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Original Article

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

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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.

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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.

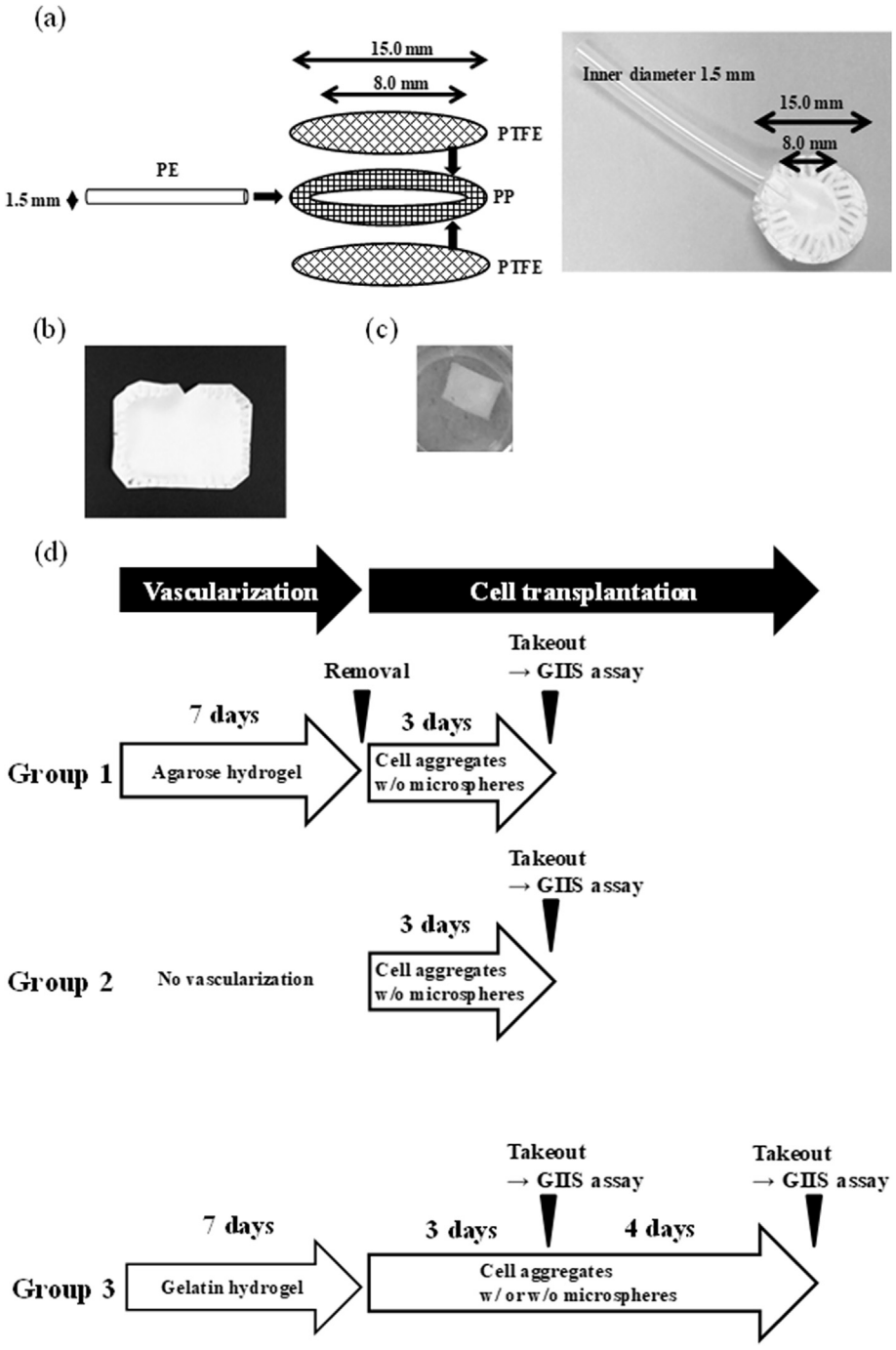
2.3. SC vascularization induction with bFGF

