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Use of precisely sculptured thin film (STF) substrates with generalized ellipsometry to determine spatial distribution of adsorbed fibronectin to nanostructured columnar topographies and effect on cell adhesion

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ABSTRACT

Sculptured thin film (STF) substrates consist of nanocolumns with precise orientation, intercolumnar spacing, and optical anisotropy, which can be used as model biomaterial substrates to study the effect of homogenous nanotopogrophies on the three-dimensional distribution of adsorbed proteins. Generalized ellipsometry was used to discriminate between the distributions of adsorbed FN either on top of or within the intercolumnar void spaces of STFs, afforded by the optical properties of these precisely crafted substrates. Generalized ellipsometry indicated that STFs with vertical nanocolumns enhanced total FN adsorption two-fold relative to flat control substrates and the FN adsorption studies demonstrate different STF characteristics influence the degree of FN immobilization both on top and within intercolumnar spaces, with increasing spacing and surface area enhancing total protein adsorption. Mouse fibroblasts or mouse mesenchymal stem cells were subsequently cultured on STFs, to investigate the effect of highly ordered and defined nanotopographies on cell adhesion, spreading, and proliferation. All STF nanotopographies investigated in the absence of adsorbed FN were found to significantly enhance cell adhesion relative to flat substrates; and the addition of FN to STFs was found to have cell-dependent effects on enhancing cell–material interactions. Furthermore, the amount of FN adsorbed to the STFs did not correlate with comparative enhancements of cell–material interactions, suggesting that nanotopography predominantly contributes to the biocompatibility of homogenous nanocolumnar surfaces. This is the first study to correlate precisely defined nanostructured features with protein distribution and cell–nanomaterial interactions. STFs demonstrate immense potential as biomaterial surfaces for applications in tissue engineering, drug delivery, and biosensing.

1. Introduction

Topographical substrate influences on protein adsorption and adhered cell behavior have been the subject of many investigations beginning with microstructured substrate features, which have been shown to influence protein adsorption, cell attachment, spreading, migration, patterning, proliferation, morphology, and differentiation, presumably by spatially confining adsorbed proteins, cell-secreted extracellular matrix proteins and cells themselves [1–5]. Recently, materials with nanotopographical features (1–100 nm feature size) have been investigated as cell culture substrates for biosensing, tissue engineering scaffolds, and therapeutic drug delivery systems; and to gain more understanding regarding nanoscale protein adsorption and cell–material interactions [6–9].

Nanoscale substrate topography and nanoroughness have been shown to influence similar cell behaviors as with microscale rough substrates, such as cell adhesion, spreading, morphology, proliferation, and differentiation in a variety of cell lines [9–15], however most of these investigations have been conducted on

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nanostructured topographies with disordered or irregular features. For example, indiscriminate distributions of carbon nanotubes have been shown to support protein adsorption [16] and the culture of neural cells [16,17] and osteoblasts [18,19]. Furthermore, nanorough surfaces prepared by sputtering TiO_2 and SiO_2 have demonstrated improvements in platelet and endothelial cell adhesion with respect to unmodified surfaces, which was found to be attributed to both the surface chemistry and nanotopography [20]. However, since these studies evaluated cell behaviors on disordered nanotopographies, the work presented here aims to investigate protein adsorption and cell behaviors using highly ordered coherent arrays of nanostructured columnar substrates with varying nanocolumn spacing/orientation and to determine the precise three-dimensional distribution of proteins either on top of or within nanocolumnar features.

Advances in substrate fabrication strategies have enabled the engineering of biomaterial surfaces possessing distinct microstructured and nanostructured topographical features [21–24]. Substrates consisting of a homogenous nanocolumnar array, referred to as sculptured thin films (STFs), can be produced by many different methods including, but not limited to, lithography, sputtering, as well as physical and chemical vapor deposition [25–27]. However, STFs are most commonly fabricated by employing glancing angle deposition (GLAD), a physical vapor deposition technique facilitated by electron beam evaporation. This approach results in STFs that possess intricate and complex architectures ranging in size from the sub-nano to micro- scales depending on the vapor flux angle, substrate rotation, and deposition duration, which affect the column height, slanting angle, and spacing [25,28–30]. The GLAD technique is applicable to a wide range of materials including metals, semiconductors, insulators, and polymers, resulting in a wide array of nanostructures with significantly different structural, mechanical, electrical, magnetic, optical, and biological properties tailored to suit specific material applications [28,29,31]. Preliminary investigations of infiltrating STFs with various organic materials such as bulk polymers [32,33], polymer brushes [34,35], and FN [36] have demonstrated that organic materials can be immobilized within the intercolumnar pore space of STFs to enable these substrates to deliver therapeutic biomolecules for biomaterial, tissue engineering, and nonviral gene delivery applications. Current industrial applications of GLAD nanostructured thin films include optical coatings for photovoltaics [37–39], sensing (chemical, biological, optical, and pressure) [25], micro- and nano-fluidics [40], nanoelectronics [29,31], and have been explored as biomaterials [41].

