Published in final edited form as:

Chem Commun (Camb). 2008 August 14; (30): 3579–3581. doi:10.1039/b805428b.

SCKs as nanoparticle carriers of doxorubicin: Investigation of core composition on the loading, release and cytotoxicity profiles

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Abstract

SCK nanoparticles having differing core thermal characteristics were designed and evaluated as thermoresponsive drug delivery systems of doxorubicin for the killing of cancerous cells.

Nanoobjects, such as polymeric micelles and nanoparticles, are considered promising vehicles for the delivery of hydrophobic or amphiphilic drugs.^{1–7} Such polymeric assemblies can be prepared from amphiphilic diblock copolymers that self assemble in aqueous solution to afford nanoscale structures with a core-shell morphology. The hydrophobic core facilitates encapsulation of the drug while the hydrophilic shell can protect the drug from its surroundings.^{1–9} It is recognized that the permeability of the shell is important to gate the uptake and release of the guest molecules and also that the incorporation of residual functionality in the shell domain of these assemblies provides a handle for the attachment of various ligands for tailored biodistribution, imaging, and targeting.^{5, 10–12} The characteristics of the core domain are also of significance.^{13,14}

In this work, we have investigated the effects of the chemical composition and thermal characteristics of the hydrophobic core polymer material for the loading and release of doxorubicin (dox). Dox is a drug widely used in chemotherapy treatments for several types of cancer.¹⁵ Liposome encapsulation of dox has been shown to both reduce toxicity and improve activity and is marketed as Doxil®.¹⁵ Several research groups have studied dox encapsulation within or conjugation onto a variety of polymeric micelles and other types of nanostructures with promising results.^{4, 16–21}

Shell crosslinked knedel-like nanoparticles (SCKs) are polymeric micelles that are crosslinked through the shell region of the particle.^{22, 23} In our laboratory, the crosslinking is performed typically by amidation of poly(acrylic acid) groups in aqueous solution and provides stability

to the nanostructure, circumventing the limitations that CMC poses for *in vivo* applications.^{10, 11}

We have selected two classes of SCKs from our library of nanostructures as hosts for dox, and evaluated a series of four SCKs, having different compositions and sizes.^{12, 24} These SCKs were constructed from amphiphilic block copolymers of either glassy amorphous poly(acrylic acid)-*b*-poly(styrene) (PAA-*b*-PS) having a high glass transition temperature, T_g ,¹² or semi-crystalline poly(acrylic acid)-*b*-poly(octadecyl acrylate-*co*-decyl acrylate) (PAA-*b*-P(ODA-*co*-DA))²⁴ having a low T_g and exhibiting melting transitions, T_m , at either well below or just above physiological temperature (Table 1 and Fig. 1). The main objective of this work was to investigate the effects of the core material on the release rate of dox under physiological temperature and pH. Four particular SCKs were prepared to test the hypothesis that nanoparticles containing core material of higher T_g and T_m characteristics would retain dox to a higher extent, resulting in slower diffusion-based release rates.

Dox was incorporated in the cores of the SCKs by the addition of a stock solution of dox suspended in CH_2Cl_2 , followed by evaporation of the organic solvent.⁴ The organic solvent was used to both solvate the dox and swell the SCK core to facilitate loading. The drug-nanoparticle solution was then washed extensively with PBS buffer in a centrifugal filter at 37 °C. By this methodology, it was possible to prepare dox-SCK, guest-host complexes with low variation in the incorporation percentages from batch to batch (Table 1). The loading capacity of the polystyrene core-based SCKs **1** and **2**, were very similar (19 % and 18 %), and were independent of the block lengths of the polymer precursors, indicating that the loading capacity is similar in a PS amorphous core. In the case of the semi-crystalline SCKs, the SCK with the highest ratio of crystalline monomer (ODA) also had the highest loading capacity, suggesting that it forms a more stable core for entrapment of hydrophobic drugs.

