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Recent developments in micellar drug carriers featuring substituted poly(ε-caprolactone)s

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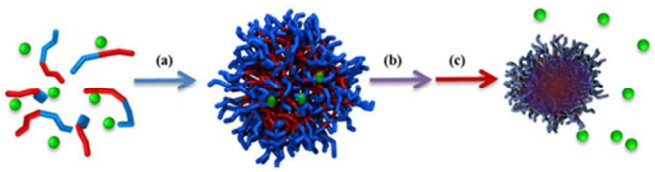
In the field of drug delivery, synthetic polymers have been widely explored due to their range of properties and functions achievable by tuning their structures. Poly(ε-caprolactone)s in particular have established themselves as excellent candidates for biomedical applications because of their biocompatibility, biodegradability, and synthetic versatility. In this review, applications of functional poly(ε-caprolactone)s in drug delivery systems are highlighted. Recent studies regarding the encapsulation or direct conjugation of drugs, bioactive molecules and moieties for targeting are discussed. Also considered are advances in amphiphilic polymers with functional poly(ε-caprolactone)s that exhibit stimuli-responsive behavior: pH-, thermo-, photo-, and reduction-sensitive. Ongoing research and development of functional poly(ε-caprolactone)s continues to expand their potential for use in micellar drug delivery systems.

Introduction

The primary objective of drug delivery systems is to improve the bioavailability of drugs at the target site.[1-3] Pharmaceutical formulations for oral or intravenous delivery, transdermal patches, novel designs for inoculations, and implantable devices for long-term delivery are a few examples of drug delivery systems.[2, 4] Polymers have demonstrated excellent utility in such biomedical applications due to their synthetic versatility.[1-3, 5] Applications in wound healing, tissue engineering, and drug delivery systems are but a subset of the biomedical technologies influenced by polymers.[1, 2, 6, 7] In the context of intravenous delivery of chemotherapeutic agents, drug carriers typically are designed to solubilize hydrophobic drug molecules, infiltrate the body with minimal immunogenic response, minimize drug loss from rapid clearance, target diseased tissues, and release the drug payload at its destination.[3, 5-14] In optimizing these drug carrier features, the negative side effects, such as damage to healthy tissues, associated with potent drugs may be curtailed.[2] Self-assembling polymers capable of encapsulating small molecules can overcome some of the challenges in chemotherapy applications.[1] By synthetic modification of their constituent polymer chains, polymeric assemblies may be tailored to address the aforementioned criteria and incorporate more specific features for the desired application.[1, 3, 15]

Synthetic polymers for such applications usually transport the drug molecules within self-assembled micelles or vesicles, illustrated in Scheme 1.[1, 3, 5] These polymeric materials must be biocompatible, i.e. non-toxic and non-immunogenic. In addition to biocompatibility, there is an emphasis on biodegradable materials, which break down over time into by-products that can be resorbed, metabolized, or excreted.[8, 16] Some of the



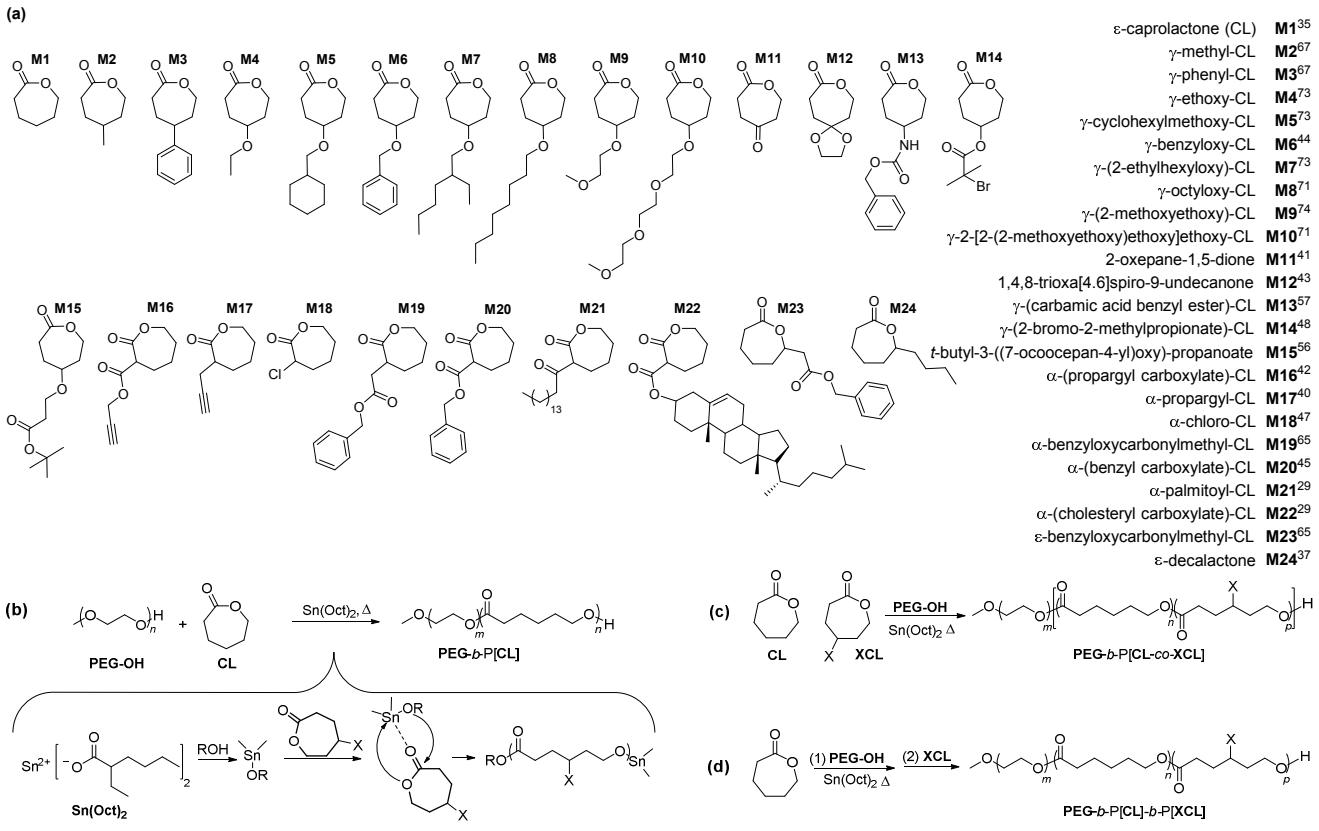
Scheme 1. Illustration of micellar drug delivery with amphiphilic block copolymers: (a) encapsulation of drug molecules by self-assembling amphiphilic block copolymers; (b) drug-loaded micelles administered to body, circulate throughout bloodstream, and accumulate at tumor site by active targeting and/or EPR effect; (c) drug release by disassembly or degradation of drug carriers, with or without stimulus.

most heavily studied biocompatible polymers for drug delivery systems are FDA-approved polyesters, poly(ethylene glycol) (PEG), and polyacrylamides.[14, 17-24] The micelle-forming block copolymers for drug delivery systems are comprised of hydrophilic and hydrophobic segments. These amphiphilic chains self-assemble in aqueous solution with the hydrophilic block forming the shell and the hydrophobic block forming to the core.[7, 15, 25] The micelle shell, often PEG-based, plays several roles; it improves the overall particle solubility, increases the circulation time of the micelle, and prevents its premature clearance and opsonization.[1, 25, 26] While the shell may be functionalized with targeting moieties, many micellar drug delivery systems exploit the enhanced permeability and retention (EPR) effect. Due to the EPR effect, particles of 10 – 200 nm tend to accumulate in tumor tissues, which typically have leaky vasculature and poor lymphatic drainage.[1, 3, 7, 14, 27] The hydrophobic micelle core, often polyester-based, houses the drug either by physical encapsulation or direct conjugation to the polymer. The hydrophobic nature of the core, along with its functional side groups, if any, can influence the micelle size, stability, and drug loading capacity.[1, 5, 14]

