Cell Durotaxis on Polyelectrolyte Multilayers with Photogenerated Gradients of Modulus

Jessica S. Martinez [†§], *Ali M. Lehaf* [‡§], *Joseph B. Schlenoff* [‡], *and Thomas C. S. Keller III* *[†]

Department of Biological Science and Department of Chemistry and Biochemistry,

Florida State University, Tallahassee, Florida 32306

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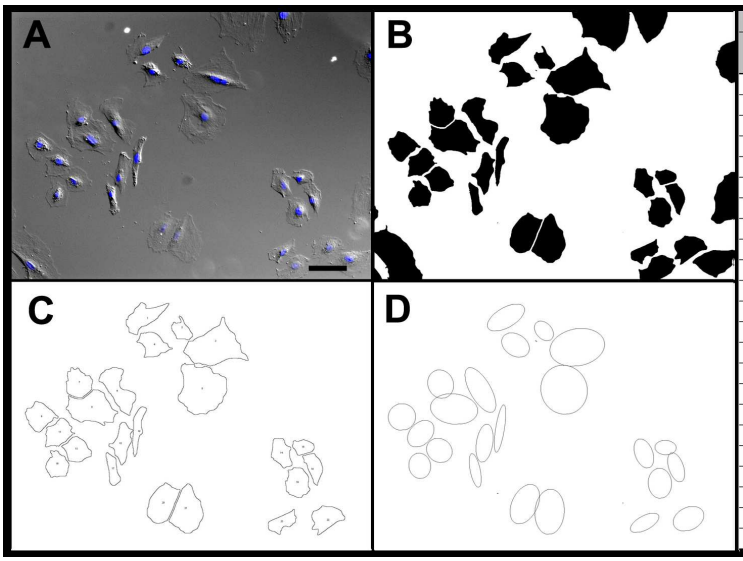
* To whom correspondence should be addressed. E-mail: tkeller@bio.fsu.edu

† Department of Biological Science.

‡ Department of Chemistry and Biochemistry.

§ *These authors contributed equally to this work.*



ImageJ Analysis of Cell Aspect Ratio and Orientation Angle					
A7r5 Cell on Glass	Major	Minor	Aspect Ratio (Major/Minor)	Fit Ellipse Angle	Absolute Angle (0-90)
1	161	77	2.1	31	31
2	87	61	1.4	132	42
3	214	144	1.5	11	11
4	116	84	1.4	147	57
5	192	179	1.1	111	21
6	196	80	2.5	120	30
7	112	98	1.1	112	22
8	184	118	1.6	173	83
9	106	104	1.0	22	22
10	186	34	5.5	81	81
11	120	84	1.4	46	46
12	149	63	2.4	81	81
13	103	90	1.1	146	56
14	118	69	1.7	111	21
15	83	56	1.5	177	87
16	99	84	1.2	100	10
17	133	39	3.4	102	12
18	119	57	2.1	109	19
19	117	90	1.3	94	4
20	164	99	1.7	64	64
21	176	114	1.6	86	86
22	125	88	1.4	26	26
23	122	55	2.2	29	29

Figure S1. Analysis of cell aspect ratio (major:minor axis) and orientation using the ImageJ EllipseFitter plugin. (A) Differential Interference Contrast (DIC) image of cells overlaid with a fluorescence image of the cells with nuclei stained with DAPI. (B) DIC image in A converted to a binary outline. (C) Cells identified by the imaging software, and (D) ellipses fitted to cell outlines in C. (Right panel) ellipse data for each cell providing the Major (longest axis), Minor (shortest axis), and Fit Ellipse Angle. Aspect ratios (major:minor) and absolute angle of orientation (0-90°C) calculated for each cell. (scale bar = 100μm).

