Original Article

The intraperitoneal space is more favorable than the subcutaneous one for transplanting alginate fiber containing iPS-derived islet-like cells

Satsuki Fukuda [1], Shigeharu G. Yabe [1], Junko Nishida, Fujie Takeda, Kiyoko Nashiro, Hitoshi Okochi*

Department of Regenerative Medicine, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama Shinjuku-ku, Tokyo, 162-8655, Japan

A B S T R A C T

Introduction: Although immunosuppressants are required for current islet transplantation for type 1 diabetic patients, many papers have already reported encapsulation devices for islets to avoid immunological attack. The aim of this study is to determine the optimal number of cells and optimal transplantation site for human iPS-derived islet-like cells encapsulated in alginate fiber using diabetic model mice.
Methods: We used a suspension culture system for inducing islet-like cells from human iPS cells throughout the islet differentiation process. Islet-like spheroids were encapsulated in the alginate fiber, and cell transplantation experiments were performed with STZ-induced diabetic NOD/SCID mice. We compared the efficacy of transplanted cells between intraperitoneal and subcutaneous administration of alginate fibers by measuring blood glucose and human C-peptide levels serially in mice. Grafts were analyzed histologically, and gene expression in pancreatic β cells was also compared.
Results: We demonstrated the reversal of hyperglycemia in diabetic model mice after intraperitoneal administration of these fibers, but not with subcutaneous ones. Intraperitoneal fibers were easily retrieved without any adhesion. Although we detected human c-peptide in mice plasma after subcutaneous administration of these fibers, these fibers became encased by fibrous tissue.
Conclusions: These results suggest that the intraperitoneal space is favorable for islet-like cells derived from human iPS cells when encapsulated in alginate fiber.

1. Introduction

Type 1 diabetic patients need frequent insulin injections due to the loss of their pancreatic β cells and consequently have a potential risk of hypoglycemia. Although islet transplantation is a very effective treatment for these patients, it is not easy to find donors. To solve the problem of donor shortage, xenografts such as porcine islets and cell-based therapy using pluripotent stem cells are considered promising as alternative cell sources [1]. Many researchers have reported the induction of insulin secreting cells from ES cells and iPS cells because of their huge proliferating capacity. D'amour et al. first reported methods for differentiating human ES cells into pancreatic β cells [2]. Rezania et al. and Pagliuca et al. showed that induced pancreatic β cells from ES cells functioned in vivo by ameliorating hyperglycemia in diabetic mice [3,4]. We previously reported our original methods employing 6 stages for inducing functional pancreatic β cells from human iPS cells [5] and very recently developed suspension culture methods using the 30 ml spinner type vessels for scaling up [6].

Abbreviations: NOD/ SCID mouse, non-obese diabetic /severe combined immunodeficiency mouse; iPS cells, induced pluripotent stem cells; ES cells, embryonic stem cells; STZ, streptozotocin.
  * Corresponding author. Department of Regenerative Medicine, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo, 162-8655, Japan.
  E-mail addresses: sfukuda@ri.ncgm.go.jp (S. Fukuda), syabe@ri.ncgm.go.jp (S.G. Yabe), jnishida@ri.ncgm.go.jp (J. Nishida), ftakeda@ri.ncgm.go.jp (F. Takeda), kyoko_chan.746@docomo.ne.jp (K. Nashiro), hokochi@ri.ncgm.go.jp (H. Okochi).
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  [1] These authors contributed equally.

For clinical applications of islet transplantation, usage of immunosuppressants for both allograft and xenograft to avoid rejection is another problem, because their long-term use has the potential risks of side effects and developing malignancies. Many papers have already reported examining encapsulation devices for islets to avoid immunological attack without using immunosuppressants [7]. The concept of the device is simple; the capsules act as semipermeable membranes which allow the diffusion of oxygen and nutrients as well as insulin, but not of immune cell movement. Strand et al. emphasized the important capsule properties of stability, permeability, and biocompatibility for encapsulated pancreatic islets and nominated alginate as one favorable material [8]. In alginate encapsulation, spherical beads are usually made by an electric bead generator. Onoe et al. reported that alginate fiber encapsulated islets reversed hyperglycemia in mice [9]. We focused on the alginate fiber because we thought it easier to retrieve fibers than beads after transplantation. When we use ES cells and iPS cells for cell therapy, there is a potential risk of forming an unwanted teratoma. Therefore, it is very important to consider retrievability, especially when transplanting a large number of these cells.

Currently, islets isolated from human donors are injected in the portal vein and survive in the liver. However, once islets are encapsulated in either spherical or cylindrical form, they are too large to fit in the portal vein. Therefore, both microencapsulated and macroencapsulated devices have been transplanted into the intraperitoneal space or the subcutaneous tissue. Examples include microencapsulated neonatal porcine islets [10] or human islets [11] transplanted in the intraperitoneal space in clinical trials. Additionally, microencapsulated ES-derived islet-like cells were transplanted in the subcutaneous tissue in another clinical trial [12]. For the alginate fiber, suitable transplantation sites have not been examined, and the number of cells required for transplantation is undetermined.

The aim of this study is to determine the optimal number of cells for transplantation and the transplantation site for human iPS-derived islet-like cells encapsulated in the alginate fiber.

