Transplant International

ORIGINAL ARTICLE

Introduction to a new cell transplantation platform via recombinant peptide petaloid pieces and its application to islet transplantation with mesenchymal stem cells

Kentaro Nakamura, Reiko Iwazawa & Yasuhiro Yoshioka

Regenerative Medicine Research Laboratories, FUJIFILM Corporation, Kanagawa, Japan

Correspondence
Kentaro Nakamura, 577 Ushijima, Kaisei-machi, Ashigarakami-gun, Kanagawa 258-8577, Japan.
Tel.: +81-465-86-1846;
fax: +81-465-86-1019;
e-mail: kentaro.a.nakamura@fujifilm.com

SUMMARY

Cell death cluster in transplanted cells remains a critical obstacle for regeneration strategies. This study describes a novel platform for cell transplantation (CellSaic) consisting of human mesenchymal stem cells (hMSCs) and petaloid pieces of recombinant peptide (RCP), which can prevent cell death by arranging the cells in a mosaic. When hMSC CellSaics were subcutaneously implanted into NOD/SCID mice, hMSC CellSaics prevented cell death and accelerated angiogenesis in the graft, compared to the findings obtained on solely implanting cell spheroids. Additionally, we examined the application of CellSaic for subcutaneous cotransplantation of 200 rat islets with 2×10^5 hMSCs into diabetic mice. As the results of blood glucose levels at 1 M, the islet-only group was 398 ± 30 mg/dl and the islets with hMSCs group were 180 ± 65 mg/dl. On the other hand, the islets with hMSCs CellSaic group showed 129 ± 15 mg/dl and significantly improved glucose tolerance ($P < 0.05$). Additionally, we showed that the surface texture of the RCP petaloid pieces played an important role in graft survival and angiogenesis. It is anticipated that CellSaic will be used as a new platform for cell transplantation and tissue regeneration.

Transplant International 2016; 29: 1039–1050

Key words
cell transplantation, islet clinical

Received: 15 March 2016; Revision requested: 14 April 2016; Accepted: 9 June 2016

Introduction

In recent years, new approaches for cell transplantation and implantation of regenerative tissues have been developed and used in the field of regenerative medicine [1–5]. Nevertheless, graft cell death remains a critical problem for various tissue and organ regeneration strategies, including cell-sheet engineering. Graft cell death mainly results from poor cell and tissue nutrition in cell clusters, which is often caused by aberration of the vascular system or failure of the metabolic waste product removal system.

The process of cell death occurring in cell clusters has been widely investigated [1–3]. It initially occurs in spheroids and is generally dependent on the typical depletion of nutrient substrates and accumulation of metabolic waste products. To demonstrate this phenomenon, Groebe and Mueller-Klieser [4–6] compared experimental and theoretical oxygen concentration data and showed that the relationship between the viable rim thickness and size of the necrotic tissue varied according to the spheroid diameter.

Cell death cluster of pancreatic islet tissues has imposed particular limitations in the progress of

doi:10.1111/tri.12810

pancreatic islet transplantation since the early stages of tissue transplantation research [7,8], including the heterotopic transplantation of pancreatic islets into organs other than the portal vein, especially via subcutaneous or intramuscular transplantation [9].

FUJIFILM Corporation, Tokyo, Japan developed a new bioabsorbable biomaterial called recombinant protein (RCP) for medical applications [10]. The evaluation of RCPs based on the alpha-1 sequence of human collagen type I showed the presence of 12 RGD (Arg-Gly-Asp) motifs in a single molecule. RCP is a recombinant protein produced by the yeast *Pichia pastoris* and differs from conventional animal collagen because there is no risk of infection, such as bovine spongiform encephalopathy [11,12].

We here present a new cell transplantation platform (CellSaic) that can prevent graft cell death in spheroids because of the use of petaloid pieces of RCP. The newly coined term 'CellSaic' is derived from 'cell' and 'scaffold'-forming 'mosaic'. Unlike spheroids, the spaces between the petaloid RCP pieces of the CellSaic platform lead to greater penetration of substances into the cells, which prevents cell death. We confirmed that the cells of the implanted CellSaic platforms showed good survival, and blood vessels could be easily formed into the grafts.

Furthermore, to investigate its applications, we examined whether CellSaic can be effectively used for cotransplanting human mesenchymal stem cells (hMSCs) with pancreatic islets. The approach of transplantation of pancreatic islets has been developed for treating patients with insulin-dependent diabetes. Currently, the liver is considered to be a favorable transplantation site, and the transplantation is performed through the portal vein. This intraportal transplantation of islets, which is referred to as the Edmonton protocol, helps in achieving long-term endogenous insulin production and glycemic stability in patients with type 1 diabetes mellitus [13]. However, the use of this protocol has thus far yielded insufficient improvements in insulin independence. One method for improving insulin independence is to use transplantation sites other than the liver; in this regard, intramuscular and subcutaneous spaces are promising candidate sites [9]. Animal studies have shown that cotransplantation of pancreatic islets with MSCs improves the function of the islets [14]. Ito *et al.* [15] showed that cotransplantation of pancreatic islets with MSCs improved the morphology and function of islet grafts. Furthermore, mice cotransplanted with pancreatic islets and MSCs showed normoglycemia, whereas mice transplanted with islets alone showed only a 30% improvement.

We expected that the CellSaic would enhance the cotransplantation effect of human mesenchymal stem cells (hMSCs) with pancreatic islets.

Materials and methods

Experimental design

The overall experimental design for this study is presented in Fig. 1. The hMSCs were used as model cells to study cell death in transplanted cells and tissues. The CellSaic platform consisting of hMSCs and micrometer-sized pieces of RCP was compared with a spheroid containing the same number of cells and a spheroid with the same volume. Furthermore, three types of micrometer-sized pieces of RCP were compared.

Figure 2 presents the experimental design for islet transplantation as an application of CellSaic. Implants including islets were transplanted subcutaneously into streptozotocin-induced diabetic mice. The transplant group was divided into three subgroups based on the implants used: (i) the CellSaic group, a mixture of hMSCs, RCP petaloid μ-pieces, and islets; (ii) the suspension group, hMSCs and islets; and (iii) the islet group, islets alone. Post-transplant glucose levels were measured in each mouse, and all mice were evaluated using the glucose tolerance test and histopathological examinations of transplant sections.

Materials

Recombinant peptide was obtained using a previously reported method [10–12]; the sequence of RCP is as follows: GAP[GAPGLQGAPGLQGMPGERGAAGLPGPKGERGDAGPKGADGAPGAPGLQGMPGERGAAGLPGPKGERGDAGPKGADGAPGKDGVRGLAGPIGPPGERGAAGLPGPKGERGDAGPKGADGAPGKDGVRGLAGPIGPPGPAGAPGAPGLQGMPGERGAAGLPGPKGERGDAGPKGADGAPGKDGVRGLAGPP]3G.

