Materials Science and Engineering C 31 (2011) 1861–1866

Contents lists available at SciVerse ScienceDirect

Materials Science and Engineering C

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

# A 2D surface morphology–composition gradient panel for protein-binding assays

Lai Wei [a], Erwin A. Vogler [b], Timothy M. Ritty [c,1], Akhlesh Lakhtakia [a,*]

[a] Materials Research Institute and Department of Engineering Science and Mechanics, Pennsylvania State University, University Park, PA 16802, USA
[b] Department of Materials Science and Engineering, and Department of Bioengineering, Pennsylvania State University, University Park, PA 16802, USA
[c] Department of Orthopaedics and Rehabilitation and Department of Pathology, College of Medicine, Pennsylvania State University, Hershey, PA 17033, USA

ARTICLE INFO

*Article history:*
Received 23 March 2011
Received in revised form 16 August 2011
Accepted 1 September 2011
Available online 10 September 2011

*Keywords:*
Gradient panel
Columnar thin film
Parylene-C
Surface oxidation
Serum protein binding
Water wettability

ABSTRACT

We designed a two-dimensional (2D) morphology–composition gradient panel, which has a gradient in surface morphology at constant chemical composition along its length and a gradient in surface chemical composition without change of surface morphology in the orthogonal direction. The panels, which exhibit a 2D gradient in surface wettability, were fabricated by thermolysis of parylene-C followed by oxygen-plasma treatment for various times. As a demonstration study, binding of three different biotinylated serum proteins (B-IgG, B-A, and B-TF) to the 2D panel was investigated. The 2D gradient panels will facilitate development of optimized binding surfaces for various biotechnological applications.



## 1. Introduction

Gradient panels are experimental vehicles that multiplex investigations of surface interactions by exposing a panel of surface properties that vary systematically with position on the panel to the phenomenon under study. In this way, a multiplicity of surface interactions is captured simultaneously in a manner that is much more efficient than sequential preparation of single-test units with a single specific surface property for one-variable-at-a-time experimentation—which is tedious and time-consuming because many different samples, each with a different value of the surface variable of interest, are required.

Gradient panels have been widely used in biotechnology to study protein adsorption [1] and cell adhesion [2] and are of use in various kinds of high-throughput screening assays [3]. The technology was introduced by Elwing et al. [4] who created a gradient in surface energy (water wettability) on glass by exposure to a concentration gradient of dimethyldichlorosilane in xylene. Pitt employed a moving radiofrequency discharge to fabricate a wettability-gradient panel by increasing oxidation along the length of a polymer strip [5]. Ueda-Yukoshi et al. engineered a gradient on poly(vinylene carbonate) by successive wet chemical reactions [6]. Roth et al. developed a colloidal-silver-nanoparticle gradient panel by drying the colloidal solution between two walls of different solvent wettability [7]. In all of these efforts, a continuous change of chemical composition in one direction on the gradient panel was realized.

Morphology, chemical composition and wettability are important properties of surfaces. In particular, surface rugosity and chemical composition can combine to produce technologically useful properties such as superhydrophobicity [8] or superoleophobicity [9]. The surface properties of a biomaterial determine the types, amounts, and conformations of adsorbed proteins [10]. The nature of adsorbed proteins in turn determines the cellular response to the biomaterial [11], thereby underscoring the essential role of surface properties in the bioactivity of biomaterials.

We report herein the design, fabrication, and testing of a two-dimensional (2D) gradient panel with mutually orthogonal gradients in surface morphology and surface chemical composition that can be used to identify optimal combinations of surface chemical and morphological characteristics. Parylene-C is thermolyzed onto an array of glass plates in such a way that a columnar thin film (CTF) is deposited with surface density and commensurate rugosity that varies along the length of the panel, thereby creating a wettability gradient that varies from hydrophobic to superhydrophobic [12]. Subsequent oxygen-plasma treatment of the surface-morphology gradient changes the wetting characteristics in relation to the treatment time in a way that permits systematic variation of the CTF wettability gradient over a 120-deg range of the advancing water contact angle. As a demonstration study, we deployed this 2D wettability-gradient panel in the study of protein

*Abbreviations:* B-A, biotinylated albumin; B-IgG, biotinylated IgG; B-TF, biotinylated transferrin; BSA, bovine serum albumin; CTF, columnar thin film; SEM, scanning electron microscope; TBS, Tris buffered saline; TCS, tissue culture substrate; PS, phosphatase substrate.

