

Supporting Information

One-Step Dynamic Fabrication of Asymmetric Multi-Layered Porous Films for Sustained Drug Delivery

Pengcheng Cui,^{a,b} Silong Wu,^{a,b} Jun Xie,^c Jiaying Ma,^c Lingyun Ding,^{a,b} Oluseyi S.

*Olasoju,^{a,b} Yongjian Cao,^{a,b} Yaqi Li,^{a,b} Liyan Shen^{*c} and Wei Sun^{*a,b}*

^aDepartment of Materials Science and Engineering, School of Materials Science and Chemical Engineering, Ningbo University, Ningbo, 315211, China

^bState Key Laboratory Base of Novel Functional Materials and Preparation Science, School of Materials Science and Chemical Engineering, Ningbo University, Ningbo 315211, China

^c Key Laboratory of Orthopaedics of Zhejiang Province, Department of Orthopaedics, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325000, China

Corresponding Author

*E-mail: sunwei@nbu.edu.cn

*E-mail: shenliyan@wmu.edu.cn

Experimental

Materials

Polystyrene (PS) ($M_w=1.92\times 10^5$), monocarboxylic acid terminated polystyrene (PS-COOH) ($M_w=2.0\times 10^5$) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Proanthocyanidins (PAC) was purchased from Beijing Solarbio Science & Technology Co., Ltd.. Didodecyl dimethyl ammonium bromide (DDAB) was purchased from Aladdin Industrial Corporation. Chloroform (CHCl_3) and acetone were purchased from Hangzhou Gaojing Fine Chemical Industry Co., Ltd.. Deionized (DI) water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was provided by a lab-water system (HK Super PW). All the chemicals were used without further purification.

Preparation of Inverse Emulsion

The oil phase was prepared by dissolving PS in CHCl_3 at the concentration of 10.0 mg/mL, and DDAB was added as emulsifier at the concentration of 0.5 mg/mL. Different amount of deionized water was then mixed with the PS solution. The resulting mixture was ultrasonicated for 20 min to form an opalescent and turbid water-in-oil emulsion by using an ultrasonic emulsification instrument (Shanghai HANUO, HN-500, China). In the case of emulsion preparation for protein-containing sample, PS and PS-COOH (1:1 of weight ratio) mixture was dissolved in CHCl_3 at the concentration of 10.0 mg/mL. BSA was dissolved in deionized water at the concentration of 40.0 mg/mL. Then the same steps were taken to fabricate the inverse emulsion.

Preparation of Ie-BF Films

Firstly, polyethylene terephthalate (PET) substrates ($2 \times 2 \text{ cm}$) were cleaned with acetone and DI water, then dried with nitrogen before use. The as-prepared inverse emulsions were cast onto the PET substrate placed in a closed chamber with adjustable temperature and humidity. The casting solution was allowed to evaporate to dryness to obtain films under the selected condition of temperature and humidity. After complete solidification, the obtained film was removed into the ambient environment for further drying.

Fabrication of Films with Planar Surfaces for stBF treatment

Polymer solutions of PS or PS/PS-COOH (1:1 of weight ratio) were prepared by dissolving the polymer or polymer mixture in CHCl_3 at the concentration of 10.0 mg/mL. The solutions were cast on PET substrate at room temperature under an environmental condition of low humidity (25 °C, $\text{RH} \approx 25\%$). After the solidification, polymeric films of PS or PS/PS-COOH with planar surface were obtained. PS film with non-planar surface was prepared using dip coating method on a capillary quartz tube (Shanghai Jiyu, China). The tube with diameter of 0.5 mm was immersed manually in the PS/ CHCl_3 solution (10.0 mg/mL) for 2 seconds and then dried at room temperature under an environmental condition of low humidity (25 °C, $\text{RH} \approx 25\%$). The aforementioned dip coating procedure was repeated for 5 times to complete the coating.

Fabrication of the Ie-BF Structure by Performing stBF Technique

The emulsions of CHCl_3 /water/DDAB and CHCl_3 /water/BSA were drop cast onto the PS and PS/PS-COOH planar films, respectively, at room temperature under the humid condition (25 °C, $\text{RH} \approx 75\%$). After complete solidification, the treated film was removed into the ambient environment for further drying. For the PS films on the tube, the stBF treatment was completed by immersing the coated tube in the emulsion of CHCl_3 /water/DDAB for 10 s. Then the sample was dried at room temperature under the humid condition (25 °C, $\text{RH} \approx 75\%$). After complete solidification, the treated film on the tube was removed into the ambient environment for further drying.