The release of the entrapped drug in the SCK nanoparticles was assessed by monitoring the decrease over time of the concentration of dox in dialysis cassettes.⁴ The dialysis was performed at 37 °C in 5 mM PBS at pH 7.4. Samples were withdrawn from the cassettes at different time points and analyzed by UV-vis spectroscopy (Abs @ 488 nm, $\epsilon = 12500 \text{ M}^{-1}\text{cm}^{-1}$). Figure 2 (lower panel) depicts the release from the amorphous SCKs **1** and **2** and the crystalline SCKs **3** and **4**. The behavior of the SCKs with PS cores are very similar, with a release of *ca.* 20 % of the therapeutic cargo in 8 h, followed by a slow release over 48 h. It is reasonable that the release profile from SCKs **1** and **2** should be similar, given that the chemical environments are the same, even though there are differences in the sizes of the amorphous cores. SCK **1** and **2** differ also in their expected shell thicknesses, as they derive from block copolymers having different PAA chain segment lengths, however, they have the same crosslinking density. For these nanoparticles of 10–38 nm overall diameters, it appears that the dimensions of the core and shell are not determining factors for the guest release profiles. In the case of the semi-crystalline SCKs **3** and **4**, release of the cargo was slightly faster than observed from the PS-cored SCKs, with a release of *ca.* 30 % in 8 h, followed by a gradual release to *ca.* 50 % after 48 h. The most significant difference can be seen in the inset in the lower diagram of Figure 2, where the low T_m SCK **4** has a much faster release rate during the first 4 h of release compared to SCKs **1–3**. Release of therapeutics from crystalline and amorphous micelles of the same chemical composition have been shown to result in different release kinetics.²⁵ In order to assess whether the loading of a small molecule therapeutic could disrupt the crystallization of SCK **3**, we conducted DSC experiments on a lyophilized sample of SCK **3** containing dox (Supplemental Information). SCK **3** has a $T_m = 42.0 \text{ °C}$ before guest loading, and the incorporation of dox resulted in a reduction of melting point to 36.1 °C, which is lower than the temperature at which the release experiments were conducted. This melting point depression is an advantage, in reducing the T_m to just below 37 °C, which provides for nanoparticles that can physically trap the dox in a crystalline matrix at room temperature and

release it from a molten core at physiological temperature. The overall release extents for each of the molten-cored SCKs were greater than for the PS core, which is expected to retain a portion of the dox within the glassy core matrices.

The cytotoxicities of the non-loaded SCKs were determined by incubating U87-MG-EGFRvIII-CBR cells (a human glioblastoma cell line modified to express mutant EGFRvIII receptor and click beetle red luciferase) with varying concentrations of SCK 1 and SCK 3 (Fig. 2 upper panel). Cell viability was assessed using the Vybrant MTT cell proliferation assay kit. After 72 h of incubation with nanoparticles containing no dox, no significant toxicity was observed at concentrations up to 10 $\mu\text{g/mL}$. Given that the IC_{50} of free doxorubicin is similar (approximately 100–200 ng/mL) on both parental U87MG cells²⁶ and on U87-MGEGFRvIII-CBR cells (Fig. 2 top), these nanoparticles are expected to allow for delivery of effective doses of dox to tumor cells with minimal toxic effects from the SCK carriers. The cytotoxic effects of dox loaded within and released from SCKs 1–4 were then tested on U87MG-EGFRvIII-CBR cells *in vitro* by the MTT assay (Fig. 2 top). All four dox-SCK complexes demonstrated cytotoxic effects on U87MG-EGFRvIII-CBR. We reasoned that the distribution, kinetics of release, and availability of dox inside the cells between dox-SCKs and free dox are different and this may account for the difference of their cytotoxic effects. FACS analysis showed that the reduced viability of the cells was caused by an increase in apoptosis (Supplemental Information).