2. Methods

2.1. Human iPSC culture and differentiation

The human iPSC line TkDN4-M was a kind gift from Dr. M. Ohtsu at The Institute of Medical Science, The University of Tokyo. Freeze-stored iPSCs were thawed and cultured as described previously [5] in hiPS medium (DMEM/Ham's F12; Wako) in the presence of 20% knockout serum replacement (KSR; GIBCO), 1x non-essential amino acids (NEAA; Wako), 55 μM 2-mercaptethanol (2-ME, GIBCO) and 7.5 ng/ml recombinant human fibroblast growth factor 2 (FGF2) (Peprotech) on mitomycin-C (Wako)-treated SNL feeder cells to maintain an undifferentiated state. Cultured iPSCs were dissociated with CTK solution and seeded at a density of 1×10^6 cells/ml in a spinner type reactor (Biott) containing 30 ml of mTeSR 1 (Veritas) with 10 μM ROCK inhibitor (Y-27632; Cayman Chemical) at a rotation rate of 45 rpm. Spheroids formed by cell aggregation during 2 day-culture and were then cultured in hiPS medium for 1 day before starting differentiation. The differentiation protocols comprise 6 stages described previously [6]; a schematic outline is summarized in Fig. 1A.
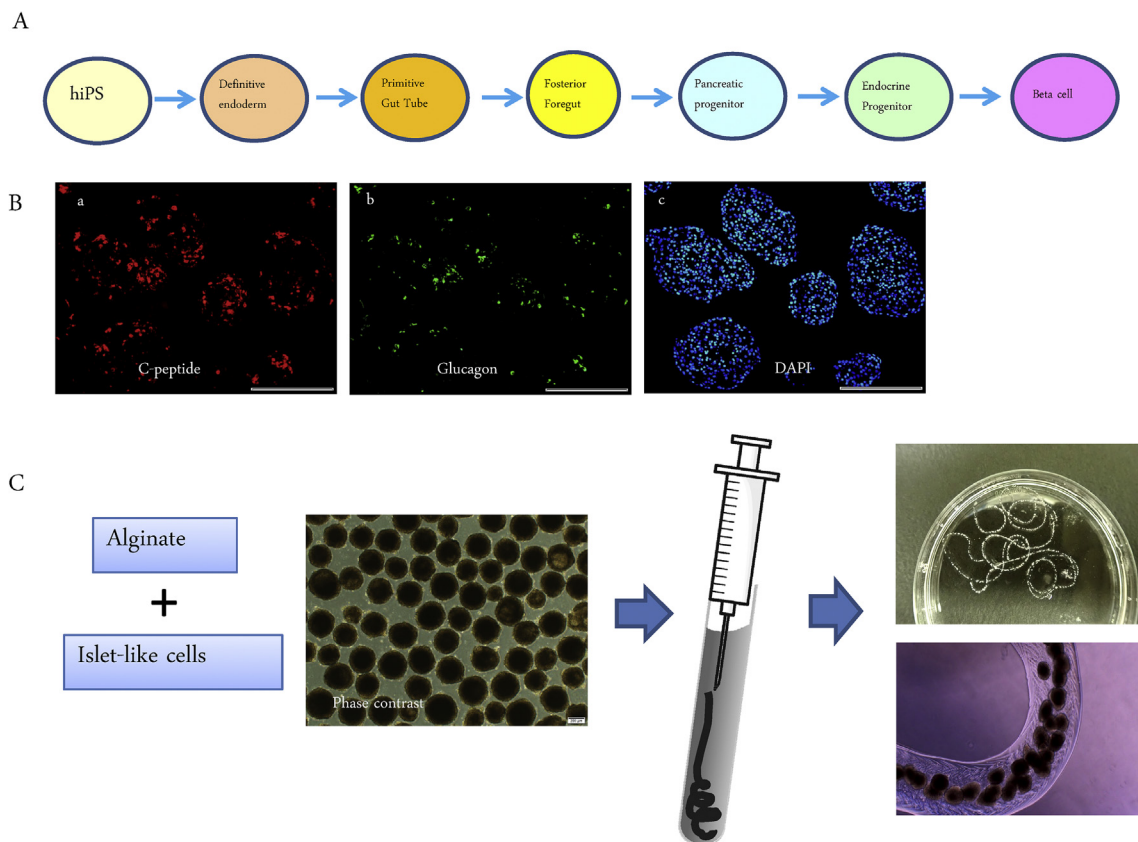
Fig. 1. Overview of the differentiation protocol for iPS cells and alginate fiber formation. A: Summary of 6 differentiation stages mimicking developmental process of pancreas. B: Immunostaining of islet-like spheroids at beta stage. a: red; human C-peptide positive cells, b: green; glucagon positive cells, c: blue; nuclear staining with DAPI. Scale bar = 200 μm. C: Alginate fiber formation. Human iPS-derived islet-like spheroids were mixed with alginate and injected into barium chloride solution, leading to the encapsulated fiber formation.

2.2. Encapsulation of spheroids into alginate fiber

After centrifugation, 6×10^3 spheroids including approximately 6×10^6 iPSC-derived cells in total were washed in saline. Then 0.75 ml of PRONOVA SLG100 (NOVAMATRIX) was added to the spheroids and mixed well with pipets. The mixture was loaded into a 1 ml syringe with 20G needle and injected into 15 ml conical tube containing 10 ml of 0.1 M barium chloride, leading to fiber formation. The fiber was immersed in the solution for 3–5 min at room temperature for cross-linking (Fig. 1C). Then it was washed with saline three times and immersed in the culture media overnight; then it was ready for transplantation experiments. For the gene expression study, 4×10^6 iPSC-derived cells in total were embedded into alginate fiber in the same way.