Due to their hydrolyzable backbones and in some cases, FDA approval, polyesters have attracted much attention in the past few decades of biomaterials research.[8, 18-24, 28-30] Among the poly(lactic acid)s (PLAs), poly(glycolic acid)s (PGAs), and poly(ε-caprolactone)s (PCLs), PCLs afford superior synthetic versatility because of the ease with which they may be substituted with functional groups. Thus, substituted PCLs continue to be explored for use in drug delivery systems.[5, 6, 21, 23, 24, 31-33] All-encompassing reports have been published on the synthesis of functional caprolactone monomers and polymers specifically for micellar drug delivery applications,[5] functional poly(ε-caprolactone)s for drug delivery in general,[34] and more broadly, unsubstituted poly(ε-caprolactone) formulations for drug delivery.[6] Highlighted in this review are advances in drug delivery systems featuring substituted caprolactones from the past five years.

Functional Caprolactones

Functional poly(ε-caprolactone)s can be synthesized by ring-opening polymerization (ROP) of substituted caprolactone monomers and/or post-polymerization modification.[5, 23, 34, 35] Shown in Scheme 2 (a) are functional caprolactone monomers, some of which feature protecting groups that could be removed after the polymerization to yield the desired functionality like hydroxy, carboxylic, and amino groups.[5] The typical route to poly(ε-caprolactone)s is the ROP of caprolactone monomer, often catalyzed by stannous 2-ethylhexanoate ($Sn(Oct)_2$), with an alcohol or other hydroxy-terminated initiator like α-methoxy-ω-hydroxy-poly(ethylene glycol) (PEG-OH), shown in Scheme 2 (b) and (c).[5, 8, 29, 34] Because of the *living* nature of the polymerization, block copolymers are achievable by sequential monomer addition as shown in Scheme 2 (d). Drug



Scheme 2. (a) Caprolactone (**M1**) and functional caprolactone (**M2** – **M24**) monomers; Ring-opening polymerization to generate diblock copolymers (b, c) and triblock copolymers (d) using PEG-OH initiator and $Sn(Oct)_2$ catalyst.

molecules, bioactive moieties, and stimuli-responsive groups can be coupled to polymer chain ends or the functional repeat units.[23] Of great interest are the effects of the hydrophilic/hydrophobic block ratios as well as the core/shell functionalities on the micelle's performance as a drug carrier.[14, 36] A measure of the thermodynamic stability of a micelle is the critical micelle concentration, or CMC.[15] The CMC is the concentration of amphiphilic polymer in water above which the free polymer chains assemble into micelles, typically in the range of 10^{-7} – 10^{-4} mol L^{-1}, or 10^{-4} – 10^{-1} g L^{-1}, for amphiphilic polymers.[1, 23] CMC values should be closer to 10^{-4} – 10^{-3} g L^{-1}, such that the micelles remain stable when diluted in the bloodstream, but not so low as to impede the release of the drug. To best exploit the EPR effect, the targeted micelle size should be 10 – 100 nm in diameter, though micelles up to 200 nm also have been reported.[1, 14] Improving the drug loading capacity of polymeric micelles, which depends on composition of both the micelle core and the drug itself, is an ongoing challenge in designing new drug delivery systems.[1]

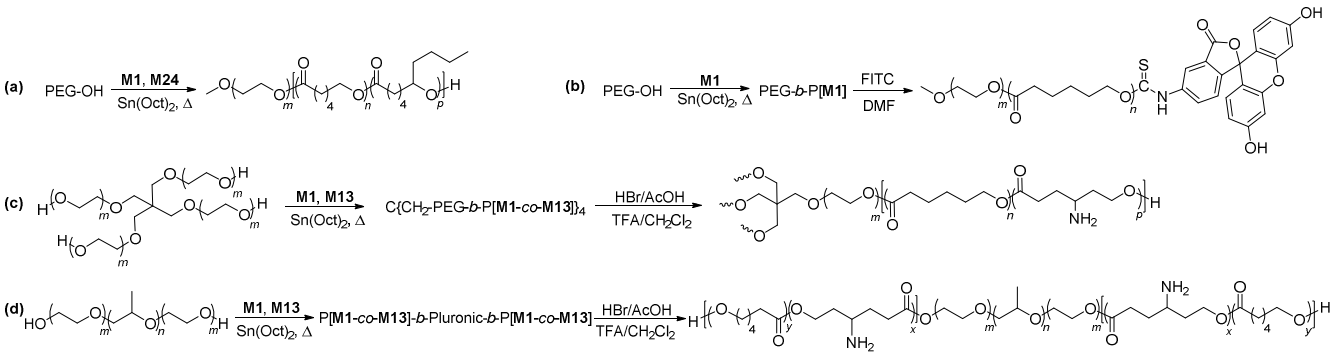
Structure-Property Relationships

The semicrystallinity of unsubstituted poly(ε-caprolactone) (P[**M1**]) has certain implications in the context of drug carrier applications.[14, 35] The effects of core crystallinity on P[**M1**]-based micelles' sizes and stability were examined by Glavas et al. in 2013.[37] By conventional ring-opening polymerization and copolymerization of **M1** and its more hydrophobic counterpart decalactone, **M24**, a series of block copolymers with varying crystallinity and hydrophobic block content were synthesized, shown in Scheme 3 (a). In this study, the polymers' CMCs decreased by increasing the core crystallinity rather than by increasing the hydrophobic ratio. A distinct relationship between micelle size and hydrophobic content was not observed.

The hydrophilic/hydrophobic ratio has been shown not only the micelle sizes and drug loading capacities, but also to affect cellular uptake of the micelles. Zhang et al. synthesized a series of PEG-*b*-P[**M1**] copolymers with molar ratios ranging from 1:1 to 1:4, shown in Scheme 3 (b).[36] In order to visualize and quantify the cellular uptake of these micelles, fluorescein isothiocyanate (FITC) was coupled to the hydrophobic end of the polymer chains. Both normal cells and tumor cells were treated with the PEG-*b*-P[**M1**]-FITC micelles. In general, the micelles with lower hydrophobicity were taken up faster. Additionally, by administering inhibitors for certain endocytic pathways, the mechanisms by which uptake occurred, such as clathrin-dependent or caveolae-mediated endocytosis, was dependent on the type of cell, HepG2 or HUVEC.

Motivated to design better carriers for gene delivery by improving the spatial charge density of cationic poly(ε-caprolactone)s, Fu et al. synthesized star-shaped block copolymers of featuring amino-functionalized caprolactone repeat units.[38] A series of star-shaped block copolymers was generated by the ROP of **M1** and γ-(carbamic acid benzyl ester)-ε-caprolactone, **M13**, with a four-armed PEG macroinitiator, shown in Scheme 3 (c). Following the polymerization, the **M13** repeat units were deprotected to generate the free amino groups. The zeta potential of the 73 nm diameter particles was as positive as 36 mV, indicating the star-shaped polymers' promising potential utility in gene delivery applications.