The calculated molecular weight for RCP is 51 600, which was reported to achieve strong cell adhesion [16].

Preparation of RCP pieces

Three types of RCP pieces, petaloid, chapped, and plain, were prepared by crushing freeze-dried RCP and fractionating the obtained pieces. The three types were grouped by the complexity of the surface texture. The details were described in Appendix S1.

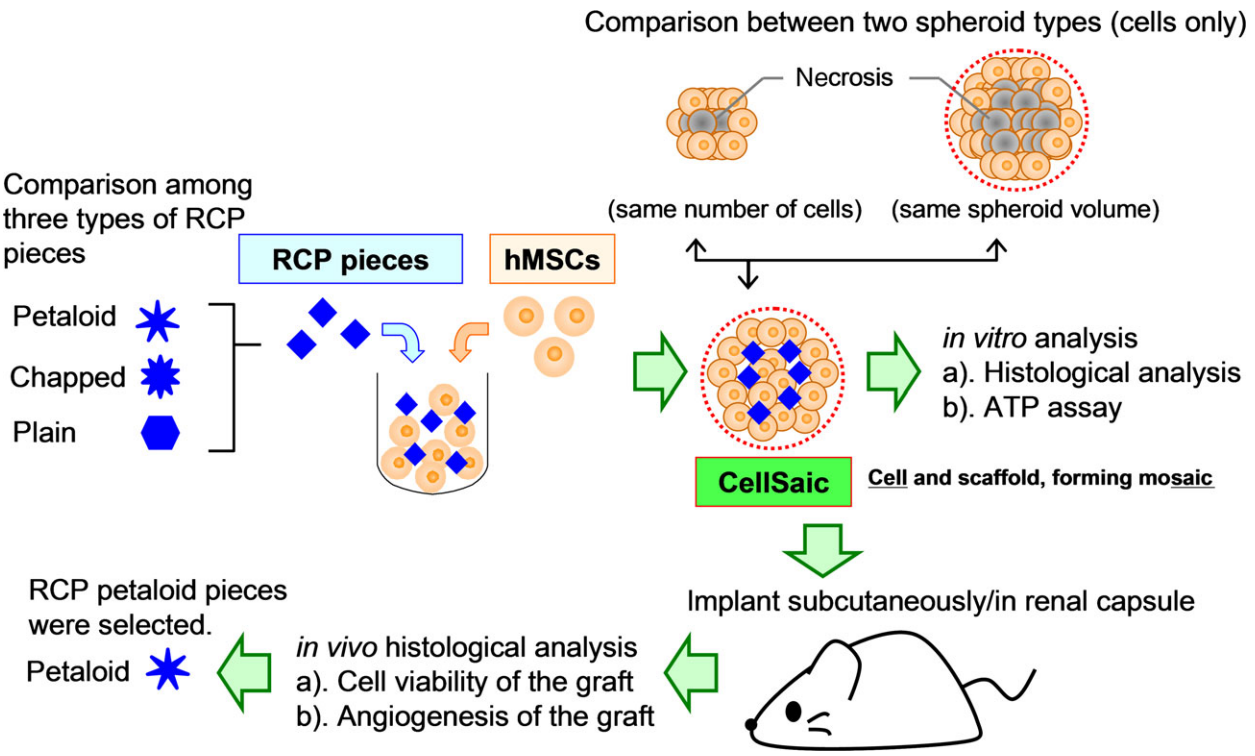

Figure 1 General experimental design. The CellSaic platform consisting of human mesenchymal stem cells (hMSCs) and micrometer-sized pieces of recombinant protein (RCP) was compared with a spheroid containing the same number of cells and a spheroid with the same volume. Three types of micrometer-sized pieces of RCP were also compared.

Formation of hMSC CellSaic platforms and hMSC spheroids

The hMSCs were purchased from Lonza (PT-2501; Basel, Switzerland) and cultured using MSCGM BulletKit medium (Lonza) at 37 °C in a 5% CO_2 humidified atmosphere. CellSaic platforms containing hMSCs were then prepared by mixing hMSCs (1×10^5 cells/ml) and RCP pieces (0.1 mg/ml) in MSCGM medium; this mixture was seeded on a PrimeSurface 96U plate (Sumitomo Bakelite Co. Ltd., Tokyo, Japan) in 200-μl wells. For spheroid formation, hMSCs in MSCGM medium were prepared using 1×10^5 cells/ml or 4×10^5 cells/ml, and the cells were seeded on a PrimeSurface 96U plate in 200-μl wells. Each plate was centrifuged using a tabletop plate centrifuge (600 *g*, 5 min) and then incubated for 24 h. The medium was changed every 3 days for both the CellSaic platforms and the spheroids.

Implantation of CellSaics and spheroids

The CellSaics and spheroids were evaluated *in vivo* in 6-week-old NOD/SCID mice (Charles River Japan Inc., Yokohama, Japan) using subcutaneous implantation under general anesthesia (isoflurane inhalation). For subcutaneous evaluation, the CellSaics and spheroids were cut in half and implanted into the lower abdomen of the mice; five samples each of the CellSaics and spheroids were implanted per animal. The Animal Care Committee of the FUJIFILM Corporation approved the experimental protocol, and all experimental procedures used in animal studies were performed in accordance with international guidelines.

Histological evaluation: cell viability and angiogenesis

To assess the tissue response over time, histological sections were prepared at 1 and 2 weeks following implantation. Cell viability and angiogenesis were scored individually for the samples using a graded scale of 0–3, as described in Table 1 [17–22]. The scorings were reviewed by a pathologist and a veterinarian at FUJIFILM Corp. The pathologic score at each endpoint represents the average of the effective values ($n = 3$).