2.3. Animal experiments

Animal studies were conducted according to the protocols approved by the Animal Care and Use Committee in the National Center for Global Health and Medicine. To induce diabetic model mice, 8-week-old male NOD/SCID mice (Japan Clea) were injected intravenously with 130 mg/kg of streptozotocin (STZ; Sigma). After blood glucose levels increased over 350 mg/dl, one or two pieces of alginate fibers were transplanted into the intraperitoneal or subcutaneous spaces of the NOD/SCID diabetic mice using forceps (n = 2 per batch). Spheroids from at least two different batches were examined for animal experiments. For the gene expression study, each alginate fiber was transplanted into intraperitoneal or subcutaneous spaces of NOD/SCID mice without STZ.

Non-fasting blood glucose levels were examined once or twice a week via the tail vein using a glucose test kit (Glutest Neo Sensor, Sanwa Chemical). Blood samples were collected in heparin-coated capillaries from tail vein every 2 or 4 weeks, and plasma was obtained after centrifugation (10 min, 4 °C, 800 g). They were kept frozen at −80 °C until use. Mice were killed 4–16 weeks after alginate fiber transplantation, and grafts were histologically analyzed.

2.4. Immunostaining and immunohistochemistry

Spheroids were collected after the differentiation process. Alginate fibers were retrieved from mice 14 or 16 weeks after transplantation. These samples were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 3-μm sections. Hematoxylin and eosin staining was performed according to the standard protocol. For immunostaining, sections were incubated with primary antibodies (diluted in phosphate-buffered saline [PBS], 1.5% goat serum) overnight at 4 °C in a humidified chamber. The primary antibodies were rat anti-C-peptide (1: 200; DSHB, University of Iowa) and rabbit anti-proglucagon (1: 300; Cell Signaling Technology). Sections were washed with PBS and then incubated with a fluorescence-conjugated secondary antibody, Alexa 594-conjugated goat anti-rat IgG (1:400; Invitrogen) or Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:400; Invitrogen), for 120 min at room temperature. Slides were counterstained with 40, 60-diamidino-2-phenylindole (DAPI; Invitrogen) prior to mounting with Fluoromount (Diagnostic Biosystems).

2.5. Quantitative RT-PCR

Using Isogen (Wako), we extracted RNA from spheroids which had been encapsulated in the alginate fibers and retrieved 4, 8 and 12 weeks after transplantation. We performed qRT-PCR analysis of several genes: UCN3, PCSK1, PCSK2, SLC30A8, ABCC8, KCNJ11 and GJD2. The primer details of those genes were listed in Table 1. The cDNA was synthesized with PrimeScript II reverse transcriptase using random nonamers and oligos (dT18). Quantitative RT-PCR reactions were carried out on CFX96Touch Deep Well (Bio-Rad) using GoTaq qPCR master mix (Promega). Relative quantification was performed against a standard curve, and the expression levels of target genes were normalized against that of the housekeeping gene, ornithine decarboxylase antizyme (OAZ1).

2.6. Measurement of human C-peptide and glucagon

Human C-peptide and glucagon concentrations in mouse plasma were determined using human ultrasensitive C-peptide ELISA kits (Mercodia) and glucagon ELISA kits (Mercodia).

2.7. Oral glucose tolerance test

Fourteen weeks after transplantation, 2 intraperitoneally transplanted mice, one subcutaneously transplanted mouse and a STZ-induced DM control mouse were fasted for 4 h, and blood glucose was measured every 5 min during the first 60 min and 70, 80, 90, 120 min after the oral administration of a glucose solution (2.0 g/kg).

2.8. Statistical analyses

Data are expressed as the mean ± SEM. For comparisons of discrete data sets, unpaired Student's t-tests were used. P < 0.05 was considered significant.

3. Results

3.1. Alginate fiber formation

The human iPSC line TkDN4-M cells were differentiated into islet-like spheroids with diameters of 200 ± 50 μm by our original 6-step suspension culture protocol as described previously (Fig. 1A) [6]. Human C-peptide positive cells and glucagon positive cells were detected in the spheroids (Fig. 1B). Approximately 6×10^3 spheroids, which presumably contained 6×10^6 iPSC-derived cells

Table 1
Primer list for qPCR.

Gene	Forward	Reverse
UCN3	TAC AGG TTG CTG CAC TGC TG	GGA AGG TTG GAC AAT ACT GCA C
PCSK1	GAC CTG CAC AAT GAC TGC AC	CAT ACT CAG AGG TCC AGA CAA CC
PCSK2	AGT TGT ATG GAG CCT CTT CTG C	TAG TTG CCG AGA AGG TGA CTG
SLC30A8	GTG GAA ACT CTG TTC TGC TTC AG	GAC AAC CAC AGG GAG AAG AGA C
ABCC8	CCA ACG TTG TCT TGG ATG	TCA CTA AGA CCA CTG TCC TCT TGT C
KCNJ11	AAG CCC AAG TTC AGC ATC TCT C	CAT ACC ACA TGG TCC GTG TGT AC
GJD2	GGA ATG GAC CAT CTT GGA GAG	AGA TCA CCA CAG TCA ACA G
OAZ1	GTC AGA GGG ATC ACA ATC TTT CAG	GTC TTG TCG TTG GAC GTT AGT TC

in total, were mixed with 0.75 ml of alginate and injected into 0.1 M barium chloride solution for cross-linking to form encapsulated alginate fiber. As shown in Fig. 1C, a single thread-like fiber whose diameter was around 2 mm was generated. The spheroids were placed in a line in the center portion of the fiber.

