Regenerative Therapy 8 (2018) 38–45

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Insulin secretion of mixed insulinoma aggregates-gelatin hydrogel microspheres after subcutaneous transplantation

Kanako Inoo [a], Hiroto Bando [b], Yasuhiko Tabata [a, *]

[a] Laboratory of Biomaterials, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan
[b] Regenerative Medicine Unit, Takeda Pharmaceutical Company Limited, Cambridge, MA, USA

ARTICLE INFO

Article history:
Received 7 September 2017
Received in revised form
31 December 2017
Accepted 9 January 2018

Keywords:
Insulinoma cell
Aggregate
Gelatin microsphere
Vascularization
Glucose-induced insulin secretion

ABSTRACT

Introduction: The objective of this study is to evaluate the insulin secretion of mixed aggregates of insulinoma cells (INS-1) and gelatin hydrogel microspheres after their subcutaneous transplantation.
Methods: Gelatin hydrogel microspheres were prepared by the conventional w/o emulsion method. Cell aggregates mixed with or without the hydrogel microspheres were encapsulated into a pouched-device of polytetrafluoroethylene membrane. An agarose hydrogel or MedGel™ incorporating basic fibroblast growth factor (bFGF) was subcutaneously implanted to induce vascularization. After the vascularization induction, cell aggregates encapsulated in the pouched-device was transplanted.
Results: The vascularization had the potential to enable transplanted cell aggregates to enhance the level of insulin secretion compared with those of no vascularization induction. In addition, the insulin secretion of cell aggregates was significantly promoted by the mixing of gelatin hydrogel microspheres even in the pouched-device encapsulated state.
Conclusion: It is possible that the microspheres mixing gives cells in aggregates better survival condition, resulting in promoted insulin secretion.

1. Introduction

Islet transplantation is one option of type I diabetes therapies [1–3], but many patients can hardly receive the medical treatment because of the serious donor shortage [4–6]. Islet-like cell aggregates formed artificially from pancreatic β cells may be potential to overcome this issue [7,8]. However, when the cell aggregates become large over 200 μm in diameter, cells inside the cell aggregates are susceptible to necrosis because of the lack of oxygen and nutrient supplies [9,10]. It is well recognized that β cells are generally sensitive to a hypoxic environment and need a large amount of oxygen to secrete insulin [11,12]. Therefore, it is indispensable for a high cell viability and function like glucose-induced insulin secretion (GIIS) to improve the condition of oxygen and nutrient supplies to cells inside cell aggregates. We demonstrate that the mixing of gelatin hydrogel microspheres enabled mesenchymal stem cells in their aggregates to improve the cell viability, proliferation, and osteogenic differentiation [13,14]. This is mainly because the microspheres promoted the oxygen and nutrients supply to cells inside. This mixing technology with hydrogel microspheres will be effective in improving β cell viability and the functions in cell aggregates.

For the site of β cells transplantation, the subcutaneous (SC) tissue is preferable because it is easy to transplant cells and remove the cells transplanted if some problems happen [15–17]. However, one of the big difficulties is to maintain the cell viability at the SC site because blood vessel networks are poor to allow cells to survive thereat. Generally, as one trial to breakthrough the problem, vascularization around the transplantation area has been tried to induce by several methods [15–20]. However, an appropriate vascularization timing remains to be fully elucidated.

In this study, gelatin hydrogel microspheres were mixed in the aggregates of insulinoma cells (INS-1) as a model β cell [21] to

Abbreviations: INS-1, insulinoma; MSC, mesenchymal stem cell; bFGF, basic fibroblast growth factor; PVDF, polyvinylidene difluoride membrane; PTFE, polytetrafluoroethylene; SC, subcutaneous.

* Corresponding author. Institute for Life and Frontier Medical Sciences, Kyoto University 53 Kawara-cho Shogoin, Sakyo-ku, Kyoto, 606-8507 Japan. Fax: +81 75 751 4646.
E-mail address: yasuhiko@infront.kyoto-u.ac.jp (Y. Tabata).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2018.01.003

expect the pathway creation of oxygen and nutrients in aggregates to evaluate whether or not the hydrogel microspheres mixing modifies the function of cell aggregates in vitro and in vivo. INS-1 cell aggregates mixed with or without gelatin hydrogel microspheres were encapsulated into a pouched-device and subcutaneously transplanted into the back of rats. Before the transplantation, vascularization by basic fibroblast growth factor (bFGF) was induced to assess the effect of vascularization on the insulin secretion from the cell aggregates encapsulated in the device transplanted.

