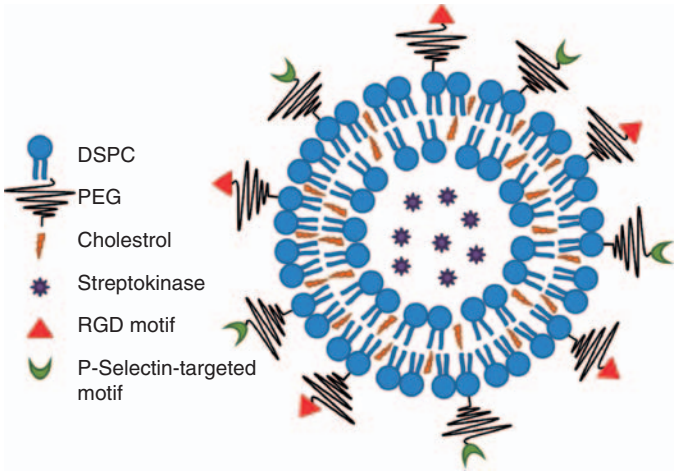

Figure 1. Schematic of SK-loaded, 'stealth' liposomes targeted to thrombus. The RGD and P-Selectin-targeted motifs provide specific targeting to activated platelets at thrombo-occulsion. The PEG provides a shell of hydration minimizing uptake by cells of the reticuloendothelial system (RES), enhancing circulation time.

MATERIALS AND METHODS

Liposome Encapsulation Volume Modeling

Theoretical encapsulation volume is defined as the volume (of buffer) contained inside all of the spheres (small, unilaminar liposomes) of 100 nm diameter formed from the starting materials (1×10^{-5} moles of liposomes). Percent encapsulation volume is defined as the theoretical encapsulation volume divided by the total volume of buffer. Briefly, theoretical encapsulation volume of liposomes was calculated using a model liposome with a 100 nm diameter and only consisting of DSPC (1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine). Surface area of a single DSPC molecule was calculated using two methods. First, average bond lengths and orbital geometry of the fatty acid tails were used to approximate a 'length' and 'width' of DSPC, modeling DSPC's effective surface area as a rectangle. Second, effective surface area was computed using the surface area of β-cyclodextrin, assuming circular geometry with a radius of 0.3 nm. Refer to Supplementary Data for further details.

Preparation of SK-loaded Liposomes

SK was entrapped in liposomes using the dehydration/rehydration method. Breifly, 10 μmol of lipid composed of 50 mol% DSPC

(1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine), 5 mol% DSPE-PEG (Distearoyl-*sn*-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000]), and 45 mol% cholesterol (Avanti Polar Lipids) were dissolved in 1 : 1 chloroform : methanol. The components were dehydrated by evaporating the solvent using a Rotovap with a water bath at 60°C, moderate rotation, and moderate negative gauge pressure. After evaporation, the lipid film was placed in a vacuum oven at 0 mmHg overnight. The lipid film was reconstituted into 1 mL of 1 × phosphate buffered saline (PBS) containing 10,000 IU of SK (Sigma Aldrich). The mixture was subjected to 10 freeze/thaw cycles of freezing with liquid nitrogen and thawing with a 60°C water bath, and it was subsequently extruded at 80°C (above the highest lipid transition temperature: 74°C for DSPE) 15 times through a 100 nm polycarbonate membrane (Avanti Polar Lipids) to maximize encapsulation efficiency [60] and produce small, unilaminar vesicles. Average particle size was determined using a DLS particle sizer (Brookhaven Instruments 90Plus/BI-MAS Multi Angle Particle Sizer). Blank solutions of liposomes were also prepared by omitting the SK from the PBS buffer.

SK Encapsulation Efficiency

Separation of free SK from SK associated with the liposomes was achieved by ultracentrifugation (Optima TLX Ultracentrifuge, Beckman Coulter) at 100,000 rpm for 20 min at 20°C. The supernatant was aspirated and placed into a Falcon tube. Fresh buffer was added to the ultracentrifugation tube, agitated to re-suspend the pellet, aspirated, and placed into another Falcon tube. Both supernatant and pellet solutions were frozen at −80°C and subsequently lyophilized.