## 3.2. Optimal number of transplanted islet-like cells

In the previous study, we confirmed that approximately 6×10^6 iPS-derived islet-like cells were enough to ameliorate hyperglycemia in diabetic mice when they were transplanted under the capsule of the kidney [6]. We also realized that more than 250 pM of human C-peptide levels in mice plasma was required to control their blood glucose without mouse insulin. We hypothesized that more cells would be needed in encapsulated fiber than in naked form to achieve such levels of human C-peptide in mice plasma, because encapsulated cells don't contact endothelial cells directly, so the secreted C-peptide reached the blood stream only by diffusion. To examine the optimal number of encapsulated cells required in animal experiments, we compared two doses, 6×10^6 cells and 1.2×10^7 cells, by transplanting one piece of fiber in the former case and two pieces of fiber in the latter into the intraperitoneal space of STZ-induced diabetic NOD/SCID mice. A mouse transplanted with 1.2×10^7 cells ameliorated hyperglycemia and maintained around 100 mg/dl of non-fasting blood glucose level up to 60 days after operation (Suppl. Fig. 1A). Human C-peptide levels in their plasma were constantly approximately 250 pM (Suppl. Fig. 1B). In contrast, a mouse transplanted with 6×10^6 cells maintained a moderately high blood glucose level which showed between 300 and 400 mg/dl, although we detected human C-peptide in their plasma at levels around 100 pM (Suppl. Fig. 1A and B).

When we compared the same number of encapsulated cells in non-treated NOD/SCID mice, human C-peptide levels in their plasma was always twice as high in the mouse that had been transplanted with 1.2×10^7 cells than in the mouse with 6×10^6 cells (Suppl. Fig. 1D). These results suggest that human C-peptide levels in mice plasma corelate with the number of transplanted cells and that 1.2×10^7 cells are optimal for transplantation.

3.3. Comparison of transplanted site between intraperitoneal and subcutaneous administration for iPS-derived islet-like cells

Next, we examined whether the transplanted site affected the efficacy of islet-like spheroids. We transplanted two pieces of fiber which contained 1.2×10^7 cells in total, either intraperitoneally or subcutaneously (n = 4 each). Blood glucose levels of intraperitoneally transplanted mice declined steadily and remained around 100 mg/dl 4 weeks after transplantation (Fig. 2A). Human C-peptide levels in their plasma were around 300pM until 12 weeks after transplantation (Fig. 2C). On the other hand, blood glucose levels of subcutaneously transplanted mice were more than 250 mg/dl, although human C-peptide levels in their plasma stayed around 200pM until 12 weeks after transplantation (Fig. 2A, C). Plasma glucagon levels were almost the same between the two groups (Fig. 2D).
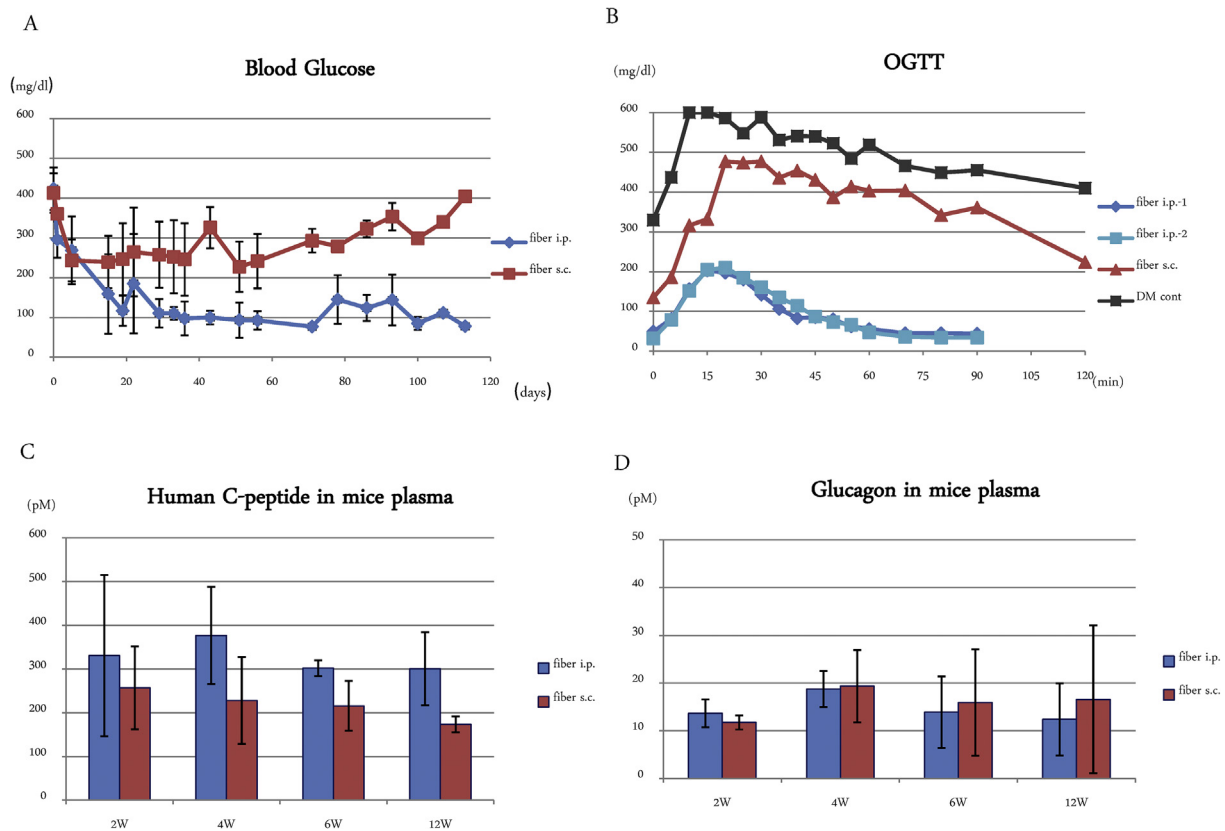
Fig. 2. Transplanted alginate fibers ameliorate hyperglycemia in diabetic mice A: Approximately 1.2×10^7 iPSC-derived cells were encapsulated in alginate fiber and transplanted into STZ-induced NOD/SCID diabetic mice intraperitoneally or subcutaneously. Non-fasting blood glucose levels were examined once or twice a week after transplantation. Two different batches of spheroids were used for transplantation experiments. fiber i. p.: intraperitoneally transplanted mice (n = 4), fiber s. c.: subcutaneously transplanted mice (n = 4). B: Oral glucose tolerance test (OGTT); Blood glucose levels were measured after oral glucose load (2.0 g/kg) at 14 weeks after transplantation. DM cont: STZ-induced NOD/SCID diabetic mouse Blood human C-peptide (C) and glucagon levels (D) in the mice plasma were measured 2, 4, 6, 12 weeks after transplantation. n = 4 each.