As reported by Zhang et al., pentablock copolymers comprised of Pluronic®, caprolactone, and amino-functionalized caprolactone demonstrated very low CMC values on the order of 10^{-4} g L^{-1}.[39, 40] These multiblock amphiphiles were synthesized using Pluronic® as the bifunctional macroinitiator in the ring-opening copolymerization of **M1** and **M13**. The protecting groups of **M13** were removed after polymerization to generate the amine-functionalized caprolactone units, shown in Scheme 3 (d). With increasing amino-caprolactone content, micelle sizes increased from about 85 to 130 nm while the crystallinity of the core decreased. Standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with human dermal fibroblast cells revealed lower cytotoxicity from these amine-functionalized pentablock copolymers compared to that of Pluronic-*b*-P[**M1**]. The high thermodynamic stability (CMC ~10^{-4} g L^{-1}) and good biocompatibility (90% cell viability after 24 hours) of the multiblock polymeric micelles have spurred future studies with similar polymer architectures.



Scheme 3. Caprolactone-based copolymers: (a) caprolactone-decalactone copolymer; (b) FITC-decorated polymer; (c) star-shaped amino-substituted copolymer; (d) pentablock amino-substituted copolymer.

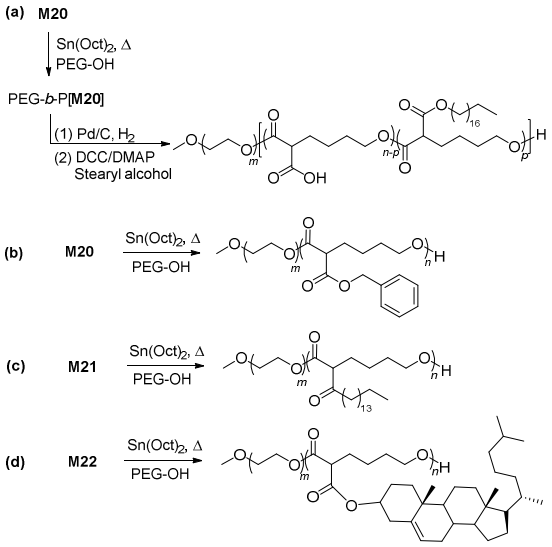
Physical Encapsulation of Small Molecules

Yueying et al. reported amphiphilic block copolymers containing ketone-functionalized hydrophobic block by the copolymerization of **M1** and **M11** (Scheme 4 (a)).[41] With increasing **M11** incorporation, the thermodynamic stability of the micelles improved by a factor of six. The micelles were loaded with up to 9 wt% DOX and released only 20% of the encapsulated drug after 70 hours in pH 7.4 buffer compared to 30% released by PEG-*b*-P[**M1**]. However, in acidic conditions the ketone-functionalized micelles released DOX at rates comparable to those of DOX-loaded PEG-*b*-P[**M1**] micelles, about 60% after 70 hours at pH 5.0, underscoring their potential utility as stable drug carriers.

Core-crosslinked polymeric micelles from alkyne-substituted poly(ε-caprolactone) cores were prepared by Garg and coworkers in 2011.[42] Synthesized by ring-opening polymerization of propargyl carboxylate caprolactone, **M16**, with PEG-OH initiator, the block copolymers self-assembled into micelles. The cores' alkyne functional groups underwent Huisgens 1,3-dipolar cycloaddition with tetraethylene glycol (bis)azide reagent to form the core crosslinks (Scheme 4 (b)). While there was no significant difference in micelle size (~57 nm) or pactlitaxel (PTX) loading content (~0.9 wt%) from non-crosslinked to crosslinked micelle cores, the core-cross-linked micelles adsorbed about 34% less bovine serum albumin than the non-crosslinked analogues did.

In 2012, Chang and coworkers designed block copolymers in which the hydrophobic blocks were comprised of a combination of hydroxy-substituted and unsubstituted caprolactone.[43] Briefly, **M1** and **M12** were copolymerized as shown in Scheme 4 (c), then **M12** cyclic ether pendant groups were converted to ketones, which in turn were reduced to generate the hydroxyl groups. The polymers self-assembled into micelles of 100-140 nm in diameter and the hydroxyl groups in the core were shown to improve the DOX loading by hydrogen bonding: up to 7.2 wt% DOX loading was achieved, compared to 4.6 wt% with only unsubstituted caprolactone in the core.

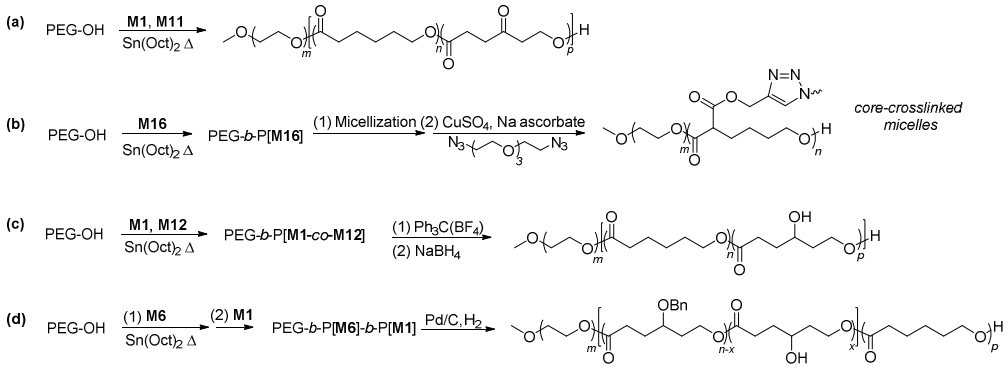
Chen and coworkers presented a study of amitriptyline-encapsulating triblock copolymers with PEG, **M6**, and **M1**.[44]



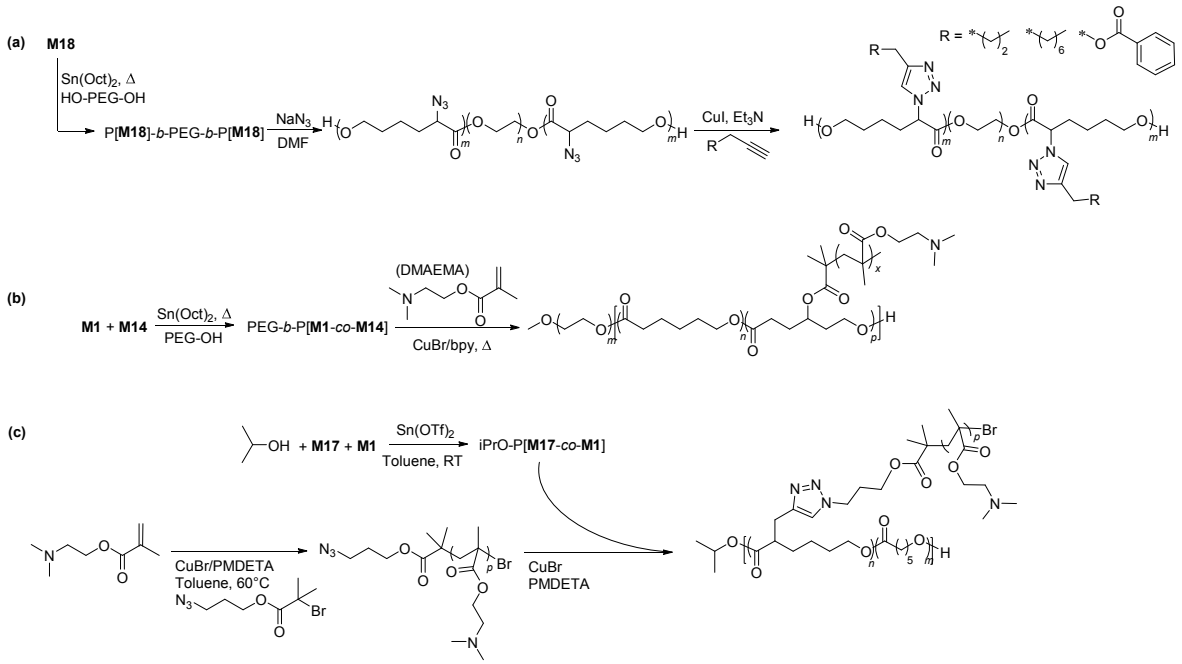
Scheme 5. Substituted poly(caprolactone)s for Amphotericin B encapsulation.

