Vapor-Phase Facile Coatings of Nanotextured Organic Biocompatible Films on Solid-State Substrates
Swati Goyal, Young-Tae Kim, and Samir M. Iqbal, Senior Member, IEEE

Abstract—Fluorinated coatings of solid surfaces are important for many applications ranging from corrosion resistance to low surface energy biological interfaces. We present a facile approach to coat solid-state surfaces directly from vapor phase without harsh chemical or plasma treatments. The coatings show nanostructures with high surface area, which is important for variety of surface functionalizations, for example, in molecule attachment and cell growth. The novel polymeric nanoporous film is achieved from the reaction and deposition of two molecules. The surface morphology and the coating on solid-state surfaces can be tuned with change in concentration of monomers in a simple reaction chamber. The X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy analyses show organic nature of the coating. Cell growth studies are done to gauge biocompatibility and adsorption of proteins and cells. The approach can be used to coat, functionalize, and treat nano- and microstructures for a variety of applications with minimal chemical footprint.

Index Terms—Biocompatible, polymeric nanostructures, solid substrates, surface coatings, vapor-phase deposition.

I. INTRODUCTION

ANO- and microstructure surfaces are often coated with various molecules to impart properties like low surface energy, selectivity, corrosion–resistance, etc. To achieve such properties, there is a constantly increasing interest in understanding the properties of organic composite films on solid-state substrates. The interface of such surfaces plays a key role, especially in biological and chemical sensing. In case of biological interfaces, the surface properties place stringent requirements for the selective detection of biological analytes [1]–[3]. Organic films are deposited, among other techniques, using plasma-enhanced chemical vapor deposition (PECVD), plasma polymerization, and self-assembled monolayers (SAMs) [4]–[6]. These films are used for chemical sensing, photoluminescence studies, biosensing, and to impart biocompatibility to surfaces [7]–[9]. Aminopropyltrimethoxy silane (APTMS) and trichloro(1H,1H,2H,2H)perfluorooctydylsilane (PFTS) are two commonly used monomers for organic coatings on silicon surfaces. APTMS is used as a linker molecule for gold nanoparticles, chemisorption, antibody attachment, and for protein/DNA attachment [10]–[13]. APTMS has also been used for the adhesion of tissues and has been shown to encourage cell growth [14], [15]. PFTS is a highly reactive hydrophobic molecule used as a releasing agent for polydimethylsiloxane (PDMS) [16]. Fluorinated surfaces have been characterized in depth for their surface energies, reduced cell adhesion, protein aggregation, chemisorption, antibody attachment, and cell-growth properties, patterning on SiO$_2$, long-term storability of hydriding alloys, etc. [17]–[23]. Attempts have also been made to create microstructured fluorinated surfaces [19]. Both APTMS and PFTS are nontoxic to the cells, carry highly reactive functional groups, and exhibit contrasting chemical behaviors [24]–[27].

PFTS is predominantly used to generate fluorine-rich coatings, and these coatings show attractive properties, such as low surface energy, heat resistance, chemical inertness, less friction, and hemocompatibility [23], [28], [29]. Several methods are used to generate fluoropolymer thin films, like plasma technique, pulsed laser deposition, CVD, and liquid injection in a UV-assisted rapid isothermal processing [30]–[34]. All these processes use harsh conditions for substrates and often require either plasma chamber at high vacuum or liquid-phase adsorption of molecules to form SAMs. We report a facile approach to coat solid-state substrates with APTMS and PFTS directly from vapor phase and under a moderate vacuum. The approach is important, as this can be easily adapted at laboratory or production scale to impart various tunable properties to solid surfaces. The room-temperature coating approach avoids any doping or leeching of the molecules to the lattice of the solid-state substrates [35].

In this study, we report thin-film coating of oxidized silicon substrates. The fluorinated films are analyzed for the surface energy and chemical composition. The coated chips are then used for growth of fibroblast cells and cardiomyocytes. The analysis from electron micrographs shows nanoporous form of coatings.

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under certain conditions, which can be used to embed various molecules that can provide corrosion resistance, biocompatibility, selectivity, etc.