Oral glucose tolerance tests were performed 14 weeks after transplantation using 2 intraperitoneally transplanted mice, one subcutaneously transplanted mouse, and one diabetic control mouse. Blood glucose levels of the intraperitoneally transplanted mice peaked at 15 min after administration of glucose and fell below 100 mg/dl after 30 min (Fig. 2B). In contrast, blood glucose levels of the subcutaneously transplanted mouse peaked at 30 min after glucose loading and gradually lowered to 200 mg/dl after 120 min (Fig. 2B). Blood glucose levels of the DM control mouse peaked at the upper limit (600 mg/dl) and didn't return to the starting level by 120 min (Fig. 2B).
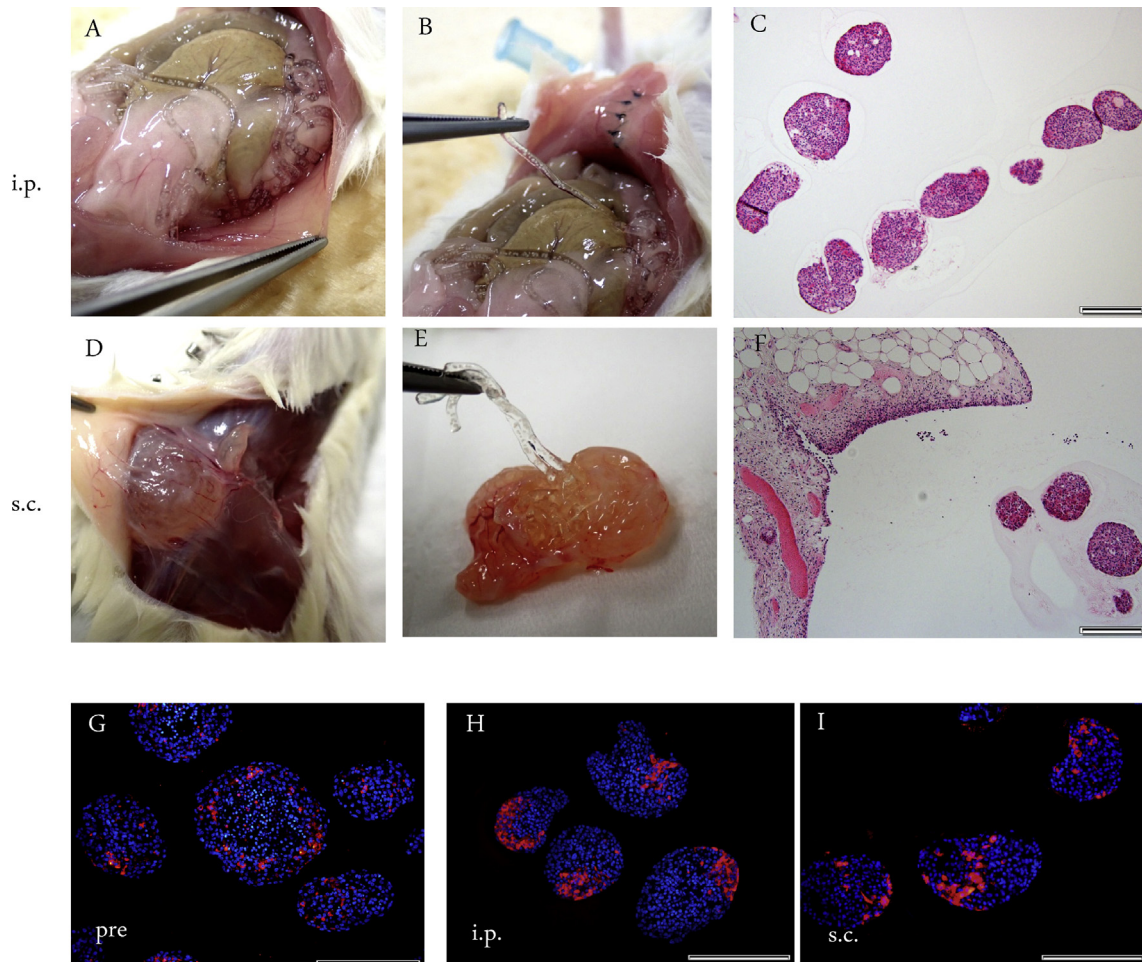
3.4. Immunohistochemical analysis of transplanted fibers

