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Differences in Nanoscale Elasticity of Planar and Nanofibrillar Tissue Cultures

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ABSTRACT

Astrocytes are cellular bridges between the neurons and capillaries in the blood brain barrier. It was recently suggested that the nanophysical properties of the basement membrane of the blood brain barrier can influence astrocyte and neuron responses. In this work, cerebral cortical astrocytes were cultured on standard poly-L-Lysine coated glass substrates, Aclar substrates, and electrospun polyamide nanofibers whose properties may recapitulate those of the basement membrane. The nanoscale elasticity of each culture environment was investigated by force curve analysis and compared. The elasticity of the individual nanofibers on nanofibrillar surfaces was also investigated. Finally, variations in elasticity of scaffolds were correlated with astrocyte responses.

INTRODUCTION

It has been shown that the elasticity of tissue scaffolds can influence cellular responses [1,2].Therefore, mechanical characterization investigations of promising tissue scaffolds are needed to optimize tissue scaffold design. The mechanical properties of tissue scaffolds can be investigated by atomic force microscopy (AFM) force curve analysis. The strategies of AFM force curve analysis have been presented by Lin et al. [3]. Force curve analysis of biological surfaces was also performed by Heinz and Hoh [4].

In this work, nanoscale elasticity investigations of poly-L-Lysine (PLL) coated glass, polyamide nanofibrillar scaffolds, and Aclar substrates are presented. PLL coated glass surfaces are conventional neural cell cultures, which were used as a control substrate in this work. The polyamide nanofibrillar matrix has demonstrated promise for the repair of the injured spinal cord in vivo investigations [5]. The polyamide nanofibrillar matrices were electrospun onto Aclar substrates, and therefore the elastic properties of this underlying substrate were investigated as well. The AFM surface height images of scaffolds provided the substrate topographies at a nanoscale. The elasticity results were then correlated with the response of cerebral cortical astrocytes to the three culture surfaces, which was investigated by epi-fluorescence microscopy with phalloidin staining for F-actin visualization. These results give insights for more accurate understanding of how the elastic properties of culture surfaces influence cellular responses.

EXPERIMENTAL PROCEDURES

Preparation of samples

Glass coverslips (12 mm, No. 1 coverglass, Fisher Scientific, Pittsburgh, PA) and Aclar coverslips (Ted Pella, Redding, CA) were used as underlying planar surfaces. Aclar is a transparent fluorinated-chlorinated thermoplastic , and manufactured by Ted Pella, Redding, CA. Glass or Aclar coverslips were placed in a 24-well tissue culture plate (one coverslip/well) and covered with 1 mL of PLL solution (50 μg PLL/mL in dH_2O) overnight. The coverslips used for the cultures were then rinsed with dH_2O and sterilized with 254 nm UV light using a Spectronics Spectrolinker XL-1500 (Spectroline Corporation, Westbury, NY). The polyamide nanofibrillar scaffolds electrospun on Aclar substrates were obtained from Donaldson Co., Inc. (Minneapolis, MN) and Corning Life Sciences (Lowell, MA). Primary astrocyte cultures were prepared as previously reported [5,6].

The astrocytes cultured on coverslips were fixed in 4% paraformaldehyde for 10 minutes and permeabilized by 0.5% Triton X-100 for 5 minutes. After a brief rinse with phosphate buffered saline (PBS), the cells were stained with Phalloidin-488 (Invitrogen, Carlsbad, CA) at a 1:100 dilution for 1 hour, mounted on microscopic slides with GelMount (Biomeda, Foster City, CA). Images of the astrocyte cultures at 24 h were captured using an Olympus IX81 inverted epi-fluorescence microscope (Olympus, Center Valley, PA). For each condition, a minimum of five images were captured from different regions of three coverslips to ensure that the results were representative for the cultures as a whole.

Analytical techniques

The AFM investigations were performed using a Nanoscope IIIa (Bruker AXS Inc, Madison WI, formerly Veeco Metrology) operated in contact mode in ambient air. An E scanner with a maximum 13x13 μm^2 x-y scan range and Veeco DNP silicon nitride probes with a $35^o \pm 2^o$ cone angle and a nominal 20 nm tip radius of curvature were used for AFM investigations. Force curve plots were performed using Nanoscope Software version 5.30r3.sr3.

