Biochemical Engineering & Bioprocess Engineering

OA Biotechnology
Open Access

Review

Fabrication and functionalization of lipid tubular microstructures

HM Bi[1], XJ Han[1,2]*

Abstract

Introduction

The fabrication methods and functionalization of lipid tubes are described. The fabrication methods of lipid tubes were classified into three categories, i.e. self-assembly of lipid molecules, microfluidic way to form tubes and stretching of giant vesicles using an electric field. The functionalization of lipid tubes was summarized as metallization and deposition of inorganic silica onto tube surface, encapsulation of nanoparticles into hollow cylindrical tube of tubular microstructures, and embedding nanoparticles into the wall of lipid tubes to form functional nanocomposites or nanostructures. The applications of these nano- and microstructures are also given. This review gives a brief overview of the interesting field of lipid tubes.

Conclusion

The fabrication of tubular microstructures demonstrated the precise self-assembly property of lipid molecules. To explore a new universal fabrication method for lipid tubes is still a direction of this field. Templating technology using lipid tubular microstructures is a promising route to make diverse nanostructures. Duo to the high biocompatibility of lipid tubes, they can be designed as drug carriers to encapsulate hydrophilic molecules inside the tube or hydrophobic molecules within the lipid bilayer for drug delivery in biomedical research.

* Corresponding author
Email: hanxiaojun@hit.edu.cn

[1] School of Chemical Engineering and Technology, Harbin Institute of Technology, No. 92, West Da-Zhi Street, Harbin, 150001 China

[2] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, No. 73 Huanghe Road, Harbin 150090, China

Introduction

Living systems are composed of various microstructures of different sizes. Lipids are the basic and important building blocks of biological membranes. Lipid tube, a self-assembled hollow cylindrical tube of lipid molecules, is widespread in nature. It plays an important role in energy and matter transmission of cell-to-cell in vivo[1]. The directed transport of ions and proteins has been monitored in a diverse range of cells, including lymphocytes, macrophages, adrenal cells and cardiomyocytes[2]. Furthermore, non-conjugative plasmids, containing hereditary genetic information, could be transferred from one cell to the recipient cells[3]. It has attracted considerable attention due to their interesting applications in many fields. Fabrication of functional composite structures using lipid tube as a template has been an exciting field in vitro. Stable and highly controlled lipid tubes can be used to synthesize nanostructures such as rods, wires and tubes[4]. After the first lipid tube was artificially created[5] a number of techniques for fabricating lipid microstructures have been developed. Coupled with advanced chemical and medicinal characterization techniques, the functionalization of the lipid nano- and microtube also made great progress[6]. These provide us with insights into the functions of the lipid nano- and microtube in living organisms[3]. Considering the recent developments and the increasing use of lipid tubes, there is a demand for documenting their preparation methods and their applications. This paper gives an overview of the methods used in the fabrication of nano- and microlipid tubes and their potential applications. The fabrication methods are categorized according to the driving force for tube formation, i.e. the manner of self-assembly of lipid molecules, the manner in which fluid flows are used, and the manner in which electric fields are used. The functionalization of lipid tube is discussed with respect to the following aspects: metallization and deposition of inorganic silica onto tube surfaces and encapsulation or embedding of nanoparticles into the tubes. This review can also be helpful to design and engineer nano- and microstructures for advanced materials and sensors using biomolecular self-assembly techniques[7].

Discussion

Methods of Lipid Tube Formation

Self-assembly of lipid molecules

Synthetic lipid tubes were first created more than three decades ago by Yager and Schoen[5]. They observed hollow tube-shaped microstructures when cooling 1,2-bis (10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine ($DC_{8,9}PC$) vesicles through its chain melting transition temperature. These tubes were approximately 0.5 μm in diameter and from 1 to over 200 μm in length. Later on, Burke[8] and co-workers improved the conversion efficiency to almost 100% by cycling the process. However it was difficult to control the required homogeneous temperature variation with the proper precision. A new process of tube formation was developed