The lyophilized powders were rehydrated into a minimum volume of water. The solutions were subjected to a commercially available colorimetric bicinchonic (BCA) acid protein assay (BCA Protein Assay, Pierce) to determine SK concentration. Since the volume of solution is known, the mass of SK in solution can be calculated from the concentration determined by the BCA assay.

Encapsulation efficiency was calculated using the principle of conservation of mass. The initial mass of SK was determined from weighing SK on a balance before being dissolved. Also, the initial mass of SK was calculated by using the BCA assay, as was the procedure to find the mass of SK in the supernatant. Subtraction of initial SK mass minus mass of SK in the supernatant yielded the mass associated with the pelleted liposomes. Encapsulation efficiency is defined as mass of SK associated with liposomes divided by the initial mass of SK multiplied by 100%.

SK Activity

To determine SK activity, SK was subjected to a modified version of a commercially available chromogenic assay (Chrom Z-PLG, Helena Labs). Briefly, specially assayed reference plasma (SARP) provides an excess amount of plasminogen (PLG). SK is added to the PLG and operates on PLG to form plasmin. Plasmin operates on its provided chromogenic substrate, liberating p-nitroaniline (pNA), which can be measured photometrically at 405 nm and whose signal is linear with respect to active SK concentration.

Ultracentrifugation Effects on SK Activity

Equimolar solutions of SK were subjected to ultracentrifugation at varying rotation speeds for 20 min at 20°C. After ultracentrifugation, the tubes were gently mechanically agitated to ensure no SK adhered to the tube wall. All solutions were aspirated, and SK activity was determined using the modified version of the Chrom Z-PLG assay.

Temperature Effects on SK Activity

Equimolar solutions of SK were subjected to varying temperatures (30–80°C with an interval of 10°C). Specifically, small, enclosed glass vials containing the SK solutions were placed in a water bath of specified temperature for 5 min. The vials were removed and allowed to cool first to room temperature and were then placed in a 4°C refrigerator. Finally, the solutions were subjected to the modified version of the Chrom Z-PLG assay to determine SK activity.

Processing Effects on SK Activity

The processing method of creating liposomes is multistage, and each step could lead to inactivation of SK. Experiments were completed with SK solutions subjected to the processing steps of making liposomes. Before and after each stage of processing – freeze/thaw, extrusion, ultracentrifugation, and lyophilization (lyophilization is a nonessential step but was used in some studies reported here) – samples were taken and SK activity was determined using the modified version of the Chrom Z-PLG assay and compared to the original stock solution before processing.

Differential Scanning Calorimetry

SK was subjected to differential scanning calorimetry (DSC) (TA Instruments DSC Q200 V23.10). The first scan began at 20°C, ramped up to 50°C, and ramped back down to 20°C. Subsequent scans advanced the maximum temperature by 10°C intervals until 80°C – the maximum temperature to which SK is subjected during processing – allowing the determination if SK undergoes a thermally induced significant transition which could potentially affect SK activity. In addition, since the SK was stabilized with bovine serum albumin (BSA), BSA was also subjected to the same DSC method.

Statistical Analysis

One sample *t*-tests were used to evaluate statistical significance between experimental SK and stock SK activities for the ultracentrifugation, temperature, and processing studies. Student's *t*-tests were used to determine any significant differences between experimental values within the ultracentrifugation, temperature, and processing studies. Also, Student's *t*-test was applied to evaluate potential significance between the balance-determined and BCA protein assay-determined encapsulation efficiencies. Results were considered statistically significant for $p < 0.05$.

RESULTS

Theoretical Encapsulation Volume

The effective surface area for a DSPC molecule was $28\,\text{Å}^2$ for the β-cyclodextrin model and $16\,\text{Å}^2$ for the bond length model, which is in good agreement with the data presented in [61]. Further, the number of liposomes predicted is on the order of 1×10^{13}, agreeing again with reported values [61].

