Cross-linked Iron Oxide Nanoparticles for Therapeutic Engineering and in Vivo Monitoring of Mesenchymal Stem Cells in Cerebral Ischemia Model

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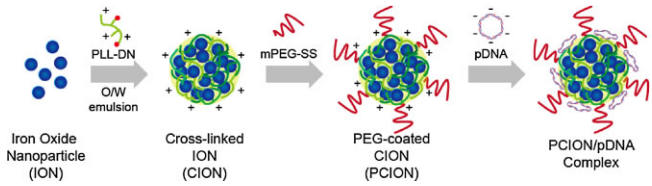
Poly(ethylene glycol)-coated cross-linked iron oxide nanoparticles (PCIONs) are developed for therapeutic engineering of mesenchymal stem cells (MSCs) and their monitoring via magnetic resonance (MR) imaging at a time. PCIONs successfully combine with plasmid DNA (pDNA) via ionic interaction. Accordingly, PCION/pDNA complexes mediate superior translocations of vascular endothelial growth factor (VEGF) pDNA into intracellular regions of MSCs under external magnetic field, which significantly elevate production of VEGF from MSCs. Genetically engineered MSCs are also clearly visualized via MR imaging after administration to rat cerebrovascular ischemia models, which enable tracking of MSCs migration from injected sites to injured ischemic area.



1. Introduction

Cerebrovascular diseases can result in stroke, which is the third major cause of death worldwide.[1] In particular, approximately 80% of all strokes are ischemic strokes, which are currently treated by thrombolytic therapy.[2] As an alternative and emerging approach for treatment, the cell therapy via transplantation of neural stem cells and

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mesenchymal stem cells (MSCs) has been greatly noticed.[3] However, several limitations of stem cell transplantation like mild therapeutic effects, unknown biological processing after injection and low survival rate have retarded successful clinical translation of cell based therapy for cerebrovascular diseases.[4] To improve therapeutic efficacy of MSCs as well as survival rate after implantation, a wide range of studies including genetic engineering of MSCs with therapeutic genes like plasmid DNA (pDNA) and siRNA have been reported.[3–5]

Due to the inherent poor transfection efficiency of primary cultured stem cells like MSCs, efficient ex vivo gene carriers are needed to transduce functional genes more safely and adjustably into target stem cells.[5,6] Not only efficient genetic engineering but also monitoring of therapeutic cells is crucial to assess the safety and therapeutic effect at the target site for clinical translation.[7] Iron oxide nanoparticles (IONs) have been greatly investigated, not only due to their biocompatibility but also superior functional versatilities including mediation of facile gene transfer via magnetofection and elevation of contrast for magneto-resonance (MR) imaging.[8] Magnetically mediated transfection has several advantages, including fast transfection within 1 h due to sedimentation of nanoparticles nearby cells in vitro. In addition, magnet-labeled cells, obtained by internalization of IONs, could allow magnet-derived retention and accumulation at target sites which could boost therapeutic effects. To elevate efficiency of magnet-derived gene transfer for genetic engineering of therapeutic cells, design and application for novel iron oxide based carriers including micelles, liposomes, and nanoparticles have been intensively studied.[9]

Among them, magnetic clusters can be a promising carrier system because they have greater magnetization per particle than single IONs while maintaining superparamagnetic properties.[8a,10] The elevated magnetization is critical for prompt and efficient response to an exterior magnetic field (MF). Previously, magnetic clusters coated with poly(ethylenimine) (PEI) as a crosslinker showed extremely enhanced cellular uptake by 6-fold for prostate cancer cells (PC3 cells) compared with single IONs. However, studies on the applications of magnetic clusters for genetic engineering of therapeutic MSCs in cerebral ischemia model have not yet been reported.

In this study, cross-linked IONs (CIONs) were designed to safely produce therapeutic MSCs expressing vascular endothelial growth factor (VEGF) via magnetically mediated gene transfection as well as selectively monitor engineered MSCs after injections at a time. According to our results, poly(ethylene glycol) (PEG)-coated cross-linked IONs (PCIONs) successfully combined with VEGF pDNA, and consequently enhanced the uptake and expression of therapeutic genes in MSCs under exterior MF. Furthermore, PCIONs enabled tracking of the MSC migration in rat cerebrovascular ischemia models via magnetic resonance (MR) imaging.

2. Experimental Section

2.1. Materials

IONs coated with oleic acid were supplied from National Creative Research Initiative Center for Oxide Nanocrystalline Materials and School of Chemical and Biological Engineering (Seoul National University, Seoul, Korea). Poly-L-lysine hydrobromide (PLL, MW 25 000), 3-(3,4-dihydroxyphenyl)propanoic acid (hydrocaffeic acid; MW 182.17), rhodamine B isothiocyanate (MW 536.08), methanol (MeOH), *N*,*N*-dimethylformamide (DMF) and Triton-X 100 were purchased from Sigma (St. Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, MW191.70) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and mPEG-succinimidyl-succinate (mPEG-SS; MW 2000) was obtained from Sunbio (Anyang, Korea). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), RPMI 1640 medium, Dulbecco's Phosphate Buffered Saline (PBS), penicillin, streptomycin and trypsin were purchased from Gibco-BRL (Grand Island, NY). Dialysis membranes were obtained from Spectrum (Houston, Texas, USA). All other chemicals and reagents were of analytical grade and used as received unless otherwise mentioned.

