Electronic Supplementary Material (ESI) for Lab on a Chip

Supplemental Information for "High-throughput quantitative imaging of cell spreading dynamics by multi-step microscopy projection photolithography based on a cell-friendly photoresist"

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Cell Culture:

HeLa cells (a gift from Dr. Sung Ho Ryu, POSTECH, Korea) were cultured in DMEM medium (Gibco) supplemented with 10% of FBS (Gibco) and 1% of penicillinestreptomycin (Invitrogen).

Synthesis of PDMP and Substrate Preparation:

Random terpolymer PDMP was synthesized and characterized as described elsewhere.[1] Clean glass coverslips were first coated with gelatin by incubating in gelatin solution (Sigma Aldrich, 0.1% in DI water). PDMP dissolved in 1,4-dioxane (3 w/v %) was spincoated onto the gelatin coated coverslips at 2000 rpm for 120 s, and the spincoated coverslips were dried in vacuo at 100°C for 24 h. The dry thickness of PDMP thin films were ~ 100 nm, and film thickness can be decreased ~ 20 nm by reducing the concentration of PDMP solution down to 0.33 w/v % without altering properties. Film baking is essential for ensuring uniformity and stability of the PDMP films under water, but can be carried out at 80°C for 2 h in case lower temperature with shorter amount of time is desirable. Under this relatively mild baking condition, fibronectin, laminin, type I collagen, and poly-l-lysine can be used instead of gelatin to promote cell adhesion.

Single cell array fabrication by Microscope Projection Photolithography (MPP):

Photomasks were printed on transparent films using a high-resolution image setter with 40,000 dpi resolution (Microtech, Korea) and inserted at the field diaphragm of the microscope. PDMP-coated coverslips were inserted into a Chamlide magnetic chamber (Live Cell Instrument, Korea) filled with PBS, and the magnetic chamber was loaded onto the microscope stage. For UV illumination, a Xenon lamp (75W, Osram) and a DAPI filter (EX. 365, BS 395) were used, and the UV exposure was automatically controlled by a MAC 5000 computer-controlled shutter system (Ludl Electronics Products). PDMP-coated coverslips immersed in PBS were exposed by UV through a transparency photomask containing arrays of circles with a projected diameter 10μm, using a 40x objective lens (Plan-Neofluar, NA=1.30), which reduces the feature size on the projection mask by 16 times, for 2 s. PDMP films in the UV exposed region spontaneously dissolved in PBS and gelatin layers underneath the PDMP films were exposed. Square arrays of 10 μm diameter circles with a center to center distance of 120 μm were generated using a motorized microscope stage automatically controlled by a

program. HeLa cells suspended in cell culture media were applied on the surface, incubated for 1 h at 37°C with 5% CO_2 to allow them to adhere on the exposed gelatin surfaces, and rinsed to remove non-adhering cells, resulting in an array of single HeLa cells. A representative low magnification image of a successfully fabricated single cell array is shown in Fig. S1. Almost identical results were obtained when fibronectin, laminin, type I collagen, and poly-l-lysine were used instead of gelatin. Meanwhile, no cell attachment occurred when bare glasses were used, indicating that a substantial fraction of adhesion molecules coated on the glasses were still functional in terms of supporting cell adhesion.