Modeling of 10 μmoles of 100 nm liposomes produced a theoretical encapsulation volume (how much volume is encapsulated by liposomes) and percent encapsulation volume (what percent of the total buffer volume is encapsulated by liposomes). For the DSPC bond-length model, the theoretical encapsulation volume is 7.9 μL, encapsulating 0.8% of the total reconstitution volume of 1 mL. The β-cyclodextrin model yielded an encapsulation volume of 14.2 μL, which reflected 1.4% encapsulation volume. This small encapsulation volume and efficiency agrees with the data for passive entrapment using small particles [61].

Experimental Encapsulation Efficiency

The ability to encapsulate SK into liposomes varied greatly. Calculations of encapsulation efficiency of samples varied from 0 to 89%. Substantial differences in stock SK buffer concentration were noticed between values obtained from the balance while weighing out SK and dissolving it into buffer and using the BCA protein assay to determine SK concentration. Hence, this disagreement produced ambiguous encapsulation efficiencies as shown in Table 1. The average encapsulation efficiency, shown in Table 2, is $45.9 \pm 34.0\%$ ($n = 9 \pm \mathrm{SD}$) as determined by the balance method and is $21.6 \pm 30.0\%$ ($n = 6 \pm \mathrm{SD}$) as determined using the values from the BCA assay. There is no difference between the calculated encapsulation efficiencies of these two methods by Student's *t*-test.

Table 1. Encapsulation efficiency of SK. Stock solution concentration was computed by using balance-determined or BCA-assay-determined SK mass. Notice the intra-method and the inter-method variability.

	Encapsulation efficiency (%)	
Trial	Balance determined	BCA assay determined
1	16.3	NA
2	19.4	NA
3	68.1	NA
4	16.9	11.8
5	87.9	87.1
6	88.8	15.9
7	88.6	14.6
8	14.3	0.0
9	13.3	0.0

Table 2. Summary of encapsulation efficiency data.

	Encapsulation efficiency summary	
	Balance	BCA assay
Average ($\bar{x}$) (%)	45.9	21.6
Standard deviation (s) (%)	34.0	30.0
$s/\bar{x} \times 100$(%)	74.0	139.1
n	9	6

Ultracentrifugation Effects on SK Activity

The effects of ultracentrifugation on SK activity were studied and are shown in Figure 2. The results show that ultracentrifugation decreases SK activity by at least 50% for all rotation speeds studied. All rotation speeds greater than 10,000 rpm showed a statistically significant decrease in SK activity compared to stock SK activity. Further, there are no significant variations between rotation speeds, indicating a relatively consistent decrease in activity across rotation speeds. A speed of 100,000 rpm has the greatest reduction in activity: only $18.8 \pm 15.3\%$ of the stock solution's activity.

Temperature Effects on SK Activity

Figure 3 shows the effects of temperature on SK activity. The 30°C sample showed no statistically significant difference in SK activity compared to the unheated stock solution. A peak of SK activity occurred for the 60°C sample, having an SK activity 16% greater than the unheated SK stock solution. Both 40°C and 80°C samples possessed significantly less SK activity compared to stock SK (single tail $p < 0.05$). Interestingly, heating to 40°C, approximately physiological temperature, resulted in roughly a decrease of SK activity by 60%.

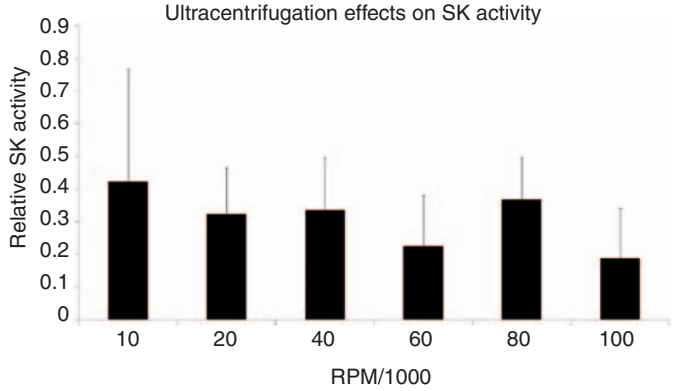

Figure 2. Relative SK activity vs ultracentrifugation rotor speed in RPM/1000. SK activity is relative to the unprocessed stock SK solution used for the trials (i.e. no centrifugation or processing). Error bars are ± standard error, and $n = 3$ for all samples.

