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Controlled synthesis of linear and comb-like glycopolymers for preparation of honeycomb-patterned films

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ABSTRACT

Honeycomb-patterned films can be facilely prepared by the breath figure method. However, it is still ambiguous how the polymer structure affects the morphologies of the films. In this work, poly(styrene-*co*-2-(2-,3-,4-,6-tetra-*O*-acetyl-β-D-glucosyloxy) ethyl methacrylate) (PS-*co*-AcGEMA) with well-defined linear and/or comb-like structures were synthesized by atom transfer radical polymerization (ATRP). These glycopolymers were used as precursors for the fabrication of pattern films by the breath figure method. The regularity and pore size of the films are greatly influenced by the polymer structure and the solution concentration. Highly ordered pattern films can be prepared from the comb-like glycopolymer and the linear block glycopolymers with relatively long PAcGEMA segment. Further studies of lectin recognition on the honeycomb-patterned films demonstrate that the glucose-containing films can specifically recognize Con A. These bioactive honeycomb-patterned films have potential applications as templates, picoliter beakers for bioanalysis and cell culture materials.

1. Introduction

Porous films with pattern structures have received increasing interest due to their potential applications as filtration membranes [1], optical materials [2], catalyst supports [3], templates [4], cell culture substrates [5], and superhydrophobic surfaces [6]. Several methods have been described in literatures for the preparation of pattern films, including lithography [7] and template techniques [8,9]. Among the template techniques, the so-called breath figure method is the most attractive one, due to its facility to prepare honeycomb-patterned films [10,11].

Francois and coworkers first described the breath figure method for preparing honeycomb-patterned films by casting a polymer solution under a humid airflow [12]. The condensed water droplets caused by solvent evaporation-induced rapid cooling act as the templates that direct the film formation [13,14]. The regularity of pore arrays and the pore sizes are strongly influenced by the polymer structures and the casting conditions [15–20]. Although the formation mechanism is still to be further elucidated, honeycomb-patterned films have been prepared from a variety of polymers including star or comb-like polystyrenes [19,21], polyimides [22], polyion complexes [14], and organometallic polymers [23].

Recently, polymers with biocompatible or bioactive moieties have received much attention for the fabrication of pattern films [24–26]. As the biomimetic counterparts of natural polysaccharides, glycopolymers (side chain carbohydrate-containing polymers) are very attractive for their protein-specific binding ability [27–29]. Glycosylated pattern films are believed to have potential applications in many fields including protein isolation and cell culture. However, the preparation of such films is only scarcely reported and the prerequisite structure of glycopolymer for the formation of honeycomb-patterned films is still ambiguous [30,31].

To prepare honeycomb-patterned films, the control over glycopolymer structure is very important. Living and controlled polymerizations, such as anionic polymerization, nitroxide-mediated radical polymerization (NMP), atom transfer radical polymerization (ATRP), and reversible addition fragmentation chain transfer (RAFT) polymerization, are useful in synthesizing well-defined glycopolymers [32–35]. Most recently, Stenzel et al. described the synthesis of a block glycopolymer via NMP and the preparation of bioactive porous films by the breath figure method [36]. They detailedly explored the polymerization kinetics and mainly focused on the synthesis of linear copolymers containing galactose moieties. Compared with NMP and other polymerization methods, ATRP is attractive and garners much more attention because of its tolerance to impurities, compatibility with a wide range of monomers, and relatively mild polymerization conditions [37,38]. In this work, well-defined glycopolymers with linear and comb-like structures were synthesized by ATRP. The obtained glycopolymers were used

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as precursors for the formation of porous films by the breath figure method and the effects of polymer structure and solution concentration on the film structure were investigated. The recognition of fluorescence-labeled lectins (concanavalin A and peanut agglutinin) by the glycosylated pattern films was also examined.

2. Experimental part

2.1. Materials

Styrene (St) was commercially obtained from Sinopharm Chemical Reagent Co. (China) and distilled at reduced pressure before use. The carbohydrate-containing monomer, 2-(2-,3-,4-,6-tetra-*O*-acetyl-β-D-glucosyloxy) ethyl methacrylate (AcGEMA), was synthesized using a procedure described previously [39]. 1-phenylethyl bromide (1-PEBr), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA), 2-hydroxylethylmethacrylate (HEMA) and 2-bromoisobutyryl bromide were used as received from Aldrich. CuBr was purified by subsequently washing with acetic acid, ethanol and drying under reduced pressure. Triethylamine (TEA) was purified by distillation. Poly(ethylene terephthalate) (PET) film was kindly provided by Hangzhou Tape Factory and cleaned with acetone for 2 h before use. Fluorescein labeled concanavalin A (FL-Con A) and peanut agglutinin (FL-PNA) (Vector, USA) were used as received. Water used in all experiments was deionized and ultrafiltrated to 18 MΩ with an ELGA LabWater system. All other reagents were analytical grade and used without further purification.

2.2. Synthesis

2.2.1. Polystyrene macroinitiator

ATRP of St was preformed according to the following procedure. St (2.3 mL, 20 mmol), 1-PEBr (13.6 μL, 0.1 mmol), CuBr (14.3 mg, 0.1 mmol), PMDETA (20.9 μL, 0.1 mmol) were added into a 25 mL round-bottomed flask with magnetic stirring bar. After degassed by three freeze–pump–thaw cycles, the flask was sealed under reduced pressure and was immersed into an oil bath at 110 °C while stirring. After a prescribed time, the flask was opened and the solution was diluted with tetrahydrofuran (THF). PS-Br **1** was isolated as a white powder by precipitation from a large excess of methanol and then dried under reduced pressure at 50 °C.

2.2.2. Linear block glycopolymer (PS-*b*-PAcGEMA ***2***)

Polystyrene-*block*-poly(AcGEMA), PS-*b*-PAcGEMA **2**, was synthesized according to the following procedure. 0.46 g (1 mmol), 0.92 g (2 mmol), 1.38 g (3 mmol) or 2.30 g (5 mmol) AcGEMA was mixed with PS-Br (**1**, 1.9 g, 0.1 mmol), CuBr (14.3 mg, 0.1 mmol), PMDETA (20.9 μL, 0.1 mmol) and chlorobenzene (10 mL) in a 50 mL round-bottomed flask with magnetic stirring bar. After degassed by three freeze–pump–thaw cycles, the flask was sealed under reduced pressure and was immersed into an oil bath at 80 °C while stirring. After a prescribed time, the reaction mixture was precipitated by pouring the solution into methanol followed by filtration and dried under reduced pressure at 50 °C to obtain the block glycopolymer.

2.2.3. Linear random glycopolymer (PS-*r*-PAcGEMA ***3***)

Polystyrene-*random*-poly(AcGEMA), PS-*r*-PAcGEMA **3**, was synthesized based on the following procedure. St (2.3 mL, 20 mmol), AcGEMA (2.3 g, 5 mmol), 1-PEBr (13.6 μL, 0.1 mmol), CuBr (14.3 mg, 0.1 mmol), PMDETA (20.9 μL, 0.1 mmol) were added into a 25 mL round-bottomed flask with magnetic stirring bar. After degassed by three freeze–pump–thaw cycles, the flask was sealed under reduced pressure and was immersed into an oil bath at 110 °C while stirring. After a prescribed time, the flask was opened and the solution was diluted with tetrahydrofuran (THF). The polymer was precipitated by pouring the solution into methanol. The random glycopolymer **3** was collected by filtration and then dried under reduced pressure at 50 °C.

