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Transplantation of insulin-secreting multicellular spheroids for the treatment of type 1 diabetes in mice

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ABSTRACT

The efficacy of cell-based therapy depends on the function and survival of transplanted cells, which have been suggested to be enhanced by spheroid formation. However, few attempts at spheroid generation from insulin-secreting cells, which may be used to treat type 1 diabetes, have been reported. We therefore developed spheroids from the mouse insulinoma cell line NIT-1 by using polydimethylsiloxane (PDMS)-based microwells with a coating of poly(N-isopropylacrylamide) (PNIPAAm). The prepared NIT-1 spheroids or dissociated NIT-1 cells were transplanted into the subrenal capsule in streptozotocin-induced diabetic mice. NIT-1 spheroids prepared using the PNIPAAm-coated PDMS-based microwells had a uniformly sized spherical structure with a diameter of 200–300 μm. The PNIPAAm coating increased cell survival in the spheroids and the recovery of the spheroids from the microwells. In diabetic mice, the transplanted NIT-1 spheroids reduced blood glucose levels to normal values faster than dissociated NIT-1 cells did. Additionally, survival was higher among NIT-1 cells in spheroids than among dissociated NIT-1 cells 24 h after transplantation. These results indicate that insulin-secreting NIT-1 spheroids prepared using PNIPAAm-coated PDMS-based microwells are more effective for the treatment of type 1 diabetes than dissociated cells in suspension.

1. Introduction

Type 1 diabetes is also known as insulin-dependent diabetes mellitus and is caused by autoimmune destruction of insulin-secreting beta cells in the islets of the pancreas [1]. Almost all type 1 diabetics require daily insulin injections for the maintenance of their blood glucose levels. Because in patients with diabetes without beta cells, a decrease in blood glucose levels by using insulin injections alone is not possible, such patients require pancreatic transplantation depending on their clinical condition [2]. Such transplantations would provide freedom from injected insulin and lead to stable blood glucose levels. However, pancreas donors are rare and the surgical risk of the transplantation procedure is high [3]. Therefore, alternative therapies are necessary.

Islet transplantation is a reasonable method for the treatment of type 1 diabetes for the following 2 reasons: (1) insulin-secreting islets are able to reduce blood glucose levels; and (2) islet transplantation is much easier and safer than pancreas transplantation [4]. The Edmonton Protocol, developed by Shapiro and Lakey of the University of Alberta, has greatly increased the success rate of islet transplantation [5,6]. However, the limited number of islet donors still prevents the widespread clinical application of islet transplantation [7].

Recent progress in techniques for cell differentiation and proliferation has greatly increased the possibility of production of large numbers of insulin-secreting cells from donor cells or patients' own cells [8–10]. Although the transplantation of insulin-secreting cells has shown some promise, the therapeutic effects are short lived because of the rapid clearance or death of transplanted cells [11]. Therefore, approaches to increase the survival of insulin-secreting cells are necessary to enhance the potency of the treatment [12,13].

Three-dimensional cell culture systems have recently been developed to culture cells under simulated *in vivo* conditions to facilitate extensive cell–cell interactions [14]. Three-dimensionally cultured spheroidal cells, *i.e.*, multicellular spheroids, have been used to evaluate the characteristics and functions of various cells, including cancer cells, hepatocytes, and pancreatic cells [15–18]. Simple and reproducible methods have been developed for the preparation of multicellular spheroids [19], and the micromolding technique has attracted great attention because of its simplicity and versatility [20]. This technique enables preparation of multicellular spheroids with controlled size, geometry, and cell organization.

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In our previous study, we demonstrated that polydimethylsiloxane (PDMS)-based microwells were useful to obtain size-controlled multicellular spheroids of fibroblasts, adenocarcinoma cells, endothelial cells, and hepatocytes [21]. In addition, we also found that the coating of the microwells with poly(N-isopropylacrylamide) (PNIPAAm), a thermoresponsive polymer, was useful to increase the quality of the multicellular spheroids, because the coating effectively prevented the cells from adhering to the PDMS-based microwells.

Based on these findings, in the present study, we used PDMS-based microwells to prepare spheroids of insulin-secreting NIT-1 cells, which are a mouse insulinoma cell line. Some functional properties of insulinoma spheroids, such as insulin secreting ability and gene expression, were evaluated in previous studies, but little has been examined about the therapeutic potential of such spheroids *in vivo*. Therefore, we first examined the effects of the PNIPAAm coating on the properties of NIT-1 spheroids and then evaluated the therapeutic potential of the insulin-secreting multicellular spheroids in a mouse model of type 1 diabetes.

