Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Biodegradable Microneedle Patch for Transdermal Gene Delivery

Moyuan Qu, Han-Jun Kim, Xingwu Zhou, Canran Wang, Xing Jiang, Jixiang Zhu, Yumeng Xue, Peyton Tebon, Shima A. Sarabi, Samad Ahadian, Mehmet R. Dokmeci, Songsong Zhu, Zhen Gu, Wujin Sun,* and Ali Khademhosseini*

*Correspondence: khademh@terasaki.org, wsun@terasaki.org

Materials and Methods

Materials

5-amino-1-pentanol, 1,4-butanediol diacrylate, dimethyl sulfoxide (DMSO), DMSO-d_6, agarose, gelatin from porcine skin, methacrylic anhydride, photoinitiator (Irgacure 2959), heparin sodium, and reagents for HE staining were purchased from Sigma-Aldrich (MO, USA). TAE buffer, loading dye, and DNA ladder were purchased from Bio-rad Laboratories (CA, USA). Cell culture media and reagents were purchased from Gibco Laboratories (NY, USA). NIH 3T3 cells were purchased from ATCC (VA, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (Japan). Lipofectamine 3000, HEPES buffer, Picogreen dsDNA Assay Kit, collagenase (type II), DAPI, Live/Dead Kit and AlexaFluor 488 conjugated GFP antibody were purchased from ThermoFisher Scientific (NJ, USA). Antigen retrieval solution was purchased from Invitrogen (CA, USA).

PBAE Synthesis

Poly(5-amino-1-pentanol-co-1,4-butanediol diacrylate) was synthesized according to protocols outlined in previous studies.[1, 2] Briefly, 5-amino-1-pentanol and 1,4-butanediol diacrylate (1.2:1 amine/diacrylate stoichiometric ratio) were weighed in a sample vial with a Teflon-lined screw cap and stirred at 1000 rpm at 90°C for 24 hours, solvent-free. Afterwards, the polymers purified by precipitation with anhydrous diethyl ether. The suspension was vortexed for 20 s and centrifuged for 5 minutes at 1000 rpm at 4°C. The supernatant was removed following centrifugation, and the precipitate was washed again with ether. The polymer was maintained under vacuum with desiccant for 5 days to remove all remaining ether. The purified polymer was then dissolved in anhydrous DMSO to a concentration of 100 mg/mL and stored with desiccant at −20°C.

^{1}H-NMR

^{1}H-NMR spectra of the polymer were obtained using dimethyl suloxide-d_6 as the solvent. All measurements were performed from Brucker AV400 broad band FT NMR spectrometer with 64 scans at room temperature.

DLS and TEM

The size and zeta potential of the PBAE/DNA NPs were measured by DLS. Briefly, 2000ng pEGFP was dissolved in 50 μL of 25 mM sodium acetate buffer (pH 5.0). Then, PBAE was dissolved in 50 μL of 25 mM sodium acetate buffer (pH 5.0) at different concentrations and added into the pEGFP solution to yield a mixture with different weight ratios of PBAE/DNA (1/1, 5/1, 10/1, 20/1, 40/1, 60/1, 80/1, 100/1). Complexes were formed after vortexing all mixtures for 15 s and subsequent incubation for 20 minutes. After that, 900 μL of 20 mM HEPES buffer was added into the PBAE/DNA mixtures. The size and zeta potential of the resultant PBAE/DNA NPs were determined by DLS and the zeta potential analyzer (Zetasizer Nano-ZS, Malvern Instruments, Ltd., United Kingdom). The morphologies of the PBAE/DAN complexes were observed using a transmission electron microscope (TEM, Tecnai G2 20S-Twin, USA).

Gel Electrophoresis

Gel electrophoresis was preformed to confirm the complexation between PBAE and DNA. PBAE/DNA complexes with 100 ng pEGFP and variable doses of PBAE with a predetermined ratio of PBAE/DNA were added into premade 8% agarose gel and run at 100V for 40 min. Before loading, loading dye was added to each sample according to the manufacturer's instructions. Following electrophoresis, the gel was visualized under UV using an imaging system (Bio-rad).

