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Thermo-responsive polymeric nanoparticles for enhancing neuronal differentiation of human induced pluripotent stem cells

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Abstract

We report thermo-responsive retinoic acid (RA)-loaded poly(N-isopropylacrylamide)-*co*-acrylamide (PNIPAM-*co*-Am) nanoparticles for directing human induced pluripotent stem cell (hiPSC) fate. Fourier transform infrared spectroscopy and ^{1}H nuclear magnetic resonance analysis confirmed that RA was efficiently incorporated into PNIAPM-*co*-Am nanoparticles (PCANs). The size of PCANs dropped with increasing temperatures (300-400 nm at room temperature, 80-90 nm at 37 °C) due to its phase transition from hydrophilic to hydrophobic. Due to particle shrinkage caused by this thermo-responsive property of PCANs, RA could be released from nanoparticles in the cells upon cellular uptake. Immunocytochemistry and quantitative real-time polymerase chain reaction analysis demonstrated that neuronal differentiation of hiPSC-derived neuronal precursors was enhanced after treatment with 1-2 μg/ml RA-loaded PCANs. Therefore, we propose that this PCAN could be a potentially powerful carrier for effective RA delivery to direct hiPSC fate to neuronal lineage.

From the Clinical Editor: The use of induced pluripotent stem cells (iPSCs) has been at the forefront of research in the field of regenerative medicine, as these cells have the potential to differentiate into various terminal cell types. In this article, the authors utilized a thermo-responsive polymer, Poly(N-isopropylacrylamide) (PNIPAM), as a delivery platform for retinoic acid. It was shown that neuronal differentiation could be enhanced in hiPSC-derived neuronal precursor cells. This method may pave a way for future treatment of neuronal diseases.

Key words: Thermo-responsive nanoparticle; Poly(N-isopropylacrylamide)-*co*-acrylamide; Retinoic acid; Human induced pluripotent stem cells; Neuronal differentiation

Induced pluripotent stem cells (iPSCs) possess great potential for therapeutic applications involving cell replacement. Epigenetically reprogrammed iPSCs have been found to have self-renewal and pluripotent capacity similar to embryonic stem cells.[1,2] Recently, iPSC-derived neurogenesis has been studied for applications in the research of neurodegenerative diseases (e.g., Parkinson's disease, Alzheimer's disease, and ischemic stroke) and neurological disorders. Diverse approaches such as genetic modifications (viral vectors, mesoporous nanoparticles),[3,4] co-culture (with astroglial cells),[5] and bioreactors (rotary suspension system)[6] have been developed to promote neuronal differentiation of iPSCs for the purpose of therapeutic applications and neuronal disease modeling. In particular, the delivery of transcription factors regulating neuronal differentiation holds great potential for the development of new populations of neuronal cell lineages from iPSCs.

Among such transcription factors, retinoic acid (RA) has drawn attention for its capacity for neuronal differentiation of stem cells. A metabolic compound derived from vitamin A, RA is well-known to be involved in neural differentiation,[7] axon outgrowth,[8] and

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patterning of the neural plate and the neural tube.[9] For those reasons, RA has been the most commonly used morphogen to induce neurogenesis.[10–16] However, RA is unstable and rapidly degraded under physiological conditions. In addition, the solubility of RA is low in the aqueous phase.[17] Therefore, nanoparticle formulations facilitating stability, solubility, and cellular uptake of RA have been sought for stem cell engineering. For example, a RA-loaded polyethylenimine (PEI) nanoparticle formulation was previously developed to enhance neuronal differentiation of neural stem cells or embryonic stem cells.[10,18,19]

Thermo-responsive polymeric nanomaterials produce great benefits for stem cell engineering. Poly(N-isopropylacrylamide) (PNIPAM), one of the representative thermo-responsive polymers, has been applied in various forms of nanogels, injectable hydrogels, particles, and surface coating films for regulating stem cell differentiation and expansion.[20–26] Given its thermo-responsive properties, PNIPAM-based biomaterials could be especially useful as delivery platforms to direct stem cell fate. For instance, the self-assembled PNIPAM-*co*-acrylic acid (AA) nanogel has been used as a gene delivery carrier for human mesenchymal stem cells.[20] PNIPAM-*co*-AA nanogel encapsulated with PEI was internalized into human mesenchymal stem cells to release plasmid DNA within the cytosol,[20] suggesting that it could efficiently deliver specific genes into stem cells.[27,28] It has also been reported that thermo-responsive PNIPAM-based delivery systems can control the release of several growth factors.[22,25,29]