2.2.4. Comb-like glycopolymer (PS-*b*-(PHEMA-*g*-PAcGEMA) ***6***)

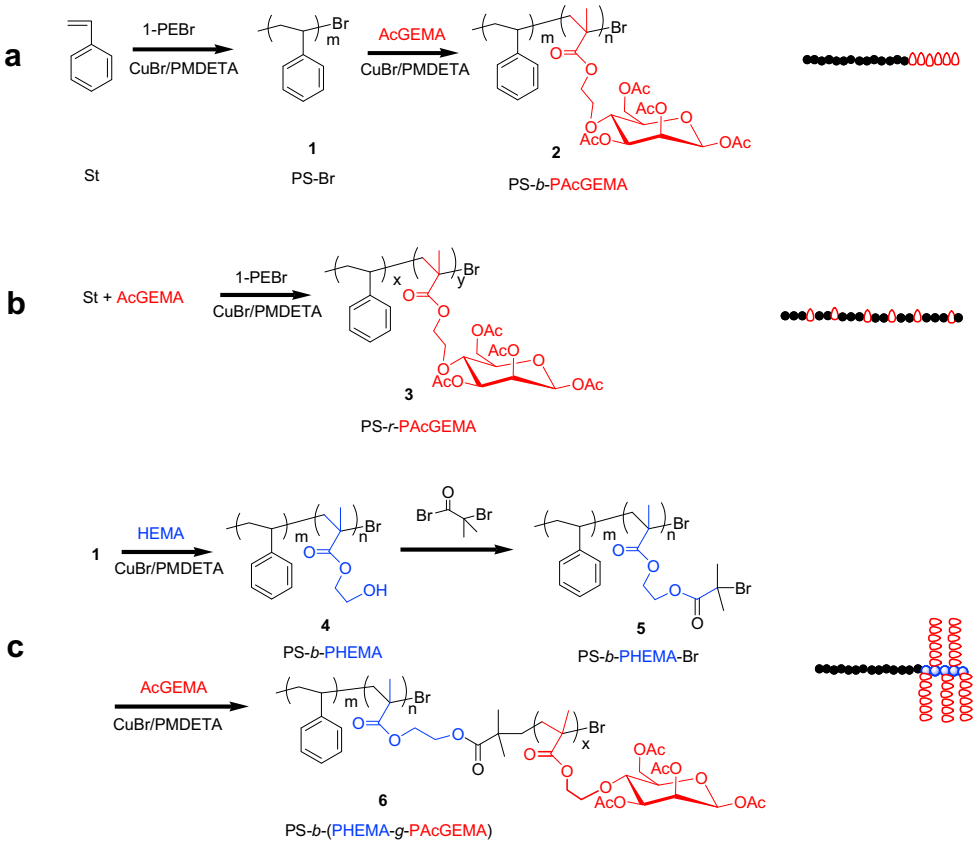
The comb-like glycopolymer, PS-*b*-(PHEMA-*g*-PAcGEMA) **6**, was synthesized as depicted in Scheme 1c. The synthesis of linear block copolymer polystyrene-*block*-poly(2-hydroxylethylmethacrylate) (PS-*b*-PHEMA) **4** was conducted in the same manner as PS-*b*-PAcGEMA **2**, except that HEMA (120 μL, 1 mmol) was added into the flask instead of AcGEMA. Block copolymer of St with 2-(2-bromoisobutyryloxy)ethyl methacrylate (PS-*b*-PHEMA-Br **5**) was synthesized based on the following procedure. PS-*b*-PHEMA (**4**, 2 g, −OH 0.6 mmol) was added into a 100 mL two-necked round-bottomed flask fitted with a N_2 inlet, dissolved in 20 mL of anhydrous dichloromethane (CH_2Cl_2), and was cooled in an ice bath. Then 0.65 mL TEA (5 mmol) was added into the flask. Thereafter, 0.32 mL (2.5 mmol) of 2-bromoisobutyryl bromide dissolved in 15 mL of anhydrous CH_2Cl_2 was then added dropwise at 0 °C in 1 h. The mixture was stirred for another 3 h at followed by stirring at room temperature for 24 h. The polymer was precipitated by pouring the solution into methanol. After filtration and washing, the polymer **5** was dried in reduced pressure. For the synthesis of the comb-like glycopolymer, PS-*b*-PHEMA-Br **5** was used as a macroinitiator to initiate the AcGEMA polymerization by a typical ATRP procedure. AcGEMA (0.46 g, 1 mmol) was mixed with PS-*b*-PHEMA-Br (**5**, 0.36 g, −Br 0.1 mmol), CuBr (14.3 mg, 0.1 mmol), PMDETA (20.9 μL, 0.1 mmol) and chlorobenzene (5 mL) in a 25 mL round-bottomed flask with magnetic stirring bar. After degassed by three freeze–pump–thaw cycles, the flask was sealed under reduced pressure and was immersed into an oil bath at 80 °C while stirring. After a prescribed time, the reaction mixture was precipitated by pouring the solution into methanol followed by filtration and dried under reduced pressure at 50 °C to obtain the comb-like glycopolymer **6**.

2.2.5. Deprotection of the glycopolymers

0.5 g of the glycopolymer (**2**, **3** or **6**) was dissolved in 50 mL of a mixed solvent of $CHCl_3$ and CH_3OH ($CHCl_3/CH_3OH$, 9/1, v/v) and freshly prepared CH_3ONa in methanol was added to yield a 0.1 M solution of CH_3ONa. The mixture was stirred at room temperature for 1 h. After the reaction, 10 mL deionized water was added in the mixture. With the evaporation of the organic solvents, the deprotected polymer with 2-(β-D-glucosyloxy) ethyl methacrylate (GEMA) units was precipitated from the solution and obtained by filtration. The product (**2**′, **3**′ or **6**′) was dried under reduced pressure at 50 °C.

2.3. Preparation of honeycomb-patterned films

The porous films were cast by the breath figure method. A ratio of 70:30 v/v% of CS_2 to CH_2Cl_2 was used for film casting. This solvent mixture was used to aid solubility of polymers, especially those with lengthy PAcGEMA blocks which are insoluble in CS_2. In a typical experiment, an aliquot of 100 μL for each polymer solution with a concentration ranging from 1 to 10 mg/mL was drop cast onto a PET substrate placed under a 1 L/min humid airflow. The humidity of the airflow was maintained to be above 80% by bubbling through distilled water and was measured by a hygrothermograph (DT-321S, CEM Corporation, Hongkong). After solidification, the film was dried at room temperature.



