Langmuir
Letter
pubs.acs.org/Langmuir

Control of Protein Adsorption onto Core−Shell Tubular and Vesicular Structures of Diphenylalanine/Parylene

Gökhan Demirel,[†,‡] Niranjan Malvadkar,[†] and Melik C. Demirel*[,†,‡,§]

[†]*Materials Research Institute,* [‡]*Department of Engineering Science, and* [§]*Huck Institutes of Life Sciences, The Pennsylvania State University, University Park, Pennsylvania 16802*

Received September 21, 2009. Revised Manuscript Received November 24, 2009

The self-assembly of peptides, specifically dipeptides, offers numerous advantages for biological applications. We describe an easy, versatile method of fabricating different types of zwitterionic Phe-Phe dipeptide structures (i.e., tubes and vesicles) through solvent-mediated assembly. The stability of the dipeptide structures is increased by thin polymer coatings of poly(chloro-*p*-xylylene), a PPX film. We also investigated protein adsorption onto PPX-coated peptide tubes and vesicles by varying the thickness of the polymer film.

Introduction

Self-assembly is a natural process that spontaneously and reversibly organizes molecular units into ordered structures through noncovalent interactions.[1−4] Among all biological molecules that can self-assemble, peptides, specifically dipeptides, offer numerous advantages such as chemical variation and biocompatibility.[5,6] In 2001, Gorbitz first reported that dipeptide molecules can self-assemble into nanotubes.[7] Reches and Gazit later showed that the self-assembly of diphenylalanine (Phe-Phe) can form long, stiff nanotubes using 1,1,1,3,3,3-hexafluoro-2-propanol as the solvent medium.[5,6] Subsequently, Song et al. found that Phe-Phe can self-assemble in water to form nanotubes.[8] Furthermore, they observed that some nanotubes became vesicles when the dispersion was diluted.[8] Yan et al. also reported that cationic dipeptides can self-assemble to form nanotubes at physiological pH values and that upon dilution of this nanotube dispersion, dipeptide vesicles can be obtained.[9−11] However, they proposed that unlike cationic dipeptides, zwitterionic dipeptides mainly formed tubular structures rather than vesicles. Although the majority of the research community agrees that the concentration of the dipeptide plays a critical role in the formation of tubular structures, the effect of the solvent as an influential factor has largely been unexplored.[5−9]

In this letter, we report the self-assembly of zwitterionic Phe-Phe dipeptides into either tubular or vesicular structures by changing the solvent (Figure 1). Moreover, these structures were coated with varying thicknesses of poly(chloro-*p*-xylylene) (PPX) thin films using vapor deposition polymerization (VDP) to control the protein adsorption onto them. Such dipeptide-PPX core/shell structures could be useful in drug or gene delivery as scaffolds for tissue engineering as well as in other biological applications.

Experimental Section

Synthesis of Peptide Tubes and Vesicles. Diphenylalanine (Phe-Phe; Bachem AG, Switzerland) was dissolved in ethanol, acetone, methanol, water, chloroform, toluene, THF, or benzene at a concentration of 2 mg/mL at 70 °C for 10 min and cooled to room temperature. To avoid any preaggregation, fresh solutions were prepared for each experiment. Each peptide solution (50 μL) was then placed onto cleaned silicon wafers or glass slides and dried until the solvent evaporated. Subsequently, the dipeptide tubes and vesicles that were obtained were coated with poly(chloro-*p*-xylylene) (PPX) by using different amounts of dichloro-di-*p*-xylylene as a precursor.[12−16] The precursor was first sublimed at 175 °C under a pressure of 0.1−10 Torr. Precursor vapor was then pyrolyzed at 650−700 °C. To control protein adsorption, PPX-coated samples having different thicknesses were first immersed into a bovine serum albumin−FITC solution (0.1 mg/mL in pH 7.4 PBS buffer) overnight at room temperature in a dark environment. The films were then washed with buffer solution several times and dried in a stream of air.