FOR CITATION PURPOSES: *Bi HM, Han XJ*. Fabrication and functionalization of lipid tubular microstructures. OA Biotechnology 2013 Jan 31;2(1):7.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Biotechnology
Open Access

Review

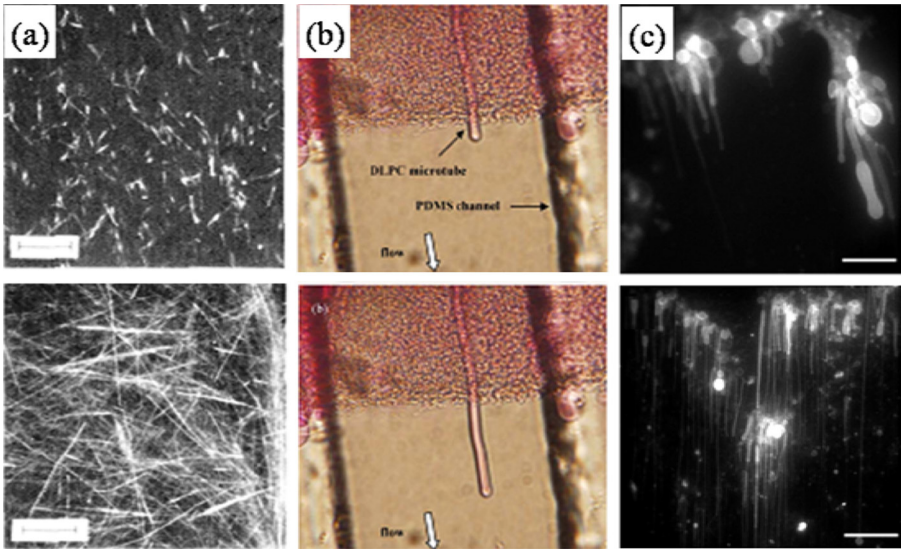

Figure 1: (a) Darkfield optical micrographs of tubes formed by addition of water to ethanol solution of $DC_{8,9}PC$. The micrographs were taken at room temperature. The final concentration of the lipid was 0.5 mg/ml. The final concentrations (v/v) of ethanol were 55% (top) and 70% (bottom). The incubation times in the ethanol–water mixtures were 10 h (top) and 6 months (bottom). Scale bars = 100 μm. Reprinted with permission from ref. 10. Copyright (1987) American Chemical Society. (b) Time-serial images of the growing single-aligned DLPC microtube in the microchannel: 0 s (top) and 7 s (bottom). Reprinted with permission from ref. 14. Copyright (2006) Elsevier B.V. (c) Fluorescence micrographs of lipid nanotubes from a liposome composition of 89:10:1 PC:PA:OG-DHPE at an applied voltage of 8 V/cm(top,) and from a liposome composition of 99:1 PC:OG-DHPE at 10 V/cm (bottom). Scale Bar = 50 μm. Reprinted with permission from ref. 20. Copyright (2009) American Chemical Society.

to use an ethanol/lipid/water solution at a constant temperature or over a narrow temperature range. Yager's group[9,10] used the monomeric lecithins $DC_{8,9}PC$ to form tubular microstructures by precipitation of the lipid from ethanol upon addition of water, as shown in Figure 1a. Lipid tubes of different sizes were obtained using this method. Self-assembly method is an easy way, but it has its limitations with respect to special lipids, such as diacetylenic phospholipid $DC_{8,9}PC$.

