Multiple morphologies from amphiphilic graft copolymers based on chitooligosaccharides as backbones and polycaprolactones as branches†

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A new route to form multiple morphologies was outlined using amphiphilic graft copolymers with interesting biological and pharmacological properties by proper adjustment of backbone and graft chain length.

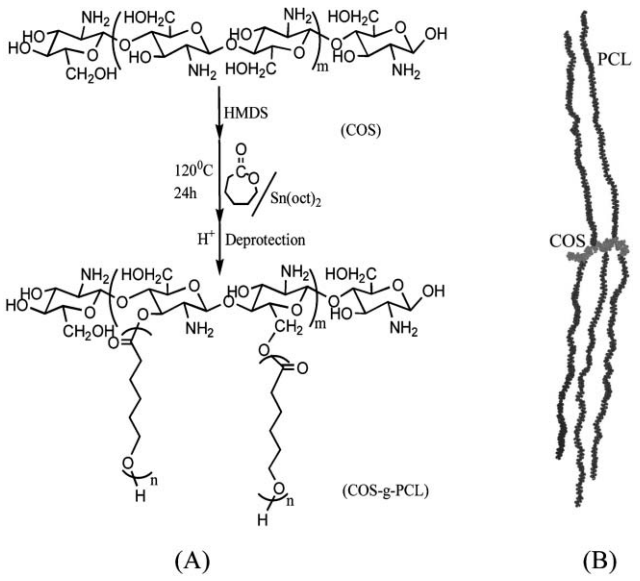
Self-assembly of amphiphilic copolymers in selective solvents has been widely investigated in terms of their micellar behavior as well as their applications in many fields such as biotechnology and pharmaceutics.[1–5] Compared with linear block copolymers, graft counterparts may provide integration of considerable functionalities onto polymer backbone that can be addressed chemically after the self-assembly process.[6,7] However, in most cases, amphiphilic graft copolymers tend to form compound spherical micelles[8–10] or unimolecular micelles,[11,12] whereas amphiphilic linear block copolymers including rod–coil and coil–coil types can organize into abundant morphologies such as sphere, rod and vesicle *etc*.[13,14] It is difficult for a graft copolymer to organize into different morphologies in a selective solvent and that the resulting aggregates can be transferred from one to another by changing the processing conditions like those of linear block copolymers. This creates the impression that the branched structure of a graft copolymer precludes morphological diversity and that the graft copolymer cannot compete with linear analogues.

So far, studies on the synthesis and the self-assembly of graft copolymer nearly all focus on the traditional comb-shaped or brush-shaped graft copolymer based on longer main chain as backbone ($M_n \approx 10000 \sim$ several tens of thousands) and shorter side chains as graft segments. It is believed that these structures easily undergo inter-molecular entanglement among the longer main chains or intra-molecular association so that they tend to form spherical micelles. If the structure of amphiphilic graft copolymer could be properly manipulated to decrease the above-mentioned influence, it should be possible for a graft copolymer to organize into abundant morphologies in selective solvents.[15] However, limited attention has been devoted to developing the kind of amphiphilic graft copolymer with a structure suitable for generating multiple self-assembled morphologies.

Here, we report a new kind of amphiphilic graft copolymer based on a shorter rigid chain as backbone and a longer chain as branch (Scheme 1B). This structure is quite different from the conventional comb-shaped or brush-shaped graft copolymers. Chitooligosaccharide (COS), the oligomer of chitosan, was chosen as the hydrophilic short rigid main chain and poly(ε-caprolactone) (PCL) as the hydrophobic long side chain in our case. It is found that these special graft copolymers COS-g-PCL can not only form some classical micelles such as sphere, rod, and vesicle like those formed from highly asymmetric block copolymers,[4,13] but also organize into a novel "petal-like" morphology. To the best of our knowledge, such aggregation behaviour has not been observed in any other graft copolymer systems

COS, containing an amount of amino and hydroxyl groups in its backbone, has been used as attractive biomaterials due to its water-solubility, biodegradation, and biological activities.[16,17] PCL, a kind of hydrophobic polymers, has been frequently applied as implantable carriers for drug delivery systems and as biomedical materials due to its biodegradation, biocompatibility and permeability to drugs.[18,19] The integration of these two kinds of biodegradable polymers may give a new functional copolymer combining the favourable properties of both COS and PCL.

In this work, amphiphilic graft copolymers COS-g-PCLs with controlled structure were synthesized using protection/deprotection of partial hydroxyl groups of COS *via* trimethylsilyl (TMS) groups and homogenous ring-opening polymerization of ε-caprolactone (CL), as shown in Scheme 1A (see Supplementary Information†).



