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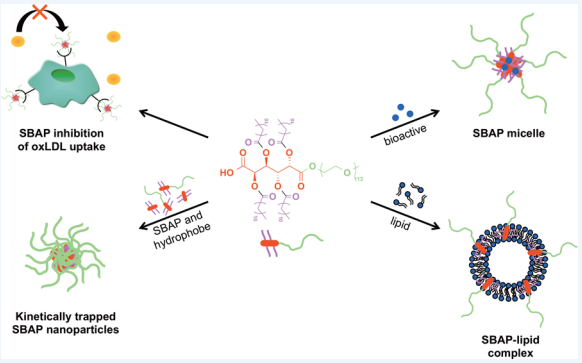
Sugar-Based Amphiphilic Polymers for Biomedical Applications: From Nanocarriers to Therapeutics

Li Gu, Allison Faig, Dalia Abdelhamid, and Kathryn Uhrich*

Department of Chemistry and Chemical Biology, Rutgers, the State University of New Jersey, 610 Taylor Road, Piscataway, New Jersey 08854, United States

CONSPECTUS: Various therapeutics exhibit unfavorable physicochemical properties or stability issues that reduce their *in vivo* efficacy. Therefore, carriers able to overcome such challenges and deliver therapeutics to specific *in vivo* target sites are critically needed. For instance, anticancer drugs are hydrophobic and require carriers to solubilize them in aqueous environments, and gene-based therapies (e.g., siRNA or pDNA) require carriers to protect the anionic genes from enzymatic degradation during systemic circulation. Polymeric micelles, which are self-assemblies of amphiphilic polymers (APs), constitute one delivery vehicle class that has been investigated for many biomedical applications. Having a hydrophobic core and a hydrophilic shell, polymeric micelles have been used as drug carriers. While traditional APs are typically comprised of nondegradable block copolymers, sugar-based amphiphilic polymers (SBAPs) synthesized by us are comprised of branched, sugar-based hydrophobic segments and a hydrophilic poly(ethylene glycol) chain.



Similar to many amphiphilic polymers, SBAPs self-assemble into polymeric micelles. These nanoscale micelles have extremely low critical micelle concentrations offering stability against dilution, which occurs with systemic administration. In this Account, we illustrate applications of SBAPs for anticancer drug delivery via physical encapsulation within SBAP micelles and chemical conjugation to form SBAP prodrugs capable of micellization. Additionally, we show that SBAPs are excellent at stabilizing liposomal delivery systems. These SBAP−lipid complexes were developed to deliver hydrophobic anticancer therapeutics, achieving preferential uptake in cancer cells over normal cells. Furthermore, these complexes can be designed to electrostatically complex with gene therapies capable of transfection.

Aside from serving as a nanocarrier, SBAPs have also demonstrated unique bioactivity in managing atherosclerosis, a major cause of cardiovascular disease. The atherosclerotic cascade is usually triggered by the unregulated uptake of oxidized low-density lipoprotein, a cholesterol carrier, in macrophages of the blood vessel wall; SBAPs can significantly inhibit oxidized low-density lipoprotein uptake in macrophages and abrogate the atherosclerotic cascade. By modification of various functionalities (e.g., branching, stereochemistry, hydrophobicity, and charge) in the SBAP chemical structure, SBAP bioactivity was optimized, and influential structural components were identified. Despite the potential of SBAPs as atherosclerotic therapies, blood stability of the SBAP micelles was not ideal for *in vivo* applications, and means to stabilize them were pursued. Using kinetic entrapment via flash nanoprecipitation, SBAPs were formulated into nanoparticles with a hydrophobic solute core and SBAP shell. SBAP nanoparticles exhibited excellent physiological stability and enhanced bioactivity compared with SBAP micelles. Further, this method enables encapsulation of additional hydrophobic drugs (e.g., vitamin E) to yield a stable formulation that releases two bioactives.

Both as nanoscale carriers and as polymer therapeutics, SBAPs are promising biomaterials for medical applications.

INTRODUCTION

Amphiphiles, composed of hydrophobic and hydrophilic segments, can self-assemble into various structures (e.g., spherical micelles, cylindrical micelles, vesicles with curved bilayers) in aqueous media above a critical micelle concentration (CMC). This self-assembly behavior is based on a hydrophobic effect in which the aggregation of hydrophobic segments increases the system's disorder by displacing water molecules that were previously organized about the hydrophobic domain.[1] This displacement results in an entropy increase and yields a thermodynamically favorable assembly.[1] Small molecular weight amphiphiles, or surfactants, are typically composed of a hydrocarbon chain and a hydrophilic headgroup. These small molecular weight surfactants are widely used as detergents, bioactive denaturing agents, microbiocides, and repellents. Pharmaceutical applications of such surfactants, however, are very limited due to their relatively high CMC values and cytotoxicity.[2] Unlike the small molecular weight amphiphiles, amphiphilic polymers (APs) have broader pharmaceutical applications, such as drug solubilization and delivery, sustained release of encapsulated therapeutics, and

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suppression of multidrug resistance, due to their lower CMC values and better cytocompatibility. APs can self-assemble into micelles with a hydrophobic core for hydrophobic drug encapsulation and a hydrophilic shell for stability under physiological conditions.[3] Furthermore, due to the nanoscale size of polymeric micelles, the micellar aggregates tend to passively accumulate in solid tumors that have a defective lymphatic drainage system, a phenomenon known as the enhanced permeability and retention (EPR) effect.[4] Over the past decades, numerous polymeric micelles have thus been developed for hydrophobic drug delivery. For example, Pluronics, composed of hydrophobic poly(propylene oxide) and hydrophilic poly(ethylene oxide), are widely used as encapsulating polymers for hydrophobic drugs.[5] Other typical hydrophobic blocks are poly(esters), poly(amino acids), etc.[6,7] Chitosan, hyaluronic acid, and poly(vinyl alcohol) are alternative hydrophilic polymers to poly(ethylene oxide).[5−10]

In this Account, we present the biomedical applications of APs in the context of one polymer family, in which the APs are derived from natural sources such as sugars and lipids. Given that APs have been well reviewed,[8−13] we will focus on the unique aspects of this particular, sugar-based AP family, particularly as a drug nanocarrier and polymer therapeutic for atherosclerosis treatment.

