

Interfacial tension governs the formation of self-organized honeycomb-patterned polymer films

Yukako Fukuhira,^{*ab} Hiroshi Yabu,^c Kuniharu Ijiro^d and Masatsugu Shimomura^{ce}

Received 26th November 2008, Accepted 26th February 2009

First published as an Advance Article on the web 30th March 2009

DOI: 10.1039/b821183c

Hexagonally packed water droplets condensed on a polymer solution are potential templates for the formation of honeycomb-patterned porous polymer films. A small number of surface-active molecules is indispensable for the stabilization of water droplets during solvent evaporation. Biocompatible surfactants; e.g., phospholipids, are required for the fabrication of biodegradable honeycomb-patterned polymer films, which can be used as novel biomedical materials, mainly *in vivo*. Among various kinds of phospholipids, dioleoylphosphatidylethanolamine (DOPE) has been reported to be the most suitable surfactant for the formation of honeycomb-patterned PLA films. Interfacial tension between a water droplet and the polymer solution is largely dependent on the chemical structure of the phospholipids. DOPE shows high interfacial tension, resulting in the stabilization of water droplets during solvent evaporation. Dierucylophosphatidylcholine (DEPC) and dierucylophosphatidylethanolamine (DEPE), both of which display high interfacial tension, were also found to be suitable biocompatible surfactants.

Introduction

A honeycomb-patterned polymer film is fabricated by casting a polymer solution the surface of which is sprayed with high humidity air. Regularly arrayed micropores are formed in the polymer film by using a self-organized array of water droplets as a template.^{1–7} To form a uniform array of water droplets, it is important that they be stabilized on the polymer solution surface during solvent evaporation. By evaporation cooling, water molecules condense to form water droplets of uniform size on the polymer solution surface. In order to prevent the coalescence of the water droplets and to stabilize them, a surfactant is added to the polymer solution. The surfactant molecules are localized at the interface between the water droplets and the polymer solution. After solvent evaporation, the regular arrangement of the water droplets is maintained and, after water evaporation, the honeycomb-patterned structure is formed. The stability and arrangement of the water droplets are strongly affected by the concentration and structure of the surfactants.

We have already reported that amphiphilic polymers, copolymers of dodecylacrylamide and ω -carboxyhexylacrylamide (CAP), as well as poly-ion complexes, are effective surfactants for the formation of honeycomb-patterned films.^{8,9} These films have potential applications as medical devices such as cell culture substrates,^{10–15} cell separators,⁹ temporary epicardial pacing wires,¹⁶ adhesion barriers,^{17,18} and superhydrophobic films.¹⁹ However, biocompatible polymers and surfactants are required for the *in vivo* use of honeycomb-patterned films.

Recently, we have focused on the use of phospholipids as biocompatible surfactants for the formation of biodegradable honeycomb-patterned films. Among the various kinds of phospholipids, we have found that dioleoylphosphatidylethanolamine (DOPE) is the most effective as a biocompatible surfactant.²⁰

In order to elucidate the effectiveness of DOPE in the formation of honeycomb-patterned films, the interfacial tension between the water droplets and polymer solution was measured, because interfacial tension is one of the key physical parameters for determining the stability and arrangement of the water droplets, and the results are presented herein.

Experimental section

Poly(D,L-lactic acid) (PLA) (MW = 200,000, Lacty #9031, Shimadzu, Kyoto, Japan) was dissolved in chloroform (Wako Pure Chemical Industries Ltd., Osaka, Japan) at a concentration of 5 mg/mL at room temperature. Phospholipids, DOPE (COATSOME ME-8181), dilauroylphosphatidylcholine (DLPC; COATSOME MC-2020), dimyristoylphosphatidylcholine (DMPC; COATSOME MC-4040), dipalmitoylphosphatidylcholine (DPPC; COATSOME MC-6060), distearoylphosphatidylcholine (DSPC; COATSOME MC-8080), dioleoylphosphatidylcholine (DOPC; COATSOME MC-8181), dierucylophosphatidylcholine (DEPC; COATSOME MC-2121AL) and dierucylophosphatidylethanolamine (DEPE; COATSOME ME-2121AL), were purchased from NOF Co. (Tokyo, Japan) and separately added to a polymer solution (0.5 wt% PLA). The honeycomb-patterned films were prepared on a 100 mm petri dish at room temperature under humid air (approximately 80% humidity). The structures of the films were observed using an optical microscope and a field-emission scanning electron microscope (FE-SEM). In this experiment, the

