Supplementary Information

Localized Neurite Outgrowth Sensing via Substrates with Alternative Rigidities

Szu-Yuan Chou,[a] Chao-Min Cheng,[b] Chih-Cheng Chen,[c] and Philip R. LeDuc[a]

[a]Departments of Mechanical Engineering, Biomedical Engineering, Computational Biology, and Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, 15213, U.S.A.

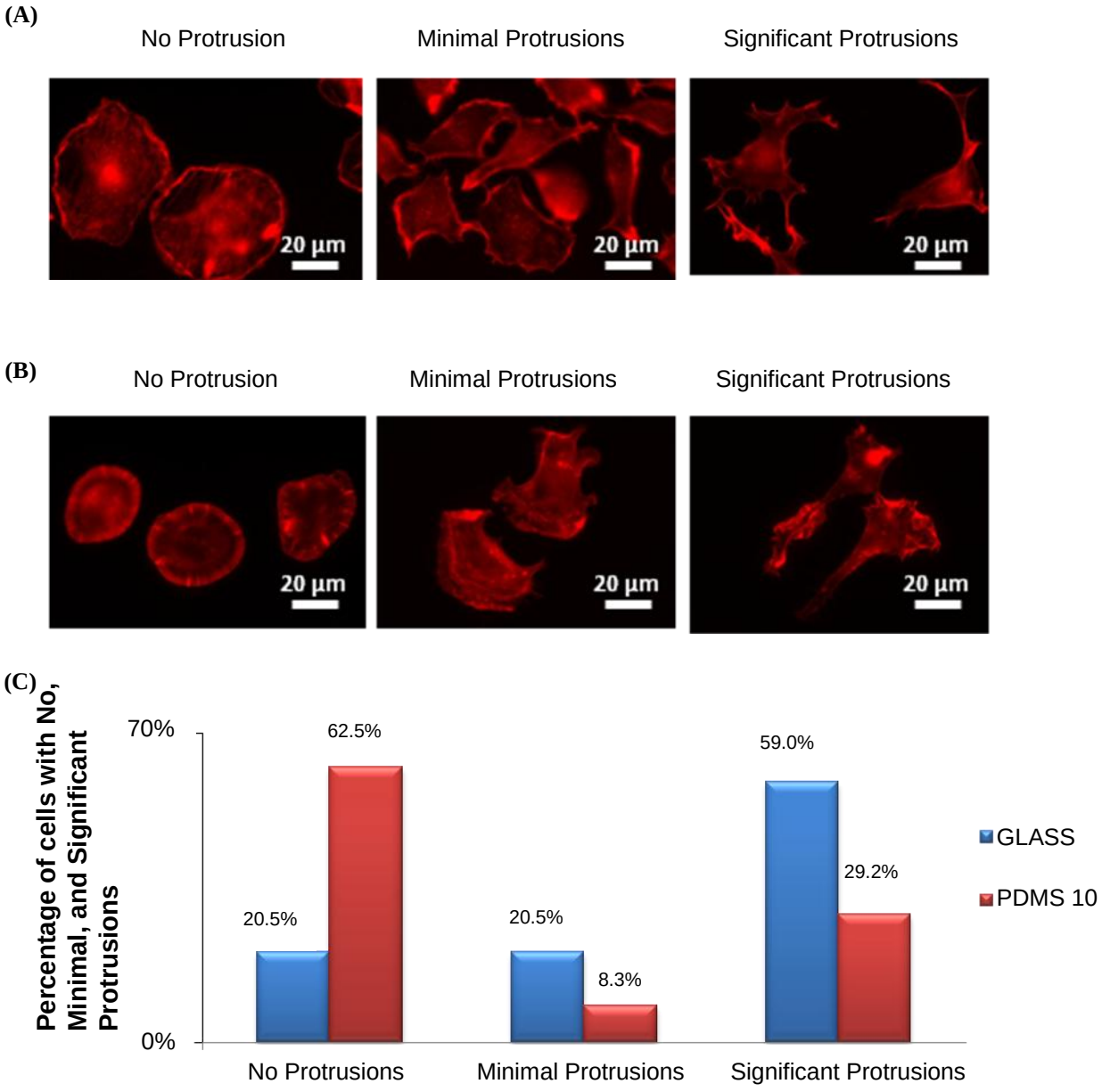
[b]Institute of Nanoengineering and Microsystems, National Tsing Hua University, Hsinchu 300, Taiwan

[c]Institute of Biomedical Sciences, Academia Sinica, Taipei, 115, Taiwan

Corresponding authors; E-mail: prl@andrew.cmu.edu & chih@ibms.sinica.edu.tw

Supplemental Figure 1.