AMPHIPHILIC POLYMERS

In contrast to many traditional APs (e.g., Pluronics), in which hydrophobic and hydrophilic *polymers* are tethered together, sugar-based amphiphilic polymers (SBAPs) synthesized by Uhrich et al.[14,15] have a smaller molecular weight hydrophobic component conjugated to a polymeric hydrophile. The hydrophobic segment of SBAPs is an alkylated sugar backbone, while a poly(ethylene glycol) (PEG) chain serves as the hydrophilic domain (Figure 1).[14,15] When micellized, the sugar-based segment provides a hydrophobic core to solubilize hydrophobic drugs, and the hydrophilic PEG shell can reduce SBAP interactions with the reticuloendothelial system (RES), prolonging the blood circulation time of SBAPs.[14,16] The original series of SBAPs are referred to as M*x*P*y*, in which M denotes a mucic acid backbone, *x* denotes each alkyl chain's total number of carbon atoms, P denotes PEG, and *y* refers to the PEG molecular weight in kilodaltons. Previous studies have shown that when the PEG molecular weight constant is kept constant, increasing the hydrophobic alkyl chain length decreases the CMC value. We concluded that alkyl chain length is a key factor influencing CMCs of SBAPs, with M12P5 showing the lowest CMC value (1.25×10^{-7} M) among the original SBAPs (Table 1).[12] We hypothesized that increasing the length and number of alkyl chains would enhance the hydrophobic interactions between unimers, leading to a larger entropic increase upon micellization and, subsequently, a lower CMC value. Lower CMCs typically indicate lower micelle dissociation rates, which lead to higher stability of hydrophobic drugs in the micelle core. Therefore, M12P5, with the lowest CMC value, was further studied for drug delivery applications.

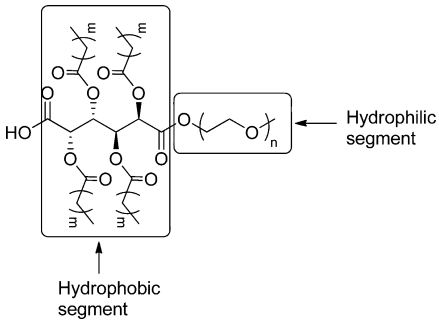

Figure 1. Chemical structure of mucic acid-based SBAPs with hydrophobic segment (alkylated mucic acid) and hydrophilic tail (PEG).

Table 1. Molecular Weights, Melting Temperatures, and CMCs of Original M*x*P*y*-Based SBAPs[15] and Their PEG Tails

sample	MW (Da)	T_m (°C)	CMC (M)
P2	2000	49.0	*a*
M6P2	2400	45.5	4.45×10^{-5}
M8P2	2500	46.9	4.76×10^{-6}
M10P2	2600	45.2	2.60×10^{-6}
M12P2	2800	45.4	1.27×10^{-6}
P5	5000	58.5	*a*
M6P5	5300	59.3	8.64×10^{-5}
M8P5	5400	57.1	7.87×10^{-6}
M10P5	5500	57.8	1.20×10^{-6}
M12P5	5900	56.4	1.25×10^{-7}

[a]Not applicable.

NANOMICELLES: SBAPs IN CANCER THERAPY

Various chemotherapeutic drugs are insoluble in aqueous media and thus difficult to effectively administer.[13] To successfully deliver hydrophobic therapeutics to target sites, numerous delivery strategies have been developed. Polymeric micelles are an important delivery system class with small size, extended release profile, good colloidal stability, and low cytotoxicity.[3,17,18] Because successful drug delivery usually requires efficient cellular internalization, the intracellular fate of SBAP micelles was assessed by incubating fluoroscein isocyanate (FITC) labeled-M12P5 (Figure 2A) with human umbilical vein endothelial cells (HUVECs). Confocal microscopy revealed that M12P5 micelles were internalized within the cytoplasm, endosomes, lysosomes, and nucleus within 60 min (Figures 2B−E).[19] The rapid internalization of the micelles indicates that SBAPs could be efficient transporters for both nucleus- and cytoplasm-targeted drugs.

To then evaluate SBAPs as hydrophobic drug carriers, we incorporated doxorubicin (DOX) and camptothecin (CPT) into the M12P5 micelle core.[20,21] The drug weight loadings were 12% and 0.5%, respectively, which may be due to the different hydrophobicities of the drugs. Although the CPT weight loading was rather low (0.5%), it was still comparable to that of commercially available polymers such as Pluronic P85 (0.7%) and Kolliphor EL (0.6%).[21] Sizes of the drug-loaded micelles, ranging from 16 to 25 nm, demonstrated that these systems maintained an appropriate size, which can evade renal clearance, penetrate tumor tissue, and avoid uptake by the RES.[13] Release studies demonstrated that drug release from M12P5 micelles was sustained for more than 2 days. Furthermore, no significant cytotoxicity of the unloaded micelle against HUVECs was detected.

In addition to physically encapsulating drugs within micelles, another approach in cancer therapy involves chemically conjugating therapeutics to polymers. Chemically conjugating

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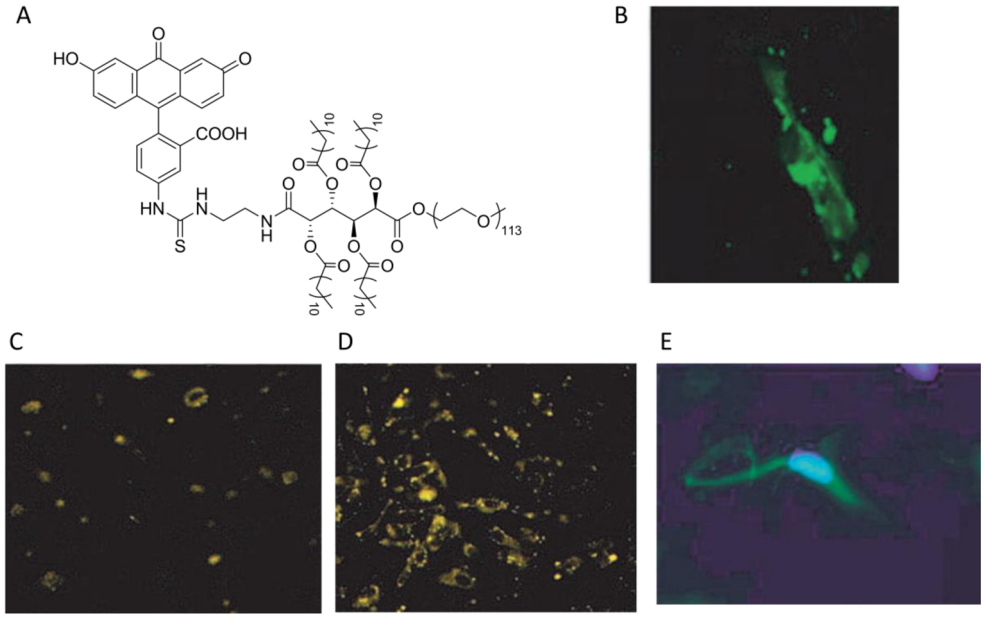

Figure 2. (A) Structure of FITC-labeled M12P5, (B) localization of SBAP (green) in cytoplasm, and colocalization of SBAP (FITC, green) and (C) endosome (Texas-Red, red), (D) lysosome (LysoTracker, red), and (E) nucleus (DAPI, blue).[19]