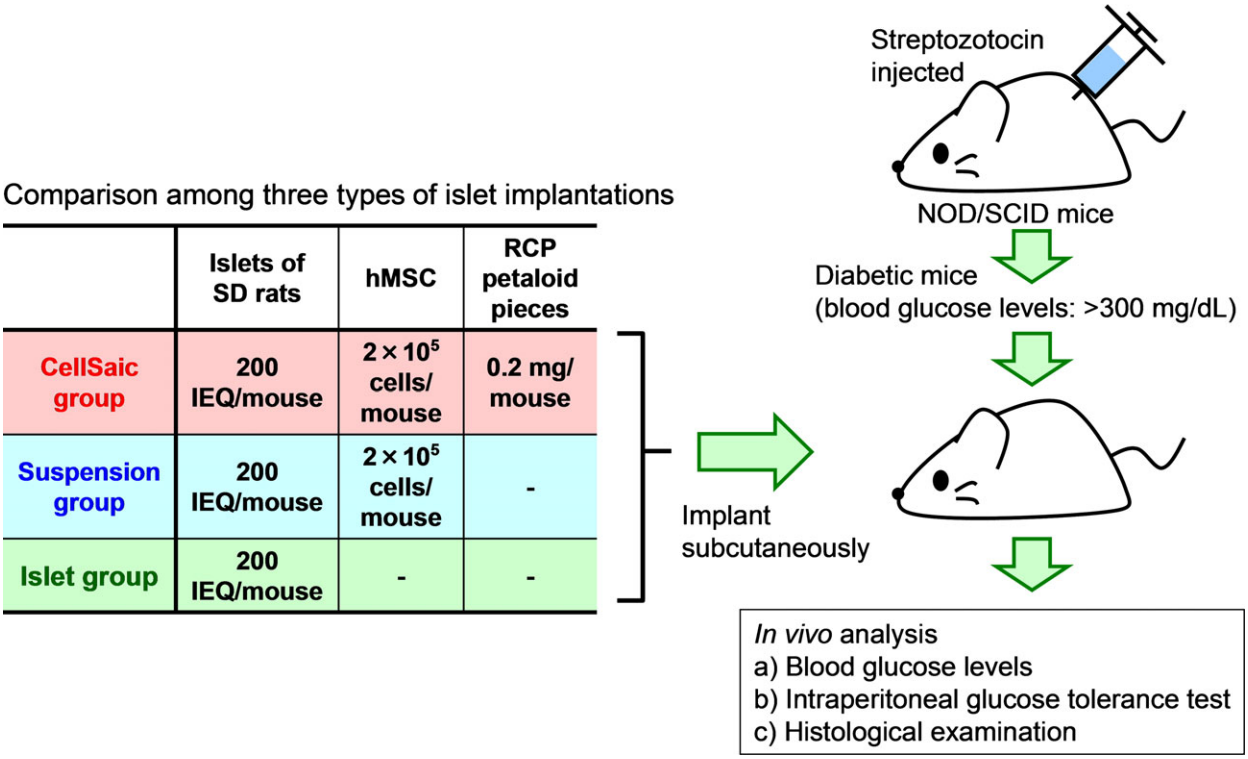

Figure 2 Experimental design for islet implantation as an application of CellSaic. Implants including islets were transplanted subcutaneously into mice in which diabetes was induced by administering streptozotocin. The transplant group was divided into three subgroups based on the implants: CellSaic group: a mixture of human mesenchymal stem cells (hMSCs), recombinant protein (RCP) μ-pieces, and islets (200 islets, 2×10^5 hMSCs, and 0.2 mg of RCP petaloid μ-pieces); suspension group: hMSCs and islets (200 islets and 2×10^5 hMSCs); and islet group: islets alone (200 islets). In each mouse, the post-transplant glucose levels were measured, and the mice were evaluated using the glucose tolerance test and by histopathological examination of transplant sections.

Table 1. Scoring system for histological evaluation.

Category	Score
Angiogenesis in the graft	
No vessel	0
Low vessel density	1
Moderate vessel density	2
High vessel density	3
Cell viability in the graft	
Scarcely distributed viable cells	0
Sparsely distributed viable cells	1
Moderately distributed viable cells	2
Graft filled with viable cells	3

Application of CellSaic: islet transplantation

Islet transplantation into the diabetic models

Diabetic mice (Appendix S1) were subcutaneously transplanted with islets alone, a mixture of islets and hMSC CellSaics or a mixture of islets and the hMSC suspension. Recipient mice were divided into the following five groups: (i) islet group ($n = 5$; 200 islets alone in 100 μl of 3 mM glucose medium); (ii) suspension group [$n = 10$; mixture of islets (200) and hMSC suspension (2×10^5 cells)]; (iii) CellSaic group [$n = 10$; mixture of islets (200) and CellSaics (total 2×10^5 hMSCs and 0.2 mg of RCP petaloid μ-pieces)]; (vi) diabetic group [$n = 4$; no transplantation (diabetic control)]; and (v) nondiabetic group [$n = 4$; no treatment with streptozotocin (nondiabetic control)]. Transplants were injected into the subcutaneous space on the backs of the recipient mice using a sterile syringe equipped with an 18-gauge needle in the case of the islet and suspension groups, and by making a skin incision under general anesthesia (isoflurane inhalation) in the CellSaic group. The experimental protocol was approved by the Animal Care Committee of FUJIFILM Corporation.

Histological and immunohistological evaluations in the diabetic models

On day 28 after transplantation, skin tissues harboring the implanted grafts were removed and fixed with a 10% phosphate-buffered formalin solution. H&E-stained

sections were prepared for histological examination. For immunostaining, deparaffinized sections were first incubated with an anti-insulin antibody (I2018; Sigma-Aldrich, St. Louis, MO, USA) and then with LSAB2 biotin-linked secondary antibody (K1015; Dako, Glostrup, Denmark) plus streptavidin–horseradish peroxidase (K1016; Dako), and signals were developed using a diaminobenzidine solution (K3468; Dako). The sections were then counterstained using hematoxylin.

Statistical analyses

The results are presented as means ± standard deviation. Blood glucose levels measured for 28 days after the transplantation and those measured during the IPGTT were analyzed using one-way analysis of variance with Dunnett's test. AUCs obtained during the IPGTT were analyzed using one-way analysis of variance with Dunn's test. $P < 0.05$ was considered to be statistically significant.

Results

Characterization of the mosaic cell structure comprising cells and scaffolds (CellSaic platform)

In the spheroids of hMSCs, a high rate of cell death (nuclear condensation and enucleation) was observed both *in vitro* and *in vivo*; Figs 3 and 4 show that there were very few normal, viable cells. The data for CellSaic platforms and spheroids were compared based on cell numbers, as well as cell-structure volumes. As shown in Fig. 4, when the spheroids were implanted subcutaneously, cell death was widely observed at 1 week following implantation, and the spheroids appeared to have shrunk after 2 weeks. After 4 weeks *in vivo*, the spheroids disappeared, making recovery impossible (data not shown).

The number of dead cells was considerably lower for the CellSaic containing 50–100 μm RCP petaloid pieces than for the spheroids; in addition, a large number of normal viable cells were observed both *in vitro* and *in vivo*. Indeed, dead cells were observed in the spheroids cultured *in vitro*, whereas elongated viable cells were visible in the CellSaic (Fig. 3). To quantify the viable cells in the spheroids and CellSaics *in vitro*, an ATP assay was performed (Fig. 3). The results confirmed considerably larger numbers of viable cells in the CellSaics compared to those in the spheroids. Moreover, cells in the CellSaic survived and the CellSaics were maintained within the subcutaneous space, appearing as thick tissue (Fig. 4). The results of the *in vivo* histological evaluations are summarized in Table 2. No blood vessels were formed in the spheroids, and viable implanted cells were scarcely visible. On the other hand, a large number of viable cells were detected in the CellSaics, with angiogenesis partially detected after 2 weeks.