One of the interesting features of PNIPAM is its lower critical solution temperature (LCST) in aqueous solutions.[30,31] Because PNIPAM chains undergo rapid structural changes like coil-to-globule transition above the LCST,[32–34] PNIPAM particles abruptly shrink.[35,36] Therefore, hydrophobic drugs such as RA can be temporally released from PNIPAM-based biomaterials above the LCST. Although previous approaches have investigated stem cell differentiation using PNIPAM-based biomaterials, there are no studies to date that have tested the delivery of RA using thermo-responsive PNIPAM-based nanoparticles for iPSC differentiation.[37]

Herein, we first report the efficacy of RA-loaded thermo-responsive PNIPAM-based nanoparticles for directing neuronal lineage differentiation from hiPSCs. Thermo-responsive PNIPAM-*co*-acrylamide (PNIPAM-*co*-Am) nanoparticles were synthesized for efficient intracellular delivery of RA and then applied to neuronal precursor cells derived from hiPSCs. Our results demonstrate that RA-loaded PNIPAM-*co*-Am nanoparticles (PCANs) significantly enhance neuronal differentiation of hiPSC-derived neuronal precursor cells.

Methods

Synthesis of PCANs

To synthesize PCANs, 500 mg NIPAM (Sigma-Aldrich, St. Louis, MO, USA), 13.5 mg N,N′-Methylenebisacrylamide (MBA, Sigma-Aldrich), 100 mg sodium dodecyl sulfate (SDS, Sigma-Aldrich), and 12.5 mg Am (Sigma-Aldrich) were added to 50 ml distilled water. The solution was mixed with nitrogen gas at room temperature for 20 minutes. Potassium persulfate (37.5 mg, KPS, Dae Jung, Siheung, Gyonggi, Korea) was added to initiate polymerization. The solution was then allowed to react with nitrogen gas at 70 °C for 5 hours. The polymerization solution was dialyzed against distilled water for 7 days to remove unreacted monomers. The distilled water was replenished every day during dialysis. The polymerization solution was subsequently lyophilized.

Preparation of RA-loaded PCANs

PCANs were dissolved in distilled water (10 mg/ml). RA (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) to prepare a stock solution (5 mg/ml). The RA solution was subsequently added drop wise to the PCAN solution. The combined solutions were stirred at room temperature in the dark for 24 hours. The RA-loaded PCAN solution was dialyzed against distilled water for 24 hours using a dialysis membrane (MWCO 6,000-8,000, Spectrum Laboratories, Inc., CA, USA) to remove unreacted RA. After dialysis, RA-loaded PCAN solutions were lyophilized (FDU-1200, Tokyo Rikakikai Co., LTD., Miyagi, Japan) at -50 °C for 4 days.

Preparation of fluorescently labeled RA-loaded PCANs

RA-loaded PCANs were dissolved in distilled water. Fluorescein isothiocyanate-dextran solution (FITC-dextran, Sigma-Aldrich) was added drop wise to the nanoparticle solution. The solution was stirred at room temperature in the dark for 12 hours. The nanoparticle solution was dialyzed against distilled water for 24 hours using a dialysis membrane. After dialysis, the nanoparticle solution was lyophilized to obtain a powder.

Transmission electronic microscopy (TEM)

PCANs were dispersed in distilled water using a sonicator (Vibra-Cell, Sonics, Newtown, CT, USA), while being chilled in an ice bath. The dispersion was applied to copper grids and left to evaporate at room temperature. The prepared samples were scanned using a TEM (JEM 1010, JEOL Ltd., Tokyo, Japan) at a voltage of 80 kV.

Fourier transmittance infrared (FTIR) spectra

PCANs and RA-loaded PCANs were dispersed in ethanol. The dispersion was dropped into the sample holder of the FTIR spectrometer. The solvent was subsequently left to evaporate at room temperature. FTIR spectra of PCANs and RA-loaded PCANs were recorded using an FTIR spectrometer (NICOLET AVATAR 330, Thermo Nicolet, Madison, WI, USA).

^{1}H nuclear magnetic resonance (NMR) spectra

PCANs and RA-loaded PCANs were dispersed in DMSO-d_6. ^{1}H NMR spectroscopy of nanoparticles was analyzed using a Unity-Inova instrument (Agilent Technologies, Santa Clara, CA, USA) at 500 MHz.

Dynamic light scattering (DLS)

Lyophilized PCAN powder was dispersed in distilled water and sonicated in an ice bath. The dispersion, placed in a DLS cuvette, was heated at a rate of 1 °C/min in the temperature range