II. MATERIAL AND METHODS

A. Composite Deposition

Chemical deposition was carried out in a cylindrical reactor at a vacuum of 25 torr. Fig. 1 shows a simple schematic of the setup. APTMS and PFTS were used as received (Sigma-Aldrich, Saint Louis, MO) without any further purification. Polished Si wafers were used as substrates for X-ray photoelectron spectroscopy (XPS) and SEM studies, and KBr crystals were used for Fourier transform infrared (FTIR) spectroscopy. After the substrate was placed in the reactor, vacuum was applied, which led to monomers volatility, reaction in vapor phase, and consecutive deposition/reaction on the substrate. The reacting monomers were volumetrically used in different ratios, as shown in Table I.

The films were named AP1, AP2, AP3, AP4, and AP5 depending on the monomer ratios (see Table I). The film AP1 and AP2 were deposited with higher concentration of PFTS. AP3 was deposited with equal ratio of monomers. AP4 and AP5 were deposited with higher concentration of APTMS. Conditions used for AP4 gave porous films; hence, subsequent depositions were done by tuning the pressure conditions to obtain AP4 films with pore sizes of ∼10–100 nm.

B. Physical and Chemical Characterization

The films were characterized with FTIR, XPS, and SEM. The FTIR spectra were recorded in transmission mode on KBr crystals at a resolution of 4 cm⁻¹ using Nicolet 6700 FTIR spectrophotometer. The XPS studies utilized Al Kα radiation at 1486.6 eV. The morphology of the films was studied using ZEISS supra 55 VP Scanning Electron Microscope. Film thickness was determined using KLA-Tencor Alpha-Step IQ Profilometer.

C. Protein Absorption and Cell Study

Experiments were performed to understand the response of biological molecules and cells to the film. Absorbed protein layers helped to determine the coagulation cascade and blood compatibility. The adsorption of two main plasma proteins: albumin and IgG (Invitrogen, Carlsbad, CA) was studied. The protein-adsorption tests were carried out using phosphate-buffered saline (PBS, pH 7.4) that contained fluorescence-labeled bovine serum albumin (BSA) and IgG at concentration of 200 µg/mL each. The incubation plates were coated with AP3 and AP4 films, and proteins were incubated for 3 h to saturate all possible protein-adsorption sites. The samples were washed with PBS to remove nonspecifically adsorbed proteins.

For cell viability on the film, two types of primary cells were tested for their growth on the films: rat-pup-derived meningeal fibroblasts (postnatal nine days) and cardiomyocytes. Both the primary cells were derived from animals and not from cell lines. Cell lines are very well known for their greater tolerance against physical and mechanical insults than primary cells. Hence, animal-derived cells were suitable to examine the biocompatibility of the film for cell and tissue applications. Postnatal nine-days-derived meningeal tissue was peeled from the cerebral cortices, and then processed by incubation for 30 min in collagenase (0.5%), 20 min in trypsin/ethylenediaminetetraacetic acid (EDTA), and triturated. Following trituration, the cells were centrifuged and plated in T-75 flasks with Dulbecco’s modified Eagles medium (DMEM/F-12) containing 10% fetal bovine serum. The cells were allowed to grow for one week to confluence. The minced ventricular tissue was incubated in trypsin solution (50 µg/mL) overnight at 4°C. After removing the trypsin solution, collagenase (1500 U in L-15 medium) was added and incubated at 37°C for 45 min. The cells were triturated, centrifuged at 1200 r/min for 5 min, and seeded in the T-75 flasks at 37°C for 45 min. To minimize fibroblasts in the cell culture, the supernatant (which contained cardiomyocytes) was removed after 45 min incubation and reseeded in new T-75 flasks.

AP4 film was deposited on substrate, placed in a vacuum overnight, and washed with PBS several times to remove any unreacted molecules. The cells were then seeded and cultured using DMEM/F-12 with 10% fetal bovine serum at 37°C. The cultured cells were fixed using 4% paraformaldehyde in 1× PBS, and their nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI) and observed under fluorescent microscope. Fibroblast cells were cultured on 35-mm culture dish. The cardiomyocytes were cultured on a culture dish and on a new class of biodegradable cross-linked urethane-doped polyester (CUPE) polymer as a control [36].
Fig. 2. Growth of AP4 film with time. (a) At $t = 10$ min, uniform film with black–white patches. (b) At $t = 20$ min, nucleations developed. (c) At $t = 30$ min, growth of film in sheet form. (d) At $t = 40$ min, uniform continuous film with nanopores. The porous morphology is highlighted in inset to (c).