EXPERIMENTAL RESULTS

AFM force curve investigation

The force curve plots were performed on nine randomly selected points on the scaffolds, and the representative results are shown in Figure 1. A Hertz model was used to evaluate Young's modulus. AFM force curves of three tissue scaffolds demonstrated that the relative elastic modulus of PLL coated glass substrate is higher than Aclar and nanofibrillar scaffolds. Aclar is less stiff than PLL coated glass, which was expected from the force curves in Figure 1 (c). The bending in the non-contact region of the Aclar and

nanofibrillar scaffolds indicated the relatively lower Young's modulus of the substrates, and the quantitative elasticity measurements supported this observation.

The tip–sample interaction can be divided into short- and long -range forces. The long-range interactions are described by the van der Waals forces. The short-range forces are between the foremost tip atoms and can be modeled by ab initio calculations where the tip is represented by a small atomic cluster [7]. The long range tip-sample interactions were observed on Aclar and nanofibrillar scaffolds.

The force curves indicate the amount of adhesion force between the probe and the sample. Figure 1 shows that the adhesion between the AFM probe and nanofibrillar scaffolds was highest amongst others. This indicated that there was a quantifiable difference in the adhesive surface properties of the nanofibrillar scaffolds that a cell could potentially respond to, although a receptor-scaffold interaction would differ from an AFM probe-scaffold interaction.

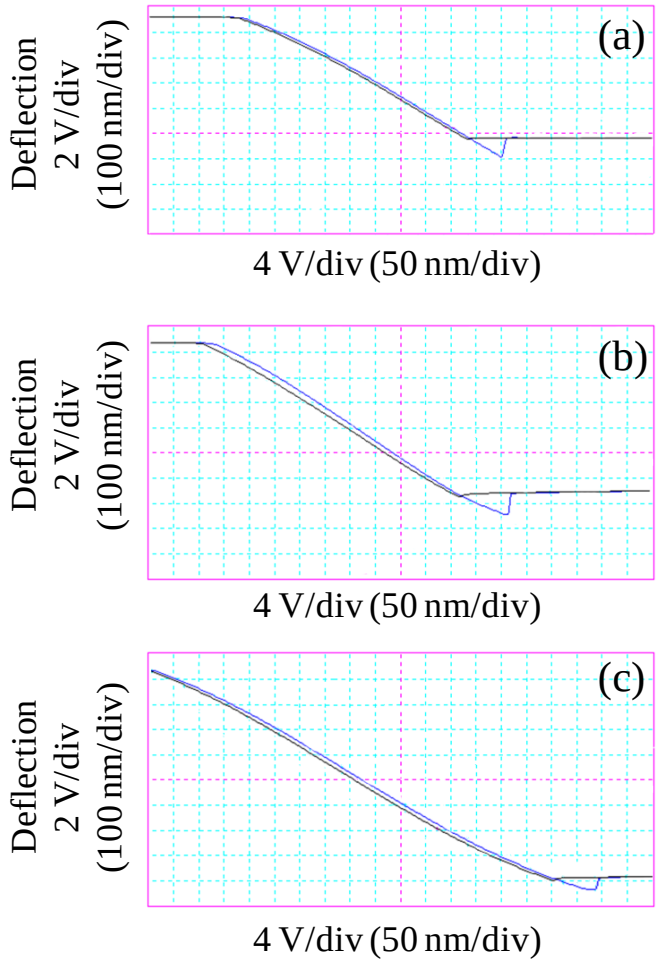

Figure 1. Force curves of PLL coated glass substrate (a), nanofibrillar scaffolds (b) and Aclar substrates (c).

In Figure 1 (a-c), the black curve corresponds to the cantilever deflection as the tip pushes into the substrate and the blue curve corresponds to the relaxation of the deflection as the cantilever straightens out. The black and blue curves were closely

overlaid on the PLL coated glass surfaces (a) but on the nanofibrillar (b) and Aclar (c) surfaces, a deviation was apparent. All measurements were carried out with the same cantilever/tip. The origin of the deviations is therefore likely due to the forces or "grab" on the tip propagated into the cantilever mechanical properties, resulting in a change in its flexure during withdrawal. We note that the greatest deviations were observed for the nanofibrillar surfaces. This could imply that this surface was more effective in restraining the tip than either the harder PLL coated glass surfaces (a) or the softer Aclar (c) surfaces.