Partial debenzylation of the **M6** units generated hydroxyl-caprolactone units in the core, shown in Scheme 4 (d). For the triblock copolymers, increasing the **M6** content changed the micelle shape from spherical to spindle-shaped. The polymers showed low amitriptyline loading (~1 to 2 wt%) which depended primarily on the polymer/drug feed ratio rather than the polymer block ratios.

Falamarzian and Lavasanifar synthesized amphiphilic copolymers with stearyl- and carboxyl-substituted hydrophobic blocks in an effort to solubilize antifungal agent Amphotericin B (AmB) and minimize its hemolytic side effects.[45] Hydroxy-terminated PEG served as initiator for the ring-opening polymerization of **M20**. Post-polymerization modification of the functional groups generated the carboxylic pendant units, a number of which were then conjugated to stearyl alcohol, depicted in Scheme 5 (a). The noncovalent interactions between AmB and the core functional groups were investigated. The stearyl/carboxylic acid micelles demonstrated better AmB loading than did an analogous polymer with unsubstituted poly(ε-caprolactone) core. The stearyl-substituted micelles also



Scheme 4. Caprolactone-based copolymers featuring: (a) ketone-functionalized caprolactone, (b) crosslinked micelle cores, (c) hydroxy-functionalized caprolactone, and (d) hydroxy- and benzyloxy-functionalized caprolactone.



Scheme 6. Poly(caprolactone)s: (a) modified by CuAAC with hydrophobic units, (b) used as macroinitiator in ATRP of DMAEMA, and (c) grafted with PDMAEMA by CuAAC.

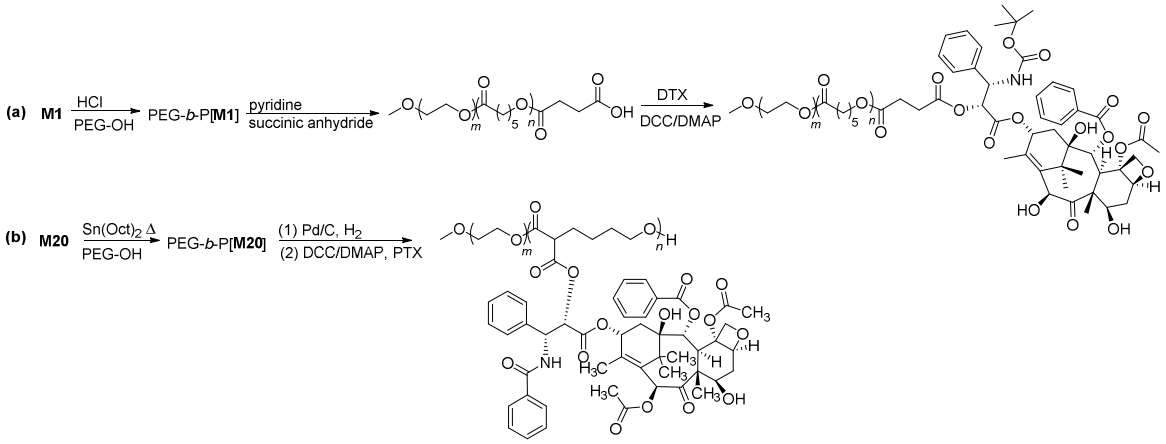
reduced the antifungal's unwanted hemolytic activity up to five times more effectively than did micelles with carboxylic acid-functionalized cores. The same group continued their research on AmB drug carriers by optimizing the hydrophobic core with other functional groups on the caprolactone block, namely benzoyl-, palmitoyl-, and cholesteryl- groups.[29] The functional monomers, **M20**, **M21**, and **M22**, were synthesized by anionic activation of caprolactone by lithium diisopropylamide and substitution with the appropriate chloroformate (benzyl-, palmitoyl-, or cholesteryl-).[46] Each of the three polymers was synthesized by ROP of the corresponding monomer initiated by PEG-OH, shown in Scheme 5 (b, c, d). Unlike the stearyl-functionalized copolymers previously reported, these required no post-polymerization modification. PEG-*b*-P[**M22**] succeeded in solubilizing AmB, and demonstrated only 7% hemolysis at 30 μg/mL AmB, compared to copolymers with P[**M20**] (40%), P[**M21**] (60%), and P[**M1**] (15%) cores.

Lee and Huang reported the synthesis of versatile triblock copolymers featuring azide-substituted caprolactones susceptible to modification by copper-catalyzed azide-alkyne cycloaddition (CuAAC).[47] **M18** was subjected to ring-opening polymerization using bifuntional initiator α,ω-dihydroxy-poly(ethylene glycol) (HO-PEG-OH). The chloro-functionality was replaced by azide in post-polymerization modification (Scheme 6 (a)). By CuAAC with propargyl benzoate, 1-hexyne, or 1-decyne, amphiphilic triblock copolymers with varying levels of hydrophobicity were formed. While increasing the length or hydrophobicity of the core increased the CMC, it improved the loading capacity and encapsulation of amitriptyline hydrochloride.

Amphiphilic cationic polymers for use as gene carriers were synthesized by Guo and coworkers.[48] To generate the diblock copolymer shown in Scheme 6 (b), PEG-OH initiated the ring-opening copolymerization of **M1** and (2-bromo-2-methylpropionate)-3-caprolactone (**M14**). This copolymer was then used as a macroinitiator for atom-transfer radical polymerization (ATRP) of *N,N*-dimethylaminoethyl methacrylate (DMAEMA), thus obtaining the amphiphilic graft-copolymer. In aqueous solution the polymers assembled into core-shell nanoparticles 55 – 85 nm in diameter with zeta potentials in the range of 10 – 18 mV. The macromolecules were thoroughly investigated for their complexes with DNA, cellular uptake, and gene transfection efficiency, and demonstrated promising results.

Another graft copolymer with ε-caprolactone and DMAEMA was reported by Darcos et al.[40] Propargyl-substituted caprolactone monomer (**M17**) was copolymerized with **M1** with $Sn(OTf)_2$ catalyst and isopropanol initiator (Scheme 6 (c)). PDMAEMA was synthesized separately by ATRP of DMAEMA using (3-azidopropyl)-bromoisobutyrate as the initiator. The azido-PDMAEMA was attached to the alkyne functionality of the **M17** repeat units by CuAAC to generate the graft copolymer. The pH-sensitive amino groups of DMAEMA were quarternized and the polymers demonstrated micellar self-assembly and successfully solubilized the hydrophobic drug clofazimine as evidenced by qualitative preliminary experimentation.