Characterization

the upper and cross-sectional surfaces of the obtained films were characterized with a field emission scanning electron microscope (FEI, Nova Nano SEM 650, USA) operating at a 5 kV accelerating voltage. The cross-sectional samples were obtained by fracturing samples in liquid nitrogen, and all samples were coated with platinum before SEM examination. Optical images were taken under a Zeiss Imager. A2m optical microscope equipped with a digital camera. The sizes of inverse emulsion droplets were measured by dynamic light scattering (DLS) at 25 °C and 90° scattering

angle on a Zetesizer Nano ZS90 (Malvern Instruments).

PAC Loading and Release

PAC was directly loaded into the film during the Ie-BF fabrication process of the film. Firstly, PAC was dissolved in deionized water at the concentration of 80 mg/mL, and the PAC aqueous solution was mixed with PS chloroform solution (10 mg/mL). The mixture was then emulsified by using the ultrasonic emulsification instrument for 20 min to make the inverse emulsion with PAC dispersed in the emulsion droplets. The as-prepared inverse emulsion was cast onto a clean substrate placed in a closed chamber with controlled humidity and temperature. After complete solidification, the obtained film was removed into the ambient environment for further drying.

Standard curves based on known concentrations (0, 0.01, 0.02, 0.039, 0.078, 0.156, 0.3125, 0.625, 1.25, 2.5, and 5 mg/mL) of PAC in phosphate buffer saline (PBS) were generated using optical densit readings obtained by ultraviolet-visible double beam spectrophotometer (Mapada UV6100s, Shanghai Mapada Instruments Co., Ltd, Shanghai, China). The subsequent PAC concentration released from the film was calculated based on the standard curve. Drug release profiling experiments were performed at 37 °C by immersing the PAC-loaded film in PBS (pH 7.4). At the predetermined time-points, PAC-loaded film was removed, and the PBS solutions were analyzed by ultraviolet-visible spectrophotometry. The PAC-loaded film was immersed in fresh PBS and incubated until the next time-point. Testing period lasted for 28 days. The percentage of PAC release was calculated by dividing the accumulated amount of released PAC by the total amount of PAC released at the end of the experimental period.