III. RESULTS

A. Microscopic Analysis

Surface morphologies of polymer films were studied using SEM. The smoothness of the films showed a trend with increasing concentration of APTMS in reaction mixture, as shown in Table I. AP1 and AP2 films showed highly nodular structure due to higher ratio of hydrophobic PFTS. The AP3 films were relatively more uniform with few pores observed. AP5 had very smooth and plain surface; however, no pores were observed. The AP4 films were continuous and porous at nanoscale. The AP4 pore diameters ranged between 10 and 200 nm. The film morphology was found to be predominantly dependent on monomer ratios. Fig. 2 shows the SEM micrographs of vapor-deposited composite organic films with respect to time (AP4). The images suggest that the growth of the films proceeded from spherical porous nucleations. The nucleations developed into porous network, and then, formed continuous film (at macroscale). These films had pores of dimensions at nanoscale, as shown in the inset to Fig. 2(c). The image at time $t = 10$ min [see Fig. 2(a)] showed a film with no pores.

The energy dispersive X-ray spectroscopy (EDAX) data showed the white area to be rich in fluorine, indicating presence of PFTS, whereas black patch gave peak characteristics of nitrogen, indicating the presence of APTMS (see Fig. 3). As the thickness of film increased, the spherical pores started developing over black patches, which gradually formed interconnected network of porous film all over the substrate.

B. Spectroscopic Analysis

The chemical composition of AP4 films was determined by FTIR analysis. The film showed multiple absorption bands. A broad stretching band was observed from 2900 to 3300 cm$^{-1}$, which is characteristic of O–H and C–H stretching. A small peak was observed at 1700 cm$^{-1}$ attributable to –C=O. In fingerprint region below 1400 cm$^{-1}$, high absorption peaks were present at 1070 and 1122 cm$^{-1}$, characteristic of siloxane (Si–O–Si bonds). The halogen peaks were generally observed in region from 800 to 600 cm$^{-1}$; there were two sharp peaks at 704 and 780 cm$^{-1}$ that can be due to Si–C or C–Cl bonds. To elucidate the surface state of polymeric surface, XPS analysis was performed for AP4 films.

The XPS spectrum is shown in Fig. 4. The elemental composition obtained by high-resolution XPS peaks of C, O, F, Si, and Cl is listed in Table II.
Fig. 4. XPS spectra of elements in AP4 film. (a) Spectrum of measured band. (b)–(e) Zoomed-in view of (a) showing characteristic peaks for F, Si, O, and C.

<table>
<thead>
<tr>
<th>Element</th>
<th>Atomic Weight</th>
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<tr>
<td>C&lt;sub&gt;1s&lt;/sub&gt;</td>
<td>38.51</td>
</tr>
<tr>
<td>O&lt;sub&gt;1s&lt;/sub&gt;</td>
<td>15.83</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.60</td>
</tr>
<tr>
<td>Si&lt;sub&gt;2p&lt;/sub&gt;</td>
<td>6.97</td>
</tr>
<tr>
<td>F&lt;sub&gt;1s&lt;/sub&gt;</td>
<td>37.65</td>
</tr>
<tr>
<td>N&lt;sub&gt;1s&lt;/sub&gt;</td>
<td>0.45</td>
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Two intense components of carbon (38.9%) and oxygen (15.8%) appeared at 280–297 eV and 531–533 eV, respectively, conferring that the films were organic. Fig. 4 shows the high-resolution peaks of different elements. The C peak is deconvoluted into five components that are located at 284.6 eV (CH<sub>2</sub>–CH<sub>2</sub>), 286.47 eV (–C–O), 288.33 eV (–C=O or –O–C–O), 289.4 eV (–C–CH<sub>3</sub>), and 291.3 eV (–C–F).

Fig. 5. Fluorescence intensity depicting protein adsorption on AP3 and AP4 coatings, which showed different surface energies, as measured from contact angle measurements. The data are average of three independent experiments.
293.15 eV (CF$_2$–CF$_2$), and 294.59 eV (CF$_3$–CF$_3$) [16]. The
data showed that 34% of C (288.33 eV) were carboxylic com-
ponent, which also accounts for high percentage of oxygen in
the film, indicating oxidation of the molecules. Two peaks at
690.81 (C–F) and 680.74 eV are assigned to fluorine, where
C–F corresponds to 88% of total fluorine present [16]. Si is
deconvoluted into two distinct peaks, at 102.22 (Si–O–Si) and
105.34 eV [37].

