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Soft nanotubes from amphiphilic ABA triblock macromonomers

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Soft, water-filled polymer nanotubes of several tens of μm in length have been prepared *via* self-assembly of amphiphilic ABA-triblock macromonomers in aqueous media; the tubes are mechanically and chemically stable and can be loaded with water-soluble substances.

Nanotube research is currently one of the most active areas of Nanoscience.[1] Carbon nanotubes could have applications in miniature electronics and for hydrogen storage, but as they are stiff, difficult to process and purify, not biocompatible, and cannot be degraded by living organisms they are not well suited for biological applications; soft nanotubes made from biocompatible organic molecules and polymers however could find application in biotechnology and biomedicine.[2] Typical examples include self-assembled lipid nanotubes,[3] and peptide-based nanotubes.[4] Recently also block copolymers have been used to prepare vesicles and spherical or rod-like micelles.[5] Polymer nanotubes however have so far only been described in organic solvents where their fabrication often requires elaborate procedures;[6–8] however, the controlled preparation of water-filled polymer nanotubes in aqueous media has remained elusive. This communication introduces a simple method for the preparation of mechanically stable, water-filled, and soft nanotubes *via* the self-assembly of amphiphilic triblock copolymers.

Amphiphilic poly(2-methyloxazoline-*block*-dimethylsiloxane-*block*-2-methyloxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA) ABA triblock copolymers were synthesized *via* cationic ring-opening polymerization of 2-methyloxazoline onto an activated telechelic PDMS block.[9] The length of each block can be adjusted by the amount of monomer added to the reaction mixture and the –OH end groups of the PMOXA blocks allow for the functionalization with methacrylic acid. These triblock macromonomers form supramolecular assemblies in aqueous solution that can be chemically crosslinked *via* polymerization of the methacrylic acid groups. For the current study, we have used symmetric triblock copolymers with molecular weights ranging from 7000 to 9000 g mol^{-1} and a PDMS block of roughly 5000 g mol^{-1}.

Polymer nanotubes were fabricated *via* film rehydration. A 1% (wt/wt) solution of the copolymer in chloroform was dried under nitrogen in a test tube; by rotating the tube during drying a polymer film forms on the glass. Drying was completed by desiccation under vacuum overnight. The film was then rehydrated by water addition and stirring for 24 hours to yield a 1wt% polymer solution, which is homogeneous and opaque. After a 10-fold dilution with water, the samples were examined with transmission electron microscopy (TEM, Hitachi H-7000). Experiments without dilution and with 1000-fold dilution yielded the same morphologies but the TEM imaging conditions were best at 10-fold dilution.

Fig. 1 shows a schematic of a self-assembled nanotube and a typical sample; the TEM image shows that the solution contains a mixture of vesicles and flexible nanotubes. The vesicles are quite polydisperse with diameters ranging from 40 to over 500 nm, but the tube diameter is astonishingly uniform at about 40 nm. The nanotubes extend up to several tens of microns in length and their concentration with respect to the number of vesicles depends on the copolymer used, that is, the formation of the tubes *vs.* vesicles depends on the relative block lengths of the hydrophilic PMOXA and hydrophobic PDMS blocks.

Table 1 summarizes the nanotube formation results. A low PDMS/PMOXA wt/wt ratio (below ~1.5) favors vesicle formation and higher ratios (~2 and higher) increasingly favor nanotube formation. This tendency is qualitatively confirmed with a control experiment where the dry polymer was directly dissolved in water and imaged in the TEM.