Microfluidic ways to form lipid tubes

Microfluidic ways are commonly used for lipid tube preparations. Brazhnik et al.[11] used microfluidic systems to create natural phosphatidylcholine (PC) micro- and nanotubes with unprecedented lengths, i.e. several centimetres in length. The hollow tubular structure was confirmed by observing fluorescent dyes inside the tube. Recently, Sugihara et al.[12] reported that lipid blocks in inverted hexagonal phase made of 1,2-dioleoyl-sn-glycero -3-phosphoethanolamine (DOPE) can protrude into lipid nanotubes upon a fluid dynamic flow in a physiological buffer solution. The outer diameter of the tubes was 19.1 ± 4.5 nm and their lengths were up to several hundred micrometres. West et al.[13] obtained lipid tubes with a major diameter of about 3.6 μm by microfluidic tweezing. Lin et al.[14] manufactured a series of phospholipid microtubes (1~10 μm in diameter) from dried thin phospholipid lms based on micro-electromechanical systems technology and micro fluidic technology. They also studied the parameters influencing the generation of lipid tubes, including flow rate, hydration temperature, pH of the aqueous solution and phospholipids concentration. The formed tubes at different stages are shown in Figure 1b. Dittrich et al.[15] applied photolithography techniques to fabricate micro-sized apertures that provide a way to form lipid structures with predictable sizes. When a vacuum pump was connected to the outlet, lipid tubes with a perfectly homogeneous diameter and extraordinary length were generated.

The aforementioned methods are all based on lipid films. In fact, the preformed vesicles were stretched into lipid tubes with flows. Heinrich et al.[16] analysed this phenomenon by means of general mathematical methods. Although a bilayer tube formed from a closed vesicle is not an ideal cylinder, it provides the theoretical basis for practical purposes. Rossier and co-workers[17] theoretically and experimentally discussed the dynamical laws that governed extrusion and retraction of tubes extracted from lipid vesicles at a high speed and under strong flows. It provides the first evidence that the tension along the tube increases from the vesicle body to the tip of the tube. The dynamical laws for retraction are in good quantitative agreement with these theoretical predictions. Recently, Sekine et al.[18] used a homogeneous laminar shear flow to prepare lipid nanotubes from surface-immobilized liposomes on a solid substrate by avidin–biotin interactions. They obtained long lipid nanotubes arranged in a well-controlled direction.

Microfluidic networks can also be used to generate lipid tube arrays. Mahajan and Fang[19] showed that lipid nano- and microtubes could be assembled into two-dimensional (2-D) parallel arrays with controlled separations by fluidic alignment. The aligned lipid tubes in the recessed channels can be transferred onto different types of substrates with

FOR CITATION PURPOSES: *Bi HM, Han XJ*. Fabrication and functionalization of lipid tubular microstructures. OA Biotechnology 2013 Jan 31;2(1):7.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Biotechnology
Open Access

polydimethylsiloxane (PDMS) stamp to form 2-D arrays of aligned lipid tubes. The method enables the alignment and patterning of lipid nanotubes into various (including curvy) shapes with a microfluidic system.

Giant vesicles stretching using an electric field

Another driving force of forming lipid tube comes from electric fields. Castillo et al.[20] fabricated lipid tubular structures by modest electric fields. They attached preformed giant vesicles onto a glass substrate between two electrodes, and then studied lipid tube formation by varying field strength and lipid composition. The liposomes were prepared with phosphatidylcholine (PC), phosphatidic acid (PA), phosphoethanolamine (PE) and cholesterol. They can form long-range (millimetres) lipid nanotubes. The formed tubes were generally aligned with the electric field. Figure 1c shows the tubular alignment under different zwitterionic-to-charged lipid ratio (by mass) and field strength. Hayes's group[21] also found that individual vesicles can be stretched into nanotubes in an electric field.

Functionalization of lipid tubes

The tubular architectures of lipids can serve as templates for mineral nucleation, deposition of inorganic silica and encapsulation or embedding of nanoparticles for making functionalized nanostructures. Due to their large geometrical aspect ratios, they are also well suited for the fabrication of magnetic and electrically conductive nanowires using template technology.

Metallization of lipid tubes

The early report utilizing phospholipid tubes as templates to fabricate metal tubular microstructures was carried out by Schnur's group[22]. The lipid tubes, derived from $DC_{8,9}PC$, acted as an excellent scaffold to create metal nanotubes by coating the lipid tubes with elegant electroless

