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Printable and flexible macroporous organosilica film with high protein adsorption capacity

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ABSTRACT

An approach for creating a flexible and macroporous silsesquioxane film using phase separation method is described. The porous film was prepared by a simple coating method where sol–gel solution containing polyacrylic acid (PAA) and 1,6-bis(trimethoxysilyl)hexane in water was applied on boehmite silica coated polymethylmethacrylate (PMMA) film. After drying, the water soluble PAA template was removed by washing the film with water revealing the porous film. With certain ratios of PAA and water, fully co-continuous pore system with open surface was obtained. Porous films with 3–4 μm thickness were found to be highly flexible. The biocompatibility of the porous film was tested by immobilizing a high affinity biotin-binding chimeric avidin (ChiAVD(I117Y)) into the porous matrix The porous film was found to adsorb higher amounts of functional chimeric avidin compared to the pure PMMA film or a boehmite silica coated PMMA film.

1. Introduction

A phase separation method to manufacture porous oxide silica materials was developed by Nakanishi and Soga in early 1990s [1,2]. The porous materials prepared by using the phase separation method are co-continuous ranging from mesoporous to macroporous [3,4] and these materials have been used in a variety of applications, for instance biomolecule immobilization [5], liquid-phase catalytic reactions [6], bioreactors [7], sample pre-treatments [8,9] and HPLC applications [3,10].

Although the porous silica materials have already been extensively studied for two decades, simple and effective methods for mass-manufacturing of porous films are still virtually non-existing. Most of the methods suitable for preparation of porous films are based on spin-coating [11,12] or dip-coating [13,14]. These coating methods are, however, not practical for large-scale production, and in addition, the reported examples were dependent on removal of the surfactant or stabilization of the silica by thermal treatment after film deposition. The discovery of an easy method for large-scale production of porous silica films would allow the use of these materials in a variety of different applications including, for example, biosensors, bioseparation materials and diagnostics.

We have previously investigated the phase separation in a closed vessel between 1,6-bis(trimethoxysilyl)hexane (BTMSH) and poly (acrylic acid) (PAA) in water to form co-continuous macroporous silsesquioxane materials where the formed pore size was easily controlled by the amount of PAA without external acid catalysis [15]. During these studies, the gelation of BTMSH was found to be very fast and the possibility to prepare thin porous films using same composition of materials was then further investigated. In the present study, we report utilization of this method to manufacture a co-continuous macroporous silsesquioxane films by using simple coating technique (Fig. 1). The sol–gel solution containing BTMSH and PAA in water solution was coated onto a boehmite silica modified polymethylmethacrylate (PMMA) film. After rapid gelation of the sol–gel solution, the PAA template was removed by quick washing with water, revealing the porous film. The porous materials prepared from bridged silanes are reported to be very hydrophilic allowing water to penetrate into the material [16]. We tested the biomolecule immobilizing properties of the prepared porous silsesquioxane films by adsorbing high affinity biotin-binding chimeric avidin [17] into the porous films. The ligand-binding capacity of adsorbed chimeric avidin was measured by fluorescence immunoassay using biotinylated antibody as a probe molecule.

2. Experimental procedures

2.1. Materials

1,6-bis(trimethoxysilyl)hexane (BTMSH, 97%) was ordered from ABCR, Germany and used without purification. (3-glycidyloxypropyl) trimethoxysilane (GPTS, 98%), tetraethoxysilane (TEOS, 98%),

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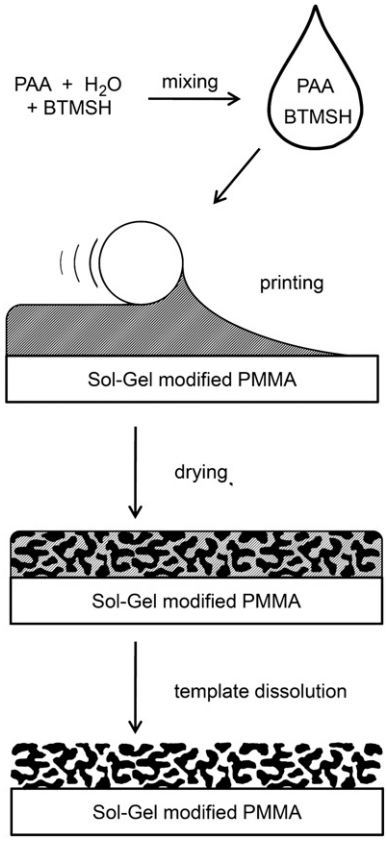

Fig. 1. Schematic illustration of the porous organosilica film fabrication by a coating method. The BTMSH and PAA in water are mixed together until homogenous suspension is formed. After aging, the suspension is coated on silica coated PMMA film using coating applicator and then air dried to form a clear film. The porous film is revealed by washing the PAA template off with water.

propyltrimethoxysilane (PTMS, 97%) and polyacrylic acid (PAA, average M_w ~100,000, 35 wt.% in H_2O) were ordered from Aldrich and used without purification. AlO(OH) solution (5 wt.% in H_2O) was ordered from PI-Kem Ltd, England. Polymethylmethacrylate film (PMMA Plexiglas 99524, thickness 125 μm) was ordered from Evonik, Germany.