Scheme 1. Synthesis routes for (a) linear block glycopolymer PS-*b*-PAcGEMA **2**, (b) linear random glycopolymer PS-*r*-PAcGEMA **3**, and (c) comb-like glycopolymer PS-*b*-(PHEMA-*g*-PAcGEMA) **6**.

2.4. Recognition and desorption of lectins

The honeycomb-patterned film was dipped into 10 mL of dry methanol containing 0.05 g sodium methoxide and vibrated for 90 min at 25 °C to deprotect the acetyl groups of glucose pentaacetate. Thereafter, the film was sequentially washed with water. After being fully wetted in water HEPES buffer solution (pH 7.5, containing 10 mM HEPES, 0.15 M NaCl, 0.1 mM Ca^{2+}, 0.01 mM Mn^{2+} (not for PNA), 0.08% sodium azide), a piece of the deprotected film (2×5 mm^2) was dipped into 200 μL of FL-Con A or FL-PNA solution with a concentration of 0.1 mg/mL in HEPES buffer solution, and incubated at 25 °C for 2 h. The film was then washed with HEPES buffer solution 6 times. The lectin-adsorbed films were put into 200 μL of free methyl α-mannopyranoside solution (0.25 M and 1 M) and incubated at 25 °C for 24 h. Then the films were washed with HEPES buffer solution 6 times. After being dried under reduced pressure at room temperature, high-resolution images of the lectin-adsorbed and lectin-desorbed films were recorded by confocal laser scanning microscopy (CLSM).

2.5. Characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker (Advance DMX500) NMR instrument at room temperature with $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet FTIR/Nexus470 spectrometer. All spectra were taken by 32 scans at a nominal resolution of 1 cm^{-1}. Attenuated total reflectance Fourier transform infrared spectroscopy (FTIR/ATR) measurements were carried out on a Nicolet 6700 FTIR spectrometer equipped with an ATR cell (ZnSe, 45°). Thirty-two scans were taken for each spectrum at a nominal resolution of

Table 1
Results for the synthesis of linear glycopolymers **2** and **3**.

No.	Sample	[*M*]/[*I*]	Conv.[a] (%)	$M_{n,th}$[b]	$M_{n,GPC}$[c]	M_w/M_n[c]	Composition St:AcGEMA (^{1}H NMR)	$M_{n,NMR}$[d]
1	PS_{187}-Br	200:1	90	18,700	19,500	1.07	–	–
2a	PS_{187}-*b*-$PAcGEMA_5$	10:1	55	22,000	20,300	1.08	187:5	21,800
2b	PS_{187}-*b*-$PAcGEMA_9$	20:1	52	24,300	22,400	1.12	187:9	23,600
2c	PS_{187}-*b*-$PAcGEMA_{20}$	30:1	58	27,500	24,300	1.24	187:20	28,700
2d	PS_{187}-*b*-$PAcGEMA_{35}$	50:1	62	33,700	27,600	1.32	187:35	35,600
3	PS-*r*-PAcGEMA[e]	200:50:1	83	36,400	23,700	1.22	84:16	–

[a] Calculated by gravimetric method.
[b] For **1**, $M_{n,th}$ was estimated by $M_{n,th} = \text{conv.} \times M_{St} \times [M]/[I]$; for **2**, $M_{n,th} = M_{n,GPC\ (PS\text{-}Br)} + \text{conv.} \times M_{AcGEMA} \times [M]/[I]$; For **3**, $M_{n,th}$ was estimated by $M_{n,th} = \text{conv.} \times M_{St\text{-}HEMA} \times [M]/[I]$, where M_{St}, M_{AcGEMA} are the molecular weights of St, and AcGEMA, and $M_{n,GPC\ (PS\text{-}Br)}$ is the number-average molecular weight of PS-Br measured by GPC.
[c] Estimated by GPC in THF with polystyrene as calibration standard.
[d] Calculated from ^{1}H NMR spectra.
[e] The polymerization of the random glycopolymer **3** was carried out at 110 °C in bulk. [St]:[AcGEMA]:[I] = 200:50:1.

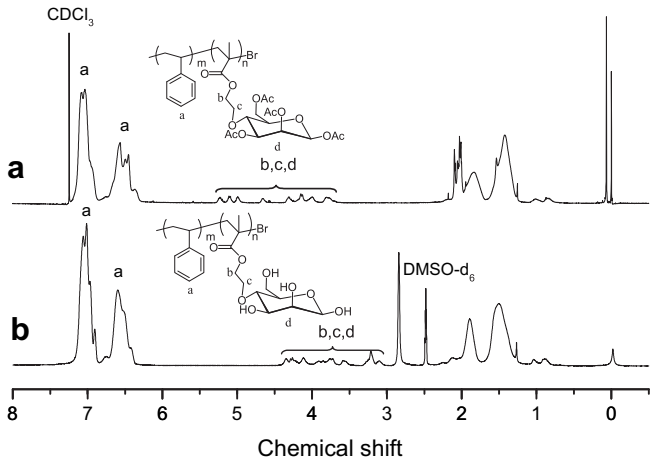


Fig. 1. Typical ^{1}H NMR spectra of (a) PS-*b*-PAcGEMA **2** in $CDCl_3$ and (b) PS-*b*-PGEMA **2′** in DMSO-d_6.

4 cm^{-1}. Molecular weight and molecular weight distribution were determined on a Waters gel permeation chromatograph (GPC) system at 25 °C, which consists of a Waters 510 HPLC pump, three Waters Ultrastyragel columns (500, 103, and 105 Å), and a Waters 410 DRI detector. THF was used as the eluent at a flow rate of 1.0 mL/min, and the calibration of the molecular weights was carried out based on polystyrene standards. Field emission scanning electron microscope (FESEM, Sirion-100, FEI, USA) was used to observe the surface morphology of films after being sputtered with gold using ion sputter JFC-1100. Confocal laser scanning microscopy (CLSM) was performed on a Leica TCS SP5 confocal setup mounted on a Leica DMI 6000 CS inverted microscope (Leica Microsystems, Wetzlar, Germany) and was operated under the Leica Application Suite Advanced Fluorescence (LAS AF) program.

3. Results and discussion

3.1. Synthesis of linear glycopolymers

As depicted in Scheme 1, three kinds of glycopolymers with controlled structures were synthesized by ATRP. For the synthesis of macroinitiator polystyrene **1**, the reaction was carried out at 110 °C with CuBr/PMDETA as the catalyst system. The conversion was controlled to be less than 95% to avoid loss of the end group functionality [40]. The number-average molecular weight and molecular weight distribution of **1** measured by GPC are 19500 and 1.07, respectively (Table 1). The block copolymerization was carried out in chlorobenzene at 80 °C and 52–62% yield was normally obtained after a polymerization time of 24 h. Block glycopolymers with different lengths of PAcGEMA block were synthesized by changing the initial monomer-to-initiator ratios. GPC analysis of the block glycopolymers confirms that the molecular weight distributions are relatively narrow ($M_w/M_n = 1.08–1.32$). The number-average molecular weights measured by GPC ($M_{n,GPC}$) are between 20300 and 27600 (Table 1). These values are slightly lower than the calculated molecular weights ($M_{n,th} = 22000–33700$). The discrepancy could be partially attributed to the differences in hydrodynamic volumes of the block glycopolymers and polystyrene standards. Fig. 1a shows a typical ^{1}H NMR spectrum of PS-*b*-PAcGEMA in $CDCl_3$. The integral of the peaks assigned to the glucose residues and $-OCH_2CH_2O-$ at 3.7–5.4 ppm is compared to the aromatic proton peak regions of polystyrene at 6.4–7.2 ppm, and the ratios of St unit to AcGEMA unit can be calculated. Since the molecular weight of PS block was accurately measured by GPC, such calculations can give the chain length of the PAcGEMA block and the number-average molecular weights of the block glycopolymers. It can be seen from Table 1 that there is a good agreement between the molar masses measured by NMR and the theoretical $M_{n,th}$ values. This supports the "living" character of the ATRP of AcGEMA using PS-Br as a macroinitiator.

