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Three-dimensional (3D) plasma micro-nanotextured slides for high performance biomolecule microarrays: Comparison with epoxy-silane coated glass slides

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ABSTRACT

Glass slides coated with a poly(methyl methacrylate) layer and plasma micro-nanotextured to acquire 3D topography (referred as 3D micro-nanotextured slides) were evaluated as substrates for biomolecule microarrays. Their performance is compared with that of epoxy-coated glass slides. We found that the proposed three-dimensional (3D) slides offered significant improvements in terms of spot intensity, homogeneity, and reproducibility. In particular, they provided higher spot intensity, by a factor of at least 1.5, and significantly improved spot homogeneity when compared to the epoxy-silane coated ones (intra-spot and between spot coefficients of variation ranging between 5 and 15% for the 3D micro-nanotextured slides and between 25 and 85% for the epoxy-silane coated ones). The latter was to a great extent the result of a strong "coffee-ring" effect observed for the spots created on the epoxy-coated slides; a phenomenon that was severely reduced in the 3D micro-nanotextured slides. The 3D micro-nanotextured slides offered in addition higher signal to noise ratio values over a wide range of protein probe concentrations and shelf-life over one year without requirement for specific storage conditions. Finally, the protocols employed for protein probe immobilization were extremely simple.

1. Introduction

Microarrays have become a routine tool in clinical diagnostics, environmental monitoring and food safety assessment. The microarray format can offer ultimate advantages in terms of low probe consumption, highly sensitive readout, and multiplex performance. Such advances hold potential of early disease diagnosis through simultaneous determination of multiple markers using minute sample quantities [1,2]. A well-known technique to place small sample volumes of antibody, protein, or other target biological material onto the microarray substrates is by employing a microarray spotting instrument. Its main advantages are accuracy, reproducibility and adequate speed. It also costs less and offers more versatility than other methods.

Over the years of microarrays development, a countless variety of surfaces has been used as substrates including glass, silicon, silicon dioxide and gold, as well as, organic polymer substrates [3–5]. Functionalized planar glass microscope slides are the support of choice for microarrays due to their low autofluorescence and cost [6–8]. However, they have some limitations including low capacity for analyte binding, and non-uniformity of spot size and intensity, thus complicating quantitative analysis by microarray scanners software. On the other hand, polymers [9–12] combine rapid and inexpensive production in high quantities with intrinsic higher binding capacity, especially of protein probes, thus circumventing the higher autofluorescence as compared to glass substrates. This higher biomolecule binding efficiency of the plastic slides is expected to lead to higher specific signals and lower detection thresholds when immunoassays are performed on these slides versus the silane-modified glass ones.

Most commonly planar surfaces are used providing reactive aldehyde-, epoxy isothiocyanate, amino-, or mercapto-groups. N-Hydroxysuccinimide (NHS) esters and aldehyde groups are amine reactive, maleiimide react with thiol groups, epoxides react with both, amine and thiol groups, and surfaces with reactive amino groups could bind to EDC/NHS activated carboxy groups [3,4]. Compared to two-dimensional (2D) substrates, three-dimensional

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(3D) substrates provide for higher probe loading capacity and ability to adjust the distribution of binding molecules. Hydrogels [13,14], gel pads [15], and particularly nitrocellulose [16] are popular 3D substrates for protein microarrays. In addition nanoparticles [17], nanotubes [18], nanopillars [19], and porous surfaces [20] have been used as 3D protein microarrays [21] to increase the amount of protein immobilized per spot and thereby the signal by increasing the area for protein immobilization.

Micro-nanotextured polymeric surfaces can offer many functionalities (i.e. wetting control [22]). They also enable high protein binding efficiency with respect to planar ones, due to increased surface area that greatly facilitates biomolecule adsorption, while they also provide for covalent binding through appropriate surface chemistry. The topic of biomolecule adsorption on superhydrophilic and superhydrophobic surfaces has been recently reviewed by Song and Mano [23]. Neto et al. [24] fabricated hydrophilic spots surrounded by superhydrophobic areas and proposed these spots as a platform for high throughput analysis of the interactions of biomaterials, proteins and cells. In addition the role of plasma processing for nanobiotechnology applications has been reviewed by Rossi et al. [25] and Colpo et al. [26]. Rucker et al. observed increased biomolecule adsorption on oxygen plasma micro-nanotextured organic polymers [27]. Vlachopoulou et al. also reported that SF_6 plasma nanotextured poly(dimethyl siloxane) (PDMS) showed a 5-fold increase in protein adsorption compared to respective flat surfaces [28,29]. Tsougeni et al. reported a similar trend in oxygen plasma nanotextured poly(methyl methacrylate) (PMMA) [30,31], with optimal etching time being 5 min. Enhancement of biomolecule binding has also been reported for 3D micro-nanotextured COP surfaces [32]. Hierarchical micro-nanotextured surfaces and their applications have been reported in a recent review article [33]. These works introduced a new type of substrate by proposing the use of micro-nanotextured sheets for development of protein [34], DNA [35], or antibody arrays [36]. These microarrays demonstrated an improvement in detection sensitivity by a factor of 100, due to higher fluorescence signal and better spot confinement and definition, as compared to respective flat substrates. They exhibited, however, a rather high background signal. In addition, since there were not commercially available PMMA slides at dimensions suitable for reading by microarray scanners their preparation was cumbersome and lacked the standards required for processing the results by the scanner software.