^aN10W8, Sapporo, 060-0810, Japan

^b4-3-2, Asahigaoka, Hino, 191-8512, Japan. E-mail: y.fukuhira@teijin.co.jp; Fax: +81 42587 5511; Tel: +81 42 586 8325

^c2-1-1, Katahira, Aobaku, Sendai, 980-8577, Japan

^dN21W10, Sapporo, 001-0021, Japan

^e2-1-1, Katahira, Aobaku, Sendai, 980-8577, Japan

shape of a water-soluble blue dye (Indigocarmine; Wako Pure Chemical Industries Ltd., Japan)-stained, millimeter-scale water droplet located on the surface of the polymer solution containing the phospholipid surfactants was observed.

The interfacial tension of each polymer solution was measured using the pendant drop technique²¹ (FAMAS CA-W software, Kyowa Interface Co., Ltd., Japan). A drop of water was obtained from a clean 22-gauge Teflon cannula immersed in the polymer solution in a 10 mL quartz glass cuvette at 20 °C. The interfacial tension (γ) was determined as follows,

$$\gamma = \Delta\rho g d_e^2 / H \quad (1)$$

where $\Delta\rho$ is the mass density difference between the drop and the fluid surrounding the drop, g is the gravitational acceleration, d_e is the equatorial diameter of the drop, and H is a dimensionless shape factor. H is a function of d_s/d_e , where d_s is the diameter of the pendant drop measured at a vertical distance d_e from the apex of the drop. The lengths corresponding to d_e and d_s of the drop were measured from digital images. The average and standard deviations of each γ value were determined by using experimental data from ten sets of measurements.

Results and discussion

In this study, DOPE and a series of phosphatidylcholines were used as surfactants, because no other phospholipids could be dissolved in the polymer solution.²⁰

Fig. 1 shows FE-SEM images of the surface of the fabricated PLA films using each phospholipid as a surfactant. Schematic views of the cross-section of the film are also shown. As reported previously,²⁰ a uniform honeycomb-patterned structure was

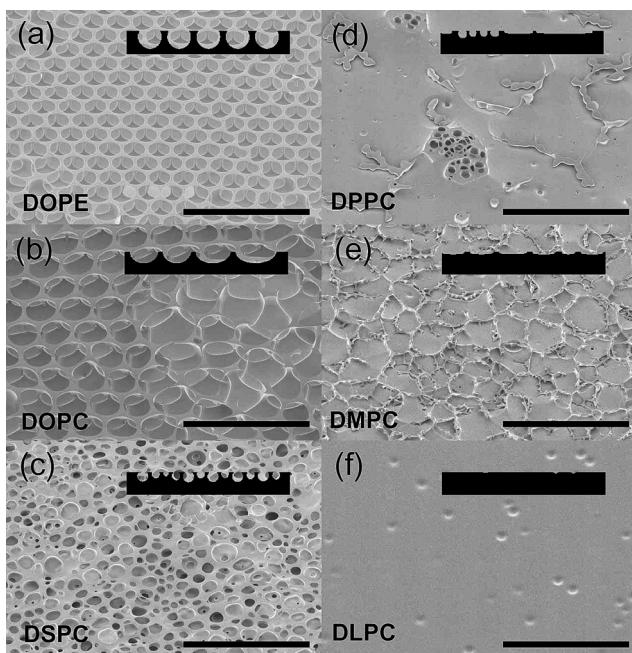


Fig. 1 FE-SEM images of film surfaces. (a) PLA/DOPE, (b) PLA/DOPC, (c) PLA/DSPC, (d) PLA/DPPC, (e) PLA/DMPC, and (f) PLA/DLPC. The scale bar represents 20 µm. Schematic views show the cross-sectional structure of each film.

formed over the entire area of the DOPE/PLA film (Fig. 1(a)), whereas uniform and nonuniform honeycomb-patterned structures coexisted in the DOPC/PLA film (Fig. 1(b)). Nonuniformly sized pores were irregularly arranged in the DSPC/PLA film (Fig. 1(c)), and small, regularly sized pores were collected in sections of the surface of the DPPC/PLA film (Fig. 1(d)). On the DMPC/PLA film surface, a random network structure of the order of several micrometers was observed (Fig. 1(e)), and randomly located 2 µm concave grooves were seen on the surface of the DLPC/PLA film (Fig. 1(f)). The honeycomb-patterned structure was only successfully formed on the PLA film surface when DOPE was used as the surfactant.

