MedCrave
Step into the World of Research

Journal of Nanomedicine Research

Architecting polymersomes from spherical to tubules by molecular alterations

Abstract

Polymersomes formed by the self-assembling of block copolymers gained huge attraction recently due to it numerous potential applications especially as nano-reactors, tunable controlled delivery carriers, templates for bio-mineralization etc. The self-assembly of these block copolymers amphiphiles into monodisperse structures in aqueous solutions is influenced by their hydrophobic and hydrophilic molecular regions. However the commonly investigated structures are spherical vesicles, micelles and worm like micelles. This study focuses on generating tubular vesicles or "Tubularsomes" by altering the amphiphilic block length of the polymer chain. Polyoxazoline (POx) is covalently conjugated with polydimethyl siloxane (PDMS) to form block copolymers and explored the self-assembling behavior with special focus on the influence of chain length forming various structures. Additionally, the current limitations in the reproducibility and biocompatibility during the synthesis and utilization of biologically important polymer vesicles are explored along with enabling the development of these nanostructures towards real-world applications.

Keywords: Polymersomes, Tubularsomes, Vesicles, Packing parameter

Research Article

Volume 7 Issue 1 - 2018

Satarupa Dhir[1,2], Sumalee Salahub[1,2], Anu Stella Mathews[1,2], Carlo Montemagno[3], Sinoj Abraham[1,2,4]

[1]Ingenuity Lab, Canada
[2]Chemical and Materials Engineering Department, University of Alberta, Edmonton, Canada
[3]Southern Illinois University, USA
[4]Mechanical Engineering Department, University of Alberta, Edmonton, Canada

Correspondence: Sinoj Abraham, Adjunct Professor, Mechanical Engineering Department, University of Alberta, Edmonton, Alberta T6G 1H9, Canada, Email sinoj@ualberta.ca

Received: February 10, 2018 | **Published:** February 26, 2018

Introduction

There is a constant demand for well-defined block-copolymers with reproducible molecular characteristics and polydispersity, which are a prerequisite for various applications including controlled biological delivery systems, hydrogels, catalysis etc.[1-3] "Polymersomes" or polymer vesicles are self-assembled structures formed from block copolymers. Compared to traditional liposomes, polymersomes provide robustness and enhanced stability both in terms of chemical and physical attributes and hence have gained popularity for numerous potential.[4-6] The hydrophilic and hydrophobic properties of the diblock-copolymers makes polymersomes a potential competitor for various nano-medical systems.[7,8] The hydrophilic corona can be utilized to encapsulate water soluble agents while the hydrophobic part can have various proteins embedded. The size and structure of polymersomes plays a crucial role in implementing functions.[9] The amphiphilic can self-assemble into simple morphologies like spherical micelles, simple vesicles, onion ring shaped vesicles, rods or more complex structures like toroid, compound micelles with inverted core shell structures.[10] Morphology of the block-copolymers is controlled by different forces such as interactive forces between hydrophobic and hydrophilic blocks, degree of stretching of core blocks and repulsive interaction among corona chains.[11,12] One of the major parameter affecting the self-assembly of block-copolymers can be explained in terms of classical dimensionless "packing parameter" or "P_c".[13] P_c is mathematically represented as: $P_c=v/a_0l_c$ where v is the volume of hydrophobic chain, a_0 is area occupied by hydrophilic head group and l_c is the length of molecule. With P_c values below 0.5, the self-assemblies are in form of spheres, cylinders or worm like micelles, whereas with increasing P_c values until 1, bilayers are formed. Factors such as solution pH, temperature, type of organic solvent, initial copolymer concentration, chemical structure of copolymer, presence of additives and hydrophilic/hydrophobic ratio determine the packing factor.[14-17] When the hydrophilic to hydrophobic ratio is greater than 1:1 the self-assembly is usually micelles. Ratios less than 1:2 usually favor vesicles while that less than 1:3 may form complex structures like inverted micro-structures along with vesicles.[18,19] Changing the block length of hydrophilic or hydrophobic chain in the copolymers may lead to switching between morphologies or make the copolymer sensitive to stimuli like pH, temperature and solvent polarity.[20] This helps in controlled release phenomena as well as in sensing applications. In earlier works by Choe et al.[21] the amphiphilic block co-polypeptide was tuned to achieve different hydrophobic lengths. Varying hydrophobic chain length led to monodisperse vesicles with low toxicity and better stability. Yingchao Chen et al.[22] blended amphiphilic block copolymers poly(acrylic acid)-block-poly-isoprene and poly(acrylic acid)-block-polystyrene to yield various self-assembly from spherical to rod like micelles. Extreme slow or medium rate of water addition to the co-polymer blends resulted in mixture of vesicle and rod like conformations. In similar study by Yoshida[23] spherical vesicles produced only by poly(methacrylic acid)-block-poly(methyl methacrylate-random-methacrylic acid) copolymer were changed into worm-like vesicles with an uneven surface upon long poly(methacrylic acid) copolymer incorporation. In the last 20 years polymer vesicles with spherical structure morphology has been the focus of extensive research. However, other interesting structures like cylindrical or rod like shape have been gaining interest particularly due to their potential application in the field of medicine and nano-technology.[24] Due its typical structure and flexibility, cylindrical vesicles or micelles can stretch and orient themselves for flow-intensive systems such as phage-mimetic or micro-pore drug delivery systems.[25,26] With spherical structures being thermodynamically stable, the cylindrical conformations were produced by blending degradable polylactic acid with inert block copolymer.[26] This morphological

