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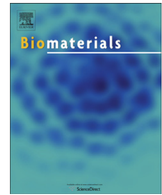

The effect of electrically charged polyion complex nanoparticle-coated surfaces on adipose-derived stromal progenitor cell behaviour

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Surface characteristics of biomaterials such as wettability, rigidity, roughness, and electrical charge affect the fate of transplanted cells such as progenitor cells or stem cells for use in regenerative medicine. Of these, the effects of surface electrical charges on cellular behaviour such as adhesion, proliferation, and differentiation are not well understood. We prepared precisely charged culture surfaces ranging from −28 mV to +21 mV, simply by surface deposition of polyion complex nanoparticles prepared by mixing a positively charged thermoresponsive homopolymer, poly(*N*,*N*-dimethylaminoethyl methacrylate), with negatively charged plasmid DNA at various charge ratios. Drastic morphological changes of adipose-derived vascular progenitor cells were generated on the positively charged surface of organized forms at +19 mV. Capillary-like networks or single aggregates of these cells were selectively created depending on cell seeding density. Our findings offer new insights that may aid develop stem cell-processing techniques for use in regenerative medicine.

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1. Introduction

Stem cell research is developing rapidly, and advances in the generation of pluripotent stem cells using embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) as well as the discovery of multipotent adult somatic progenitor cells (SPCs) suggests that the application of stem cell-based therapies in medical fields such as regenerative medicine and drug discovery is highly expected [1–3]. Regenerative medicine is based on tissue engineering technology, in which the transplanted tissues are prepared using three elements: stem cells, biomaterials, which act as cellular scaffolds, and cellular growth factors. One of the most difficult processes in this tissue engineering process is proper control of the proliferation and differentiation of stem cells (ESCs, iPSCs, or SPCs) in order to obtain a sufficient number of functional cells. Maintaining the quality of differentiated transplant cells is also crucial because these cells change or lose their phenotypes depending on the physiological and chemical properties of the biomaterials used in the cell culture substrate [4,5].

It has been recognized that surface characteristics of biomaterials such as rigidity [6,7], wettability (hydrophilicity/hydrophobicity) [8,9], roughness [10,11], electrical charge [12,13], and chemistry [14,15] affect cell functions such as adhesion, proliferation, and differentiation. For example, Yang et al. reported that the rigidity of poly(sodium styrene sulphonate) hydrogel significantly affected the antithrombotic function of human coronary artery endothelial cells following their expression of endothelial cell-specific genes [6]. On the other hand, the influence of surface electrical charge on cellular attachment, proliferation, and differentiation have also been confirmed using surface ionization techniques such as corona discharge treatment or using ionized hydrogels prepared by polymerization of charged monomers [12,13,16]. However, it is difficult to quantitatively vary surface charge densities in such methods without altering other surface properties such as rigidity and chemistry. Therefore, cell behaviour on charged surfaces is inadequately understood at present.

Recently, we developed a bioactive surface having antithrombotic or gene transfectable properties by surface immobilization of antithrombotic heparin or plasmid DNA (pDNA) using poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMA), which is a surfactant polymer with both cationic and thermoresponsive characteristics [17,18]. In this surface modification system, the nanoparticles of a polyion complex (PIC) were electrostatically formed by mixing PDMAEMA with negatively charged anionic heparin or pDNA. They get deposited on cell culture surfaces via the hydrophobic interaction between PIC and the culture surface by the

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thermal modulation of hydrophilic/hydrophobic transition of PDMAEMA. Therefore, in our PIC-based surface modification, we could easily modulate surface charge characteristics simply by changing the mixing ratio of PDMAEMA and anionic polymers.

To date, few studies have reported the effects of surface electric charge characteristics on the attachment, growth, and differentiation of ESCs, iPSCs, and SPCs. Therefore, in this study, we prepared surfaces having various charge characteristics with wide range from negative to positive by using PIC nanoparticles of cationic PDMAEMA and anionic pDNA at various mixing ratios. The behaviour of adipose-derived stromal progenitor cells (ADSCs) seeded on these charged surfaces was observed.