Supporting figures

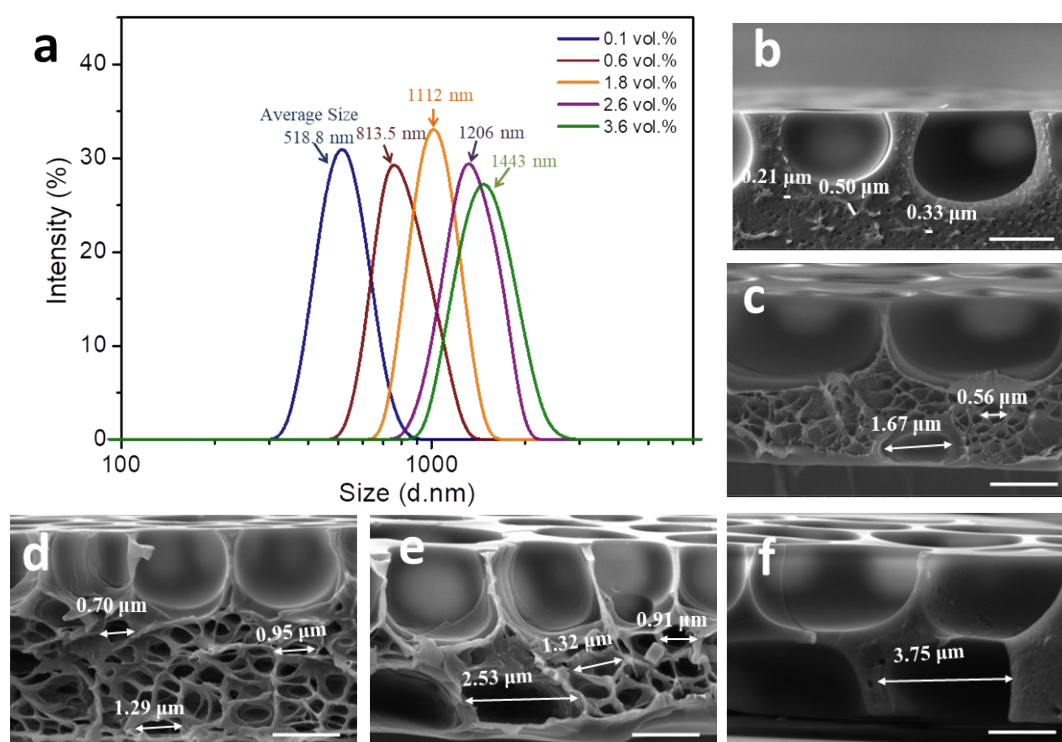


Fig. S1. a) Size distribution of inverse emulsion droplets with different water ratios. b-f) Cross-sectional SEM images of the films fabricated from inverse emulsions with different water ratios (same as in a). b) 0.1 vol%; c) 0.6 vol%; d) 1.8 vol%; e) 2.6 vol% and f) 3.6 vol%. The environmental conditions of the film casting: 25 °C, RH≈75%. Scale bar: 2 μm.

Discussion on S1: The sizes of the selective sublayer pores in 5 different samples (Fig. S1b-f) were measured for comparison with the sizes of the corresponding emulsion droplets recorded by DLS (Fig. S1a). It could be seen that the sizes of the pores in the bulk grew larger with the size increase of the emulsion droplets, showing fine correspondence.

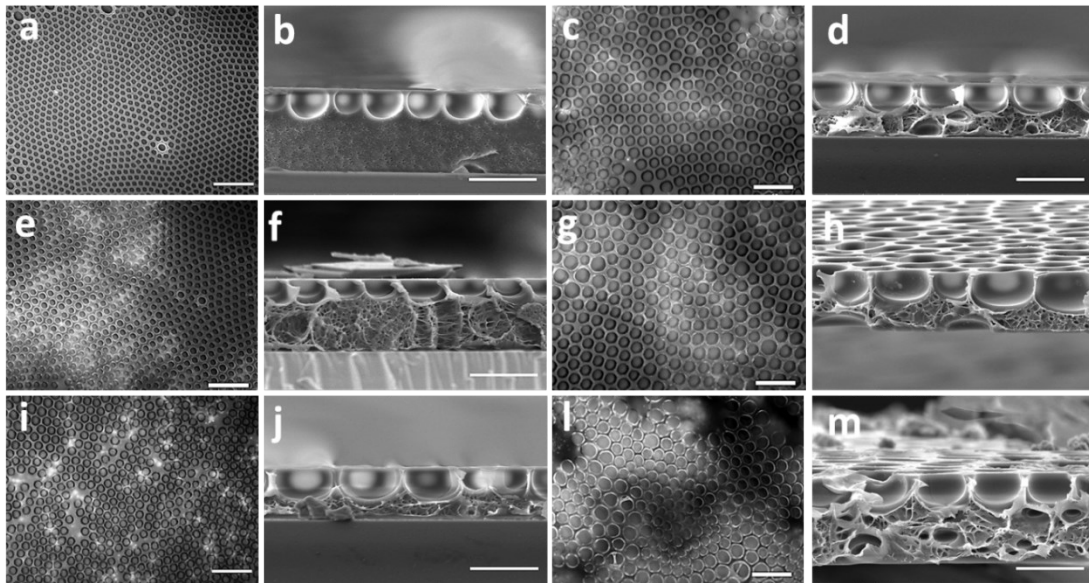


Fig. S2. Top-view optical (a,c,e,g,i,l) and cross-sectional SEM (b,d,f,h,j,m) images of the films fabricated from inverse emulsions with different water/oil ratio. a,b) 0.1 vol%; c,d) 1.8 vol%; e,f) 1.0 vol%; g,h) 2.2 vol%; i,j) 1.4 vol% and l,m) 3.2 vol%. The environmental conditions of the film casting: 25 °C, RH \approx 75%. Scale bar: Optical images 10 μ m; SEM images 5 μ m.