* Corresponding author at: Department of Engineering Science and Mechanics, Pennsylvania State University, University Park, PA 16802, USA. Tel.: +1 814 863 4319.
*E-mail address:* akhlesh@psu.edu (A. Lakhtakia).
[1] Present address: Genomics Division, Thermo Fisher Scientific, 2650 Crescent Drive, Lafayette, CO 80026, USA.

0928-4931/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.msec.2011.09.001

binding using immunoglobulin G (B-IgG, 160 kDa), albumin (B-A, 66 kDa), and transferrin (B-TF, 76 kDa).

## 2. Materials and methods

### 2.1. Fabrication of 1D surface morphology gradient panels

Parylene-C is the polymeric form of para-chloro-xylylene $C_8H_7Cl$. It has been approved by the US Food and Drug Administration for medical implants inside the human body and exhibits excellent biocompatibility in many biotechnical/medical applications [13].

A physicochemical vapor deposition technique, used for several decades to make dense thin films of parylene-C for implantable devices [14–16], was recently modified to deposit fibrous thin films with specific volumetric morphology [12, 17, 18]. In this technique, the raw material is parylene-C dimer (SCS Coatings, Indianapolis, IN). This material is first vaporized at ~150 °C and then thermolyzed at ~650 °C so that each dimer is cleaved into two reactive monomers. The vapor comprising reactive monomers is then directed through a nozzle onto a flat platform on which a specially prepared substrate is mounted. Room-temperature polymerization of the collimated reactive-monomer flux occurs on the exposed surface.

The platform must be held stationary for a CTF to be deposited. As shown in Fig. 1, the reactive-monomer flux was directed at an angle $\chi_v = 10$ deg with respect to the platform plane. The 48 mm × 9 mm substrate comprised 3 mm × 3 mm square plates of glass assembled together as a rectangular panel on a 16 × 3 lattice. This configuration was chosen mainly for ease of conducting protein-binding experiments. The rectangular panel was mounted on the platform with its long axis, identified as the $x$ axis in Fig. 1, parallel to the projection of the direction of the reactive-monomer flux on the platform plane, and deposition took place at a pressure of approximately 26 mTorr for 10 min. As a result, CTFs of parylene-C [12, 18] were formed on each of the 48 square plates of glass, the plates close to the proximal end (x = 0 mm) of the rectangular panel having thicker CTFs than those close to the distal end (x = 48 mm).

The CTF-thickness gradient along the $x$ axis on the rectangular panel yielded a lengthwise gradient in surface morphology [12], as discussed in Section 3.1, which therefore exhibits a lengthwise gradient in surface hydrophobicity [12], as discussed in Section 3.2.

### 2.2. Fabrication of 2D surface morphology–composition gradient panels

Oxygen-plasma treatment is a nondestructive process to introduce functional groups that interact with water to overcome the inherent hydrophobicity of many polymers. Oxygen-plasma-treated dense films of parylene-C have hydrophilic surfaces [19], and so do their CTF counterparts [12].

As shown in Fig. 1, each morphology-gradient panel consists of three rows of 16 square plates of glass. Two such panels (six rows altogether) were fabricated. One row was not treated with oxygen plasma, but each of the remaining five rows was separately treated with oxygen plasma for a specific duration $t$. The chosen values of $t$ are 0 s, 5 s, 10 s, 20 s, 40 s and 60 s. We used the oxygen-plasma-treatment system Metroline M4L Plasma Etcher with the radiofrequency power set at 50 W, while oxygen flow was maintained at 50 sccm with the chamber pressure set at 400 mTorr.