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



Scheme 1. (a) A schematic illustration of a pouched-device to encapsulate cell aggregates. Two PTFE membranes were adhered by a heat-adhesion with a ring-shaped PP membrane. (b) A picture of agarose hydrogel covered by a PVDF membrane. (c) A picture of gelatin hydrogel (MedGel™). (d) Experimental schedule to evaluate the insulin secretion of implanted cell aggregates in the pouched-device: Group1, an agarose hydrogel was implanted to induce vascularization for the initial vascularization for the initial 7 days. After the hydrogel was removed, cell aggregates encapsulated in the pouched-device were transplanted. Cell aggregates were taken out 3 days later and their insulin secretion was evaluated. Group 2, without any vascularization treatment, cell aggregates encapsulated in the pouched-device were transplanted. Cell aggregates were taken out 3 days later and their insulin secretion was evaluated. Group 3, a gelatin hydrogel (MedGel™) was implanted to induce vascularization for the initial 7 days. After the hydrogel was removed, cell aggregates encapsulated in the pouched-device were transplanted. Cell aggregates were takenout 3 or 7 days later and their insulin secretion was evaluated.

pouched-device containing cell aggregates with or without (Group 3) gelatin hydrogel microspheres was subcutaneously transplanted after vascularization treatment for 7 days and the insulin concentration in the supernatant was measured. Experiments were performed for four mice.

2.5. Statistical analysis

All the statistical data are expressed as mean ± standard error of the mean (SEM). The data were analyzed using the Student's t-test and the statistical significance was accepted at $P < 0.05$ or 0.01.

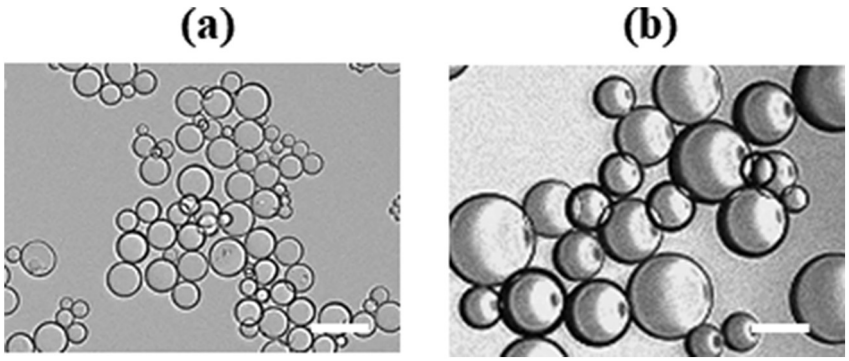


Fig. 1. Light microscopic pictures of gelatin hydrogel microspheres dispersed in the culture medium. The diameter of gelatin hydrogel microspheres is (a) 47.0 ± 11.0 or (b) 91.0 ± 19.0 μm. Scale bar is 100 μm.

3. Result

3.1. Characterization of gelatin hydrogel microspheres

Fig. 1 shows the typical microscopic pictures of gelatin hydrogel microspheres. The microspheres were of spherical shape and had a smooth surface. The microspheres could be fractionized in size by using the sieve.

3.2. Characterization of INS-1 cell aggregates with or without gelatin hydrogel microspheres and insulin secretion in vitro

Fig. 2 shows the microscopic pictures of cell aggregates with or without gelatin hydrogel microspheres 5 or 7 days after cell culture. Cell aggregates without gelatin hydrogel microspheres were obtained at any initial seeding density. When the initial seeding density was 1×10^4 cells/well, cell aggregates uniformly containing gelatin hydrogel microspheres were not formed. The gelatin hydrogel microspheres were located at the centre of a cell aggregate. On the other hand, the cell aggregates uniformly containing gelatin hydrogel microspheres were successfully formed at the initial seeding density of 1×10^3 cells/well. Therefore, based on this, 1×10^3 cells/well of the initial seeding density was used for the following experiment. The formation speed of cell aggregates with gelatin hydrogel microspheres of 47.0 μm in diameter of was higher compared with that of cell aggregates containing hydrogel microspheres of 91.0 μm in diameter.