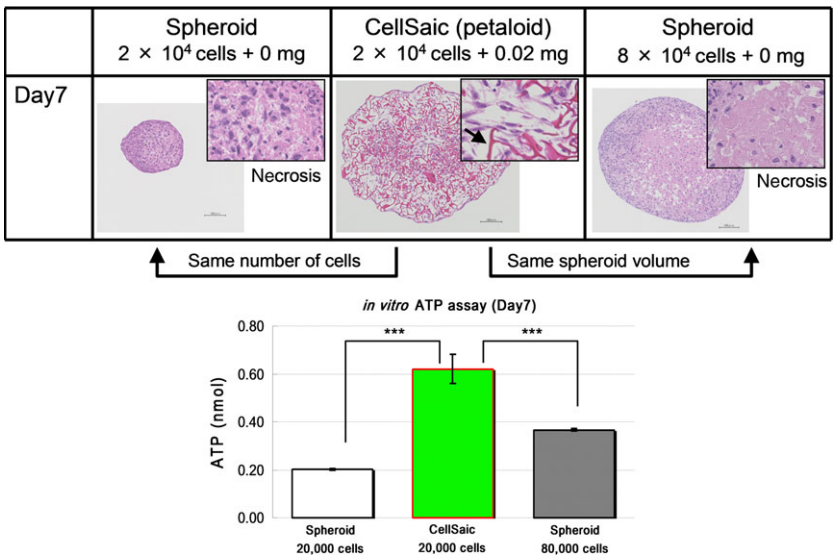

Figure 3 Hematoxylin and eosin (H&E)-stained sections of the CellSaic and spheroids cultured *in vitro*, and results from the ATP assay. Spheroids composed of human mesenchymal stem cells (hMSCs; 2×10^4 cells), spheroids composed of hMSCs (8×10^4 cells), the CellSaic containing hMSCs (2×10^4 cells), and petaloid pieces were cultured *in vitro*. The images show H&E-stained sections prepared after 7 days of culture. Arrows indicate the recombinant protein (RCP) petaloid pieces.

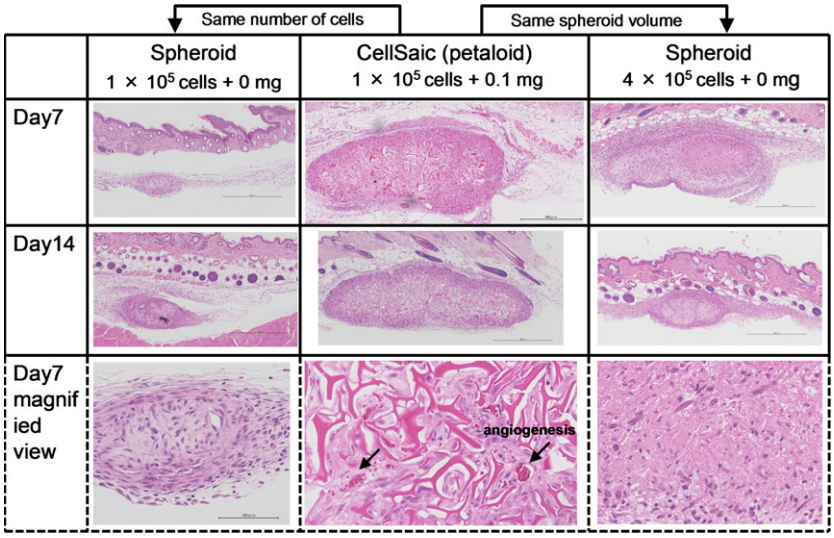

Figure 4 CellSaics and spheroids subcutaneously implanted into NOD/SCID mice. Spheroids composed of human mesenchymal stem cells (hMSCs; 1×10^5 or 4×10^5 cells), and CellSaics composed of 1×10^5 hMSCs and petaloid pieces (0.1 mg) were subcutaneously implanted *in vivo* into NOD/SCID mice. Hematoxylin and eosin-stained sections of the implants are shown. Images show the stained sections at 7 and 14 days postimplantation. Enlarged images of the stained sections 7 days after implantation are also shown.

Table 2. Scores for the histological evaluation of subcutaneously implanted grafts (spheroids and CellSaic platforms).

	Spheroid				CellSaic (petaloid)	
	4×10^5 cells + 0 mg		1×10^5 cells + 0 mg		1×10^5 cells + 0.1 mg	
	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
Angiogenesis	0.0	0.0	0.0	0.0	1.0	1.7
Cell viability	1.0	0.0	0.0	0.0	2.0	2.3
	CellSaic containing 1×10^5 cells + 0.1 mg					
	Plain pieces		Chapped pieces		Petaloid pieces	
	1 week	2 week	1 week	2 weeks	1 week	2 weeks
Angiogenesis	0.0	0.0	0.0	0.7	1.0	1.7
Cell viability	1.0	0.5	1.0	1.3	2.0	2.3

Cell viability of the CellSaics implanted *in vivo* according to RCP piece shape

As described above, cell viability improved considerably when the cells were cultured in the CellSaic containing RCP petaloid pieces. Furthermore, the results confirmed that cell viability depends on the structure of the RCP piece. We prepared three types of RCP pieces: plain, petaloid, and chapped; the chapped shape is intermediate to the plain and petaloid shapes. Figure 5 shows the scanning electron microscope images and H&E-stained sections of these pieces, and each sample type was evaluated *in vivo*. Cell viability in the CellSaics containing the plain pieces was poor, with fewer viable cells retaining a normal appearance; however, cell viability improved when the RCP piece was chapped and improved even further when the piece was petaloid (Fig. 6 and Table 2).

