Supporting Information

"Polyethyleneimine-Functionalized MXenes as Stable Two-Dimensional Platforms for Gene Delivery Systems"

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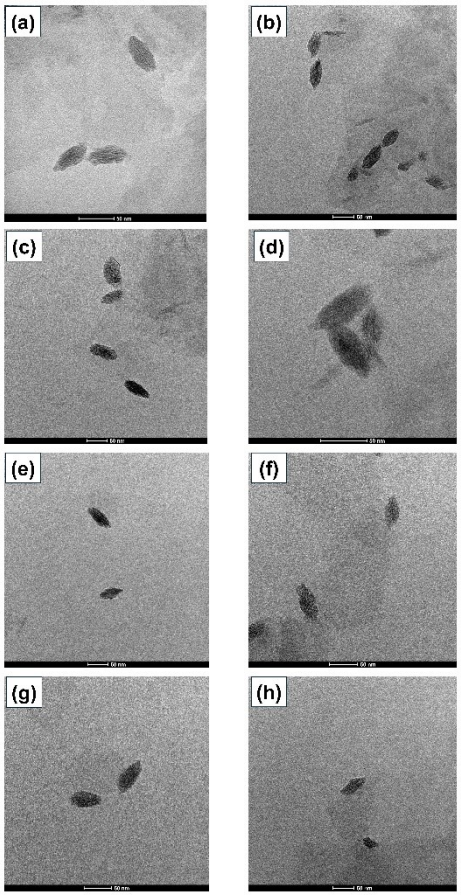
Sample	Nitrogen %	Carbon %	Hydrogen %	Sulfur %
MP1	4.22	14.60	2.49	0.09
MP2	4.59	15.49	2.61	0.09
MP3	6.96	18.76	3.35	0.06
MP4	6.96	19.02	3.22	ND

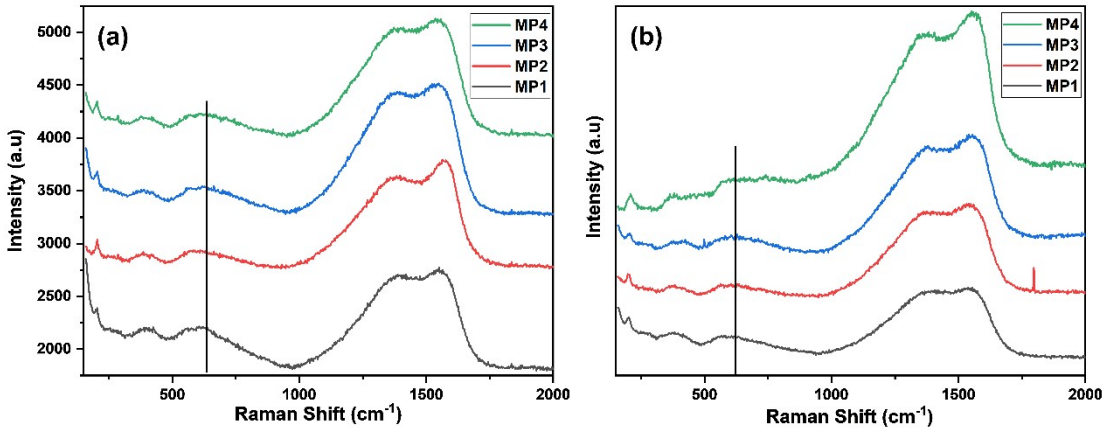
Table.S1. CHNS elemental composition of MP1-4

Stability of MP1-4 samples in PBS:

The stability of the PEI–MXene complexes was evaluated by incubating the samples in phosphate-buffered saline (PBS, pH 7.4) at room temperature for 14 days to assess their structural integrity under physiological conditions. Transmission electron microscopy (TEM) analysis revealed that the morphology and lateral size of the PEI–MXene particles remained largely unchanged compared to the pristine samples, with no observable formation of spherical nanoparticles typically associated with TiO_2 formation from oxidation of $Ti_3C_2T_x$ MXene.



S1. Tem images of MP1 (a) fresh (b) after 14 days, MP2 (c) fresh (d) after 14 days, MP3 (e) fresh (f) after 14 days and MP4 (g) fresh (h) after 14 day



S2. Raman Spectroscopy of (a) Fresh and (b) after 14 days MP1-4 samples

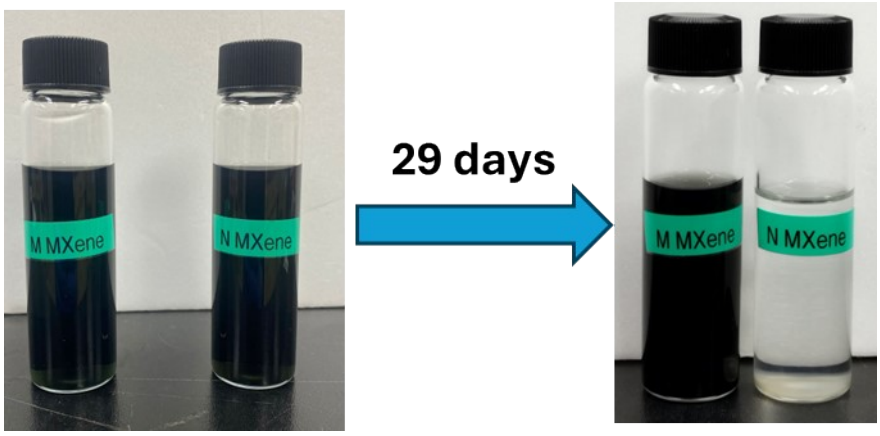
Raman spectroscopy further confirmed the structural stability, as the characteristic vibrational modes of MXene (150–700 cm^{-1}) and PEI (1000–1700 cm^{-1}) were preserved after incubation. Importantly, no additional Raman peak near ~630 cm^{-1}, which is commonly attributed to TiO_2, was detected. These results collectively demonstrate that the PEI coating effectively stabilizes MXene against oxidation and structural degradation in physiological buffer environments for at least 14 days.