To compare with the linear block glycopolymer **2**, a random glycopolymer PS-*r*-PAcGEMA **3** was also synthesized by ATRP. The molecular weight measured by GPC is 23,700 and the molecular weight distribution is 1.22. The copolymer composition of PS-*r*-PAcGEMA **3** was determined by ^{1}H NMR spectroscopy. It is found that the molar ratio of St unit to AcGEMA unit is 84:16.

3.2. Synthesis of comb-like glycopolymer

In view of the fact that hydroxyl groups can be conveniently converted into ATRP initiators [41,42], PS-*b*-PHEMA **4** was used as a precursor for the synthesis of comb-like glycopolymers. PS-Br **1** was used as a macroinitiator to initiate the block copolymerization of HEMA. GPC analysis of the copolymer **4** confirms a slight increase in the number-average molecular weight ($M_{n,GPC}$) (Table 2 and Fig. 2). The chemical composition was also determined by ^{1}H NMR spectroscopy. As shown in Fig. 3a, the peaks at 3.6–4.0 ppm are assigned to the group $-OCH_2CH_2O-$ in HEMA. Accordingly, it can be calculated from the ^{1}H NMR results that the number of HEMA unit in the copolymer **4** is about 6.

The HEMA units were then converted into 2-(2-bromoisobutyryloxy)ethyl methacrylate units by the reaction of hydroxyl groups with 2-bromoisobutyryl bromide. Fig. 4 shows the typical FTIR spectra. The peak at 1727 cm^{-1} is due to C=O stretching in the carbonyl group of HEMA units. After bromination, the intensity of this peak strengthens because of the introduction of more carbonyl groups. The peak also shows a blue shift from 1727 cm^{-1} to 1737 cm^{-1}. Moreover, the complete disappearance of the −OH peak (3100–3600 cm^{-1}) indicates a nearly quantitative conversion of the hydroxyl groups into brominated esters. The reaction of HEMA units was also ascertained by ^{1}H NMR (Fig. 3). Chemical shifts of the methylene protons *b* and *c* of PS-*b*-PHEMA-Br **5** change to 4.4 and 4.2 ppm from 4.0 and 3.6 ppm, respectively. Based on the NMR results, the calculated number of 2-(2-bromoisobutyryloxy)ethyl

Table 2
Results for the synthesis of comb-like glycopolymer **6**.

No.	Sample	Conv.[a] (%)	$M_{n,th}$[b]	$M_{n,GPC}$[c]	M_w/M_n[c]	Composition St:AcGEMA (^{1}H NMR)	$M_{n,NMR}$[d]
4	PS_{187}-*b*-$PHEMA_6$	45	20,100	20,000	1.14	187:6	20,300
5	PS_{187}-*b*-$PHEMA_6$-Br	–	–	22,000	1.19	187:6	21,100
6	PS_{187}-*b*-($PHEMA_6$-*g*-$PAcGEMA_{32}$)	53	36,600	25,200	1.43	187:32	35,900

[a] Calculated by gravimetric method.
[b] for **4**, $M_{n,th} = M_{n,GPC\ (PS\text{-}Br)} + \text{conv.} \times M_{HEMA} \times [M]/[I]$; for **6**, $M_{n,th} = M_{n,GPC\ (PS\text{-}b\text{-}PHEMA\text{-}Br)} + \text{conv.} \times M_{AcGEMA} \times [M]/[I]$, where $M_{n,GPC\ (PS\text{-}b\text{-}PHEMA\text{-}Br)}$ is the number-average molecular weight of PS-*b*-PHEMA-Br **5** measured by GPC.
[c] Estimated by GPC in THF with polystyrene as calibration standard.
[d] Calculated from ^{1}H NMR spectra.

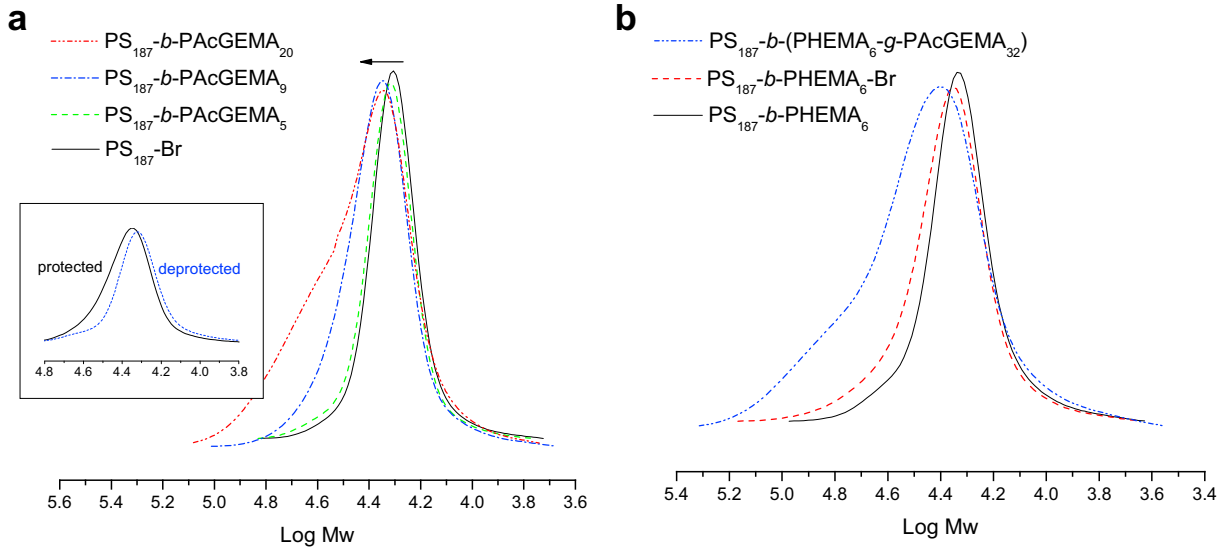


Fig. 2. GPC curves of (a) the linear block glycopolymers, (b) the comb-like glycopolymer and its precursors. Inset of (a) shows the curves before and after deprotection of the glycopolymer **2b**.

methacrylate units is also about 6, which is the same with the number of HEMA unit in PS-*b*-PHEMA **4**. Therefore, the conversion of hydroxyl groups into brominated groups is almost 100%.

