Journal of Colloid and Interface Science 466 (2016) 150–161

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

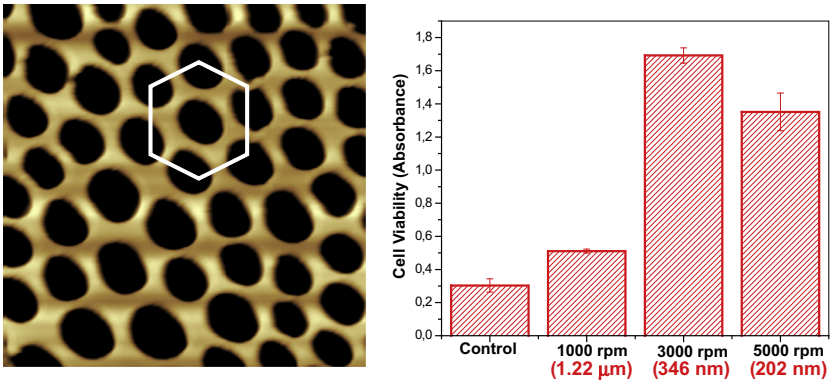
journal homepage: www.elsevier.com/locate/jcis

Controlling cell growth with tailorable 2D nanoholes arrays

Vanessa H. Fragal [a], Thelma Sley P. Cellet [a], Elizângela H. Fragal [a], Guilherme M. Pereira [a], Francielle P. Garcia [b], Celso V. Nakamura [b], Tewodros Asefa [c,d], Adley F. Rubira [a,*], Rafael Silva [a,*]

[a] Departamento de Química, Universidade Estadual de Maringá, Avenida Colombo 5790, CEP: 87020-900 Maringá, Paraná, Brazil
[b] Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Avenida Colombo 5790, CEP: 87020-900 Maringá, Paraná, Brazil
[c] Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, United States
[d] Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, United States

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 September 2015
Revised 8 December 2015
Accepted 8 December 2015
Available online 9 December 2015

Keywords:
Nanopore arrays
Breath figure method
Nanofabrication
Cell growth
Polystyrene

ABSTRACT

A facile and reproducible route that can lead to two-dimensional arrays of nanopores in thin polymer films is demonstrated. The formation of the pores in the polymer films involves breath figure phenomenon and occurs during the film deposition by spin coating. The formation of nanoporous thin films takes only few seconds, and the method does not require complex equipment or expensive chemicals. This method also constitutes a straightforward approach to control the size of the pores formed in thin films. Besides allowing control over the average pore size of the porous films, the use of dynamic deposition with the breath figure phenomenon causes the reduction in the pore size to nanometer scale. The nanoporous arrays obtained by the breath figure are applied as substrates for cell growth, and the effect of their nanopore size on cell growth was evaluated. Notably, it is found that cell viability is related to pore size, where 2D nanoporous structure is more beneficial for cell culture than 2D microporous structures. The change in the average pore size of the polymer films from 1.22 µm to 346 nm results in a threefold increase in cell viability.

1. Introduction

Nanoporous thin films are highly desired because they have numerous potential applications in areas ranging from catalysis to more advanced systems such as in antireflection coatings

* Corresponding authors.
E-mail addresses: afrubira@uem.br (A.F. Rubira), rsilva2@uem.br (R. Silva).

http://dx.doi.org/10.1016/j.jcis.2015.12.016
0021-9797/

[1–4], optoelectronic devices [5], sensors [6], microlenses [7,8], membranes [9], and cell culture media [10–14]. However, the large-scale synthesis of high quality and large area nanoporous thin films through fast, reproducible and low cost preparation methods still remain a huge challenge [15,16].

Over the past two decades, various methods based on different lithographic techniques and self-assembly processes that can result in patterned films have been reported [17–19]. Despite their great capabilities, such methods are time consuming and demand complexes setups and/or expensive chemical reagents, limiting their application for large scale production of nanostructured polymers [20].

The breath figure method, which was first demonstrated by Francois et al. [21–24], is a straight forward methodology to obtain microporous polymer surfaces and membranes. The method allows the formation of ordered hexagonal arrays of pores on polymeric surfaces through a simple, quick, dynamic, economical and reproducible way [9,25–27]. However, the method has also some drawbacks, notably the inability to tune the size of pores, which are constrained to only few micrometers; this is due to the method's inability to control the kinetics of water condensation. Consequently, an improvement of the method to enable the formation of smaller nanometer scale pores would be an important step forward to utilize it for nanofabrication of porous thin films with controlled structures and pore sizes.

Even though the breath figure method and its experimental procedures to generate ordered porous surfaces can easily be carried out, there are several parameters that can affect the final outcomes. This is mainly to do with the synthetic parameters involved in the synthetic method.