Gene transfection with PBAE/pDNA NPs *in vitro*

Murine embryonic fibroblasts (NIH 3T3) were used to test pEGFP transfection efficacy with PBAE/DNA NPs. Cells were seeded in 96 well plates at a density of 5000 cells per well. After incubation for 24 h, the medium was changed to 100 μL Opti-mem with PBAE/DNA NPs with 100ng pEGFP and variables amounts of PBAE (PBAE/DNA: 0/1, 40/1, 60/1, 80/1 and 100/1). After a 4h incubation, the medium was changed to 100 μL complete medium. Following an additional 24h incubation, cells were stained with DAPI and EthD-1 and counted using fluorescence microscope and flow cytometry to assay viability. The commercialized non-viral gene vector, Lipofectamine 3000, was used according to the manufacturer's protocol to generate the control group.

Cytotoxicity assay

To assay the cytotoxicity of PBAE/DNA NPs, the cell viability of transfected cells was measured using the CCK-8 assay. Briefly, cells were transfected with PBAE/pEGFP NPs with different ratio of PBAE/DNA as previously described. Lipofectamine 3000 was used according to the manufacturer's protocol as a control group. At pre-determined time points, the medium was removed, and complete medium supplemented with 10% CCK-8 reagent (v/v) was added. After a 2h incubation, the absorbance of the medium was measured at 450 nm using a microplate reader (Varioskan Flash Multimode Reader, Thermo Scientific). Cell viability was reported as a relative percentage compared to untreated samples.

Preparation of GelMA

GelMA was prepared according to a previously published protocol.[3] Briefly, 10g of type A gelatin from porcine skin was dissolved in 100ml DPBS at 50 °C. 0.25ml methacrylic anhydride (MA) (0.25 volume%) was gradually stirred into the gelatin solution at a rate of 0.5 mL/min at 50 °C for 1h. To stop the reaction, 500ml of warm (40 °C) DPBS was added. Then, the unreacted salts and MA were removed by dialysis in

40 °C distilled water using 12–14 kDa cutoff dialysis tubing while stirring for one week. The resulting GelMA was lyophilized for one week and stored at -80 °C for further use.

Preparation of MN/PBAE/DNA

To fabricate the PBAE/pEGFP NP-loaded MNs, premade PBAE/pEGFP complexes with a PBAE/DNA ratio of 80/1 and photoinitiator (Irgacure 2959) were added into a GelMA solution to yield a final concentration of 15% GelMA with 30 μg/ml pEGFP and 0.5% photoinitiator. Then, 100 μL of solution was added into each MN mold. After centrifugation (3000 rpm for 5 min at 37 °C), the solution was exposed to 350 mW/cm^2 UV light for defined exposure durations (0, 5, 10, and 30s). After dried in the dark, the MN was removed from the mold and kept at -20 °C until further use. In this procedure, the reagents and devices we used were sterilized and DNase free to protect the pDNA from degradation.

SEM

MNs were coated with gold using a sputter coater (Pelco, SC-7) and the surface morphology was characterized using a field emission scanning electron microscope (ZEISS Supra 40VP SEM).

Mechanical properties of the MN/PBAE/DNA

The mechanical strength of the MN/PBAE/DNA with different UV exposure times was measured under dynamic force using a low-force mechanical testing system (5943 MicroTester, Instron, USA). During testing, the applied force and the corresponding deformation were recorded. In the test, MNs were pressed against a stainless-steel plate at a speed of 0.5mm/min with a maximum loading force of 50.0 N. To demonstrate that the MNs could penetrate mouse skin, MNs were pushed into the cadaver skin with 20 N

of force for 30 seconds. Then, the penetrated skin was stained for 10 min using 0.5% trypan blue solution. After washing three times, trypan blue-stained samples were imaged.