2.2. Synthesis of PLL-DN

Catechol-grafted poly-L-lysine (PLL-DN) was prepared by carbodiimide coupling chemistry between a primary amine group of PLL and a carboxylic acid group of hydrocaffeic acid. PLL (100 mg, 4×10^{-6} mol) dissolved in 2 mL of MeOH was added into the solution containing hydrocaffeic acid (55.2 mg, 350×10^{-6} mol) dissolved in 2 mL of DMF and EDC (65 mg, 350×10^{-6} mol) in 2 mL of MeOH. The reactants were stirred for 12 h, dialyzed against acidic HCl solution (pH 4) using a dialysis membrane (MW cut-off: 3000) for 2 d, and then freeze-dried. The degree of substitution in PLL with catechol groups was assessed by analysis of ^{1}H NMR spectroscopy (Bruker DRX 400 spectrometer operating at 400 MHz).

2.3. Preparation of PCIONs and PCION/pDNA Complexes

CIONs were prepared by oil-in-water (O/W) single emulsion/evaporation method according to the previous study.[8a] First, 10 mg of IONs in 1 mL of chloroform were mixed with 20 mg of PLL-DN in 1 mL of deionized water. The mixture was added into 10 mL of deionized water and emulsified using a tip-type sonicator (Branson Sonifier, Branson Ultrasonics Corp., tip diameter: 0.5 inches) with a duty cycle of 30 and output 3 for 5 min. The chloroform and unreacted PLL-DNs were eliminated by evaporation at 40 °C and filtration through an Amicon® Ultra-4 Centrifugal Filter (MW cut-off: 100 000), respectively.

To prepare PCIONs, remnant amine groups of PLL exposed on the surface of CIONs were reacted with mPEG-SS. The mPEG-SS (200 μg) dissolved in 1 mL of deionized water was reacted with 2 mg of CIONs in 3 mL of the solution for 24 h. To remove the remaining

mPEG-SS, PCIONs were purified using an Amicon Ultra-4 Centrifugal Filter. For production of rhodamine B-labeled PCIONs, 2 μg of rhodamine B isothiocyanate dissolved in 200 μL of deionized water was reacted with 200 μg of PCIONs. After 24 h, the reactant was rinsed with deionized water using an Amicon Ultra-4 Centrifugal Filter to remove unreacted dyes. The PCION/pDNA complexes were prepared by mixing of PCIONs and VEGF plasmid DNA (0.2 μg) at different PCION/pDNA weight ratios (0, 1, 2, 4, 8 and 16), and then incubated for 20 min at room temperature.

2.4. Physical Characterization of PCIONs and PCION/pDNA Complexes

The physical characterizations of PCIONs were performed using transmission electron microscopy (TEM) (Tecnai F20, Philips) and dynamic light scattering (DLS) instrument (Zetaplus, Brookhaven, NY, USA) equipped with a He-Ne laser at a wavelength of 632.2 nm. The DLS instrument was calibrated using a Nanosphere size standard (Duke scientific Corporation, CA, USA) with a multimodal particle size distribution pattern. The binding capacity of pDNA to PCION was determined by agarose gel retardation assay. The PCION/pDNA complexes formed at the desired weight ratios of PCION to pDNA (0, 1, 2, 4, 8 and 16) were loaded onto a 1% of agarose gel containing GelRed (Biotium Inc., Hayward, CA). Electrophoresis was performed with 100 V current for 30 min in 1 × TEA buffer solution [0.010 M tris(hydroxymethyl)aminomethane (Tris)/HCl, 1 vol% acetic acid, 1.0×10^{-3} M ethylenediamine tetraacetic acid (EDTA)]. The retardation of pDNA bands was visualized with an image analyzer equipped with a ChemiDoc gel documentation system (Syngene, Cambridge, UK). The data were representative of three independent experiments.

2.5. Intracellular Uptake of PCIONs into MSCs

The rhodamine B-labeled PCIONs (1 μg mL^{-1}) were treated into rat MSCs seeded at a density of 0.5×10^5 cells per well in a four-chamber culture slide. After incubation with or without external MF for 15 min, the cells were washed twice with PBS, and then fixed with 1% formaldehyde in PBS for 30 min. The fixed cells were visualized by confocal laser microscopy (LSM510, Zeiss Pascal, Jena, Germany). The experiments were independently carried out three times, and representative images were presented.

2.6. Measurement of VEGF Expression in MSCs Transfected by PCION/VEGF-pDNA Complexes

For in vitro transfection study, rat MSCs were seeded at a density of 1×10^5 cells per well in a 6-well plate. After 24 h of incubation, the culture medium was replaced with fresh serum-free medium. Cells were transfected with the PCION/VEGF-pDNA complexes at a PCION/pDNA weight ratio of 8 for 15 min with and without external MF at 37 °C. Then, the transfection medium was exchanged with fresh culture medium. After further incubation for 24 h, the conditioned medium was harvested to measure the amount of released VEGF from MSCs using an enzyme-linked immunosorbent assay (ELISA) kit for quantitative detection of VEGF protein (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. The data were presented as the mean ± standard deviation (SD) of triplicate samples for three independent experiments. Statistical analysis was carried out by means of one-way ANOVA, and a p-value less than 0.05 was considered statistically significant.