We retrieved fibers 14 or 16 weeks after transplantation. As shown in Fig. 3A, intraperitoneally transplanted fibers were intermingled with intestinal tracts but were retrieved easily with the forceps without any adhesion (Fig. 3B). In contrast, subcutaneously transplanted fibers were surrounded by a fibrous membrane (Fig. 3D), and fibers were retrieved after cutting the membrane (Fig. 3E). Encapsulated fibers were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 3-μm sections. Hematoxylin and eosin staining revealed that more than 90% of the spheroids survived in the intraperitoneally transplanted fibers (Fig. 3C). In contrast, about 70% of the spheroids survived in the subcutaneously transplanted fibers (Fig. 3F). Immunohistochemical analysis revealed that there was no difference in the frequency of human C-peptide positive cells between intraperitoneally and subcutaneously transplanted fibers (Fig. 3G–I).

3.5. Comparison of serial gene expression and hormone secretion between intraperitoneally and subcutaneously transplanted fibers

We compared the gene expression profiles of iPS-derived islet-like cells after transplantation between intraperitoneal and subcutaneous administration. We extracted RNA from encapsulated fibers which were collected 4, 8, and 12 weeks after transplantation. We performed qRT-PCR analysis of several genes, UCN3, PCSK1, PCSK2, SLC30A8, ABCC8, KCNJ11 and GJD2, all of which are related with the function of pancreatic β cells. We examined three different batches, and we basically obtained almost the same expression pattern in both intraperitoneal and subcutaneous samples (Fig. 4A and B). Although we did not find a clear difference in plasma glucagon levels between the two groups, the intraperitoneally transplanted mice had higher human C-peptide levels in their plasma than subcutaneously transplanted ones (Fig. 4C, D).

Fig. 3. Retrieved fibers and immunohistochemical analysis of grafts Representative images of the fiber 16 weeks after transplantation. A, B: intraperitoneally transplanted fiber, D, E: subcutaneously transplanted fiber. Representative images of H&E staining. C: intraperitoneally transplanted fiber, F: subcutaneously transplanted fiber. Scale bars = 200 μm. Immunofluorescent staining of the spheroids. G: pre; before transplantation, H: i. p.; intraperitoneally transplanted spheroids, I: s. c.; subcutaneously transplanted spheroids, red; human C-peptide, blue; DAPI. Scale bars = 200 μm.
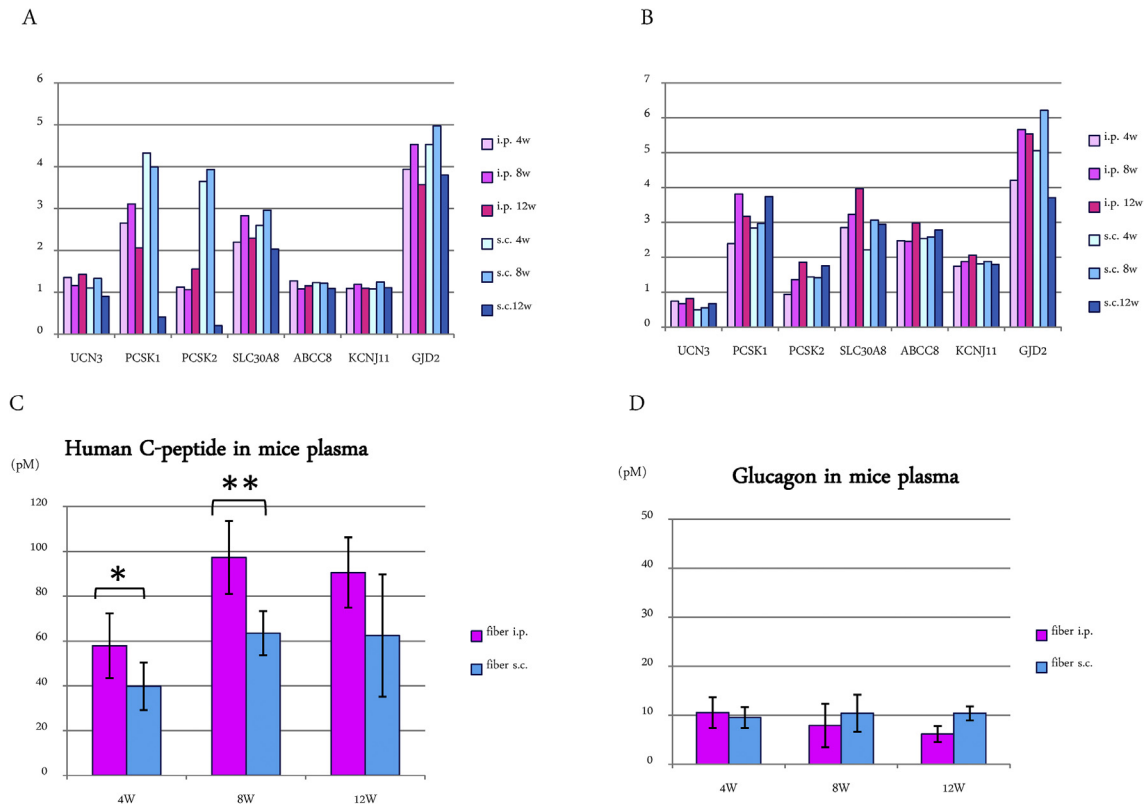
Fig. 4. Comparison of serial gene expression and hormone secretion A, B: Results of qPCR. In these experiments, 4.0×10^6 cells were encapsulated in the alginate fiber and transplanted. We compared the gene expression profiles of spheroids collected 4, 8, 12 weeks after transplantation. The expression levels of target genes were normalized against that of OAZ1. Each bar reflects the fold induction comparing with the expression level before transplantation. Three different batches of the cells were determined, and two sets of representative data were shown. A: experiment 1, B: experiment 2. i. p.; intraperitoneally transplanted spheroids, s. c.; subcutaneously transplanted spheroids C, D: Human C-peptide and glucagon concentrations in mouse plasma were determined 4, 8, 12 weeks after transplantation using human ultrasensitive C-peptide ELISA kits and glucagon ELISA kits. n = 3 each. C: Human C-peptide, D: glucagon. P < 0.05 was considered significant. *: P < 0.05, **: P < 0.01.