To evaluate the relationship between the number of cells and shapes of the RCP pieces, we visualized and compared the CellSaics containing plain, chapped, and petaloid pieces *in vitro* using H&E staining. The results showed fewer cells in areas within the CellSaic containing plain pieces than in the platform containing petaloid pieces (Fig. 7). In addition, in areas within the platform containing plain pieces where the cells were

Transplant International 2016; 29: 1039–1050

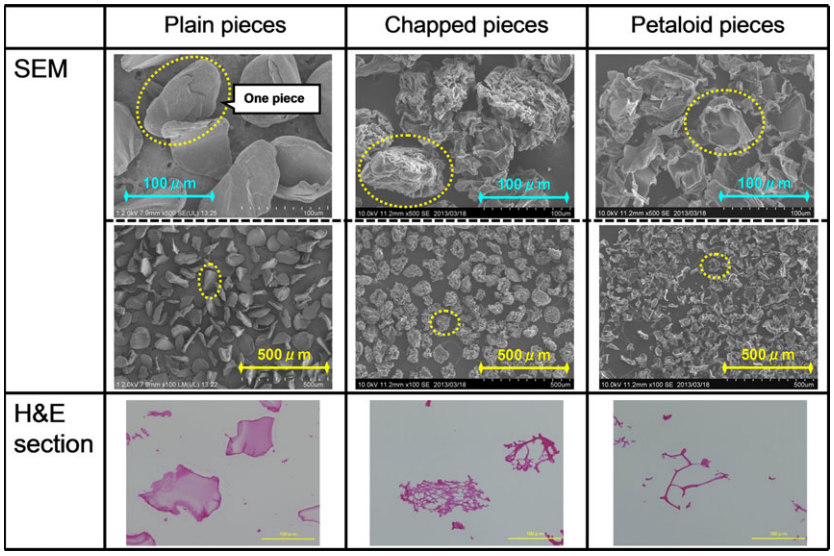

Figure 5 Structure and morphology of three different types of recombinant protein (RCP) pieces. Scanning electron microscope images and hematoxylin and eosin (H&E)-stained sections of CellSaics using plain, chapped, or petaloid RCP pieces are shown.

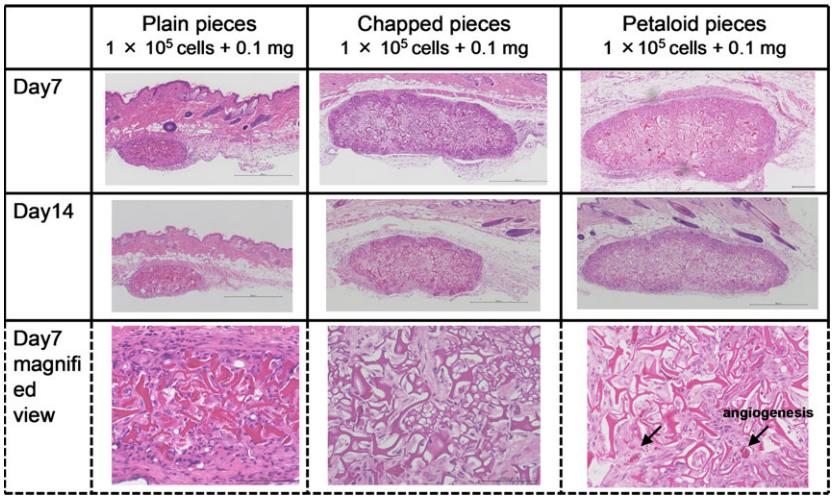

Figure 6 Hematoxylin and eosin (H&E)-stained sections of CellSaics with three different types of recombinant protein (RCP) pieces subcutaneously implanted *in vivo*. CellSaics formed using plain, chapped, or petaloid pieces were subcutaneously implanted into NOD/SCID mice. The images shown are of sections stained with H&E after 7 and 14 days postimplantation.

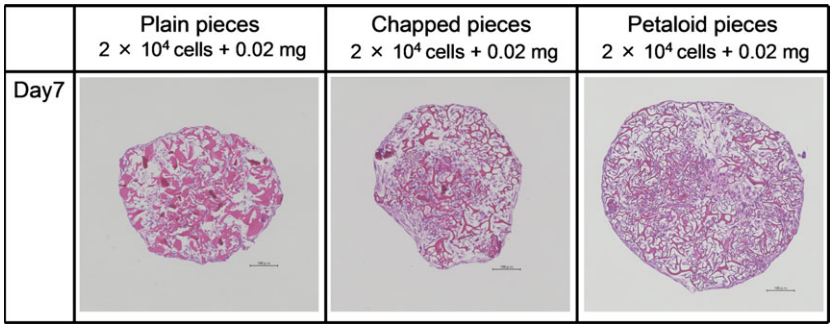

Figure 7 Hematoxylin and eosin (H&E)-stained sections of CellSaics with three different types of recombinant protein (RCP) pieces cultured *in vitro*. CellSaics formed using plain, chapped, or petaloid pieces were cultured *in vitro* for 7 days, and images of the stained sections are shown.

densely distributed, the pieces and cells were unevenly distributed; however, in the CellSaic containing petaloid pieces, the cells and pieces were evenly distributed.

To analyze the complexity of the pieces, we evaluated the spaces formed between the RCP pieces using tapped density calculations; the lower the tapped density, the easier it became to form the spaces between pieces (Table 3). The boundary length to square root of the area ratio for each piece was calculated using a section stained with H&E. The results of these calculations are shown in Table 3 and were used as a measure of the complexity of the individual structures of the pieces.

Fusion and the snowball effect of the CellSaic

Similar to spheroids, CellSaics naturally fuse when they are aligned next to each other. Figure 8a shows microscopic images and H&E-stained sections of three CellSaics within a 1-mm-diameter space; these CellSaics were aligned, cultured for 2 days, and fused. The images show that the three CellSaics visibly fused to each other, while the hMSCs from these platforms proliferated and adhered to each other at the fusion sites. As another method to increase the CellSaic size, RCP petaloid pieces were added to the culture medium each time the medium was refreshed (0.1 mg every 3 days), which resulted in a snowball effect (Fig. 8b). The size of the CellSaic could increase further as long as the scaffolds were constantly provided, whereas the size of the CellSaics did not change when no additional petaloid pieces were added.

Application of CellSaic: islet transplantation

As an application of CellSaic (petaloid), implants including islets were transplanted subcutaneously into

Table 3. Effect of tapped density and complexity on the *in vivo* viability for three types of recombinant peptide (RCP) pieces.

	Plain	Chapped	Petaloid
Tapped density	523.8 ± 7.2	189.0 ± 4.5	135.3 ± 3.1
The boundary length to square root of the area ratio	7.8 ± 0.3	14.1 ± 2.3	18.6 ± 1.9
In vivo cell viability (2 weeks)	0.5	1.3	2.7