Fig. 3 shows the insulin secretion of INS-1 cell aggregates 7 days incubation with or without gelatin hydrogel microspheres. Cell aggregates with gelatin hydrogel microspheres of 91.0 μm in diameter secreted significantly larger amount of insulin than those without the gelatin microspheres or with the microspheres of 47.0 μm in diameter. The following experiment was performed by using the gelatin hydrogel microspheres of 91.0 μm in diameter unless otherwise mentioned.

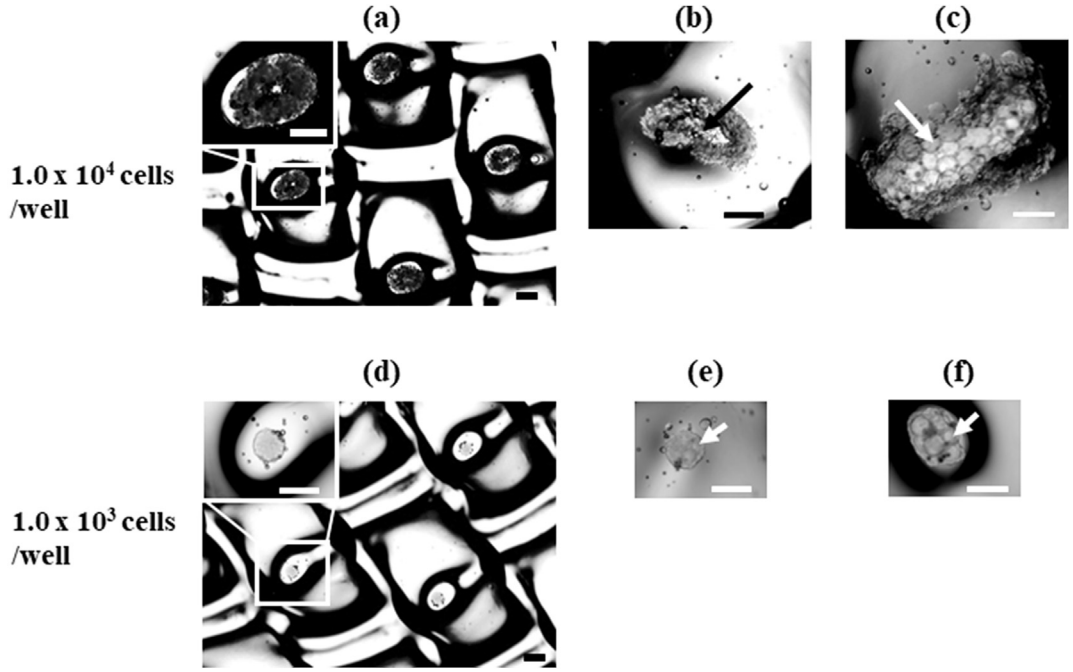


Fig. 2. Light microscopic pictures of INS-1 cell aggregate 5 (a, b, c) and 7 days (d, e, f) after incubation with or without gelatin microspheres. INS-1 cell aggregates were cultured with gelatin hydrogel microspheres of 47.0 μm (b, e) and 91.0 μm in diameter (c, f) or without gelatin microspheres (a, d). The numbers of cells initially added are 1×10^4 and 1×10^3 for 47.0 μm and 91.0 μm diameter microspheres, respectively. Scale bar is 200 μm. Arrows indicate gelatin hydrogel microspheres.