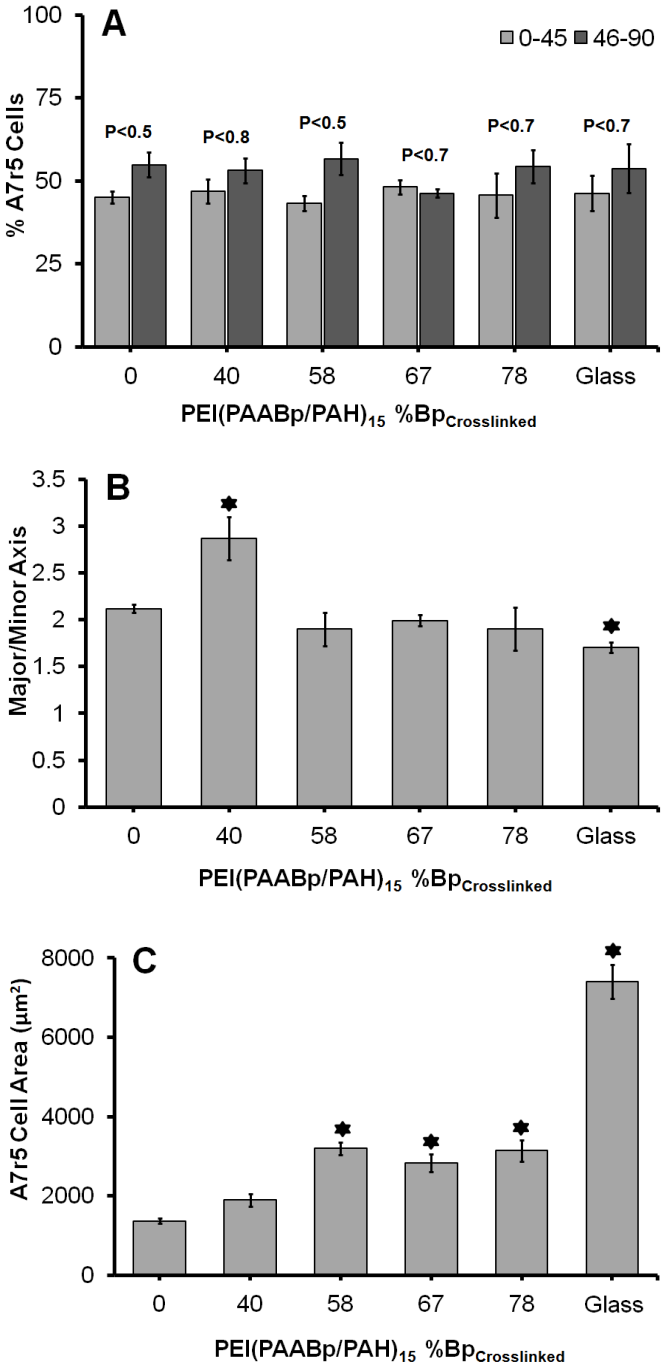


Figure S2. Orientation, elongation, and surface area of A7r5 cells on uniform modulus PEI(PAABp/PAH)$_{15}$ PEMUs photocrosslinked for various UV exposure times. A7r5 cells were cultured for 24 hours on PEI(PAABp/PAH)$_{15}$ PEMUs crosslinked with: no UV exposure (0% crosslinked), 2 min of UV exposure (40% crosslinked), 10 min of UV exposure (58%

crosslinked); 35 min of UV exposure (67% crosslinked), 100 min of UV exposure (78% crosslinked), or directly on a glass coverslip with no PEMU, as in Figure 5. The morphology of each cell (n=40 cells; 20 cells in each of two independent trials) on each indicated surface was fit with a model ellipse using the ImageJ EllipseFitter plugin. (A) Percentage of A7r5 cells with an absolute major axis angle orientation to the gradient axis of between 0-45° or 46-90°, with corresponding Chi Squared P values for the significance of difference from a 50:50 (i.e. random) distribution. (B) Aspect ratio (major:minor axis) of the ellipse fitted for each A7r5 cell. The area of each cell (C) was measured with ImageJ. Asterisks indicate Student's T-test P values of <0.05 for significance of difference compared to the aspect ratio of cells on the native, uncrosslinked PEMU for each condition.