Conjugation to Drugs and Bioactive Molecules

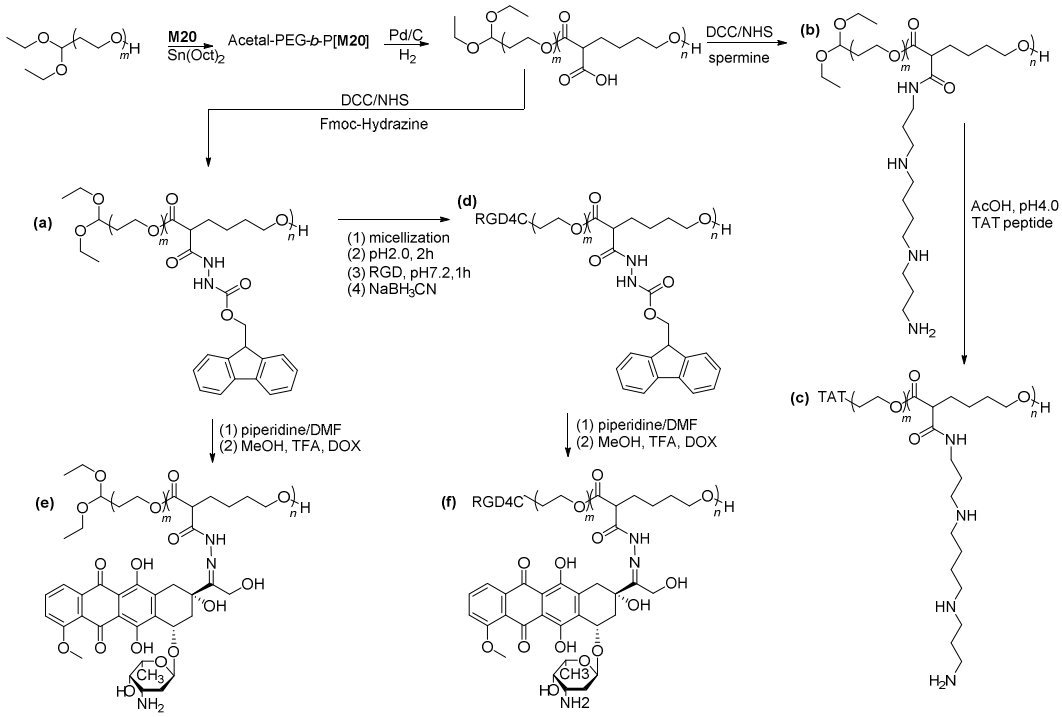


Scheme 7. Polymer-drug conjugates with (a) docetaxel (DTX) and (b) paclitaxel (PTX).

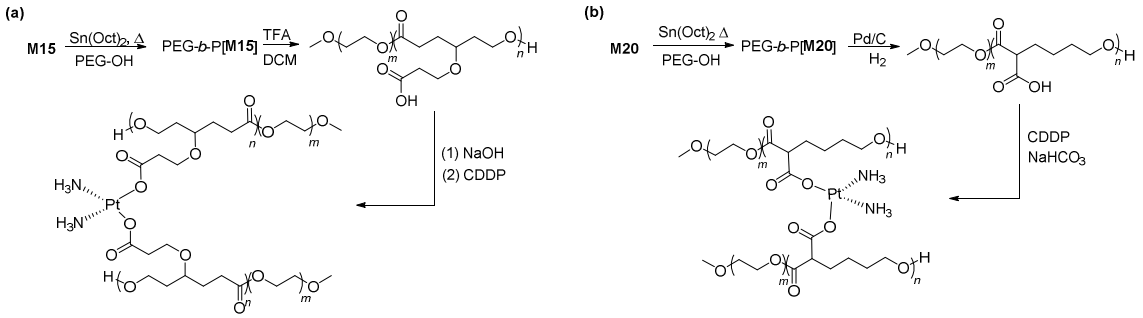
In 2010 Mikhail and Allen reported block copolymers end-capped with docetaxel (DTX) for use as micellar drug carriers.[49] As outlined in Scheme 7 (a) PEG-*b*-P[**M1**] was end-functionalized by reaction with succinic anhydride to generate a carboxylic acid terminated hydrophobic block. Further reaction with anticancer drug docetaxel (DTX) generated the PEG-b-P[**M1**]-DTX polymer-drug conjugate. The self-assembly, size, shape, physical encapsulation of free DTX, and drug release of the copolymer-drug conjugate were analyzed and compared to those of unmodified PEG-*b*-P[**M1**]. Compared to unmodified PEG-*b*-P[**M1**], the polymer-drug conjugate demonstrated lower CMC, 1.4 x 10^{-2} g/L versus 2.1 x 10^{-2} g/L, and better DTX loading, 6.7 wt% versus 1.7 wt%.

Shahin and Lavasanifar reported diblock copolymer with chemically-conjugated paclitaxel (PTX) on the caprolactone backbone.[50] This was achieved by the ring-opening polymerization of **M20**. Removal of benzyl protecting groups generated carboxylic acid functionalities which then were coupled to PTX as shown in Scheme 7 (b). The polymer-drug conjugates were characterized for PTX encapsulation, loading capacity, and release properties. The polymer-drug conjugates were found to better encapsulate PTX (2.2 wt%) than analogous block copolymers with only benzylcarboxylate-functionalized (1.0 wt%) or unsubstituted (1.3 wt%) caprolactone cores.

Multifunctional polymer nanoparticles featuring targeting moieties and either DOX or polyamine on the poly(ε-caprolactone) block were designed by Lavasanifar group.[51-54] For each of the reported polymers the synthesis began in the same manner. **M20** was subjected to ROP initiated by α-acetal-ω-hydroxy-PEG, shown in Scheme 8. After the protecting



Scheme 8. Multifunctional amphiphilic block copolymers featuring doxorubicin, spermine, and targeting moieties.



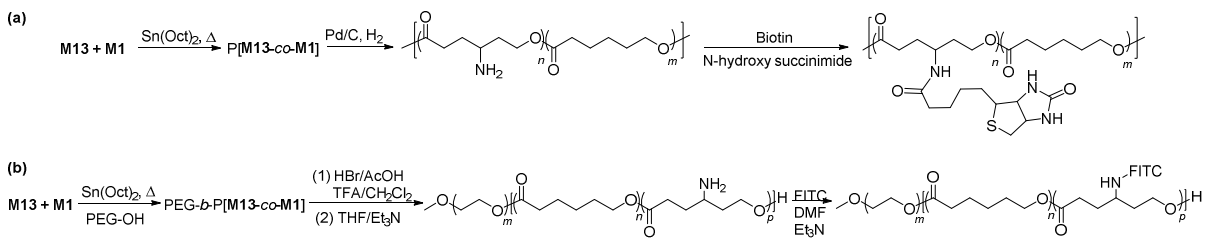
Scheme 9. Functional poly(caprolactone)s for cisplatin delivery.

groups were removed, the carboxylic groups were reacted further to generate either 9-fluorenylmethoxycarbonyl (Fmoc) (Scheme 8 (a)) or spermine (Scheme 8 (b)) functionalities. The spermine-substituted polymer was modified with TAT peptide on the shell to facilitate cell penetration (Scheme 8 (c)). The PEG block of the Fmoc-functionalized polymers was decorated with RGD4C ligand for cancer targeting (Scheme 8 (d)). The Fmoc-substituted polymers were reacted with DOX to generate polymer-drug conjugates shown in Scheme 8 (e) and (f). These amphiphilic copolymers formed well-defined micelles (93 – 108 nm diameter) in aqueous solution and demonstrated promising preliminary results: 6 wt% DOX loading, triggered drug release in endosomal conditions (pH 5), and successful intracellular accumulation.