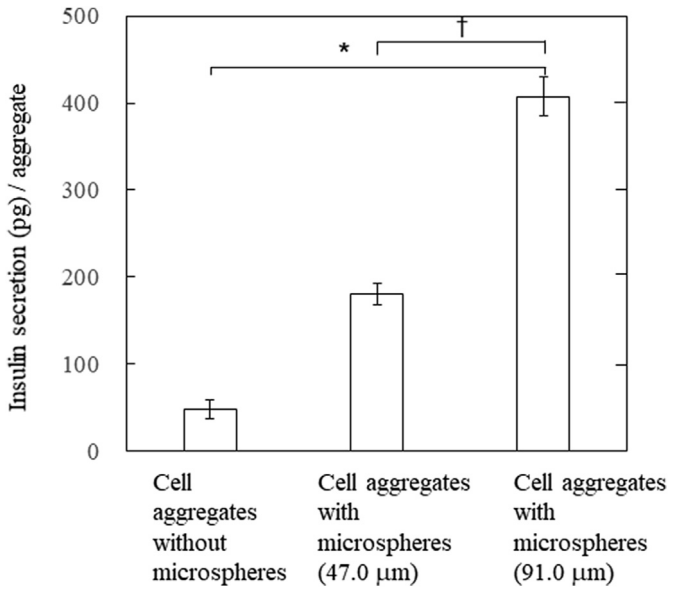


Fig. 3. Insulin secretion of INS-1 cell aggregates 7 days incubation with or without gelatin hydrogel microspheres. *P < 0.01: significant different between the groups. †P < 0.01: significant different between the groups.

3.3. *Effect of vascularization on insulin secretion of cell aggregates*

Fig. 4 shows that tissue appearance 7 days after implantation of an agarose hydrogel without bFGF (a) or containing 50 μg of bFGF (b) into the back subcutis of rats. (c) Tissue appearance of normal rat. Vascularization was successfully induced by the implantation of agarose hydrogels containing bFGF. Less vascularization was seen for bFGF-free agarose hydrogel.

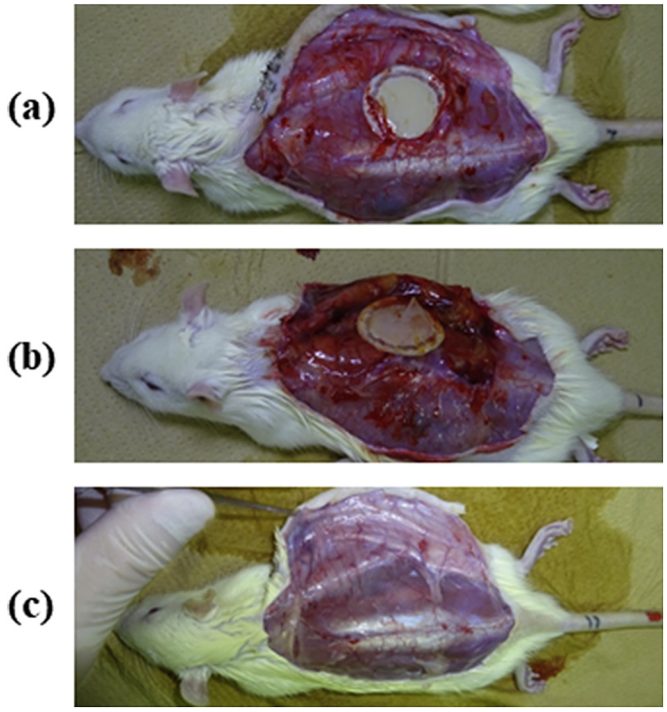


Fig. 4. Tissue appearance 7 days after implantation of an agarose hydrogel without bFGF (a) or containing 50 μg of bFGF (b) into the back subcutis of rats. (c) Tissue appearance of normal rat.

Fig. 5 shows that insulin secretion of INS-1 cell aggregates encapsulated in the pouched-device with or without 3 days vascularization by the agarose hydrogel implantation. Cell aggregates transplanted into the site which had been vascularized by the implantation of agarose hydrogel containing bFGF (Group 1) showed the tendency of higher than amount of insulin secretion compared with those transplanted into non-vascularized site (Group 2), although the was no significant difference. The potential that vascularization treatment enables the cell aggregates to enhance the amount of secreted insulin secreted.

3.4. *Effect of gelatin hydrogel microspheres mixing and vascularization timing on insulin secretion cell aggregates in vivo*

Fig. 6 shows that insulin secretion of INS-1 cell aggregates with or without gelatin hydrogel microspheres encapsulated in the pouched-device before SC transplantation, and 3 and 7 days transplantation. Cell aggregates with gelatin hydrogel microspheres in the pouched-device secreted the amount of insulin to a significant higher extent than those without gelatin hydrogel microspheres mixing 3 and 7 days after transplantation. The amount of secreted insulin decreased compared with that before transplantation. However, there was no significant difference in the insulin secretion between 3 and 7 days after transplantation.