Stability of Al-$Ti_3C_2T_x$ (MXene):

The enhanced stability of Al-rich $Ti_3C_2T_x$ (M-MXene) compared to stoichiometric $Ti_3C_2T_x$ (N-MXene) originates from a synergy of thermodynamic, kinetic, and microstructural effects, as well as distinct oxygen incorporation mechanisms during synthesis. The critical difference lies in the precursor stoichiometry: Al-rich Ti_3AlC_2 is synthesized with an Al molar ratio of 2.2, compared to 1.05 for the stoichiometric phase. During synthesis, excess Al (m.p. 660 °C) functions as a reactive flux, promoting atomic diffusion and enabling the sequential formation of intermetallic phases ($TiAl_3 \rightarrow TiAl \rightarrow Ti_3Al$). This flux-assisted pathway facilitates the growth of high-purity Ti_3AlC_2 at lower temperatures, while simultaneously compensating for Al evaporation losses. The surplus Al also suppresses secondary phase formation (Ti_2AlC, TiC) and mitigates carbon vacancies, both of which commonly occur in conventional routes. Consequently, Al-rich Ti_3AlC_2 exhibits superior crystallinity, enhanced grain growth, and reduced defect density, thereby minimizing high-energy sites such as vacancies and grain boundaries that typically serve as initiation points for oxidation or decomposition. Thermodynamically, excess Al saturates the A-sites of the MAX lattice and shifts the equilibrium toward Ti_3AlC_2 formation, while kinetically, the low-defect lattice raises the energy barriers for oxidation and degradation. As a result, the Al-rich phase achieves near-ideal stoichiometry and phase purity (>99 wt.%), which directly translates to MXenes with improved oxidation resistance and long-term structural integrity—features crucial for applications such as gas sensing, energy storage, and catalysis.

Oxygen chemistry further distinguishes the stability of Al-rich systems. In stoichiometric Ti_3AlC_2, oxygen readily incorporates into carbon vacancy sites under high-temperature conditions, forming $Ti_3Al(C,O)$ phases that degrade long-term durability. By contrast, in Al-rich Ti_3AlC_2, the surplus aluminum acts as an

oxygen getter during sintering, preferentially reacting with oxygen to produce discrete α-Al_2O_3 particles. This gettering mechanism diverts oxygen away from the MAX lattice, suppressing vacancy formation and limiting oxygen substitution. While such alumina inclusions may slightly reduce the surface area of the resulting MXene, their inert nature ensures significant improvements in bulk stability and oxidation resistance. Collectively, the optimized synthesis pathway, reduced structural defect density, and controlled oxygen incorporation impart superior phase purity, microstructural robustness, and functional durability to Al-rich Ti C_2T_x, positioning it as a highly promising candidate for advanced applications requiring long-term stability under demanding conditions.

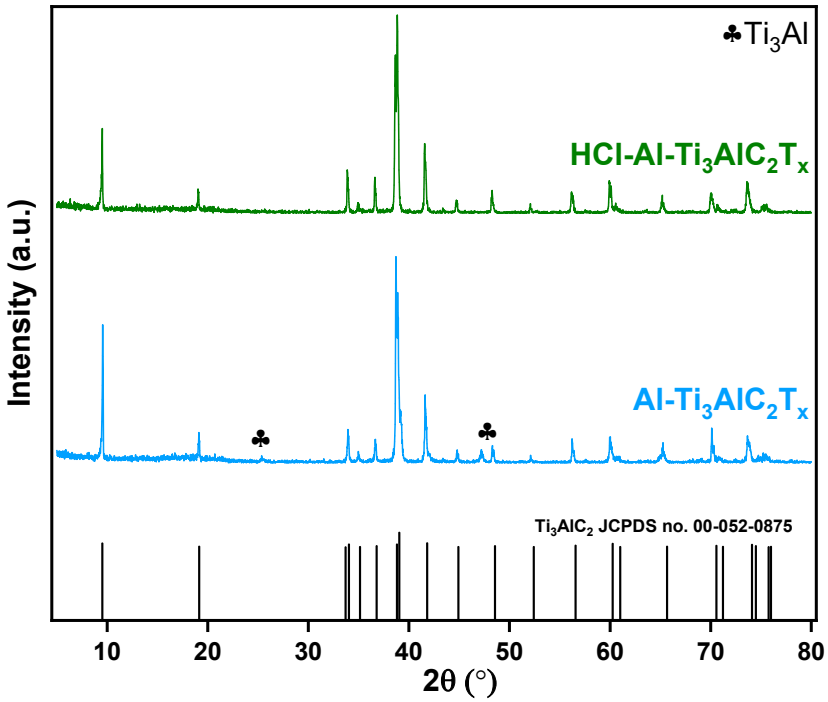


S3. Stability test of N and M MXenes in aqueous solution (0.3 mg/ml) under room temperature.

