[0105]

First, a warmed agarose solution (Lonza agarose, 2.5% agarose/physiological saline) was poured into the mold. Next, the mold was cooled to room temperature, and after gelation of the agarose, the agarose microwells were removed from the mold. The agarose microwells were transferred to a 12-well polystyrene plate for cell culture, and a medium (DMEM/F12) was added in the vicinity of the agarose microwells to immerse the agarose microwell plate therein. The plate was placed in an incubator (37°C, 5% CO_2) for one night or more to equilibrate the agarose microwell plate with the medium in its vicinity. Thus, agarose microwells having 256 wells, each of which had a cylinder part (diameter: 400 μm) and a hemispherical bottom, were obtained. The above operation was performed under aseptic conditions in a clean bench.

[0106]

Formation of Aggregates, and Differentiation Induction into Pancreatic Progenitor Cells

iPS cells (253G1, obtained from Riken Cell Bank) were cultured in E8 medium (Thermo Fisher Scientific) for 3 to 4 days using a culture container coated with Geltrex (Thermo Fisher Scientific). After treatment using TrypLE (Thermo Fisher Scientific) under 70 to 80% confluent conditions, the cells were collected as single cells. The cells were suspended in E8 medium containing 10 μM Y-27632 (ROCK inhibitor, Wako Pure Chemical Industries, Ltd.), and seeded at 2500 cells/well on average in the 256-well agarose microwell plate, which was prepared as described above and placed in one well of the 12-well polystyrene plate.

[0107]

After the agarose microwell plate was left to stand for 10 minutes to precipitate the cells in the bottom, a medium (E8 medium + ROCK inhibitor) was added in the vicinity of the agarose microwell plate to immerse the plate in the medium. The cells were cultured at 37°C with 5% CO_2 for 24 hours (5% CO_2, 37°C) to

aggregate the cells, and then cultured for a certain period of time to induce their differentiation. Specifically, medium replacement was performed in such a manner that the medium was sucked out every day, and a new medium was added, as described below. In addition, the medium composition was changed on predetermined days. The medium composition and the number of days of culture in each medium were determined according to the description of A Rezania et al. Reversal of diabetes with insulin producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014 Nov; 32 (11): 1121-33.

First Stage (3 days)

MCDB131 (Thermo Fisher Scientific) + 1.5 g/L $NaHCO_3$ (Nacalai Tesque, Inc.) + 0.5% fat-free BSA (Wako Pure Chemical Industries, Ltd.) + 2 mM GlutaMax (Thermo Fisher Scientific) + 10 mM D-Glucose (Nacalai Tesque, Inc.) + 3 μM CHIR99021 (Tocris Bioscience) + 100 ng/mL Activin A (R&D Systems) (CHIR99021 was added to the medium only on the first day).

Second Stage (2 days)

MCDB131 + 1.5 g/L $NaHCO_3$ + 0.5% fat-free BSA + 2 mM GlutaMax + 10 mM D-glucose + 50 ng/mL fibroblast growth factor 7 (FGF-7, PeproTech) + 0.25 mM ascorbic acid (Sigma-Aldrich)

Third Stage (2 days)

MCDB131 + 1.5 g/L $NaHCO_3$ + 0.5% fat-free bovine serum albumin (fat-free BSA) + 1/200 ITS supplement (Thermo Fisher Scientific) + 2 mM GlutaMax + 20 mM D-glucose + 50 ng/mL FGF-7 + 0.25 μM SANT-1 (Wako Pure Chemical Industries, Ltd.) + 0.1 μM LDN193189 (Wako Pure Chemical Industries, Ltd.) + 1 μM retinoic acid (Sigma-Aldrich) + 0.2 μM TBP (PKC activator; Catalog No. 565740; EMD Chemicals Inc.) + 0.25 mM ascorbic acid

Fourth Stage (2 days)

MCDB131 + 1.5 g/L $NaHCO_3$ + 0.5% fat-free BSA + 1/200 ITS supplement + 2 mM GlutaMax + 20 mM D-glucose + 50 ng/mL FGF-7 + 0.25 μM SANT-1 + 0.2 μM LDN193189 + 0.1 μM retinoic acid + 0.1 μM TBP + 0.25 mM ascorbic acid

TBP: (2S,5S)-(E,E)-8-(5-(4-(trifluoromethyl)phenyl)-2,4-

Differentiation Induction into Pancreatic Progenitor Cells Derived from Other iPS Cell Lines

454E2 line (obtained from Riken Cell Bank), RPChiPS771-2 line (ReproCELL, Inc.), and P11025 line (Takara Bio, Inc.) were used as human-derived iPS cells.

[0126]

The 454E2 line was cultured in E8 medium (Thermo Fisher Scientific) for 3 to 4 days using a culture container coated with Geltrex (Thermo Fisher Scientific). After treatment using TrypLE (Thermo Fisher Scientific) under 70 to 80% confluent conditions, the cells were collected as single cells. The cells were suspended in E8 medium containing 10 μM Y-27632 (ROCK inhibitor, Wako Pure Chemical Industries, Ltd.), and seeded at 1.2 to 1.5 x 10^5 cells/cm^2 in a culture container coated with Geltrex (Thermo Fisher Scientific). The 454E2 line was adhesion-cultured to induce differentiation into pancreatic progenitor cells. Differentiation induction was performed using the same culture solution for the same culture period as described above, except that culture of the fourth stage was performed for 3 days.

[0127]

The 771-2 line was cultured in StemFit AK02N medium (Ajinomoto Co., Inc.) for 3 to 4 days using a culture container coated with Geltrex (Thermo Fisher Scientific). After treatment using TrypLE (Thermo Fisher Scientific), the cells were collected as single cells. Then, the cells were suspended in StemFit AK02 medium (Ajinomoto Co., Inc.) containing 10 μM Y-27632 (ROCK inhibitor, Wako Pure Chemical Industries, Ltd.), and seeded at 1.2 to 1.5 x 10^5 cells/cm^2 in a culture container coated with Geltrex (Thermo Fisher Scientific). The 771-2 line was adhesion-cultured to induce differentiation into pancreatic progenitor cells. Differentiation induction was performed using the same culture solution for the same culture period as described above, except that culture of the fourth stage was performed for 3 days.

[0128]

The P11025 line was cultured using DEF-CS Culture