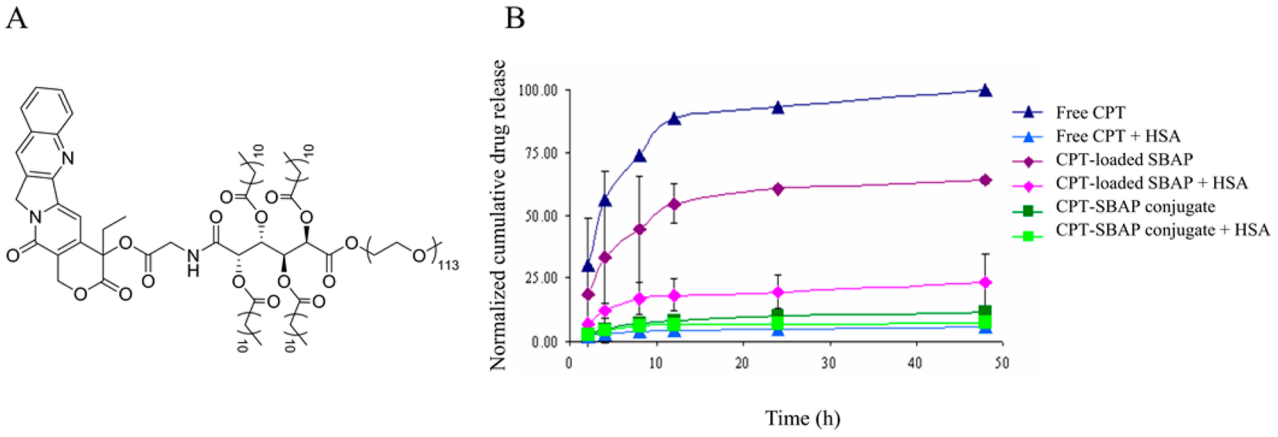

Figure 3. (A) Structure of CPT-conjugated M12P5 via glycine linkage and (B) CPT release from free CPT (▲), CPT-loaded micelles (◆), and CPT-conjugated micelles (■) in the absence (dark blue, dark pink, and dark green) and presence (light blue, light pink, and light green) of HSA.[21]

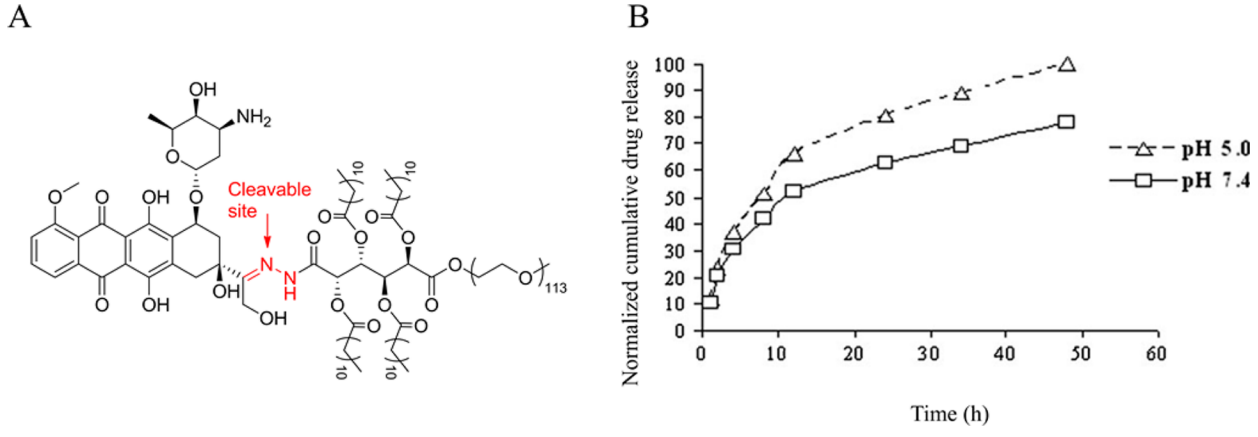

Figure 4. (A) Structure of DOX-conjugated SBAP via the cleavable hydrazone linkage and (B) drug release profile of DOX−SBAP conjugate at two relevant pHs: physiological (□) and acidic (△).[20]

drugs to the hydrophobic core of the amphiphiles has advantages, including enhanced drug loading, improved drug protection, and increased size resulting in slower renal excretion and longer blood circulation time. Several different approaches

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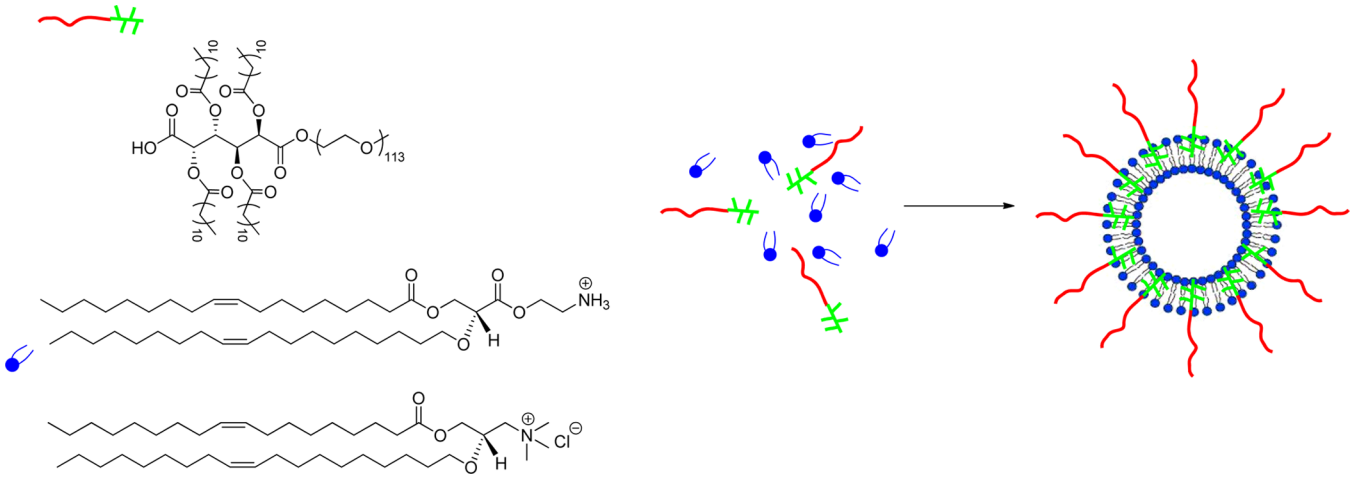

Figure 5. Chemical structures of M12P5 (top left)and lipids (DOPE and DOTAP, bottom left) and graphical illustration of SBAP–lipid complex (right)[22]

were developed to obtain drug-conjugated SBAPs as pro-drug micelles. With glycine as a linker molecule, CPT was conjugated to the carboxylate group of M12P5 (Figure 3A).[21] Because CPT can be converted from the active lactone form to an inactive carboxylate form under neutral and basic conditions and in the presence of human serum albumin (HSA), chemically conjugating CPT to a SBAP will further enhance its encapsulation and lactone stability compared with physical encapsulation within micelles. The chemically conjugated CPT–SBAP micelle showed a higher weight loading (3%) than the physically encapsulated CPT micelle (0.5%). In the presence of HSA, the release of free CPT (i.e., no SBAP system present) significantly decreased. Because our assay only detected the active form of CPT, this decrease indicated that the presence of HSA converted the active lactone form to the inactive CPT form. When CPT was physically encapsulated within M12P5 micelles in the presence of HSA, an enhanced release (23%) at 48 h compared with that of free CPT (6%) was observed (Figure 3B). Release from the conjugated CPT–SBAP micelle in the presence of HSA was less than that of CPT-loaded micelles at 48 h, likely due to the slower ester hydrolysis rate, yet shows no significant difference compared with free CPT (Figure 3B). While CPT-conjugated micelles showed increased drug loading, their ability to protect CPT remains inconclusive. CPT-loaded micelles, however, showed enhanced protecting abilities against HSA compared with CPT alone.[21]

