Electronic Supplementary Information

Biofunctionalized Viral Delivery Patch for Spatially Defined Transfection

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Experimental section

Preparation of lentiviral vectors

HEK-293T cells were cultured on cell culture plate in DMEM (HyClone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS) (Biowest, Nuaille, France), 1% L-glutamine (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA). When cells were confluent enough to cover 90-95% plate surface, cells were trypsinized, washed by 1x phosphate buffer solution (PBS) (Gibco, Grand Island, NY, USA) and counted. After that, cells were resuspended in 100μL resuspension buffer per 1 million cells, containing lentiviral expression plasmid (PLV-eGFP, 30μg) (Addgene Plasmid 36083), packing plasmid (psPAX2, 5μg) (Addgene Plasmid 12260), enveloping plasmid (pMD2.G, 15μg) (Addgene Plasmid 12259) per 5 million cells. The cell/DNA mixture was carefully pipetted without introducing bubbles and electroporated under 1100V, 2ms, 2 pulses conditions. The transfected cell/DNA mix was immediately transferred into fresh medium in cell culture plate, and gently mixed it to stabilize the cells. Lentivirus supernatant medium was collected every 24hr for 72hr after electroporation and filtered through 0.45μm cellulose acetate filter to remove cell debris. Collected virus in medium was concentrated by using 5x PEG-it ™ Virus Precipitation Solution (System Biosciences, Mountain View, CA, USA) and after overnight or 1 day at 4 °C,

virus medium was centrifuged under 1500xg, 30 min, at 4 °C. Lentivirus pellet was resuspended by 100 fold PBS to original volume of collected virus medium and stored at -80℃.

Fabrication of chemically modified substrates

Glycidyl methacrylate (GMA) (97%) was used as monomer and *tert*-butyl peroxide (TBPO) (98%) was used as initiator without another purification process. Both materials were purchased from Sigma-Aldrich (St. Louis, MO, USA). The GMA (pGMA) was polymerized on the substrate in an iCVD vacuum reactor chamber (Daeki Hi-Tech Co., Daejeon, Korea). For the vaporization of GMA and TBPO, GMA was heated at 35℃ and TBPO was maintained to room temperature. GMA and TBPO were fed into the iCVD reactor at the flow rate of 1.9 sccm and 0.9 sccm respectively. The pressure of the iCVD reactor was maintained at 200mTorr during the polymerization reaction of GMA. The substrate temperature was maintained at 25℃ for adsorption of monomers and the filament was heated to 180℃ for the formation of free radicals from TBPO initiator. The deposition thickness was observed using He-Ne laser (JDS Uniphase, Milpitas, CA, USA).

Immobilization of viral vector on functionalized substrate

To immobilize viral vector on substrate, 100 fold concentrated viral vectors (100 μl) in PBS/0.01% Tween 20 were applied onto functionalized substrate for 6 h at 4 °C. After coating process, substrate was washed vigorously with PBS/0.01% Tween 20 for 3 times.

Fabrication of fluorescent labelled viral vectors

The viral vectors were visualized via tethering fluorescein isothiocyanate (FITC). 100 fold

concentrated lentivirus (100 μl) in PBS was mixed with 100 μl of 1mg/ml FITC solution overnight at 4 °C. To concentrate FITC coated lentivirus, PEG-it ™ Virus Precipitation Solution was diluted with mixture at 1:5 ratio, and stored overnight at 4 °C. The mixture was centrifuged at 1500 x g for 30 minutes at 4 °C. After removing supernatant, PBS/0.01% Tween 20 was added to mixture. An unreacted FITC was then removed by dialysis using Spectra/Por® Dialysis membrane (MWCO 12,000 to 14,000) at 4 °C for 2 days. The final solution was stored at -80 °C until further use.

SEM imaging

To confirm micro / nano scopic images of substrate, each substrate (bare glass, pGMA, pGMA-PEI coated glass) was dehydrated in vacuum chamber overnight. To fix viral vectors on substrate, the pGMA-PEI-LV coated glass was treated with 4% (w/v) paraformaldehyde (PFA) (Sigma-Aldrich, St. Louis, MO, USA) overnight at 4 °C, and dehydrated in vacuum chamber overnight. The samples were then mounted on the conductive carbon tape coated SEM Specimen Mount, and then observed using by FE-SEM (JSM-6700F, JEOL, Tokyo, Japan).

X-Ray Photoelectron Spectroscopy (XPS) analysis

The amount of coated PEI on substrate was measured by the chemical composition using XPS (K-alpha, Thermo VG Scientific). The sample was irradiated by a monochromator X-ray source (200W, 12kV, kinetic energy (KE) = 1,486.6 eV) under an ultra-high vacuum (10^{-10} Torr), and spectra was measured ranging from 0 to 1,100 eV. The atomic composition of sample was calculated using each area of peaks for C1s, O1s, and N1s in the survey scans multiplied by the atomic sensitivity factors.