pDNA release properties

At the optimal ratio of heparin/PBAE, the amount of DNA in solution after dissociation should be approximate to the amount in solution before being complexed with PBAE. Heparin sulfate solutions with predetermined weight ratios between heparin and PBAE were added to the PBAE/DNA complexes and incubated for 15 min. Then, Picogreen was used (according to the manufacturer's protocol) to quantify the amount of dissociated DNA. In order to detect the release of NPs from the MNs, the MN/PBAE/DNA were soaked in DPBS with or without collagenase (2U/ml). At predetermined time points, a 50 μL suspension of each sample was incubated with heparin to dissociate the DNA, and Picogreen was added to quantify the concentration of DNA released from the MNs.

Gene transfection with the MN/PBAE/DNA in 2D cell models

NIH 3T3 cells were seeded in 12 wells plates at a density of 1×10^5 cells per well. After incubation for 24 h, the medium was changed to medium with 2U/ml collagenase. Transwell inserts were placed in the well plate and sterilized MN/PBAE/DNA applied to the upper membrane of the Transwell.[4] After 3 days of incubation, the cells were stained with DAPI and EthD-1 and counted using a fluorescence microscope and flow cytometry.

Gene transfection with MN/PBAE/DNA in 3D cell model

NIH 3T3 cells were 3D-cultured in a GelMA hydrogel matrix. Briefly, 200 μL of 10% GelMA solution with 2×10^6 cells was added into a PDMS mold and crosslinked under UV light for 10 seconds. After one

day of incubation, the previously prepared MN/PBAE/DNA (10s crosslinking) were applied to the upper surface of the hydrogels. After further incubation for three days, the hydrogels were imaged using a Leica Confocal SP8-STED/FLIM/FCS following staining with DAPI and EthD-1.

In vivo transdermal gene transfection model

All animal experiments were approved by the UCLA Animal Research Committee (UCLA ARC #2018-003-01E). Eighteen 7-week-old, C57BL/6J male mice (average weight: 20 grams) were purchased from Jackson Laboratory (Sacramento, CA, USA). All animals were treated in compliance with the National Research Council criteria as outlined in the "Guide for the Care of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health. At the day of application (Day 0), the MNs were applied to the dorsum of the mice topically under inhalational anesthesia (1.5% isoflurane in 100% O_2). Blank MNs (n=6), MN/DNA (n=6) and MN/PBAE/DNA (3μg pEGFP and 240μg PBAE, n=6) were insert into the dorsum skin. On day 3, the mice were euthanized using CO_2 for further evaluation.

Histological analysis and immunofluorescence staining

Skin specimens including the MN application site and surrounding skin were fixed in 10% neutral buffered formalin (Leica Biosystems, IL, USA). Then, skin samples were further processed for histological analysis and embedded in paraffin. Routine hematoxylin and eosin (HE) staining was conducted on 4 μm tissue sections. A Nikon inverted microscope was used to image the histology samples and AmScope image analysis software (AmScope, Irvine, CA, USA) was used for analysis. For immunostaining, the tissue sections were deparaffinized, antigen retrieved (heat-induced), permeabilized in PBST (0.3% Triton in PBS), and incubated with goat serum for 30 min. Then, the sections were incubated overnight at 4℃ with AlexaFluor 488 conjugated GFP antibody. The slides were rinsed with PBST and counterstained with DAPI

for 5 min. The fluorescent images were imaged via Nikon Eclipse Ti-S Inverted Phase Contrast Fluorescent Microscope. The transfection rates of cells in interest region were analyzed using ImageJ (NIH).

Statistics

All data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed with Graph Pad Software (San Diego, CA, USA). The differences among the groups were analyzed by one-way ANOVA. Statistical significance was set at $p<0.05$.