2.7. Characterization of MSC Phenotype under Magnetic Field

To characterize the phenotype of the genetically modified MSCs, the isolated MSCs and PCION/pDNA treated MSCs were analyzed by flow cytometry after staining with fluorescence-labeled monoclonal antibodies for cluster of differentiation 34-phycoerythrin (CD34-PE), CD45-PE, and CD90-fluorescein isothiocyanate (CD90-FITC) (BD Biosciences, San Diego, CA). The harvested cells (1×10^6 cells · mL^{-1}) were incubated with each antibody (0.5 mg mL^{-1}) for 15 min at room temperature and then washed twice with cold PBS. Fluorescent intensities of cells labeled with antibodies were measured by fluorescence-activated cell sorting analysis (Becton Dickinson, San Jose, CA). The data were representative of three independent experiments.

2.8. In Vitro MR Imaging of Engineered MSCs

For in vitro magnetic resonance imaging (MRI), 2.0×10^6 cells of MSCs were seeded in a 35 mm tissue culture dish and incubated with PCION/pDNA complexes for 15 min in the absence or presence of external MF. After cell harvesting in an Eppendorf tube, MR imaging were visualized using a 1.5 T clinical MRI instrument with a micro-47 surface coil (Intera; Philips Medical Systems, Best, The Netherlands). T2-weighted images were measured by the Carr-Purcell-Meiboom-Gill (CPMG) sequence: TR = 10 s, 32 echoes with 12 msec even echo space, number of acquisition = 1, point resolution of 156 × 156 μm, section thickness of 0.6 mm.

2.9. Prussian Blue Staining of Cultured MSCs

The existence of an intracellular iron was visualized by Prussian Blue staining using freshly prepared 5% potassium ferrocyanide in 5% HCl 1:1. Rat MSCs were plated at a density of 0.5×10^5 cells per well in a four-chamber culture slide 24 h prior to transfection. The MSCs transfected by the PCION/pDNA complexes with and without external MF were stained by Prussian Blue solution for 30 min. Then, the cells were washed with deionized water three times. For 3,30-diaminobenzidine (DAB) enhancement of Prussian Blue staining, the MSC culture slides were incubated in 3% H_2O_2 for 3 min prior to staining. Subsequently, Prussian Blue staining was performed, which were incubated in 0.05% DAB in PBS for 5 min and followed by another incubation in 0.05% DAB in PBS and 0.03% H_2O_2 for 3 min.

2.10. Animal Preparation and Ischemic Surgery

The use of animals was in accordance with the International Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Animal Research Committee of Yonsei University College of Medicine. Sprague-Dawley (SD) rats

were obtained from CoreTech (Seoul, Korea). Cerebrovascular ischemia models were prepared as follows. After periods of adaptation for one week, SD rats (250–280 g) were anesthetized by intraperitoneal injection of Rompun (10 mg kg^{-1}) and Zoletil (30 mg kg^{-1}, Yuhan Corp., Seoul, Korea). Common and external carotid arteries in neck were knotted with silk suture to block blood flow after galvanocautery and amputation of superior thyroid artery. After the detachment of occipital artery from internal carotid artery, galvanocautery and amputation were conducted and proximal popliteal artery was tied with a silk suture. Then, internal carotid artery was loosely tied and blood flow was blocked by a micro clip. Slight scratch was made at a split site of common carotid artery using a micro scissor and middle cerebral artery occlusion (MCAO) suture (Doccol Corporation, diameter: 0.35 ± 0.02 mm) was inserted into the scratch to be collusion with internal carotid artery. When MCAO suture reached to micro clip (ca. 24 mm), the micro clip was removed, and then the loosely tied silk suture was rapidly tightened. The incised skin was stitched up with silk suture and sterilized. After 2 h from blockade, MCAO suture was removed and operated rat was stabilized in a cage. Next day, the infarction site was evaluated by MR imaging.[11]

2.11. Cell Transplantation in Ischemic Stroke Rat Model

The PCION/pDNA complexes (weight ratio = 8) at an iron concentration of 25 μg mL^{-1} were placed in a tube containing serum-free DMEM medium. The solution containing PCION/pDNA was added to the MSCs (6×10^5 cells per well) and incubated for 2 h, and subsequently an equal volume of complete medium was added. After overnight incubation, the genetically modified rat MSCs in 5 μL solution were injected into SD rats using an infusion syringe pump 2 weeks after intraluminal MCAO. As a control group, an equivalent volume of PBS was injected into other rats (PBS group) ($n = 8$ per group).

2.12. Monitoring of Transplanted MSCs

For MR imaging studies, an animal coil (Shanghai Chenguang Medical Technologies Co., LTD, China) was plated at the head of each rat after anesthesia, and MR imaging was performed with a 3T clinical instrument (Philips, The Netherlands). To examine the extent of ischemic lesions, FLAIR images were visualized using the spin-echo technique (TR = 11 000 ms and TE = 125 ms) between the vertex of the head and the bottom of the brain. Other imaging parameters were as in the following: 0.7 mm slice thickness, in-plane resolution of 284×286 (voxel size of 0.0569 mm^3), and number of acquisitions = 1. The infarct area of brain slice was visualized by hematoxylin and eosin (H&E) staining, and the existence of iron-labeled cells was determined by Prussian Blue staining, as described in 2.9.