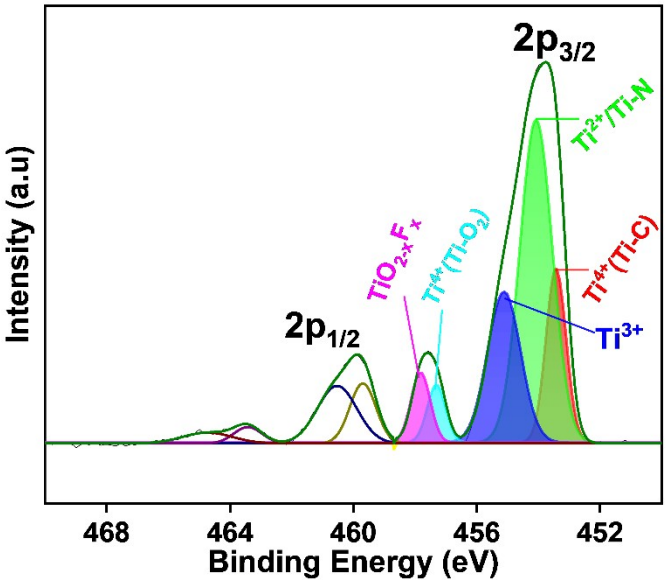
Synthesis of Al-Ti_3AlC_2 (MAX Phase)

Titanium carbide (TiC), titanium (Ti), and aluminum (Al) powders were weighed in a molar ratio of 2:1.25:2.2 and thoroughly mixed in an agate mortar for approximately 45 min to ensure homogeneity. The mixed precursor powder was pressed into pellets using a uniaxial hydraulic press and placed in an alumina crucible, which was then transferred to a Alumina tube furnace. Prior to sintering, the furnace was purged with high-purity argon (99.999%) for 10 min to eliminate residual oxygen. The samples were then heated to 1450 °C at a rate of 5 °C/min, held for 4 h, and subsequently cooled to room temperature at the same controlled rate under continuous argon flow. Dense sintered compact pallet containing Al–Ti_3AlC_2 MAX phase were thus obtained.

The sintered products were ground into fine powders using agate mortar and subjected to acid washing in 9 M HCl for 18 h at room temperature under constant stirring to remove intermetallic impurities such as. This treatment resulted in a mass reduction of approximately 20–30 wt%, in line with the dissolution of non-MAX secondary phases. During the washing process, the supernatant gradually developed a deep purple coloration, which is consistent with the formation of soluble Al- and Ti-based intermetallic. The acid-treated powders were repeatedly washed by centrifugation with deionized water until reaching close to neutral pH, followed by drying in a vacuum oven at 50 °C for at least 12 h.



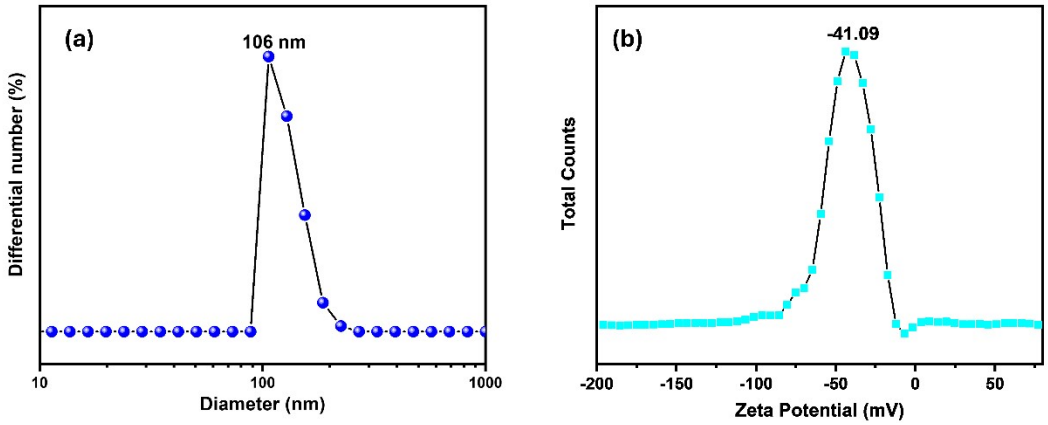
S4. shows the XRD pattern of as prepared and HCl washed MXene.



S5. shows the XPS of MP2 sample.

DLS and Zeta Potential

Collectively, the Z average of (166.4 nm) and strong negative zeta potential (−41.09 mV) of MXene demonstrate that the processing route yields small, well-dispersed $Ti_3C_2T_x$ nanoflakes that are colloidally stable and structurally suited as cores for cationic polymer coating. Such dimensions fall within the sized window typically associated with efficient cellular internalization, while the negative surface charge is advantageous for subsequent charge inversion and gene complex formation.



S6. Fig (a) DLS of MXene and Fig (b) Zeta potential of MXene

Characterization of MP series and MP/pDNA complexes

The hydrodynamic size and zeta potential of the samples were measured using a Zetasizer ZS90 (Malvern Instruments, Malvern, UK). For the MXene raw material, measurements were performed at a concentration of 0.01 mg/mL. The MP series (PEI/MXene nanocomplexes) were prepared by complexing MXene with PEI (0.8k, 2k, 10k, and 25k) at PEI/MXene weight ratios of 5, 10, 15, and 20, as mentioned above. The measurements were conducted at a final MXene concentration of 0.01 mg/mL. For the MP/pDNA complexes, 0.5 µg of pDNA was added per sample and the complexes were formulated at weight ratios (MP : pDNA) ranging from 5 to 50. Both hydrodynamic size and zeta potential were measured three times, and the results were presented as the average values.

Synthesis of Al-$Ti_3C_2T_x$ (MXene)

A 20 ml of 9 M hydrochloric acid (HCl) was initially added into a polypropylene container, followed by the addition of 1.6 g of lithium fluoride (LiF). The mixture was stirred using magnetic stirrer until the LiF was completely dissolved, forming a homogeneous etchant solution. Then, 1 g of Ti_3AlC_2 MAX phase powder was gradually added to the acid-LiF solution at a controlled rate of approximately 1 g/min under continuous stirring. The suspension was stirred at room temperature for 24 hours for selective etching of the aluminum layers from the MAX structure. The entire process was conducted inside a fume hood to safely manage any hazardous gases released during the reaction.