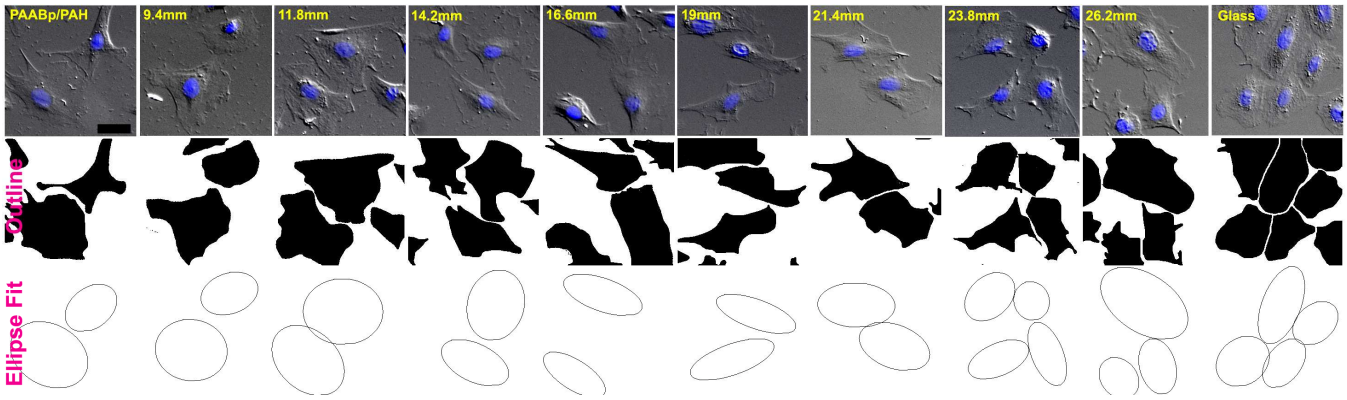


Figure S3. A7r5 cells on shallow UV-photocrosslinked PEI(PAABp/PAH)$_{15}$ PEMU modulus gradient. (Top) Enlargement of A7r5 cell DIC images along indicated regions on a shallow PEMU modulus gradient. (Middle) Binary outline of each cell. (Bottom) Ellipse fitted by ImageJ for each cell. (scale bar = 50 μm).

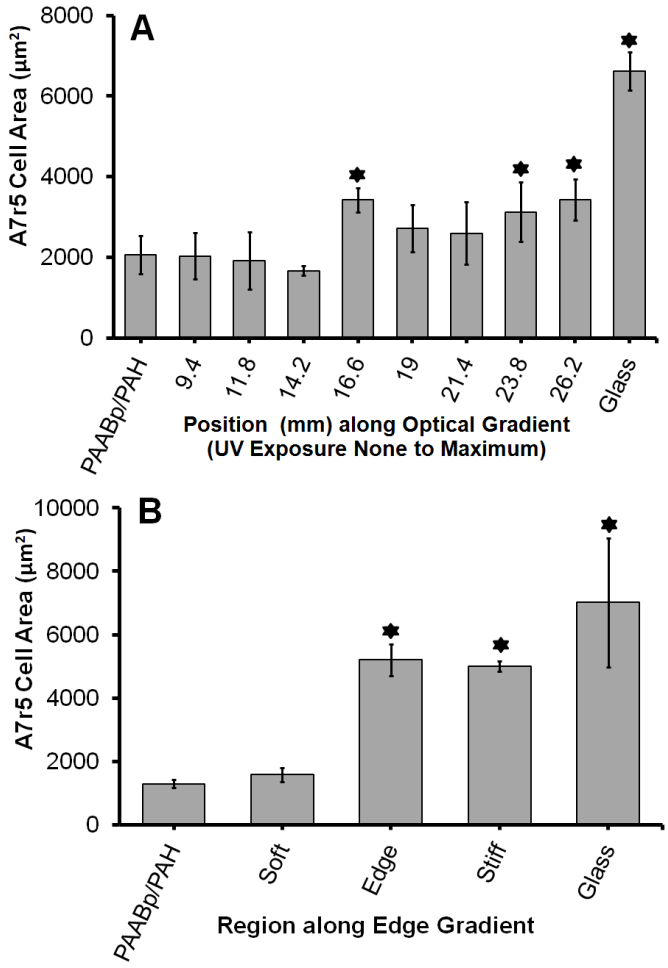


Figure S4. Area of A7r5 cells on shallow and steep photocrosslinked PEI(PAABp/PAH)$_{15}$ PEMU modulus gradients. The area of cells (n=40 cells; 20 cells in each of two independent trials) on uniform native, uncrosslinked PEI(PAABp/PAH)$_{15}$ PEMUs (PAABp/PAH), on uncoated glass coverslips (Glass), and on each indicated region of shallow (A, UV-photocrosslinked through a neutral density gradient optical mask) and steep (B, UV-photocrosslinked using an edge mask) modulus gradients. Asterisks indicate Student's T-test P values of <0.05 for significance of difference compared to the area of cells on the native, uncrosslinked PEMU (PAABp/PAH) for each condition.