3. Results and Discussion

3.1. Synthesis of PLL-DN

PLL was adopted as a crosslinking material of IONs due to its high cationic charge and biocompatibility.[12] PLL has been facilitated as a coating material of citrate-IONs via ionic interaction.[13] For the strong attachment of PLL onto IONs via catechol-iron coordination, herein the amine group of PLL was modified with catechol group via carbodiimide coupling chemistry to prepare PLL-DN (Figure 1A).[14] The degree of catechol groups per single PLL in PLL-DN was quantitatively analyzed by ^{1}H NMR (Figure 1B). Approximately 17.4% of total amine groups on single PLL molecule were modified with the catechol groups.

3.2. Preparation and Characterization of PCIONs

As shown in Figure 1C, the catechol groups of PLL-DN were reacted with IONs due to the strong catechol-iron coordination during oil-in-water (O/W) emulsion process, which resulted in cross-assembled IONs (CIONs). To enhance serum stability of CION, the remnant amine groups of PLL-DN on the CION were further modified with mPEG-SS. In this study, it was hypothesized that PEG-coated CIONs (PCIONs) could successfully incorporate therapeutic target gene via ionic interaction, which might mediate efficient gene transfection into MSCs by external MF. The size and surface charge of PCIONs were examined by DLS (Figure 2A). PCIONs had the hydrodynamic radius of 185.8 ± 2.1 nm and the surface charge of $+28.6 \pm 8.6$ mV, which mainly attributed to the positive charge of PLL-DN molecules on the surface of IONs. According to the TEM analysis (Figure 2B), a spherical shape of PCIONs was observed, indicating that IONs with an average size of 11 nm were self-aggregated and cross-linked to form a nanocluster having diameters in the range from 150 to 180 nm, in good agreement with the DLS measurements.

3.3. Preparation of PCION/pDNA Complexes

To examine whether cationic PCIONs could be complexed with VEGF pDNA, a gel retardation assay was performed as shown in Figure 3A. After mixing VEGF pDNA with different amounts of PCIONs, the migration profile of VEGF pDNA in each mixture was visualized by agarose gel electrophoresis. Increasing amounts of PCIONs resulted in a gradual retardation of the mobility of the DNA band. The gel band of free DNA had finally disappeared at the PCIONs to VEGF pDNA weight ratios above 8, indicating that VEGF pDNA was successfully incorporated onto PCIONs. The VEGF pDNA complexation made no big difference in particle size (196.0 ± 10.4 nm), although the particle surface charge was almost neutralized at the PCION/pDNA weight ratio of 8 (0.3 ± 4.4 mV). The increased cationic surface charge density in PCIONs via PLL-DA crosslinking may provide the main driving force in the formation of polyelectrolyte complexes with VEGF

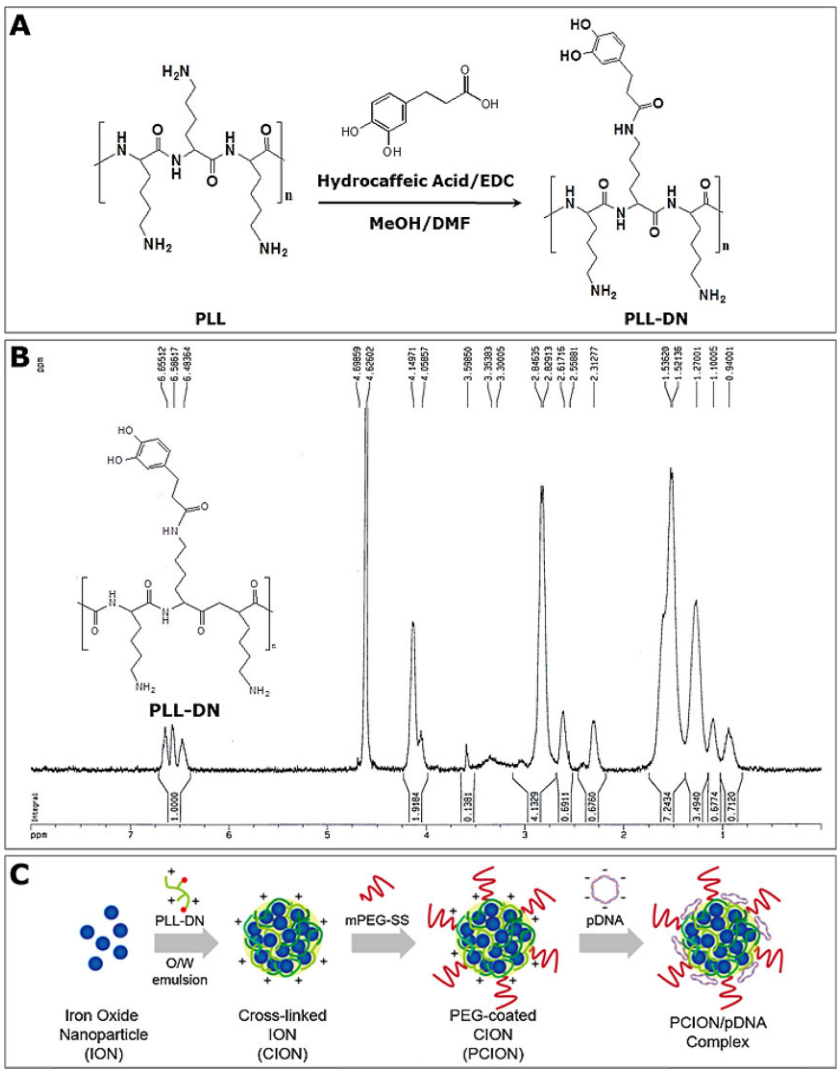

Figure 1. A) Synthetic scheme for the preparation of a catechol-grafted poly-L-lysine (PLL-DN). B) ^{1}H NMR spectrum of PLL-DN in D_2O. C) Schematic illustrations to prepare PCION/pDNA complexes.

pDNA through the electrostatic attractions between oppositely charged molecules.