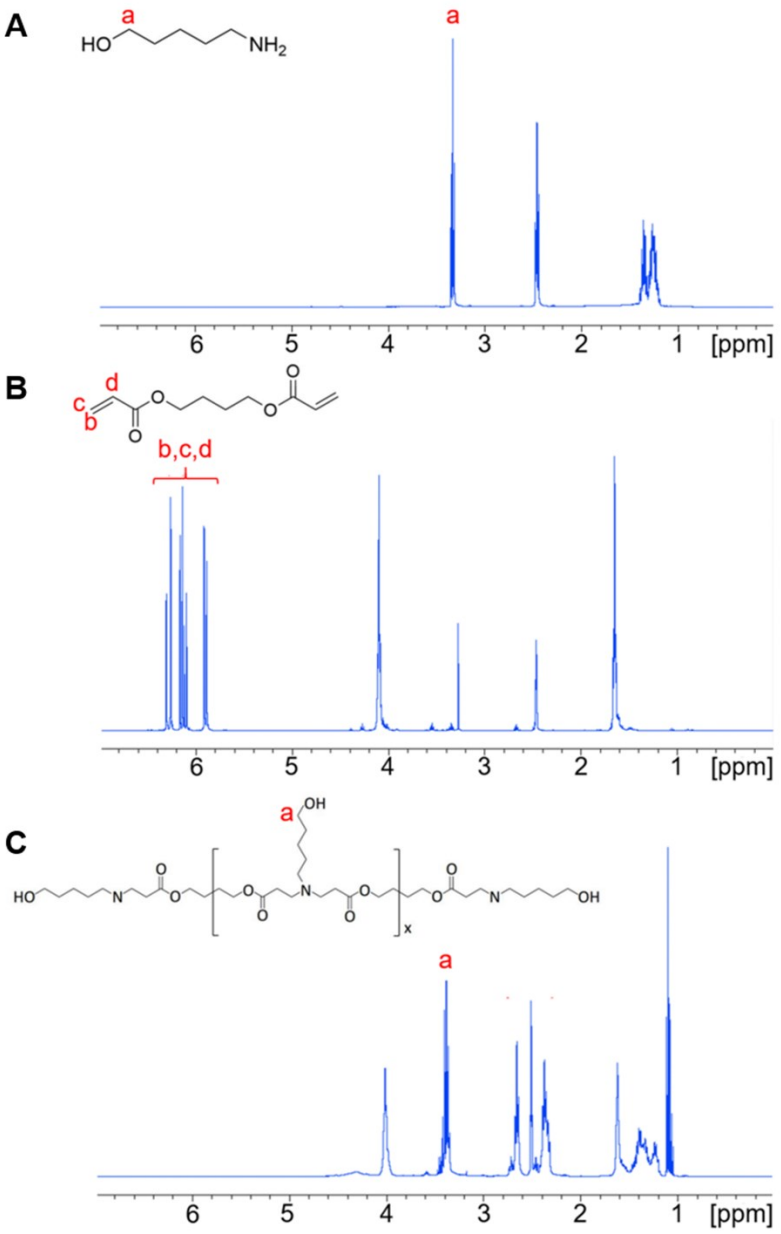

Fig. S1 ^{1}H-NMR spectra of reactants and products in the synthesis of PBAE. A. ^{1}H-NMR spectra of 5-amino-1-pentanol. B. ^{1}H-NMR spectra of 1,4-butanediol diacrylate. C. ^{1}H-NMR spectra of synthesized poly(5-amino-1-pentanol-co-1,4-butanediol diacrylate). The disappearance of peak b, c, and d and the presence of peak a indicate the successful synthesis of PBAE.