By a different approach, DOX was chemically conjugated to the hydrophobic segment of M12P5 via a hydrazone linkage to yield a DOX–SBAP pro-drug micelle (Figure 4A).[20] We hypothesized that due to the pH-sensitive nature of the degradable hydrazone linkage, the DOX–SBAP pro-drug micelle would yield a pH-dependent DOX release profile. DOX release from the conjugate micelle reached 100% at pH = 5 compared with 78% at pH = 7.4 (Figure 4B). This enhanced DOX release at pH = 5 was attributed to the cleavage of hydrazone in the DOX conjugate. Further studies indicated that the DOX-conjugated SBAP was more potent than free DOX and physically encapsulated DOX against human liver cancer cells after 72 h. The DOX–SBAP conjugate was likely internalized by the cell, and this micellar system then released DOX within the cell's acidic environment leading to an enhanced efficacy in cancer cells.[20]

M12P5 micelles exhibit great potential as hydrophobic drug delivery systems. Their low CMC values and hydrophobic core enables the physical encapsulation of various drugs. Furthermore, while only CPT- and DOX-conjugated systems were explored, the presence of a carboxylic acid on the hydrophobic segment of M12P5 provides options for chemical conjugation of various anticancer drugs.

LIPOSOMES: STABILIZATION AND DRUG DELIVERY

Aside from polymeric micelles with a core/shell structure, liposomes are another important delivery vehicle composed of bilayer membranes. Under physiological conditions, however, liposomes can undergo aggregation, fusion, and flocculation due to interactions with serum proteins. To circumvent this colloidal instability, we hypothesized that SBAPs could stabilize liposomes by providing a steric PEG barrier to prevent serum protein binding.[22] M12P5 was therefore incorporated into heterogeneous liposomes containing a 1:1 weight ratio of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (DOTAP) (Figure 5) via the hydrophobic interactions between the branched alkyl chains of M12P5 and the aliphatic tails of the lipids. To investigate the thermodynamic interaction between SBAP and lipid, Langmuir film balance (LFB) was used to monitor the compression isotherm of a monolayer comprised of different compositions of M12P5 and lipid. The attractive intermolecular interaction between SBAP and lipid was illustrated by the observed condensation effect, in which the experimental molecular area negatively deviates from the ideal molecular area (i.e., no intermolecular interaction). These results indicated that SBAPs and lipids formed a stable mixture.[23] To evaluate how serum impacts the size of SBAP–lipid complexes, dynamic light scattering (DLS) was used to monitor the complexes' sizes over time. Complexes with low SBAP content underwent visible aggregation upon serum addition. With increasing SBAP content, however, the SBAP–lipid complexes exhibit enhanced tolerance to serum-induced aggregation.[22]

SBAP–LIPID COMPLEXES FOR CANCER THERAPY

Given the hydrophobic layer within liposomes, we investigated SBAP–lipid complexes for hydrophobic anticancer drug

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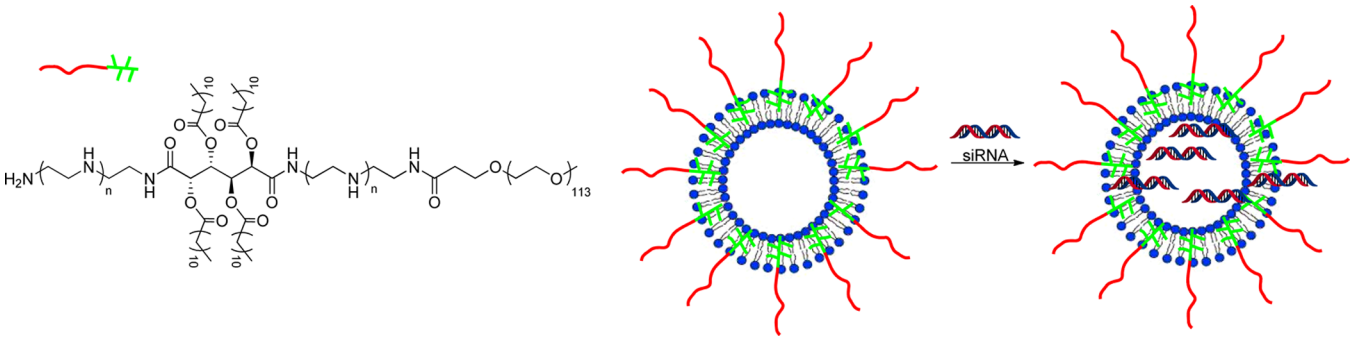

Figure 6. Structure of oligoethylenimine-modified SBAP (left) and graphical illustration of siRNA/SBAP–lipid complex formation (right).[31]