2.2. Preparation of porous films

2.2.1. Boehmite silica coating of PMMA films

Boehmite silica solution was prepared by mixing hydrogen chloride (0.1 M, 5.1 mL) with ethanol (4.6 mL). Precursor silanes were added to the HCl/ethanol solution in the order of GPTS (6.6 mL), TEOS (4.0 mL) and PTMS (5.3 mL). The mixture was cooled down to near 0 °C and the AlO(OH) solution (14.0 g, 5% solution in water) was slowly added to the mixture. The temperature was slowly raised to room temperature (RT) and the mixture was stirred for 1 h at RT. After mixing, the mixture was refluxed for 18 h. The cooled mixture was stored at 4 °C. PMMA slides were O_2 plasma etched with Tepla 440-G plasma etcher for 5 min (200 W). Boehmite silica was applied with a coating applicator, *K202 Control Coater*, with a wet film thickness of 12 μm onto PMMA slides and dried at 60 °C for 1 h. The silica coated PMMA slides were stored at RT.

2.2.2. Fabrication of porous films

PAA (35% w/w in water) was diluted with water in a 1.5 mL vial to obtain the desired ratio (Table 1) between PAA and water. BTMSH (200 mg) was added to the vial through the septum using a syringe and the mixture was immediately shaken vigorously with a vortex mixer keeping the vial closed. After homogenous suspension was obtained, the suspension was aged for two minutes while still stirring vigorously. After aging, the suspension was coated onto a boehmite silica coated PMMA using a coating applicator, *K Control Coater* (12 μm wet thickness). The film was air dried at RT for ten minutes and then immersed in water for 5 minutes to remove the water soluble PAA template. The film was dried and stored at room temperature prior characterization or chimeric avidin immobilization.

Table 1
Characteristics of the porous materials generated by using varying sample compositions.

Sample	PAA [mg]	H_2O [mg]	BTMSH [mg]	ECD[a] [nm]
A	35	165	200	–[b]
B	40	160	200	–[c]
C	45	155	200	–[c]
D	50	150	200	103[d]
E	55	145	200	95[d]
F	60	140	200	109[d]
G	65	135	200	124[d]

[a] ECD = equivalent circular diameter, calculated from SEM images.
[b] No porous film was formed.
[c] Porous film with closed surface was formed.
[d] Porous film with completely open surface was formed.

2.2.3. Characterization of porous films

Field emission scanning electron microscopy (FESEM) images were used as the main characterization method for the fabricated porous films using 5.0 kV operating voltage. Pore diameters were calculated from planar view SEM images of the films having an open and porous surface using image evaluation technique. The samples for cross-section SEM images were prepared by immersing films in liquid N_2 for few seconds and then bending the films until they crack to form a sharp cut. Cross-section SEM images were measured by tilting the sample to 70° angle and the images were used to measure the thickness of the porous films.

2.3. Immobilization of biomolecules to porous films

2.3.1. Immobilization of chimeric avidin

The porous film was deposited on both sides of the silica coated PMMA film. All tested films (unmodified PMMA film, boehmite silica coated PMMA film and porous silsesquioxane film, sample E) were O_2 plasma etched with Tepla plasma etcher to increase the hydrophilicity of the films and to assure identical conditions for immobilization of chimeric avidin [17]. Films were cut to 1 cm × 1 cm in size and placed to 24-well plate. 350 μL of a solution containing 2.6 μg/mL chimeric avidin in PBS (Phosphate-Buffered Saline: 0.137 M sodium chloride, 2.8 mM potassium chloride, 11.9 mM phosphate buffer, pH 7.4) was added to each well and all films were incubated in this chimeric avidin solution for one hour at 37 °C. After incubation, the films were washed five times with 500 μL of PBST (PBS containing 0.05% TWEEN® 20).

2.3.2. Determination of biotin binding activity of films functionalized with chimeric avidin

The biotin binding activity of films functionalized with chimeric avidin was measured by fluorescence immunoassay immediately after the functionalization. All films were blocked with 350 μL of 3% BSA (Bovine serum albumin) solution in PBS for one hour. After that the films were washed three times with 350 μL of PBST. Films were then incubated for one hour in 250 μL of biotinylated anti CRP Mab 6404 antibody solution in 1% BSA in PBS using the following antibody concentrations: 0 μg/mL, 0.204 μg/mL, 0.408 μg/mL and 0.816 μg/mL. For all antibody concentrations, three parallel samples were prepared. After incubation, all samples were washed three times with 350 μL of PBST. Alexa Fluor® 488-labelled goat anti-mouse IgG (H + L) fluorescent dye (2 mg/mL) solution was then used as a secondary antibody (diluted to 1:1500 with 1% BSA in PBS) and all films were