Electronic Supplementary Material (ESI) for RSC Advances.


# Astrogliosis in a Dish: Substrate Stiffness Induces Astrogliosis in Primary Rat Astrocytes

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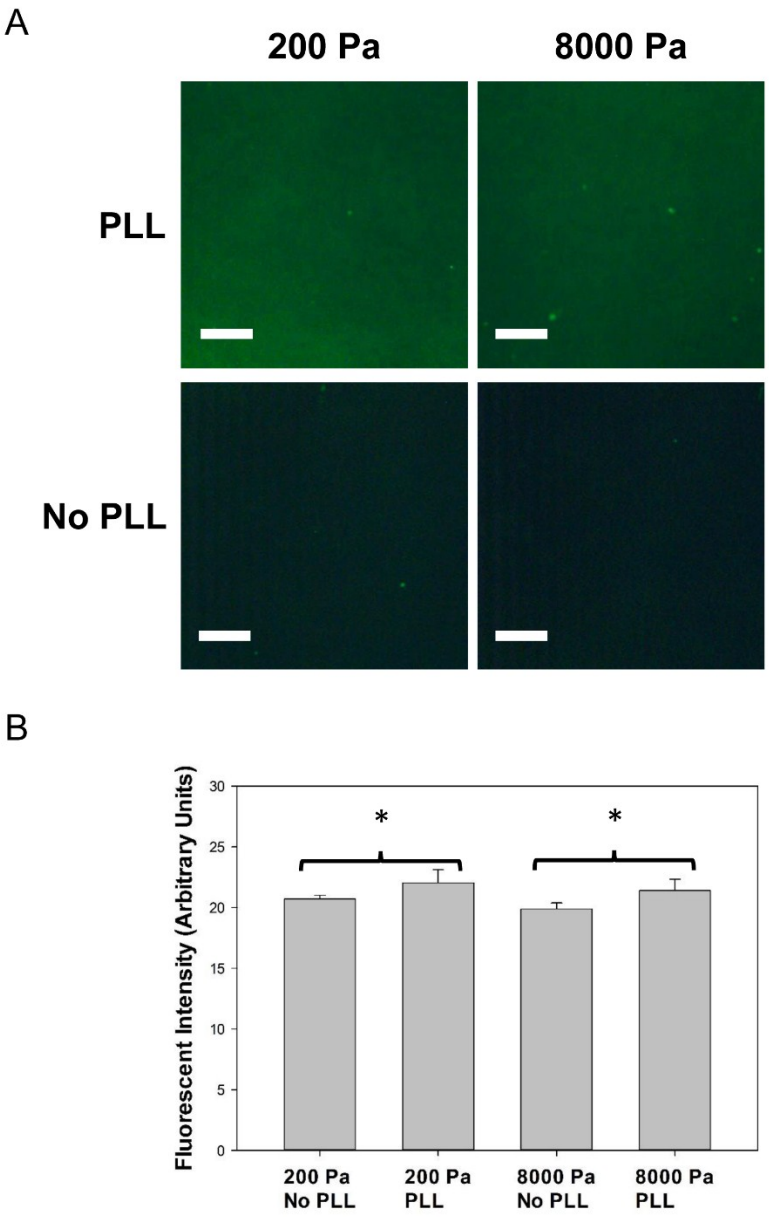
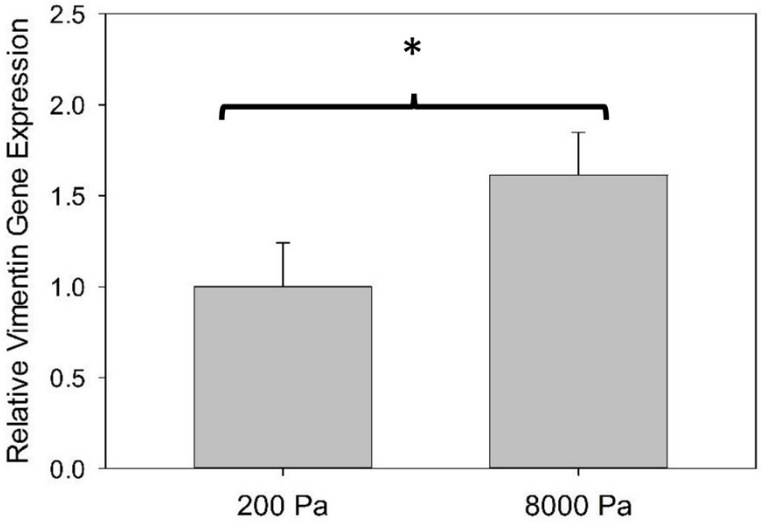
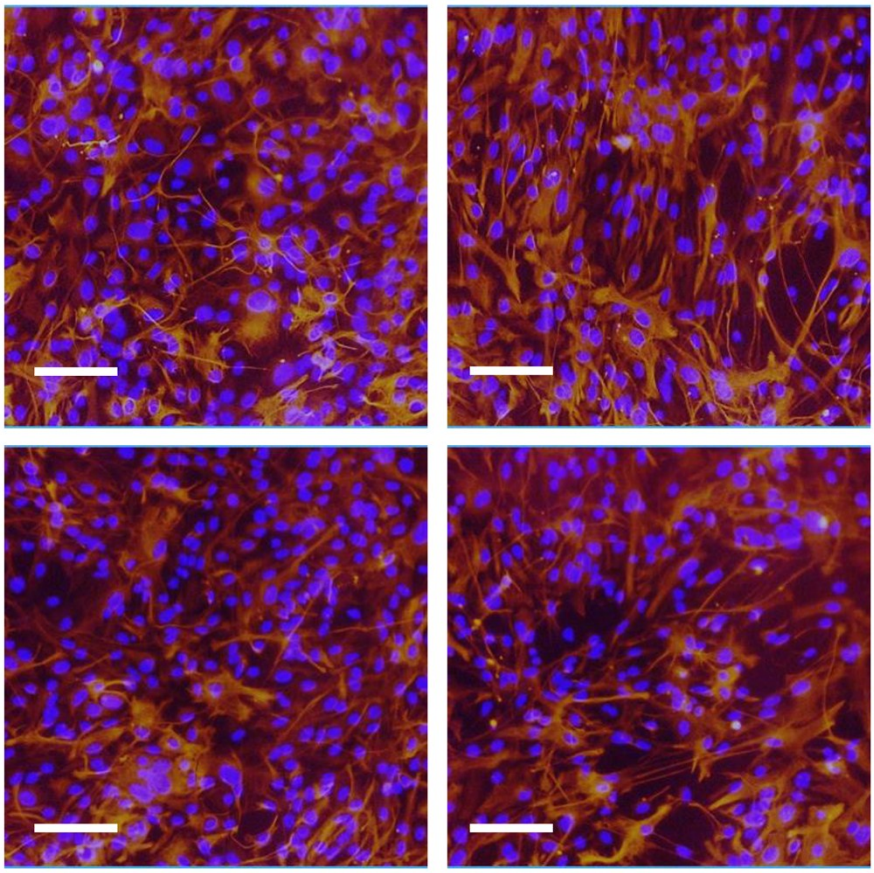



