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# Biocompatibility of printed paper-based arrays for 2-D cell cultures

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ABSTRACT

The use of paper-based test platforms in cell culture experiments is demonstrated. The arrays used for two-dimensional cell cultures were prepared by printing patterned structures on a paper substrate using a hydrophobic polydimethylsiloxane (PDMS) ink. The non-printed, PDMS-free areas formed the array for the cell growth experiments. Cell imaging was enabled by using a lipophilic staining agent. A set of coated paper substrates was prepared to study the effect of the physicochemical properties of the substrate (topography, roughness and surface energetics) on cell attachment and growth. The studied paper substrates were found to be cell-repellent or cell-supporting. Cell growth was supported by substrates with a large bearing area, low surface area ratio ($S_{dr}$), high total surface free energy and an intermediate electron donor surface energy component. The cells were grown to full confluency within 72 h.



## 1. Introduction

There is a growing interest in using paper as a substrate for low-cost analytical tests in healthcare applications. Various printed paper-based diagnostic and pharmaceutical applications have recently been demonstrated [1–5]. Fields in drug research, chemical toxicity and tissue engineering benefit from the development of fast cell assays which can be used in high-throughput screening. Several ways of producing platforms for two-dimensional (2-D) cell culture have been introduced [6]. Generally, smooth polymer substrates are used as cell culture scaffolds. In order to prepare scaffolds with certain topographical features for cell studies, microfabrication methods such as hot embossing [7] and soft lithography [6] have been used, but they are often time consuming and are not well suited for mass production. Special surface treatments, e.g. protein coatings, are also often needed [8]. Fast and cost-efficient methods to produce biocompatible assays can be realized by using roll-to-roll compatible fabrication methods such as printing.

Surface properties of the substrate affect cell growth, viability and proliferation [9] and, consequently, cell growth can be governed by surface modification and patterning [8,10,11]. Different cell types respond in different ways to surface topography and roughness of the substrate [11]. Surface energy and its components are important factors that influence the attachment of cells [12–15]. Surface chemistry has a self-evident impact on surface energy. It also plays a role in specific adhesive binding of cells. Optimal cell adhesion is dependent on the distribution of the beneficial chemical groups (i.e. binding sites) on the surface. Specific interactions of extracellular matrix (ECM) proteins with cells are important for cell growth [9]. The presence of serum proteins in the cell medium and their adhesion to the surface of the substrate have an important effect on cell attachment [14]. In addition, cell growth can be directed into desired areas by using cell-repellent materials. Polydimethylsiloxane (PDMS) has been proven to hinder the attachment of several cell types due to its low surface energy [16,17]; furthermore, it has been shown to be nontoxic for ARPE-19 cells [18].

The influence of the characteristics of systematically patterned surfaces on the attachment, spreading and alignment of cells has been investigated at the subcellular level [7,9,19]. Paper, being flexible and porous, offers an interesting alternative to the conventional substrates, such as polystyrene and glass, that are often used in cell studies. Uncoated paper has been used in 3-D cell culture applications [20]. The topography and surface chemistry of coated paper substrates can be tailored by changing the composition of the coating formulations and by using different coating methods. Paper can be readily adapted for printing of various functional ink materials [4,21]. For example, the fabrication of printable planar reaction arrays that are applicable for diagnostics has been recently demonstrated [4]. In addition, paper is an excellent substrate for the printing of drug substances [2], and can thus be potentially exploited, for example, in high-throughput screening

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of target formulations for cytotoxicity. Paper is also an economical and renewable alternative for glass and oil-based plastic materials.

In this study, the applicability of paper-based arrays in 2-D ARPE-19 cell culture was examined. The array was constructed through flexographic printing of a PDMS ink by using a patterned printing plate. Four different types of coated paper substrates with characteristic roughness and surface energy were chosen for analyzing the substrate surface factors that influence the 2-D cell culture.

## 2. Materials and methods

### 2.1. Materials

The fabrication procedures of the different paper substrates (P-1, P-2 and P-3) have been described in detail elsewhere [21,22]. Briefly, the main components in the top coating layer of the paper substrates are: (P-1) precipitated calcium carbonate pigments and latex binder; (P-2) a mixture of two different kaolin pigments and latex binder; and (P-3) kaolin pigments and latex binder. The sample P-4 is a paper coated with an aqueous latex blend consisting of a 3:2 weight ratio of low-$T_g$ film-forming ("soft") styrene butadiene and high-$T_g$ nonfilm-forming ("hard") polystyrene. The hard latex pigments (particle size 100–200 nm) provide blocking resistance, mechanical strength and integrity to the film, while the soft latex particles act as a film-forming component. The latex-coated paper was dried with an infrared (IR) lamp (4 s) and calendered at 70 bars and 35 °C before use. The latex coating was further IR cured for a total of about 1 min in order to thermally modify the morphology of the latex particles.

A solventless and hydrophobic three-component ink (referred to as PDMS ink), consisting of PDMS with vinyl groups (Dehesive® 920), Wacker® catalyst OL and a crosslinker V24 (mixing ratio: 100:2.5:1 wt.%, supplied by Wacker Chemie, AG, Germany), was flexographically printed and IR-cured [4]. The printed and IR-cured PDMS layer is referred to as PDMS film. The areas remaining unprinted by PDMS formed a patterned 2-D array, i.e. a matrix of circles with a diameter of 2 or 5 mm (Fig. 1). These unprinted, PDMS-free circles acted as the test areas for cell culture.