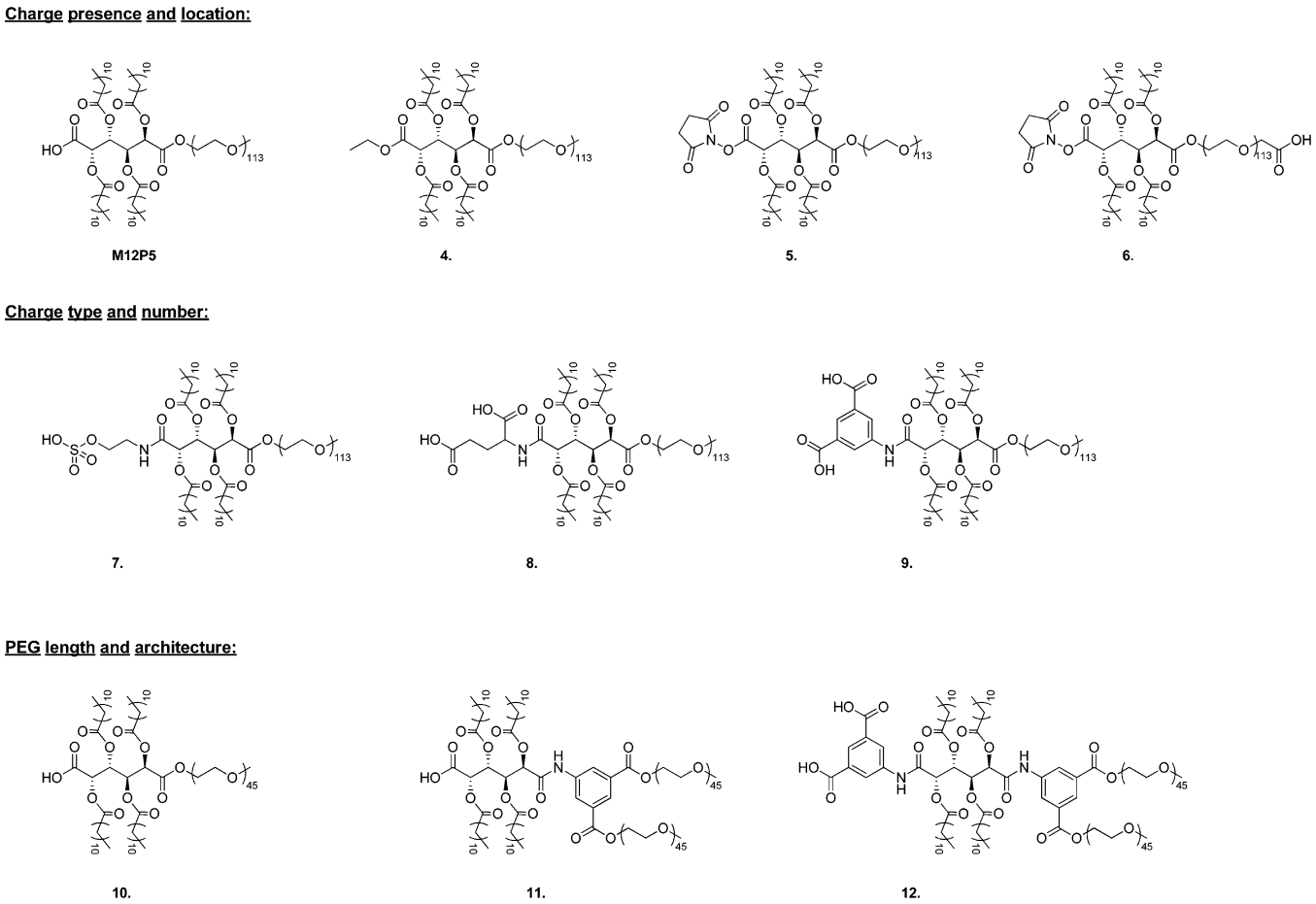

Figure 7. Chemical structures of SBAPs with systematically varied charge and PEG features.[43,44]

delivery.[22] Because intracellular uptake is a key step for drug delivery, we studied the cell uptake of the SBAP–lipid complexes. While evaluating this cell uptake, we observed preferential uptake in carcinoma cells compared with fibroblast cells. Instead of using a targeting motif to obtain preferential carcinoma cell uptake, the SBAP–lipid complex provided a unique passive cell targeting system in heterogeneous carcinoma cultures, possibly due to the unique structures of the SBAP–lipid complex. In-depth investigations are needed to illustrate the cell targeting mechanism of these complexes. Because the hydrophobic bilayer of the complex provides a compartment for hydrophobic drugs, paclitaxel (PTX) was encapsulated in the SBAP–lipid complex. PTX was chosen as the anticancer drug because, while highly potent, its formulation for delivery remains a challenge. Clinically, Kolliphor EL and Abraxane are used as PTX formulations.[21] Kolliphor EL suffers serious side effects including neurotoxicity, nephrotoxicity, and hypersensitivity due largely to the Kolliphor EL component.[24] Abraxane is an albumin-bound PTX nanoparticle, which is formulated in a solvent-free fashion and eliminates solvent-associated toxicity;[25] however, Abraxane exhibits rapid elimination of PTX from the blood.[22] In our studies, incorporating SBAP did not affect the encapsulation efficiency compared with the PTX-loaded liposomes. Cytotoxicity studies using the carcinoma cells indicated that PTX-loaded SBAP–lipid complexes showed similar efficacy to PTX-loaded liposomes alone.[22] Given their preferential uptake by carcinoma cells and inherent biocompatibility, SBAP–lipid complexes are promising carriers for anticancer drugs and the subject of further investigations.

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SBAP–LIPID COMPLEXES FOR GENE THERAPY

Because SBAP–lipid complexes have demonstrated efficient cellular uptake, we investigated other intracellular environment-targeted drugs such as small interfering ribonucleic acid (siRNA). Since the discovery of RNA interference, siRNA has been widely viewed as a promising gene-based therapeutic approach for many diseases.[26] Due to the anionic nature of siRNA and the presence of RNases in the bloodstream, naked siRNA delivery is limited by inadequate cellular uptake and poor stability under physiological conditions. Therefore, improved efficiency of siRNA delivery vehicles is necessary.[27−29] To address this need, novel SBAPs were synthesized with linear ethylenimines to obtain cationic SBAPs capable of complexing with and delivering siRNA (Figure 6, left).[30] Moderate transfection efficacy with low cytotoxicity was shown when SBAPs were used as the siRNA carrier. To further improve the transfection efficacy, cationic SBAP–lipid complexes were designed, given that SBAPs can stabilize liposomes and cationic lipid head groups can provide enhanced siRNA protection (Figure 6, right).[31] Because both the SBAPs and lipids possessed cationic groups, LFB was used to confirm the net attractive interactions between the cationic SBAPs and lipids. Isothermal titration calorimetry demonstrated that SBAP–lipid complex formation is a thermodynamically favorable process. A significantly higher transfection efficacy compared with the commercial gold standard, Lipofectamine, was demonstrated when SBAP–lipid complexes were used with 1:10 weight ratios. Further studies showed that siRNA bound favorably to the complexes at pH 7.4 in contrast to the minimal binding of siRNA at pH 5.[31] This data indicated that the SBAP–lipid complexes protect siRNA at physiological pH but undergo a physicochemical change at pH 5 to release siRNA. These results suggest that SBAP–lipid complexes can serve as potent siRNA carriers with tunable release.

SBAPs AS THERAPEUTICS

Besides their promising drug delivery applications, SBAPs also demonstrate capabilities to manage atherosclerosis. Atherosclerosis, a major cause of cardiovascular diseases, is characterized by inflammatory responses that lead to plaque formation in the arterial walls.[32−34] This inflammation results from increased levels of oxidized low-density lipoproteins (oxLDL) in the vascular intima. Accumulation of oxLDL triggers monocyte recruitment, differentiation into macrophages, and the subsequent upregulation of macrophage scavenger receptors, specifically scavenger receptor A (SRA) and cluster of differentiation 36 (CD36).[35−40] Through SRA and CD36, macrophages rapidly uptake oxLDL and this uptake stimulates inflammatory cytokine secretion and additional monocyte recruitment. Uncontrolled oxLDL uptake and intracellular accumulation leads to the formation of lipid-filled foam cells that eventually form arterial plaque, narrowing blood vessels and restricting blood flow.