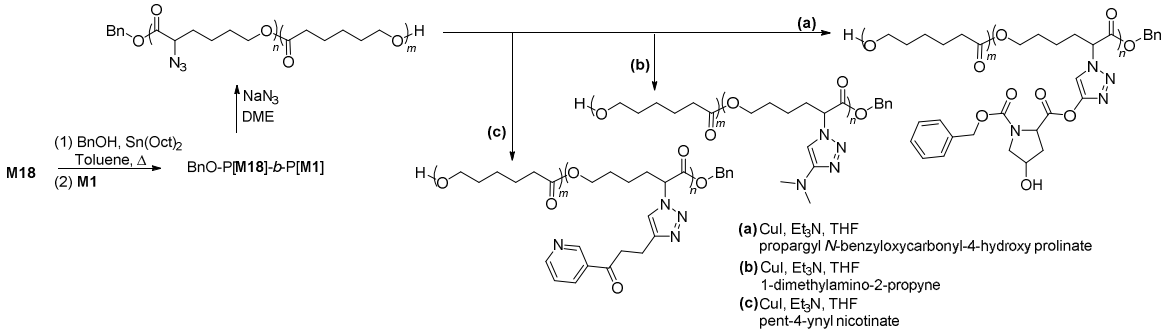
Recently, the anticancer drug *cis*-diamminedichloroplatinum(II) (cisplatin or CDDP) was loaded into polymeric assemblies by complexation with carboxyl-functionalized hydrophobic block as reported by two research groups in 2014.[55, 56] A highly potent chemotherapeutic agent, cisplatin has been the subject of laborious research. Efforts are focused primarily on minimizing severe side effects by incorporating cisplatin within drug carriers like micelles which could accumulate in tumor tissues by the EPR effect. For maximum therapeutic effect, the drug must be retained within its carrier and released upon arrival at the target site; thus stimuli-responsive carriers are highly sought after. Jayakannan et al. synthesized a new monomer, *t*-butyl-3-((7-oxooxepan-4-yl)oxy)-propanoate (**M15**), and subjected it to ring-opening polymerization and subsequent deprotection to generate the carboxyl functional groups as shown in Scheme 9 (a).[56] The copolymers successfully complexed with cisplatin, achieving 16 wt% loading. The polymeric assemblies were hypothesized to release cisplatin by exposure to saline or enzymatic conditions. In saline conditions, 35% of the cisplatin was released in a burst, then about half of the remaining cisplatin was released gradually over six days. In the presence of esterase enzyme, 100% release of the loaded cisplatin was achieved in ten hours by hydrolysis of the poly(ε-caprolactone) backbone.

Also in 2014, Shahin et al. prepared PEG-*b*-P[carboxylic acid caprolactone] by hydroxy-terminated PEG-initiated ROP of α-benzylcarboxylate-ε-caprolactone followed by deprotection using H_2/Pd.[55] The PEG-*b*-P[carboxylic acid caprolactone] diblock copolymers were investigated as carriers for cisplatin. The polymer-cisplatin conjugate was generated according to Scheme 9 (b). The pH-sensitive self-assembling block copolymers achieved up to 12.6 mol% (~1 wt%) cisplatin loading.

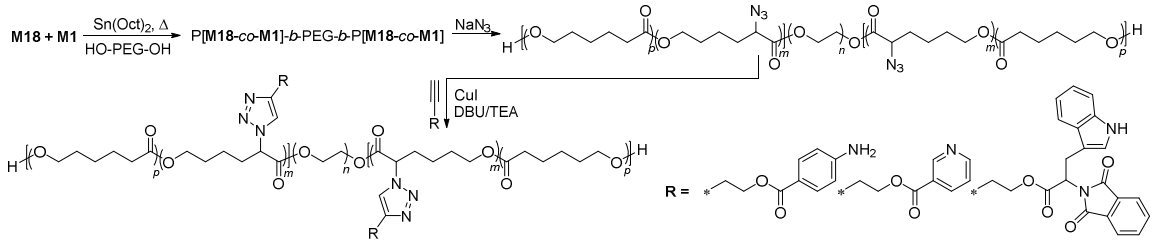
Yan et al. were the first to report the γ-(carbamic acid benzyl ester)-ε-caprolactone monomer (**M13**). Initial studies reported the synthesis and deprotection of P[**M1**-*co*-**M13**] copolymer to generate the amino-substituted hydrophobic block, as shown in Scheme 10 (a).[57] The amino groups were then functionalized with biotin, vitamin H, to demonstrate the substituted polymer's potential utility in bioconjugation. Later, amphiphilic block copolymers of PEG-*b*-P[**M1**-*co*-**M13**] were synthesized and deprotected to generate the amino groups shown in Scheme 10 (b).[58] As proof-of-concept to demonstrate and visualize the micelles' cellular uptake, the amino-poly(ε-caprolactone) was functionalized with fluorescein using FITC. The fluorphore-labeled polymers self-assembled into micelles with good biocompatibility which were taken up successfully by human fibroblast cells. In a subsequent report, PEG-*b*-P[**M1**-*co*-**M13**] was used directly as a carrier for DOX.[59] Taking into consideration the Flory-Huggins interaction parameters between DOX, poly(ε-caprolactone), and the proposed PEG-*b*-P[**M1**-*co*-**M13**], two block copolymers were synthesized with the new core-forming block, a mix of **M1** and



Scheme 10. Polymers from γ-(carbamic acid benzyl ester)-ε-caprolactone featuring (a) biotin and (b) FITC.



Scheme 11. Functional poly(caprolactone)s grafted with bioactive moieties.



Scheme 12. Triblock copolymers with *p*-amino benzoic acid, nicotinic acid, and phthaloyltryptophan.

M13. Micelle size, stability, core crystallinity, and DOX loading capacity were analyzed and correlated with the **M1**:**M13** content. As hypothesized, the drug loading capacity increased from 10 wt% to 25 wt% with increasing **M13** content in the core.

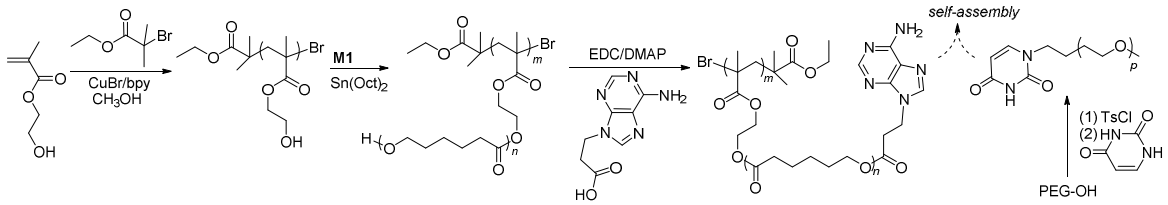
Huang and coworkers grafted bioactive molecules onto azide-functionalized poly(ε-caprolactone).[60] Bioactive moieties included 4-hydroxy-$_L$-proline, dimethylamino propyne (DMAP), and nicotinic acid, as shown in Scheme 11 (a, b, c). Biocompatible with no significant cytotoxicity to HeLa cells, these amphiphilic block copolymers were shown to self-assemble into micelles with CMC values on the order of 10^{-3} – 10^{-2} g L^{-1} and hydrodynamic diameters around 100 – 160 nm. The drug loading efficiency was probed using indomethacin, a non-steroidal anti-inflammatory drug. In general, the DMAP and nicotinic acid functionalized micelles best encapsulated indomethacin (36 – 59 wt% loading), with the loading capacity increasing as the hydrophilic/hydrophobic block ratio increased.