Scheme 1 (A) Synthesis of amphiphilic graft copolymer COS-g-PCL; (B) schematic illustration of graft copolymer COS-g-PCL containing long PCL branches.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b5/b504428f/

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Briefly, the trimethylsilylation of COS took place first onto the hydroxyl and amino groups, in which the number of protected-OH group was controlled by the adjustment of the molar ratio of COS to hexamethyldisilazan (HMDS). The introduction of the TMS groups was confirmed by IR and H-NMR analyses (See Supplementary Information†). Then, the grafting polymerization of ε-caprolactone onto the modified COS backbone was successfully performed in a mixed medium of chloroform and xylene using stannous octoate ($Sn(Oct)_2$) as a catalyst. Finally, TMS groups of the resulting graft copolymers were removed by the incubation of the polymer samples in an isopropyl alcohol–H_2O–HCl mixture. The obtained COS-g-PCLs were characterized with IR and NMR (solvents without TMS). The removal of TMS groups was confirmed by the disappearance of methyl proton signals from TMS at 0.10 ppm. The methylene proton signals of PCL can be observed at 4.1, 2.3, 1.7, and 1.4 ppm. The methine and methylene proton signals of COS are at 3.0–5.0 ppm. D_p (average degree of ε- caprolactone grafted on every glucose unit of COS backbone) was calculated from the ratio of the integral areas of the methylene signal of PCL at 2.3 ppm to the methine proton signal (H-2) of COS at 3.0 ppm. Under the conditions that we used, no occurrence of the degradation of COS was detected.

Molecular weight (M_n) and molecular weight distribution (M_w/M_n) were determined with a GPC instrument.† As shown in Scheme 1B, by adjusting the length of the grafted PCL branches, we obtained graft copolymers containing shorter COS backbones and longer PCL branches with different lengths (table 1).

The aggregation behaviour of COS-g-PCL was studied by an indirect method as follows, where COS_{11}-g-PCL_{253} and COS_{11}-g-PCL_{520} were considered (Table 1). COS-g-PCL copolymer was initially dissolved in THF. Deionised water was added dropwise to the COS-g-PCL solution under vigorous stirring until a predetermined water contents was reached. After that, a large amount of water was added to the solution in order to quench the resulting morphologies. The solution was then dialyzed against water to remove the organic solvent. Transmission electron microscopy (TEM) samples were prepared by dropping 10 μl aliquots of copolymer aqueous solution onto a carbon coated copper grid. After staining with phosphotungstic acid (PTA), morphologies were observed using TEM JEOL-1200.

Fig. 1 shows the TEM images of the resulting self-assembled aggregates of COS_{11}-g-PCL_{253}. It appears that COS_{11}-g-PCL_{253} self-organized into spherical micelles at first. As more water was added to the THF solution of the graft copolymer, spherical micelles were transformed into rod and then vesicular morphologies. Noticeably, two kinds of different spherical micelles were unexpectedly obtained as shown in Fig. 1A and 1B. One is a small normal sphere (about 75 nm) and the other is a large "petal-like"

Table 1 Preparation and characterization of the COS-g-PCL

Sample	[CL]/[glucose unit of COS] (molar ratio)[a]	$M_n \times 10^4$ (M_w/M_n)[b]
$g_1(COS_{11}$-g-$PCL_{66})$	6.0	0.9(1.4)
$g_2(COS_{11}$-g-$PCL_{132})$	12.0	1.4(1.4)
$g_3(COS_{11}$-g-$PCL_{253})$	23.0	4.1(1.3)
$g_4(COS_{11}$-g-$PCL_{520})$	47.3	8.5(1.6)

[a] Measured by ^1H NMR spectroscopy. [b] Estimated by GPC in THF.