3.4. Intracellular Uptake of PCIONs into Rat MSCs with Exterior MF

In the previous study, exterior MF (MagnetoFACTOR plate, Chemicell) could greatly increase intracellular uptake of IONs and ION-based carriers for cancer cells.[8a] Compared to fast-growing cancer cells, primary cultured MSCs undergo very slow proliferation, which are not apt for intracellular delivery of genetic materials using cationic carriers.[15] To assess whether MF could enhance intracellular uptake of PCIONs in rat MSCs, rhodamine-labeled PCIONs were incubated with MSCs under the influence of external MF for 15 min and intracellular PCIONs were visualized by confocal microscopy (Figure 3B). Without applying exterior MF to MSCs, only a negligible amount of PCIONs were taken up to MSCs despite their strong positive surface charge ($+28.6 \pm 8.6$ mV). In the applied external MF, however, much higher amount of PCIONs was internalized into MSCs. This result supports that PCIONs could be useful as simple and rapid magnetofection-based transfection reagents for genetic engineering of the primary cultured rat MSCs.

3.5. VEGF Production from Engineered MSCs

It has been known that VEGF can mediate therapeutic angiogenesis in clinic, which can be a promising strategy for ischemic diseases like stroke.[16] In addition, VEGF has been demonstrated to improve survival rates in various cell lines,

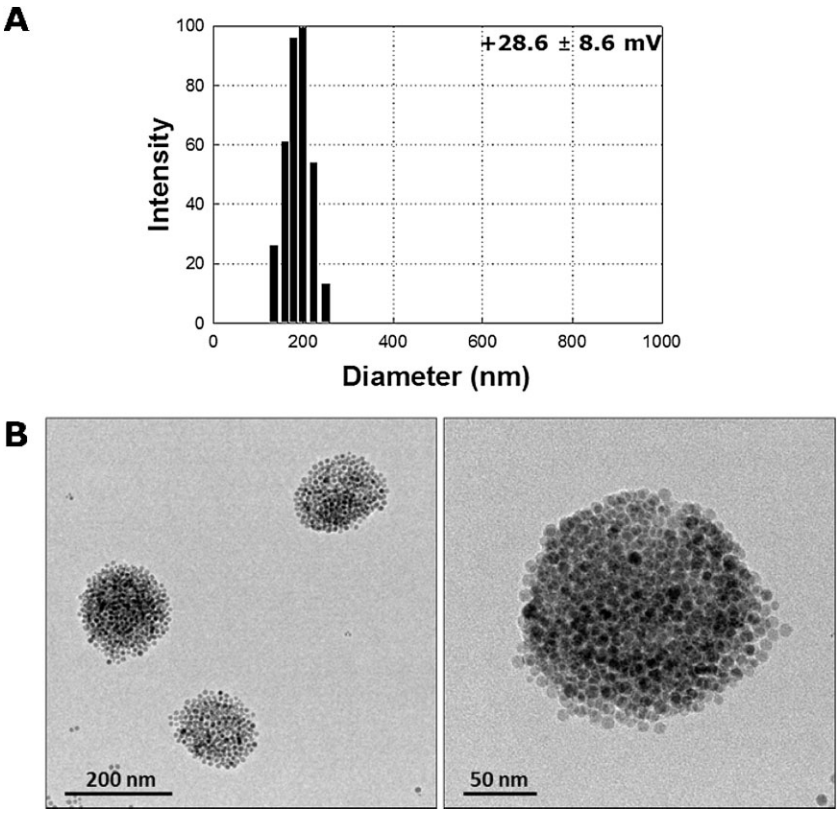

Figure 2. A) The hydrodynamic size and surface charge of PCIONs. B) TEM images of PCIONs.