After etching, the mixture was washed repeatedly with deionized water via centrifugation until the supernatant reached a neutral pH of approximately 6–7, indicating removal of spent acid and soluble

byproducts. The suspension was then vigorously shaken to delaminate the multilayer $Ti_3C_2T_x$ MXene. This was followed by centrifugation at 3,900 rpm for 30 minutes to separate single layer MXene from multilayer MXene and unetched MAX. The dark (ink like), colloidal supernatant containing single-layer MXene nanosheets was carefully collected for further use.

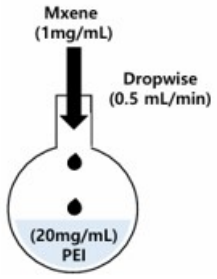
Synthesis of PEI/MXene Nanocomplexes

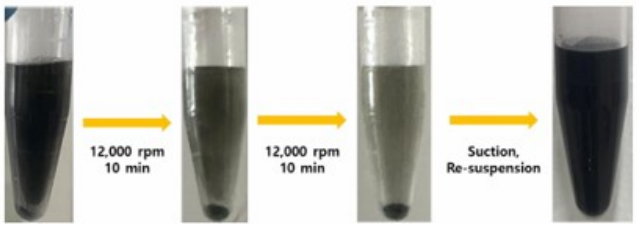
Polyethylenimine (PEI) with different molecular weights was used in this study. PEI 0.8k and PEI 25k were purchased from Sigma Aldrich (St. Louis, MO, USA). PEI 2k was obtained from Polyscience (Warrington, PA, USA), and PEI 10k was purchased from Alfa Aesar (Haverhill, MA, USA).

The PEI/MXene nanocomplexes were synthesized through a solution mixing approach using polyethyleneimine (PEI) of various molecular weights (0.8k, 2k, 10k, and 25k). A 20 mg/mL PEI stock solution was prepared in deionized water, while a 1 mg/mL aqueous dispersion of delaminated MXene was obtained separately. The MXene dispersion was then added dropwise into the PEI solution at a controlled rate of 0.5 mL/min to ensure gradual interaction between the positively charged amine groups of PEI and the negatively charged MXene surface terminations, thereby preventing uncontrolled aggregation. The mixture was stirred at room temperature for 24 h under nitrogen protection to suppress oxidation of MXene. Different PEI-to-MXene weight ratios (5, 10, 15, and 20) were employed by varying the volume of PEI solution (1–4 mL) while keeping the MXene volume constant (4 mL), allowing systematic evaluation of the influence of polymer loading on surface modification.

After complexation, the mixtures were centrifuged at 12,000 rpm for 30 min to remove excess unbound PEI, and the supernatant was carefully discarded. The resulting pellet containing PEI/MXene nanocomplexes was washed with deionized water and centrifuged again under the same conditions, a step repeated twice to ensure thorough removal of free polymer. Finally, the purified pellet was re-suspended in deionized water to obtain a stable aqueous dispersion of the PEI/MXene nanocomplexes. The combination of controlled dropwise mixing, nitrogen protection, and repeated washing ensured uniform PEI functionalization of MXene surfaces, yielding dispersions with improved stability and suitability for further physicochemical and functional studies.

WR (PEI/MX)	5	10	15	20
PEI (20mg/mL)	1	2	3	4
MXene (1mg/mL)	4	4	4	4
Total (mL)	5	6	7	8



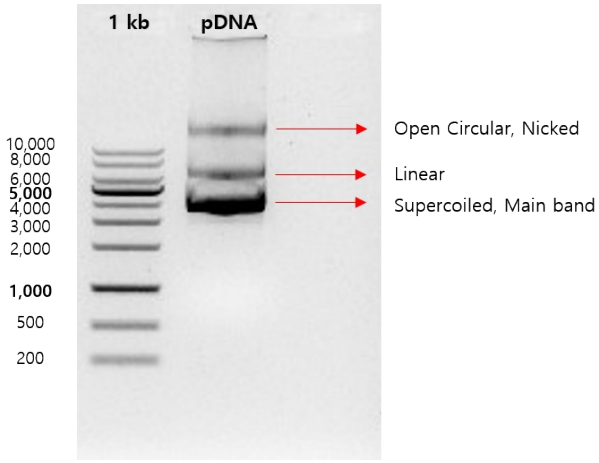


Preparation of pDNA

pDNA (pCN-Luci) was provided by Prof. Jong Sang Park. pCN-Luci was constructed by subcloning cDNA of *Photinus pyralis* luciferase together with a 21 amino acid nuclear localization signal from SV40 large T antigen into pCN (Gene Therapy, 2002, 9, 859–866). It was amplified by using Dyne DH5α Chemically Competent *E.coli* ver.2 (DYNEBIO, Sungnam, Republic of Korea) and purified by using NucleoBond Xtra Maxi (Macherey-Nagle, Duren, Germany).