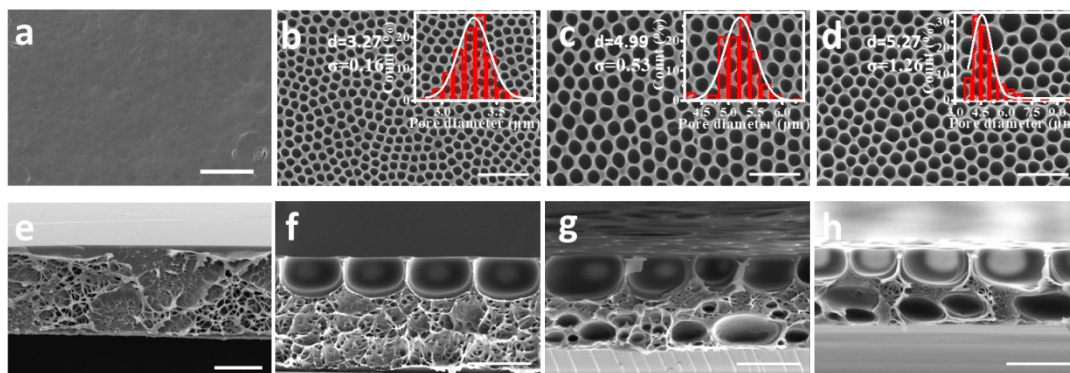


Fig. S3. Top-view (a-d) and cross-sectional (e-h) SEM images of the films fabricated from inverse emulsions with 1 vol% water ratio under different conditions of RH. a,e) 45% RH; b,f) 75% RH; c,g) 85% RH and d,h) 95% RH. Average pore diameters of the top-surface pores and the corresponding standard deviations (σ) for each sample were presented as insets of b-d. The environmental temperature of the film casting was 25 °C. Scale bar: a-d) 20 μm ; e-h) 5 μm .

Discussion on S3: Another basic factor to be evaluated is humidity. We cast the emulsion with water ratio of 1 vol% under different humidity and characterized the morphologies of the fabricated films (Fig. S3). Under the condition of rather low humidity (45%), casting of the emulsion could be able to generate sub-micrometer sized pores in the bulk layer (Fig. S3e), yet no pores on the surface (Fig. S3a). When the condition of humid environment (75%) was provided, regularly arranged BF pores appeared on the top surface of the obtained film (Fig. S3b). While with further elevation of the humidity, the surface pores were enlarged, and the uniformity of the pore sizes became poorer (Fig. S3c,d). It was in accordance with common observations in the previous reports for BF pore morphology with the increase of humidity.¹ On the other hand, compared with the case on the top surface, much greater change took place for the pores in the bulk when the humidity increased. Sub-micrometer sized pores were formed under the relative humidity of 75% just like the case of low humidity (Fig. S3f). While pores grew into sizes of several micrometers with 85% and 95% of RH (Fig. S3g,h). We would assume that during the Ie-BF process, the emulsion droplets, dispersed within the cast emulsion, would serve as

droplet nuclei to grow into larger droplets when the humidity reach rather high value (higher than 85% RH). Furthermore, when the droplets began to grow, it would be easier for the growing droplets in the bulk layer to coalesce into each other, resulting in the formation of bulk pores with the size as shown in the Fig. S3h. It is one of the unique features of Ie-BF process.

It could be concluded that the emulsion droplets served as main origin of the bulk pores, while adsorption of water vapor from the humid air facilitated the growth of the templating water droplets in the bulk. Hence, both humidity and the water ratio of the fabricated emulsion could be used to manipulate the porosity of the bulk layer significantly.

