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Mammalian fibroblast cells avoid residual stress zone caused by nanosecond laser pulses

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ABSTRACT

This study investigates the effects of laser irradiation on crystalline silicon and its application in biomaterials. We used an analytical model to predict the ablation depth and pit size resulting from laser exposure of silicon samples. The temperatures generated are predicted correlate with laser power, and to result in the formation of a residual stress zone bordering the ablated groove. Different crystal orientations found in the substrate confirm that there was crystal distortion, which consequently induces these residual stress zones. Mouse embryonic fibroblasts avoid the stress areas and accumulate outside of these zones. Higher laser power results in broader residual stress zone and a larger zone of cellular exclusion. We argue that residual stress resulting from high-energy laser ablation of silicon may be a promising avenue to explore as a method for patterning cell growth on these materials.

1. Introduction

Silicon has shown tremendous promise in the field of materials engineering due to its versatility and ease of manipulation. It is the most commonly used material in the microelectronics and photovoltaic industry (Jeong et al., 2012; Kiani et al., 2009; Green et al., 2001; Kiani et al., 2011; Rahmani et al., 2012; Schweicher and Desai, 2014). However, research in using silicon for biomedical applications has been scarce because the human body rejects crystalline silicon in its pure form (Shaoqiang et al., 2004; Buckberry and Bayliss, 1999). Researchers have attempted to change the biological characteristics of silicon via topographical and chemical modifications. For example, Myllymaa et al. investigated the feasibility of packaging Si-based material in a biocompatible material such as a titanium coating (Myllymaa et al., 2010). Porous silicon has been achieved though electrochemical etching and has proven to be biocompatible (Santos et al., 2014; Lin et al., 1997). It is possible to create ample Si fibers (porous structure) with a more affordable nanosecond laser under ambient conditions without any other chemical or physical manipulations (Colpitts et al., 2016; Colpitis and Kiani, 2016). The use of such nanofibrous silicon layering is not limited to internal implants, but can be beneficial for applications outside the body, such as bio-plates and lab-on-a-chip (LOC) devices.

The interaction of cells with non-biological materials such as silicon is modulated by several factors. Rhee (Rhee, 2009) investigated the importance of biomechanical conditions in addition to biochemical cues for cell signaling and migration in 3-dimensional (3D) collagen matrices. Tan et al. Tan et al., (2003) demonstrated the control of cell adhesion and spreading with combination of microcontact printing and photolithography to engineer surfaces with a wide range of mechanical properties. They showed both biochemical and mechanical signals paly important role in cell adhesion and cell migration mechanisms. Keshavarz et al. Keshavarz et al., (2016), showed that the cytocompatibility in terms of cell migration and directional cell alignment in fibroblast and HeLa cancer cells can be affected by mechanical forces. Finally, cells (including fibroblasts) are sensitive to electric fields, exhibiting either positive or negative galvanotaxis (Sugimoto et al., 2012; Huang et al., 2016).

Generally, laser processing modifies both the chemical and mechanical state of the substrate as well as its topography (Colpitts et al., 2016; Colpitis and Kiani, 2016; Keshavarz et al., 2016). However, there has not been substantial focus on the mechanical changes caused by laser pulses and how these changes modulate cell interaction with the substrate (Wolf, 1999; Legant et al., 2010; Charras and Sahai, 2014).

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We predict that cell signaling and migration may be affected by mechanical loading of the substrates to which they adhere, similarly to the texture and other properties that are known to affect cell biology (Tan et al., 2003). We have previously shown that cells accumulate in the hybrid amorphous and crystalline silicone lined groove left by laser ablation of silicon substrates (Colpitts et al., 2016). Here we focus on the region flanking the groove. We use Raman and XRD spectroscopy to demonstrate mechanical stresses within the substrate in the region flanking the ablation groove, and we find that cells are largely excluded from these regions of residual stress, suggesting that fibroblast cells can somehow perceive these changes in the crystalline structure of their substrates. The combination of shockwave formations and crystal distortion caused by the laser pulses, induces residual stress on a surface of single crystalline substrate which can be used for fabrication of bio-templates with functionalized surface properties without any modifications in chemicals or topographic structures. This method can lead to technologies for cell patterning through subsurface stress caused by laser pulses.

2. Experimental setup

2.1. Laser processing and generation of treated pattern

A nanosecond Nd:YAG pulsed laser with a wavelength of 1064 nm is used in this study to ablate a simple line pattern on silicon wafers with an orientation of < 100 > . EZCAD software was used to design and control the laser the pattern above the ablation threshold at a sub-micro scale. The pulse powers were 7 W, 10 W, and 15 W. The scanning speed of the laser was set to 400 mm/s and the pulse frequency was 100 kHz.