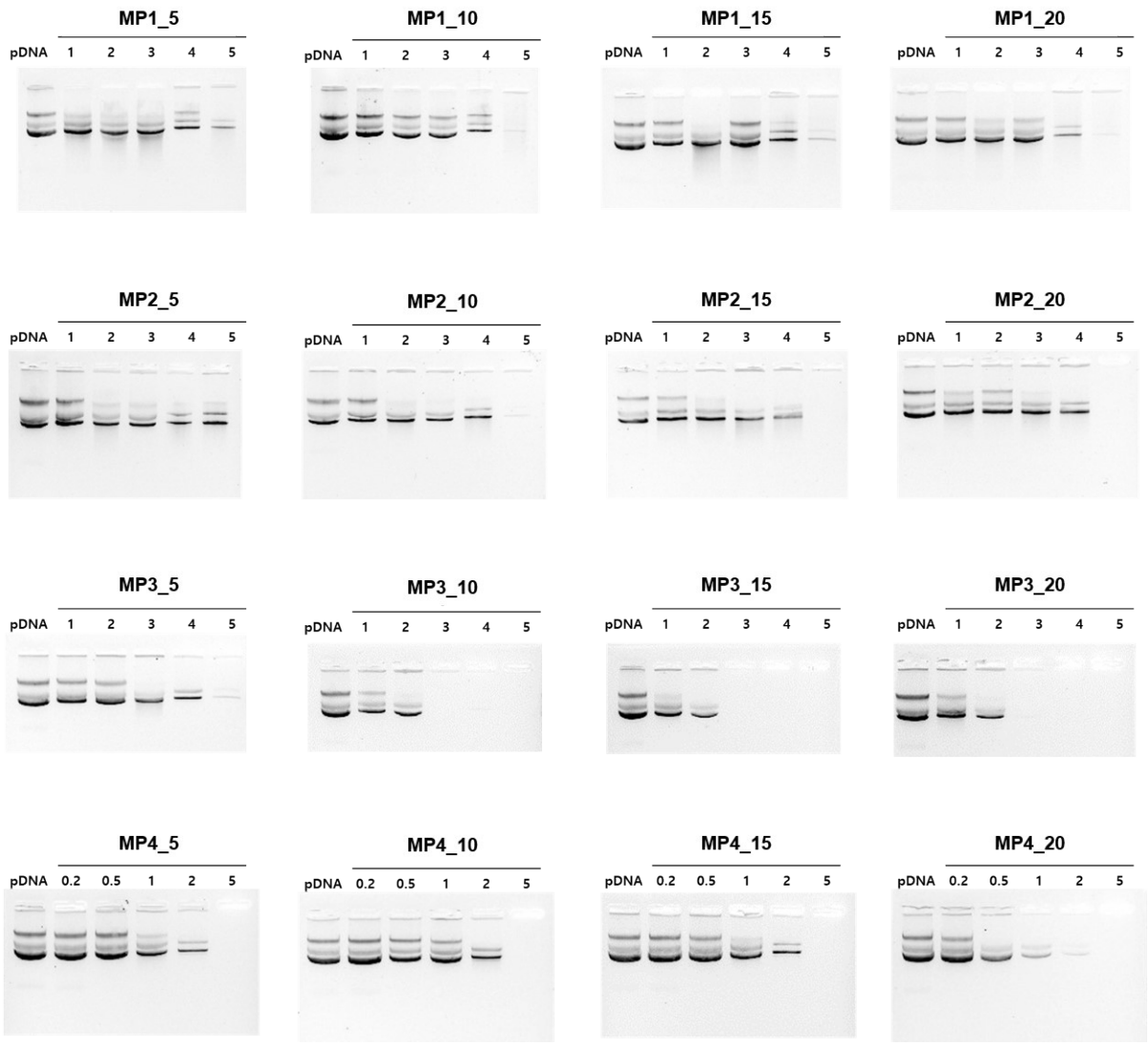
Agarose gel electrophoresis of pDNA

Agarose gel electrophoresis was performed to examine the size of pCN-Luci. pDNA was electrophoresed with 1 kb DNA ladder (Enzynomics, Daejeon, Republic of Korea) in 0.7% agarose gel prepared in TEA buffer containing EtBr (0.5 μg/mL), at 90 V for 20 min.

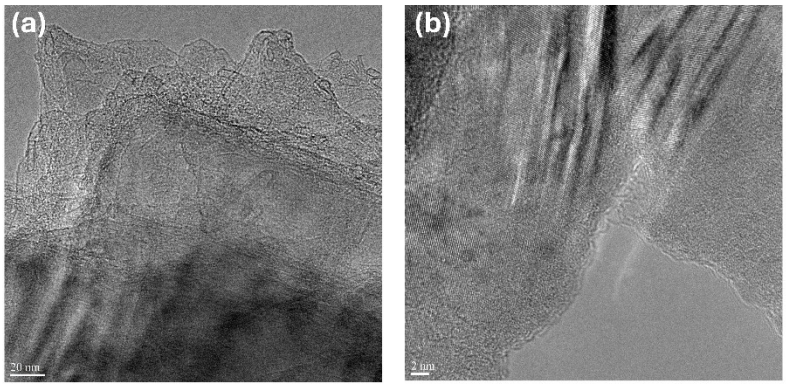


Agarose gel electrophoresis of MP series

Agarose gel electrophoresis was performed to analyze the pDNA condensation ability of MP series. 0.7% agarose gel was prepared in TEA buffer containing EtBr (0.5 μg/mL), and electrophoresis was conducted at 90 V for 12 minutes. All samples were prepared in HEPES buffer (pH 7.4) with the addition of 2 μL of 15% (w/v) Ficoll solution to improve loading efficiency. The complexes of MP1, MP2, and MP3 with pDNA were prepared at weight ratios of 1, 2, 3, 4, and 5, while MP4/pDNA complexes were prepared at weight ratios of 0.2, 0.5, 1, 2, and 5.



S7. Agarose gel electrophoresis results of MP series. The numbers above bar (5–20) mean the mixing ratios of MP series (PEI : MXene, w/w). The numbers below bar (0.2–5) mean the weight ratios of MP/pDNA complexes (MP : pDNA, w/w).