The Young's moduli of the culture surfaces are given in Figure 2 as the mean value ± standard deviation. The Young's moduli calculations were done based on a Hertz model [8], with Poisson's ratio for the PLL coated glass, polyamide, and Aclar assumed to be 0.2, 0.35 and 0.4, respectively. The Poisson's ratio for PLL coated glass was assumed to be 0.2 since the PLL coating was thin and not expected to significantly change the value for the underlying glass substrate, which is 0.2. Aclar is a thermoplastic material, which typically have Poisson's ratios close to 0.4. A standard Poisson's ratio of 0.35 for polyamide was used for the nanofibrillar scaffolds [9]. PLL coated glass had the highest mean Young's moduli values (10.6 ± 0.7 MPa), whereas Aclar had the lowest value (3.9 ± 0.03 MPa). A comparison between the Young's modulus values acquired from nine well separated regions and from nine points directly along a single nanofiber indicated that these values were close at 5.8 ± 0.6 MPa and 5.8 ± 0.9 MPa, respectively.

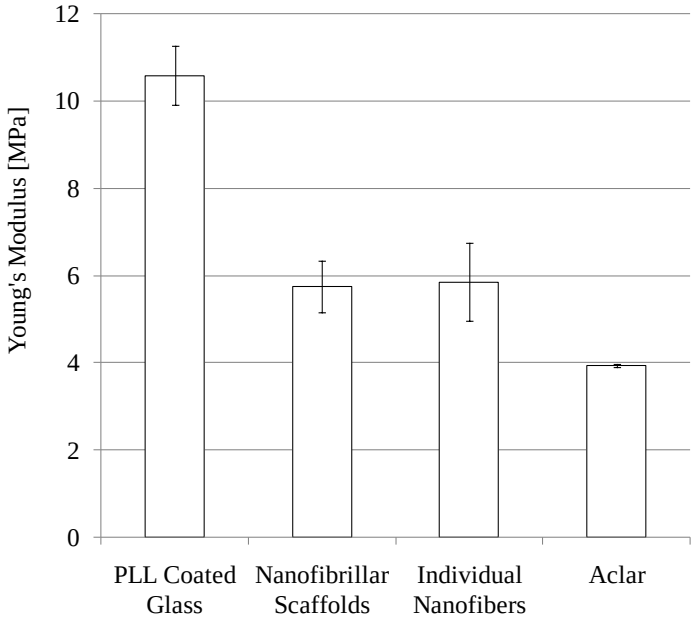

Figure 2. Young's modulus measurement results of tissue scaffolds. Error bars show standard deviation.

AFM Height Images for Qualitative Surface Roughness Comparison

Contact mode AFM height images of the scaffolds are given in Figure 3. The AFM height images demonstrated that the PLL coated glass substrates were smoother than Aclar substrates and nanofibrillar scaffolds.

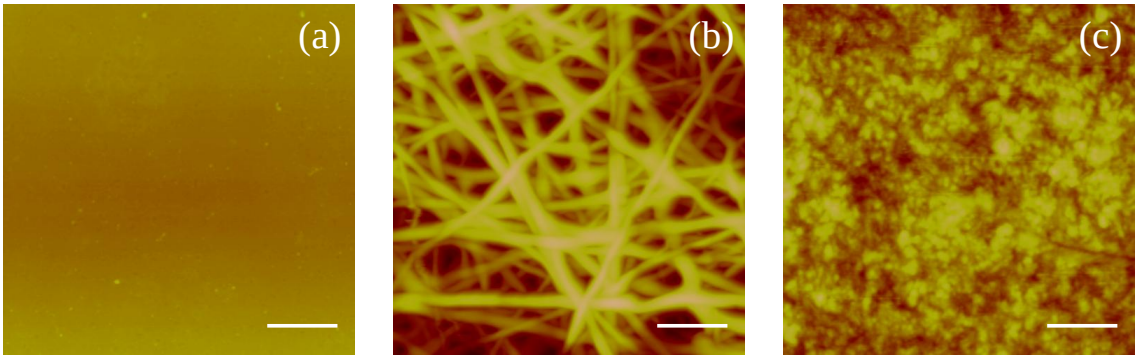

Figure 3. Contact mode AFM height images of PLL coated glass (a), nanofibrillar scaffold (b) and Aclar (c) surfaces. The height scales are 200 nm (a), 1 µm (b), and 200 nm (c), and scale bars show 2 µm.

