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# Effectiveness of bioengineered islet cell sheets for the treatment of diabetes mellitus

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ABSTRACT

*Background:* The present study aimed to evaluate whether bioengineered mouse islet cell sheets can be used for the treatment of diabetes mellitus.

*Methods:* Isolated mouse pancreatic islets were dispersed, and cells were plated on temperature-responsive culture plates coated with iMatrix-551. On day 3 of culture, the sheets were detached from the plates and used for further analysis or transplantation. The following parameters were assessed: (1) morphology, (2) expression of β-cell–specific transcription factors and other islet-related proteins, (3) methylation level of the pancreatic duodenal homeobox-1 (*Pdx-1*) promoter, as determined by bisulfite sequencing, and (4) levels of serum glucose after transplantation of one or two islet cell sheets into the abdominal cavity of streptozotocin-induced diabetic severe combined immunodeficiency mice.

*Results:* From each mouse, we recovered approximately 233.3 ± 12.5 islets and $1.4 \pm 0.1 \times 10^5$ cells after dispersion. We estimate that approximately 68.2% of the cells were lost during dispersion. The viability of recovered single cells was 91.3 ± 0.9%. The engineered islet cell sheets were stable, but the messenger RNA levels of various β-cell-specific transcription factors were significantly lower than those of primary islets, whereas *Pdx-1* promoter methylation and the expression of NeuroD, Pdx-1, and glucagon proteins were similar between sheets and islets. Moreover, transplantation of islet cell sheets did not revert serum hyperglycemia in any of the recipient mice.

*Conclusions:* Engineering effective islet cell sheets require further research efforts, as the currently produced sheets remain functionally inferior compared with primary islets.



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## Introduction

Islet transplantation is considered a viable option for the treatment of type 1 diabetes mellitus. The use of this treatment has progressed considerably over the past 12 y, and more than 750 patients around the world received islet transplants during this period.[1] However, the proportion of patients displaying insulin-independence 5 y after transplantation is less than 15%.[1,2]

The liver, via the portal vein, is currently the site of choice for clinical islet transplantation, although it is not an ideal location. Low oxygen tension and the induction of inflammatory responses impair islet implantation and lead to the significant early loss of transplanted cells. As a result, most recipients revert to insulin dependence.[3,4] Because of these unresolved issues, the search for an ideal site for islet transplantation has continued with varying degrees of success. Several transplant sites have been tested in an experimental setting, including the spleen,[5] abdominal cavity,[6] omentum,[7] testes,[8] and renal subcapsular space.[9] Although transplantation into these sites has shown some modicum of success for the regulation of blood glucose levels, this has not undergone clinical testing.[10]

Recently, a unique and effective experimental tissue engineering approach has emerged as a potential treatment modality for diabetes mellitus. It entails the creation of cell sheets by plating dispersed islet cells onto temperature-responsive polymer culture dishes.[11-13] When multiple layers of engineered islet cell sheets were transplanted into the subcutaneous spaces of streptozotocin-induced diabetic severe combined immunodeficiency (SCID) mice, the hosts successfully reverted from diabetic status to normal glycemic levels.[12] However, subcutaneous spaces are less effective islet transplantation sites compared with the liver, possibly because of major differences between the skin and the liver in terms of the immune environment. Subcutaneous spaces might be associated with more challenges in suppressing alloimmunity.[14,15]

In this study, we evaluated whether the transplantation of islet cell sheets into the abdominal space is an effective treatment option for diabetes mellitus *in vivo*, by examining the function of one or two engineered islet cell sheets following transplantation into the abdominal cavity of streptozotocin-induced diabetic SCID mice. In addition, the quality of the engineered sheets was assessed based on morphology, expression of β-cell transcription factors, and other islet-related proteins, and promoter methylation of the pancreatic duodenal homeobox-1 (*Pdx-1*) gene.

We included methylation analysis of the *Pdx-1* promoter in our study because we hypothesized that this would be downregulated if the dispersion primary islet cells affected the characteristics and function of the β-cells. Epigenetic regulation including DNA methylation and histone modification was recently shown to be involved in the control of endocrine cell fate decision and β-cell identity.[16,17] Regarding DNA methylation, it has been long known that mammalian genes contain cytosine-guanosine dinucleotide (CpG) sites that play an important role in the maintenance of lineage identity by sustaining patterns of somatic tissue-specific gene expression.[16-20] The methylation of cytosine residues can fulfill this role because it regulates transcription both directly by inhibiting the binding of specific transcription factors and indirectly by recruiting methyl-CpG-binding proteins that exert repressive chromatin-remodeling activity.[18-20]

## Materials and methods

### Animals

Adult Institute of Cancer Research and Institute of Cancer Research SCID mice, aged 8-10 weeks, were obtained from CLEA Japan, Inc (Tokyo, Japan). All mice were bred conventionally in an air-conditioned room with free access to tap water and standard pelleted chow. All animal experiments were approved by the Institutional Animal Care and Use Committee of Meiji University (IACUC 12-0019 and 12-0020).

### Primary islet isolation

Mice were anesthetized using a mixture of ketamine (Fujita Pharmaceutical Co, Ltd, Tokyo, Japan) and xylazine (Bayer Yakuhin, Ltd, Osaka, Japan). Animals underwent laparotomy, after which the pancreas was distended with 2 mL of cold Hank's balanced salt solution (HBSS; Life Technologies, Carlsbad, CA) containing 1000 U/mL of Collagenase-Yakult (Yakult Pharmaceutical Industry Co, Ltd, Tokyo, Japan), which was administered through the common bile duct.[21] The pancreas was then excised and incubated in a stationary bath at 37°C. Once the pancreas was excised, cervical dislocation was performed manually, which resulted in euthanasia within approximately 10 s. After separating islets by density gradient centrifugation (Histopaque-1077; Sigma–Aldrich, St. Louis, MO), they were handpicked using a stereomicroscope. Isolated islets were then cultured for 24–36 h in RPMI1640 (Life Technologies) containing 10% inactivated fetal bovine serum (FBS; Life Technologies) and antibiotics in Corning Ultra-Low Attachment flasks (Corning Inc, New York, NY) at 37°C, in a humidified atmosphere containing 5% $CO_2$. Islets were either immunostained, used for RNA or genomic DNA extraction, transplanted into mice, or dispersed into separate cells for use in the engineering of islet cell sheets.

### Islet cell sheet engineering

After treating isolated primary islets with trypsin-EDTA (Invitrogen, Carlsbad, CA) for 5 min with occasional stirring (10 s vortex on, 10 s vortex off) at 37°C, dispersed cells were centrifuged at 124 × *g* for 7 min and plated on temperature-responsive 24-well plates (UpCell; CellSeed Inc, Tokyo, Japan) coated with iMatrix-551 (rLN511 E; Nippi, Tokyo, Japan) at a density of $1.2–1.4 \times 10^6$ cells/well. Cells were cultured in RPMI-1640 (GIBCO #11875; Life Technologies) supplemented with 10% FBS (#171012; Nichirei Biosciences, Tokyo, Japan) at 37°C in a humidified atmosphere of 5% $CO_2$. On day 3 of culture, the temperature was lowered to 20°C for 20 min to detach the cells as a uniformly connected tissue sheet. Detached cell sheets were morphologically observed, immunostained, used for RNA or genomic DNA extraction, or transplanted into mice.