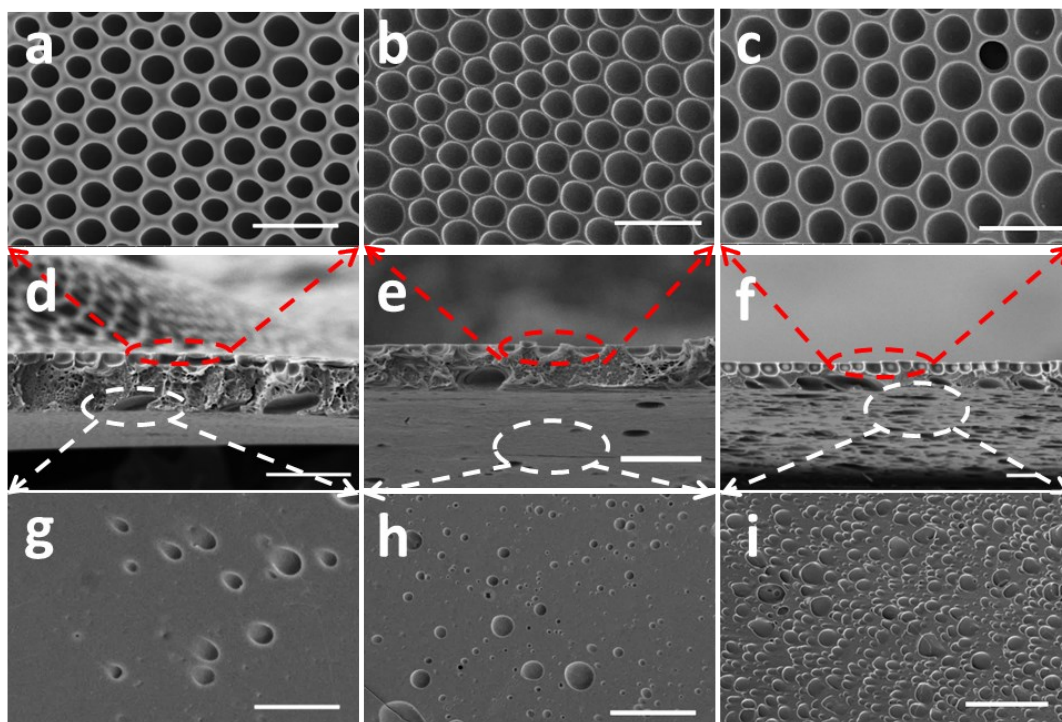


Fig. S4. Top-view (a-c), cross-sectional (d-f) and bottom-view (g-i) SEM images of the films fabricated from inverse emulsions with different water/oil ratio. a,d,g) 1.0 vol%; b,e,h) 2.0 vol% and c,f,i) 3.2 vol%. The environmental conditions of the film casting: 25 °C, RH \approx 75%. Scale bar: a-f) 10 μ m; g-i) 50 μ m.

Discussion on S4: By performing the Ie-BF procedure, one could fabricate thin films with the top surface patterned with honeycomb-structured pore arrays and the bulk layer structured into randomly porous matrix. The asymmetry is quite obvious between regular array and irregular porous layer from the cross-sectional view. On the other hand, pore openings were found to be formed on the bottom line of the film when relatively high water ratio was adopted to prepare the casting emulsion. We have examined the morphologies of both faces of the films made from emulsions with different water ratios. As shown in Fig. S4g, there were only a few pore openings on the bottom face of the obtained film under the condition of 1 vol% of water content. More pores emerged with water content been increased to 2 vol% (Fig. S4h). While porous structures were obtained with 3.2 vol% water content (Fig. S4i). In contrast of hexagonally arranged BF pore arrays on the top surface (Fig. S4a-c), the pores on the

bottom side were rather irregular both in shapes and arrangement. Hence, in addition to the asymmetric pore layers in the cross section, the two-faced asymmetry was also established for the Ie-BF films.