The 3D micro-nanotextured slides, presented in this work, aim to eliminate most of the problems routinely encountered in microarrays employing either glass or polymer substrates by combining a glass slide as support for a thin polymer layer that is micro-nanotextured using the cutting edge technology of gaseous plasma processing with simultaneous micro-nanotexturing [37]. The 3D micro-nano topography created on the PMMA coating is highly uniform and reproducible offering increased surface area for biomolecule immobilization. Due to the use of the glass slide as support, the 3D micro-nanotextured slides are compatible with all commercial microarray printers and scanners. In addition, the implementation of a PMMA film (approx. 7 μm) instead of a thick polymer sheet leads to significant reduction of auto-fluorescence signal. The 3D micro-nanotextured slides were compared in terms of spot morphology, reproducibility, and protein binding capacity with glass slides that have been modified with an epoxy-silane layer, a modification that is applied by many companies manufacturing slides for protein arrays. Both surfaces were then employed as substrates for the immunochemical determination of C-reactive protein and Salmonella lipopolysaccharides, and the advantages of 3D micro-nanotextured slides in terms of detection sensitivity were demonstrated. Moreover, the shelf-life of 3D micro-nanotextured slides under storage at room temperature was determined, showing stability for over 12 months. All the results presented here for the 3D micro-nanotextured slides refer to protein immobilization through physical adsorption, accompanied by partial covalent binding on the carbonyl groups created on the surface upon plasma treatment (see Fig. S1 of Supporting information for chemical reactions leading to covalent binding of proteins onto 3D micro-nanotextured as well as on the epoxy-silane modified slides). Nevertheless, pure covalent binding is also possible through activation of stable carboxyl-groups created on the surface upon plasma treatment [36].

2. Materials and methods

2.1. Materials

Microscope glass slides with dimensions of 25 × 75 × 1.0 mm (WxLxH) were purchased from Knittel Glässer (Braunschweig, Germany). PMMA with molecular weight of 120 kDa, (3-aminopropyl)triethoxysilane (APTES), (3-glycidyloxypropyl) trimethoxysilane (GOPTS), propylene glycol methyl ether acetate (PGMEA), mouse gamma-globulins (MgG), bovine serum albumin (BSA), and Salmonella liposaccharides (LPS) were purchased from Sigma (St. Louis, MO). Goat anti-mouse IgG antibody and streptavidin labelled with fluorescent dye CF555 were purchased from Biotium Inc. (Fremont, CA, USA). Affinity purified goat polyclonal antibody against C-reactive protein (CRP) and CRP for preparation of calibrators were from Scripps Laboratories Inc. (San Diego, CA, USA). Affinity purified polyclonal rabbit anti-Salmonella group antigen antibody was purchased by AbD Serotec (Oxford, UK). The biotinylation of bovine serum albumin (b-BSA), goat anti-CRP and rabbit anti-Salmonella antibodies was performed according to a published method [38].

2.2. Preparation of epoxy-coated glass slides

The glass slides were cleaned and hydrophilized, prior to chemical activation with GOPTS, by immersion in Piranha solution (1:1 (v/v) H_2O_2/H_2SO_4) for 20 min. Then they were extensively washed with distilled water and dried under a N_2 stream. The slides were then immersed into a 1% (v/v) GOPTS solution in anhydrous toluene and left overnight. After that, they were rinsed repeatedly with toluene and absolute ethanol and sonicated for 20 min in absolute ethanol prior to drying under a N_2 stream. The epoxy-silane coated slides were used either immediately for biomolecules deposition or kept under vacuum at room temperature (RT) until use. Identical performance of these in-house made epoxy slides and commercial epoxy slides was confirmed using model binding assays (biotinylated-BSA or mouse-IgG).

2.3. Preparation of 3D micro-nanotextured slides

To improve the adhesion of the PMMA film onto the glass slides, they were firstly modified with an amine-silane layer. For this purpose, the glass slides were cleaned and hydrophilized in Piranha solution as described above and then immersed in a 2% (v/v) aqueous APTES solution for 20 min at RT. After gentle washing with distilled water, the surfaces were dried under N_2 stream and baked in an oven at 120 °C for 20 min. PMMA was dissolved in anhydrous PGMEA to prepare a 25% (w/v) solution which was spin-coated on the APTES-modified microscope glass slides (1500 rpm for 30 s with acceleration of 1500 rpm s^{-2}) resulting in a uniform film with thickness of ~7 μm (thickness variation less than 1% across the slide). After spin-coating, the glass slides were baked at 170 °C for 2 h. Plasma processing was performed in a Nextral RIE parallel plate plasma reactor with an RF source of 13.56 MHz. The PMMA-coated glass slides were treated in O_2 plasma under highly anisotropic etching conditions (plasma power 400 W, pressure 10 mTorr, 100 sccm). After that, a thermal annealing step at 100 °C