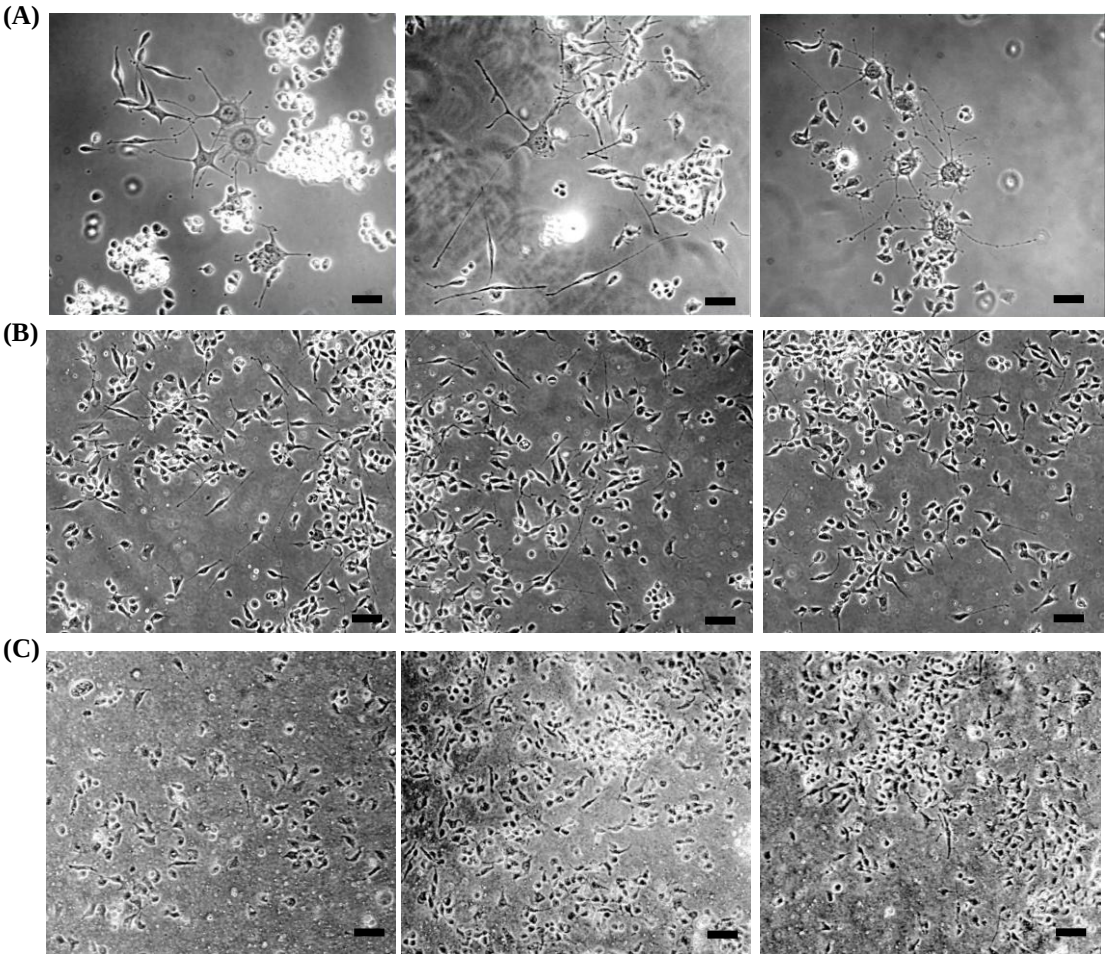
Soma responses to the substrate with distinct elasticities. (A, B) Immunofluorescent staining of actin filaments in neuroblasts attached on substrates with different elasticities of (A) glass and (B) PDMS (10:1) soft substrate. There were three main characteristics of the cells observed for these neuroblasts: no protrusion, minimal protrusions, and significant protrusions. These three panels indicate the cells observed in the same cell culture substrate. (C) The percentages of cells expressing each characteristic on glass (N = 39) and base to curing ratio of 10:1 PDMS (N = 24) substrate. The cells having their longest protrusion to be greater than 25% of the soma length were considered to have significant protrusions. The cells were considered to have minor protrusions if their longest protrusion was less than 25% of the soma length. The cells were considered no protrusions if their longest protrusion was less than 1% of the soma length. For cells attached on the glass substrate, the percentages of cells that had no protrusion, minimal protrusions, and significant protrusions were 20.5%, 20.5% and 59.0%, respectively; for cells attached to a PDMS substrate with a base to curing ratio of 10:1, the percentages were 62.5%, 8.3%, and 29.2%, respectively. Cells attached on the hard substrates appeared to have more protrusions.



Supplemental Figure 1.

Supplemental Figure 2.