change enabled the rod-like micelles to penetrate nano-porous gels, thereby indicating the capability of tissue permeation and controlled drug release. In the current work, we synthesized amphiphilic poly (2-ethyloxazoline-block-dimethylsiloxane-block-2-ethyloxazoline) (PEOXA-b-PDMS-b-PEOXA) with varying PEOXA chain length and poly (2-methyloxazoline-block-dimethylsiloxane-block-2-methyloxazoline) (PMOXA-b-PDMS-b-PMOXA). The change in morphologies due to different hydrophilic chain length and chemical structure were studied via transmission electron microscopy (TEM) and dynamic light scattering (DLS) (Figure 1).

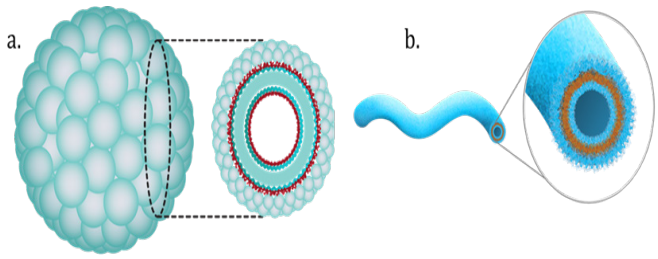

Figure 1 Schematic representation of a. Polymersome; b. Tubularsome.

Materials and methods

PEOXA-b-PDMS-b-PEOXA was synthesized via cationic ring opening polymerization of 2-ethyl oxazoline. The preliminary step had monomer 1,3-bis(4-hydroxybutyl)tetramethyldisiloxane initiated with one of the Brönsted acid, trifluoromethanesulfonic acid anhydride to form a living polymer chain. The length of PEOXA block was adjusted by addition of varying the amount of freshly distilled 2-ethyl-2-oxazoline. Finally, the polymerization was terminated by addition of 0.5 M KOH in ethanol. For the this study the hydrophobic PDMS block was maintained at 5600 $gmol^{-1}$while the molecular weight of the hydrophilic end group PEOXA was varied from 1088 to 1396 $gmol^{-1}$. For synthesis of PMOXA-b-PDMS-b-PMOXA, the PDMS block was maintained at 5600 $gmol^{-1}$ while the hydrophilic PMOXA end group was kept at 1500 $gmol^{-1}$. Synthesis of PMOXA was accomplished using a propargyl tosylate initiator.[27] PDMS was converted into tosylate end groups TsO-PDMS-OTs. This tosylation made it easier to be transformed into azides N_3-PDMS-N_3 via nucleophilic substitution. Finally, copper-catalyzed alkyne-azide cycloaddition (CuAAC) click reaction was utilized to achieve high yield PMOXA-b-PDMS-b-PMOXA.