Characterization. Scanning electron microscope (SEM) images were recorded with a Philips XL-40 system after the samples were coated with gold. To obtain topographical information about dipeptide tubes and vesicles, samples were characterized by a Nanoscope-E atomic force microscope (AFM) (Veeco Metrology, CA). Topographical images were collected in ambient air at room temperature with silicon nitride (SiN) triangular cantilevers having contact mode tips (DNT-20, Veeco Metrology, CA). Fluorescence microscopy images were taken using an Olympus Fluoview 300 confocal laser scanning microscope with a 50× objective. Static contact angle experiments were performed on an FTA-1000 (Firsttenangstrom Inc., VA) apparatus using

(1) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. *Science* **1991**, *254*, 1312–1319.
(2) Fernandez-Lopez, S.; Kim, H. S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature* **2001**, *412*, 452–455.
(3) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684–1688.
(4) Ozin, G. A.; Hou, K.; Lotsch, B. V.; Cademartiri, L.; Puzzo, D. P.; Scotognella, F.; Ghadimi, A.; Thomson, J. *Mater. Today* **2009**, *12*, 12–23.
(5) Reches, M.; Gazit, E. *Science* **2003**, *300*, 625–627.
(6) Reches, M.; Gazit, E. *Nano Lett.* **2004**, *4*, 581–585.
(7) Gorbitz, C. H. *Chem.−Eur. J.* **2001**, *7*, 5153–5159.
(8) Song, Y. J.; Challa, S. R.; Medforth, C. J.; Qiu, Y.; Watt, R. K.; Pena, D.; Miller, J. E.; van Swol, F.; Shelnutt, J. A. *Chem. Commun.* **2004**, *9*, 1044–1045.
(9) Yan, X. H.; He, Q.; Wang, K. W.; Duan, L.; Cui, Y.; Li, J. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 2431–2434.
(10) Yan, X. H.; Cui, Y.; He, Q.; Wang, K. W.; Li, J. B.; Mu, W. H.; Wang, B. L.; Ou-yang, Z. C. *Chem.−Eur. J.* **2008**, *14*, 5974–5980.
(11) Yan, X. H.; Cui, Y.; He, Q.; Wang, K. W.; Li, J. B. *Chem. Mater.* **2008**, *20*, 1522–1526.
(12) Lahann, J. *Polym. Int.* **2006**, *55*, 1361–1370.
(13) Demirel, M. C. *Colloids Surf., A* **2008**, *321*, 121–124.
(14) Boduroglu, S.; Cetinkaya, M.; Dressick, W. J.; Singh, A.; Demirel, M. C. *Langmuir* **2007**, *23*, 11391–11395.
(15) Cetinkaya, M.; Boduroglu, S.; Demirel, M. C. *Polymer* **2007**, *48*, 4130–4134.
(16) Gorham, W. F.; New, A. *J. Polym. Sci., Part A: Polym. Chem.* **1966**, *4*, 3027–3035.

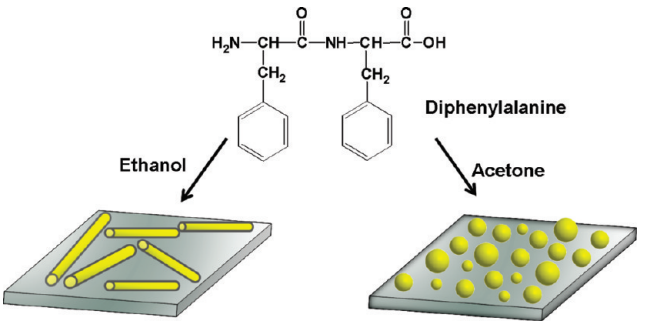

Figure 1. Schematic representation of the self-assembly of Phe-Phe dipeptides into tubes and vesicles in ethanol and acetone, respectively.

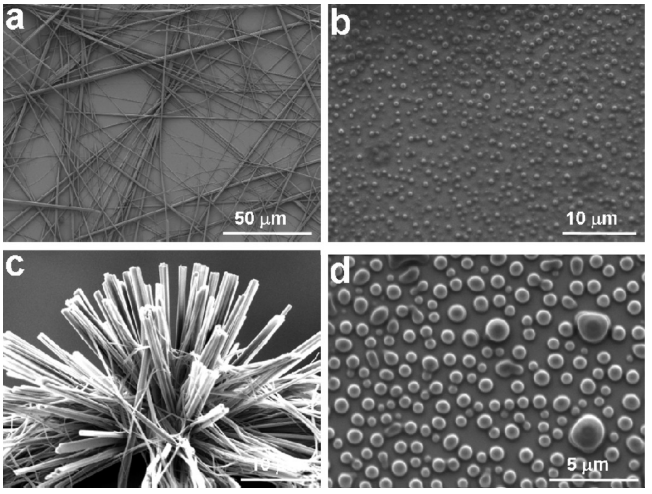

Figure 2. SEM images of Phe-Phe tubes and vesicles: (a) 2 mg/mL dipeptide in ethanol vaporized at 25 °C, (b) 2 mg/mL dipeptide in acetone vaporized at 25 °C, (c) 2 mg/mL dipeptide in ethanol vaporized at 80 °C, and (d) 2 mg/mL dipeptide in acetone vaporized at 80 °C.