Atomic-Force Microscopy imaging

The surface morphologies of the bare wafer, the pGMA deposited wafer and the lentivirus

immobilized wafer were observed using atomic force microscopy (AFM) (PSIA XE-100).

Cell area analysis on substrate

To test cellular activity on each substrate, HeLa cells (density : 4 x 10^4 cells / ml) were seeded on bare, pGMA, pGMA-PEI coated glass (radius : 5mm). After 4 hr and 24 hr, samples were fixed with 4 % (w/v) PFA overnight at 4 °C, and then incubated with Alexa Fluor 594 Phalloidin (1:200 dilution; Thermo Fisher Scientific) overnight at 4°C, and counterstained with DAPI (1:250 dilution; Invitrogen). Cell area was calculated with Image J.

Cell viability test

To test cell viability on each substrate, HeLa cells (density : 4 x 10^4 cells / ml) were seeded on bare, pGMA, pGMA-PEI coated glass (radius : 5mm). After 24 hr of seeding, each sample was stained with Live/Dead assay reagents (Invitrogen) to quantify cell viability according to the manufacturer's instructions.

Cell seeding efficiency test

To test cell seeding efficiency on each substrate, HeLa cells (density : 4 x 10^4 cells / ml) were seeded on bare, pGMA, pGMA-PEI coated glass (radius : 5mm). After 4 hr of seeding, each sample was trypsinized to detach cells, and collected to detect DNA content using a Quanti-iT PicoGreen dsDNA Assay Kit (Invitrogen) following the manufacturer's instructions.

Cell proliferation test

To test cell proliferation on each substrate, HeLa cells (density : 4 x 10^4 cells / ml) were seeded on bare, pGMA, pGMA-PEI coated glass (radius : 5mm). Each sample was incubated with Alamar blue solution diluted with DMEM (Gibco, USA) containing 10 % (v/v) fetal bovine

serum (FBS), 1% (v/v) L-glutamine, and 1% (v/v) penicillin and streptomycin at 5:1 ratio for 3 hr. The alamar blue solution was then collected and measured the absorbance at 570nm, using 600nm as a reference wavelength. The cell proliferation test was performed at day 1, 3, 5, 7.

Transduction of immobilized viral vectors into cells

Each sample (bare glass, pGMA, pGMA-PEI coated glass) was coated with 100-fold LV-eGFP in PBS/Tween 20 for 6 h at 4 °C, and rinsed with PBS three times. HeLa cells were then plated onto each substrate at a density of 2 x 10^4 cells / ml. After cultured for 2 days, cells were treated with trypsin-EDTA (Gibco), and analyzed transduction efficiency using Flow cytometry.

	% C	% O	% N	O/C Ratio
TCP-pGMA-PEI	74.77 %	15.32 %	9.9 %	0.20
TCP-pGMA	74.56 %	25.43 %	-	0.34
TCP-PEI	85.57 %	8.77 %	5.65 %	0.10
TCP	89.68 %	10.33 %	-	0.11

Table S1. Summary of quantitative analysis of the XPS survey scan results for substrates: TCP, TCP-PEI, TCP-pGMA, and TCP-pGMA-PEI.

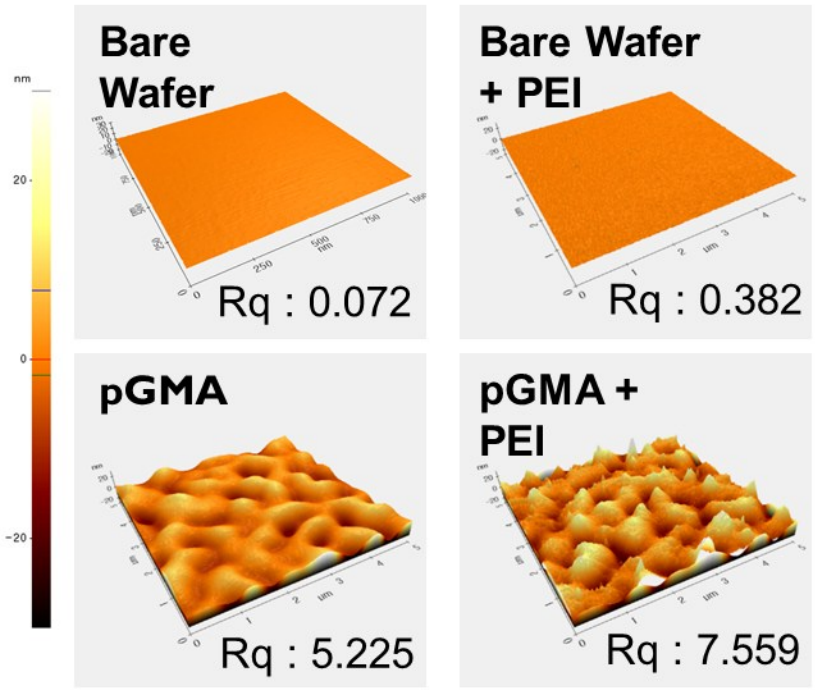

Figure S1. AFM images of substrate : Bare wafer, PEI, pGMA, and pGMA-PEI treated substrate.