While investigating SBAPs as delivery systems for atherosclerosis treatments, we discovered that SBAPs have a unique ability to intervene in the atherosclerotic cascade. As such, we explored the SBAP's ability to inhibit oxLDL uptake by macrophages, using IC21 macrophages and fluorescently labeled oxLDL.[41,42] Comparison of anionic and neutral SBAPs (Figure 7, M12P5 and **4**, respectively) indicated that anionic charge is essential for activity. Investigations using antibody blocking assays indicated that carboxylate-terminated SBAPs (M12P5) competitively block SRA and CD36, while their neutral analogs (**4**) do not. Given that inflammatory cytokine secretion and foam cell formation lead to atherogenesis, we next investigated the effect of SBAPs on atherogenic pathways by cell morphology observations and TNF-α secretion studies. Anionic SBAP, M12P5, was found to significantly reduce cholesterol accumulation, foam cell formation, and consequent TNF-α secretion.[41] SBAPs thus differ from current atherosclerosis therapies, which rely on lowering blood cholesterol levels through sequestration or inhibition of cholesterol synthesis, yet fail to alter or reverse inflammatory reactions. In contrast, the SBAPs inhibit oxLDL uptake and essentially prevent the atherosclerotic cascade.

SBAP STRUCTURE–ACTIVITY RELATIONSHIPS IN oxLDL UPTAKE

To determine which SBAP structural components are necessary for oxLDL uptake inhibition, novel SBAPs were synthesized, and their bioactivity (namely, inhibition of oxLDL uptake) was assessed under physiological conditions.[43] Within these initial studies, six structural elements were varied including type of anionic charge, location of anionic charge, number of anionic charges, rotational freedom of the anionic charge, PEG length, and PEG architecture (Figure 7). Based on previous findings that suggested anionic charge can facilitate oxLDL uptake inhibition, we hypothesized that increasing the net anionic charge within the hydrophobic domain would enable stronger SBAP interactions with scavenger receptor binding domains. Further, we anticipated that two short-chain linear PEG tails would better shield SBAPs from serum proteins, thus enhancing their stability under physiological conditions.

In comparing the various SBAPs synthesized (Figure 7), we was ascertained that SBAPs with one, rotationally restricted carboxylic acid on the their hydrophobic portion (i.e., M12P5) enabled effective inhibition of oxLDL uptake. Decreased efficacy was observed when the anionic charge was placed on the hydrophilic PEG tail (Figure 7, **6**), indicating that this negatively charged group was not well shielded from serum proteins. The improved efficacy of the carboxylic acid over a sulfate group (Figure 7, **7**) may result from the sulfate group's decreased steric hindrance around the anionic charge, which could promote hydrogen-bonding interactions with areas remote from the active site. Given that increasing the net anionic charge (Figure 7, **8** and **9**) did *not* improve SBAP bioactivity, it is plausible that only one anionic charge can interact with the scavenger receptor active site at a time. Further, restriction of the rotational motion of this charged group (Figure 7, M12P5 or **9**) may result in improved binding to the active site. While PEG length and architecture (Figure 7, **10**−**12**) did not significantly impact oxLDL uptake inhibition, a 5000 MW PEG tail appeared to sterically stabilize SBAPs in the presence of serum proteins. Finally, in comparing M12P5 to control molecules (Figure 8), including an anionic PEG derivative (PEG-COOH) and commercially relevant amphiphiles (Kolliphor EL and Pluronic P85), we find that SBAPs' branched hydrophobic domain is the key structural component that significantly influences bioactivity.[43]

Because initial SAR studies suggested that the branched hydrophobic domain of SBAPs was necessary to inhibit oxLDL uptake, further SAR studies focused on systematically varying this backbone architecture. This aspect was investigated through modifying the sugar-based backbone to investigate how stereochemistry and backbone conformation (i.e., linear,

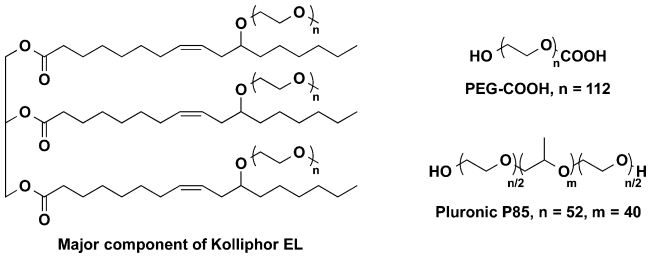

Figure 8. Chemical structures of control molecules, Kolliphor EL, PEG-COOH, and Pluronic P85.

cyclic, or aromatic) influenced biological activity (Figure 9).[45] By alteration of one of M12P5's stereocenters, a saccharic acid-based SBAP (Figure 9, **13**) was synthesized that produced micelles of a similar hydrodynamic diameter and CMC value to M12P5 itself. Despite these similarities, however, both modeling and *in vitro* studies indicated that **13** was not nearly as effective at associating with SRA and, subsequently, inhibiting oxLDL uptake (Figure 9, right). Using an aromatic backbone (Figure 9, **14**) yielded SBAPs that formed larger micelles yet had a similar CMC and bioactivity to M12P5, further suggesting that structurally rigid SBAPs effectively inhibit oxLDL uptake. With a cyclic core (Figure 9, **15**), SBAPs formed micelles with hydrodynamic diameters similar to **14** but with higher CMCs, indicating lowered stability under physiological conditions. These uncharged cyclic analogs were not as effective at inhibiting oxLDL uptake, which may have resulted from their lack of anionic charge, higher CMCs, or different spatial orientation. This study on backbone architecture demonstrated that small changes in the hydrophobic domain, such as a single stereocenter, can significantly impact biological efficacies of SBAPs.[45]

To better understand how changes in the hydrophobic domain may impact SBAP physicochemical and biological properties, novel SBAPs were synthesized with varied lipophilicity and stereochemistry (Figure 10).[46] Because hydrophobic interactions significantly contribute to protein−polymer complexation, it was hypothesized that increasing SBAP lipophilicity would yield SBAPs that better prevented oxLDL uptake. To test this concept, SBAPs based on an L-tartaric acid (L-TA) sugar backbone (Figure 10, **16**) were synthesized, and their lipophilicity was increased by coupling two acylated sugar backbones together or growing dendrons from the L-TA backbone. In addition to investigating the L-TA-based SBAPs, a meso analog (Figure 10, **17**) was also evaluated; this exhibited similar stereochemistry but decreased lipophilicity compared with M12P5. It was ascertained that the overall lipophilicity, including the number of hydrophobic arms and the length of the hydrophobic domain, impacts the hydrodynamic diameters of the micelles, while both lipophilicity and stereochemistry influence SBAP self-assembly and, in turn, CMC values. Furthermore, it was demonstrated that **17**, having decreased hydrophobicity compared with M12P5 and analogous stereochemistry, exhibited the highest degree of oxLDL uptake inhibition (Figure 10, right). Given that L-TA-based SBAPs were not as biologically efficacious, these findings suggest that stereochemistry, not lipophilicity, significantly impacts the ability of SBAPs to block scavenger receptors.[46]

SBAP-BASED NANOPARTICLES TO ENHANCE SERUM STABILITY

The SBAP applications discussed thus far have involved thermodynamic assemblies, including polymeric micelles. When SBAP concentrations exceed their CMC, SBAPs spontaneously self-assemble into such micelles.[12,47] SBAP micelles can be beneficial delivery systems because their hydrophilic shell helps prevent nonspecific protein adsorption while their nanoscale size can reduce renal clearance and RES uptake.[48,49] Furthermore, SBAPs administered in this micelle form have demonstrated the ability to inhibit oxLDL uptake by macrophages, indicating their potential use as antiatherosclerotic therapies. Despite these advantageous properties enabling SBAPs to serve as both bioactives and delivery systems, research has suggested that polymeric micelle stability can be compromised in physiological environments.[50−52]