10 μL of ultrapure water. ATR-FTIR (Thermo Nicolet IR) data was also collected with respect to a silicon wafer reference in air and was recorded at 4 cm^{-1} resolution.

Results and Discussion

We prepared Phe-Phe tubes and vesicles by using ethanol and acetone as the respective solvents. In a typical experiment, 10 mg of dipeptide was dissolved in 5 mL of solvent (either ethanol or acetone) at 70 °C for 10 min and cooled to room temperature. The solution (50 μL) was then placed on a cleaned silicon wafer or glass slide and dried until the solvent evaporated. Figure 2a,b shows scanning electron microscopy (SEM) images of dipeptide tubes and vesicles, respectively. The dense tubular and vesicular structures evident from the SEM images illustrate the high yield of the preparation method. Upon rapid evaporation of ethanol, forestlike structures were also formed on the substrate, as previously suggested by Reches and Gazit (Figure 2c).[17] Unlike with ethanol, we could not observe any difference in the structure when acetone was rapidly evaporated, although there was an increase in diameter (Figure 2d). To obtain direct topographical information, the samples were investigated using atomic force microscopy (Figure S1 in Supporting Information). The AFM images show that the diameters of the dipeptide tubes and vesicles were 0.3−3 μm and 0.2−2 μm, respectively.

(17) Reches, M.; Gazit, E. *Nat. Nanotechnol.* **2006**, *1*, 195–200.

Table 1. Solvent Effect on the Self-Assembly of Phe-Phe

solvent	dielectric constant	peptide formation
water	80.1	tubular
methanol	32.6	tubular
ethanol	24.3	tubular
acetone	20.7	vesicular
tetrahydrofuran (THF)	7.5	none
chloroform	4.8	none
toluene	2.4	none
benzene	2.3	none

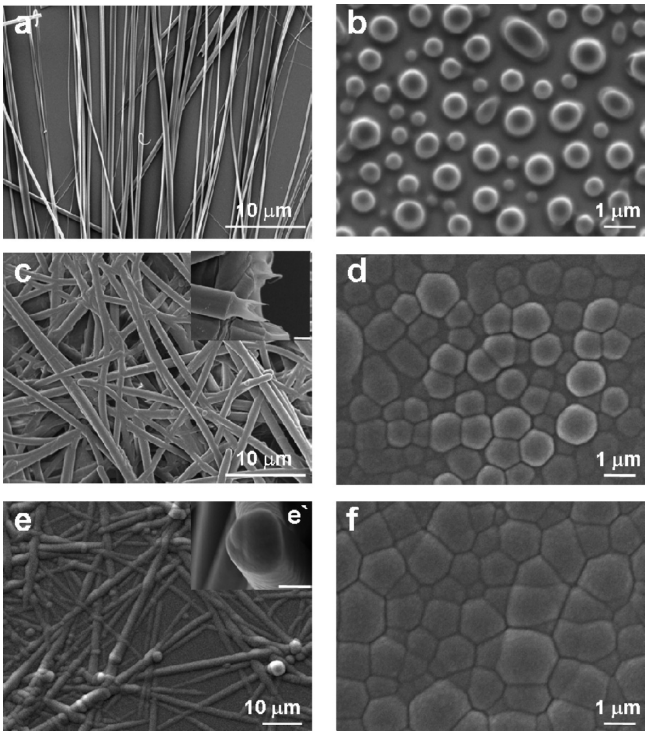

Figure 3. SEM images of PPX-coated Phe-Phe tubes and vesicles: (a, b) without PPX, (c, d) 0.8 μm of PPX-coated dipeptide structures (an open-pore PPX-coated dipeptide tube at high magnification in the inset), and (e, f) 3.6 μm of PPX-coated tubes and vesicles (a closed-pore PPX-coated tube at high magnification in the inset, scale bar 1 μm).