2.2. NIH 3T3 cell culturing

In this study, we used mouse embryonic fibroblast cells (NIH 3T3) obtained from American Type Culture Collection, Rockville, MD, USA to explore cellular interactions with the laser-treated Si samples. Samples were rinsed in ethanol and autoclaved prior to plating. Cells were seeded in triplicate at 2400 cells/cm^2 onto each variation of laser-treated silicon samples and incubated for 72 h at 37 °C under 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated calf serum. Silicon substrates were rinsed with PBS to remove non-adherent cells and fixed in 4% formaldehyde in PBS overnight at 4 °C before staining with phalloidin (1:2000 dilution) and draq5 (1:10,000 dilution) over night and subsequently fluorescence imaging.

3. Materials and methods

3.1. Microscopy

3.1.1. Scanning Electron Microscopy (SEM)-

The JEOL 6400 SEM equipped with Geller dPict digital image acquisition software and a Gatan ChromaCL Cathodoluminescence imager for capturing high resolution images.

3.1.2. 3D optical microscopy

A Zeta-20 Optical Profiler was used to obtain surface profiles and 3D images of each silicon sample.

3.2. Spectroscopy

3.2.1. Energy Dispersive Spectroscopy (EDS)

Elements in the laser-treated silicon substrate were characterized with the use of the Energy Dispersive Spectroscope (EDS). The Hitachi SU-70 Field Emission Gun (FEG) SEM was the model used in this research.

3.2.2. Micro-raman spectroscopy

Chemical composition of the laser-treated samples was characterized in addition to elemental analysis by EDS. The Renishaw inVia micro-Raman spectrometer with a maximum power of 150 W was used.

3.2.3. X-ray diffraction

X-ray Diffraction is used to characterize crystal structure of the laser-treated silicon material. The data was collected using a Bruker D8 Advance spectrometer. The X-ray source maintained a voltage of 40 kV at 30 mA.

3.3. Characterization of adherent cells

To study the distribution and morphology of the cells adhering to surface of silicon samples and their cytoskeleton, cells were stained with Alexa Fluor 546-conjugated phalloidin (Thermo Fisher) to visualize microfilaments and Draq5 as nuclear counterstain. Cells adhering to silicon substrates were imaged at low power using a Leica M205 stereo epifluorescence microscope to analyze distribution of cells with respect to laser-treated regions of the silicon substrates. Images were processed and analyzed using Fiji 1.6.0 24 (64 bit) (Schindelin et al., 2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676–682, PMID 22743772. To analyze cell surface coverage in relation to distance from the ablated area, we demarcated five zones at distances of 0 µm to 50 µm (zone 1), 50 µm to 100 µm (zone 2), 100 µm to 150 µm (zone 3), 150 µm to 200 µm (zone 4) and 200 µm to 250 µm (zone 5) from the edge of the laser ablated area (surface adjacent to groove resulted by laser treatment). To measure cell density in epifluorescence micrographs, the greyscale threshold function of Fiji was used to contrast the surface (black) and cells (white) (Schindelin et al., 2012). The percent area covered by cells was then determined for each zone.

3.4. Statistical analysis

A minimum of 10 measurements were taken to obtain the mean height and width values of the sample profiles. Also, a p-test was performed to compare the density at each distance from the ablation. Cell density data was analyzed in excel using scattered points for each set of data to find the best fit sigmoid function. Using each function, 300 points were generated to show expected cell coverage values from the edge of the groove to the 300-µm distance from the edge of texture for each data set. These points are represented as a trend line for each set of data.

4. Results & discussion

3D optical micrographs and scanning electron micrographs illustrate the shape of the groove made by the laser ablation (Fig. 1). The groove width and depth increases with power with ranging from 5.9 µm to 7.7 µm for depth and 19 µm to 20 µm for width.

An analytical model was also used to predict the shape of the groove with respect to the radius, as seen in Eq. (1) (Hendow and Shakir, 2010):

$$h(r) = \sqrt{-4\kappa\tau \ln\left\{\frac{\beta K \Delta T_B}{\gamma I_{max}}\sqrt{\frac{\pi}{\kappa\beta\tau}}\left(1+\frac{8\beta\kappa\tau}{W^2}\right)\right\} - \frac{r^2}{1+\frac{W^2}{8\beta\kappa\tau}}} \leq h(0) \quad (1)$$

Where β is a correction factor of 0.5 and ΔT_B is silicon's boiling temperature of 2972 K. Solving Eq. (1) at the surface ($h(r) = 0$), the ablation pit radius can be found with Eq. (2) (Hendow and Shakir, 2010):

$$r_o = \sqrt{-(4\kappa t_o + 0.5W^2)\ln\left\{\frac{\beta K \Delta T_B}{\gamma I_{max}}\sqrt{\frac{\pi}{\kappa\tau}}(1+\frac{8\kappa\beta\tau}{W^2})\right\}} \quad (2)$$

With the use of Eqs. (1) and (2), the ablated groove can be

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