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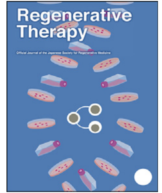
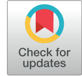


Original Article

Enhanced survival and insulin secretion of insulinoma cell aggregates by incorporating gelatin hydrogel microspheres



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ABSTRACT

Introduction: The objective of this study is to evaluate the survival and glucose-induced insulin secretion of rat-derived insulinoma cells (INS-1) from their aggregates incorporating different size of gelatin hydrogel microspheres comparing with microspheres-free cell aggregates.
Methods: The gelatin hydrogel microspheres were prepared by the conventional w/o emulsion method. The INS-1 cells were cultured in a V-bottomed well, combining with or without the gelatin hydrogel microspheres to form their aggregates with or without microspheres.
Results: When the cell viability, the live cell number, the reductase activity, and the insulin secretion of cell aggregates were evaluated 7 or 14 days after incubation, the cell aggregates incorporating gelatin hydrogel microspheres showed higher cell viability, reductase activity and a larger number of live cells. The cell aggregates incorporating larger size and number of gelatin hydrogel microspheres secreted a larger amount of insulin, compared with those incorporating smaller size and number of microspheres or without microspheres.
Conclusion: It is conceivable that the incorporation of gelatin hydrogel microspheres in cell aggregates is promising to improve their survival and insulin secretion function.
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1. Introduction

Islet transplantation has been investigated as a treatment of type 1 diabetes for patients with insufficient glucose control [1–3]. However, a big problem of islet transplantation therapy is the serious donor shortage [4–6]. To circumvent this issue, it has been reported to reconstitute islet-like aggregates of insulin secreting cells [7,8]. However, for this approach, when the cell aggregates become larger than 200 μm in diameter, the cells in the center of cell aggregates tend to die because of a lack of oxygen and nutrients supply [9,10]. It is well known that insulin secreting cells show a decreased function of insulin secretion under a hypoxic environment [11,12]. Therefore, to achieve sufficient therapeutic effect with the insulin secreting cell aggregates, it is necessary to develop a method for the promotion of oxygen and nutrients supply.

Previous studies demonstrated that the incorporation of gelatin hydrogel microspheres in mesenchymal stem cells (MSC) aggregates enabled the cells to improve the viability, proliferation and osteogenic differentiation. This is because the microspheres improved the state of oxygen and nutrients supply for cells [13,14]. In this study, the gelatin hydrogel microspheres technology was introduced to insulin secreting cell aggregates to assess the cell viability and insulin secretion function comparing with microspheres-free cell aggregates. Gelatin hydrogel microspheres with different sizes were prepared by the conventional w/o emulsion method previously reported [15]. Rat insulinoma cells (INS-1), the model of insulin secreting cells, were incubated with or without the gelatin hydrogel microspheres in a V-bottomed well to form the cell aggregates with or without the microspheres. We examined the effect of microspheres size and number on the cell viability, reductase activity, and insulin secretion ability in the aggregates.

Abbreviations: INS-1, insulinoma; MSC, mesenchymal stem cell.

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2. Materials and methods

2.1. Preparation of gelatin hydrogel microspheres

Gelatin hydrogel microspheres were prepared by the chemical cross-linking of gelatin in a water-in-oil emulsion state according to the method previously reported [15]. Briefly, an aqueous solution (20 ml) of 10 wt% gelatin (isoionic point 5.0 (pI 5), weight-averaged molecular weight = 1,00,000, Nitta Gelatin Inc., Osaka, Japan) was preheated at 40 °C, and then added dropwise into 600 ml of olive oil (Wako Pure Chemical Industries Ltd., Osaka, Japan) at 40 °C, followed by stirring at 200 rpm for 10 min to prepare a water-in-oil emulsion. The emulsion temperature was decreased to 4 °C for the natural gelation of gelatin solution to obtain non-crosslinked microspheres. The resulting microspheres were washed three times with cold acetone in combination with centrifugation (5000 rpm., 4 °C, 5 min) to completely exclude the residual oil. Then, they were fractionated by size using sieves with apertures of 20, 32, and 53 μm (Iida Seisakusyo Ltd., Osaka, Japan) and air dried at 4 °C. The non-crosslinked and dried gelatin microspheres (200 mg) were treated in a vacuum oven at 140 °C and 0.1 Torr for 48 h for the dehydrothermal crosslinking of gelatin. Pictures of gelatin hydrogel microspheres in a dispersed state in RPMI medium 1640 containing L-glutamine (Invitrogen Ltd., Carlsbad, CA), were taken with a light microscope (BZ-X710, KEYENCE Corp., Osaka, Japan). The size of 100 microspheres for each sample was measured using the computer program of microscope (BZ-X710) to calculate the average diameter.