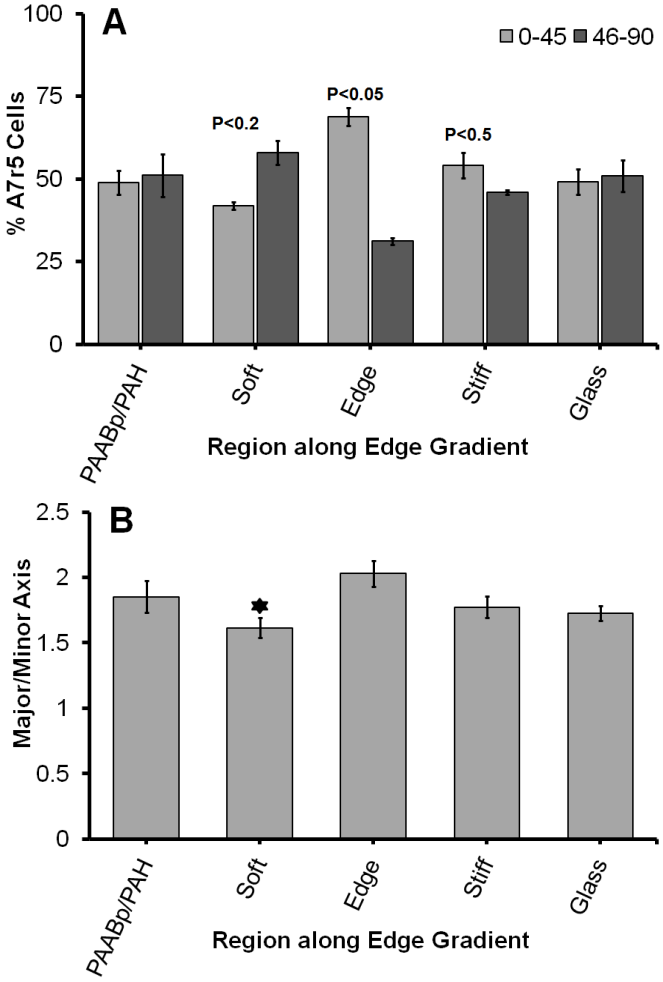


Figure S5. Orientation and elongation of A7r5 cells on a steep UV-photocrosslinked PEI(PAABp/PAH)$_{15}$ PEMU modulus gradient. The morphology of each cell (n=40 cells; 20 cells in each of two independent trials) on a uniform native, uncrosslinked PEMU (PAABp/PAH), on an uncoated glass coverslip (Glass), and on each indicated region of the modulus gradient was fit with a model ellipse using the ImageJ EllipseFitter plugin. (A) Percentage of A7r5 cells with an absolute major axis angle orientation to the gradient axis of between 0-45° or 46-90°, with corresponding Chi Squared P values for the significance of difference from a 50:50 (i.e. random) distribution. (B) Aspect ratio (major:minor axis) of the ellipse fitted for each A7r5 cell. Asterisks indicate Student's T-test P values of <0.05 for significance of difference compared to the aspect ratio of cells on the native, uncrosslinked PEMU with a uniform modulus.

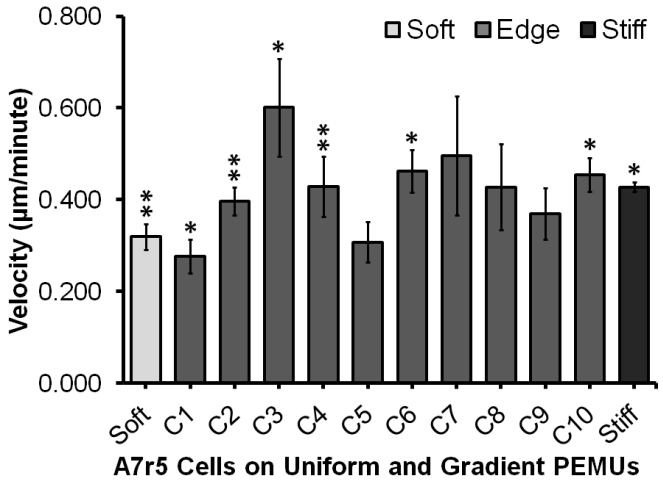


Figure S6. Velocities of the A7r5 cell movements on the steep photocrosslinked modulus gradient and uniform soft and stiff modulus PEI(PAABp/PAH)$_{15}$ PEMU in Figure 8. Velocities (means and SD) were determined by measuring the distance between the locations of each cell at 10-minute intervals over 24 hours. (Soft, Stiff) Velocities of cells (n=10) on the soft and stiff uniform modulus gradients were pooled separately. (C1-C10) Velocities of the ten individual A7r5 cells on the steep modulus gradient in Fig. 8 are the mean and standard deviation for 144 images of each cell. Single asterisks (*) indicate Student's T-test P values of <0.05 for significance of difference compared to the velocity of cells on the native, uncrosslinked PEMU with a uniform modulus. Double asterisks (**) indicate Student's T-test P values of <0.05 for significance of difference compared to the velocity of cells on the crosslinked PEMU with a uniform modulus.