To investigate cellular uptake and trafficking of dox-SCKs in U87-MG-EGFRvIII-CBR cells, we performed microscopic studies based on red autofluorescence from dox. Most of the autofluorescence from dox-SCKs localized in the cytoplasm, specifically in the perinuclear region, while cells treated with free-dox demonstrated significant auto-fluorescence in the nucleus (Supplemental Information).

Red auto-fluorescence was also found from dox-SCKs that were co-localized with a fluorescent lysosome marker (lysotracker, data not shown), suggesting these dox-SCKs were indeed endocytosed into lysosome organelles. Lysosomal trapping of dox-SCKs may control the release of dox inside the cells, thus, limiting their cytotoxic effects, compared with free dox. We are currently testing several lysosome perturbant agents combined with these dox-SCKs to enhance their therapeutic efficacy on human glioma cell lines.

In this work, we have demonstrated the successful incorporation of dox in nanoparticle vehicles for drug delivery. Release of the incorporated therapeutic was demonstrated in a dialysis model; the release rate was shown to be dependent on the core material. Theoretical studies have indicated that enthalpic interactions between the core material and drug are an important factor governing the release behavior.¹⁴ Intermolecular interactions in combination with thermal properties could account for the reduced release of dox in the PS-cored particles, since the core is capable of forming π - π interactions with the drug. However, the overall shapes of the release curves are similar, which suggests that the release behavior from these two classes of SCKs is predominantly governed by the general nanoparticle core-shell morphology and is less dependent on the composition of the core domain (PS *vs.* P(ODA-co-DA)). We have achieved excellent loading capacities and release extended over several hours. Burst release was avoided by the purification protocol employed. The dox-SCK complexes were shown to kill U87-MG-EGFRvIII-CBR cancer cells. The SCKs themselves did not negatively affect the viability of these cells. These SCKs show promise as a nanoparticle platform for drug delivery. Further investigation of additional SCKs with broader core compositions and characteristics tailored for particular drugs are needed to investigate the ability to attract and retain the guest molecules to differing degrees. We are currently modifying our nanoparticle carriers for targeted drug delivery by introducing poly(ethylene glycol) chains for increased *in vivo* circulation time, tags for imaging, and targeting moieties for tissues specific binding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This material is based upon work supported by The Children's Discovery Institute of St. Louis Children's Hospital and Washington University School of Medicine, and the National Heart Lung and Blood Institute of the National Institutes of Health as a Program of Excellence in Nanotechnology (HL080729). Postdoctoral and assistant professor fellowship provided by the Knut and Alice Wallenberg Foundation is gratefully acknowledged (A.M.N.).

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Fig. 1.
Schematic representation of the SCKs prepared in the study.

Fig. 2. Top, MTT assay of SCKs 1 and 3 without doxorubicin loading at 72 h incubation, and MTT assay of SCKs 1–4 with doxorubicin loading. Bottom, release kinetics of SCK 1, 2, 3, and 4 (average of $n = 2$).

Table 1

Summary of the prepared SCK-drug conjugates.

Sample	Polymer	<i>D</i> [nm] ^a	<i>D_h</i> [nm] ^b	<i>T_m</i> [°C]	Dox [μM]	Loading [%] ^c
1	PAA ₆₀ - <i>b</i> -PS ₃₀	11±1	17±2	-	89±10	19±2
2	PAA ₁₀₀ - <i>b</i> -PS ₁₄₀	25±1	34±4	-	91±4	18±1
3	PAA ₉₇ - <i>b</i> -P(ODAA ₆₉ - <i>co</i> -PDA ₂₃)	29±6	54±5	42.0	82±3	18±1
4	PAA ₉₇ - <i>b</i> -P(ODAA ₂₄ - <i>co</i> -PDA ₆₄)	50±6	58±6	1.5	69±10	14±2

^aBy transmission electron microscopy.

^bBy dynamic light scattering.

^cAverage weight percent loading of doxorubicin per nanoparticle mass from two loading batches. Preparation of SCK 1, 2, 3 and 4 is described elsewhere.^{12, 24} Nominal crosslinking extents of the shells were 50 % in 80 each case.