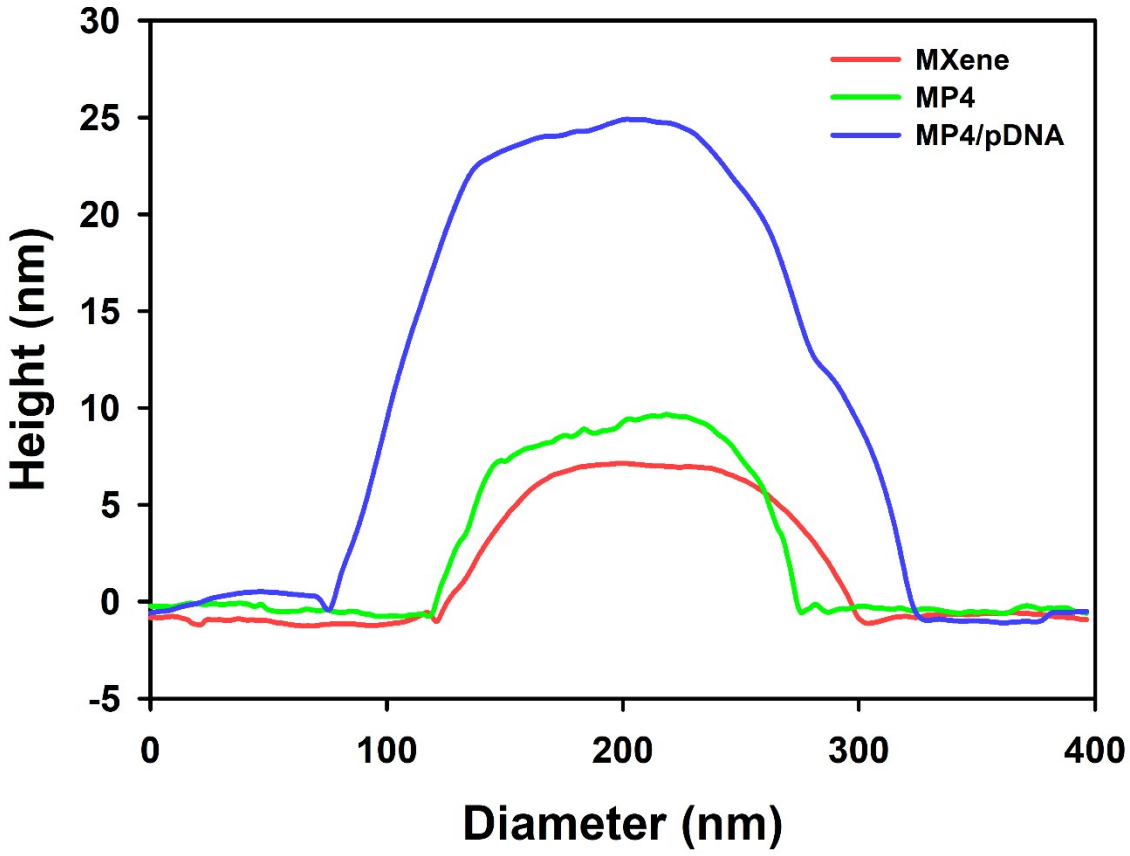
S8. TEM images of $Ti_3C_2T_X$ MXene.

Morphological Analysis by TEM

Transmission electron microscopy (TEM) was performed to analyze the morphology of MXene, MP4 (PEI : MXene = 20:1, w/w), and MP4/pDNA (MP4 : pDNA = 20:1, w/w). TEM images were obtained using a 200 kV TEM instrument (JEM-2100, JEOL, Japan). Samples were prepared by loading each solution once onto a 300-mesh copper grid and washing twice. After sample preparation, the grids were completely dried before imaging.

Atomic Force Microscopy (AFM) Analysis

The surface morphology of MXene, MP4 (PEI : MXene = 20 : 1, w/w), and MP4/pDNA (MP4 : pDNA = 20:1, w/w) complexes was characterized using an atomic force microscope (NX10, Park Systems, Suwon, Republic of Korea) operated in non-contact mode. A silicon cantilever (PPP-NCHR 10M, Park Systems) was used for imaging. Samples were prepared at a concentration of 0.1 μg/mL (based on MXene) by depositing each complex onto a P-type boron-doped Si wafer (Carlit, Tokyo, Japan). Prior to sample loading, the wafer surface was washed sequentially with water and ethanol. After sample deposition, the wafers were completely dried at room temperature and subsequently washed with water to remove loosely bound particles. AFM images were obtained using the SmartScan software (Park Systems) at a scan rate of 0.5 Hz over scan areas of 3 × 3 or 5 × 5 μm with a resolution of 256 × 256 pixels. Post-processing, including size measurements and height profile analysis, was performed using the XEI software (Park Systems).



S9. Height profile of MXene, MP4, and MP4/pDNA complex from AFM images (Fig. 4)