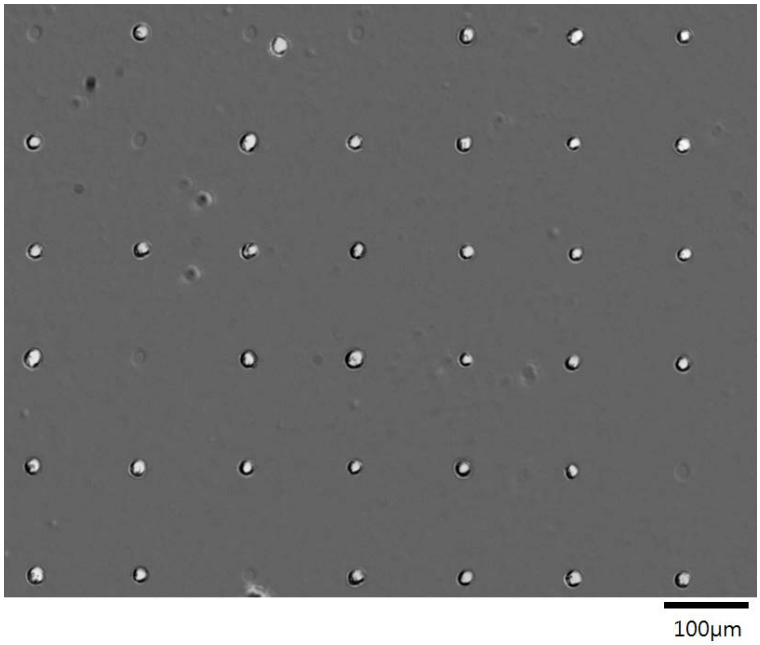

Figure S1. Representative low magnification image of a successfully fabricated single cell array.

Interference Reflection Microscopy(IRM):

IRM was performed using a modified Zeiss Axio Observer.Z1 epi-fluorescence microscope with 100x (Plan-Neofluar, NA = 1.30) objective lens and a CoolSNAP HQ2 (Photometrics) CCD camera. Fluorescence filters were replaced with a linear polarizer, a narrow band-pass filter (EX BP 633/10), a beamsplitter (20/80) and a crossed analyzer. The microscope was automatically controlled by Metamorph 7.7 (Molecular Devices).

Cell Spreading Experiments:

Among cells in the single cell arrays fabricated as described above, 20 neighboring cells were selected, and their positions were memorized in the multi- dimensional acquisition module of Metamorph. Each position was

sequentially exposed to UV using a 100x objective lens without a photomask, which generates a circular beam with a diameter of 100 μm at the focal plane, for 1 sec using a motorized stage automatically controlled by Metamorph. Subsequently, cell spreading dynamics were recorded by acquiring IRM images every 2 min for 90 min of all the positions exposed to UV by automatically translocating the stage for every acquisition. Since IRM is extremely sensitive to focus, focus was maintained by running a software autofocusing algorithm of Metamorph before each IRM image acquisition. For pharmacological inhibition, cells in the single cell array were treated with one of the following inhibitors for 1 h: 0.1 μM Latrunculin A (Sigma Aldrich), 20 μM ML-141 (R&D Systems), 30 μM ML-7 (Sigma Aldrich), 100 μM NSC23766 (Tocris), 50 μM Y-27632 (Sigma Aldrich), 10 μM Nocodazole (Sigma Aldrich). Cell spreading experiments were then initiated while inhibitors were still remaining in the media.

Image processing:

Acquired IRM images were processed with MATLAB R2011b (MathWorks), ImageJ (NIH) and Metamorph (Molecular Devices) by implementing the previously developed algorithm.[2] IRM images acquired using an arc lamp light source suffer from a shading effect due to a non-uniform illumination pattern of arc, as shown in Fig. S2a. To remove the shading effect, shading correction was performed by subtracting the acquired image from a shading reference image. Shading reference images were generated by suppression of information except the shading effect by applying A Gaussian blur filter with a large sigma value (80 pixels, which corresponds to 10.32 μm) to the original image (Fig. S2b). A shading reference image of the first frame of the time-lapse images was used to correct the shading effect of remaining frames. The first frame of the time-lapse shading corrected images (Fig. S2c) was then analyzed to extract the distribution of IRM signal intensities (Fig. S2d) for determination of the threshold value of binarization. Cells adhering on 10 μm diameter circlar patches, which correcponds with about 3% of the area of the entire field of view, were used for spreading experiments, and the intensities of IRM signals of cell-contact sites were typically lower than that of background regions, and thus an intensity of pixels at low 3% was selected as the threshold intensity for the binarization. Using the threshold intensity value determined in Fig. S2d, time-lapse shading corrected images were binarized in such a way that pixels with intensities higher than the threshold value were set to 0 or black and pixels with intentisies lower than the threshold value were set to 1 or white. As shown in Fig. S2e, the majority of contact area was converted to white. Noise and holes in the contact area were then removed by open and fill holes functions in MATLAB, resuting in the final image shown in Fig. S2f.