No peak was observed at 101.6 eV, which is characteristic of
Si–O–C, indicating that all Si–O–C bonds in APTMS are bro-
ken, and Si–O–Si bonds are formed as a result of condensation
of silane molecules.

High-resolution oxygen peaks were observed at 532.15 and
534.7 eV corresponding to oxygen and a small Cl peak at 200 eV.
A weak nitrogen peak was recorded, indicating that either the
PFTS was predominantly present in the top layer or the amine
group of APTMS reacted with Cl of PFTS leaving least nitrogen
in top layer. The PFTS is more volatile than APTMS, and it is
expected to have higher ratio of PFTS than APTMS in film
under same pressure.

C. Protein Adsorption

Surface energy and roughness are the two main parameters
that affect thrombogenicity [37]. Rough surfaces activate co-
agulation system and studies suggest that very hydrophobic or
very hydrophilic surfaces can be hemocompatible [37]–[39].
The careful choice of parameters enabled formation of smooth
films, yet porous at nanoscale with very high surface energy.
AP3 film had higher percentage of PFTS than that in AP4, and
therefore had higher contact angle and less surface energy.
The amount of protein adsorbed was much higher on rough AP3
films having micron-size pores as compared to AP4 films with
relatively much smoother surface having nanometer size pores
(see Fig. 5). Three independent experiments were performed
with two chips in each run (total of six chips for each type of
coating). The microporous surface provided much large area for
protein adsorption in case of AP3.

D. Cell Growth

The effect of film/pore structure on cell’s proliferation and
growth was compared with the control samples, as described
in Section II. Fig. 6 shows higher density of primary fibroblast
cells on polymer film (number of cells 412 ± 57; n = 3) than
those on control surface of culture dish (number of cells 188 ± 32; n = 3). The presence of pores increased the overall surface
area for cell growth, providing more adherent surface space
for fibroblast protrusions that resulted in better growth of cells.
Fig. 7 shows the cardiomyocytes over AP4 film and culture dish
after 48 h.

Cardiomyocytes were cultured on CUPE polymer and CUPE
coated with AP4. The CUPE is known to be biodegradable and
have mechanical strength better than polyester due to incorpo-
ration of amide bond. Cardiomyocyte cells were grown for four
days and stained using DAPI on this polymer. Fig. 8 shows the
fluorescence images of control and treated samples. AP4 film
being porous in nature provided more surface area for better
attachment and higher growth of cardiomyocytes.

IV. DISCUSSION

The data show that the ratio of PFTS and APTMS, and the to-
tal reactor pressure are important parameters to achieve desired
morphology of the films. The AP1, AP2, and AP3 produced
very low surface energy films with high hydrophobicity, and
the degree of substrate roughness may have induced different
levels of protein aggregation on the surfaces. Roughness and
relative hydrophilicity are known to increase thrombogenesis,
as discussed by Clarotti et al. [22]. The AP3 films have lower
surface energy and hence higher contact angle than AP4. Just
increased hydrophobicity of surface may be enough to explain
higher level of protein adsorption on AP3 [40]; however, the sur-
face roughness of the coating may also be a contributing factor,
leading to different levels of albumin and IgG adsorption.

Cell study involving growth of primary fibroblast cells and
cardiomyocytes on different substrates reveal the possibility of
using the polymer-coated surfaces for cell growth. There can
be several applications for this kind of surfaces beyond already

explored, such as to store/encapsulate signal molecules, growth factors, and other molecules to direct the cell differentiation. However, there will be only a range of molecules that can participate, owing to low surface energy of treated substrate. As the surface coating is done in vapor phase, it can also find applications in the coating of the inner surfaces of implants with desired polymeric films.

V. CONCLUSION

A simple method to produce vapor-phase polymer coating has been demonstrated. Chemically, the coatings are rich in fluorine and have nanopores that provide high surface area. The coatings support growth of primary fibroblast and cardiomyocytes.

The vapor-phase coating of solid-state devices can have important implications in producing biocompatible, fluorine-rich, low-energy, and smooth polymer composite surfaces that can support cell growth. There are many approaches to produce novel fluorine coatings, but the presented method requires no special equipment. The coating of nanotextured films at room temperature is not only well-suited for processing of materials in biomedical applications, it also extends the range of temperature-sensitive substrates that can be successfully coated, especially the flexible polymer-based substrates. Nano- and microstructure devices can be evenly coated with uniformly thick layers of the polymers for a variety of applications, for example, to coat biological implants or for in vivo studies.

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REFERENCES

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