In addition to the precise control over STF fabrication, the nanocolumnar architecture provided by STFs allows for sensitive optical detection by GE due to the optical anisotropy provided by the nanocolumn orientation of the STFs [28,33,36,42,43]. Recently, investigations of infiltrating STFs with various organic materials such as bulk polymers [32,33], polymer brushes [34,35], and fibronectin (FN) [36] have demonstrated that organic materials can be immobilized within the intercolumnar pore space of STFs. Generalized ellipsometry has been used in conjunction with quartz crystal microbalance with dissipation (GE/QCM-D) to characterize the dynamic adsorption processes of organic molecules to both flat and nanostructured STF substrates [34–36,44–46]. While protein loading has been evaluated extensively on flat surfaces, evaluation of protein adsorption to/within nanostructured thin films is sparse due to the lack of effective methods to evaluate the three-dimensional distribution of proteins within nanostructured features.

The FN protein adsorption studies described in this paper aim to elucidate the influences of highly ordered nanostructured columnar features, such as column orientation, nanocolumn surface density (spacing), and total surface area, on FN adsorption and loading. FN was chosen for protein adsorption studies since FN is a commonly used ECM protein to coat biomaterial substrates for the purpose of enhancing cell adhesion and cell–material interactions [23,47–53]. The present investigation is unique relative to previous GE/QCM-D investigations of biomolecule adsorption since GE is used here to discriminate between FN adsorption to the top of three-dimensional nanostructured columnar substrates and the FN loading within the intercolumnar pore spaces of nanostructured substrates. Since both surface adsorbed proteins and substrate nanotopographies have been previously shown to independently enhance cell behaviors, such as cell adhesion, spreading, and proliferation, the current investigation also aims to evaluate cell–material interactions and biocompatibility as a function dependent on both precisely defined substrate nanotopographies and enhanced protein loading within nanostructured surfaces [1,40,41,54,55]. These three-dimensional nanostructured STFs loaded with FN are hypothesized to be excellent candidates for use as biomaterial substrates to load and release biomolecules and to enhance cell–substrate interactions for applications in drug delivery, tissue engineering, and diagnostics.

2. Materials and methods

2.1. Sample preparation

STFs were fabricated by electron beam evaporation of titanium (Ti) pellets (Super Conductor Materials, Inc., Tallman, NY) onto either gold-coated quartz crystal microbalance (QCM) sensors (Q-Sense Inc., Linthicum Heights, MD) for protein adsorption studies or onto silicon wafers (University Wafer, South Boston, MA) for cell culture studies. Details regarding specific STF deposition parameters are included in the Supplemental methods Section S1.1. Immediately following the fabrication of STFs, generalized ellipsometry (GE) measurements of STFs were acquired using a Woollam M-2000 spectroscopic ellipsometer (J.A. Woollam, Co., Inc., Lincoln, NE) to confirm deposited film thicknesses and column slanting angles. The procedures for acquiring GE measurements of STFs to confirm film thickness, column slanting angle, and volume fractions (related to column spacing) are similar to previously published STF ellipsometric procedures [28,33,36,43] and specific details regarding GE data acquisition and modeling are included in the Supplemental methods Section S1.2.

2.2. Combined generalized ellipsometry and dissipative quartz crystal microbalance

The combinatorial GE/QCM-D setup consists of an E1 QCM-D (Q-Sense, Inc.) module, mounted to the sample stage of a M-2000 spectroscopic ellipsometer (J.A. Woollam Co., Lincoln, NE). QCM-D wafers containing either STFs or flat Ti thin films were mounted within the QCM-D liquid chamber, which contains windows for ellipsometer's light beam to pass through the cell at a 65° angle of incidence, allowing for dynamic molecular adsorption analysis using both instruments simultaneously. Upon acquiring baseline GE measurements that are necessary for subsequent modeling of spectral data, 1× phosphate buffered saline (PBS) (Life Technologies, Carlsbad, CA) solution was introduced into the liquid chamber at a constant rate of 0.1 mL/min using an Ismatec IPC 8 peristaltic pump (IDEX Health and Science GmbH, Wertheim-Mondfeld, Germany) and Tygon tubing (U.S. Plastic Corp., Lima, OH) connected to the liquid chamber. Then, 10 μg/mL human plasma FN (Sigma–Aldrich, St. Louis, MO) flowed through the liquid chamber for 90 min. Following FN adsorption, 1× PBS was used

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