Since the honeycomb-patterned film was fabricated using a self-organized array of water droplets as a template, the shape of the water droplets would influence the structure of the pores in the film. The effect of phospholipids on water droplet shape was estimated by observation of a millimeter-scale water droplet in the polymer solutions containing each phospholipid, because direct observation of the shape of micrometer-scale water droplets is difficult (Fig. 2). Two types of polymer solutions, a low concentration solution (5 mg/mL) and a high concentration solution (100 mg/mL), were prepared, as the polymer solution was concentrated during the honeycomb-patterned film fabrication process. Side views of the water droplet in the low concentration solution are shown in Fig. 2(I) for each phospholipid. The shape of the water droplet in the low concentration solution was almost globular. In the high concentration DOPE/PLA solution, the globular shape of the water droplet was maintained (Fig. 2(a)-(II)). In contrast, in the high concentration DOPC/PLA solution, the droplet expanded slightly and its shape changed (Fig. 2(b)-(II)). The expansion of the water droplet in the high concentration DSPC/PLA solution was significantly increased (Fig. 2(c)-(II)), and this trend was observed in the high concentration solutions containing DPPC, DMPC and DLPC (Fig. 2(d)-(II), 2(e)-(II) and 2(f)-(II)). Fig. 2 clearly shows that

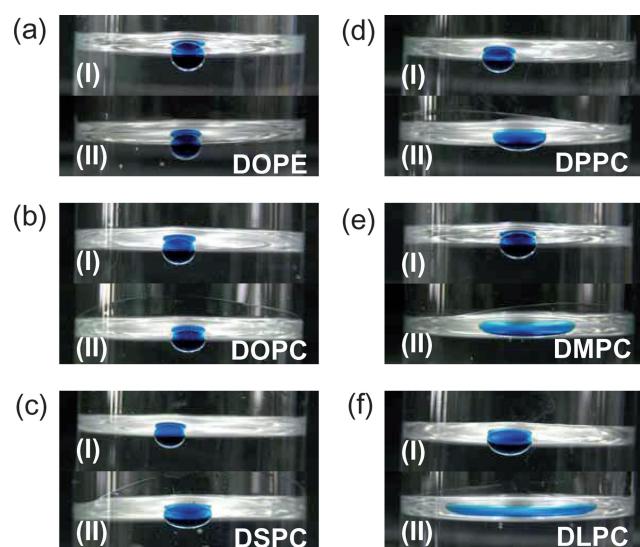


Fig. 2 Appearance of water droplet in the polymer solution. (a) DOPE/PLA, (b) DOPC/PLA, (c) DSPC/PLA, (d) DPPC/PLA, (e) DMPC/PLA, and (f) DLPC/PLA. (I) Phospholipid/PLA/chloroform = 0.025 mg/5 mg/ml. (II) Phospholipid/PLA/chloroform = 0.5 mg/100 mg/ml.

Table 1 HLB data of phospholipids

Surfactant	RCOO	HLB
DOPE	C18:1	6.5
DOPC	C18:1	7.2
DSPC	C18:0	7.2
DPPC	C16:0	7.7
DMPC	C14:0	8.3
DLPC	C12:0	9.1

when the alkyl chain length of the phospholipid became shorter, the water droplet spread in the high concentration solution. These results indicate that phospholipids strongly influence the shape and stability of water droplets in the polymer solution.

The ability of the phospholipid to dissolve the water droplets in the polymer solution can be predicted from the hydrophilic-lipophile balance (HLB). The HLB values of the phospholipids were calculated using Griffin's method (Table 1).