2.2. Preparation of INS-1 cell aggregates with or without gelatin hydrogel microspheres

A cell line 832/13, derived from INS-1 rat insulinoma cells, was obtained from Dr. Christopher B. Newgard (Duke University Medical Center, Durham, NC) [16]. Cells were grown in RPMI medium 1640 containing L-glutamine (Invitrogen Ltd.), 1 mM sodium pyruvate (Invitrogen Ltd.), 10 mM HEPES (Invitrogen Ltd.), 10 vol% heat-inactivated fetal bovine serum (Thermo Fisher Scientific Inc., Waltham, MA), 55 μM 2-mercaptoethanol (Invitrogen Ltd.), 100 IU/ml penicillin (Gibco, Grand Island, NY), and 100 μg/ml streptomycin (Gibco). Cells were cultured in a humidified atmosphere containing 5% CO_2/95% air at 37 °C.

Gelatin hydrogel microspheres and INS-1 cells were separately suspended in the culture medium under different conditions (Table 1). Gelatin microsphere suspensions (100 μl) were added to each well of a 96-well culture plate with V-bottomed wells, followed by 50 μl of INS-1 cell suspensions at the initial density of 1.0×10^3 or 1.0×10^4 cells/well and 1.0×10^1, 0.5×10^2, 1.0×10^2 or 1.0×10^3 microspheres/well. The cells/microspheres number ratio is 10/1, 100/1, or 200/1. Pictures of INS-1 cell aggregates with or without gelatin hydrogel microspheres were taken with the microscope as described above.

Table 1
Preparation conditions of INS-1 cells aggregate with or without gelatin microspheres incorporation

Code	Number of INS-1 cells (cells/well)	Number of gelatin hydrogel microspheres (/well)	Size of gelatin hydrogel microspheres (mm)
a	1.0×10^3	0	–
b	1.0×10^3	1.0×10^1	46.0 ± 14.0
c	1.0×10^3	1.0×10^1	82.0 ± 18.0
d	1.0×10^4	0	–
e	1.0×10^4	1.0×10^2	22.0 ± 9.0
f	1.0×10^4	1.0×10^3	22.0 ± 9.0
g	1.0×10^4	1.0×10^2	46.0 ± 14.0
h	1.0×10^4	0.5×10^2	82.0 ± 18.0
i	1.0×10^4	1.0×10^2	82.0 ± 18.0

2.3. Evaluation of cell viability, reductase activity, and live cell number

The number of live cells in INS-1 cell aggregates with or without gelatin hydrogel microspheres was determined by trypan-blue staining. After 7 or 14 days incubation, cell aggregates were collected into a 1.5 ml microtube and washed by 100 μl of phosphate-buffered saline solution (PBS, Gibco) once. After centrifugation and the supernatants removal, the aggregate pellets were incubated in 100 μl of trypsin–EDTA solution (Sigma–Aldrich Company Ltd., St. Louis, MO) for 15 min at 37 °C. Following the incubation, 100 μl of culture medium was added to stop the trypsin action. Live cells in the cell suspension were counted by a cell counter (Countess II FL, Thermo Fisher Scientific Inc.). Live/dead assays were conducted using a Live/Dead Viability/Cytotoxicity Assay (Invitrogen) according to the manufacturer's protocol. The cell aggregates were rinsed once with PBS 7 or 14 days after incubation, and then incubated with the mixed solution of 2 mM calcein AM and 4 mM ethidium homodimer-1 solution in PBS for 15 min at room temperature in the dark, followed by observation using a microscope (BX-X710).

Reductase activity of INS-1 cell aggregates with or without gelatin hydrogel microspheres was determined by colorimetric assay using water-soluble tetrazolium salt as one measure to assess a cell metabolic activity. After 7 or 14 days incubation, cell aggregates were collected into a well of 96 well flat-bottomed plate and the culture medium was adjusted 100 μl in each well. WST-8 reagent (10 μl, Dojindo Laboratories, Kumamoto, Japan) was added into each well, and then cell aggregates were incubated for 4 h at 37 °C. After the incubation, the absorbance at 450 nm was measured by SpectraMax M2/M2e Microplate Reader (Molecular Devices, LLC, CA). The reductase activity was normalized by the live cell number.

2.4. Evaluation of glucose-induced insulin secretion

After 7 or 14 days incubation, INS-1 cell aggregates with or without gelatin hydrogel microspheres were transferred to each well of a 12 mm Transwell (#3402, Coring Inc. Corning, NY) and washed once by PBS. Then, Krebs-Ringer-bicarbonate HEPES (KRB) buffer solution containing 10 mM glucose [16] was added to each well, and the cell aggregates were incubated for 1 h at 37 °C. The concentration of secreted insulin in the supernatants was measured by ELISA kit (Rat Insulin ELISA KIT, Shibayagi Co. Ltd., Gunma, Japan). The insulin secretion was normalized by the live cell number.

2.5. Statistical analysis

All the statistical data are expressed as mean ± standard error of the mean (SEM). The data were analyzed using the Tukey's test and the statistical significance was accepted at $P < 0.05$ or 0.01.

3. Result

3.1. Characterization of gelatin hydrogel microspheres

Fig. 1 shows the microscopic images of gelatin hydrogel microspheres. The microspheres had spherical shape and smooth surface.

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