Epi-Fluorescence Microscopy Results

The cerebral cortical astrocytes cultured on the three tissue scaffolds were investigated by epi-fluorescence microscopy and are shown in Figure 4.

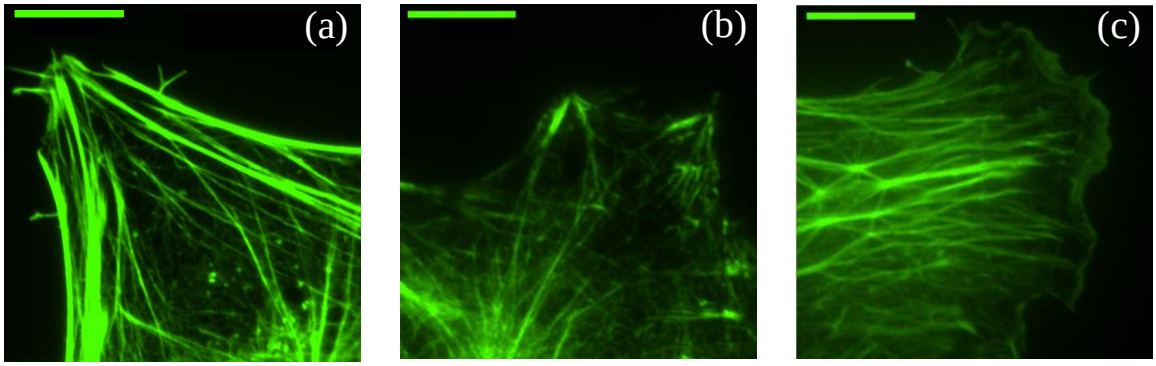

Figure 4. Astrocytes cultured on PLL coated glass (a), nanofibrillar scaffolds (b), and Aclar (c) were stained with phalloidin and shown in green. Scale bars, 10 µm.

The astrocytes cultured on PLL coated glass surfaces have a roughly parallel-oriented F-actin organization to cell membrane whereas the astrocytes cultured on nanofibrillar scaffolds and Aclar surfaces have F-actin expression throughout the cytoplasm. Furthermore, F-actin is more oriented on nanofibrillar scaffolds, but more random on Aclar.

DISCUSSION

Elasticity is one of the physical properties of the tissue scaffolds that could induce cell development, differentiation, and regeneration. Recently, AFM force curve analysis has been used for elasticity measurements of different materials. In this work, qualitative elasticity comparison of PLL coated glass, nanofibrillar surfaces and Aclar surfaces was

performed. AFM force curve analysis also showed that the highest adhesion force was observed between the AFM probe and nanofibrillar surfaces.

AFM height images reveal the three dimensional topography of samples at nanoscale. In this work, surface roughness investigations of PLL coated glass, polyamide nanofibrillar scaffolds and Aclar substrates were performed by contact mode AFM. Nanofibrillar scaffolds are relatively new type of biomaterials which have unique surface topography that mimics the basement membrane at the blood brain barrier [10].

Immunocytochemistry is used to localize specific protein antigens within cells. It is known that cytoskeletal organization of muscle cells depend on scaffold stiffness. In this work, the F-actin organization of astrocytes cultured on tissue scaffolds having different surface roughness and elasticity were investigated by immunocytochemistry. It is shown that PLL coated glass surfaces have roughly parallel oriented F-actin organization whereas the others are more randomly organized. This result suggests that the F-actin organization may be induced by elasticity of the substrate. Further investigations are required in order to elucidate the signaling pathways that induce cytoskeletal organization.

CONCLUSIONS

Elasticity and surface roughness investigations of PLL coated glass, polyamide nanofibrillar scaffolds and Aclar substrates were performed in this work. Force curve elasticity measurements showed that PLL coated glass surfaces have a higher Young's modulus than the other scaffolds investigated in this work. AFM force curve analysis has shown that adhesion force between the AFM probe and nanofibrillar scaffolds was highest amongst other scaffolds. Qualitative surface roughness investigation by contact mode AFM height images showed that the Aclar surface roughness is greater than the PLL coated glass substrates. Phalloidin staining of astrocytes cultured on the scaffolds demonstrated that the astrocytes cultured on PLL coated glass substrates have roughly parallel oriented F-actin organization to cell membrane. These results present examples for different astrocyte responses due to different physical environment.

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