In general, surfactants with HLB values between 3 and 6 partially disperse in water and are used as water-in-oil (W/O) emulsifiers. Surfactants with HLB values between 7 and 9 disperse well in water and are used as wetting agents and oil-in-water (O/W) emulsifiers. Surfactants with such high HLB values are not suitable for fabricating honeycomb-patterned films, because these surfactants are dissolved into the water droplets and the surface tension of the water droplets is decreased. Harbins expresses the spreading coefficient (S) as follows,

$$S = \gamma_a - (\gamma_b + \gamma_{a/b}) \quad (2)$$

where γ_a is the surface tension of the polymer solution, γ_b is the surface tension of the water droplet, and $\gamma_{a/b}$ is interfacial tension between the polymer solution and the water droplets in this case. This means that a water droplet with low surface tension cannot maintain a globular shape. Therefore, DOPE, which has a low HLB value (6.5), can maintain water in the form of droplets in a polymer solution.

It is essential to investigate the relation between the chemical structure of the phospholipid and interfacial tension in order to quantitatively discuss the estimated size of the millimeter-scale water droplets in the polymer solution containing the phospholipid, because the stability of the water droplets in the polymer solution is governed by interfacial tension. In the film fabrication process, the concentrations of the polymer and surfactant in the solution were gradually increased with solvent evaporation. Therefore, the interfacial tension was measured at four simulated stages of evaporation (0.025 mg/5 mg/mL (phospholipid/PLA/chloroform), 0.05 mg/10 mg/mL, 0.25 mg/50 mg/mL, and 0.5 mg/100 mg/mL); *i.e.*, from the initial concentration state to a highly concentrated state (Fig. 3). The interfacial tension decreased with increasing phospholipid concentration for each phospholipid. Among the phospholipid-containing solutions used in our study, the DOPE/PLA solution showed the highest interfacial tension, as DOPE is the most hydrophobic of the phospholipids owing to its having the longest unsaturated alkyl chains and a small head group.

To investigate the relation between interfacial tension and honeycomb-patterned structure formation, *in situ* observation of the honeycomb-patterned film fabrication process was carried out for DOPE/PLA and DMPC/PLA films. The time course of

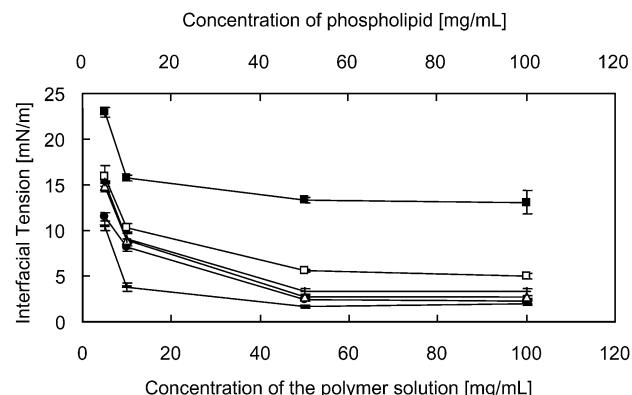


Fig. 3 Interfacial tension of phospholipids at the polymer solution–water interface as a function of total polymer concentration. ■: DOPE, □: DOPC, +: DSPC, Δ: DPPC, ●: DMPC, —: DLPC.

the interfacial tensions of the DOPE/PLA films, the polymer solution concentration, and the film surface appearance are shown in Fig. 4(a). The polymer solution concentration was calculated from the polymer solution weight, which was monitored *versus* time. Optical interference due to the regularly formed array of the water droplets was observed on the surface of the polymer solution approximately 60 s after the start of the film fabrication process (Fig. 4(a)-(I)). The interference spread to the entire area of the petri dish by approximately 120 s (Fig. 4(a)-(II)). The solidification of the film surface occurred at approximately 180 seconds (Fig. 4(a)-(III)). At this point, the concentration of the polymer solution was approximately 10 mg/mL. Interference due to the honeycomb-patterned structure was observed after the evaporation of chloroform and water droplets from the film surface (Fig. 4(a)-(IV)). The value of the interfacial tension was maintained above 10 mN/m after film solidification. On the other hand, in the case of the DMPC/PLA films, interference was observed only in the initial stage (Fig. 4(b)-(I)) and disappeared with time (Fig. 4(b)-(II)). This variation in interference time course between the two phospholipids can be explained by the fact that as the interfacial tension at the initial concentration of the polymer solution was higher than 10 mN/m, water droplets remained stable at the polymer solution surface. During solvent evaporation, the interfacial tension decreased below 10 mN/m, causing the water droplets to be deformed as they coalesced on the polymer solution. In all polymer solutions containing phospholipids, with the exception of that containing DOPE, the interference appeared only once and then disappeared. When the interfacial tension was higher than 10 mN/m, at the time at which solidification of the polymer solution occurred, the water droplets were sufficiently stable to cause stable interference, thereby yielding good-quality honeycomb-patterned films.

