Applied Surface Science 305 (2014) 375–381

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Deposition and alignment of cells on laser-patterned quartz

Sajan D. George[a,*], Uma Ladiwala[b,1], John Thomas[a], Aseefhali Bankapur[a], Santhosh Chidangil[a], Deepak Mathur[c]

[a] Centre for Atomic and Molecular Physics, Manipal University, Manipal 576 104, India
[b] UM-DAE Centre for Excellence in Basic Sciences, Kalina Campus, Mumbai 400 098, India
[c] Tata Institute of Fundamental Research, 1 Homi Bhabha Road, Mumbai 400 005, India

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 27 February 2014
Accepted 15 March 2014
Available online 24 March 2014

Keywords:
Femtosecond laser writing
Wettability
Cell alignment
Micro-Raman spectroscopy

ABSTRACT

Linear grooves have been laser-written on quartz surfaces using ultrashort (50 fs) pulses of 800 nm light. Measurements of water contact angle indicate that laser patterning makes the quartz surface more hydrophilic. Fibroblast cells were cultured on such laser-written surfaces; they were observed to align preferentially along the direction of the laser written grooves (width ~2 μm. Raman spectroscopy results indicate that there are no chemical changes induced in the surface upon our laser writing. Most unexpectedly, there are also no chemical changes induced in the cells that are spatially aligned along the laser-written grooves. Atomic force microscopy measurements confirm that our laser-writing induces dramatic enhancement of surface roughness along the grooves, and the cells appear to respond to this. Thus, cell alignment seems to be in response to physical cues rather than chemical signals.

1. Introduction

Surface properties appear to be playing an increasingly important role in studies aimed at understanding interactions involving cells and bio-materials. It also appears imperative to take cognizance of surfaces in any rational design of bio-compatible devices, such as those used for implantation, microfluidics, directed cell growth for cell-based biosensors, and imaging. Such devices require a study of the effects of various surface properties, like surface topography, surface and bulk chemistry, charge, rigidity, porosity and wettability on cells, not only to predict and control the behavior of cells for efficient function but also to ascertain that cell/tissue integrity and homeostasis is maintained. The growing realization that surface wettability and topography influence cell behavior makes this an area of intense current interest and debate.

Wettability of surfaces can be modulated by surface patterning using approaches like electron beam lithography [1], photolithography [2], soft lithography [3], and chemical patterning [4,5]. In a biomedical environment, we note that these techniques require chemicals that may be harmful to biological cells. Direct writing of patterns on surfaces using high intensity laser beams obviates this difficulty [6–10]. Compared to nanosecond lasers, the use of a femtosecond laser offers additional advantages such as applicability to transparent and opaque materials, rapid energy deposition and lower timescale for ablation, minimum heat-affected zone, high spatial resolution, and precise ablation threshold [9–11]. Further, the use of femtosecond lasers precludes the use of photomasks and requirements of a special environment, thereby making them more acceptable from an industrial point of view. Although the physical mechanism of surface modification with femtosecond pulses in not yet well understood, it is considered to be a non-thermal phenomenon, as the phase transformation occurs on timescales that are much faster than conventional thermal processes [8]. Moreover, femtosecond laser patterning of periodic structures has been shown to produce up to two length scales of surface modification [7]. Moreover, femtosecond laser patterning of surfaces has been shown to regulate cell dynamics on various substrates, such as silicon [12], electrospunpoly(ε-caprolactone) gelatin [13], stainless steel, plastic [6] parylene-C surfaces [14] and titanium [15]. However, the correlation between laser-induced substrate modification and cell response is not yet well understood. For instance, there are insufficient insights into whether femtosecond laser modification results in chemical alterations that, in turn, affect cell dynamics on the modified surfaces. Or are the altered dynamics simply a manifestation of physical changes induced on a surface by a femtosecond laser beam?

In this paper, we present results of experimental studies of cell behavior on femtosecond laser patterned substrates. The substrates

* Corresponding author. Tel.: +91 820 2925072; fax: +91 820 2570062.
E-mail addresses: sajan.george@manipal.edu (S.D. George), brainwave@cbs.ac.in (U. Ladiwala).
[1] Tel.: +91 22 26524976.

http://dx.doi.org/10.1016/j.apsusc.2014.03.095
0169-4332/

we have focused our attention on in this study are commonly used quartz coverslips. Cell adhesion on quartz is important for imaging and in the quartz crystal microbalance used to study the functional responses of cells to external biochemical stimulation [16]. To the best of our knowledge, little work has been done on modulating the wettability of quartz surfaces by femtosecond laser patterning, nor on investigating the cell response to such laser created patterns. The results of our experiments demonstrate a pronounced increase in the wettability of quartz upon femtosecond laser patterning; we also show a proclivity of cultured fibroblast cells to align along the grooves of laser-created patterned areas on the quartz surface. Furthermore, we have utilized high-resolution micro-Raman spectroscopy to ascertain whether femtosecond laser patterning induces chemical changes in the quartz or in the cells that align along the patterned grooves of the quartz substrates. We unequivocally establish that femtosecond laser patterning in quartz does result in surface modification but such modifications are not accompanied by, or a result of, any chemical change. Nor are there any chemical changes observed in the biological cells that are aligned along these laser-induced patterns: these cells retain their pristine biochemical identity. We have characterized the topography of the laser-modified quartz surfaces using atomic force microscopy (AFM). Our AFM studies indicate that femtosecond laser-induced periodic patterning creates two-scale roughness that appears to play the key causative role in the directional alignment of cells, thus confirming the importance of surface topography in the modulation of cell behavior at the interface of bio-materials.