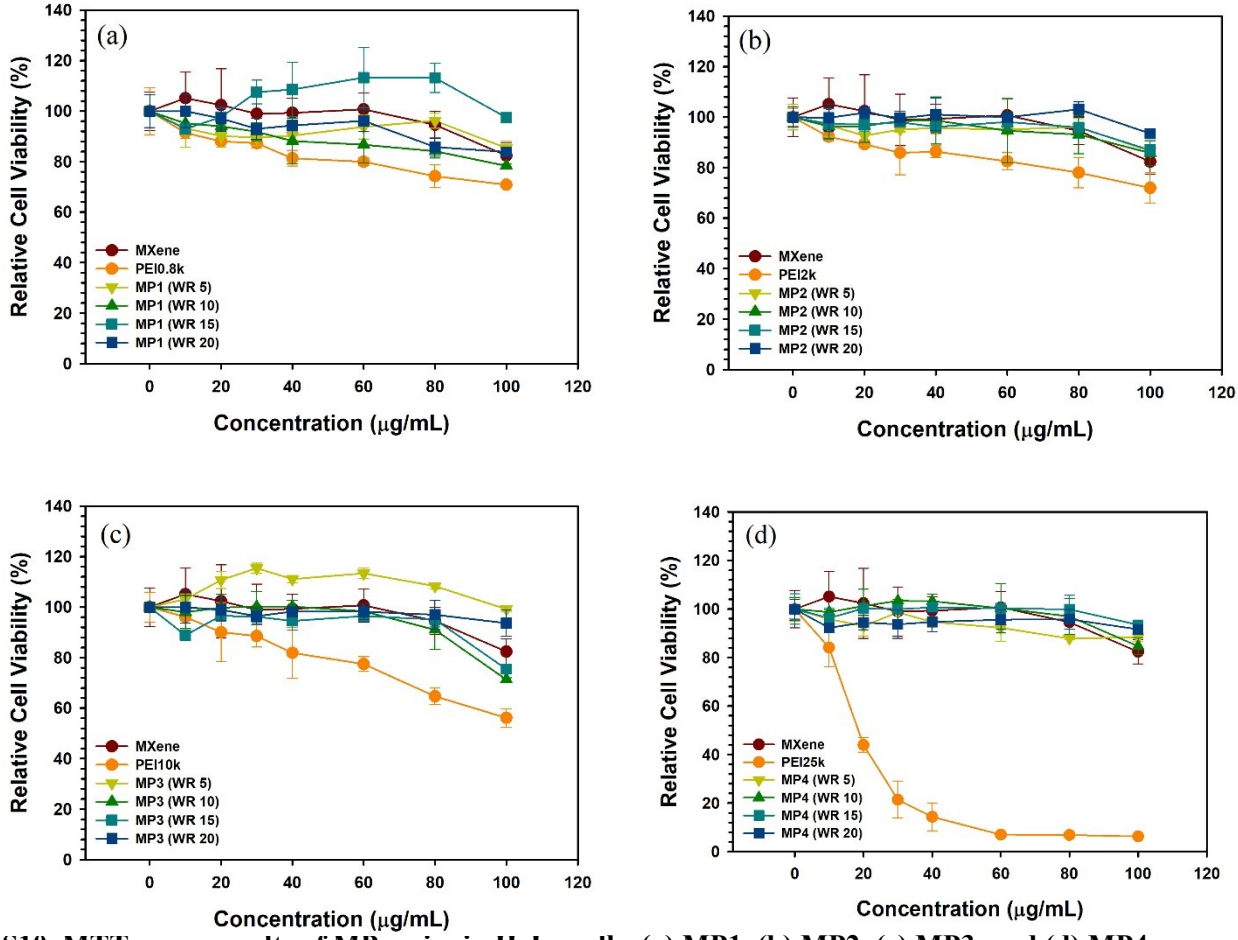
Cell culture

Human cervical adenocarcinoma cell line (HeLa, origin: human cervix, uterine, Korean Cell Line Bank) was used for in vitro experiments. Cells were cultured in a medium supplemented with 10% FBS and 1% penicillin/streptomycin (DMEM+GlutaMAX™). All cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 ℃.

MTT assay

The cytotoxicity of PEI, MXene, and MP series was evaluated using an MTT assay on HeLa cells. HeLa cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h to reach 70–80% confluency. For the cytotoxicity evaluation, PEI and MXene were treated at concentrations of 0–100 μg/mL, while MP series (weight ratios of PEI : MXene = 5–20 : 1, w/w) were treated based on MXene content (0–100 μg/mL). After treating the cells with 100 μL of sample solutions (serum-free medium) for

4 h, the media were exchanged with fresh medium containing 10% FBS. Following 24 h of incubation, 25 µL of MTT solution (2 mg/mL in DPBS) was added to each well and incubated for 2 h at 37 °C. The media were then aspirated off and 150 µL of DMSO was added to dissolve the purple formazan crystals formed by metabolically active cells. Absorbance was measured at 570 nm using a microplate reader. The relative cell viability (% RCV) was calculated as a percentage of untreated control cells.

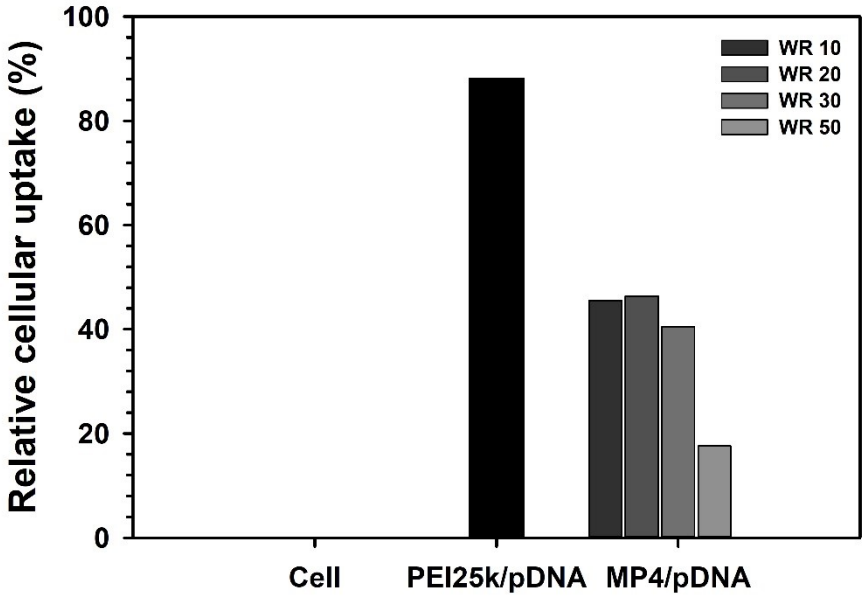


S10. MTT assay results of MP series in HeLa cells. (a) MP1, (b) MP2, (c) MP3, and (d) MP4.

Cellular uptake of MP4/pDNA complexes

HeLa cells were seeded in a 6-well plate at a density of 2×10^5 cells per well and cultured until 70–80% confluency was reached. For the assay, MP4 (PEI : MXene = 20 : 1, w/w) /pDNA complexes were prepared with weight ratios (MP4 : pDNA) ranging from 10 to 50 in serum-free medium. pDNA used was pre-labeled with YOYO-1 dye at a ratio of one dye molecule per 50 base pairs of pDNA, and the labeling was performed at room temperature for 30 min in TE buffer. Treatment of 2 mL sample solutions was conducted under serum-free conditions for 4 h and the treated-cells were washed with Trypan blue (0.1 mg/mL) for 5 min to quench any extracellular fluorescence. After washing twice with DPBS, cells were then trypsinized for

2 min and collected by centrifugation at 1500 rpm for 3 min. The cell pellet was resuspended in DPBS for flow cytometry analysis. The cellular uptake efficiency was examined by using a BD Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) at a minimum of 1×10^4 cells gated per sample. The analysis was performed by using BD Accuri C6 software.