Both polymersomes and tubularsomes were prepared by popular "film rehydration method". Film rehydration technique involved dissolving the block copolymers in organic solvent like chloroform with subsequent film formation by evaporation of the solvent using a rotavaporator. The film was further placed under high vacuum overnight to remove any traces of organic solvent. Consequently Milli-Q water was added to the dry film followed by continuous stirring for 4 hours to rehydrate the film. The suspension obtained was then extruded through two polycarbonate membrane filters (Whatman nucleopore membrane) with nominal diameter of 400 nm held in an extruder using 200 psi nitrogen gas. The number of times the sample was extruder was maintained at 10 passes to reach a uniform size distribution. The scanning electron micrograph in TEM mode was acquired on Hitachi S4800 field emission microscope operating at 30 kV and 20 μA. The diluted suspension of vesicles were dropped on carbon-coated TEM grids followed by removal of excess using Kim wipes. One drop of staining solution 2% uranyl acetate was placed on the grid for 30 seconds which was also removed by Kim wipes. The TEM grids were placed under vacuum for 1 hour to completely remove the solvent after which they were analyzed under microscope. Dynamic light scattering (DLS) study was carried out on Malvern Nano ZS zetasizer at room temperature with back scattering angle θ = 173°.

Results and discussion

Figures 2&3 shows the TEM images of tubularsomes of various PEOXA chain length block copolymers and confirm that the major population is of tubular morphology with dark regions corresponding to uranyl acetate staining of PDMS domains and lighter areas corresponding to hydrophilic POx segments. The individual tubular vesicle formed from $PEOXA_{11}$-*b*-$PDMS_{72}$-*b*-$PEOXA_{11}$ exhibited a uniform external diameter of 29 nm and length 50~250 nm. A tubular vesicle formed from the $PEOXA_{14}$-*b*-$PDMS_{72}$-*b*-$PEOXA_{14}$ had a diameter of 36 nm and length of 50~450 nm. The hydrodynamic diameters obtained from DLS, Figure 5, for $PEOXA_{14}$-*b*-PDMS-*b*-$PEOXA_{14}$ were 100-500 nm and 110-600 nm for $PEOXA_{11}$-*b*-PDMS-*b*-$PEOXA_{11}$. As illustrated in Figure 4&5, the vesicle formed from $PMOXA_{18}$-b-PDMS-b-$PMOXA_{18}$ triblock was spherical in structure with an average diameter of 250 nm and hydrodynamic diameter of 150-250 nm.The triblock copolymers formed worm like tubular to spherical structures upon varying the experimental parameters. The morphology change depended on the hydrophilic weight fraction, suggesting a strong effect of the PEOXA block length as well as change of PEOXA into PMOXA on self-assembly of the vesicles. Due to the amphiphilic nature of POx-*b*-PDMS-*b*-POx having both hydrophobic and hydrophilic domains, the volume fractions in aqueous solution formed a micro-phase separated regime of the polymer domains which self-assembled into lamellae structures. The triblock copolymer in the films showed only limited micro-phase separation and on contact with water or buffer solution resulted in micro-phase separation into hexagonally packed rods, with further hydration leading to the formation of lamellae. The spontaneous curvature formation of the triblock copolymer occurs when the effective hydrophilic area increases. This change in morphology is critical while designing vesicles for applications such as long tubular vesicles provide much needed surface area for encapsulation of drugs and easier transport through fine blood vessels (Figure 2-5).

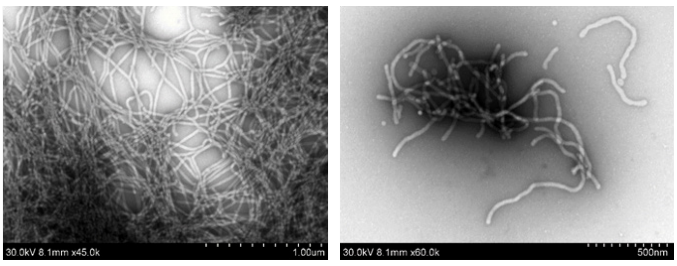

Figure 2 Scanning electron microscopy images of $PEOXA_{11}$-b-PDMS-b-$PEOXA_{11}$.

