Electronic Supplementary Information

Thermally-modulated cell separation columns using a thermoresponsive block copolymer brush as a packing material for the purification of mesenchymal stem cells

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S.1 Cell culture

Cell culture was performed based on the following procedure using cell culture mediums (Table S1).

MSCs were cultured on a 100-mm tissue culture polystyrene dish (TCPS) at 37°C in 5% CO_2 using a CO_2 incubator (9000EX, Wakenbtech, Kyoto, Japan). The cell culture medium was changed every three days. At 80% confluency, the medium was removed by aspiration. Then, the cells were rinsed with PBS (3 mL), and trypsin solution (4 mL) was added to the dish. The dish was incubated for 2 min. After the detachment of the cells from the dish, the cell culture medium (4 mL) was added to the dish, and the cell suspension was collected to a centrifuge tube. Centrifugation was performed at 1000 rpm for 5 min. The supernatant was removed, and the medium was added to the cells. 10 μL of the cell suspension were collected, and the cells in the cell suspension were counted. The cells were seeded to 3700 cells/cm^2 to the 100-mm TCPS for the passage culture.

NHDFs were cultured in a similar procedure to that of MSCs using the cell culture medium of NHDF (Table S1), except that centrifugation was performed at 1200 rpm for 3 min. The cells were seeded to 3500 cells/cm^2 to the other 100-mm TCPS for the passage culture.

Jurkat was cultured using the cell culture medium for Jurkat (Table S1) at 37°C in 5% CO_2 using a CO_2 incubator, and the medium was changed every three days. Passage culture was performed for the seeding cells (1.0×10^6 cells/mL).

BM-CD34$^+$ was cultured using a cell culture medium (Table S1) at 37°C in 5% CO_2 using a CO_2 incubator, and the medium was changed every three days. Passage culture was performed once for the seeding cells (1.0×10^6 cells/mL).

Table S1. Cell culture media.

Cells	Culture media	Additives
Mesenchymal Stem Cells: MSC	DMEM, low glucose, pyruvate	10% FBS 1% Pen-Strep 3 ng/mL bFGF
BM-CD34$^+$	X-VIVO™ 20 Hematopoietic Growth Medium	ITES 5 mL 25 ng/mL SCF 50 ng/mL TPO 50 ng/mL Flt-3 Ligand
Normal human dermal fibroblasts: NHDF	DMEM, high glucose	10% FBS 1% Pen-Strep 1% NEAA
Jurkat	RPMI medium 1640	10% FBS 1% Pen-Strep 1% NEAA

S.2 Zeta potential measurement

The zeta potentials of the cells were measured to investigate their electrostatic properties. One mL of PBS (1 mL; pH 7.4, 0.1 M) was diluted to 10 mL. Then, sucrose was added to the solution, and the concentration became 0.25 M. The cells were added to the prepared solution at a density of 1.0×10^5 cells/mL. The zeta potential was measured using an ELSZ2KOP zeta potential analyzer (Otsuka Electronics, Osaka).

Table S2. Zeta potential of the cells.

Cells	Zeta potential (mV) [a)]
Mesenchymal Stem Cells: MSC	−24.5
BM-CD34$^+$	−6.70
NHDF	−3.3
Jurkat	−2.5

a) Measured by zeta potential analyzer.

