Electronic Supplemental Information:

Fluorescence-Based Assessment of Plasma-Induced Hydrophilicity in Microfluidic Devices via Nile Red Adsorption and Depletion

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Materials and Methods

Cell Culture

Two different cell types were used in this study: (1) human umbilical vein endothelial cells (HUVECs) were used in both the main study and the supplemental materials, and were cultured in EBM-2 media (#CC-3156 and #CC-4176, Lonza, Basel, Switzerland) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S); (2) NIH-3T3 mouse fibroblasts were tested for results in the supplemental materials only, and were cultured in MEM-α (#12561-056, Life Technologies, Carlsbad, CA) with 10% FBS and 1% P/S. Cells were seeded at a surface density of ~800 cells/mm^2, and cultured for 24 h before being fixed with 4% paraformeldahyde (PFA) (#43368, Alfa Aesar, Ward Hill, MA). Cells were subsequently permeabilized with 0.1% Triton X-100, and stained with Hoechst nuclear dye (#33342, Invitrogen, Carlsbad, CA) and Alexa Fluor 488 phalloidin (#A12379, Invitrogen) for 15 min each. Stained cells were analyzed for morphology and adherent fraction.

To obtain a baseline for cell morphology and adhesion in microchannels, native (non-NR-adsorbed) PS substrates were passively bonded to cured PDMS layers with microchannels. Prior to bonding, PS substrates were exposed to three different levels of oxygen plasma treatment: (i) highly hydrophilic (<15˚), (ii) moderately hydrophilic (30-60˚), and (iii) hydrophobic (~85˚, untreated PS). Cured PDMS layers containing microchannels were then passively bonded to the PS substrates to create enclosed channels for cell culture. As a positive control for HUVECs, a fourth surface treatment involved coating the PS with fibronectin (FN) at 0.1 mg/mL to promote cell adhesion via attachment to adsorbed matrix proteins that are typically found in endothelial basement membranes[1].

To characterize the effect of plasma treatment patterns on cell adhesion and growth in microchannels, native (non-NR-adsorbed) PS substrates were passively bonded to cured PDMS layers with microchannels to create enclosed conduits. Plasma treatment was applied to each sample at 2 mbar, 50% power, and for 50 seconds. The PDMS was then removed, and cells were seeded over the entire exposed PS surface.

Cell Imaging and Analysis

Images of cell stains of phalloidin (green) and nuclear Hoechst dye (blue) were acquired with a 10x objective, and overlaid using ImageJ. All cell analysis and quantification was performed using ImageJ, where images were converted to binary images using the threshold function. Binary images were used to quantify overall area coverage and number of cells in each microchannel condition. To quantify penetration distance with cell, fluorescence intensity along channel length was averaged across channel width, and the resulting intensity profile was analyzed to determine the location where the intensity reached half of its maximum.

Supplemental Results and Discussion

Cell Adhesion: The Need for in situ Measurements

The need to develop a fast, convenient, and informative method for determining surface hydrophilicity within microchannels was motivated in part by observations of inadequate cell adhesion and spreading in microchannels in cases when the plasma treatment was not optimized. Further, little is known about how plasma treatment occurs in confined environments though plasma in microchannels has been used to perform analog computing[2]. To illustrate how surface conditions, after oxygen plasma treatment, can affect cell adhesion, we cultured two established

adherent cell types in microchannels with PS substrates, human umbilical vein endothelial cells (HUVECs) (Figure S4A), and NIH-3T3 fibroblasts (FBs) (Figure S4B), quantified the number of adhered cells and the cell spreading area (Figure S4C), and normalized this data against their respective control conditions (Figure S4D), as a metric of compatibility with the surface. For HUVECs, the control surface was PS with FN coating at 0.1 mg/mL, and for FBs, the control surface was untreated PS.