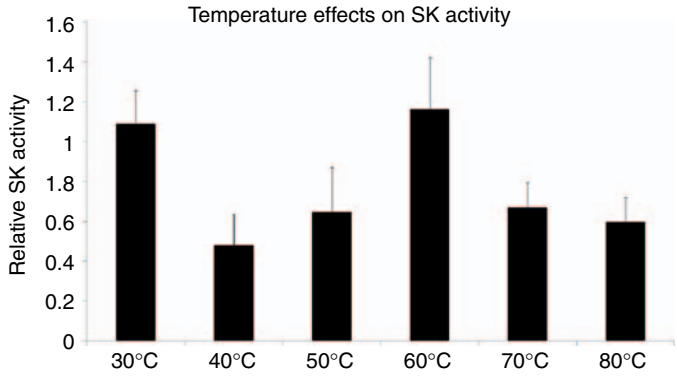

Figure 3. Relative SK activity vs temperature. SK activity is relative to the unprocessed stock SK solution used for the trials (i.e. no heating or processing). Error bars are ± standard error, and $n = 3$ for all samples.

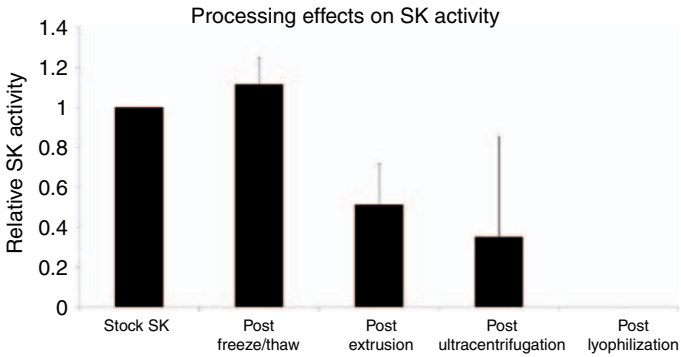

Figure 4. Relative SK activity vs processing stage. SK activity is relative to the unprocessed stock SK solution (left) used for the trials. Error bars are ± standard error, and $n = 3$ for all samples.

Processing Effects on SK Activity

The different processing steps in the preparation of liposomes were conducted sequentially with SK solution to determine their cumulative effects on SK activity, the results of which are summarized in Figure 4. No statistically significant decrease in SK activity was observed due to freeze/thaw, extrusion (although it decreases SK activity by approximately 50% of stock SK activity), or ultracentrifugation at 100,000 rpm (although it further decreases the activity to 35% of stock SK activity).

After lyophilization, SK activity is essentially zero, which is statistically significantly less than stock SK activity, although lyophilization is not a necessary or even preferred step in liposome preparation.

Differential Scanning Calorimetry

Performing DSC on SK with increasing temperature reveals a transition at approximately 61°C as shown in Figure 5(a) and (b) (zoomed). Decreasing temperature from above the transition temperature displays a transition at approximately 41°C – Figure 5(c). Since no liposome procedure occurs at temperatures greater than 80°C, DSC did not consider temperatures greater than 80°C. Only the transition at 61°C, as shown in Figure 5(b), was observed, and this transition is in good agreement with the literature [62,63]. DSC was also performed on BSA, and no transition was observed (data not shown).