The brominated copolymer, PS-*b*-PHEMA-Br **5**, was used as a macroinitiator for the ATRP of AcGEMA. Fig. 4d shows the FTIR spectrum of the comb-like glycopolymer **6**. It is obvious that the peak at 1756 cm^{-1} assigned to acetyl group of AcGEMA units is very strong, indicating the successful grafting of AcGEMA by ATRP. The ^1H NMR spectrum for the comb-like glycopolymer is presented in Fig. 3c. The peaks at 3.7–5.4 ppm are attributed to the glucose residues and the methylene protons (*b*, *c*, *d*, *e*). Therefore, the chemical composition of the comb-like glycopolymer **6** can be calculated, which has a 32 polymerization degree of AcGEMA. Assuming the initiator efficiency of bromine groups is 100%, a PS_{187}-*b*-($PHEMA_6$-*g*-$PAcGEMA_{32}$) molecule contains six PAcGEMA branches and the length of each branch is 4–5 (it is to be noted that the terminal bromine group at the backbone could also initiate the block polymerization of AcGEMA).

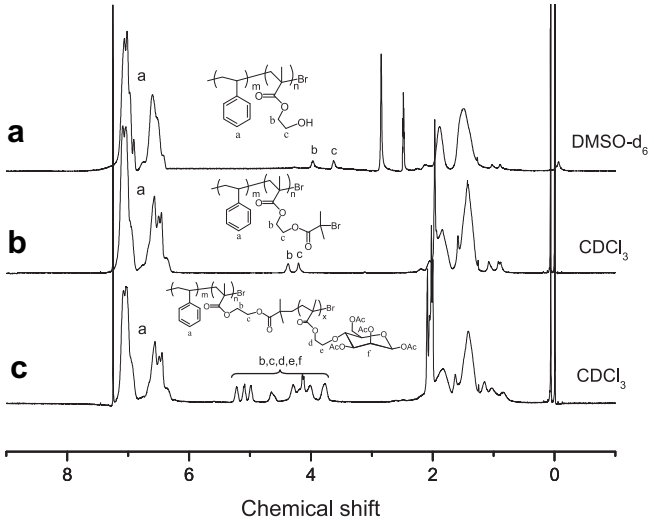


Fig. 3. ^1H NMR spectra of (a) PS-*b*-PHEMA **4** in DMSO-d_6, (b) PS-*b*-PHEMA-Br **5** in $CDCl_3$, and (c) PS-*b*-(PHEMA-*g*-PAcGEMA) **6** in $CDCl_3$.

3.3. Deacetylation of the glycopolymers

The *O*-protecting acetyl groups in PS-*b*-PAcGEMA **2**, PS-*co*-PAcGEMA **3** and PS-*b*-(PHEMA-*g*-PAcGEMA) **6** were removed with freshly prepared CH_3ONa in the mixed solvent of chloroform and methanol (v/v, 9/1) to obtain the amphiphilic copolymers (**2′**, **3′** and **6′**) [34]. Deprotection leads to the change of solubility of the glycopolymers. With the proceeding of the reaction, the initial clear solution gradually became turbid, especially for those with relatively long PAcGEMA segments. After deprotection, the glycopolymers (**2c**, **2d**, **3** and **6**) are insufficiently soluble in organic solvents such as $CHCl_3$, CH_2Cl_2 and CS_2, which is caused by the strong hydrophilicity of the PGEMA block. However, the glycopolymers with shorter PGEMA segments (**2a′** and **2b′**) can still be dissolved in $CHCl_3$, CH_2Cl_2, and partially in CS_2. Fig. 5 shows the FTIR spectra of PS_{187}-*b*-$PAcGEMA_9$ **2b** before and after *O*-deacetylation. It can be clearly seen that the carbonyl absorption peak of acetyl group at 1755 cm^{-1} disappears after *O*-deacetylation, while the absorption peak at 1726 cm^{-1} ascribed to the ester bond connecting to the main chain remains unchanged. A broad absorption peak at

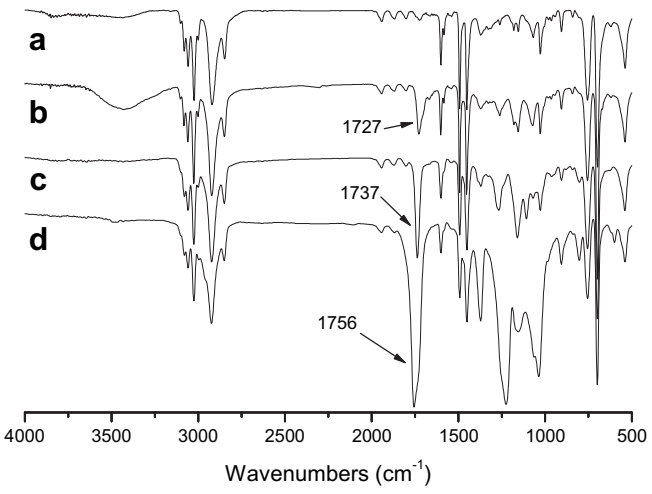


Fig. 4. FTIR spectra of (a) PS-Br **1**, (b) PS-*b*-PHEMA **4**, (c) PS-*b*-PHEMA-Br **5**, and (d) PS-*b*-(PHEMA-*g*-PAcGEMA) **6**.

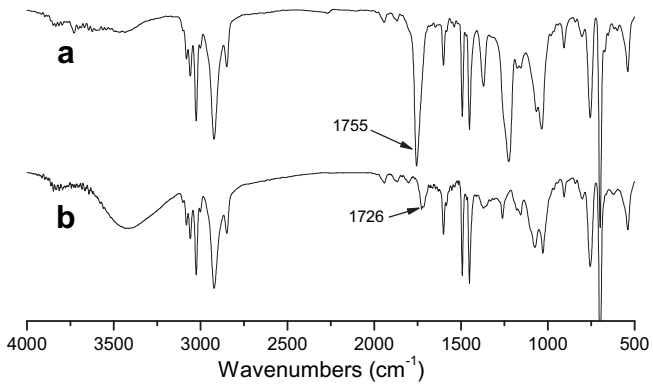


Fig. 5. FTIR spectra of (a) PS-*b*-PAcGEMA **2b** and (b) PS-*b*-PGEMA **2b′**.

3100–3600 cm^{-1} corresponding to hydroxyl groups appears after deprotection. The ^1H NMR spectrum of **2b′** in DMSO-d_6 is shown in Fig. 1b. Compared with Fig. 1a, the peak arising from the *O*-protecting acetyl groups at 1.9–2.1 ppm is almost absent, while those due to the protons of glucopyranose and the $-OCH_2CH_2O-$ spacer can still be clearly observed at 3.0–4.4 ppm. Integrals from the spectrum indicate an estimate of 91% successful deacetylation of glucose moieties. The calculated molar ratio of St unit to glucose unit is 187:9, which is the same with that before deprotection. Therefore, it can be concluded that under the present conditions, the *O*-protecting acetyl groups have been removed nearly quantitatively, while the ester bonds connecting with the main chain remain unaffected.