When administered to an *in vivo* environment or exposed to physiological conditions, polymeric micelles can undergo

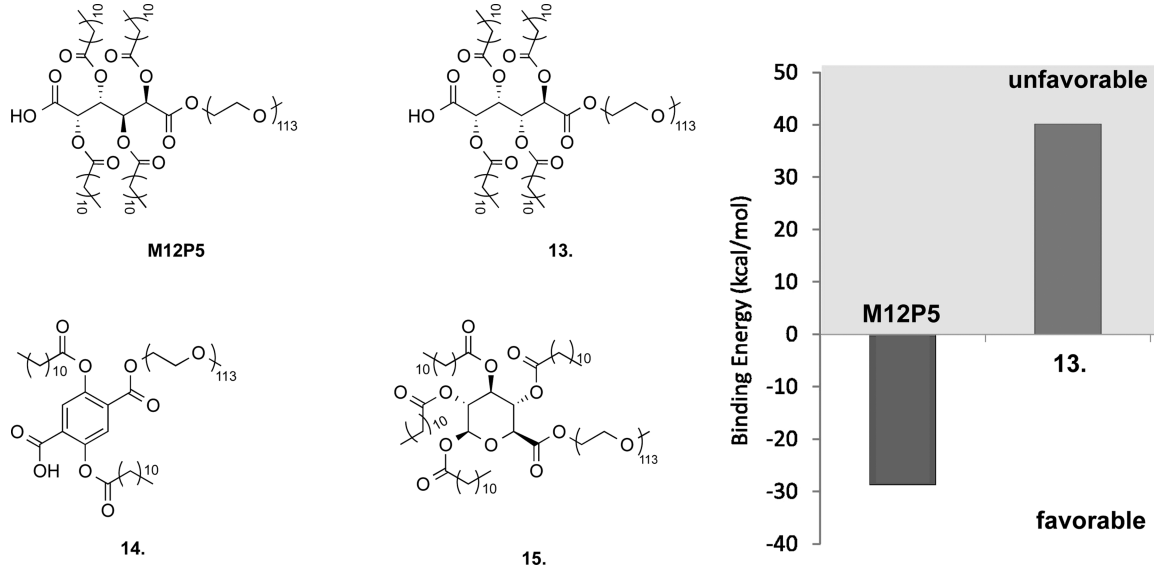

Figure 9. Chemical structures of SBAPs with altered sugar backbone architectures (left) and key results of modeling studies, indicating binding energies between SBAPs and SRA (right)[45]

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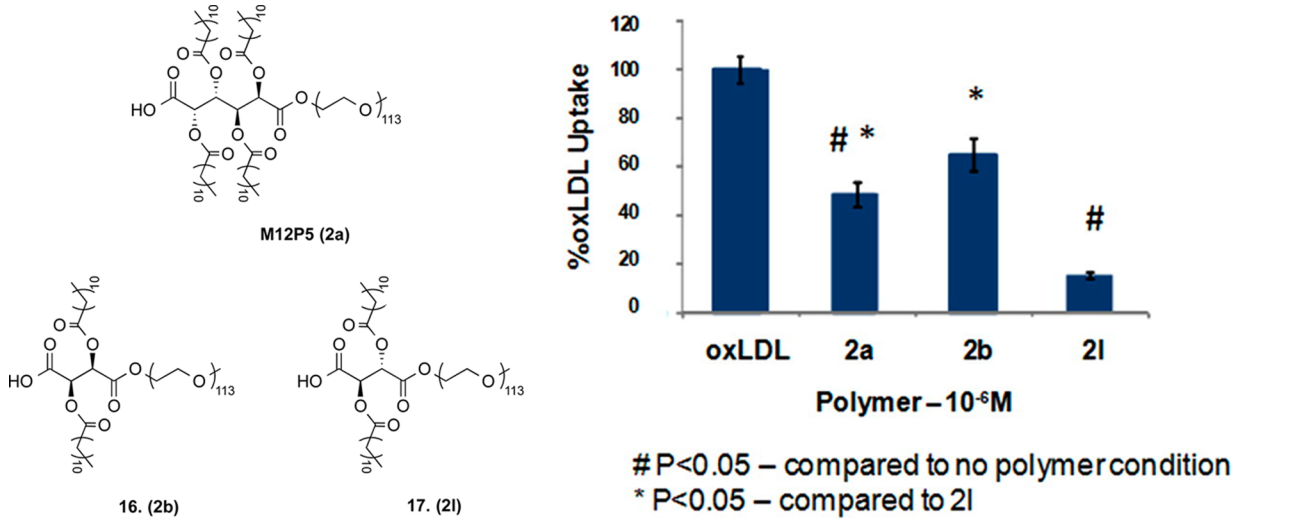

Figure 10. Chemical structures of SBAPs with variable stereochemistry and lipophilicity (left) and their corresponding inhibition of oxLDL uptake (right). Reproduced from ref 46. Copyright 2013 American Chemical Society.

dilution to concentrations below their CMC, resulting in gradual micelle dissociation.[48,51] This disintegration may prevent polymeric micelles from reaching their target sites and reduce their biomedical efficacies. In addition to instability associated with *in vivo* dilution, polymeric micelles, regardless of their concentration, can lose their integrity in the presence of serum components.[49−53] Studies have indicated that lipophilic serum proteins often extract the micelle's hydrophobic solutes or the hydrophobic segment of the unimer itself, resulting in micelle dissociation and subsequently limiting clinical applications of polymeric micelles.[50,51,54,55] Thus, while SBAP micelles have exhibited promise for drug delivery and atherosclerotic treatment applications, their dynamic nature could compromise the system's stability in physiological environments.[52] Further, because SBAP unimers are comprised of ester bonds, their stability in the presence of esterases under physiological conditions is a concern. Preliminary *in vitro* studies indicated that M12P5 undergoes rapid cleavage of PEG in the presence of lipase, while **11** (Figure 7) undergoes cleavage of alkyl chains first followed by the cleavage of PEG.[56] Given the dynamic equilibrium of these micellar systems, we hypothesize that enzymes can easily interact with SBAP unimers and subsequently cleave the ester bonds.