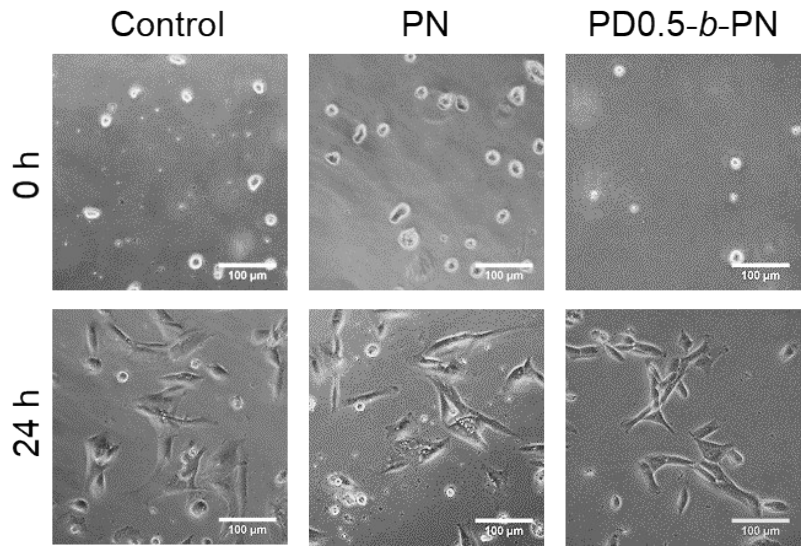

Fig. S1. Cell adhesion behavior of the recovered cells. Scale bar: 100 μm

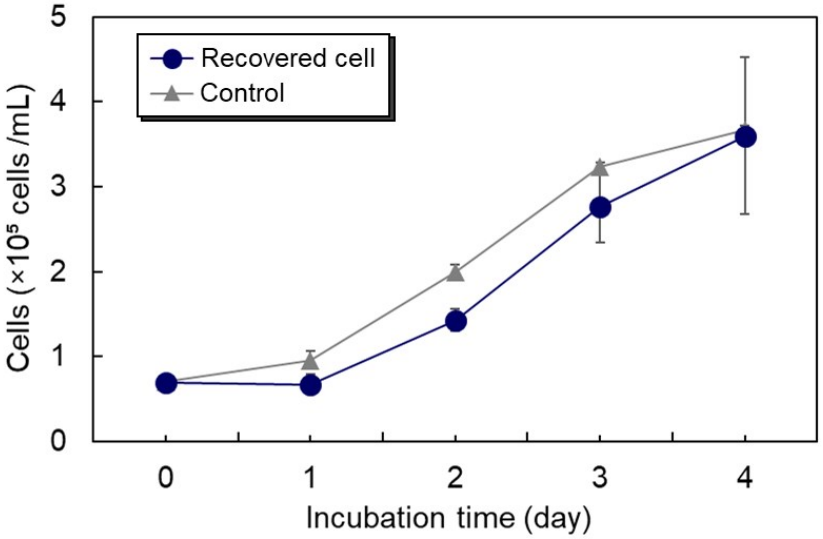

Fig. S2. Cell proliferation curve of the eluted mesenchymal stem cell (MSC) from PD0.5-*b*-PN.

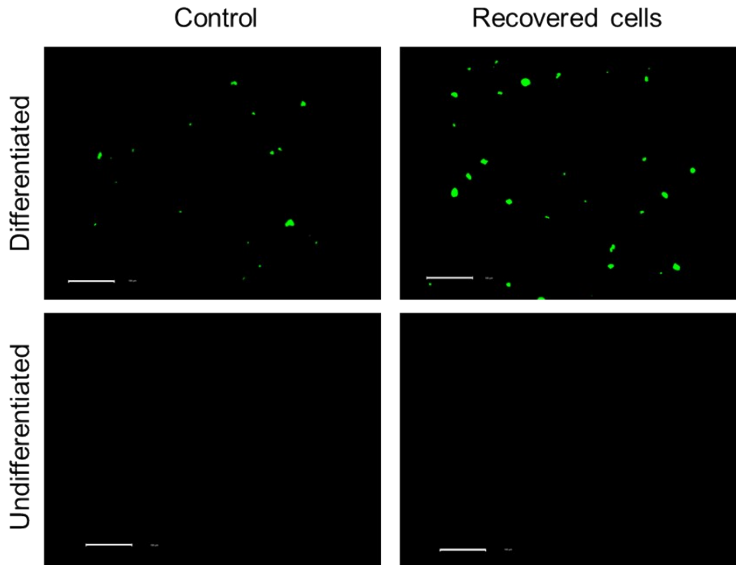

Fig. S3. Immunofluorescent staining of osteocalcin in MSCs after osteogenic differentiation. Scale bar: 100 μm