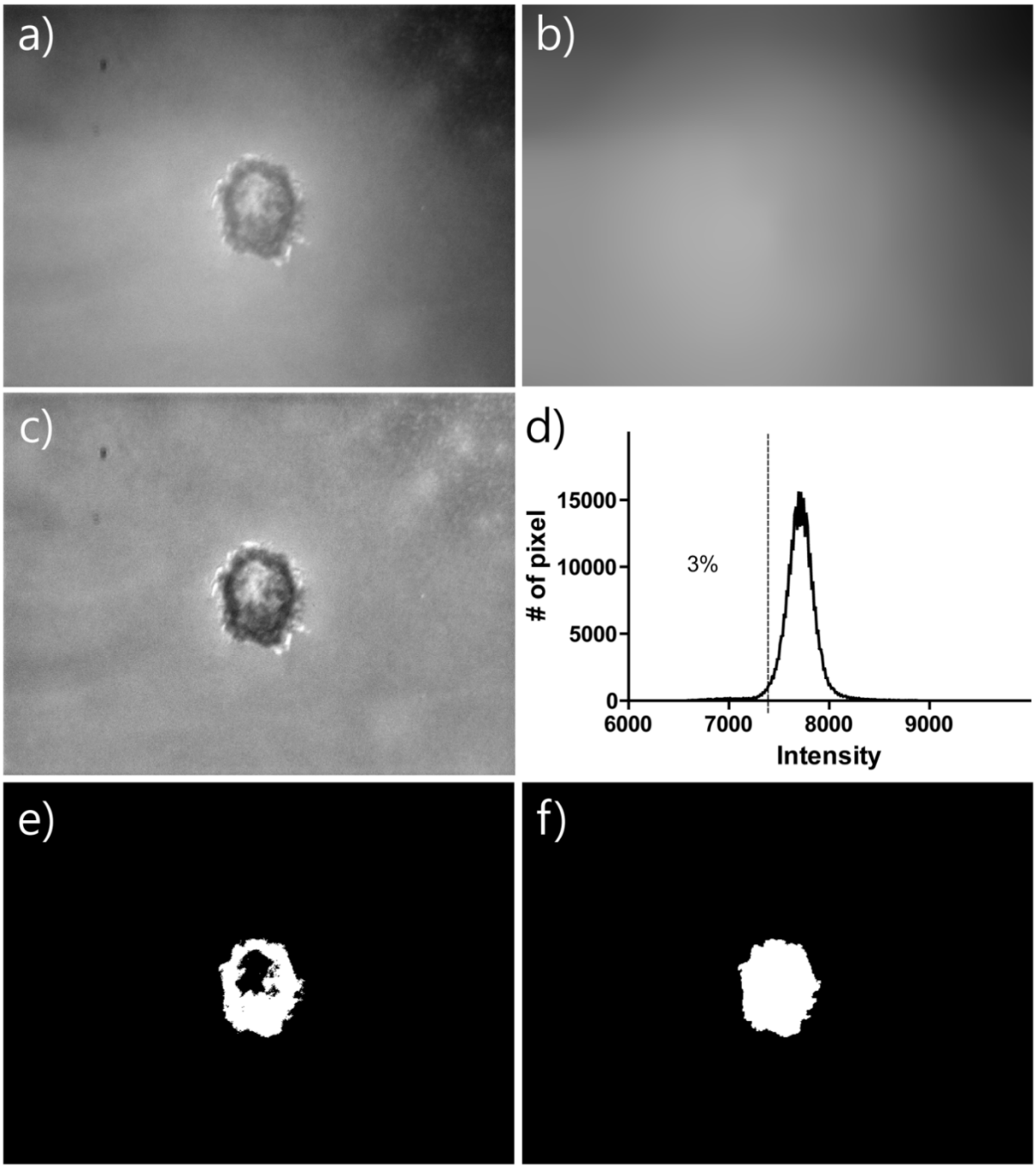

Figure S2. Representative images of each step of image processing of IRM data. a) Original IRM image. b) Shading reference image generated by applying a Gaussian blur filter to the original image. c) Shading corrected image obtained by subtracting the shading reference image from the original IRM image. d) Intensity distribution of shading corrected image and determination of a threshold intensity for binarization. e) Binarized image applying the threshold to the shade corrected image. f) The final image obtained by applying an open and fill holes algorithm to the binarized image.