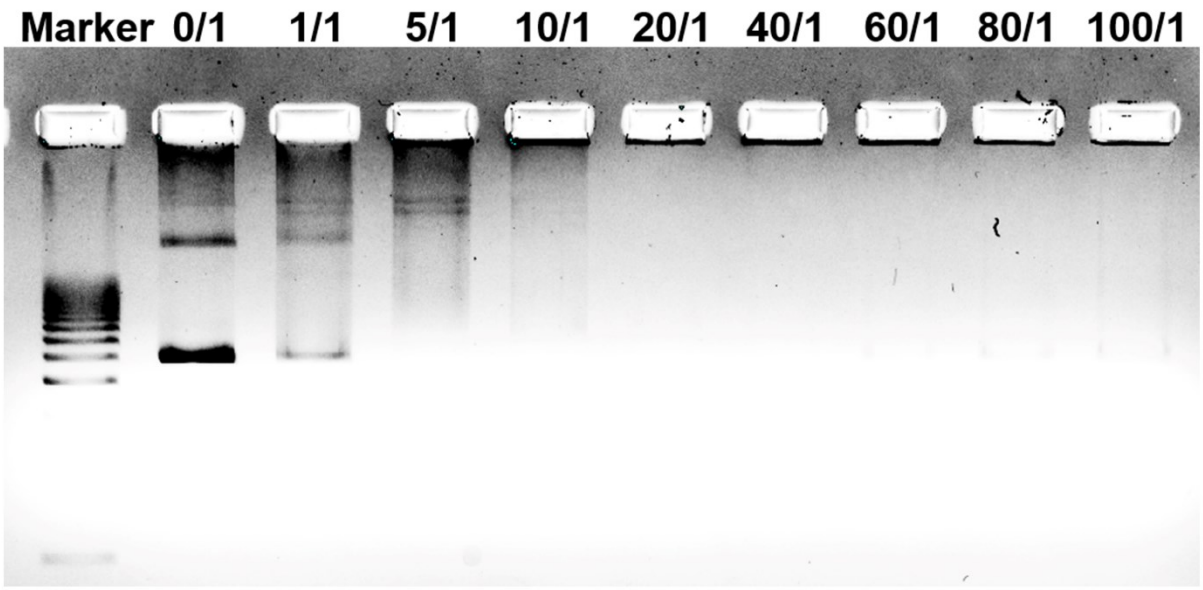

Fig. S2 Agarose gel electrophoresis of PBAE/DNA NPs with predetermined ratios of PBAE/DNA (w/w).