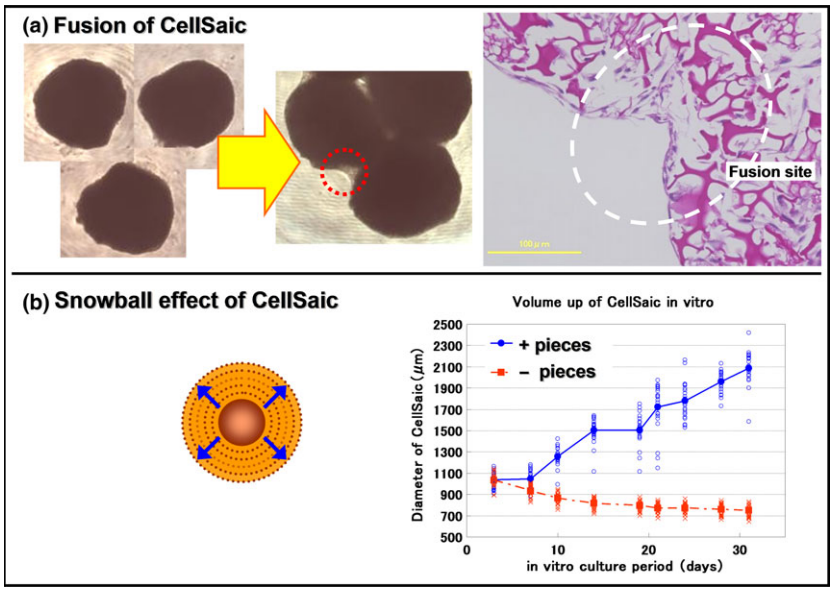

Figure 8 Fusion and volume increase (snowball effect) for the CellSaic. (a) Fusion of CellSaics. Optical microscopy and images of hematoxylin and eosin (H&E)-stained sections are shown. Dotted circles show the fusion sites. (b) Method for increasing the CellSaic volume. The CellSaic size was gradually increased by adding recombinant protein (RCP) pieces when the medium was changed (snowball effect). The figure on the left shows a schematic image of this principle. The graph on the right shows the change in the diameter of the CellSaics to which pieces were added, as well as that of the platforms to which no pieces were added, relative to the culture time.

streptozotocin-induced diabetic mice. The transplant group was divided into three groups based on the implants: (i) CellSaic group, a mixture of hMSCs, RCP μ-pieces, and islets; (ii) suspension group, the hMSCs and islets; and (iii) islet group, islets alone. Post-transplant glucose levels were measured in each mouse, and all mice were evaluated using the glucose tolerance test and histopathological examinations of transplant sections.

Blood glucose levels in the diabetic model

In the islet group, the blood glucose level measured for 28 days after transplantation was 398 ± 30 mg/dl (Fig. 9), which was similar to that in the diabetic group (402 ± 51 mg/dl). By contrast, the blood glucose level in the suspension group was 393 ± 45 mg/dl at the time of transplantation, but decreased to 245 ± 85 mg/dl by 7 days after the transplantation, and remained at that level until 28 days after the transplantation (180 ± 65 mg/dl). In the CellSaic group, the blood glucose level was 386 ± 33 mg/dl at the time of transplantation and then decreased to 154 ± 45 mg/dl by 7 days after transplantation, and remained at that level until 28 days after the transplantation (129 ± 15 mg/dl). In the nondiabetic group, blood glucose levels remained in the range of 100–150 mg/dl for the 28 days after the beginning of the treatment. Both the CellSaic group and the suspension group showed statistically significant differences in blood glucose levels on days 7, 14, 21, and 28 after transplantation, as compared with the islet group ($P < 0.05$).

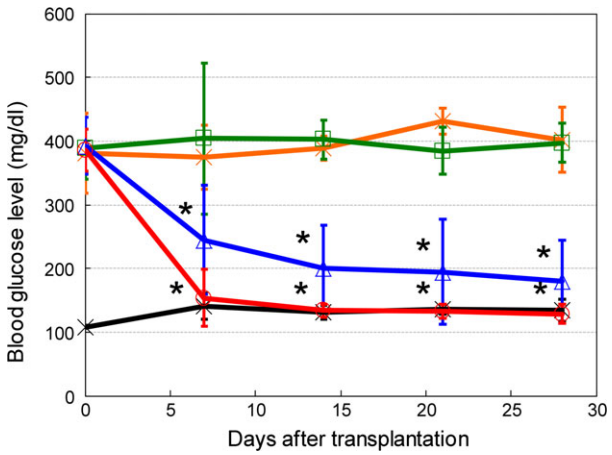

Figure 9 Nonfasting blood glucose levels of diabetic NOD/SCID mice after islet transplantation. On Day 0, diabetic mice were subcutaneously transplanted with a mixture of islets and human mesenchymal stem cell (hMSC) CellSaics [CellSaic group, $n = 10$ (red); 200 islets, 2×10^5 hMSCs, and 0.2 mg of recombinant protein (RCP) petaloid μ-pieces], a mixture of islets and the hMSC suspension [suspension group, $n = 10$ (blue); 200 islets and 2×10^5 hMSCs], or islets alone [islet group, $n = 5$ (green); 200 islets]. Diabetic mice [diabetic group, $n = 4$ (orange)] and nondiabetic mice [nondiabetic group, $n = 4$ (black)] were used as controls. *$P < 0.05$. The CellSaic and suspension groups were each compared with the islet group using one-way analysis of variance with Dunnett's test.

IPGTT in the diabetic model

The IPGTT was performed at 28 days after transplantation to confirm the effect of cotransplantation of hMSC CellSaics with islets (Fig. 10a). In the diabetic group, intraperitoneal administration of glucose increased the blood glucose level to 436 ± 31 mg/dl, and this level remained at 309 ± 38 mg/dl up until 180 min after the start of the test. In the islet group, glucose administration increased the blood glucose level to 301 ± 82 mg/dl, and this level decreased to 211 ± 78 mg/dl at the 180-min time point. In the suspension group, glucose administration increased the blood glucose level to 311 ± 49 mg/dl, which decreased to 168 ± 81 mg/dl at the 180-min time point. Last, in the CellSaic group, the blood glucose level rose to only 218 ± 27 mg/dl after glucose administration and then decreased to 123 ± 23 mg/dl at the 180-min time point. At all time points examined after glucose administration, except 15 min, the blood glucose levels in the CellSaic group were significantly different from those in the islet group ($P < 0.05$). By contrast, the blood glucose levels in the suspension group were significantly different from those in the islet group only at 180 min after glucose administration. Comparison of the AUCs (Fig. 10b) showed statistically significant differences between the CellSaic group and the islet and suspension groups ($P < 0.05$). However, no significant differences were observed between the suspension and the islet groups. The IPGTT results indicated that mice cotransplanted with hMSC CellSaics and islets showed the most favorable glucose responses.

Histological examination in the diabetic model

The volume of the islets in the CellSaic group was greater than that in the suspension group (Fig. 11a and d), which indicated a comparatively higher level of blood vessel formation in the CellSaic group (Fig. 11b and e). Immunostaining with the anti-insulin antibody confirmed that insulin-positive areas were larger in the CellSaic group than in the suspension group (Fig. 11c and f). In contrast, no transplanted grafts were observed in the islet group.