Citation: Dhir S, Salahub S, Mathews AS, et al. Architecting polymersomes from spherical to tubules by molecular alterations. *J Nanomed Res.* 2018;7(1): 00176. DOI: 10.15406/jnmr.2018.07.00176

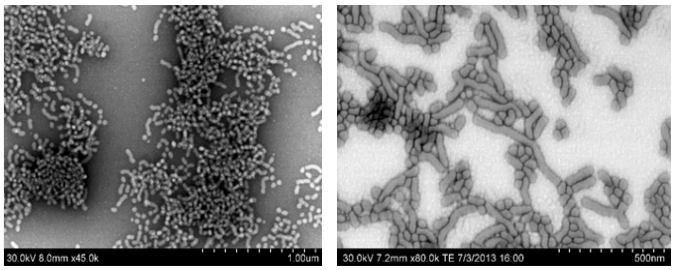

Figure 3 Scanning electron microscopy images of $PEOXA_{14}$-b-PDMS-b-$PEOXA_{14}$.

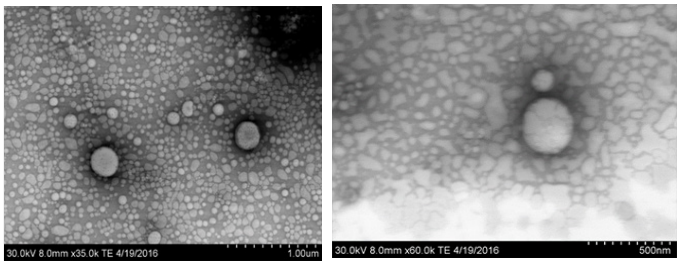

Figure 4 Scanning electron microscopy images of $PMOXA_{18}$-b-PDMS-b-$PMOXA_{18}$.

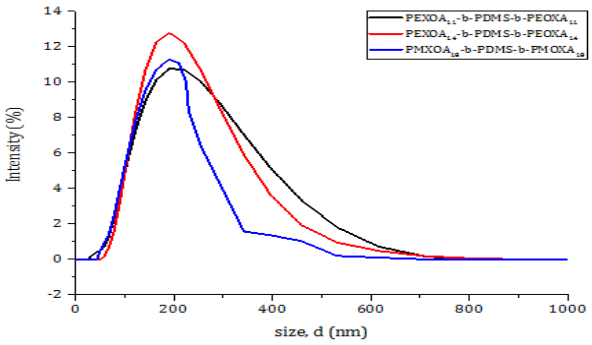

Figure 5 Dynamic light scattering plot of POx-b-PDMS-b-POx.

Conclusion

In summary, changing the hydrophilic chain length of the block copolymer constitutes an efficient an elegant way of controlling the stability, morphology and size of vesicles formation. The spontaneous formation of vesicles was simply achieved by changing the block length of same appropriately chosen hydrophilic material. Longer the hydrophilic chain, more pronounced spherical is structure of vesicles. This important research work helps us to tailor make the vesicles based on the applications in pharmacy, drug-delivery and cosmetics industry.

Acknowledgements

The authors would like to acknowledge the National Institute for Nanotechnology (NINT) and Nanofab of University of Alberta for the equipment and instrumentation. This work was supported by the Province of Alberta, Alberta Innovates Technology Futures (AITF) and the National Research Council.

Conflict of interest

There is no conflict of interest.