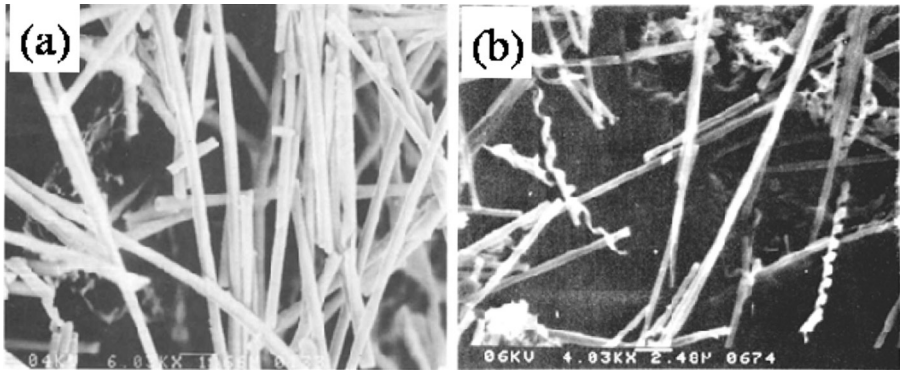

Figure 2: (a) SEM of tubes plated with electroless nickel. Reprinted with permission from ref. 22. Copyright (1987) published by Elsevier. (b) SEM of tubes and helices formed from $DC_{8,9}PC$ at 50% 2-propanol in water that were subsequently coated with copper metal. Note that all helical structures are right handed and that the pitch of the helices is somewhat variable. Scale bar = 2.48 μm. Reprinted with permission from ref. 10. Copyright (1987) American Chemical Society.

plating processes. The electroless metallization technique allows the nanotubes to be clad with a uniform layer of metals on the exterior and interior surfaces. The thickness of the metal layer can be controlled by change of plating solution concentration and plating time. The lipid tubes can be metallized with any metal capable of being plated, such as Ni^{2+}. The formed metal tubes are shown in Figure 2a. Almost at the same time, Georger et al.[10] applied this technique to deposit a thin copper layer on the lipid tube surface. Figure 2b shows a scanning electron micrograph (SEM) of copper-coated $DC_{8,9}PC$ tubes and helices. These robust metal-covered objects are three-dimensional. It can also be seen that the helices formed from the L stereoisomer of the lipid are right handed. Banerjee et al.[23] also fabricated Cu nanotubes using sequenced histidine-rich peptide lipid nanotubes. The sequenced histidine-rich peptide lipid molecules were assembled as nanotubes that can bind Cu ions on the nanotube surface. Those nanotubes showed significant change in electronic properties by varying the nanocrystal diameter. Therefore, this system may be developed to a conductivity-tunable building block for microelectronics and biological sensors. Besides the electroless plating technique, using the capacity of lipid bilayers to bind metal cations from aqueous solution, particularly divalent and trivalent species, aluminium mineral microtubes were prepared on the nanotube of $DC_{8,9}PC$. Mann's group[24] reported a nanometre-thick, continuous coating of a magnesium phyllo(organo) silicate clay using self-assembled nanotubes of $DC_{8,9}PC$.

Considering the stability of tubes, the metallization of lipid tubes is mainly restricted to the diacetylenic phospholipid. The above examples of metallization mainly used lipid of $DC_{8,9}PC$. It is a knotty problem to realize this application with natural lipids such as egg PC.

Deposition of silica on lipid tubes

After Schnur's group[22] reported that silica films grew on the nanotube of $DC_{8,9}PC$, Baral and Schoen[25] applied sol–gel methods to deposit thin silica films (Figure 3a) on self-organized lipid tubes to stabilize the tubular structure. The silica film on the tube surface remained continuous after extensive washing with water, indicating a strong adherence of the silica film

FOR CITATION PURPOSES: *Bi HM, Han XJ*. Fabrication and functionalization of lipid tubular microstructures. OA Biotechnology 2013 Jan 31;2(1):7.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Biotechnology
Open Access