The stability of a free, thin liquid film against small, spontaneous fluctuations in thickness has been previously explored.²² The liquid film is unstable with respect to fluctuations with a wavelength larger than the critical wavelength. The condition under which the film coalesces is described by the Vrij theory as follows,

$$\frac{d^2V}{dh^2} < -2\pi\gamma/A_c \quad (3)$$

where $V(h)$ is the free energy of the interaction as a function of the film thickness h , γ is the interfacial tension, and A_c is the

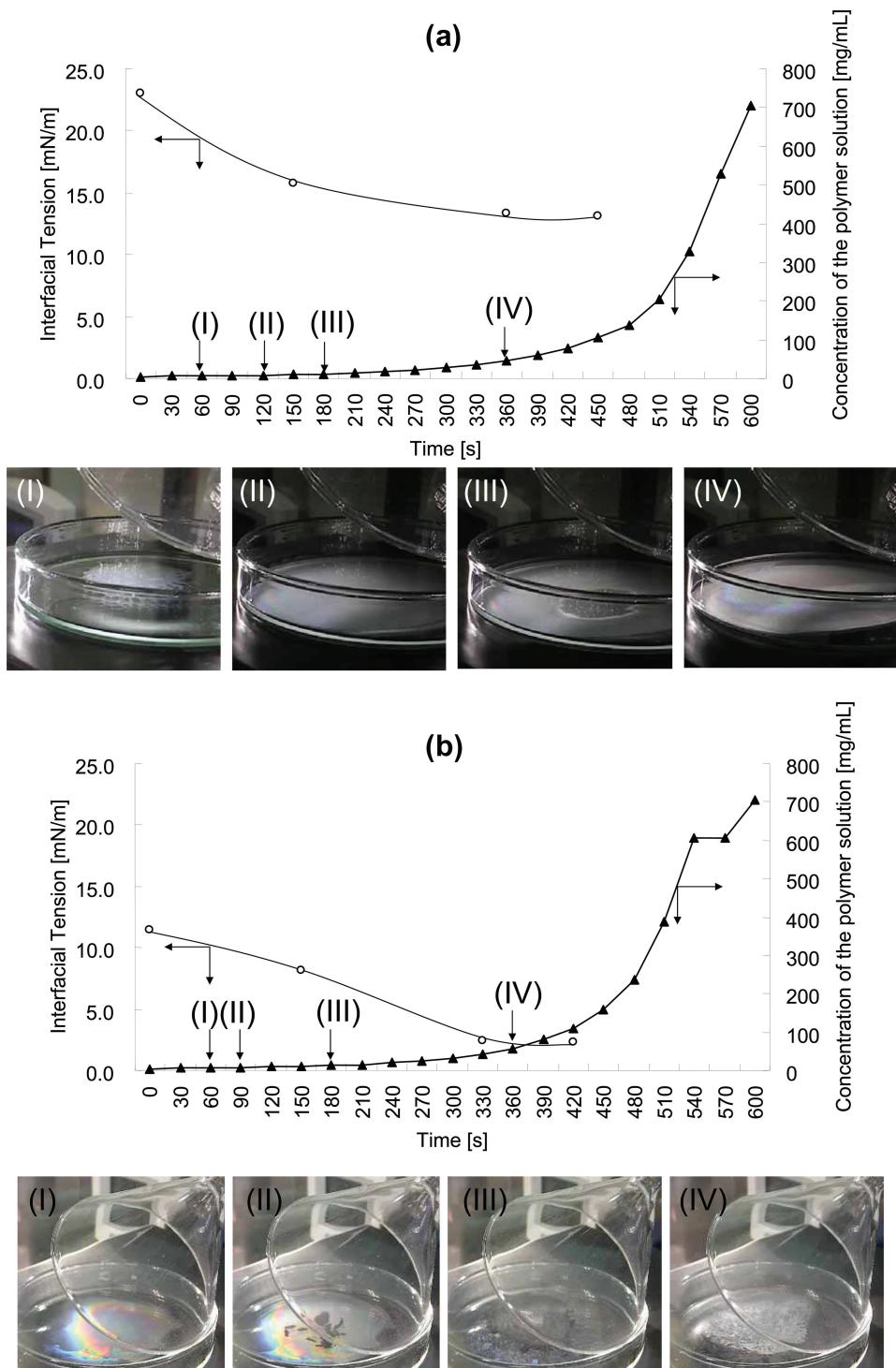


Fig. 4 (a) Change in interfacial tension of DOPE (\circ) at the polymer solution–water interface and the concentration of the polymer solution (\blacktriangle) as a function of film formation time. Video frames captured at 60, 120, 180, and 360 s after the start of the experiment are also shown. (b) Change in interfacial tension of DMPC (\circ) at the polymer solution–water interface and the concentration of the polymer solution (\blacktriangle) as a function of film formation time. Video frames captured at 60, 90, 120, 180, and 360 s after the start of the experiment are also shown.

wavelength of the critical fluctuation. $V(h)$ includes van der Waals attraction and double-layer repulsion. Equation (3) explains why a low interfacial tension has a tendency to break the liquid film.²³ In our study, surfactants having a low interfacial tension could not maintain the coalesced water droplets, thus our

results are in excellent agreement with the results predicted using equation (3).