Suksiriworapong et al. grafted *p*-amino benzoic acid, nicotinic acid, and phthaloyltryptophan onto the azide functionalized caprolactone, shown in Scheme 12.[61, 62] In a subsequent report, the use of these amphiphiles as drug carriers for indomethacin and ibuprofen was explored.[63] While the nicotinic acid improved indomethacin loading (21 wt%) compared to non-nicotinic acid functionalized micelles (11 wt%), varying the quantity of nicotinic acid functional groups on the hydrophobic segment did not appear to affect loading capacity. Different release profiles for each drug indicated indomethacin's release being more influenced by core crystallinity whereas ibuprofen's release was also affected by hydrogen bonding with the hydrophobic block.

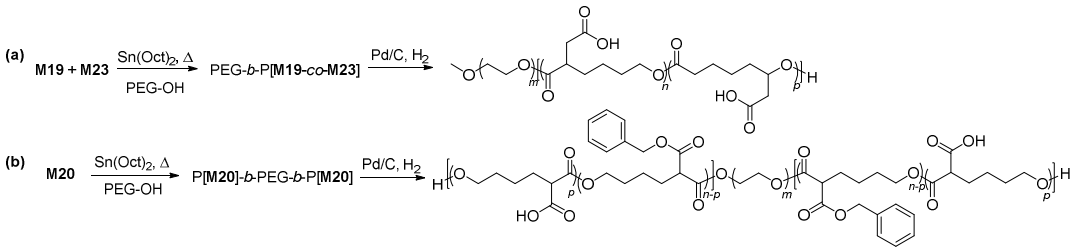
Stimuli-Responsive Block Copolymers

The incorporation of stimuli-responsive functionalities into polymers for drug delivery has been a common strategy in the development of drug delivery systems with controlled release.[7] Such drug carriers experience a change in response to a stimulus, such as a decrease in pH, an increase in temperature, or exposure to a biochemically reducing environment.[14, 15]

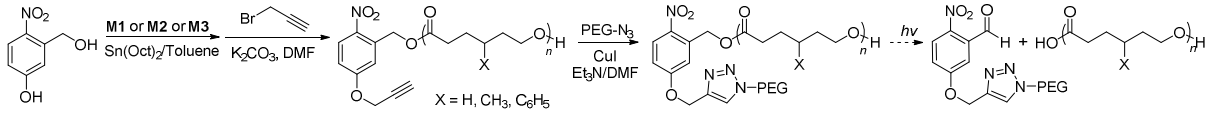
New brush-like copolymer micelles were designed by Wang et al. to improve the controlled release of DOX in the high-salt low-pH environment of tumor tissue.[64] Shown in Scheme 13 is their multi-step preparation. First, poly(2-hydroxyethyl methacrylate) (PHEMA) was synthesized by ATRP. Next, P[**M1**] was grafted from PHEMA and end-capped with the nitrogenous base adenine, while PEG-OH was coupled to uracil. The noncovalent interactions between the complementary bases drove the self-assembly of PHEMA-*g*-P[**M1**]-adenine with PEG-uracil. Up to 7 wt% DOX loading capacity was achieved, as was stimuli-responsive behavior *in*



Scheme 13. Self-assembling polymers for salt/pH responsive drug delivery.



Scheme 14. Diblock (a) and triblock (b) pH-responsive amphiphilic copolymers.



Scheme 15. Photosensitive amphiphilic block copolymer.

vitro. Increasing salt concentration and/or decreasing pH contributed to the destabilization of the micelles, which triggered the release of encapsulated DOX.

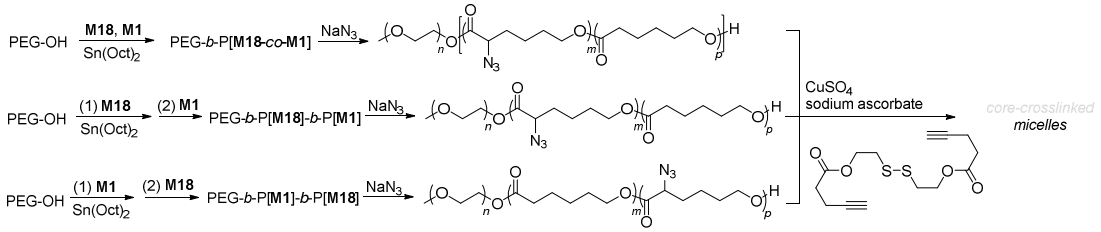
Zhang and coworkers synthesized new pH-sensitive copolymers from α- and ε-benzyloxycarbonylmethyl-caprolactone (**M19** and **M23**).[65] The new monomers were copolymerized using PEG-OH as macroinitiator, and were deprotected subsequently to generate the carboxylic functionalities, Scheme 14 (a). The monomer to PEG molar ratio was varied such that the series featured a range of hydrophobic block lengths. Self-assembly, solubility, and micelle size were largely pH-dependent as the carboxyl groups were protonated or deprotonated. Below the polymer's pKa, the protonation of the carboxylic groups drove the self-assembly of the chains into well-defined micelles of 40 – 50 nm in diameter.

Nikouei reported a series of triblock copolymers synthesized by the ROP of **M20** initiated by the bifunctional HO-PEG-OH.[66] The substituted caprolactone repeat units were partially debenzylated, resulting in triblock copolymers with varying ratios of benzylcarboxylate to carboxylic acid pendant groups on the hydrophobic blocks (Scheme 14 (b)). Because of the carboxylic functional groups, pH-dependent behavior was observed, with a decrease in the size of micellar assemblies from ~160 nm at pH 3.0 to ~50-80 nm at pH 9.0. Also reported was a thermal transition around 29 °C, manifested as either an increase or decrease in micelle size, depending on the ratio of deprotected/protected functional caprolactone repeat units.

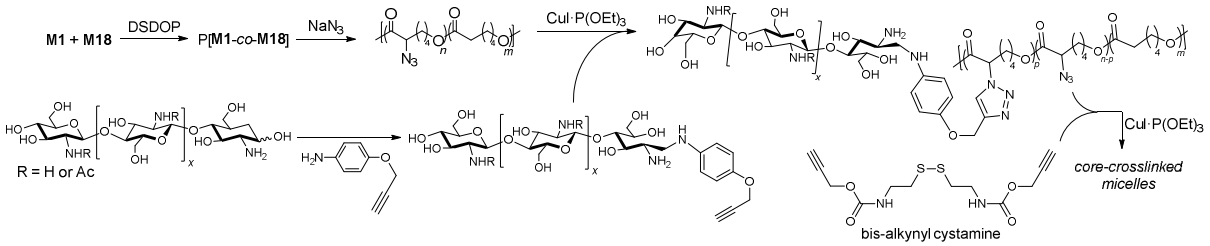
A series of amphiphilic block copolymers with a photoresponsive moiety linking the hydrophilic and hydrophobic blocks was synthesized by Peng et al.[67] The ring-opening polymerization of **M1**, **M2**, or **M3** was initiated by 5-hydroxy-2-nitrobenzyl alcohol. As depicted in Scheme 15, post-polymerization modification was performed by reacting the hydroxy group on the initiator with propargyl bromide, followed by the cycloaddition of azide-terminated PEG. The photocleavable polymer chains self-assembled and encapsulated indomethacin with ~40 wt% efficiency for substituted cores (**M2**, **M3**), and 29 wt% efficiency for unfunctionalized (**M1**) cores. Exposure to UV radiation caused the loaded micelles to release 30 – 40% more indomethacin over 24 hours than did the non-irradiated micelles.