4. Discussion

Generally, multi-well U or V-bottomed plates or the hanging-drop method are used to prepare cell aggregates in lab-scale [25–27]. However, in this study, the EZSPHERE was selected considering the large-scale preparation of cell aggregates aiming at their transplantation [28,29]. The EZSPHERE was effective in forming cell aggregates with or without the gelatin hydrogel microspheres mixing of cell aggregates. The mixing of gelatin hydrogel microspheres significantly promoted the insulin secretion of cell aggregates (Fig. 3). The formation of cell aggregates was influenced by the initial seeding density of cells. At the initial seeding density of 1×10^4 cells/well, gelatin hydrogel microspheres

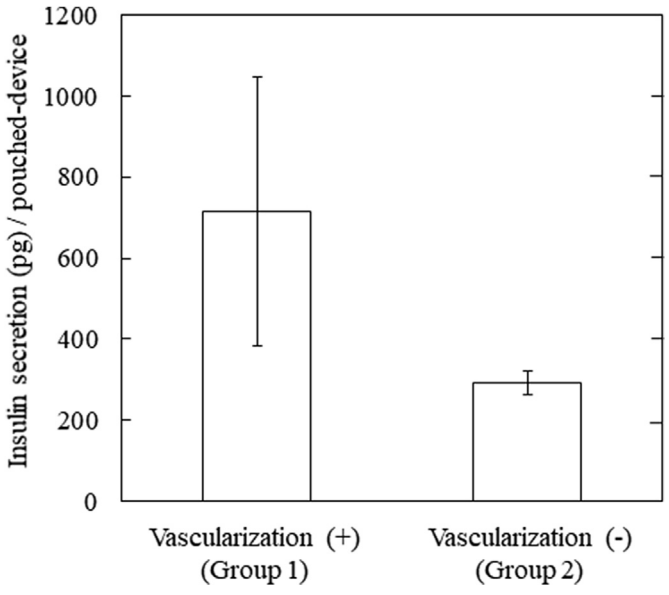


Fig. 5. Insulin secretion of INS-1 cell aggregates encapsulated in the pouched-device without or with 3 days vascularization by the agarose hydrogel implantation. Vascularization (+) or (−) respectively shows the group with or without vascularization before transplantation.

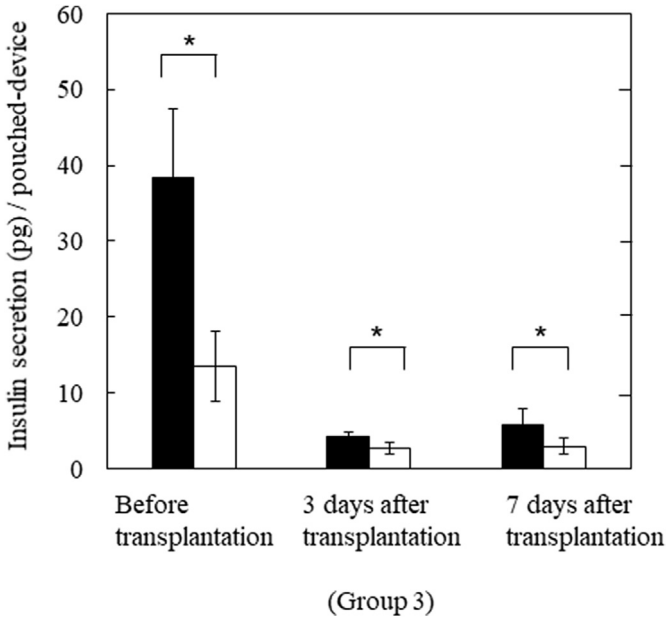


Fig. 6. Insulin secretion of INS-1 cell aggregates with (black bars) or without gelatin hydrogel microspheres (white bars) encapsulated in the pouched-device before SC transplantation, and 3 and 7 days transplantation. Cell aggregates were subcutaneously transplanted after the vascularization for 7 days.