To confirm this, we next investigated surfactants with interfacial tension higher than 10 mN/m in a 10 mg/mL polymer solution. The interfacial tension of DEPC containing

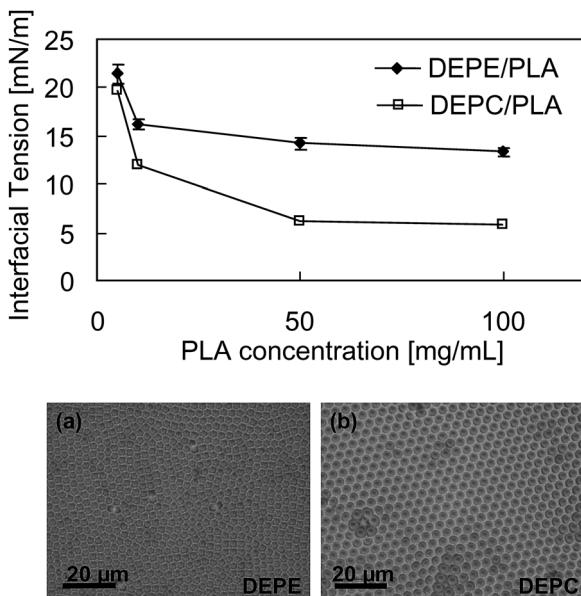


Fig. 5 Interfacial tension of DEPC and DEPE at the polymer solution–water interface as a function of total polymer concentration. Optical microscope images of film surfaces. (a) PLA/DEPE, and (b) PLA/DEPC.

unsaturated alkyl chains having 22 carbon atoms and 1 double bond was 12 mN/m in 10 mg/mL of the PLA polymer solution (0.5 wt% of PLA), and the interfacial tension of DEPE containing unsaturated alkyl chains having 22 carbon atoms and 1 double bond was 16 mN/m in 10 mg/mL of the PLA polymer solution (0.5 wt% of PLA). The HLB values of DEPC and DEPE were 6.3 and 5.6, respectively, which compare favorably with that of DOPE (6.5). Thus both the DEPC/PLA and DEPE/PLA solutions were predictably effective in the fabrication of the honeycomb-patterned films (Fig. 5).

Conclusion

The stabilization of the water droplets on a polymer solution surface was necessary for the formation of a honeycomb-patterned structure. The stability of the water droplets in the solution is affected to a large degree by the surfactants. The HLB value and interfacial tension are important parameters affecting droplet stability. DOPE, which is effective as a surfactant for honeycomb-patterned film formation, has a low HLB value and can maintain high interfacial tension (>10 mN/m) during chloroform evaporation. Therefore, the results of this study have aided us in formulating guidelines for selecting surfactants to be used in the fabrication of honeycomb-patterned films.