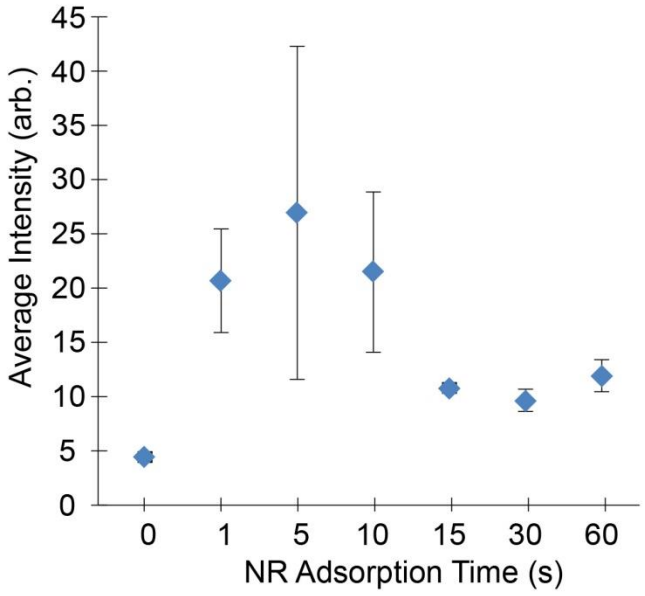

Figure S1. Fluorescence intensity is a function of adsorption time, and longer adsorption times yield better coating homogeneity (lower variability in intensity between images) (n = 3).

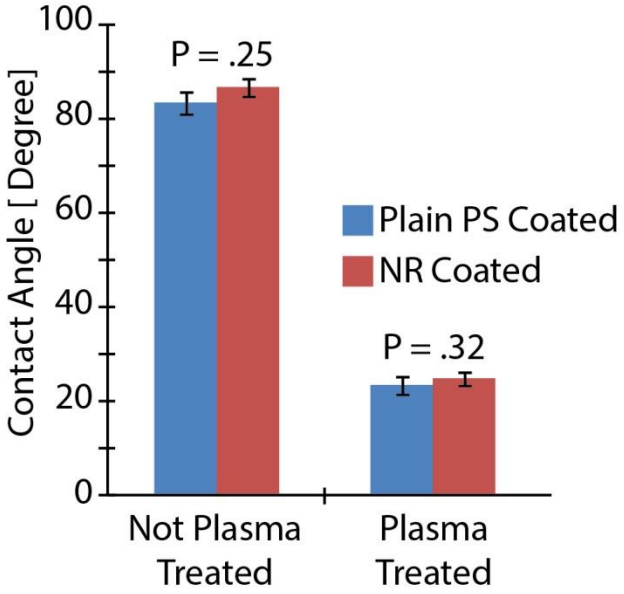

Figure S2. The NR applied to the PS surface does not significantly affect the contact angle for non-plasma treated PS (p = .25) or plasma treated PS (p = .32) (Student T-test, n = 3), as measured using a goniometer.