Porous polymer films created by the breath figure method can find some interesting potential applications in biology and tissue engineering, as recently demonstrated for number of various polymeric materials [27–29]. In particular, the development of materials with special surface features that can assist cell growth is an important area, as such materials can help with development of specific tissues for various medical applications [30–34]. For example, Kawano et al. showed that a poly(dimethylsiloxane) (PDMS) substrate with polystyrene possessing honeycomb porous structure produced by the breath figure method could induce the differentiation of human mesenchymal stem cells [35]. The author also evaluated stem cell differentiation over the substrates with pore diameters in the range of 1.6–4.8 μm, and their results indicated that the substrate's pore size affected the cell differentiation process.

However, the lack of substrates that provide high cell viability for a variety of cells remains an important issue on cell culture [36–38]. Heng et al. studied the growth of anticancer cell on ordered honeycomb structures prepared by breath figure method and concluded that porous structures are not beneficial for cell growth [39]. In fact, porous surfaces can be used to inhibit the proliferation of microorganism. Manabe et al. demonstrated that porous surface prepared by breath figures with controlled average pore size could suppress *Pseudomonas aeruginosa* biofilm formation [40]. In particular, films with pores in the range of 5–11 μm could effectively prohibit bacterial adhesion and growth.

Herein we demonstrate that breath figure method combined with spin coating process can be used to obtain uniform thin polystyrene films with thickness and pore diameter both as low as 100 nm that are conducive for growth of cells. In addition, we show that the film preparation procedure can be executed using only readily available chemicals and can easily be extended to a great variety of substrates. The spin coating process to produce the porous film under high humidity conditions is relatively fast, and can take as little as only 10 s. These advantages thus make the method a strong candidate for the preparation of nano-filtration membranes, cells culture substrates as well as substrates for other surface applications. We also show the ability to control of the pore size of the porous polystyrene thin films by varying the deposition spin speeds on different substrates. Finally, the ability of produce nanoporous arrays with controlled pores size by the breath figure method and the effects of the so-obtained different substrates on the growth of Vero cells is included, reforcing the influence of topographic scale on cell proliferation [41,42]. The average pore size of the nanoporous substrates formed by the breath figure is found to be strongly correlated with cell viability.

2. Experimental section

2.1. Materials

Low density polyethylene (LDPE) (Sigma–Aldrich), poly(ethylene terephthalate) (PET) (Mylar Commercial), and polydimethylsiloxane (PDMS) (Sylgard 184) were purchased and used as received. Glass slides with dimensions of 3 cm × 3 cm were used as substrates for deposition of the polymer films. Polystyrene (PS), with an average molecular weight of 35,000 Da (Sigma–Aldrich, Catalog Number: 331651), and tetrahydrofuran (THF) (Nuclear) were used as received.

2.2. Spin coating and humidity control

The experiments were carried out using a SCS G3 Spin Coater, Model G3P-8Spincoat Specialty Coating Systems. The humidity in the chamber was controlled using a saturated solution of ammonium sulfate. A beaker containing a saturated solution of ammonium sulfate was placed inside the spin coater chamber two days prior to the chamber's use to ensure stabilization of the humidity inside. A hygrometer, placed inside the chamber, was used to measure the humidity. The room temperature was kept at 25 °C.

2.3. Preparation of nanoporous PS films on PET and effect of spin speed

PET film placed in spin coater and on top of it was added 150 μL of a solution of 10% (w/v) PS in THF. The film was made by spin coating at 1000 rpm for 10 s. The same procedure was repeated with a spin speed of 3000, 5000 and 9000 rpm.

2.4. Preparation of nanoporous PS films and effect of substrate

Pristine PET, LDPE, PDMS, and plasma-treated polymer films were used as substrates, and on each substrate 150 μL of a solution of 10% (w/v) PS/THF was deposited. The plasma-treated films were prepared by exposing the pristine polymer films to oxygen plasma for 1 min in a chamber (Harrick Plasma cleaner). This process led to more hydrophilic polymer films. The PS films were deposited on these different substrates following the same procedure as above and using a spin speed of 3000 rpm for 10 s. All the experiments were carried out at 25 °C and relative humidity (RH) of 81%.

2.5. Culture of Vero cells on the scaffolds

The cell line used in this *in vitro* study was the *Cercopithecus aethiops* (African green monkey) Kidney (Vero) epithelial-ATCC® CCL-81™ – American Type Culture Collection. The cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) medium supplemented with 10% fetal bovine serum (FBS) at 37 °C and an atmosphere of 5% CO_2. Cells were separated and counted in a Neubauer chamber hemacytometric to obtain a suspension of 2.5×10^5 cells/mL.