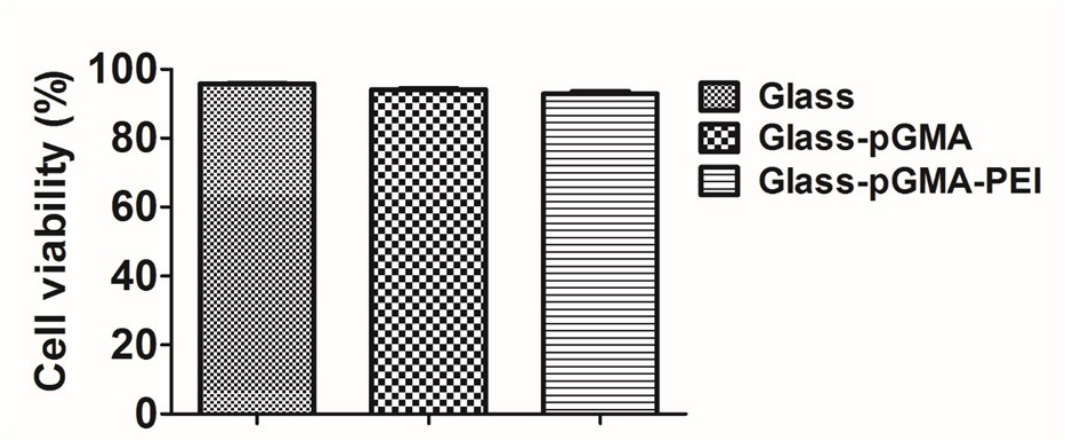

Figure S2. Cell viability test according to substrates.

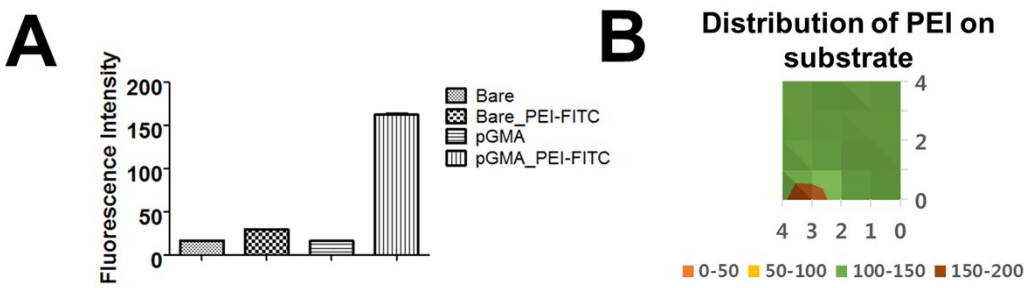

Figure S3. (A) Quantification of PEI coating uniformity using FITC labelled PEI, and (B) distribution of PEI on substrate.

Figure S4. SEM images of each substrate: bare wafer, pGMA, and pGMA-PEI.

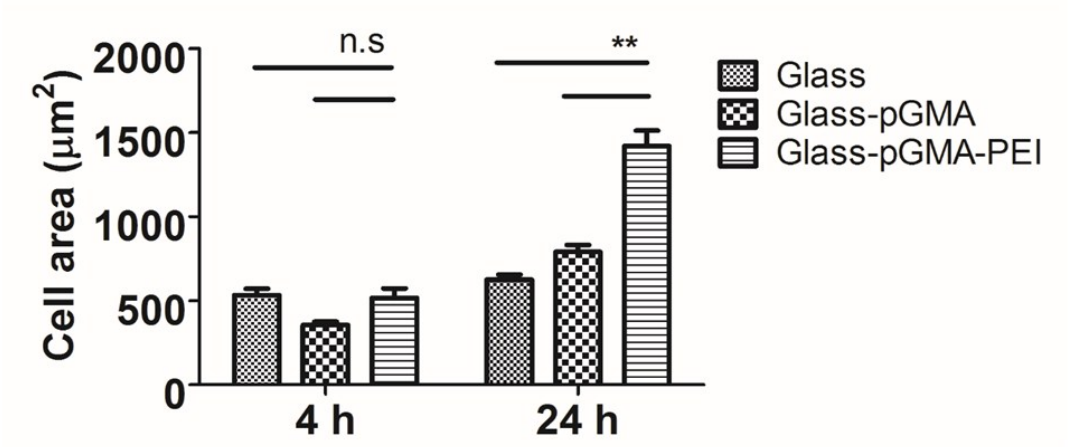

Figure S5. Cell attachment areas on each substrate