2. Materials and methods

2.1. Materials

N,N-Dimethylaminoethyl methacrylate (DMAEMA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). The other chemical reagents were also commercially obtained from Wako. DMAEMA was distilled under reduced pressure before use to remove the stabilizer. The other reagents were purified before use as required.

2.2. General methods

^{1}H NMR spectra were recorded in deuterium oxide (D_2O) using a 300 MHz NMR spectrometer (Gemini 300; Varian, Palo Alto, CA) at room temperature. Gel permeation chromatography (GPC) analyses using *N,N*-dimethylformamide as a solvent were carried out using an HPLC-8320 instrument (Tosoh, Tokyo, Japan) using Tosoh TSKgel SuperAW-4000 and SuperAW-5000 columns. The columns were calibrated with narrow distribution poly(ethylene glycol) standards.

2.3. Synthesis of PDMAEMA

PDMAEMA was synthesized according to the procedure described in our previous report [18]. Briefly, DMAEMA (7.0 g; Tokyo Kasei, Tokyo, Japan) was poured into a glass tube (35 × 65 mm; Maruemu Co., Osaka, Japan) under N_2 gas atmosphere and irradiated for 21 h by using 18-W fluorescent light (FCL20BL; NEC, Tokyo, Japan). After irradiation, re-precipitation was carried out 6 times with chloroform solution in hexane (Kanto Chemical, Tokyo, Japan). The final precipitate was dried under reduced pressure to obtain PDMAEMA (4.3 g, 61.4% conversion). The molecular weight of PDMAEMA was determined to be 9.7×10^4 g mol^{-1} (polydispersity: 4.1) by GPC analysis. ^{1}H NMR: δ 0.8–1.2 ppm (br, $-CH_3$), 1.6–2.0 (br, $-CH_2-CH_3$), 2.2–2.4 (br, $N-CH_3$), 2.5–2.7 (br, CH_2-N), 4.0–4.2 (br, $O-CH_2$).

2.4. Preparation and characterization of PIC

A saline solution (20 μl) of PDMAEMA were dissolved in a DNase-free pure water (Invitrogen, CA, USA) solution (30 μl) of the firefly luciferase-encoding plasmid DNA (pGL3-control vector; Promega, WI, USA) or Cy3-labelled plasmid DNA (*Label* IT® plasmid delivery control-Cy3; Mirus, WI, USA) (concentration: 300 μg/ml) to obtain polymer/DNA ratios from 1 to 16, which corresponded to cation/anion (C/A) ratios. The solutions (total volume, 50 μl) were then mixed using a pipette to generate PIC.

The mean diameters and *z*-potentials of the PIC in a saline solution of the same concentration as that used for cell culture experiments were determined by employing dynamic light scattering (DLS) on Zetasizer Nano S (Sysmex, Kobe, Japan) equipped with a 10-mW He–Ne laser. The data are presented as means ± S.D. ($n = 5$).

2.5. Surface characterization

An aqueous solution of the PIC (50 μl; 160 ng DNA at 20 μg/ml) was diluted with 150 μl of saline, and then added into each well of a 96-well polystyrene (PS) dish (Asahi Glass, Tokyo, Japan). After incubation at 37 °C for 6 h, the culture surfaces were observed by AFM (SPM-9700; Shimadzu, Kyoto, Japan) and fluorescent microscopy, respectively.

The *z*-potential of the PIC-coated PS surface was measured with an ELSZ-1000Z electrophoretic light scattering spectrophotometer (Otsuka Electronics, Osaka, Japan). The PIC having C/A ratio of 1–16 in 580 μl of a saline solution were added to PS culture plates (Asahi Glass) that were cut into 17 mm × 32 mm size and dried at 40 °C for 24 h. After drying, the plates were placed at the bottom of electrophoretic cells filled with distilled water containing surface charge-neutralized PS latex particles, and then, *z*-potential was measured at 37 °C.