were located at the bottom of wells while cells proliferated around them, which caused no formation of cell aggregates mixed with microspheres. It is likely that heavier microspheres sunk down to the well bottom faster than cells, and consequently placed the well surface. In addition, the well size of EZSPHERE would be too small to form cell aggregates (diameter; 1400 μm, depth; 600 μm) because microspheres occupied the well. On the other hand, cell aggregates were successfully formed at the initial seeding density of 1×10^3 cells/well, and an uniform distribution of cells and microspheres was observed in the cell aggregates. The in vitro insulin secretion assay revealed that at 1×10^3 cells/well, the microspheres mixing improved the insulin secretion from cell aggregates, and the improvement was significantly higher for larger microspheres than smaller microspheres. The same result was obtained in preparing cell aggregates with the gelatin microspheres in 96 well V-bottomed plates. We can say that the microspheres of larger volume were more efficient to create the pathway of oxygen and nutrients supply to cells inside the cell aggregates than smaller microspheres. This is because oxygen and nutrients are generally permeated through the water phase of homogeneously mixing hydrogels.

Although we have no data on the maturity and amount of blood vessels, we believe that it is apparent that bFGF incorporation promoted vascularization in vivo to a significantly great extent compared with no incorporation from Fig. 4. The vascularization ability of bFGF was known well and there have been reported on the bFGF-induced vascularization [30–32]. However, less vascularization for bFGF-free hydrogel was induced. This may be due to the hydrogel property to induce inflammation. To assess the inflammation caused by agarose hydrogels, agarose hydrogels containing bFGF with different sizes were subcutaneously implanted. When the tissue was evaluated 1 week after the implantation, larger hydrogels induced severe edemas. As the size of hydrogels decreased, the edema tended to reduce (Supplemental Fig. 1 b–d). Based on this, in this study, the agarose hydrogel of 1.5×3.0 cm was selected for vascularization induction to evaluate the effect of vascularization on the insulin secretion in vivo. Considering the clinical treatment of diabetes by cell transplantation, it is desired to suppress the inflammation caused by the transplantation and in addition, simplify the transplantation protocol. The gelatin hydrogel is degraded with time in vivo [30,31]. Therefore, the hydrogels are not required to be removed after vascularization. We experimentally demonstrated that gelatin hydrogels were degraded about 1 week after the SC implantation. To reduce inflammation, the smaller size (0.5×0.5 cm) to incorporate bFGF solution was selected for the following in vivo study (Supplemental Fig. 1a).

Cell aggregates transplanted into the site which had been vascularized by the implantation of agarose hydrogel containing bFGF, showed high amount of secreted insulin compared with those transplanted into non-vascularized site, although there was no significant difference (Fig. 5). This may be due to the large fluctuation of experimental values. However, this finding strongly suggests that the vascularization induction was required to maintain the function of cell transplantation. When calculated per one aggregate without gelatin hydrogel microspheres, the amount of insulin secretion even after vascularization remained only around 1/50 comparing with that before transplantation. There are various reasons for this phenomenon. Firstly, low oxygen tension in the site to be transplanted deteriorates the condition of cells. Secondly, the viability of cell aggregates was lost when encapsulated into the pouched-device, because the oxygen permeability of PTFE membrane is limited. In addition, an absorption test demonstrates that the insulin absorption to the PTFE membrane was detected (data not shown). As one trial to tackle the absorption, it is necessary to optimize the materials for the pouched-device in terms of low absorption property and high biocompatibility [33–35]. It is reported that the pouched-devices have a property to induce vascularization in the transplantation site [35–37]. Base on them, the PTFE membrane was used in the present study. In addition, to suppress the crumping of encapsulated cell aggregates in the pouched-device and uniformly distribute oxygen to the aggregates in the device, the inner shape of the device and the cell encapsulation method should be modified [38–40]. Because the uniform distribution of cell aggregates would supply a good survival condition to aggregates in the pouched-device. If the cell aggregates are heterogeneously located in the device, even if the vascularization around the device is induced, the blood supply to cells is not sufficient for their survival. Therefore, the method of SC vascularization should be optimized for the SC transplantation. In addition, it is necessary to develop other strategies for improved blood supply for cells by making use of chemical compound [41], biological protein [42], and mechanical device [43]. There have been reported on other technologies studied to overcome this cell function decrease. One of the technologies is to use a hydrogel in which cells are embedded as the transplantation substrate [44–46]. Matrigel is the most famous hydrogel for cell transplantation [47,48]. Although Matrigel improves the retention rate and viability of transplanted cells, Matrigel is a solubilized basement membrane extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma, and it is difficult to apply for the clinical study. Furthermore, it is highly conceivable that the presence of hydrogels suppresses the biological response of glucose-induced insulin secretion because glucose and insulin have to diffuse in hydrogels. To overcome this problem on the diffusion of biological factors, the conformal coating method was developed, which utilized the chemical crosslinking on the surface of cell aggregates [49,50]. However, this chemical crosslinking reaction and crosslinking agents sometimes decrease the cell viability. On the other hand, our microspheres enable biological factors to interact with cells directly, and the technology does not cause the chemical damage.