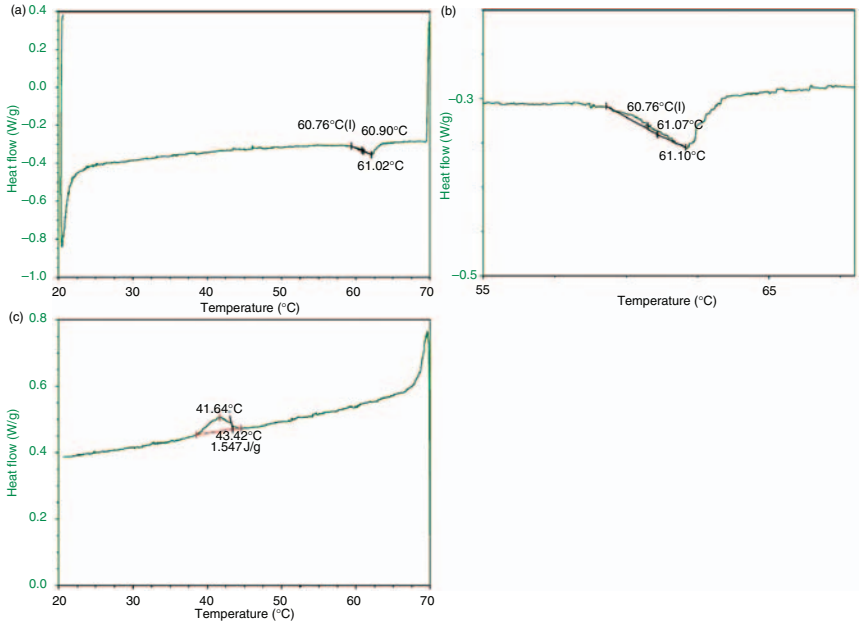

Figure 5. DSC of SK. (a) Increasing temperature from 20°C to 70°C. SK undergoes a transition at approximately 61°C, which is also the temperature at which SK's activity increases; (b) Zoomed-in view of the transition shown in (a); (c) Decreasing temperature from 70°C to 20°C. SK undergoes a transition at ≈41°C, above physiological and room temperatures.

DISCUSSION

The theoretical encapsulation volume and efficiency agree well with theoretical predictions in the literature [61]. Since the reconstitution volume of 1 mL is relatively high, particle diameter is relatively small, and encapsulation is passive, it is expected that encapsulation should be quite small. At the nanoscale, the surface area to volume ratio increases, requiring much material to create a particle with a small volume. Further, the volume of buffer is large and there is no mechanism to aid encapsulation.

The theoretical encapsulation volume is $\approx 1\,\%$ of the total reconstitution volume, and assuming a well-mixed drug-loaded buffer, only 1% of dissolved drug would be encapsulated by our drug delivery vehicles. At this loading efficiency, the delivery platform may be sub-optimal to incorporate sufficient concentration of drug for a clinically useful therapeutic effect. However, many studies in the literature report substantially higher experimentally determined encapsulation efficiencies of similar platforms relative to the theoretical calculation [39–44], similar to the findings in this study.

One possible mechanism of increased encapsulation is that hydrophobic regions of amphiphilic drugs like SK can associate with the lipid components of the liposome bilayer during the formulation in addition to that encapsulated within the aqueous core. Also, some SK could associate with the PEG 'forest' due to inter-molecular forces or steric entrapment within the forest as the lipid molecules assemble into liposomes. These additional modes of drug incorporation in the liposomal system can effectively increase the 'encapsulation efficiency' of the drug delivery platform.

The experimental encapsulation efficiency of this study varied greatly. Large differences existed between balance-determined and BCA protein assay-determined SK stock solution concentrations, which contributed to differing encapsulation efficiencies; however, the difference in calculated encapsulation efficiency between the two methods is not statistically significant. Intra-method differences also were nontrivial. The authors think that lipid could be interfering with the assay, or during ultracentrifugation, SK could irreversibly adsorb to the tube or have its domains separated, affecting quantification. Because of all the numerous steps in generating liposomes, it may be that small variations of each step magnify themselves across many procedures, resulting in variable results. For example, variations in the formation of the lipid cake and its rehydration could substantially affect encapsulation of SK. Further, anisotropic local concentration of particular lipids, anisotropic

local aggregate concentration within films, or anisotropies across different lipid films could affect the thermodynamics of the self assembly, thereby substantially affecting encapsulation. By virtue of how the particles align during extrusion could result in liposome rupture and release of encapsulated SK.

This high variability suggests that encapsulation efficiency is affected to a great degree because of processing conditions. Between the two methods, the balance-determined method is preferable as it had a smaller standard deviation relative to the mean and is not subjected to additional potential sources of error encountered with a colorimetric assay. These methods have been reported in the literature to give consistent data. Even with high variability in the data found in this study, each batch could be quantified to draw reasonable conclusions regarding encapsulation efficiency and the effects of processing.