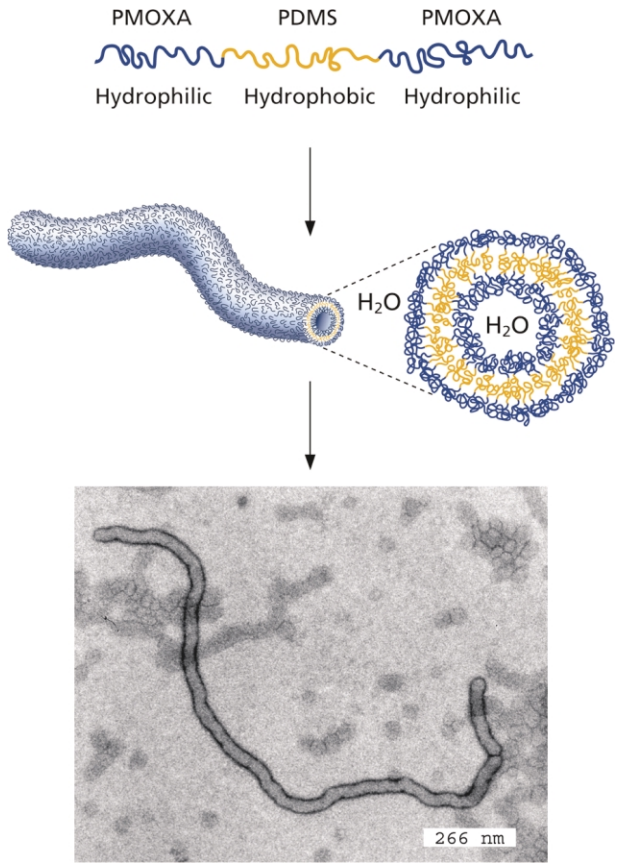

Fig. 1 Self-assembly of ABA triblock copolymers in aqueous solution; TEM image of a polymer nanotube.

Table 1 Approximate volume fraction of tubes as a function of polymer composition and sample preparation[a]

Copolymer[b]	PDMS/PMOXA Ratio (wt/wt)	Tube volume fraction Rehydration	Tube volume fraction Dissolution
$A_{11}B_{62}A_{11}$	2.4	90 ± 5	30 ± 5
$A_{16}B_{72}B_{16}$	2.1	85 ± 5	20 ± 5
$A_{15}B_{62}B_{15}$	1.9	75 ± 5	25± 5
$A_{21}B_{72}B_{21}$	1.5	5 ± 5	1 ± 5
$A_{21}B_{69}B_{21}$	1.5	10 ± 5	5 ± 5

[a] Volume fractions were estimated from TEM images by assuming that the area occupied by tubes is directly related to their volume fraction. [b] A: PMOXA, B: PDMS.

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Although we observe a trend in both sets of experiments, a quantification of this phenomenon is difficult from TEM data alone and the data in Table 1 thus have an error of about 10%. Nevertheless, these findings suggest that the formation of the nanotubes is largely controlled by the geometry of the PMOXA blocks: at a ratio of ~1.5 the PMOXA block is, relatively speaking, larger than at a ratio of ~2 and more and it hence favors vesicles over tubes because each chain occupies a larger hydrodynamic volume.

The results in Table 1 also indicate that not only the PDMS/PMOXA ratio but also the preparation procedure influences the formation of the nanotubes; film rehydration is thus a more efficient pathway to polymer nanotubes than simple dissolution of the dry polymer in water. It is hence an elegant additional method to enhance nanotube over vesicle formation.

A major reason for the interest in polymer vesicles and nanotubes is their high mechanical stability compared to lipid nanotubes. For example, our nanotubes are resistant to repeated filtration trough 0.45 μm filters and to size exclusion chromatography. The nanotubes also remain stable over 8 months if stored at 4 °C.

As the polymers can be functionalized with methacrylate end groups that polymerize upon UV-irradiation, chemical cross-linking can further increase nanotube stability. As in earlier experiments[10] we have seen by Nuclear Magnetic Resonance that the conversion of the methacrylates is almost complete and that the nanotube shapes are therefore permanently fixed. This is also confirmed by dissolution experiments: only the crosslinked nanotubes preserve their structural integrity after dispersion in ethanol or chloroform (which are good solvents for both blocks of the polymers). This is confirmed in Fig. 2 that shows polymerized nanotubes isolated from ethanol. Obviously the incorporation of methacrylate end groups and subsequent UV polymerization does not change the shape of the aggregates but yields a mechanically even more stable structure.

To prove the formation of water-filled tubes (as opposed to rodlike micelles with a hydrophobic interior) we have encapsulated carboxyfluorescein (CF), a water-soluble fluorescent dye, into the nanotubes. To that end, a copolymer film was prepared as before, but was rehydrated with a 10 mM solution of CF in PBS buffer.† The final copolymer concentration was again 1 wt%. Non-encapsulated CF was removed by size exclusion chromatography (Sepharose® 4B, Sigma). The purified sample was re-concentrated by centrifugation (Centricon® YM-100, 100'000 Da nominal molecular weight limit cut-off) and examined with fluorescence microscopy (Leica DMIRE2).