References

1. Bellomo EG, Wyrsta MD, Pakstis L, et al. Stimuli-responsive polypeptide vesicles by conformation-specific assembly. *Nat Mater*. 2004;3:244–248.
2. Litvinchuk S, Lu Z, Rigler P, et al. Calcein release from polymeric vesicles in blood plasma and PVA hydrogel. *Pharm Res*. 2009;26(7):1711–1717.
3. Meng F, Zhong Z, Feijen J. Stimuli-Responsive polymersomes for programmed drug delivery. *Biomacromolecules*. 2009;10(2):197–209.
4. Discher BM, Won YY, Ege DS, et al. Polymersomes: Tough vesicles made from diblock copolymers. *Science.* 1999;284(5417):1143–1146.
5. Bermudez H, Brannan AK, Hammer DA, et al. Molecular weight dependence of polymersome membrane structure, elasticity and stability. *Macromolecules*. 2002;35(21):8203–8208.
6. Nardin C, Winterhalter M, Meier W. Giant free-standing ABA triblock copolymer membranes. *Langmuir*. 2000;16:7708–7712.
7. Langowska K, Palivan CG, Meier W. Polymer nanoreactors shown to produce and release antibodies locally. *Chem Commun*. 2013;49(2):128.
8. Sauer M, Haefele T, Graff A, et al. Ion-carrier controlled precipitation of calcium phosphate in giant ABA triblock copolymer vesicles. *Chem Commun.* 2001;23:2452–2453.
9. Nallani M, Benito S, Onaca O, et al. A nanocompartment system (Synthosome) designed for biotechnological applications. *J Biotechnol.* 2006;123:50–59.
10. Du J, Reilly RKO. Advances and challenges in smart and functional polymer vesicles. *Soft Matter*. 2009; 5(19):3544–3561.
11. Hassouneh W, Zhulina EB, Chilkoti A, et al. Elastin-like polypeptide diblock copolymers self-assemble into weak micelles. *Macromolecules*. 2015;48(12):4183–4195.
12. Glavas L, Olsén P, Odelius K, et al. Achieving micelle control through core crystallinity. *Biomacromolecules*. 2013;14(11):4150–4156.
13. Israelachvili J. *Intermolecular & Surface Forces.* 2nd ed. London: Academic Press, 1991.
14. Tsitsilianis C, Sfika V. Heteroarm star-like micelles formed from polystyrene-block-poly(2-vinyl pyridine)-block-poly(methyl methacrylate) ABC triblock copolymers in toluene. M*acromol Rapid Commun*. 2001;22(8):647–651.
15. Fernyhough C, Pantazis D, Pispas S, et al. The micellar behavior of linear triblock terpolymers of styrene (S), isoprene (I), and methyl methacrylate (MMA) in selective solvents for PS and PMMA. *Eur Polym J.* 2004;40:237–244.
16. Chu B, Zhou Z. Physical chemistry of polyoxyalkylene block copolymer surfactants. In Nonionic surfactants: polyoxyalkylene block copolymers. M Dekker, editor. New York: Surfactant science series; 1996.
17. Booth C, Attwood D. Effects of block architecture and composition on the association properties of poly(oxyalkylene) copolymers in aqueous solution," Macromol. *Rapid Commun*. 2000;21(9):501–527.

18. Smart T, Lomas H, Massignani M, et al. Block copolymer nanostructures. *Nano Today*. 2008;3(3–4):38–46.
19. Blanazs A, Armes SP, Ryan AJ. Self-assembled block copolymer aggregates: From micelles to vesicles and their biological applications," Macromol. *Rapid Commun*. 2009;30(4–5):267–277.
20. Burke S, Shen H, Eisenberg A. Multiple vesicular morphologies from block copolymers in solution. *Macromol Symp*. 2001;175(1): 273–284.
21. Choe UJ, Rodriguez AR, Li Z, et al. Characterization and minimization of block copolypeptide vesicle cytotoxicity using different hydrophobic chain lengths. *Macromol Chem Phys*. 2013;214(9): 994–999.
22. Chen Y, Zhang Ke, Wang X, et al. Multigeometry nanoparticles: Hybrid vesicle/cylinder nanoparticles constructed with block kinetic control. *Macromolecules*. 2015;48(16):5621–5631.
23. Yoshida E. Morphology control of giant vesicles by composition of mixed amphiphilic random block copolymers of poly(methacrylic acid)-block-poly(methyl methacrylate-random-methacrylic acid). *Colloid Polym Sci*. 2014;293(1):249–256.
24. Karayianni M, Pispas S. Fluorescence Studies of Polymer Containing Systems. 2016.
25. Dalhaimer P, Bates FS, Discher DE. Single molecule visualization of stable, stiffness-tunable, flow-conforming worm micelles. *Macromolecules*. 2003;36(18):6873–6877.
26. Kim Y, Dalhaimer P, Christian DA, et al. Polymeric worm micelles as nano-carriers for drug delivery. *Nanotechnology*. 2005;16(7): S484–S491.
27. Isaacman MJ, Barron KA, Theogaraj LS. Clickable amphiphilic triblock copolymers. *Journal of polymer science part A: Polymer chemistry*. 2012;50(12):2319–2329.

Citation: Dhir S, Salahub S, Mathews AS, et al. Architecting polymersomes from spherical to tubules by molecular alterations. *J Nanomed Res*. 2018;7(1): 00176. DOI: 10.15406/jnmr.2018.07.00176