To overcome stability issues associated with micelle systems and potentially minimize SBAP accessibility to esterases, we formulated kinetically trapped SBAP-based nanoparticles (NPs).[52] These NPs were generated using a flash nanoprecipitation (FNP) method, in which an aqueous-miscible organic stream containing SBAPs and hydrophobic core solutes was rapidly mixed with an aqueous stream (Figure 11). As the two streams mix, the aqueous solution becomes supersaturated, resulting in the formation of core−shell NPs comprised of SBAP shells and hydrophobic cores. All SBAP-based NPs exhibited uniform particle sizes ranging from 150 to 200 nm,[52] within the ideal size range for enhanced bloodstream stability.[48]

SBAP-based NPs were next assessed for their stability and bioactivity. After incubation in the presence of serum proteins, NPs exhibited constant particle sizes, suggesting their ability to withstand dissociation and aggregation. In comparing SBAP release from micelle and NP formulations in serum, NPs retained a significantly higher quantity of SBAP compared with micelles (Figure 12). This SBAP retention demonstrates

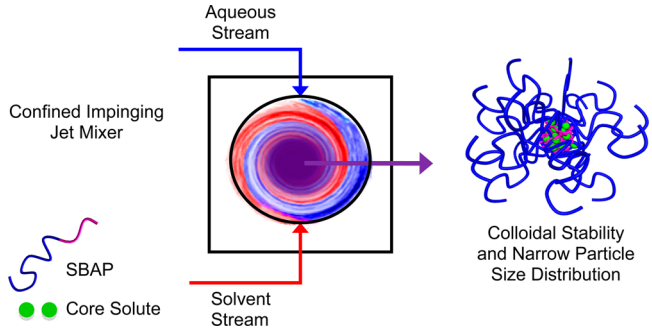

Figure 11. Schematic showing FNP methodology used to formulate SBAP-based nanoparticles.[52]

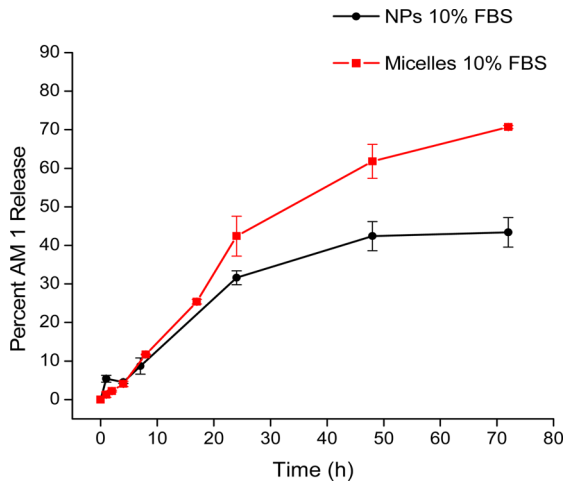

Figure 12. SBAP retention of NP and micelle systems under serum conditions (10% fetal bovine serum, FBS).[52]

resistance of the NPs to unimer extraction by serum components and enhanced stability under physiological conditions.[52] Because the success of colloidal carriers depends on their ability to remain intact until they reach a specific target site,[50] this improved stability could benefit both drug delivery and diagnostic applications. Previous research has shown that hydrophobic fluorophores, drugs, peptides, and inorganic

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species can be encapsulated using the FNP method.[57] Drug molecules or imaging agents could, therefore, serve as the hydrophobic solutes during NP fabrication to generate SBAP-based NPs for therapeutic, diagnostic, or theranostic applications.

While stable, cytocompatible SBAP-based NPs could be used to deliver hydrophobic drugs and imaging agents, they may also retain the antiatherosclerotic activity of SBAP micelles. Consequently, we investigated their inherent bioactivity for atherosclerotic treatment applications.[52] In comparison of the oxLDL uptake inhibition of NP and micelle systems, NP systems showed enhanced retention of bioactivity as serum concentrations were increased, likely due to reduced instances of unimer extraction. Furthermore, under similar conditions, NPs exhibited a significant reduction in foam cell formation whereas micelle systems exhibited no such reduction.[52] *In vivo* experiments also indicated that SBAP-based NPs have a long half-life (29 h) and show localization in the atherosclerotic lesions of a diseased mouse model.[53] The enhanced NP bioactivity in the presence of serum, in conjunction with improved stability of NPs over micelle systems and localization at atherosclerotic lesion sites *in vivo*, makes SBAP-based NPs viable and novel atherosclerotic treatments. By maintaining their efficacy under physiological conditions, SBAP-based NPs show promise in drug delivery, diagnostic, and cardiovascular applications.

CONCLUSION

APs are polymeric surfactants with hydrophilic and hydrophobic domains that self-assemble into a variety of structures useful for various biomedical applications. SBAPs synthesized by Uhrich et al., consisting of a branched sugar-based hydrophobic domain conjugated to a hydrophilic PEG tail, constitute one family of APs. Given their ability to spontaneously self-assemble into polymeric micelles with low CMC values, SBAPs were initially investigated as nanoscale carriers for drug delivery applications. SBAP micelles, having a hydrophobic core, can solubilize water-insoluble chemotherapeutics through physical encapsulation or chemical conjugation techniques, enabling tunable drug release rates. SBAPs can also spontaneously incorporate into liposomes, improving colloidal stability of the liposomes through steric shielding. While investigating SBAPs for drug delivery applications, we discovered that SBAP micelles possess a unique ability to intervene in the atherosclerotic cascade through binding to macrophage scavenger receptors and preventing oxLDL uptake. To ascertain which SBAP structural features were critical to SBAP bioactivity, SBAP chemical structures were systematically modified, and their bioactivity (specifically, ability to hinder oxLDL uptake) was studied. Although SBAP micelles are promising antiatherosclerotic bioactives, their poor serum stability could hamper their clinical use. Recent work demonstrates that formulating SBAPs into kinetically trapped nanoparticles enhances stability under physiological conditions, maintains bioactivity for atherosclerotic applications, and retains ability to encapsulate additional hydrophobic therapeutic or diagnostic agents. Given SBAPs' promise as delivery systems and bioactives, future research aims to extend SBAP applications through solving biomedical problems.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

Biographies

Li Gu received his B.S. in Chemistry from Peking University in 2009. He earned his Ph.D. degree in the Department of Chemistry and Chemical Biology (CCB) at Rutgers University. His Ph.D. research focused on developing pH-responsive amphiphilic macromolecules for drug delivery applications. He is now a postdoctoral associate in the Hammond group at MIT.

Allison Faig is a Ph.D. candidate in the Department of CCB at Rutgers University. She received her B.S. from Wake Forest University in 2011. Her research interests include the design and synthesis of novel, bioactive amphiphilic macromolecules.

Dalia Abdelhamid is an assistant professor at the College of Pharmacy, Minia University, Egypt. She received her B.S. from Cairo University in 2000 and her Ph.D. from The Ohio State University in 2011. Thereafter she worked as a postdoctoral fellow under Dr. Kathryn Uhrich at Rutgers University. Her research interests encompass organic synthesis of natural products and chemical modification of drug leads to establish structure−activity relationship studies, which guide the development of safer and more potent drugs.

Kathryn Uhrich is a professor in the Department of CCB at Rutgers University. She received her B.S. from the University of North Dakota in 1986 and her Ph.D. in Chemistry from Cornell University in 1992, working under Dr. Jean M. J. Fréchet. Following her Ph.D. studies, she worked as a postdoctoral fellow under Dr. Robert Langer at the Massachusetts Institute of Technology, studying biodegradable biomaterials. Dr. Uhrich's research interests are broadly in the areas of polymeric biomaterials for medical applications.

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