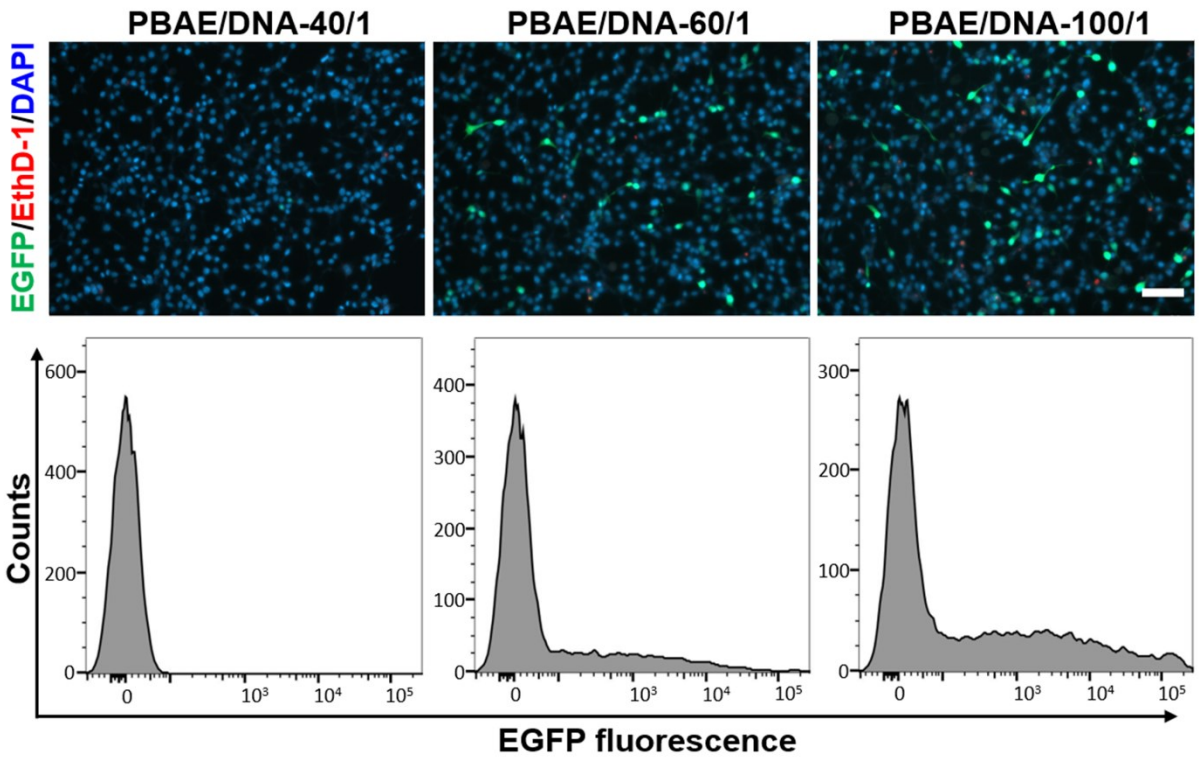

Fig. S3 *In vitro* gene transfection using PBAE/pEGFP NPs with predetermined ratios of PBAE/DNA (w/w). Transfection rate and cytotoxicity of each group were analyzed by flow cytometry (Fluorescence and flow cytometry results of free DNA, 80/1, and Lipofectamine group are shown in Fig. 2C).

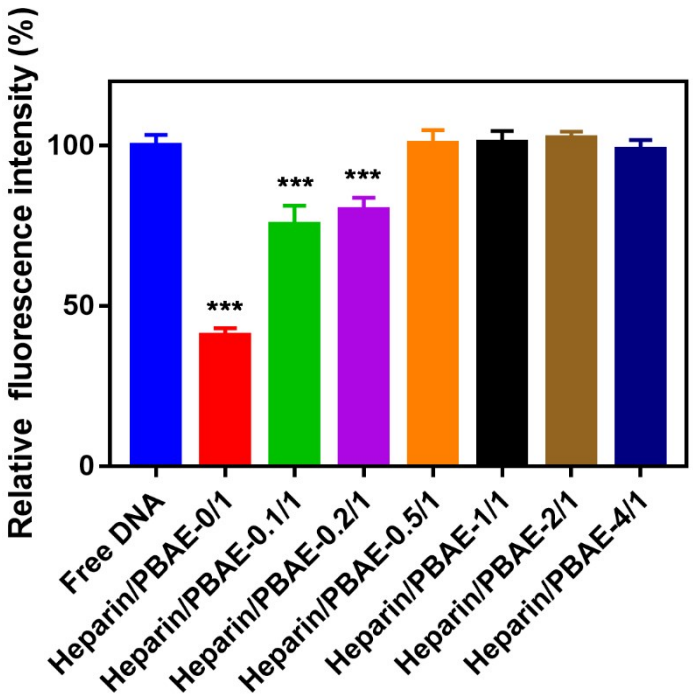

Fig. S4 Relative fluorescent intensity of DNA solutions quantified with Picogreen. The binding of DNA with PBAE in the formation of PBAE/DNA NPs affected the binding and coloration of PicoGreen. Heparin can disrupt the polyplexes and completely free the DNA when the mass ratio of heparin/PBAE was over 0.5:1. Data are shown as mean ± SD (n = 3). (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