Determination of parameters for cell spreading dynamics from A vs. t curves:

Average slope <S> and instantaneous slope S_t at a specific time t were defined as <S> = (A(T) – A(0))/T and S_t=A(t+Δt)-A(t)/Δt, where T is the total experimental time (90 min in our experiments) and Δt is the time interval for each data acquisition (2 min in our experiments). Since S_t values at stages I and III are close to 0, S_t values of stages I and III will be much smaller than the <S> value while S_t values of stage II will be much greater than the <S> value. Therefore, by comparing S_t values with the <S> value, τ_0 and τ_p were determined. For the determination of τ_0, S_t values were compared with <S> from t = 0 by increasing t, and when five consecutive S_t values were greater than the <S> value, or $S_{t+i\Delta t}$ > <S> for i = 0, 1, 2, 3, and 4, we set that time point to τ_0. Similarly, when five consecutive S_t values for t > τ_0 were smaller than <S>, we set that time point to τ_p. The rate of spreading r_s was determined by calculating the slope at τ_0 < t < τ_p by least-square fitting, and the adhesion area at plateau A_p was calculated by time-averaging A at t > τ_p.

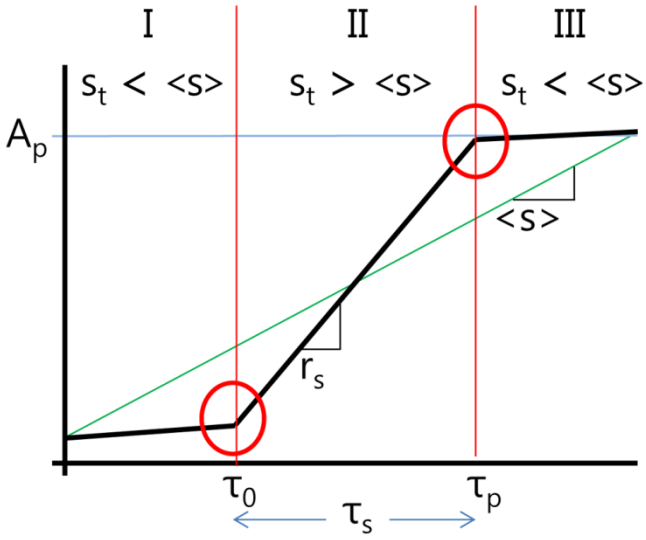

Figure S3. Determination of parameters and stages of cell spreading dynamics.