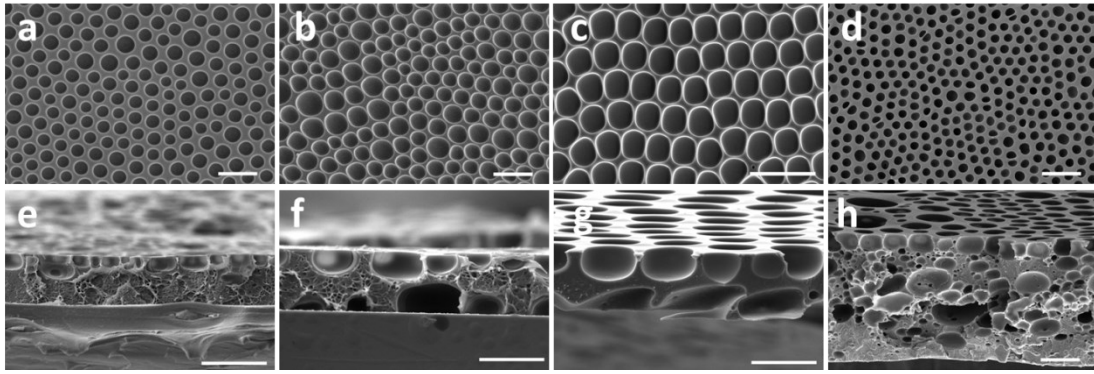
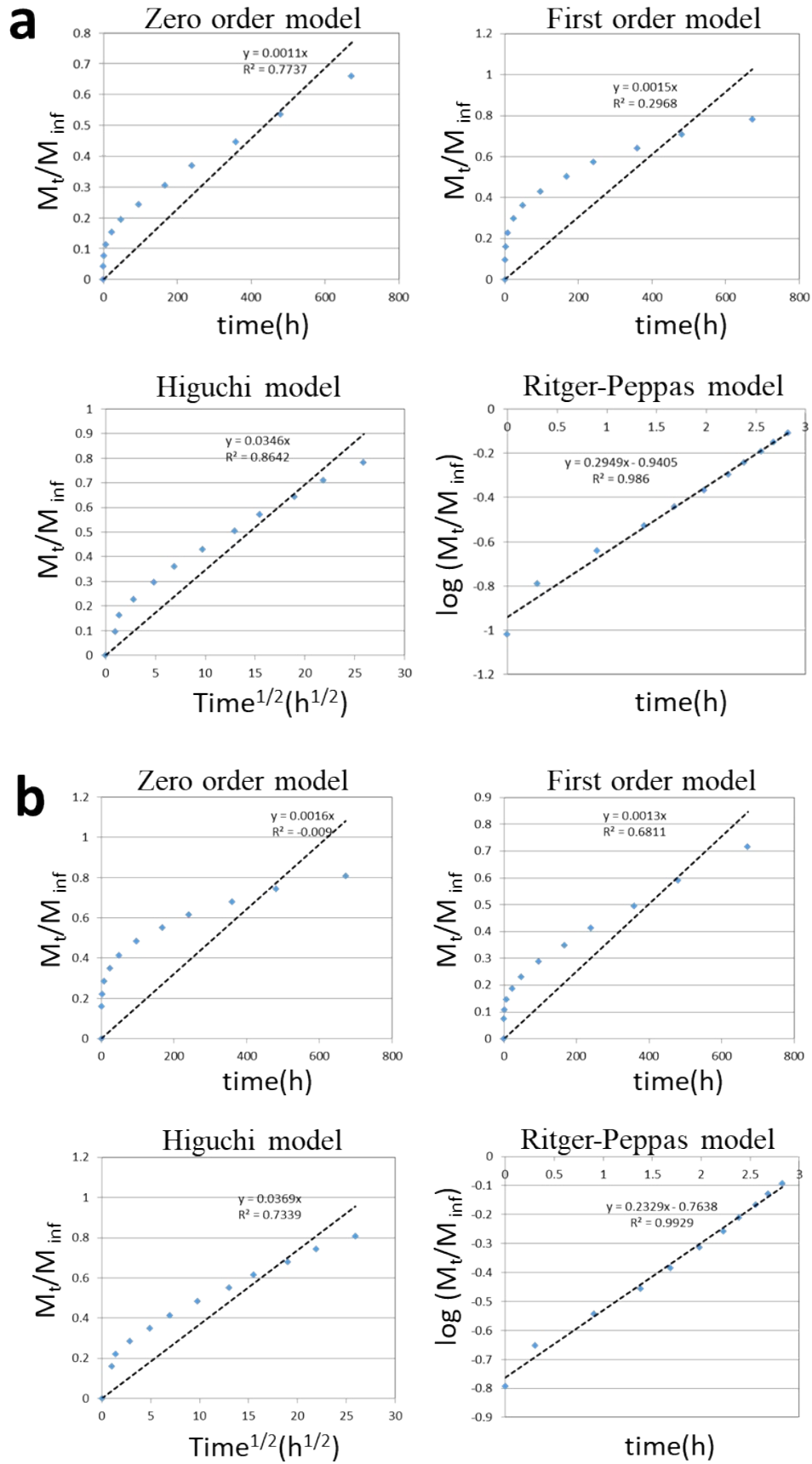


Fig. S5. Top-view (a-d) and cross-sectional (e-h) SEM images of stBF sample made from PS (a-c) and PS/PS-COOH (d) thin film with planar surfaces. The films were treated by emulsions with different water ratios. a,e) 0.6 vol%; b,f) 2.6 vol%; c,g) 3.6 vol% and d,h) 2 vol% (BSA-containing). The environmental conditions of stBF treatment: 25 °C, RH \approx 75%. Scale bar: 10 μ m.