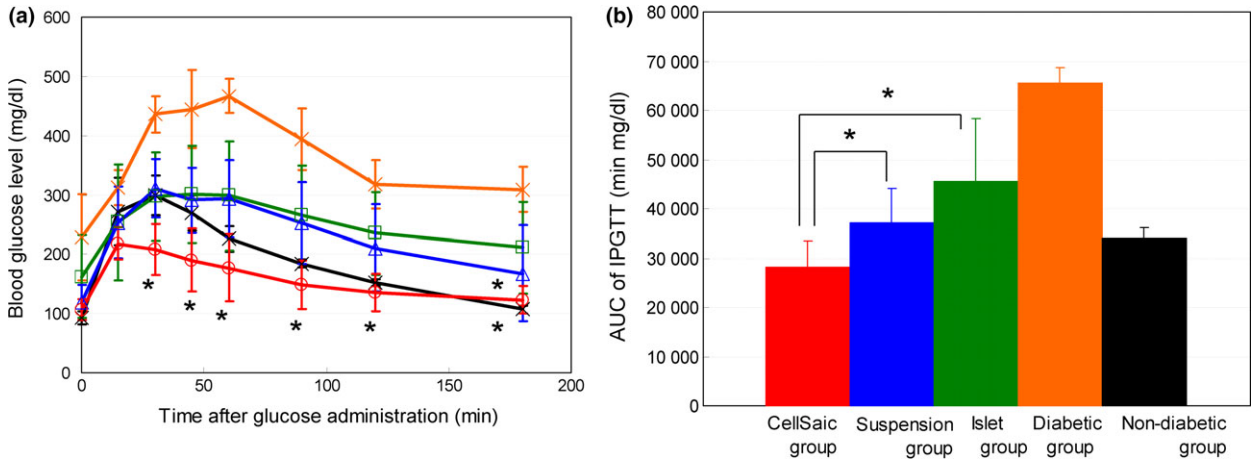

Figure 10 Results of the intraperitoneal glucose tolerance test (IPGTT) obtained for each group at 28 days after transplantation. Diabetic NOD/SCID mice were subcutaneously transplanted with a mixture of islets and human mesenchymal stem cell (hMSC) CellSaics [CellSaic group, $n = 10$ (red); 200 islets, 2×10^5 hMSCs, and 0.2 mg of recombinant protein (RCP) petaloid μ-pieces], a mixture of islets and the hMSC suspension [suspension group, $n = 10$ (blue); 200 islets and 2×10^5 hMSCs], or islets alone [islet group, $n = 5$ (green); 200 islets]. Diabetic mice [diabetic group, $n = 4$ (orange)] and nondiabetic mice [nondiabetic group, $n = 4$ (black)] were used as controls. (a) Glucose tolerance profiles of diabetic NOD/SCID mice after islet transplantation. The CellSaic and suspension groups were individually compared with the islet group by one-way analysis of variance with Dunnett's test (*$P < 0.05$). (b) The area under the curve (AUC) was measured during the IPGTT from 0 to 180 min at 28 days after transplantation. The CellSaic, suspension, and islet groups were compared using one-way analysis of variance with Dunn's test (*$P < 0.05$).

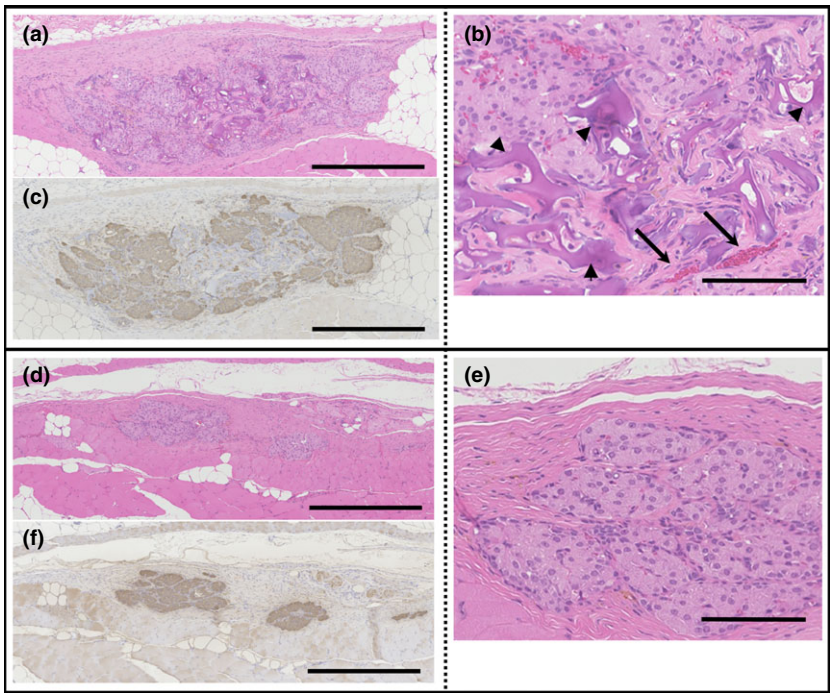

Figure 11 Histological and immunohistological analyses of subcutaneous transplants at 28 days after transplantation. Skin sections obtained from mice transplanted with the mixture of islets and human mesenchymal stem cell (hMSC) CellSaics (CellSaic group; a–c), and from mice transplanted with the mixture of islets and the hMSC suspension (suspension group; d–f). Hematoxylin and eosin (H&E) staining (a, b, d, and e) and immunohistological staining with anti-insulin antibody (positive areas are shown in brown; c and f). Arrows indicate new blood vessels. Arrowheads indicate recombinant protein (RCP) petaloid μ-pieces. Scale bars = 500 μm (a, c, d, and f) and 100 μm (b and e).

Transplant International 2016; 29: 1039–1050

Discussion

We hypothesized that cell death could be prevented for a specific volume and thickness of the cell structure by providing pathways for nutrients to enter the cell structure. Based on this hypothesis, we developed and evaluated a newly developed cell structure (CellSaic) incorporated with biodegradable RCP petaloid pieces. Here, use of the phrase 'RCP petaloid pieces' refers to pieces with an uneven surface, resulting in concavities and convexities whose size is similar to that of the cells.

The results indicated that the CellSaic platform could prevent central necrosis, even when the diameter of the cell structure was 400 μm or greater. Cell viability in the CellSaic platform was higher than that in the spheroid, both *in vitro* and *in vivo*. This effect was considered to be due to the formation of structures by the RCP pieces within the spaces; specifically, the addition of the petaloid-shaped pieces should result in the formation of spaces for waste products to form and nutrient transport to occur. Collectively, our results suggest that the permeability of the CellSaic leads to the creation of pathways for nutrients to enter the cell structure via spaces that are formed between the cells and the pieces. The data also showed that the tapped density of the piece is negatively correlated with cell viability, whereas complexity is positively correlated with viability. These findings suggest that the shape of the RCP pieces greatly affects cell viability and that the petaloid shape is well suited for future applications.