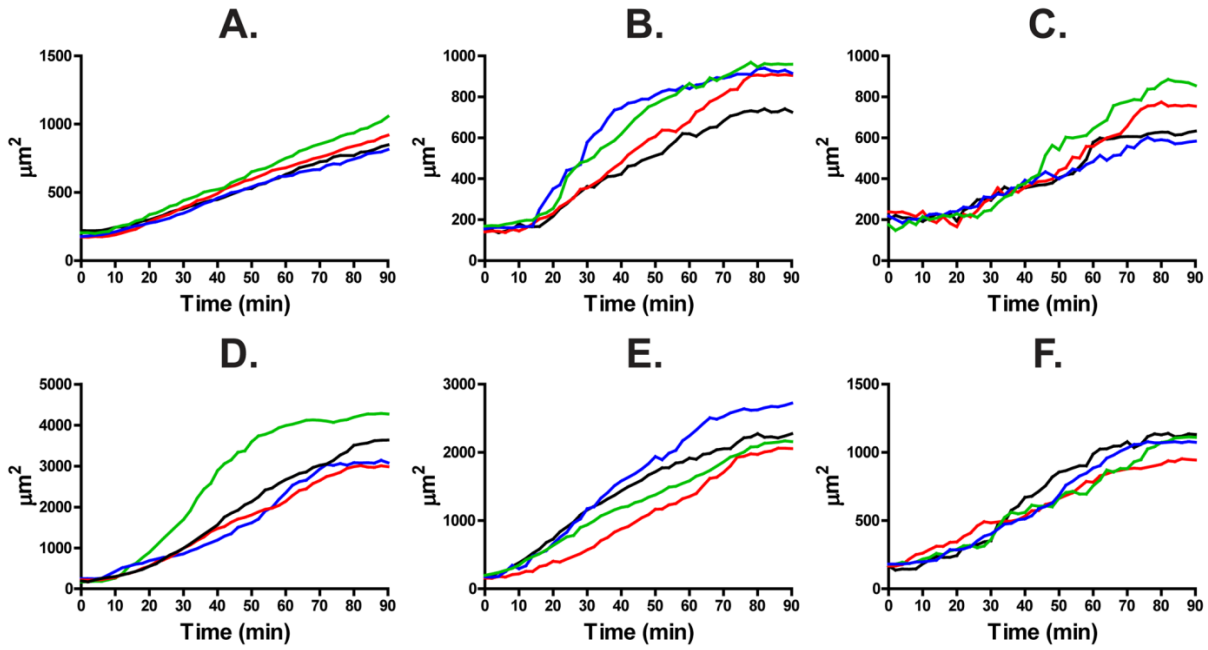

Figure S4. Representative A vs. t curves of cells treated with (A) Latrunculin A, (B) NSC 23766, (C) ML-141, (D) ML-7, (E) Y-27632, and (F) Nocodazole.

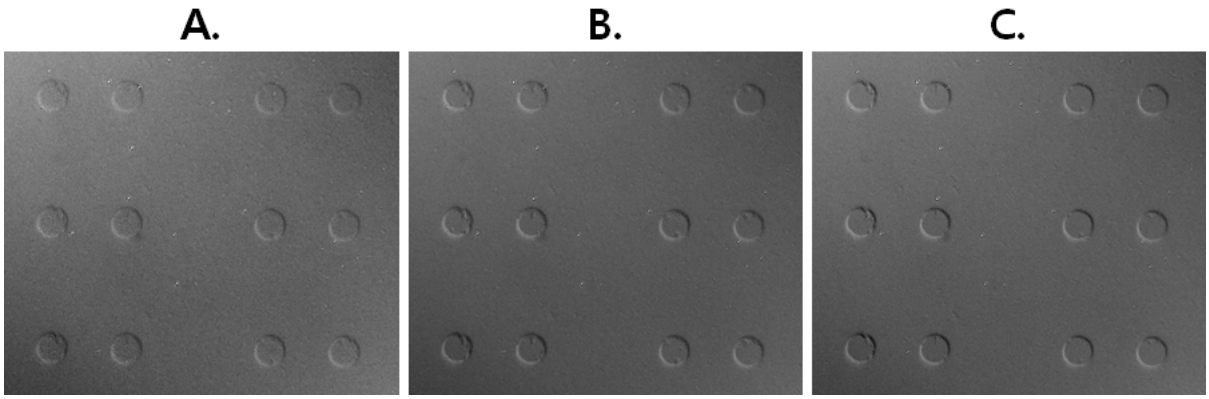

Figure S5. DIC images of PDMP pattern after (A) MPP process, (B) blue (λ = 470 nm) light exposure for 1 min and (C) green (λ= 550 nm) light exposure for 1 min. Patterns are remained unchanged by blue and green light exposure.

Effect of adhesion molecules on cell spreading dynamics:

Glass coverslips coated with various cell adhesion molecules such as gelatin, type I collagen, laminin, poly-l-lysine, and fibronectin were spincoated with PDMP, and cell spreading experiments were performed as described above. Four representative data of A vs. t curves (Fig. S6A-E) and τ_s, r_s, and A_p values of cells spreading on various adhesion molecules are plotted in Fig. S6A-E and Fig. S6F, respectively.