Discussion on S5: Emulsions with water ratio of 0.6, 2.6 and 3.6 vol% as well as 2 vol% (BSA-containing) were prepared without the inclusion of PS and cast onto the PS thin films with planar surfaces. Fig. S5 showed the top-down and cross-sectional morphologies of the four stBF samples. The honeycomb-structured pore arrays were obtained for all the samples. For the bulk layer, porous morphologies with different pore features were formed (Fig. S5e-g), which were rather similar for the corresponding BF samples using the same water ratio (Fig. 1f-h). While for the protein-containing samples, a thick porous bulk layer was formed (Fig. S5h). Comparing to the morphology shown in Fig. 2e, the polymeric matrix of the protein-containing stBF sample was not completely “foamed” into cellular pores. We would assume that by drop casting, the solvation effect upon the PS film from the emulsion was not thorough enough to reconstruct the whole bulk as in the case of the casting of polymer-containing emulsion.



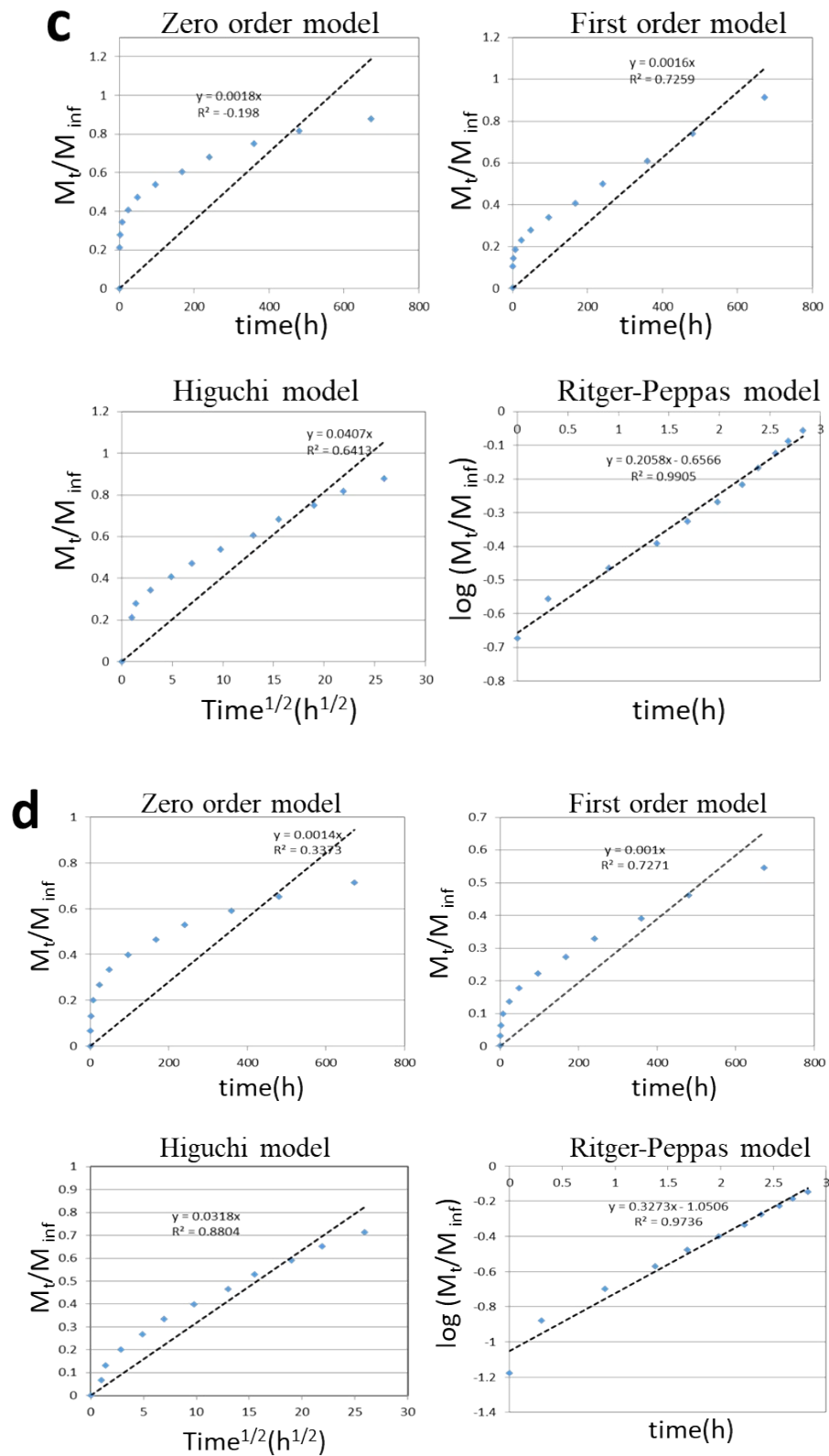


Fig. S6. Fitted PAC release data of four different film S1-S4 obtained using different release models. a) S1; b) S2; c) S3; d) S4.

Discussion on S6: To further reveal the release kinetics, the release profiles of S1-S4 were fitted by four commonly adopted mathematical models (i.e., zero-order equation (1), first-order equation (2), Higuchi equation (3) and Ritger-Peppas equation (4)).³⁻⁶

$$\frac{M_t}{M_{inf}} = kt \quad (1)$$

$$\log\left(1 - \frac{M_t}{M_{inf}}\right) = - (k/2.303)t \quad (2)$$

$$\frac{M_t}{M_{inf}} = kt^{0.5} \quad (3)$$

$$\frac{M_t}{M_{inf}} = kt^n \quad (4)$$

where M_t is the cumulative amount of drug released at time t ; M_{inf} is the cumulative amount of drug released at infinite time; M_t/M_{inf} is the fractional drug release at time t ; k is the kinetic constant; n is the release exponent related to the release mechanism.