Cell aggregates with large gelatin hydrogel microspheres in the pouched-device secreted a significantly higher amount of insulin than those without microspheres mixing. This technology was experimentally confirmed both for the in vitro and in vivo systems (Fig. 6). However, the amount of insulin secretion from cell aggregates decreased after their transplantation when compared with that of in vitro culture condition. This can be explained in terms of oxygen tension. It is conceivable that in the subcutaneous site, the oxygen concentration is not high enough to maintain the survival and function retention of cells externally transplanted. To break through the low oxygen concentration at the site to be transplanted, it is of prime importance to design and develop the material and structure of devices for cell aggregates encapsulation. The vascularization method should be also modified to allow the transplanted cell aggregates to supply oxygen. There was no significant difference in the level of insulin secretion between 3 and 7 days post-transplantation. This suggests that the oxygen supply by the vascularization treatment was efficient to maintain the function of cell aggregates. However, a longer-term evaluation should be performed in the future. To optimize the timing of vascularization, the gelatin hydrogel containing bFGF was subcutaneously implanted at the same or different time of cell transplantation. The pouched-device containing cell aggregates with or without gelatin hydrogel microspheres was transplanted into the subcutaneous site of rat after vascularization (Group 3) or at the same time as cell transplantation (Group 4) (Supplemental Fig. 2). There was no difference between Group 3 and 4 after 3 or 7 days transplantation. It has been demonstrated that the bFGF release initiated immediately after implantation, resulting in the subsequent generation of bFGF-induced vascularization [30,31]. The similar result was obtained by the spontaneous implantation of the gelatin hydrogel with the cell aggregates. This finding indicates that the removal of vascularization device for the subsequent cell transplantation is not required.

The present study demonstrates that the vascularization or the mixing with gelatin hydrogel microspheres and the microspheres size affect the behavior of insulin secretion for cell aggregates. The technology of hydrogel microspheres mixing and vascularization treatment is promising to achieve the cost reduction and high therapeutic effect in cell transplantation therapy. Since the purpose of this study was to show the potential of gelatin hydrogel microspheres to improve the insulin secretion in vivo, we did not perform in vivo study using a diabetes model animal and the measurement of blood glucose levels. It is demonstrated that vascularization development with growth factors like bFGF, is known to be impaired in diabetes models [51–53]. It is easily easy to imagine the phenomenon that the transplanted cells are exposed to a harsher environment in diabetes models. Therefore, we strongly believe that the present microspheres improve elicit the effect on insulin secretion improvement in vivo. In the future, we plan to evaluate not only the insulin secretion in vivo from cell aggregates with or without microspheres, but also the blood glucose level.

5. Conclusions

The vascularization induced by bFGF release enabled transplanted cell aggregates to enhance the level of insulin secretion compared with no vascularization induction. In addition, the insulin secretion of cell aggregates was significantly promoted by the mixing of gelatin hydrogel microspheres both in vitro and vivo systems. It is concluded that the microspheres mixing had the potential to give cells in aggregates a better survival condition, resulting in the promoted insulin secretion.

Conflicts of interest

There is no conflict of interest to disclose.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.reth.2018.01.003.

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