To understand the formation of tubular and vesicular structures, we investigated the self-assembly of Phe-Phe using various solvents having different dielectric constants (i.e., ethanol, acetone, methanol, water, chloroform, toluene, THF, and benzene, which have dielectric constants of 24.3, 20.7, 32.6, 80.1, 4.8, 2.4, 7.52, and 2.28, respectively). It is known that molecules interact with each other in solution during self-assembly and form structures ranging from simple dimers to complex mesoscopically sized structures.[9] These interactions often depend on the solvent properties (e.g., polarity and dielectric constant). If self-assembly occurs through hydrogen bonding, then it may not be stable in hydrogen-bonding solvents such as water or alcohols. At the same time, complexes formed via a hydrophobic effect as the driving force usually dissociate in hydrophobic solvents (e.g., benzene). In our study, the dipeptide concentration was kept constant at 2 mg/mL and experiments were performed at room temperature. Although dipeptide tubes or vesicles were obtained using ethanol, acetone, methanol, and water, we could not observe any regular structures when THF, benzene, toluene, or chloroform was used

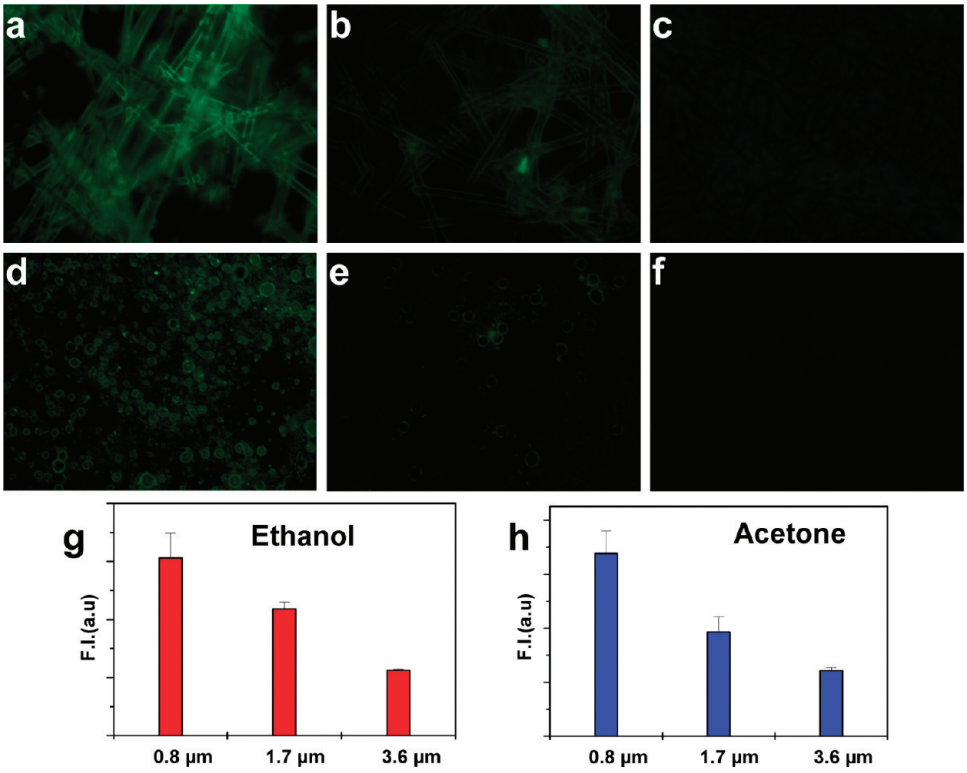

Figure 4. Fluorescence microscopy images of BSA-FITC-adsorbed PPX-coated dipeptide tubes and vesicles prepared using different amounts of PPX monomer: (a, d) 2.0 g, (b, e) 1.0 g, and (c, f) 0.5 g. (g, h) Fluorescence intensities for dipeptide tubes and vesicles, respectively.

as the solvent (Table 1 and Figure S2 in Supporting Information). These differences may be attributed to the dielectric constants of these solvents. Solvents having a dielectric constant >4 tend to solvate the hydrophilic headgroups of the Phe-Phe molecule in the medium, allowing the self-assembly process to proceed. Solvents having a dielectric constant <4 may, in contrast, force the peptide molecules to form reversed micelles or network structures in the solution, thereby reducing the concentration of hydrophilic groups in the medium.[18] We note that the interaction between the solvent and the dipeptide molecules is also very important to the understanding of the morphology of the structures. It is known that ethanol is a protic solvent and strongly solvates ionic molecules via hydrogen bonding. Acetone is an aprotic solvent and has less of a tendency to form hydrogen bonds with dipeptides. In the case of acetone, the dominant force for self-assembly is a hydrophobic interaction and intramolecular hydrogen bonding between dipeptide molecules. It is assumed that a hydrophobic interaction between aromatic moieties of Phe-Phe provides an energetic contribution as well as order and directionality for the initial interaction, forming an extended pleated sheet that is stabilized by hydrogen bonds and hydrophobic interactions in the beginning of the self-assembly.[6] These structures are then transformed into different structures (e.g., tubular or vesicular) to minimize their energy, depending on the interactions between molecules. In the case of ethanol, the formation may occur by the closure of the extended sheet along one axis of the 2D layer as a result of intermolecular hydrogen bonding between ethanol and dipeptide molecules. Alternatively, in acetone, as mentioned above, hydrophobic interactions and intramolecular hydrogen bonding between the dipeptide molecules may force the formation of vesicular structures as a result of the closure of the sheet along two axes. However, modeling and simulation studies of these processes are still needed for further understanding.