The effect of fusion on the CellSaic suggests that it will be possible to form larger CellSaics (up to several centimeters). It is speculated that larger transplantation tissue samples can be formed via the fusion method or the snowball effect that was observed using the CellSaics.

In the cotransplantation of isles with hMSCs using CellSaic, more effective islet engraftment in the suspension group was observed than in the islet group. In the islet group, islet engraftment could not be confirmed at the graft site, either macroscopically or microscopically, at 28 days after transplantation. By contrast, in the suspension group, transplanted grafts could be observed at 28 days after the transplantation; islet engraftment was confirmed, and clear insulin-positive areas were detected in this group. Similar results have been reported previously, and the conclusion was that cotransplantation of islets with hMSCs improved the engraftment of islets [23].

The volume of islets in the grafts in the CellSaic group was higher than the suspension group. H&E staining at 28 days after the transplantation showed more blood vessels in the grafts in the CellSaic group than in the suspension group. Subcutaneous transplantation of hMSC CellSaics alone in nondiabetic NOD/SCID mice resulted in the formation of blood vessels, but transplantation of hMSCs alone in these mice did not lead to blood vessel formation. As mentioned above, CellSaic transplantation induces the formation of blood vessels, and successful formation of blood vessels in the transplanted grafts promotes the engraftment of the transplanted islets. This observation could account for the greater volume of islets in the CellSaic group than in the suspension group. These results indicated that cotransplantation of islets with hMSC CellSaics increased the formation of blood vessels in transplanted grafts, as compared to the formation of blood vessels observed after transplantation of hMSCs alone.

In conclusion, we have described a new cell transplantation platform, CellSaic, which incorporates RCP petaloid pieces. Use of this CellSaic will enable the transplantation of larger cell-based tissues than those transplanted using conventional methods, with an added benefit of preventing cellular necrosis. This platform is expected to be advantageous in many fields, including the development of cell therapies and the transplantation of regenerated tissues and organs.

Authorship

KN and RI: collected and analyzed data and wrote the article. FW: collected and analyzed data. KN, RI and YY: designed the study.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no conflicts of interest.

Acknowledgements

The authors would like to thank members of the technical staff of Regenerative Medicine Research Laboratories, FUJIFILM Corporation, whose opinions and knowledge were very helpful throughout the completion of this work.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Materials and methods.

Table S1. The difference of angiogenesis-related growth factors release between CellSaic (MSCs + RCP petaloid) and MSCs only.

Table S2. Number of vessels, histological evaluation of subcutaneously implanted grafts (spheroids and CellSaic platforms).

Figure S1. *in vitro* insulin release test. Stimulation indexes of islets, islets + MSCs, and islets + MSCs + RCP petaloid.

REFERENCES

1. Shirakawa K, Tsuda H, Heike Y, *et al.* Absence of endothelial cells, central necrosis, and fibrosis are associated with aggressive inflammatory breast cancer. *Cancer Res* 2001; **61**: 445.
2. Mueller-Klieser W. Three-dimensional cell cultures: from molecular mechanisms to clinical applications. *Am J Physiol* 1997; **273**: C1109.
3. Folkman J, Hochberg M. Self-regulation of growth in three dimensions. *J Exp Med* 1973; **138**: 745.
4. Groebe K, Mueller-Klieser W. On the relation between size of necrosis and diameter of tumor spheroids. *Int J Radiat Oncol Biol Phys* 1996; **34**: 395.
5. Mueller-Klieser W, Freyer JP, Sutherland RM. Influence of glucose and oxygen supply conditions on the oxygenation of multicellular spheroids. *Br J Cancer* 1986; **53**: 345.
6. Groebe K, Mueller-Klieser W. Distributions of oxygen, nutrient, and metabolic waste concentrations in multicellular spheroids and their dependence on spheroid parameters. *Eur Biophys J* 1991; **19**: 169.
7. MacGregor RR, Williams SJ, Tong PY, Kover K, Moore WV, Stehno-Bittel L. Small rat islets are superior to large islets in in vitro function and in transplantation outcomes. *Am J Physiol Endocrinol Metab* 2006; **290**: E771.
8. Giuliani M, Moritz W, Bodmer E, *et al.* Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. *Cell Transplant* 2005; **14**: 67.
9. Sakata N, Aoki T, Yoshimatsu G, *et al.* Strategy for clinical setting in intramuscular and subcutaneous islet transplantation. *Diabetes Metab Res Rev* 2014; **30**: 1.
10. Nakamura K, Tabata Y. A new fluorescent imaging of renal inflammation with RCP. *J Control Release* 2010; **148**: 351.
11. Chang RC, Kivirikko KI, Neff TB, Olsen DR, Polarek JW. Recombinant gelatins. US Patent 6992172, 2006.
12. De Boer AL, Van Urk H, Bouwstra JB, Van Asten PFTM. Recombinant gelatins. US Patent US20100119574 A1, 2008.
13. Shapiro AM, Ricordi C, Hering BJ, *et al.* International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; **355**: 1318.
14. Sakata N, Goto M, Yoshimatsu G, Egawa S, Unno M. Utility of co-transplanting mesenchymal stem cells in islet transplantation. *World J Gastroenterol* 2011; **17**: 5150.
15. Ito T, Itakura S, Todorov I, *et al.* Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. *Transplantation* 2010; **89**: 1438.
16. Nakamura K, Tabata Y. Targeting agent to newly formed blood vessels. US Patent US8, 877, 185, 2010.
17. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. *Hepatology* 1996; **24**: 289.
18. Degott C, Zafrani ES, Callard P, Balkau B, Poupon RE, Poupon R. Histopathological study of primary biliary cirrhosis and the effect of ursodeoxycholic acid treatment on histology progression. *Hepatology* 1999; **29**: 1007.
19. Kuipers EJ, Uyterlinde AM, Peña AS, *et al.* Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet* 1995; **345**: 1525.
20. Weidner N, Folkman J, Pozza F, *et al.* Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. *J Natl Cancer Inst* 1992; **84**: 1875.
21. Yeh WC, Li PC, Jeng YM, *et al.* Elastic modulus measurements of human liver and correlation with pathology. *Ultrasound Med Biol* 2002; **28**: 467.
22. Leitner SP, Swern AS, Weinberger D, Duncan LJ, Hutter RV. Predictors of recurrence for patients with small (one centimeter or less) localized breast cancer (T1a,b N0 M0). *Cancer* 1995; **76**: 2266.
23. Berman DM, Willman MA, Han D, *et al.* Mesenchymal stem cells enhance allogeneic islet engraftment in nonhuman primates. *Diabetes* 2010; **59**: 2558.