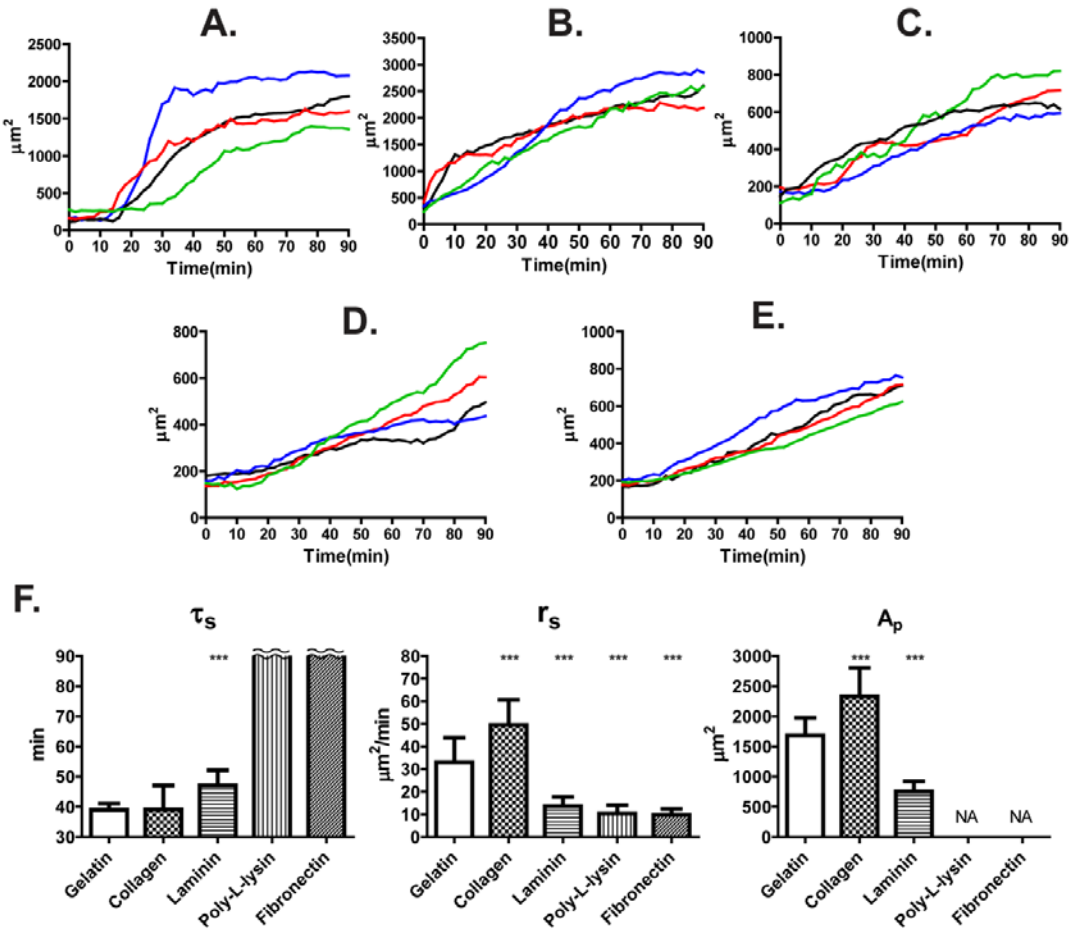

Figure S6. Representative A vs. t curves of cells on (A) gelatin, (B) collagen, (C) laminin, (D) poly-l-lysin, (E) fibronectin coated surfaces and (F) key parameters of spreading dynamics of those cells.

References:

1. M. Kim, J. C. Choi, H. R. Jung, J. S. Katz, M. G. Kim and J. Doh, *Langmuir*, 2010, **26**, 12112-12118.
2. A. Pierres, P. Eymeric, E. Baloche, D. Touchard, A.-M. Benoliel and P. Bongrand, *Biophysical journal*, 2003, **84**, 2058-2070.