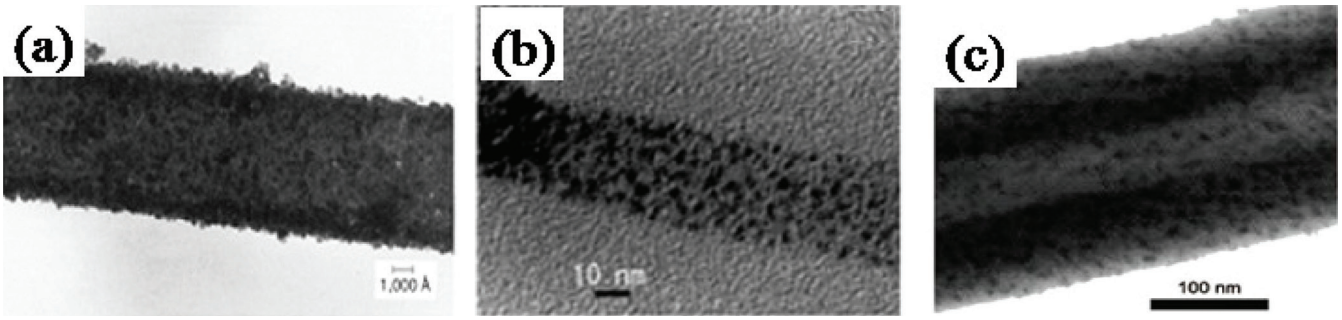

Figure 3: (a) Transmission electron micrograph (TEM) of a silica coated $DC_{8,9}PC$ phospholipid tube. Reprinted with permission from ref. 25. Copyright (1993) American Chemical Society. (b) TEM of a lipid nanotube loaded with 2–4 nm silver nanoparticles. Reprinted with permission from ref. 29. Copyright (2004) American Chemical Society. (c) STEM for the lipid nanotube of synthetic peptide lipid where the CdS nanodots are embedded all over the lipid bilayer membranes. STEM was reproduced with permission from ref. 31. Copyright 2007 Wiley-VCH.

to the tube surface. Ji et al.[26] utilized a glycolipid nanotube as a template to form silica-lipid-silica laminated structures by sol–gel polymerization of tetraethyl orthosilicate (TEOS). They obtained silica tubular structures with different diameters. Moreover, by changing the template morphology by addition of ethanol in a silica/lipid xerogel, they obtained tube-in-tube silica nanotubes after calcination[27]. Because of the excellent biocompatibility of silica, deposition or coating of lipid tubes offered them potential applications in long-term sustained-release systems in agriculture, environment and medicine[28].

Encapsulation or embedding of nanoparticles in lipid tubes

Apart from the surface modification of lipid tubes, encapsulation and embedding of appropriate nanoparticles into hollow cylinder and membrane lamella of lipid tubes, respectively, were studied by Shimizu's group[29,30]. They have succeeded in filling gold and silver nanoparticles into lyophilized lipid nanotubes to realize the 1-D arrangement of the nanodots that are shown in Figure 3b. It shows the remarkable size effect of the nanodots in the 1-D organization profile in the lipid nanotube hollow cylinder. The bilayer membrane wall also serves as an embedding matrix of nanoparticles to form functional nanocomposites or nanostructures. Shimizu's group[31] embedded CdS into bilayer membranes of a synthetic peptide lipid to obtain fluorescent nanotubes, as shown in Figure 3c. Cd^{2+} coordinates to two negatively charged COO -groups of the lipid to form Cd-complexes. Upon exposure to H_2S vapour, the Cd^{2+} in the Cd-lipid nanotubes was released to react with S^{2-}, subsequently to initiate CdS nuclei and finally growing to the CdS QDs all over the lipid bilayer membranes. Encapsulation and embedding of nanoparticles into hollow cylinder and membrane lamella of lipid tubes achieved the integration of inorganic particles into organic matrices, which enabled us to produce tailor-made-type nanotube hosted with the desired functionalities not only in biological material but also in other nanocomposite fields.

Conclusion

The fabrication of tubular microstructures demonstrated the precise self-assembly property of lipid molecules. To explore a new universal fabrication method for lipid tubes is still a direction of this field. Templating technology using lipid tubular microstructures is a promising route to make diverse nanostructures. The current achievements of this route have spurred intense and rapid progress. Mineralization of lipid tube can be applied to fabricate various metallic and semiconductor nanotubes. Besides the existing elements of lipid tubular modification, it should also be possible to decorate these structures with other molecules to exploit particular functionalized microstructures. The recognition ability of lipid molecules enables us to manipulate 1-D nanostructures into 3-D or new super molecular architectures in the near future.