4. Discussion

In this study, we demonstrated that intraperitoneally transplanted cells encapsulated in alginate fibers ameliorated hyperglycemia and maintained constant human C-peptide levels in the mice plasma until 12 weeks after transplantation. Moreover, these alginate fibers were retrieved easily with forceps without any adhesion. In contrast, subcutaneously transplanted cells did not reverse hyperglycemia, because the fibers were wholly surrounded by a fibrous membrane.

Our previous results showed that 6×10^6 islet-like cells derived from human iPS cells were sufficient for reversal of hyperglycemia in diabetic mice when transplanted under the kidney capsule in a naked form. In this study, twice as many cells were needed for reversal when encapsulated in alginate fibers and transplanted intraperitoneally. Dulong and Legallais estimated that, in fiber devices whose diameter was 2 mm, two to eight times more cells would be needed for implantation than naked cells [13]. We were able to achieve the minimal cell number required for the device based on their calculation, because we didn't lose the transplanted cells to necrosis.

Notably, in the subcutaneously transplanted cases, we were able to detect human C-peptide (about 180 pM) in the mouse plasma 12 weeks after transplantation, indicating that the transplanted cells were still functional. HE staining confirmed that encapsulated cells survived even if they were covered with a fibrous membrane. In addition, the immunohistochemical analysis revealed that C-peptide positive cells remained viable 12 weeks after transplantation. Iwata et al. focused on the oxygen diffusion in a device and

indicated that spherical bioartificial pancreas allows for selecting a wider range of membrane thicknesses than cylindrical and planar ones [14]. We didn't find any central necrosis, one sign of hypoxia, in the encapsulated spheroids, suggesting that a minimal oxygen concentration was maintained. The results of OGTT showed that the subcutaneously transplanted cells did not respond quickly to the elevation of blood glucose, and it took a long time to revert to the basal blood glucose level; this pattern clearly differed from that of intraperitoneally transplanted cells. The difference may be due to the fibrous membrane and/or to the functional inferiority of individual cells. In intraperitoneally transplanted mice, the blood glucose levels were already very low just before OGTT after 1.5 h fasting. We stopped measuring the blood glucose concentration at 90 min and then administered the glucose to the mice, because we should avoid further fasting considering the animal welfare. The low blood glucose levels in intraperitoneally transplanted mice were explained by the overproduction of insulin from the encapsulated cells. One possible reason of the overproduction of insulin might be the immature state of beta cells. We previously reported that differentiated islet-like cells produce certain amount (more than 2000 pM/h/2 × 106 cells) of C-peptide even in low glucose condition (2 mM) in vitro before transplantation [6]. Glucose stimulated insulin secretion assay revealed that cells didn't respond well to the high glucose, suggesting the immature state. These cells in alginate fiber may continue to produce insulin in vivo even in low blood glucose due to the delay of maturation. Another possibility is the delay of glucose sensing and response because encapsulated cells don't contact endothelial cells directly. It will take some time for insulin to be incorporated into blood stream by diffusion after

secreted from the fiber and therefore beta cells may produce much more insulin than needed. When we transplanted naked islet-like cells differentiated with the same protocols as this experiment under the kidney capsule where cells could contact endothelial cells directly, the peak of blood glucose levels in OGTT was at 15 min [6]. In contrast, the peak of blood glucose levels in OGTT in case of intraperitoneally transplanted mice was at 30 min as shown in Fig. 2B. These results support the delay of glucose sensing and response in alginate fiber.