2.6. Cell preparation

ADSCs were isolated from rat fat tissue by the method described in our previous study [18]. Briefly, approximately 1 g of fat tissue was obtained from its subcutaneous fatty layer and digested using 0.1% collagenase type I solution (Wako) at 37 °C for 1 h with gentle agitation. After filtering the digest through a 100-μm nylon mesh (BD Biosciences, NJ, USA) and centrifuging it at 1300 rpm for 4 min, the cell pellet was collected. The cell pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen, Carlsbad, CA) containing 10% foetal bovine serum (Hyclone Laboratories, Logan, UT), penicillin (200 units/ml; ICN Biomedicals, Aurora, OH), and streptomycin (200 mg/ml; ICN) (growth medium; GM). Cell suspensions were then filtered through a 70-μm nylon mesh (BD Biosciences). The cells were placed on a dish (55 cm^2; Asahi Glass) with the growth medium, and cultured in an atmosphere of 5% CO_2 at 37 °C. When the culture was nearly confluent, it was harvested and subcultured at 1.0×10^4 cells/cm^2. The cells were used for experiments before they reached the third passage. Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (CC-2517; Walkersville, USA), and cultured in endothelial cell basal medium supplemented with 2% FBS and endothelial growth supplements (endothelial medium; EM, CC-3124, Lonza).

2.7. ADSC and HUVEC culture

ADSCs and HUVECs were cultured on the PIC-coated culture surface. Briefly, an aqueous solution of PIC (50 μl; plasmid concentration, 20 μg/ml) having C/A ratios from 1 to 16 was diluted with 150 μl of saline, and then added into each well of 24-multiwell dishes. After incubation at 37 °C for 6 h, the ADSCs and HUVECs (0.8–3.2×10^5 cells/well) in 1.0 ml of GM and EM respectively were seeded and cultured in an atmosphere of 5% CO_2 at 37 °C for 2–10 days observed with optical microscope.

2.8. Capillary formation assay

Tube formation assays for ADSCs and HUVEC were performed using Matrigel™ basement membrane matrix (10.2 mg/ml; BD Biosciences) as a positive control. A 24-multiwell dish was coated with 100 μl of Matrigel™ in each well. After incubation at 37 °C for 3 h, ADSCs and HUVECs suspended in GM and EM, respectively, were seeded to each well with 8.0×10^5 cells/well. The culture was incubated in an atmosphere of 5% CO_2 at 37 °C for 2–10 days before being observed with an optical microscope.

2.9. Determination of rat VEGF production

The concentration of rat VEGF in cell culture supernatant was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine Immunoassay Kits; R&D Systems, Minneapolis, MN). The concentration was expressed as the production of VEGF per 10^5 cells at the time of harvest.

3. Results

3.1. Surface modification by PIC

As demonstrated in our previous study, the diameter of PDMAEMA particles in water was approximately 72 ± 4 nm [18], and it was increased more than two-fold when mixed with a pGL3-control pDNA solution, indicating the formation of PIC of PDMAEMA and pDNA (Fig. 1A). The diameter of the PIC increased with the amounts of PDMAEMA ranging from 116 ± 1 nm to 222 ± 10 nm for C/A ratios of 2 and 16, respectively. On the other hand, a significantly larger particle diameter of the PIC (357 ± 79 nm) was observed at a C/A ratio of 1, in which the number of positive and negative charges were equal.

Although *z*-potential of the PIC had a negative charge of approximately −30 mV at the lowest C/A ratio of 1, it increased drastically and shifted to a positive charge of approximately +19 mV at C/A ratios of 2 (Fig. 1B white symbol). A C/A ratio higher than 2 did not significantly affect *z*-potential value, indicating saturation of positive charges surrounding the PIC particles. The *Z*-potential of PIC-coated PS surfaces increased gradually with C/A ratio from negative (−28 mV) to neutral (±0 mV) and positive charges (+19 mV) for C/A ratios of 1, 2, and 4, respectively (Fig. 1B; black symbol). The positive charge on the surface was saturated at around +20 mV when the PIC having a C/A ratio higher than 4 was coated.

The topography of the PIC-coated PS surface was observed using AFM. At relatively low C/A ratios of 2 and 4, AFM images showed

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