3.4. Regularity of honeycomb-patterned films

Honeycomb-patterned films can be prepared by the breath figure method, which is based on evaporative cooling and subsequent water-droplet templating to form an ordered array of breath figures [10]. Many reports have emerged showing the versatility of the method, and many polymers can be used in this process. However, it is still ambiguous how the polymer structure affects the morphology of the film [11,18]. In this study, the linear and comb-like glycopolymers with different structures were utilized in the casting process. From these results, some tentative conclusions can be drawn on the relationship between the structure of the glycopolymers and film formation by the breath figure method.

The polymers were cast from CS_2/CH_2Cl_2 mixture under a humid airflow. To obtain reproducible and reliable results, each polymer was cast up to 20 times with ranging conditions. The obtained films show different pore sizes and qualities depending on the polymer used. As shown in Fig. 6, PS-Br **1** is difficult to form regular pores, which is in accordance with the result reported previously [12]. Films of good quality (narrow pore size distribution, high regularity, and large film area) are obtained from linear block glycopolymer with relatively long PAcGEMA segments (**2c** and **2d**). Glycopolymers with short PAcGEMA segments (**2a** and **2b**) result in smaller pore size, but lower regularity (Fig. 6b and c). It seems that

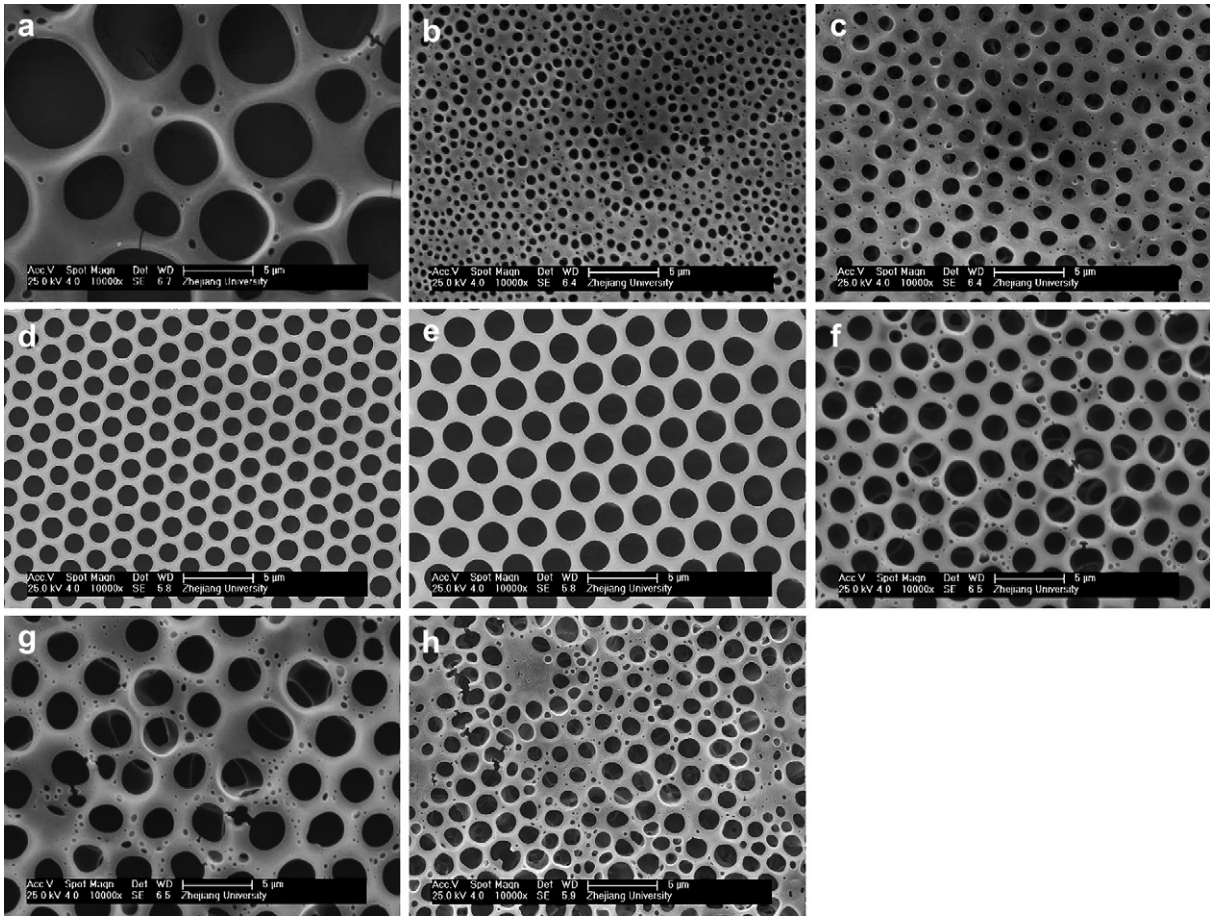


Fig. 6. SEM images of the porous films fabricated from (a) PS-Br, (b) PS_{187}-*b*-$PAcGEMA_5$ **2a**, (c) PS_{187}-*b*-$PAcGEMA_9$ **2b**, (d) PS_{187}-*b*-$PAcGEMA_{20}$ **2c**, (e) PS_{187}-*b*-$PAcGEMA_{35}$ **2d**, (f) PS_{187}-*b*-$PGEMA_5$ **2a′**, (g) PS_{187}-*b*-$PGEMA_9$ **2b**, (h) PS-*r*-PAcGEMA **3**.

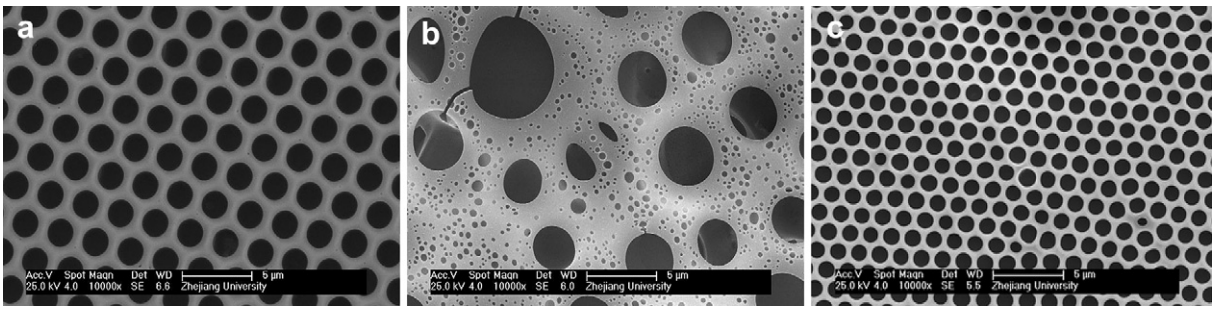


Fig. 7. SEM images of the porous films fabricated from (a) PS-*b*-PHEMA **4**, (b) PS-*b*-PHEMA-Br **5**, and (c) PS-*b*-(PHEMA-*g*-PAcGEMA) **6**.

increasing the PAcGEMA block length tends to assist the formation of regular films. The deacetylated glycopolymers (**2a′**, **2b′**) lead to films with unsatisfied quality and glycopolymers **2c′**, **2d′**, **3′** and **6′** cannot be fully dissolved. Moreover, the random glycopolymer **3** with similar molecular weight and molar fraction of PAcGEMA to the glycopolymers **2c** and **2d** cannot generate honeycomb-patterned films. Surprisingly, the block copolymer PS-*b*-PHEMA **4** with relatively short PHEMA block is able to form films of almost prefect regularity but the brominated copolymer **5** cannot (Fig. 7a and b). The comb-like glycopolymer **6** yields films with high regularity.