In addition, because of the high biocompatibility, flexibility and easy modification of the surfaces of lipid tubes, they were designed as drug carriers to encapsulate hydrophilic molecules inside the tube or hydrophobic molecules within the lipid bilayer for drug delivery in biomedical research[32,33]. Once the drug carrier reaches the specific target, one can control the drug release by applying extrinsic stimulation. The lipid tubular and functional lipid tubular microstructures have a potential application in the field of biomedicine.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 21003032, 21273059), program for New Century Excellent Talents in University (Grant No. NCET-09-0054).

FOR CITATION PURPOSES: *Bi HM, Han XJ*. Fabrication and functionalization of lipid tubular microstructures. OA Biotechnology 2013 Jan 31;2(1):7.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

OA Biotechnology
Open Access

Review

References

1. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. Science. 2004 Feb;303(5660):1007–10.
2. Liu HQ, Bachand GD, Kim H, Hayden CC, Abate EA, Sasaki DY. Lipid nanotube formation from streptavidin-membrane binding. Langmuir. 2008 Apr;24(8):3686–9.
3. Dubey GP, Ben-Yehuda S. Intercellular nanotubes mediate bacterial communication. Cell. 2011 Feb;144(4):590–600.
4. Tanaka M, Critchley K, Matsunaga T, Evans SD, Staniland SS. Fabrication of lipid tubules with embedded quantum dots by membrane tubulation protein. Small. 2012 May;8(10):1590–5.
5. Yager P, Schoen PE. Formation of tubules by a polymerizable surfactan. Mol Cryst Liq Cryst. 1984;106(3–4):371–81.
6. Nakashima N, Asakuma S, Kunitake T. Optical microscopic study of helical superstructures of chiral bilayer-membranes. J Am Chem Soc. 1985 Jan;107(2):509–10.
7. Schnur JM. Lipid tubules–A paradigm for molecularly engineered structures. Science. 1993 Dec;262(5140):1669–76.
8. Burke TG, Rudolph AS, Price RR, Sheridan JP, Dalziel AW, Singh A, Schoen PE. Differential scanning calorimetric study of the thermotropic phase-behavior of a polymerizable, tubule-forming lipid. Chem Phys Lipids. 1988 Oct;48(3–4):215–30.
9. Chappell JS, Yager P. Formation of mineral microstructures with a high aspect ratio from phospholipid-bilayer tubules. J Mater Sci Lett. 1992 May;11(10):633–6.
10. Georger JH, Singh A, Price RR, Schnur JM, Yager P, Schoen PE. Helical and tubular microstructures formed by polymerizable phosphatidylcholines. J Am Chem Soc. 1987 Sep;109(20):6169–75.
11. Brazhnik KP, Vreeland WN, Hutchison JB, Kishore R, Wells J, Helmerson K, Locascio LE. Directed growth of pure phosphatidylcholine nanotubes in microfluidic channels. Langmuir. 2005 Nov;21(23):10814–7.
12. Sugihara K, Chami M, Derenyi I, Voros J, Zambelli T. Directed self-assembly of lipid nanotubes from inverted hexagonal structures. Acs Nano. 2012 Aug;6(8):6626–32.
13. West J, Manz A, Dittrich PS. Lipid nanotubule fabrication by microfluidic tweezing. Langmuir. 2008 Jun;24(13):6754–8.
14. Lin YC, Huang KH, Chiang JT, Yang CH, Lai TH. Manipulating self-assembled phospholipid microtubes using microfluidic technology. Sensor Actuat B. 2006 Oct;117:464–71.
15. Dittrich PS, Heule M, Renaud P, Manz A. On-chip extrusion of lipid vesicles and tubes through microsized apertures. Lab Chip. 2006 Apr;6(4):488–93.