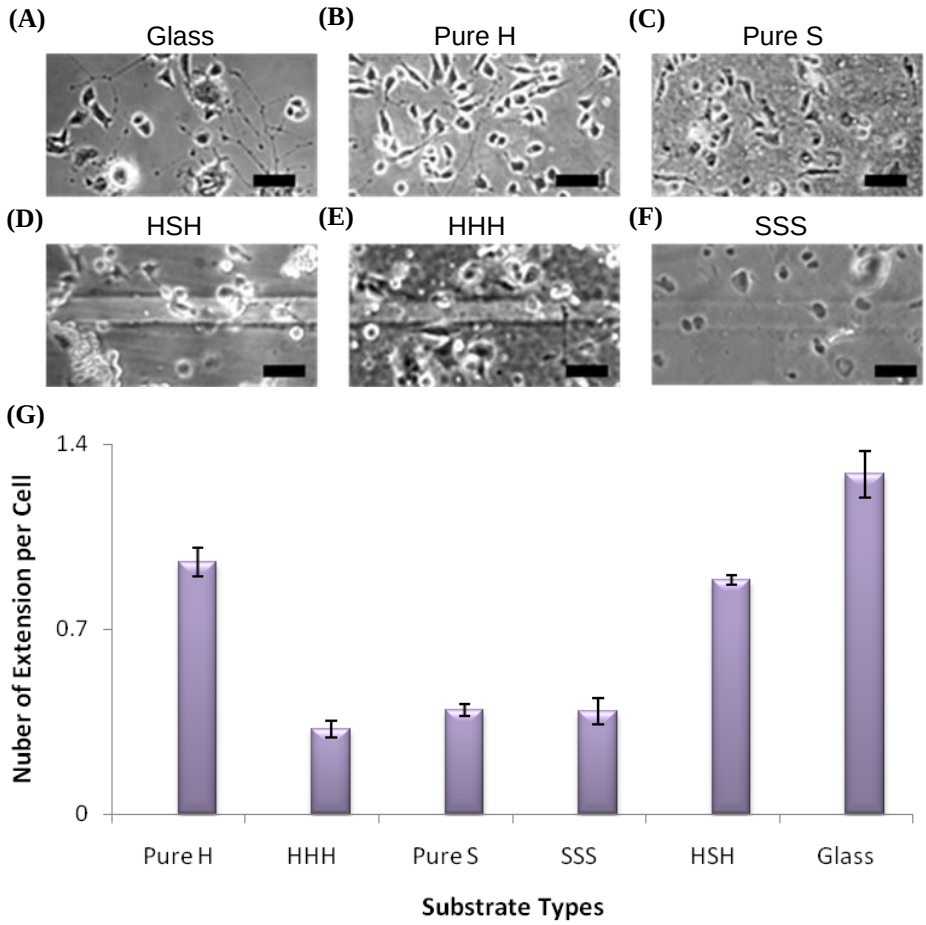
Neurite outgrowth of Neuro-2A cells in response to substrates with homogenous elasticities. Supplemental figures supporting Figure 4 for neurite outgrowth of Neuro-2A cells in response to substrates with homogenous elasticities. Neuro-2A cells after treating with retinoic acid for 8 days in response to (A) glass substrates, (B) PDMS substrates with a base-to-curing agent ratio of 5:1 and (C) PDMS substrates with a base-to-curing agent ratio of 30:1. To quantify the Neuro-2A response to substrates with homogenous elasticities, 8 samples of images were selected for each type of substrate. The longest identifiable neurites from each cell were measured, and the lengths of the 40 longest neurites from the 8 samples of each type of substrate were analyzed, which is shown in Figure 4D. For each substrate, three representative images are shown. Bars = 50 μm.



Supplemental Figure 2.

Supplemental Figure 3.

The comparison of the average neurite extensions per Neuro-2A cells in response to the substrates with either homogenous elasticities or localized different elasticities. Neuro-2A cells after treating with retinoic acid for 8 days in response to (A) glass substrates, (B) PDMS substrates with a base-to-curing agent ratio of 5:1 (Hard; H), (C) PDMS substrates with a base-to-curing agent ratio of 30:1 (Soft; S), (D) composite PDMS substrates with the base-to-curing agent ratio of 30:1 as the middle pattern, and the base-to-curing agent ratio of 5:1 as the surrounding substrate (Hard-Soft-Hard; HSH), (E) composite PDMS substrates with the base-to-curing agent ratio of 5:1 as the middle pattern, and the base-to-curing agent ratio of 5:1 as the surrounding substrate (Hard-Hard-Hard; HHH), (F) composite PDMS substrates with the base-to-curing agent ratio of 30:1 as the middle pattern, and the base-to-curing agent ratio of 30:1 as the surrounding substrate (Soft-Soft-Soft, SSS), Bars = 50 μm. (G) The quantification of neurite extensions to the substrates of (A) to (F). The neurite extensions were calculated from 40 cell samples for each type of substrate, and then divided by the number of cells to get the number of extensions per cell plot. The neurite were considered as the distinct extensions when the length is longer than ½ of their soma. The analysis shows that there is a significant difference of cell response in neurite extensions to the HSH substrates with either the HHH or the SSS substrates. Data are presented as mean ± standard deviation.



Supplemental Figure 3.