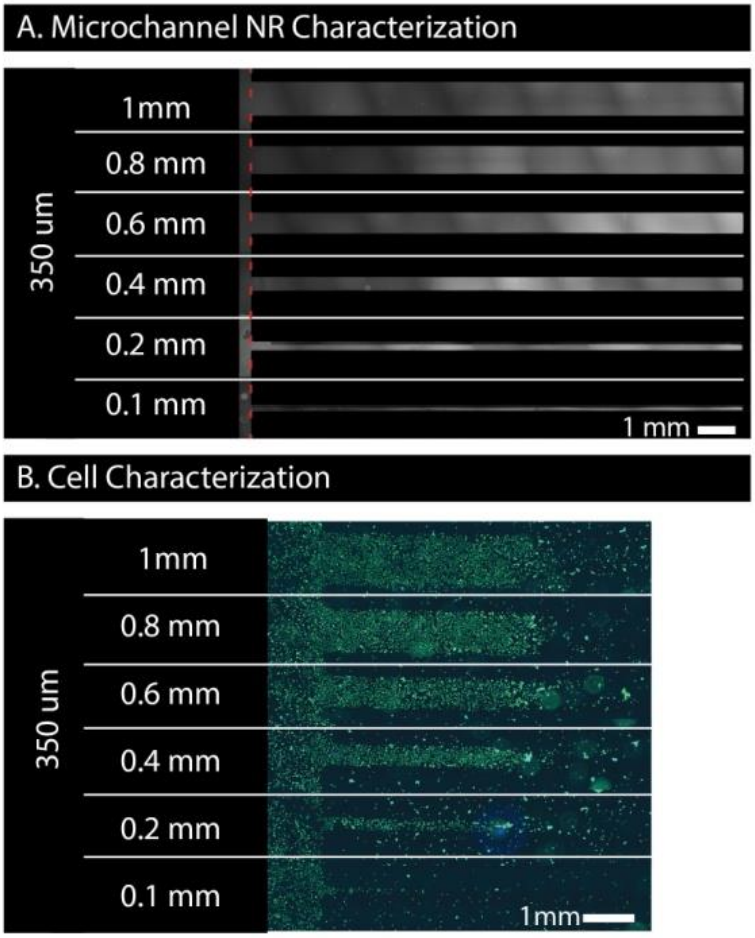

Fig S3. (A) Nile Red characterization of 350 μm tall microchannels. There was significant variability in the fluorescent output after plasma treatment. The periodic fluctuations of fluorescence intensity are an artifact of the image stiching. This phenomena could be improved by using higher end microscopy setups that have more even illumination or automated stages to take pictures with more overlap, however, it should not change the overall trend observed in the data. (B) HUVECs cultured on a PS substrate after plasma treatment with 350 μm tall microchannels. Channels wider than 0.4 mm had a δ of ~4 mm, while the 0.1 and 0.2 mm wide channels had a δ of 0.2 and 1.2 mm, respectively.