16. Heinrich V, Bozic B, Svetina S, Zeks B. Vesicle deformation by an axial load: From elongated shapes to tethered vesicles. Biophys J. 1999 Apr;76(4): 2056–71.
17. Rossier O, Cuvelier D, Borghi N, Puech PH, Derenyi I, Buguin A, Nassoy P, Brochard-Wyart F. Giant vesicles under flows: Extrusion and retraction of tubes. Langmuir. 2003 Feb;19(3):575–84.
18. Sekine Y, Abe K, Shimizu A, Sasaki Y, Sawada S, Akiyoshi K. Shear flow-induced nanotubulation of surface-immobilized liposomes. RSC Advances. 2012 Apr;2(7):2682–4.
19. Mahajan N, Fang JY. Two-dimensional ordered arrays of aligned lipid tubules on substrates with microfluidic networks. Langmuir. 2005 Mar;21(7):3153–7.
20. Castillo JA, Narciso DM, Hayes MA. Bionanotubule formation from surface-attached liposomes using electric fields. Langmuir. 2009 Jan; 25(1):391–6.
21. Hayes MA, Pysher MD, Chen KP. Liposomes form nanotubules and long range networks in the presence of electric field. J Nanosci Nanotechnol. 2007 Jul ;7(7):2283–6.
22. Schnur JM, Price R, Schoen P, Yager P, Calvert JM, Georger J, Singh A. Lipid-based tubule microstructures. Thin Solid Films. 1987 Sep;152(1–2):181–206.
23. Banerjee IA, Yu L, Matsui H. Cu nanocrystal growth on peptide nanotubes by biomineralization: Size control of Cu nanocrystals by tuning peptide conformation. PNAS. 2003 Dec;100(25):14678–82.
24. Patil AJ, Muthusamy E, Seddon AM, Mann S. Higher-order synthesis of organoclay pipes using self-assembled lipid templates. Adv Mater. 2003 Nov;15(21):1816–9.
25. Baral S, Schoen P. Silica-deposited phospholipid tubules as a precursor to hollow submicron-diameter silica cylinders. Chem Mater. 1993 Feb;5(2):145–7.
26. Ji QM, Kamiya S, Jung JH, Shimizu T. Self-assembly of glycolipids on silica nanotube templates yielding hybrid nanotubes with concentric organic and inorganic layers. J Mater Chem. 2005;15(7):743–8.
27. Ji QM, Iwaura R, Shimizu T. Regulation of silica nanotube diameters: Sol-gel transcription using solvent-sensitive morphological change of peptidic lipid nanotubes as templates. Chem Mater. 2007 Mar;19(6):1329–34.
28. Zhou Y, Shimizu T. Lipid nanotubes: A unique template to create diverse one-dimensional nanostructures. Chem Mater. 2008 Feb;20(3):625–33.
29. Yang B, Kamiya S, Shimizu Y, Koshizaki N, Shimizu T. Glycolipid nanotube hollow cylinders as substrates: Fabrication of one-dimensional metallic-organic nanocomposites and metal nanowires. Chem Mater. 2004 Jul;16(14):2826–31.
30. Shimizu T. Self-assembled lipid nanotube hosts: The dimension control for encapsulation of nanometer-scale guest substances. J Polym Sci Part A: Polym Chem. 2006 Sep;44(17):5137–52.
31. Zhou Y, Kogiso M, He C, Shimizu Y, Koshizaki N, Shimizu T. Fluorescent nanotubes consisting of CdS-embedded bilayer membranes of a peptide lipid. Adv Mater. 2007 Apr;19(8):1055–8.
32. Nappini S, Al Kayal T, Berti D, Norden B, Baglioni P. Magnetically triggered release from giant unilamellar vesicles: Visualization by means of confocal microscopy. J Phys Chem Lett. 2011 Apr;2(7):713–8.
33. Furt F, Moreau P. Importance of lipid metabolism for intracellular and mitochondrial membrane fusion/fission processes. Int J Biochem Cell Biol. 2009 Oct;41:1828–36.

FOR CITATION PURPOSES: *Bi HM, Han XJ*. Fabrication and functionalization of lipid tubular microstructures. OA Biotechnology 2013 Jan 31;2(1):7.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.