Cajot and coworkers synthesized three block copolymers from the PEG-OH initiated ring-opening polymerization of **M1** and **M18**, depicted in Scheme 16.[68] The chloro groups were converted to azides and by co-solvent micellization the cores were crosslinked with disulfide-containing alkyne compound by Huisgens 1,3-dipolar cycloaddition. The micelles were stabilized by the core-crosslinks, demonstrating no signs of disassembly or aggregation over 30 days, while non-crosslinked micelles appeared to aggregate after only ten days.

Novel poly(ε-caprolactone)-*g*-(chitosan oligosaccharide) amphiphilic copolymers with reduction-sensitive crosslinked cores were reported by Guerry et al.[69] Ring-opening copolymerization of **M18** and **M1** using 2,2-dibutyl-2-stanna-1,3-dioxepane (DSDOP), followed by reaction with sodium azide generated the copolymer shown in Scheme 17. The chitosan oligosaccharides (CO) were modified with alkyne functional groups, enabling the CuAAC coupling of CO with the azide substituted poly(ε-caprolactone). Only a fraction of the N_3 pendant groups were grafted with the alkyne-terminated



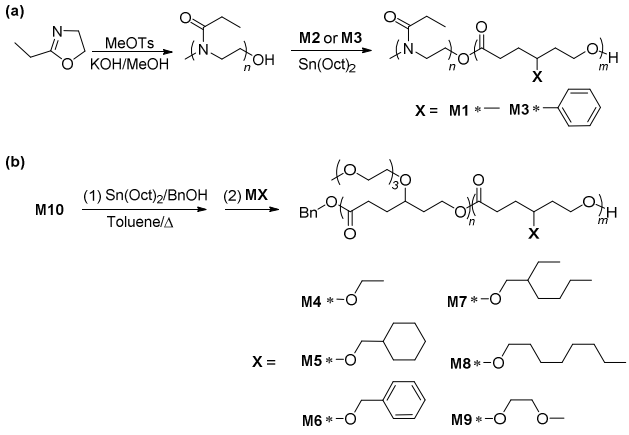
Scheme 16. Diblock and triblock copolymers with reduction-sensitive core crosslinks.



Scheme 17. Poly(caprolactone)-*g*-(chitosan oligosaccharide) with reduction sensitive crosslinked micelle core.

oligosaccharide so that the remaining azide groups could be crosslinked by the reduction-sensitive crosslinker *bis*-alkynyl cystamine. Micelles were formed and encapsulated DOX with 8.6 wt% loading capacity. To investigate the reduction-triggered release of DOX, the loaded micelles were exposed to glutathione (GSH), a reducing agent, to mimic extracellular (2 μM GSH) and intracellular conditions (10 mM GSH). The micelles exposed to higher concentration of GSH demonstrated 66% DOX release compared to about 30% for micelles exposed to the lower concentration of GSH.

Thermoresponsive block copolymers comprised of poly(2-ethyl-2-oxazoline) and methyl- (**M2**) or phenyl- (**M3**) substituted caprolactone, were investigated by Peng et al. (Scheme 18 (a)).[70] Temperature-responsive polymers like these are soluble in water below their lower critical solution temperature (LCST), but are insoluble once the solution is heated above the LCST. Incorporation of the substituted caprolactone in the hydrophobic block reduced the LCST as low as 38 °C, compared to the poly(2-ethyl-2-oxazoline) homopolymer with LCST around 90 °C. In general, as the hydrophobic block length was reduced, the drug loading improved. Indomethacin was loaded more efficiently in the phenyl substituted cores (60 wt%) than the methyl-substituted version (23 wt%) due to pi-pi stacking. The mechanism of DOX-loaded micelle uptake by HeLa cells was investigated systematically with different uptake inhibitors and was found to be largely endocytotic by constitutive macropinocytosis.



Scheme 18. Thermoresponsive block copolymers featuring (a) 2-ethyl-2-oxazoline and substituted caprolactone, or (b) functional caprolactones with hydrophilic or hydrophobic substituents.

While most of the reported biocompatible thermoresponsive polymers are polyacrylamide-based and non-degradable,[7, 17] new thermoresponsive, biodegradable all-caprolactone block copolymers have been developed recently. The Stefan group first synthesized γ-2-[2-(2-methoxyethoxy)ethoxy]ethoxy-ε-caprolactone (**M10**) and reported a series of thermoresponsive polymers with P[**M10**] as the hydrophilic block.[71] Homopolymer P[**M10**] was soluble in aqueous conditions below its LCST of 48 °C, but underwent a coil-to-globule phase transition in which the pendant methoxy(ethoxy)$_3$ chains dehydrated and the polymer's solubility diminished substantially. By synthesizing a series of diblock copolymers comprised of P[**M10**] as the hydrophilic block and P[**M4**], P[**M5**], P[**M6**], P[**M7**], P[**M8**], or P[**M9**] serving as the hydrophobic block, highly tunable LCSTs from 31 °C to 44 °C were achieved (Scheme 18 (b)). The thermoresponsive behavior was largely influenced by the block ratios and nature of the core block's functional groups. [71-74] Special focus was given to copolymers with thermal transitions in the 38 °C to 40 °C range, as these could exist as micellar assemblies in physiological conditions and release their drug payload upon local heating or mild hyperthermia. Block copolymer micelles were subjected to degradation, biocompatibility, stability, and drug loading studies and demonstrated promising potential, with maximum DOX loading of 2.5 wt% for P[**M10**]-*b*-P[**M8**]. This unique family of poly(ε-caprolactone)s constitutes a novel approach in micellar drug delivery due to their fully hydrolyzable backbones and thermoresponsive properties.

Conclusions

Recent advances in functional poly(ε-caprolactone)s exemplify their wide range of tunable properties and utility in drug delivery systems. Synthetic modification of caprolactone monomers and polymers provides a route to self-assembling block copolymers with various functionalities for the physical encapsulation or direct conjugation of anticancer agents and bioactive molecules. Systematic studies on amphiphilic block copolymers with substituted caprolactones have helped elucidate the relationships between polymer structures and micelle properties. The hydrophilic/hydrophobic block ratios, core crystallinity, and nature of the pendant groups along the polymer backbones can influence micelle size, stability, drug loading capacity, and cellular uptake. Targeting peptides and stimuli-responsive moieties have been incorporated into micelle shells or cores to facilitate the controlled release of cargo drug at the desired target. Considering the range of substituted

monomers already synthesized, substantial progress in this field is expected to stem from the fine-tuning of functional poly(ε-caprolactone)s. Of particular interest will be the continuing development and optimization of stimuli-responsive substituted poly(ε-caprolactone)s to attain highly sensitive, responsive materials for triggered drug release. In sum, ongoing research on functional poly(ε-caprolactone)s continues to demonstrate their excellent tunable properties and great potential in micellar drug carrier formulations.

Notes and references

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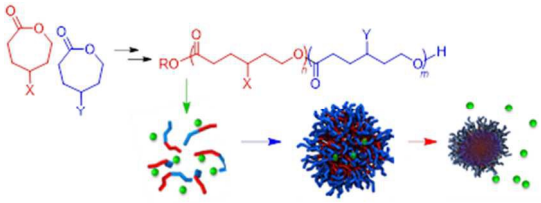
Graphical Abstract for Manuscript ID PY-REV-11-2014-001628 entitled "Recent developments in micellar drug carriers featuring substituted poly(ε-caprolactone)s"



Synthetic modification of caprolactone monomers and polymers provides a route to self-assembling block copolymers for use in drug carrier applications.