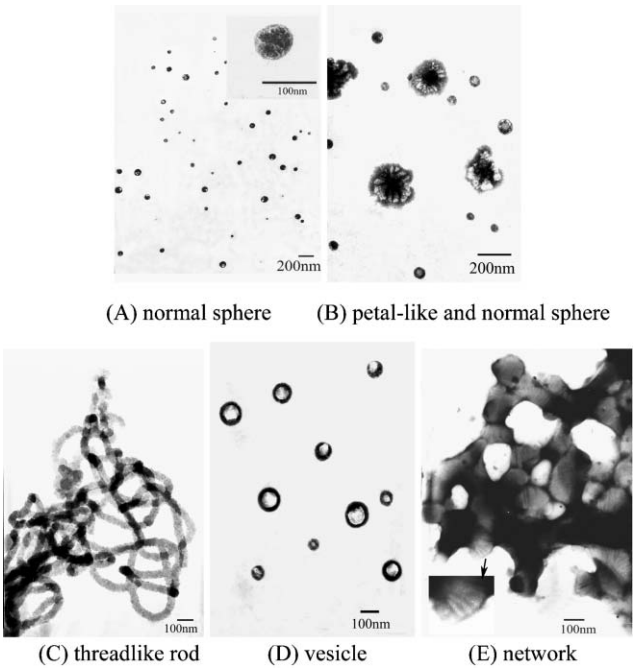

Fig. 1 Transmission electron micrographs of the aggregates formed under different conditions. (A) and (B) 5wt% H_2O, (C) 25wt% H_2O, (D) 30wt% H_2O (A–D, 0.5% COS_{11}-g-PCL_{253}) (E) 5wt% H_2O (0.5% COS_{11}-g-PCL_{520}).

spherical aggregate (about 200 nm). To the best of our knowledge, the latter has not been observed in any other block or graft copolymer systems. The "petal-like" spheres often coexist with small spherical micelles, and their spherical cores are not entirely filled with the PCL segments (Fig. 1B). Interestingly, at high magnification, even in most small normal spheres, it was observed that the spherical cores were not completely filled with the PCL segments (Insert in Fig. 1A).

For the graft copolymer COS-g-PCL, the short hydrophilic COS backbones in aqueous solution are rigid and the long PCL branches are hydrophobic. Fig. 2 displays the possible self-assembled mechanism of these novel graft copolymers in aqueous solution. Before the addition of water, the graft copolymers COS-g-PCL are unwound unimolecules in good solvent THF (Fig. 2A). Upon the addition of the water to the THF solution of the copolymer, the solvent becomes progressively more incompatible with the PCL graft segments. At a certain water content, the PCL graft segments start to associate and the molecular structure behaves like a bundle of highly asymmetric linear block copolymer as shown in Fig. 2B. Several kinds of solvophobic forces exist in system, such as the intra-shrinkage of every PCL branch and the intra- or inter- aggregation of the different PCL graft chains in the graft copolymer macromolecules. Spherical micelles are formed as the mentioned interaction forces reach dynamic equilibrium (Fig. 2C). Due to the special branched structure of the copolymer based on a short and rigid COS as backbone, as well as long PCL as grafts, the spherical micelles are transferred into rod-like and vesicular morphologies with the gradually increasing water content (Fig. 2D and 2E).

The self-assembly behavior of all synthesized copolymers is not the same, especially for polymers with large differences in

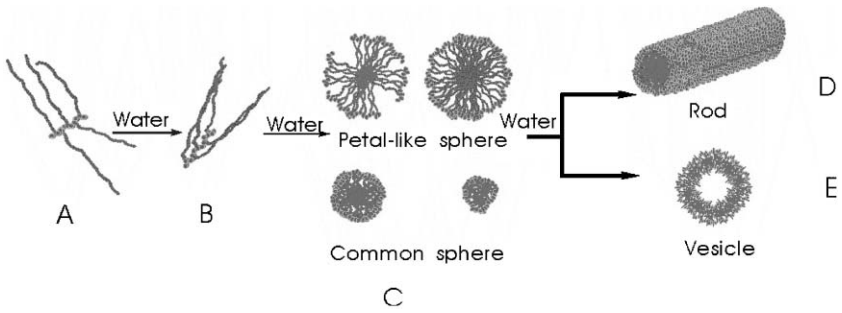

Fig. 2 A proposed mechanism leading to the self-assembled morphologies of amphiphilic graft copolymer COS-g-PCL in water.

COS/PCL ratios. In the case of the polymer (COS_{11}-g-PCL_{66}), due to the very low solubility of this polymer in THF, no self-assembly experiment was performed. In the case of the polymer (COS_{11}-g-PCL_{132}), however, under the same self-assembly conditions as those for the polymer (COS_{11}-g-PCL_{253}), only spherical and rodlike micelles were observed. And when the selective solvent is acid aqueous solution, the polymer COS-g-PCL can also form a vesicular morphology (TEM images in the supplementary information†). For the copolymer (COS_{11}-g-PCL_{520}) with longer PCL branches, a complicated network morphology with the PCL side chains vertical to the network surface was observed, as a result of the fusion of spheres (Fig. 1c).

In summary, a new kind of amphiphilic and asymmetric graft copolymer was synthesized, which exhibits multiple morphologies similar to those of linear block copolymers. Since these special graft copolymers not only contain amino and hydroxyl groups on the COS backbone but are also biocompatible, the self-assembled system may be useful to design attractive functional nano-hybrid materials with potential applications in biotechnology.

Further studies on the mechanism of formation of the petal-like morphology, the transformation among the different morphologies, and the application of micelles in drug encapsulation are under way.

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