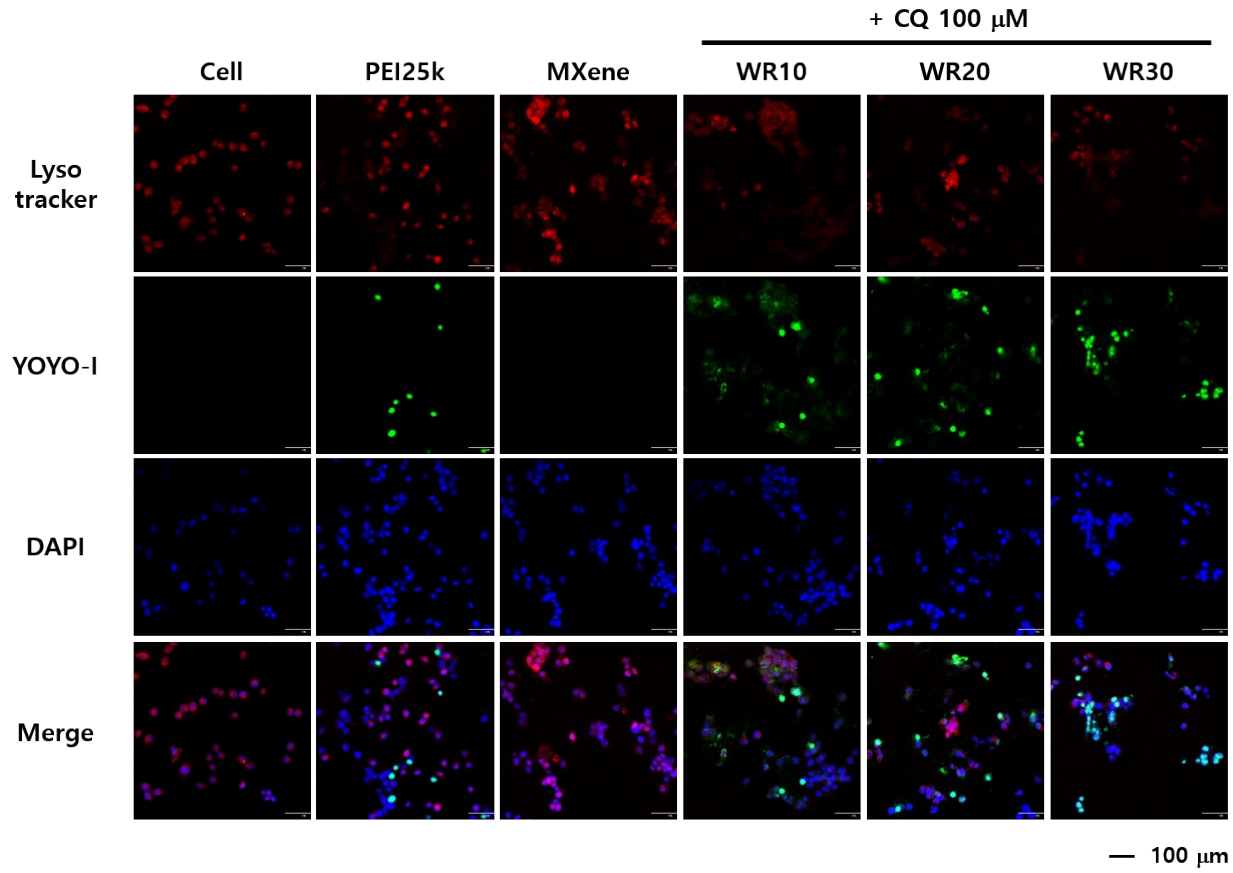


S11. Cellular uptake efficiency analysis of MP4/pDNA complexes by flow cytometry

Intracellular trafficking observation by confocal microscopy

HeLa cells were seeded in a confocal dish at a density of 3×10^5 cells per well and cultured until 70–80% confluency was reached. For the assay, MP4 (PEI : MXene = 20 : 1, w/w) /pDNA complexes were prepared with weight ratios (MP4 : pDNA) ranging from 10 to 30 in serum-free medium. PEI25k polyplexes (PEI25k : pDNA =1 : 1,w/w) and MXene complexes (MXene : pDNA = 10 : 1, w/w) were used as controls. pDNA used was pre-labeled with YOYO-1 dye at a ratio of one dye molecule per 50 base pairs of pDNA, and the labeling was performed at room temperature for 30 min in TE buffer. Sample solutions (1 μg pDNA/dish) were treated to the cells under serum-free conditions for 4 h after 30 min pretreatment of 100 μM chloroquine diphosphate salt (CQ). After washing twice with DPBS, cells were then stained with

Lysotracker red (1 μM) for 30 min, fixed with 4% paraformaldehyde for 10 min. Finally, 4′,6-diamidino-2-phenylindole (DAPI, 0.125 μg/mL) staining was performed for 10 min. Thereafter, 1 mL of DPBS was filled and cells were observed by confocal fluorescence laser scanning microscopy (K1-Fluo, Nanoscope Systems, Daejeon, Republic of Korea). Images were processed with FlowView software.



S12. Intracellular trafficking observation by confocal microscopy. Endolysosomes of cells were stained by Lysotracker (red). pDNA was labeled by YOYO-1 iodide (green). Cell nuclei were stained by DAPI (blue).