It generally accepted that the crucial point for the formation of a regular honeycomb-patterned film is preventing the coalescence of water droplets [11]. This can be achieved either by kinetic control or by thermodynamic control [43]. The kinetic control is important for star or comb-like polymers. They precipitate at the organic–water interface very fast so that the water droplets have no time to coalesce before the solidification of the film [44]. Therefore, the star and comb-like polymers have broader “window” for the formation of honeycomb-patterned films compared with their linear analogues. This is also confirmed by our results that comb-like glycopolymer **6** forms regular porous arrays (Fig. 7c).

The thermodynamic control involves the stabilization of water droplets by interfacial-active compounds, e.g. amphiphilic linear polymers. The polymer with hydrophilic component tends to aggregate at the organic–water interface and to stabilize the water droplets [11]. It was reported that many amphiphilic block copolymers could yield regular porous films [17,18,45]. However, an optimum balance between the hydrophilic block and the hydrophobic block is expected to be a prerequisite [18]. In this study, glucose groups have been attached on the side chain of the styrene-based copolymers and served as the hydrophilic component. Nevertheless, the hydrophilicity of the PAcGEMA segment is relatively weak because of the protection of the hydroxyl groups. The glycopolymers with short PAcGEMA segment do not have sufficient hydrophilicity to stabilize the water droplets well. Therefore, the regularity of the porous films prepared from linear block glycopolymer **2** increases with the length of the PAcGEMA segment (Fig. 6). After deprotection, the glucose-containing blocks of the glycopolymers (**2a′** and **2b′**) become highly hydrophilic. As pointed out by Stenzel, copolymers with highly hydrophilic blocks have marked tendency to take up water [18]. The water droplets increase in size so rapidly that the glycopolymers cannot keep them from coalescence. As a result, the regularity of the porous films decreases. Compared with the glucose-containing segments, the PHEMA segment has a moderate hydrophilicity. This segment becomes hydrophobic after bromination. Therefore, it is reasonable that the copolymer PS-*b*-PHEMA **4** forms regular films while PS-*b*-PHEMA-Br **5** cannot. For comparison, the random glycopolymer **3** was studied also but could not be processed into honeycomb-patterned films with high regularity. It can be concluded that block glycopolymers can aggregate at the organic–water interface much easier than random glycopolymers. Although some random copolymers were reported to generate regular films [31,46], the casting condition need to be carefully optimized (the humidity, the concentration, and the composition of the polymer).

3.5. Structure underneath and pore size

The top layer of the honeycomb-patterned film can be easily removed with an adhesive tape to expose the honeycomb structure underneath (Fig. 8a). The pores beneath the top layer also organize into a hexagonal array with a bigger diameter, which are isolated from each other by the continuous thin wall. The top layer of the ordered pores is supported by this “polymer ring” structure, indicating that the pores are disconnected. The

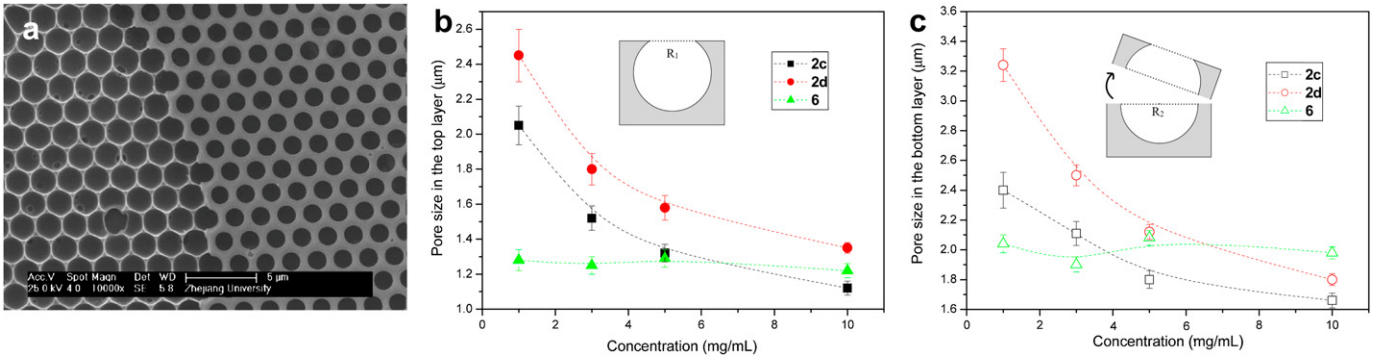


Fig. 8. (a) Typical SEM image of the porous film after the removal of the top layer; (b, c) Correlation between pore size and concentration of casting solution of polymers with different structures.