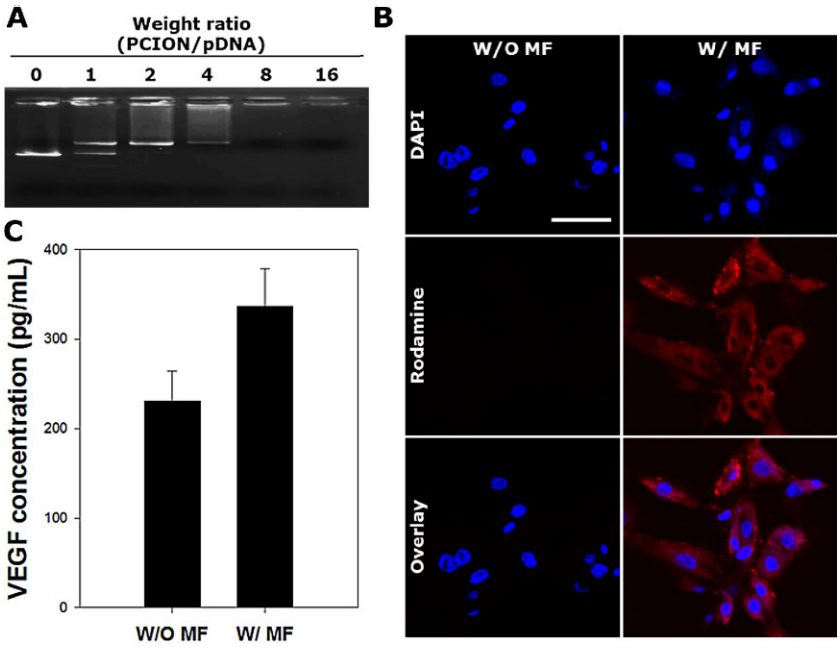

Figure 3. A) Migration of pDNA after incubation with PCIONs at different weight ratios. B) Intracellular uptake of rhodamine B-labeled PCIONs into MSCs without (W/O) or with (W/) an external magnetic field (MF) for 15 min (Scale bar: 20 μm). C) Amount of released VEGF from MSCs after transfection of PCION/pDNA complexes without and with MF. There was a statistically significant difference between W/O MF and W/MF at $p < 0.05$.

including stem cells, after their transplantation into damaged tissue.[17] Thus, herein VEGF-expressing plasmid DNA vector (VEGF-pDNA) was used to genetically modify rat MSCs. Although the biological mechanism of recovery of stroke patients by MSCs transplantation is still unclear, it is considered as one strong reason that growth factors such as VEGF and HGF released from MSCs might boost repair mechanisms.[18] Therefore, it might be crucial to induce MSCs to secrete lots of neurotrophic factors including VEGF for advanced therapeutic effects. To modulate rat MSCs to release a high amount of VEGF, the PCION/VEGF-pDNA complexes were transfected into MSCs by applying exterior MF. The released amount of VEGF protein from the genetically engineered MSCs was measured by ELISA (Figure 3C). The amounts of secreted VEGF from the MSCs treated by PCION/VEGF-pDNA without and with exterior MF were 228 ± 25.3 and 337 ± 79.6 pg mL^{-1}, respectively. According to the previous studies, the VEGF amounts released from intact MSCs were at very low levels, even below the detection range of ELISA.[19] These results indicate that magnetofection of PCION/pDNA complexes could significantly increase the transfection efficiency of VEGF gene into primary cultured rat MSCs, leading to elevated VEGF production compared with the innate VEGF release from MSCs. It should also be noted that MF is exposed to MSCs for only 15 min, which shows sufficient transfection efficiency of the PCION/VEGF-pDNA complexes probably due to high magnetization of clustered IONs.

3.6. Phenotypic Characterization of Genetically Engineered MSCs with Magnetic Field

In order to exclude a possibility of undesirable change in MSCs by adoption of alien gene and external stimulus like MF, phenotypic characteristics of rat MSCs were assessed after magnetofection of the PCION/VEGF-pDNA complexes. As shown in Figure 4, expression profile of MSC surface markers, that is, CD90 as a positive selectable marker, CD34 (an oval stem cell marker) and CD45 (a hematopoietic stem cell marker) as negative selectable markers, was examined in engineered MSCs before and after exposure of MF using fluorescence-activated cell sorting (FACS) analysis.[20] There were negligible