Surface wettability is expected to increase with $t$, as longer treatment introduces more functional groups (such as C=O, C–O, and O–C=O) to the surface [19]. Thus, a 2D surface morphology–composition panel comprising six rows of 16 square plates each was assembled to function as a 2D wettability gradient panel.

### 2.3. Characterization of 2D wettability gradient panels

A Hitachi S3500 scanning electron microscope (SEM) was used to qualitatively evaluate the surface of a 1D surface morphology gradient panel. A thin layer of gold was sputtered on the surface before visualization at 5 kV by the SEM. The CTF thickness was measured by a Tencor 500 profilometer.

For surface wettability measurements, linear arrays of 3 mm × 3 mm square plates from the proximal to the distal were re-assembled without intervening spaces. The surface wettability was quantified with respect to surface morphology by measuring the advancing water contact angle θ [20], which is defined as the maximum angle allowable without increasing the water–solid interfacial area by adding volume

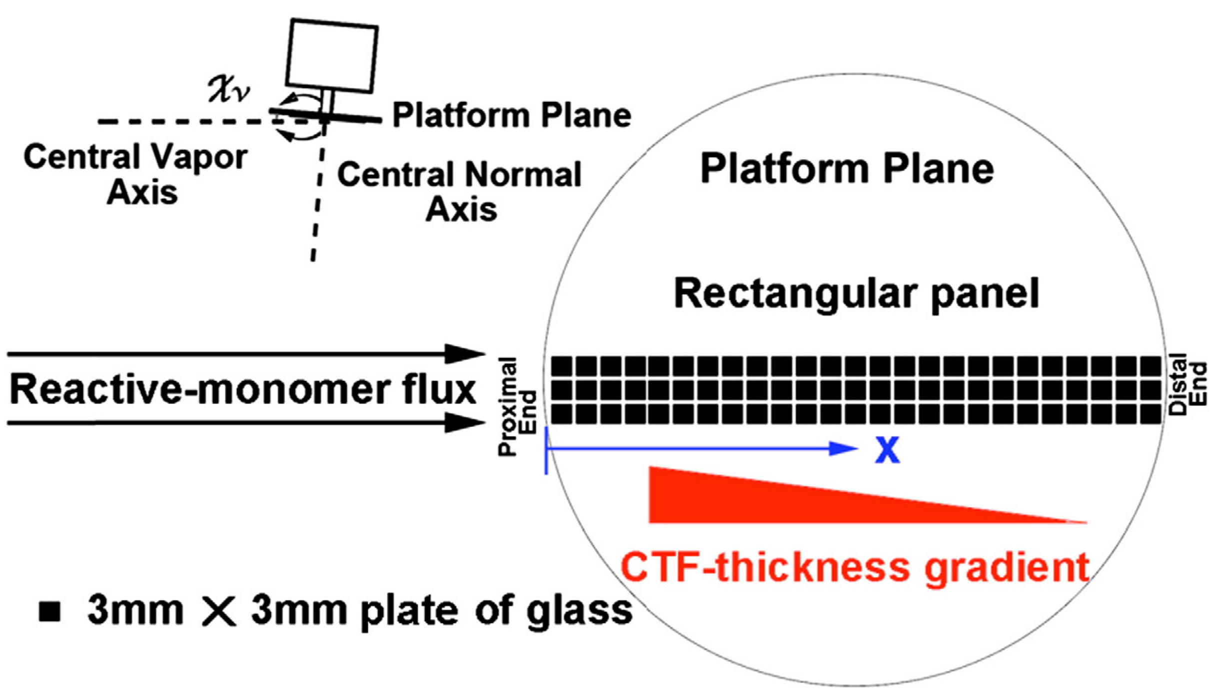


**Fig. 1.** Schematic of the fabrication method for a panel comprising 3 rows of 16 3 mm × 3 mm plates of glass placed adjacent to each other with virtually no space between neighbors. The $x$ axis begins at the proximal end.