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# Cell patterning using molecular vapor deposition of self-assembled monolayers and lift-off technique

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ABSTRACT

This paper reports a precise, live cell-patterning method by means of patterning a silicon or glass substrate with alternating cytophilic and cytophobic self-assembled monolayers (SAMs) deposited via molecular vapor deposition. Specifically, a stack of hydrophobic heptadecafluoro-1,1,2,2-tetrahydrodecyltrichlorosilane SAMs and a silicon oxide adhesion layer were patterned on the substrate surface, and a hydrophilic SAM derived from 3-trimethoxysilyl propyldiethylenetriamine was coated on the remaining non-treated areas on the substrate surface to promote cell growth. The primary characteristics of the reported method include: (i) single-cell resolution; (ii) easy alignment of the patterns with the pre-existing patterns on the substrate; (iii) easy formation of nanoscale patterns (depending on the exposure equipment); (iv) long shelf life of the substrate pattern prior to cell culturing; (v) compatibility with conventional, inverted, optical microscopes for simple visualization of patterns formed on a glass wafer; and (vi) the ability to support patterned cell (osteoblast) networks for at least 2 weeks. Here, we describe the deposition technique and the characterization of the deposited layers, as well as the application of this method in the fabrication of multielectrode arrays supporting patterned neuronal networks.


## 1. Introduction

Patterned growth of cultured cells, pioneered by Kleinfeld et al. at AT&T Bell Laboratories [1], is a technique gaining importance in a variety of applications and fields, such as cell-based sensors, neurobiology and tissue engineering [2,3]. The critical step in cell patterning is the formation of alternating patterns of permissive (cytophilic) or non-permissive (cytophobic) surface regions for respectively promoting and suppressing cell growth. It is well known that, in vivo, most mammalian cells require extracellular matrices (ECM), containing cytophilic proteins such as fibronectin and collagen [2], and chemical growth factors for physical attachment, survival and growth. The ECM plays a critical role in multiple cellular functions, ranging from migration to proliferation to apoptosis [2–4]. Thus, patterning of the ECM or chemicals which can promote cell attachment on a substrate is a prerequisite for the growth of patterned cells, in vitro.

A number of research groups have developed various methods to pattern cytophilic and cytophobic chemicals on a substrate [5–7]. Such methods usually combine microfabrication, chemical surface modification and material processing. Among them, micro-contact printing (μCP) is the most frequently used [7–9]. This simple and cost-effective technique first requires the fabrication of desired topographical features on a silicon wafer using well-developed semiconductor processing technology. Polydimethylsiloxane is then molded onto the patterned silicon surface structure and peeled off as a stamp. Finally, the stamp, coated with a specific chemical which is favorable or non-favorable for cell growth, is brought into contact with a substrate, transferring the corresponding cytophilic or cytophobic molecules to the substrate, similar to traditional ink printing. Other patterning techniques include dip-pen nanolithography [10,11], the parylene-based dry lift-off technique [12], inkjet printing for cell patterning [13], laser scanning lithography [14] and microfluidic patterning [15].

Recently, the patterning of self-assembled monolayers (SAMs) has been intensely explored as an alternative to protein patterning. Patterning SAMs is an attractive alternative because the SAM molecules are highly orderly and exhibit controllable properties for a variety of desired applications, depending on the functionality of the terminal group (such as hydrophobic or hydrophilic control) or the chain length [16,17]. Most frequently, self-assembled monolayers have been deposited using micro-contact printing [18] or adsorption [19,20]. They have been used, among other applications, for biosensors [16,21] and making arrays of proteins and cells on chips [19,22].

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However, most patterning techniques, whether based on patterning proteins or patterning self-assembled monolayers, have some limitations, such as an inability to combine single-cell resolution and simple alignment of the pattern to features and/or structures already existing on the substrate. For example, our target application is the fabrication of multielectrode arrays (MEAs) capable of supporting patterned neuronal networks with single neurons positioned on top of underlying electrodes, and defined narrow pathways connecting the electrodes, capable of supporting the outgrowth of neurites but not the attachment of neuronal cell bodies. For that application, the desired cell patterning technique must be capable of providing single-cell resolution and must be easily aligned with the underlying electrodes. Additionally, it is desirable for this technique to generate patterns which are both suitable for visualization by inverted microscopy (both prior to and during cell culture) and able to support patterned neuronal networks for extended time periods (2 weeks or more) to allow longer-term experiments. The surface patterns should also have a relatively long shelf life, making them more convenient for utilization.

We sought to develop a technique that satisfied all of the requirements mentioned above at the same time. To accomplish this, we built upon several significant and relevant studies. For example, several patterning techniques have achieved single-cell patterning, such as μCP [23,24], using magnetic microposts [25] and dielectrophoresis [26]. Two related, and particularly relevant, publications describe techniques developed by Stenger et al. [27] and Ravenscroft et al. [28], whereby high-resolution substrates for cell patterning were created, based on self-assembled monolayers; our choice of surface treatment was based on these important studies. However, these previously developed cell patterning methodologies required deep UV lithography, which requires specialized equipment that was not available in our laboratory.