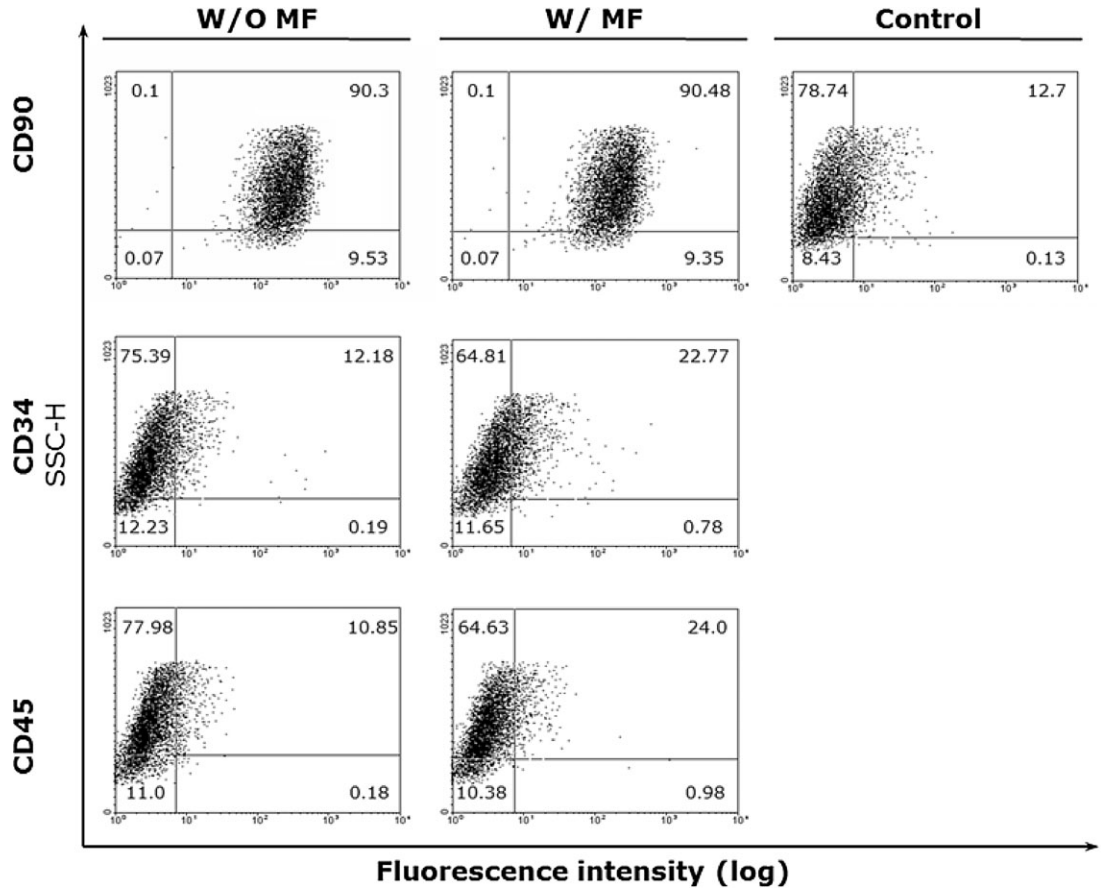

Figure 4. FACS analysis for expression profiles of stem cell markers.

differences in the expression patterns of both positive and negative selectable markers between MSCs before and after genetic modification under the influence of exterior MF. These results indicate that the magnetofection of the PCION/pDNA complexes does not influence the phenotypes of rat MSCs. Considering exposure of MF up to 10 tesla for 4 d to mammalian cells showed insignificant safety issues including cell growth, MF (0.3 tesla) used in this study seems to be free from side-effects like non-specific toxicity to MSCs.[21] In particular, several studies have been recently reported that genetically engineered MSCs can maintain transgene expression without additional genetic modification in vitro and in vivo.[22] Thus, this genetic stability is one of the positive attributes of MSCs for development of cell-based gene therapy.

3.7. In Vitro MR Imaging of MSCs Treated with PCION/pDNA Complexes

Magnet-labeled cells can have significantly higher contrast in MR imaging.[14b] The rat MSCs treated with the PCION/pDNA complexes were visualized using MR imaging (Figure 5, upper panel). As expected, cells exposed to MF showed a significantly darker image than cells without MF, suggesting that the PCION/pDNA complexes were successfully translocated into intracellular regions of MSCs by applying external MF and they could play the exact role in MR imaging. To further confirm that the higher contrast in MR image was attributed to the magnetofection of the PCION/pDNA complexes, Prussian Blue staining was adopted (Figure 5, lower panels). The blue-stained region was evidently observed in the PCION/pDNA treated MSCs with exterior MF, whereas MSCs without MF did not show any iron contents. Taken together, these results indicate that PCIONs located in intracellular region of MSCs via MF may improve the signal in T2-weighted images.

3.8. Monitoring of MSC Migration to Ischemic Area

MSCs modulated with PCION/pDNA were injected to the right lateral cerebral ventricle in a left middle cerebral artery (MCA) stroke rat model and visualized by MR imaging. Interestingly, a significant portion of injected MSCs was observed in the area of left cerebral infarction at 2 d post-injection, which suggests that the PCION/pDNA treated MSCs were efficiently migrated from the site of implantation (blue arrow) into the ischemic brain regions (red circle) (Figure 6A). In the previous study, MSCs labeled with PLL-IONP could be successfully and superiorly visualized by MR images at the injected site compared with those labeled with Endorem.[13] However, they could not observe migration and translocation of MSCs from injected site to target injury site. In this study, we could evidently observe that migration of injected MSCs to ischemic area in cerebral ischemia model. Migration of engineered MSCs was also confirmed by Prussian Blue staining in histological sections. As shown in Figure 6B, the magnet-labeled MSCs were observed as blue-stained region (black arrows) in the cerebral infarction (red inset), a right pallor area in H&E stained images (top right panel), not in the injection site (blue inset). According to the previous literature, MSCs move to ischemic region after injection through interactions between a chemokine, stromal derived factor-1 (SDF-1) and the chemokine receptor, CXCR4.[18] Our results show that PCION-based transfection methodology may be applicable for non-invasive tracking of injected cells in cell therapy to monitor the behavior of therapeutic target cells after transplantation. Unfortunately, the PCION/VEGF-pDNA transfected MSC treatment was insufficient to attenuate cerebral infarction in a MCA stroke rat model even with direct injection of the engineered MSCs into the infarct injury site. It is probably due to the low cell engraftment rate of trypsinized single cells after their transplantation in the unfavorable environment for cell survival. Previous studies revealed that transplantation of a group of cells by using microfabricated cell clusters or cell aggregates markedly improved cell survival rates and transplantation efficiencies compared to injection of single cells.[23] Since the assembled cell clusters maintain a supportive microenvironment during transplantation, the development of the PCION/VEGF-pDNA