2. Materials and methods

2.1. Chemicals

Hank's balanced salt solution and phosphate-buffered saline were obtained from Nissui Pharmaceutical (Tokyo, Japan). PNIPAAm was obtained from Polysciences (Warrington, PA, USA). Ham's F-12K (Kaighn's modification) medium and streptozotocin (STZ) were obtained from Wako Pure Chemicals Industry (Osaka, Japan). Fetal bovine serum (FBS) was obtained from GIBCO-Invitrogen (Carlsbad, CA, USA). A PDMS prepolymer and the curing agent (Silpot 184) were purchased from Toray-Dow Corning Co. (Tokyo, Japan). Trypan blue was obtained from Nacalai Tesque (Kyoto, Japan). Non-enzymatic cell dissociation buffer was obtained from Sigma Chemical Company (St. Louis, MO, USA). Trypsin was obtained from Becton Dickinson (Mansfield, MA, USA). Carboxyfluorescein diacetate succinimidyl ester (CFSE) was obtained from Dojindo Laboratories (Kumamoto, Japan). All other chemicals were of the highest grade commercially available.

2.2. Animals

Male BALB/c mice (8–10 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan), and maintained on standard food and water under conventional housing conditions. The protocols for the animal experiments were approved by the Institutional Animal Experimentation Committee.

2.3. Cell culture

The mouse insulinoma cell line NIT-1 was purchased from ATCC (Rockville, MD, USA) and grown in Ham's F-12K medium supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in humidified air containing 5% CO_2.

2.4. Preparation of PDMS-based microwells

Micropillar arrays were fabricated as previously described [21]. A 10:1 mixture of PDMS prepolymer and the curing agent was poured onto micropillar arrays placed in a cell culture dish. The mixture was cured by 2 h of incubation at 60 °C after degassing for 30 min in a vacuum chamber. Subsequently, the PDMS-based microwell sheet was peeled off from the micropillar arrays and trimmed to fit a well in 6-well culture plates. The structure of the microwells was observed under a Biozero microscope (Biozero BZ-8000, Keyence, Osaka, Japan).

2.5. PNIPAAm coating of PDMS-based microwells

The PDMS-based microwell sheet in a well of a 6-well culture plate was immersed in 5% PNIPAAm solution in anhydrous ethanol. Air bubbles in the solution were removed under vacuum, and the excess PNIPAAm solution was removed by pipetting. Subsequently, the PNIPAAm-coated culture plates were dried in a 60 °C chamber for 2 h.

2.6. Adhesion of NIT-1 cells to culture plates

PNIPAAm solution in anhydrous ethanol or a solution containing the precursor of PDMS and the catalyst was added to wells of 12-well culture plates. The culture plates were dried by incubation at 60 °C for 2 h. Thereafter, 1×10^5 NIT-1 cells suspended in Ham's F-12K medium were added to each well and incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator. Culture plates were incubated statically or shaken at a speed of 65 rpm. At 24 h after incubation, floating NIT-1 cells were removed, and the number of NIT-1 cells adhering to the culture plates was measured by an MTT assay as described previously [22].

2.7. Preparation of NIT-1 spheroids

NIT-1 cells (5×10^6 cells) suspended in Ham's F-12K medium were added to PNIPAAm-coated PDMS-based microwells placed in 6-well culture plates and incubated for 48 h at 37 °C in a humidified 5% CO_2 incubator. The culture plates were shaken at a speed of 65 rpm. To maintain the PNIPAAm coating on the microwells, the coated microwells were kept at 37 °C during the experiments. After incubation, the temperature of the plates was allowed to decrease spontaneously to room temperature, at which point the PNIPAAm coating was dissolved. Then, NIT-1 spheroids were recovered from PDMS-based microwells and washed twice with PBS to remove possibly remaining PNIPAAm. Thereafter, NIT-1 spheroids were picked up using micropipettes. The diameter of the NIT-1 spheroids was measured under the Biozero microscope. The number and viability of NIT-1 cells in 1 spheroid were measured by staining the cells with trypan blue solution after dispersion of the spheroid by using a trypsin-EDTA solution (0.25% w/v).

2.8. Recovery of NIT-1 spheroids from PDMS-based microwell sheet

The PDMS-based microwell sheet with NIT-1 spheroids was detached from the culture plates, soaked in PBS on ice or at 37 °C, and shaken back and forth using forceps at a rate of 3 shakes per second as previously reported [21]. The remaining cell spheroids in the PDMS-based microwell sheet were counted.

2.9. Insulin secretion from NIT-1 cells

Ten NIT-1 spheroids that contained a total of approximately 73,000 were added to a 6-well culture plate and pre-incubated in Krebs-Ringer bicarbonate (KRB) buffer (140 mM NaCl, 2 mM $CaCl_2$, 0.15 mM Na_2HPO_4, 5 mM $NaHCO_3$, 1 mM $MgSO_4$, 0.05% BSA, 4.6 mM KCl, and 30 mM HEPES, pH 7.4) containing 3 mM glucose for 30 min. Subsequently, the solution was replaced with fresh KRB buffer containing 3 or 20 mM glucose. After 30 min of incubation, the supernatants were collected and centrifuged at 4000 rpm for 5 min. The amount of insulin secreted from the NIT-1 cells was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden). Monolayered NIT-1 cells treated as described above were used as a control.

2.10. Transplantation of NIT-1 spheroids into STZ-induced diabetic mice

BALB/c mice received an intravenous injection of STZ dissolved in citrate buffer (pH 4.5) at a dose of 120 mg/kg of body weight. The non-fasting blood glucose level of the mice was measured, and mice