To improve the structural stability of the peptide tubes and vesicles, we coated them with a protective PPX thin film using VDP polymerization. This method has several advantages compared to solvent-based polymerization techniques, including uniform modification, conformal coating, high accuracy, excellent adhesion, and avoiding impurities associated with the use of solvents, initiators, or plasticizers.[12−16] VDP was carried out using 0.15−2.0 g of dichloro-di-*p*-xylylene as the precursor. The resulting PPX-coated protein structures were analyzed by SEM (Figure 3). SEM images of PPX-coated dipeptide structures show a slightly rugged surface and an increase in diameter (Figure 3c−f) compared to pristine dipeptide structures (Figure 3a,b). Moreover, when more than 0.5 g (i.e., 0.8 μm in thickness) of the PPX precursor was used, the open ends of the tubes were fused, as evident in high-magnification SEM images (Figure 3c,e insets). We also note that a thinner PPX coating ($\sim$200 nm) provides a conformal film of peptide structures (Figure S6). It is known that peptide-based structures are mostly stable in organic solvents but decompose under proteolytic attack. PPX-coated peptides remained stable in proteinase solution (proteinase, 0.2 mg/mL for 6 h at 25 °C).

We also investigated protein adsorption onto PPX-coated peptide tubes and vesicles. It is known that protein adsorption onto a biomaterial surface plays an important role in the biological response.[19,20] In this study, we attempted to control protein adsorption onto dipeptide structures by coating them with PPX layers of varying thickness. Fluorescence microscopy

(18) Nie, H. Y.; Walzak, M. J.; McIntyre, N. S. *J. Phys. Chem. B* **2006**, *110*, 21101–21108.

(19) Khademhosseini, A.; Jon, S.; Suh, K. Y.; Tran, T. N. T.; Eng, G.; Yeh, J.; Seong, J.; Langer, R. *Adv. Mater.* **2003**, *15*, 1995–2000.

(20) Mao, C.; Qiu, Y. Z.; Sang, H. B.; Mei, H.; Zhu, A. P.; Shen, J.; Lin, S. C. *Adv. Colloid Interface Sci.* **2004**, *110*, 5–17.

images confirmed the control of protein adsorption onto the PPX-coated dipeptide tubes and vesicles (Figure 4). When the thickness of the PPX layer increased (i.e., the roughness of the film decreased), protein adsorption dramatically decreased. This result is consistent with PPX coatings on glass substrates where minimal protein adsorption is observed (not shown).

Conclusions

Using zwitterionic Phe-Phe dipeptides and straightforward experimental procedures, we have succeeded in creating tubular and vesicular dipeptide structures. These structures can be readily manufactured and easily decorated chemically. We also have shown that the stability of these structures can be improved by VDP of PPX onto their surfaces. Moreover, protein adsorption can be controlled by changing the thickness of the PPX layer on the dipeptide structures. Given its simplicity, efficiency, and generalizability, we believe that these types of materials can be useful for a wide range of biological applications, such as drug or gene delivery and tissue engineering. Future work will proceed in this direction.

Acknowledgment. We thank the Office of Naval Research for financial support of this work through a Young Investigator's Program Award.

Supporting Information Available: AFM images of self-assembled Phe-Phe vesicular and tubular structures. Optical microscope images of self-assembled Phe-Phe in various solvents. X-ray diffraction of self-assembled Phe-Phe tubular and vesicular structures on a glass substrate. Thicknesses of PPX thin film layers as a function of the amount of starting dimer ([2.2]dichloro-paraxylylene, PCP). ATR-FTIR spectra of a bare PPX film and PPX-coated Phe-Phe tubular structures. Electron microscopy images of PPX-coated vesicular and tubular Phe-Phe structures on silicon substrates. This material is available free of charge via the Internet at http://pubs.acs.org.