A standard polystyrene cell culture dish (Thermo Scientific Nunclon™ 12-well MultiDish®, sterile) was used as a reference substrate (referred to as PS dish).

ARPE-19 cells (ATCC, Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM/F-12, Gibco). PBS (Dulbecco's modified phosphate buffer without calcium and magnesium), fetal bovine serum (FBS), L-glutamine and penicillin/streptomycin mixture were purchased from Gibco. 3,3′-Dioctadecyloxacarbocyanine perchlorate (DiO) used in cell staining was purchased from Sigma.

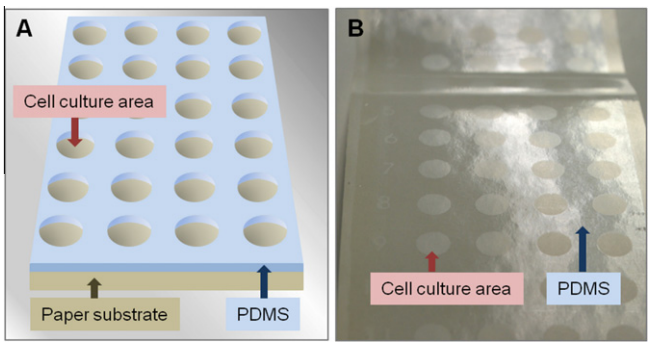


**Fig. 1.** A printed 2-D array. A schematic illustration (A) and a photograph of a sample with a printed translucent PDMS film pattern (B).

### 2.2. Atomic force microscopy (AFM) measurements

An NTEGRA Prima (NT-MDT, Russia) atomic force microscope was used to analyze the topography of the used paper substrates. The images (1024 × 1024 pixels) were recorded using uncoated rectangular silicon cantilevers (MikroMasch, model DP16/GP/AlBS) in ambient conditions (RH = 30 ± 10%, RT = 26 ± 2 °C) in an intermittent-contact mode with a damping ratio of 0.6–0.7 and a scan speed of 0.2–0.4 Hz. The Scanning Probe Image Processor (SPIP; Image Metrology, Denmark) software was used for roughness analysis of the images. Four AFM images with an image size of 5 × 5 μm were measured for each of the samples. Roughness parameters were calculated from the topographical raw data in order to quantify the surfaces. The parameters are described in the following. The bearing area ratio is a measure of the ratio between the top area and the total area of the image (estimated from height histograms; see Supplementary data, Figs. S1 and S2). The root-mean-square roughness ($S_q$) is a standard deviation of height values. The surface area ratio ($S_{dr}$) describes the roughness-induced increment of the interfacial surface area relative to the area of the projected flat plane.

### 2.3. Contact angle measurements and surface energy determination

A CAM 200 contact angle goniometer (KSV Instruments Ltd) was used to measure the apparent contact angles ($\theta_a$) of three probe liquids (water, diiodomethane (DIM) and ethylene glycol (EG); droplet volume: 1–2 μl) on the different substrates in ambient conditions (RH = 15 ± 5%, RT = 24 ± 1 °C). The apparent contact angle values $\theta_a$ lower than 90° (average values of three parallel measurements) were normalized by taking the surface roughness into account by Wenzel's equation ($\cos\theta_a = r\cos\theta_Y$) [23], where $\theta_Y$ is Young's contact angle and $r = 1 + S_{dr}/100$ [24]. The total surface free energy ($\gamma^{tot}$) was then determined by the Van Oss–Chaudhury–Good method ($(\gamma^{tot} = \gamma^{LW} + 2(\gamma^+\gamma^-)^{1/2}$, where $\gamma^{LW}$ is the apolar Lifshitz–van der Waals component, $\gamma^+$ is the electron acceptor component and $\gamma^-$ is the electron donor component) using the probe liquid surface tension component values suggested by Volpe and Siboni [25]. The percentage value of polarity describes the ratio of the polar components ($\gamma^-$ and $\gamma^+$) to the total surface free energy ($\gamma^{tot}$).

### 2.4. Cell culture and seeding

Human ARPE-19 cells (ATCC CRL-2302) were cultured in DMEM-F12 (1:1) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U $ml^{-1}$ penicillin and 100 μg $ml^{-1}$ streptomycin. Cell lines were cultured at 37 °C in a humidified incubator equilibrated with 7% $CO_2$. ARPE-19 cells were seeded on 2-D cell arrays at a cell density of 160,000 cells $cm^{-2}$. Three replicates were made and incubated in a standard cell culture dish at 37 °C and 7% $CO_2$ in an incubator for 24, 48 and 72 h.

### 2.5. Cell fixing and staining

Cells were fixed in 2% paraformaldehyde for 15 min at room temperature and washed with PBS for three times. A lipophilic stain DiO was used to label the membranes. DiO (green fluorescence emission at the wavelength region 500–550 nm) was chosen, since the autofluorescence of the paper samples did not cause disturbing background at this region. A hydrophilic dye adheres to the paper substrate and causes a background; therefore a hydrophobic dye was used. DiO was dissolved in ethanol (35 μg $ml^{-1}$) and diluted in purified milliQ water to the working concentration of 5 μg $ml^{-1}$. Two-dimensional cell arrays were incubated in a DiO