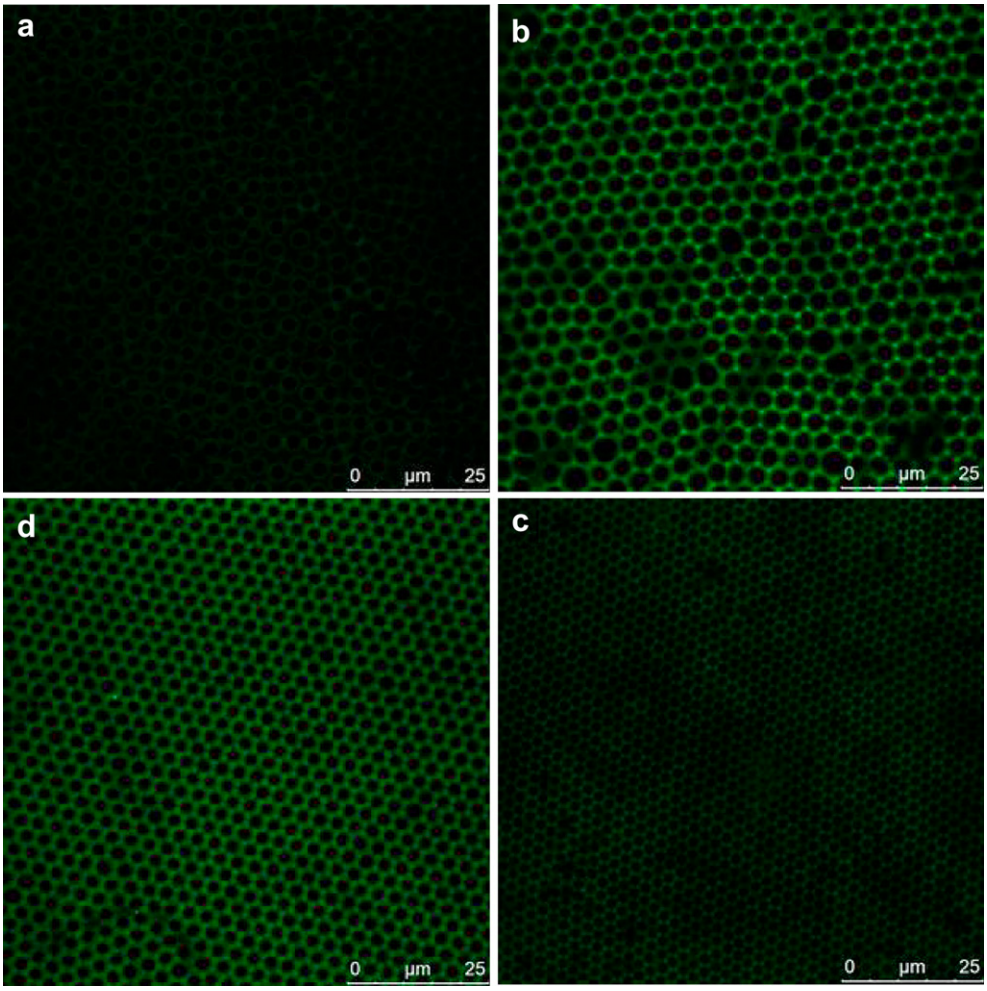


Fig. 9. Fluorescence images of honeycomb-patterned films after adsorption of fluorescent-labeled lectins. (a) Adsorption of FL-Con A on PS_{187}-*b*-$PAcGEMA_{35}$, (b) adsorption of FL-Con A on PS_{187}-*b*-$PAcGEMA_{35}$ after deprotection, (c) adsorption of FL-Con A on PS-*b*-(PHEMA-*g*-PAcGEMA) after deprotection, and (d) adsorption of FL-PNA on PS-*b*-(PHEMA-*g*-PAcGEMA) after deprotection.

pore diameters in both the top and bottom layers of the films are dependent on the polymer structure and the solution concentration. Amphiphilic block copolymers are hygroscopic and interact with water during the casting process as discussed. With increasing the length of the hydrophilic block the hygroscopic capacity of the copolymer increases. The copolymer tends to take up more water and the water droplets become larger. Thus, for the linear block glycopolymers, an increase in the pore size with PAcGEMA block length can be observed. The solution concentration also has a great influence on the pore size. As shown in Fig. 8b and c, the average pore diameter of **2c** and **2d** decreases obviously with the concentration. The pore size of the film is mainly determined by the growth time of the water droplets. With increasing polymer concentration, more polymers

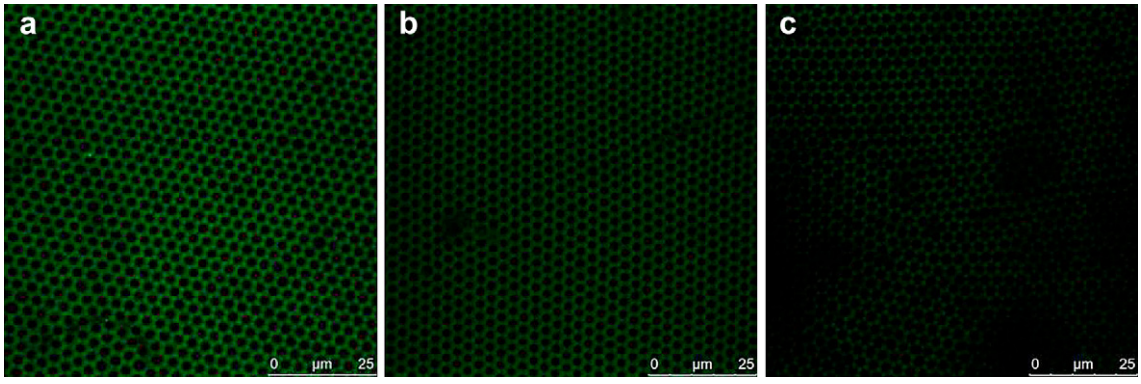


Fig. 10. Fluorescence images of honeycomb-patterned films before and after desorption of FL-Con A. (a) FL-Con A adsorbed film, and the corresponding desorbed film by immersing the film in (b) 0.25 M and (c) 1 M methyl α-mannopyranoside solution at 25 °C for 24 h.

precipitate at the organic–water interface. As a result, the water droplets are stabilized in a short time with smaller size. However, the comb-like glycopolymer **6** is less concentration sensitive. The pore sizes are almost the same with varying polymer concentration and keep in a smaller diameter. This is due to the fast precipitation of the comb-like glycopolymer as mentioned above. The water droplets would be stabilized quickly even at very low polymer concentration.

3.6. Recognition and desorption of lectins

The specific recognition to lectins was performed on the honeycomb-patterned films. Films fabricated from linear glycopolymer **2d** and comb-like glycopolymer **6** with comparable molecular weight and molar fraction of PAcGEMA were utilized in this study. The immersion of films from **2d** (Fig. 9a) and **6** into a fluorescent-labeled Con A (FL-Con A) solution followed by a standard washing step does not result in obvious adsorption of Con A. This is because of the *O*-acetylation of the glucose residues. The acetyl groups were removed by dipping the films into sodium methoxide/methanol solution, which can be confirmed by FTIR/ATR spectra (See Supplementary Content). After deprotection, the honeycomb pattern of green fluorescence emission was observed from both **2d′** and **6′**. The specifically bound Con A can be effectively desorbed from the film using 1 M methyl α-mannopyranoside solution (Fig. 10). Moreover, the fluorescence microscopy image of the films that was immersed into the fluorescent-labeled PNA (FL-PNA) solution only shows very weak fluorescence (Fig. 9d), indicating that the glucose-based honeycomb-patterned films have specific interactions with Con A.

4. Conclusion

Three kinds of glycopolymers, that is linear PS-*b*-PAcGEMA and PS-*co*-PAcGEMA, comb-like PS-*b*-(PHEMA-*g*-PAcGEMA), were synthesized by ATRP from St and AcGEMA. Results confirm that the polymerization processes can be well controlled and the resultant glycopolymers have well-defined structures. The glycopolymers were used as the precursor for the formation of honeycomb-patterned films by the breath figure method. The stabilization of water droplets is a key factor in forming the ordered porous structure. Honeycomb-patterned films can be only prepared from the comb-like glycopolymer and the linear block glycopolymers with relatively long PAcGEMA segment. The pore diameters in both the top and bottom layers can be controlled by varying the concentration of the casting solution or polymer structure. Furthermore, the preliminary studies on lectin recognition demonstrate that the glucose-containing pattern films have specific interactions with Con A. These bioactive honeycomb-patterned films have potential applications in many fields such as templates, picoliter beakers for bioanalysis and cell culture materials.

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Appendix. Supplementary content

FTIR/ATR spectra of honeycomb-patterned film before and after deacetylation are available.

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.polymer.2010.03.021.

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