In consideration of the clinical translational potential of the formulation, it is concluded that loading of biologically active SK within actively targeted liposomes is feasible, but the encapsulation efficiency and activity are very much dependent upon processing parameters. Further studies in this area will deal with optimization of the processing parameters to maintain a consistent dosage-dependent activity of the encapsulated SK. It is anticipated that following further optimization, such formulations will be available in the clinic in the form of lyophilized powder, which can be reconstituted right before administration and hence will maintain a reasonably consistent activity.

DSC demonstrated that the SK used undergoes a transition around 61°C and corresponds to an increase in SK activity. Therefore, it is contended that one (or more) of SK's chains are liberated from the globular structure, and that either the liberated chains and the remaining structure are able to participate in fibrinolytic activities or the liberation exposes to a greater extent the active site of fibrinolysis. Therefore, it is expected that at the transition temperature, SK activity should increase, which is shown in Figure 3. Further, the same reasoning also provides evidence as to why the encapsulation efficiency data is variable. Since SK readily undergoes a transition due to temperature alone, it is extremely hard to predict if it undergoes the same or a different transition(s) due to other processing parameters. Extrusion occurs at 80°C, slightly above the transition temperatures of the lipids. Hence, SK should certainly experience a transition during this stage. Predicting other transitions and the percent of recrystallization and inactivation is essentially impossible. Therefore, this complex phenomenon could play an important role in the variability of encapsulation efficiency.

All of the processing experiments investigated free SK in buffer, as encapsulation would inherently subject SK to all steps in the liposomal encapsulation and purification process, preventing a step-by-step analysis of the effects of processing. Using SK in buffer allowed for precise control of SK concentration and averted having to disrupt liposomes to assess their contents which could potentially reduce SK activity.

The temperature, ultracentrifugation, and sequential processing activity experiments all demonstrate that SK is inactivated during processing. It can be extrapolated that processing is a physically harsh process on SK, resulting in processing-induced SK conformational alterations, potentially including the dissociation of subunits or uncharacteristic rotations and/or breaking of chains, which could substantially alter SK activity. SK associated with liposomes could possibly be protected from some of the mechanical agitations through SK-liposome interactions or simply by being sequestered into the aqueous core; however, encapsulated SK would still be exposed to a similar processing environment as conditions such as temperature and centrifugal force transcend the liposomal carrier.

Even with processing-induced SK inactivation, Figure 4 shows that after the last step of liposome processing, ultracentrifugation, over 30% of SK remains active. (Lyophilization is an auxiliary step which was investigated but is not necessary and is not even preferred for the generation of liposomes.) Since SK is associated with liposomes, it is protected from the vascular environment, preventing the rapid inactivation seen with free SK injected in the bloodstream. Further, this liposomal platform has been shown to be targeted specifically to the site of thrombo-occlusion. Therefore, a higher percentage of these targeted liposomes reach the thrombo-occlusion than nontargeted liposomes. Thus, a substantial amount of the active SK remains active while in circulation and is specifically delivered to its intended site of action. Therefore, even though processing reduces SK activity, the nature of the delivery system should provide a more than adequate therapeutic level targeted to the site of thrombo-occlusion as supported by other reports in the literature demonstrating the enhancement of thrombolytic activity that arises from encapsulation and targeting [20,40, 41,44,64,65].

The fundamental knowledge of the effects of processing on encapsulation efficiency and bioactivity of therapeutic molecules in general could direct future studies of formulating these molecules in nanoparticle platforms. For example, new ultracentrifugation methods or using sonication instead of extrusion could be investigated. Also, now that it is

shown that SK can be encapsulated inside these 'stealth', targeted liposomes, future work could involve *in vivo* clot dissolution assays and eventually evaluate the performance of these SK-loaded liposomes in *in vivo* animal models of thrombo-occlusion.

CONCLUSIONS

This study has shown that encapsulation efficiency of SK into liposomes is variable and that processing of SK-loaded liposomes decreases SK fibrinolytic activity; however, for all processing conditions, over 30% of SK remains active after processing. Considering that liposomes prevent SK inactivation in circulation and that this formulation can specifically target the site of thrombo-occulsion, this SK-loaded, thrombus-targeted, 'stealth' liposomal delivery platform is potentially feasible for strategies in vascular nanomedicine.

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