As indicated by the fitting results in Fig. S6 and Table S1, the release behavior of all four samples of S1-S4 best fitted with Ritger-Peppas model, reaching a much higher R^2 value compared to the other models. For S1-S4, n was less than 0.5, suggesting that the drug release followed Fickian diffusion mechanism.² The kinetics results using Ritger-Peppas model indicated the fact that the Ie-BF thin film was ideal candidate for drug delivery material with feature of long-term release (Fig. 4b-e). While by comparing the value of n between S1-S4 using Ritger-Peppas model, one would conclude that S4 ($n=0.33$) showed the ability of most sustained release. While for S1-S3, S1 ($n=0.29$), made from emulsion with lowest water ratio, showed more

prolonged release behavior than S2 ($n=0.23$) and S3 ($n=0.21$). Such conclusion based on the values of n matched the result of the release profile in Fig. 4a well.

Table S1. Correlation coefficients R^2 for PAC released from the films of S1-S4 after fitting the release profile obtained using different release models.

Release at different water/oil ratio	Zero-order Model R^2	First-order Model R^2	Higuchi Model R^2	Ritger-Peppas Model	
				N	R^2
S1	0.7737	0.2968	0.8642	n=0.29	0.986
S2	-0.009	0.6811	0.7339	n=0.23	0.9929
S3	-0.198	0.7259	0.6413	n=0.21	0.9905
S4	0.3373	0.7271	0.8843	n=0.33	0.9736

REFERENCES

- 1 O. Karthaus, N. Maruyama, X. Cieren, M. Shimomura, H. Hasegawa and T. Hashimoto, *Langmuir*, 2000, **16**, 6071-6076.
- 2 T. F. Huang, P. Manchanda, L. W. Zhang, O. Shekhah, N. M. Khashab, M. Eddaoudi and K. V. Peinemann, *J. Membr. Sci.*, 2019, **584**, 1-8.
- 3 T. Higuchi, *J. Pharm. Sci.*, 1963, **52**, 1145-1149.
- 4 R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri and N. A. Peppas, *Int. J. Pharm.*, 1983, **15**, 25-35.
- 5 R. Machin, J. R. Isasi and I. Velaz, *Eur. Polym. J.*, 2013, **49**, 3912-3920.
- 6 L. Serra, J. Domenech and N. A. Peppas, *Biomaterials*, 2006, **27**, 5440-5451.