Acknowledgements

The authors thank Hisao Moriya of the Institute for Structure Analysis, Teijin Limited, for his assistance with the FE-SEM analysis, and Sadakazu Matsubara for his helpful input.

References

- M. Shimomura and T. Sawadaishi, *Current Opinion in Colloid & Interface Science*, 2001, **6**, 11–16.
- M. Srinivasarao, D. Collings, A. Philips and S. Patel, *Science*, 2001, **292**, 79–83.
- U. H. F. Bunz, *Advanced Materials*, 2006, **18**, 973–989.
- G. Widawski, M. Rawiso and B. François, *Nature*, 1994, **369**, 386–389.
- O. Karthaus, N. Maruyama, X. Cieren, M. Shimomura, H. Hasegawa and T. Hashimoto, *Langmuir*, 2000, **16**, 6071–6076.
- B. de Boer, U. Stalmach, H. Nijland and G. Hadzioannou, *Advanced Materials*, 2000, **12**, 1581–1583.
- Y. Xu, B. Zhu and Y. Xu, *Polymer*, 2005, **46**, 713–717.
- H. Yabu, M. Tanaka, K. Ijiro and M. Shimomura, *Langmuir*, 2003, **19**, 6297–6300.
- M. Tanaka, M. Takebayashi, M. Miyama, J. Nishida and M. Shimomura, *Biomed Mater Eng*, 2004, **14**, 439–446.
- Y. Fukuhira, H. Kaneko, M. Yamaga, M. Tanaka, S. Yamamoto and M. Shimomura, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2008, **313–314**, 520–525.
- T. Nishikawa, J. Nishida, R. Ookura, S. Nishimura, S. Wada, T. Karino and M. Shimomura, *Materials Science and Engineering: C*, 1999, **8–9**, 495–500.
- K. Sato, K. Hasebe, M. Tanaka, M. Takebayashi, K. Nishikawa, M. Shimomura, T. Kawai, M. Matsushita and S. Todo, *International Journal of Nanoscience*, 2002, **1**, 689–693.
- M. Tanaka, K. Nishikawa, H. Okubo, H. Kamachi, T. Kawai, M. Matsushita, S. Todo and M. Shimomura, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2006, **284–285**, 464–469.
- A. Tsuruma, M. Tanaka, N. Fukushima and S. Masatsugu, *e-Journal of Surface Science and Nanotechnology*, 2005, **3**, 159–164.
- M. Tanaka, A. Takayama, E. Ito, H. Sunami, S. Yamamoto and M. Shimomura, *J Nanosci Nanotechnol*, 2007, **7**, 763–772.
- Y. Narita, Y. Fukuhira, H. Kagami, E. Kitazono, H. Kaneko, Y. Sumi, A. Usui, M. Ueda and Y. Ueda, *Ann Thorac Surg*, 2006, **82**, 1489–1493.
- Y. Fukuhira, M. Ito, H. Kaneko, Y. Sumi, M. Tanaka, S. Yamamoto and M. Shimomura, *J Biomed Mater Res B Appl Biomater*, 2008, **86B**, 353–359.
- T. Okuda, T. Higashide, Y. Fukuhira, Y. Sumi, M. Shimomura and K. Sugiyama, *Journal of Glaucoma*, 2008, in press.
- H. Yabu, M. Takebayashi, M. Tanaka and M. Shimomura, *Langmuir*, 2005, **21**, 3235–3237.
- Y. Fukuhira, E. Kitazono, T. Hayashi, H. Kaneko, M. Tanaka, M. Shimomura and Y. Sumi, *Biomaterials*, 2006, **27**, 1797–1802.
- K. E. Anderson, J. A. Rogers and D. Li, *J Pharm Pharmacol*, 1997, **49**, 587–591.
- A. Vrij, *Discussions of the Faraday Society*, 1966, **42**, 23–33.
- E. Dickinson, *Pure & Appl. Chem.*, 1992, **64**, 1721–1724.