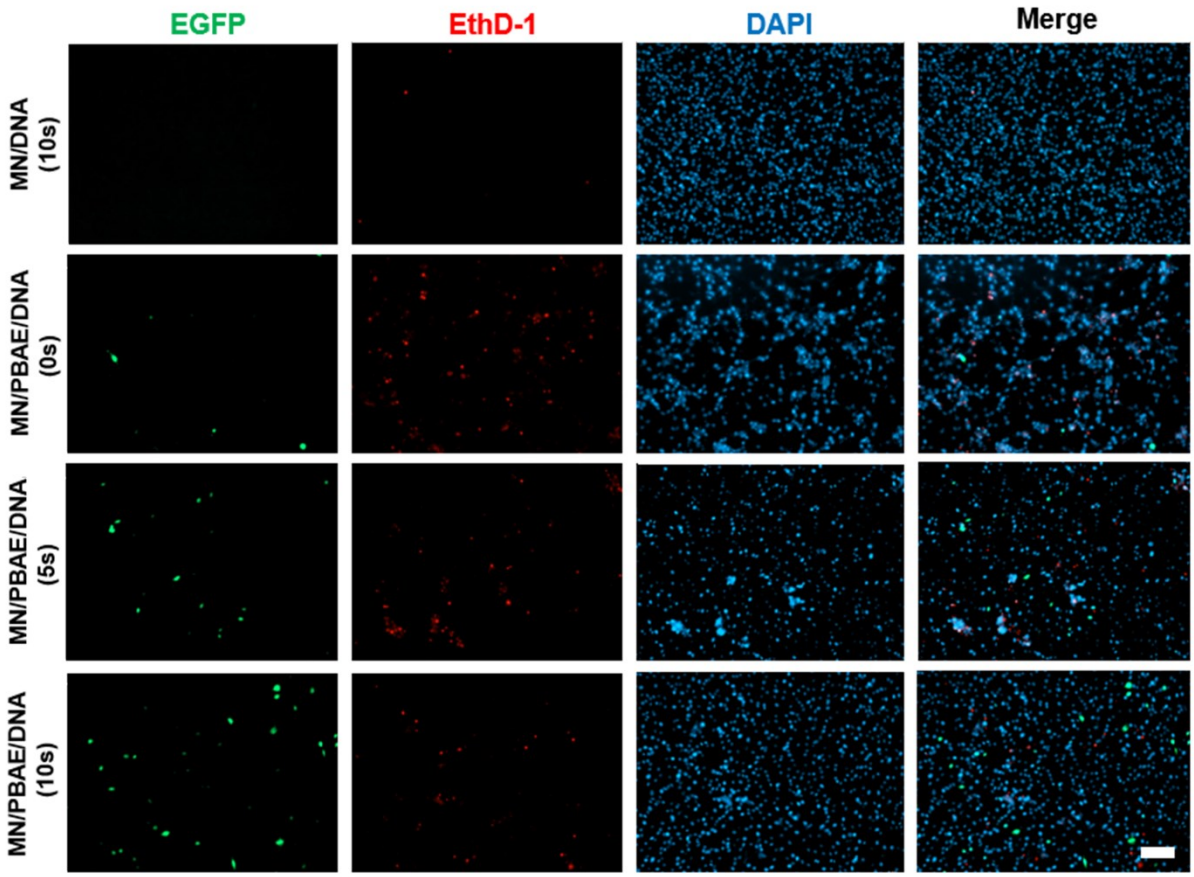

Fig. S5 Individual channel and merged images of transfected cells of each group in 2D cell transfection with MN/PBAE/DNA. Dead cells and cell nuclei were labeled with EthD-1 and DAPI, respectively. Scale bar: 100 μm.

References:

1. D. G. Anderson, A. Akinc, N. Hossain and R. Langer, *Mol Ther*, 2005, **11**, 426-434.
2. Y. Liu, J. Chen, Y. Tang, S. Li, Y. Dou and J. Zheng, *Mol Pharm*, 2018, **15**, 4558-4567.
3. J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar and A. Khademhosseini, *Biomaterials*, 2010, **31**, 5536-5544.
4. N. Segovia, M. Pont, N. Oliva, V. Ramos, S. Borros and N. Artzi, *Adv Healthc Mater*, 2015, **4**, 271-280.