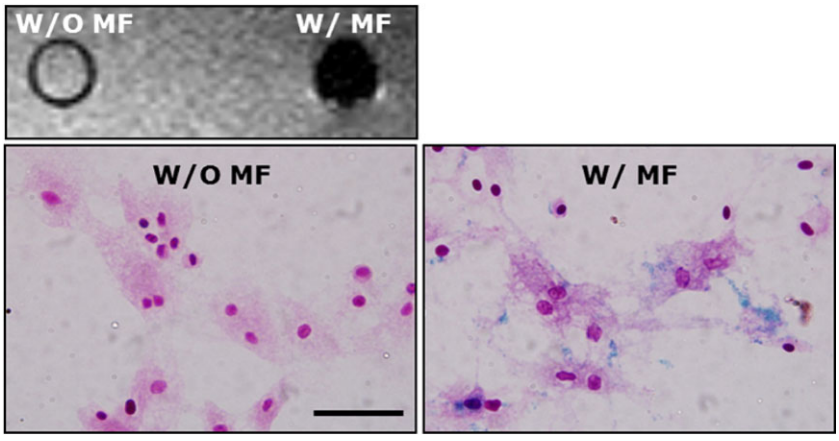

Figure 5. MR image (upper panel) and Prussian blue staining image (lower panel) of MSCs after treatment of PCION/pDNA complexes without and with MF (Scale bar: 20 μm).

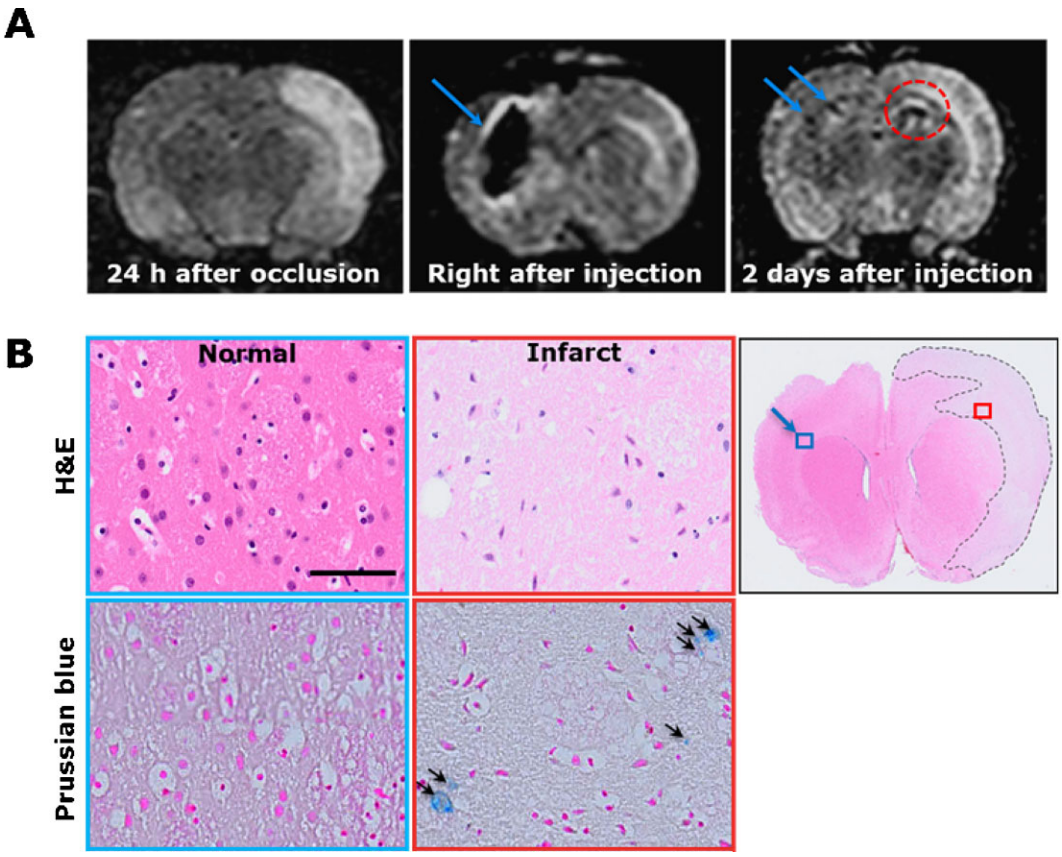

Figure 6. A) In vivo monitoring via MR imaging of rat cerebrovascular ischemia models before and after transplantation of MSCs transfected with PCION/pDNA complexes via magnetofection. B) H&E staining (upper panel) and Prussian blue staining (lower panel) of brain slice from rat cerebrovascular injury model 2 d after transplantation (Scale bar: 20 μm). The left and middle panels are enlarged images of blue and red insets of right panel, respectively.

transfected MSC clusters by a microfabrication technique may provide more therapeutic benefits. To achieve two goals, that is, stem cell tracking and cell-based gene therapy, simultaneously, the use of a group of engineered MSCs needs to be further considered.

4. Conclusion

In this study, novel PLL-based magnetic nanoclusters, PCIONs, were formulated for genetic engineering of MSCs via magnetofection. VEGF-expressing pDNA could be successfully delivered to MSCs by short time exposure of MF, which enabled significantly elevated release of VEGF from MSCs. The genetically engineered and magnet-adopted MSCs could be clearly visualized in vitro and in vivo, which allows monitoring their migration from injected site to ischemic area in cerebrovascular ischemia rat model. In addition, it is conceivable that efficiently adopted PCIONs could potentially mediate magnet-derived accumulation and retention at target location, which could also increase the accuracy of in vivo cell tracking. However, for potential clinical application of the PCION/pDNA complexes to cell tracking and cell-based therapy, use of an appropriate methodology for the fabrication of MSC clusters should be further considered to provide prompt and durable engraftment of the PCION/VEGF-pDNA transfected MSCs.

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