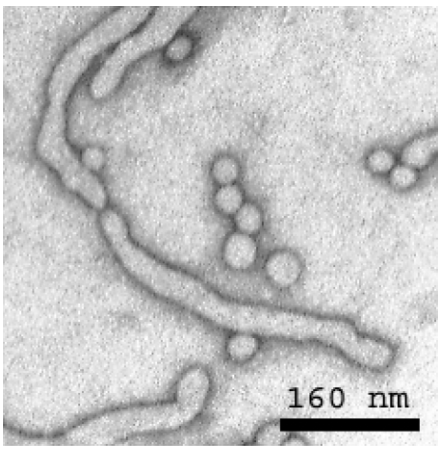

Fig. 2 TEM image of cross-linked nanotubes and vesicles.

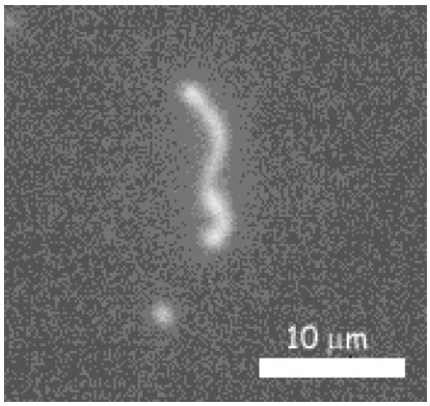

Fig. 3 Fluorescence micrograph of a CF-loaded nanotube.

Fig. 3 shows an intense fluorescence from the interior of a tube (and a vesicle), but no measurable intensity outside. This proves that the inside of the tubes really contains water and CF. This in turn rules out the formation of rodlike micelles. The fluorescence microscopy also demonstrates the exceptional stability and low permeability of the nanotubes because the fluorescence dye CF was kept in the tube during the whole purification process.

In conclusion, we have demonstrated the direct formation of soft water-filled nanotubes from ABA-triblock copolymers by direct self-assembly from a dry film. The nanotubes are mechanically stable and can be loaded with water-soluble substances. The possibility to add reactive groups to the copolymer enables further stabilization by chemical cross-linking; other functional groups may enable specific targeting or molecular recognition. Self-assembled polymer nanotubes may thus find applications as smart drug delivery devices, as highly specific templates for inorganic synthesis, and in nanofluidics, where they could act as connections between different, spatially separated compartments. As an extension of this current study, we are exploring asymmetric ABC triblock copolymers for the fabrication of nanotubes with chemically different inner and outer surfaces.[11]

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Notes and references

† PBS = Phosphate Buffer Saline, pH = 7.4.

1 M. L. Cohen, *Mater. Sci. Eng., C*, 2001, **C15**, 1.
2 C. R. Martin and P. Kohli, *Nature Rev. Drug Discovery*, 2003, **2**, 29.
3 A. I. Smirnov and O. G. Poluektov, *J. Am. Chem. Soc.*, 2003, **125**, 8434.
4 J. M. Buriak and M. R. Ghadiri, *Mater. Sci. Eng., C*, 1997, **C4**, 207.
5 Y.-Y. Won, A. K. Brannan, H. T. Davis and F. S. Bates, *J. Phys. Chem. B*, 2002, **106**, 3354.
6 S. Stewart and G. Liu, *Angew. Chem. Int. Ed.*, 2000, **39**, 340.
7 K. Yu and A. Eisenberg, *Macromolecules*, 1998, **31**, 3509.
8 J. Raez, R. Barjovanu, J. A. Massey, M. A. Winnik and I. Manners, *Angew. Chem. Int. Ed.*, 2000, **39**, 3862.
9 C. Nardin, T. Hirt, J. Leukel and W. Meier, *Langmuir*, 2000, **16**, 1035.
10 W. Meier, T. Hirt and C. Nardin, *PCT Int. Appl.*, 2001, 28.
11 R. Stoenescu and W. Meier, *Chem. Commun.*, 2002, 3016.