**Supplemental Figure 1**: PLL coating characterization on 200 Pa and 8000 Pa surfaces through images (A) and quantification of fluorescence using image J (B) reveals similar uniform coating on soft and stiff surfaces. Scale Bar 100 μm. "*" $P < 0.05$




**Supplemental Figure 2**: RT-PCR gene expression quantification of Vimentin on soft and stiff surfaces, N = 3. "*" $P < 0.05$



**Supplemental Figure 3.** Representative images of the astrocyte culture. The astrocyte culture utilized for experiments was characterized by immunostaining with anti-glial fibrillary acidic protein (GFAP, red) and DAPI nuclear staining (blue). Cells were fixed with 4% paraformaldehyde for 20 min, permeabilized in 0.1% Triton X-100 for 15 min and background blocked with 1% bovine serum albumin (BSA) in PBS for 1 hr at room temperature. Cells were stained in primary antibody solution (1:1000 anti-GFAP [DAKO] in 1% BSA in PBS) at 4 °C overnight, secondary antibody solution (1:500 anti-Rabbit rhodamine [Millipore] in PBS) for 2 hr at room temperature and DAPI staining solution (1 µg/ml DAPI in PBS) for 5 min at room temperature. Images were obtained using Axiovert 40 CFL [Zeiss] and images taken with a Progres C3 [Jenoptick] camera with an X-Cite series 120Q [Lumen Dynamics] lamp and a CY3 or DAPI filter [Chroma]. Culture purity was determined to be > 90 % astrocytes by counting the number of GFAP positive nuclei over the total nuclei using Image J cell counter, N = 8 images, Scale bar 100 µm.

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