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 [13]. Briefly, an aqueous solution (20 ml) of 10 wt% gelatin (isoelectric point 5.0, 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 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 and insulin secretion evaluation (GIIS assay)

The cell line 832/13, derived from INS-1 rat insulinoma cells, was obtained from Dr. Christopher B. Newgard (Duke University Medical Center, Durham, NC) [21]. 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. The initial seeding density of cells was 1×10^3 or 1×10^4 cells/well at the cells/microspheres number ratio of 100/1. The mixed suspensions of microspheres and INS cells (14 ml) were added to EZSPHERE (4000-905, AGC Techno Glass Co. Ltd., Shizuoka, Japan). Pictures of INS-1 cell aggregates with or without the gelatin hydrogel microspheres incorporation were taken with the microscope as described above.

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 phosphate-buffered saline solution (PBS, Gibco). Then, Krebs-Ringer-bicarbonate HEPES (KRB) buffer solution containing 10 mM glucose [21] was added to each well, and the cell aggregates were incubated for 1 h at 37 °C. The concentration of insulin secreted in the supernatants was measured by ELISA kit (Rat Insulin ELISA KIT, Shibayagi Co. Ltd., Gunma, Japan). Experiments were performed on three wells for each sample.

2.3. SC vascularization induction with bFGF

Male Wistar rats were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited.

An agarose hydrogel was prepared [22]. Briefly, 4.5 wt% agarose (SeaKem GTG Agarose; Camrex Bio Science Rockland, Inc., Rockland, ME) solution in water was prepared by autoclave, followed by the solution cooling to 25 °C for the natural gelation. Next, the hydrogel was freeze-dried to obtain the agarose hydrogel. The agarose hydrogel was formulated into a round shape (3.0 cm in diameter) or an oval shape with 1.5 × 3.0 cm, and then covered by a polyvinylidene difluoride membrane (PVDF) to suppress the adhesion to the surrounding subcutaneous tissues [23]. bFGF (500 μg of Fiblast Spray, Kaken Parmaceutical Co., Ltd., Tokyo, Japan) was dissolved in 500 μl of PBS. The bFGF solution (50 μl) was dropped onto the dried agarose hydrogel, following leaving at 25 °C for 30 min to prepare the agarose hydrogel containing 50 μg of bFGF. The agarose hydrogel containing bFGF was implanted into the back subcutis of rats. The hydrogel was taken out 7 days later to visually observe the induction of vascularization around the implanted site. As another material, a gelatin hydrogel (0.5 × 0.5 cm, pI 5, MedGel, Tokyo, Japan) was used to induce the vascularization in vivo. Similarly, 50 μg of bFGF was impregnated into the gelatin hydrogel. The gelatin hydrogel containing bFGF was implanted to induce vascularization for 7 days.

2.4. In vitro evaluation of insulin secretion for cell aggregates encapsulated in the pouched-device after in vivo transplantation (GIIS assay)

Several in vivo experiments were performed to evaluate the effect of vascularization and gelatin hydrogel microspheres mixing on the insulin secretion for cell aggregates encapsulated in the pouched-device (Scheme 1). To retain the cell aggregates at the transplanted site, a pouched-device was made of polytetrafluoroethylene membrane (PTFE) (Omnipore Membrane Filter, Hydrophilic, 0.45 μm, Millipore Corp., Bedford, MA), which is a biocompatible material clinically available [24]. The 2 PTFE membranes were adhered by a heat-adhesion with a ring-shaped polypropylene membrane (Prefilter, Hydrophobic, 0.6 μm pore, Millipore Corp., Bedford, MA). A tube (1.5 mm in inner diameter, PE Tubing, Natsume Manufacturing, Tokyo) was inserted into the pouched-device (Scheme 1a). The cell aggregates without gelatin hydrogel microspheres were infused into the pouched-device by insertion of an 18 G needle (TERUMO Corp., Tokyo, Japan) (750 cell aggregates/pouched-device). Then, they were subcutaneously transplanted into the SC site of rats which the vascularization treatment had been performed with bFGF containing the agarose hydrogel (Group 1) or had not been done (Group 2). The pouched-device was taken out 3 days later and the glucose-KRB solution was added to the pouched-device, followed by incubation for 2 h at 37 °C. Experiments were performed for six mice. Similarly, the