Some authors have recommended the intraperitoneal space as the transplantation site [15–18]; others, the subcutaneous space [19,20]. Generally speaking, from the safety point of view, it is easier and preferable to retrieve devices from the subcutaneous space than from the intraperitoneal space. The development of laparoscopic surgical procedures has made abdominal surgery less invasive. An et al. has reported that they were able to retrieve reinforced alginate fiber by laparoscopic surgery in a dog model [21]. In both sites, foreign body reaction is the major problem of devise implantation, because inflammatory foreign body responses leading to pericapsular fibrotic overgrowth often cause micro-encapsulated islet-cell death and graft failure [7,22,23]. Foreign body reaction is usually provoked by macrophages and leads to pericapsular fibrotic overgrowth. Paredes Juarez et al. noted that we must consider immunological and technical aspects in the application of alginate-based microencapsulation systems, because the immune reaction differs among species against danger-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPs) [24]. We used immuno-compromised mice (NOD/SDID) for the transplantation experiments, because we wanted to avoid inflammation caused by the xeno-antigens [25,26]. Judging from the results of intraperitoneally transplanted fibers, which were retrieved without any adherent cells, we succeeded in preventing such inflammations. Therefore, the reason for the pericapsular fibrotic overgrowth in our case of subcutaneously transplanted fibers cannot be explained by xeno-antigens. One possible cause may be the shape of the fibers. Intraperitoneally transplanted fibers were scattered in the abdominal cavity by peristaltic movement, and they intermingled with visceral organs without adhesion. In contrast, the subcutaneously transplanted fibers remained in a group at the transplanted site; thus they might be recognized as a giant foreign body by the macrophages.

Although we proved the effectiveness of transplanted cells up to 16 weeks and retrieved fibers without breakage, another concern is how to maintain the long-term effect of transplanted fibers. Alginate fibers are known to become fragile gradually, because they slowly lose the cations which are indispensable for maintaining gel. It is impossible to follow their longer effectiveness in our current experimental procedures, because the NOD/SCID mice only live around 6 months due to thymoma. We need to employ other mice, such as NOG, and to modify the alginate fiber to increase its strength for long-term use. Moreover, Morch et al. reported that high-G alginate beads accumulated higher concentration of barium after exposure of gelling solution containing barium than high-M alginate beads [27]. According to their results from mice, they estimated the barium leakage when they give 60 ml alginate to 70 kg person. The value was 0.03 mg barium/kg, which was about 1.5 times higher than the tolerable intake value recommended by WHO. We will need to consider the gelling methods because they recommended to make alginate beads gelled with a combination of calcium (50 mM) and low concentration of barium (1 mM) to reduce the leakage of barium [27].

Recently ViaCyte performed a clinical trial using pancreatic beta cell precursors (PEC-01™) differentiated from human ES cells with subcutaneous implantation in a retrievable medical device (Encaptra® cell delivery system). They reported in a press release

"The first-in-human STEP ONE trial is evaluating the safety and efficacy of ViaCyte's PEC-Encap (a.k.a. VC-01™) product candidate, a stem cell-derived, encapsulated cell replacement therapy. Although limited by low levels of engraftment due to a foreign body giant cell response, differentiation into endocrine islet cells was observed in both 12-week and two-year explants. ViaCyte is modifying the Encaptra Cell Delivery System to improve the potential for long-term engraftment."

Although ViaCyte does not use alginate for the macro-devices, several reports have examined avoiding foreign body reaction against alginate devices [28–30]. Vegas, A. J et al. reported that alginate beads with three triazole-containing analogs substantially reduced foreign body reactions in both rodents and, for at least 6 months, in non-human primates [31,32]. Very recently, Alagpulinsa, D. A. et al. reported that alginate-microencapsulation of human stem cell-derived beta cells with CXCL12 prolonged their survival and function in immunocompetent C57BL/6 mice without systemic immunosuppression [33].

Barron and He reported that the most prominent and successful method in clinical application was the coaxial electrospray method whose system consisted of two syringe pumps for pushing the cell-containing fluid and the alginate solution, leading to a microcapsule composed of an aqueous core fluid with living cells surrounded by an alginate hydrogel shell [34]. Basta et al. reported the 4 cases of type 1 diabetic patients who received intraperitoneal transplant of microencapsulated human islets. They used the alginate/poly-aminoacidic encapsulation system and the injected total graft volume didn't exceed 100 ml. All the patients showed positive for serum C-peptide response throughout 3 years [35]. If we aim at a clinical trial, we will need 2000 times more cells than we do in a mouse, which means we have to provide a huge number of cells and alginate. We will need 3000 ml of alginate for the fibers, which doesn't seem realistic. However, in the first in human clinical trial, if we set a goal to maintain a base-line levels of insulin secretion in the type 1 diabetic patients, one thirtieth of therapeutic dose will be needed for that purpose, approximately 100 ml of alginate which is comparable to the previous clinical trial [35]. It is very important for those patients to keep a base-line levels of insulin secretion in order to reduce the risk of hypoglycemic episodes. On the other hand, we have to consider possible teratoma formation when we use a large number of cells, especially ones derived from pluripotent stem cells. We observed that the size of spheroid didn't increase in the alginate fiber after transplantation into mice, suggesting that proliferating cells are few, if any. Moreover, the spheroids didn't fuse with each other in the alginate fiber, which should also lower the risk of teratoma formation. In addition, we were able to remove the alginate fiber easily, which is advantageous, because retrievability is also important in cell-therapy using devices. These results suggest that using alginate fiber is promising, because it is not only immunosuppressive but also safer in terms of retrievability. In the future we have to further improve the function of iPS-derived β cells to reduce the number of the cells required and the costs of preparing cells.

5. Conclusions

The intraperitoneal space is more favorable for encapsulated alginate fibers containing iPS-derived islet-like cells than the subcutaneous space.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2019.05.003.

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