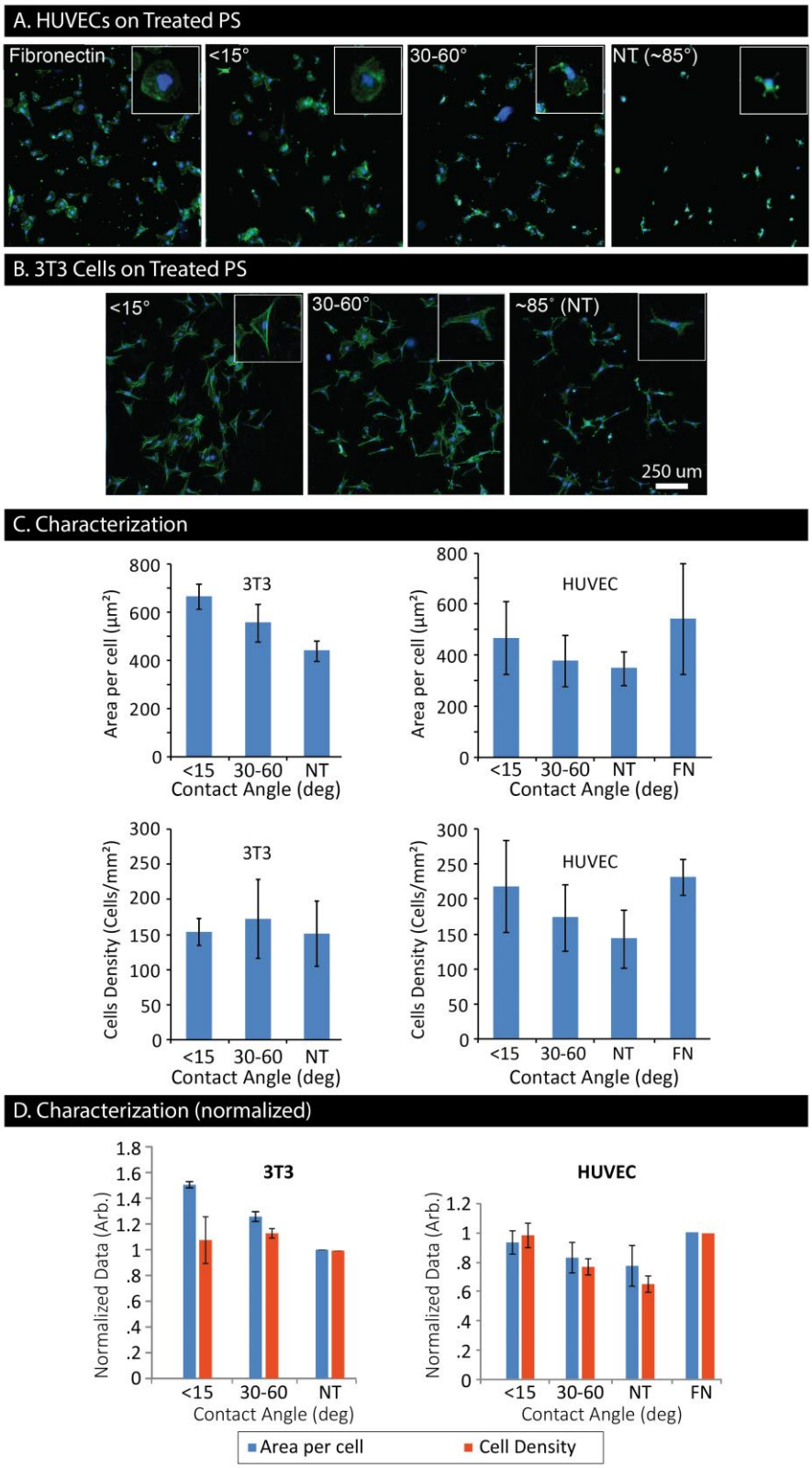

Figure S4 (see caption below).

Figure S4. Culturing (A) HUVECs and (B) FBs on non-NR-coated PS samples demonstrate the effect of hydrophilicity on morphology and cell adhesion. (C) Average area per cell, and number of cells per unit area (cell density) were quantified for both HUVECs and FBs. (D) Normalized data for average area per cell, and number of cells per unit area. FBs were normalized to the untreated PS surface, and HUVECs were normalized to the FN treated surface (n = 9). Both cell types follow the trend of decreasing average area per cell as the level of plasma treatment decreases.

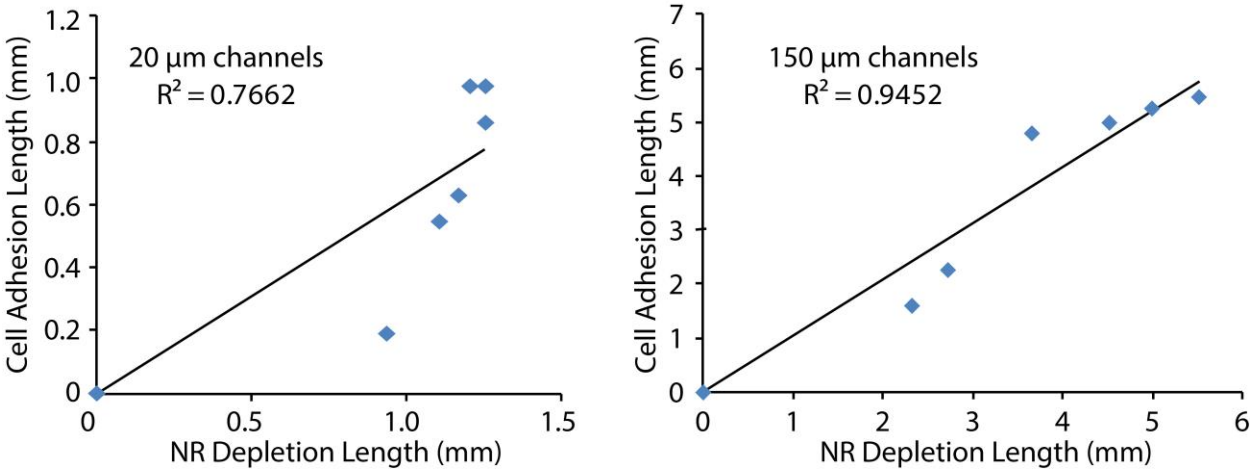

Fig S5. Comparison between penetration distances based on the half maximums for NR-depletion and cell culture in 20 µm and 150 µm tall plasma treated microchannels.

Fig S6. Comparison between plasma treated channels with one open (top) verse two open ends (bottom). Penetration distance of the channel with one open end is 3.67 mm and penetration distance into the channel with two open ends is 3.77 mm (penetration into the channel from the other end was not characterized).

References

(1) Young, E. W. K.; Wheeler, A.; Simmons, C. Lab Chip, 2007, 7, 1759-1766.

(2) Reyes, D. R.; Ghanem, M. M.; Whitesides, G.M.; Manz, A. Lab Chip, 2002, 2, 113-116.