It should also be mentioned that some earlier cell patterning techniques have demonstrated a good alignment of μCP-created patterns with respect to the underlying features, such as microelectrodes in MEAs [29,30], where modified optical aligners were utilized. Finally, extended-time experiments have been performed successfully using certain patterning methods [22,31,32]. Indeed, Branch et al. [32] have demonstrated long-term monitoring of electrical signals on patterned substrates via microelectrode arrays for four weeks. In doing so, two important goals were achieved: the formation of relatively coarse neuronal networks patterned on top of microelectrode structures, and the maintenance of the survival and activity of these networks for longer time periods. This previous study provided important groundwork; however, our envisioned networks have a finer resolution (single cell level) than that demonstrated by the previous work. In the current study, we implement, for the first time in cell patterning efforts, a recently developed SAM deposition technique from the gaseous phase (rather than the traditional method of soaking) via molecular vapor deposition (MVD®). Vapor-phase deposition has a potential advantage of small feature formation that would be more difficult to achieve from the traditional liquid-phase deposition. Additionally, because of its repeatability, it might have an advantage in mass manufacturing conditions.

Here, we report a new, simple and effective cell patterning technique that accomplishes all of our desired goals. Using this technique, predefined patterns for cell growth can be visualized prior to cell culturing, single-cell patterning resolution is achieved and the patterns can be used to form a complicated biosensor system with accurate alignment using a conventional aligner. This method uses a patterned bilayer of a hydrophobic fluorine-based SAM, heptadecafluoro-1,1,2,2-tetrahydrodecyltrichlorosilane (FDTS), on a silicon oxide ($SiO_2$) adhesion layer, using the MVD process to create a cytophobic surface that prevents cell growth. A hydrophilic SAM, 3-trimethoxysilyl propyldiethylenetriamine (DETA), which has been shown to be cytophilic and encourages cell adhesion [27,28,33], is then backfilled on the open silicon oxide surface to promote cell growth in alternating regions. The predefined patterns we obtain can be conveniently visualized prior to cell culturing. As this method is photolithography-based, it also achieves high resolution and single-cell positioning and patterning, which can be maintained for extended culture periods. Additionally, the deposited materials display long-term reliability. Using this method, we have successfully patterned immortalized mouse hypothalamic (GT1–7) neurons and mouse (MC3T3) osteoblast cells, with osteoblast patterns being maintained for at least 14 days in vitro.

## 2. Materials and methods

### 2.1. Microfabrication of substrate

The microfabrication process, illustrated in Fig. 1, starts from a single crystal silicon wafer, three inches in diameter. First, a silicon oxide layer of 100 nm thickness was deposited at 130 °C using a plasma-enhanced chemical vapor deposition (PECVD) method (Fig. 1a). Then positive photoresist (Shipley 1818, Shipley Company Inc, MA) was spin-coated at 3000 rpm for 30 s to obtain a 2.2 μm thick thin film (Fig. 1b). After prebaking at 90 °C for 60 s, the photoresist was exposed to UV light (wavelength $\lambda = 405$ nm, power density = 12 mW $cm^{-2}$) for 6.4 s using an EV620 mask aligner (EV Group Inc., NY), developed in Shipley MF321 developer for 90 s, then flood-exposed for 60 s to assist photoresist stripping later (Fig. 1c).

Once photolithography was completed, the substrate with patterned photoresist was coated successively with a 10 nm thick silicon oxide adhesion layer followed by a self-assembled organosilane functional monolayer of FDTS (chemical structure illustrated in Fig. 1) using an MVD system that was initially developed for applying anti-stiction coatings to micromechanical systems (MEMS) devices. The MVD system (schematically shown in Fig. 2) and the deposition procedure have been described in detail elsewhere [34] and are also presented in the "Supplementary Material" to this paper.

Following the MVD processing (Fig. 1d), the whole 3 inch wafer was cut into dies, 1.5 × 1.5 cm in size, using a K&S 7100 dicing saw (Kulicke & Soffa, PA). This die size was chosen so that the die would fit in a well of a six-well culture plate (BD Biosciences, NJ) during subsequent cell culturing. Next, the photoresist was stripped in acetone for 5 min, and the wafers were cleaned by an ultrasonic cleaner (VWR, PA; Fig. 1e), immersed in a 9 mM solution of DETA (Gelest, Inc., PA) for 1 h and withdrawn. The DETA has been shown to function in a manner similar to poly-D-lysine, and promotes cell attachment and growth [33]. Due to the high DETA/FDTS inter-surface energy, DETA does not rest on the FDTS SAM film. The post-MVD immersion and withdrawal resulted in a cytophilic DETA SAM pattern complementary to the cytophobic pattern of the MVD-produced FDTS SAM/$SiO_2$ bilayer. The substrates were rinsed with ethanol and deionized (DI) water and dried with a stream of nitrogen to remove any residual solvent (Fig. 1f) before cell culturing (Fig. 1g).

While the EV620 contact aligner can reach line widths of approximately 2 μm, projection optics in an Autostep stepper (AS200, GCA Corporation, MA) allowed us to achieve a 0.5 μm line width of patterned photoresist. In this case, we used OiR 620-7i *i*-line photoresist (Fuji films, Japan), 0.45 μm thick, soft-baked at 90 °C for 1 h, exposed for 0.168 s and baked at 115 °C for 1 min, followed by development in AZ 300 MIF developer (AZ Electronics Materials US Corporation, NJ) for 1 min. Then FDTS SAM/$SiO_2$ bilayer was deposited using the MVD process and subsequently