2. Experimental

2.1. Laser patterning

The schematic of the experimental set-up employed by us for laser patterning of quartz substrates is shown in Fig. 1. Laser induced periodic patterns (with 50 and 100 μm spacing) were created on quartz coverslips that were mounted on a XYZ translation stage coupled to a computer-controlled stepper motor with a minimum step size of 1 μm.

The samples were irradiated in air by a Ti:sapphire laser oscillator (Femtolasers, Austria) producing 50 fs long pulses of 800 nm wavelength at 5.1 MHz repletion rate. A single-shot autocorrelator, fiber optics spectrometer, and photodetector coupled to a fast oscilloscope were used to monitor the pulse width, spectrum, and repetition rate, respectively. Our laser beam energy (typically 200 nJ) was controlled using a half wave plate and thin film polarizer before it impinged on the quartz substrate through a microscope objective (40×, 0.75 NA). The samples were irradiated over an area of 8 mm × 8 mm at a laser scanning speed (v) of 1 mm/s. At first, the line patterns were written by moving the sample along a single axis (x-axis) with desired separation between the successive scan lines. A second scan, directly over the thus created line pattern (along the orthogonal y-axis), was employed to fabricate two-dimensional grid patterns, keeping the focusing conditions unchanged. The laser exposure intensity at the sample surface was fixed at $\sim 1.5 \times 10^{14}$ W cm^{-2} A typical optical microscope image of laser fabricated patterns with inter-groove separation of 100 μm is shown in the inset of Fig. 1. The focal beam spot size ($2w_0$) for the patterning was estimated to be ~1.3 μm.

2.2. Raman spectroscopy

Micro-Raman spectra of patterned and unpatterned regions of quartz coverslips were acquired using a diode laser source (Starbright Diode Laser, Torsana Laser Tech, Denmark) with an output beam at 785 nm wavelength. The laser beam was optically expanded and steered, through a 1:1 telescopic arrangement comprising two convex lenses of equal focal length, into the oil immersion objective (Nikon Plan Flour, 0.85 NA, 60×) of an inverted microscope (Nikon Eclipse Ti-U, Japan) through a dichroic mirror which had high reflectivity at 785 nm. The sample was positioned such that the focused laser beam impinged on a region located within the patterned area. Backscattered light from the sample was collected by the same objective and was focused onto the entrance slit of our Raman spectrometer by an f/4 lens coupled to one of the exit ports of our microscope. A 785 nm high-pass edge filter (Razor Edge LP02-785RU-25, Semrock, USA) was used to exclude the intense Rayleigh scattering signal that inevitably accompanies the weak Raman signal. The Raman signals were spectrally dispersed using a Horiba JobinYvon *i* HR320 spectrometer (0.6 nm resolution at 435 nm wavelength) using a grating with 1200 grooves/mm blazed at 750 nm. The spectrometer signal was detected using a liquid-nitrogen-cooled charge coupled detector (Symphony CCD-1024 × 256-OPEN-1LS) with 1024 × 256 pixels operating at a temperature of 140 K. Spectra were acquired for patterned and non-patterned regions of the quartz substrates under identical illumination conditions.

Raman spectra of cells cultured on laser-patterned coverslips (as below) were also acquired using the same experimental set-up.

2.3. Atomic force microscopy (AFM)

The surface topology of the laser patterned quartz coverslips was measured using a BrukerInnova® SPM Atomic Force Microscope. The system is capable of scanning a maximum cross sectional area of 100 μm × 100 μm and a minimum area of 1 μm × 1 μm. Our high resolution system typically had an rms value of noise less than 1.2 nm in the lateral direction (XY) and an rms value of noise less than 50 pm in the axial direction (Z). The measurements we report here were performed with the AFM cantilever in tapping (non-contact) mode.

2.4. Cell culture on laser patterned quartz substrates

The laser patterned quartz coverslips were cleaned by immersing them in mild detergent; they were washed with three changes of sterile distilled water and air dried in a sterile atmosphere. NIH-3T3 fibroblast cells suspended in DMEM with 10% FCS (both Invitrogen, USA), were plated on the patterned regions at a density of 250 cells in 20 μl. Cells on patterned regions of the quartz coverslips were counted at regular time intervals – 0, 6, 12 and 24 h – and the percentage of cells unaligned, aligned along the grooves and those at an angle to the grooves were counted.

Raman spectra of cells aligned along the grooves and unaligned were acquired using the apparatus depicted in Fig. 2.

3. Results and discussion

3.1. Wetting studies

We quantified the influence of periodic laser patterning on the wettability of quartz substrate in terms of water contact angle (WCA). The WCA of a 3 μl liquid droplet deposited on non-patterned as well as laser-patterned quartz substrates was measured using a commercial contact angle measuring instrument (Holmarc, India). Before making WCA measurements, the pristine quartz substrates were cleaned in ethanol and distilled water followed by drying in N_2 gas. Laser patterned substrates were ultrasonicated in distilled water for 30 min to remove any debris present due to laser ablation of the quartz. Five measurements were taken