Proliferation and functional assessment of pseudo-islet cells using pancreatic endocrine cells

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Proliferation and functional assessment of pseudo-islet cells using pancreatic endocrine cells

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Doctoral Dissertation
submitted to the Department of Medicine
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

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June 2012

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June 2012

ACKNOWLEDGEMENTS

I would like to express profound gratitude to my teacher, Professor Myoung Soo Kim, for his invaluable support, encouragement, supervision and useful suggestion throughout this research work. His moral support and continuous guidance enabled me to complete my work successfully. I would like to thank my committee members, Professor Yu Seun Kim, Professor Hwal Suh, Professor Seung Woo Park, and Professor Hyung Joon Ahn for their valuable suggestions throughout this study.

I am as ever, especially indebted to my parents for their love and support throughout my life. I also wish to thank my wife for her support and understanding for my study and job. My Sincere thanks go to Sang Jun and Seo Jun, for sharing their unconditioned love with me.

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ABSTRACT

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Pancreas islet cell transplantation is a new therapy for type 1 diabetes mellitus. However, there are many obstacles precluding the use of islet transplantation as a conventional treatment. Particularly, insufficient islet mass may limit the use of this approach. We have developed a new technology that could produce pseudo-islets. Recently researches concerning co-culture with pancreatic islet and bone marrow mesenchymal stem cells (BMSCs) have been reported that low differentiated BMSCs demonstrated the positive effect on pancreatic islet functions. The purpose of this study is Morphologic and functional evaluation was performed to test the feasibility of using these cells for transplantation and to prove the mutual effect between pancreatic islet cells and BMSCs. A three-step procedure known as disaggregation-expansion-reaggregation (DER) is the method employed for pseudo-islet preparation. Islets were isolated from 200-250 g male Lewis rats by collagenase digestion and separated into single cells by trypsinization. These pancreatic endocrine cells (PECs) were expanded by serial passages in culture and were then aggregated at a high cell-density in a suspension state. For BMSCs collection, bone marrow was aspirated from the rat femur and adherent mononucleated cells were further expanded by passaged cultures. Subsequently, PECs and BMSCs at a high density were placed on low cell binding culture dish, and kept suspended state using shaking culture and maintained. The mixed cell complex were evaluated its function and characteristics with Glucose Challenge Test, insulin ELISA Analysis, RT-PCR

and Immunohistochemistry. Through expansion for 2 weeks in continuous culture passages, approximately one million PECs were recovered after aggregation. They presented with a spherical shape and a similar size when compared to naïve islets (50–800 μm) by phase-contrast microscopy. RT-PCR results indicate expression of insulin, glucagon, and PDX-1, which were observed in primary isolated islets as well. The insulin secretion capacity of pseudo-islets was confirmed by ELISA. The cellular aggregates of pancreatic islet cells and BMSCs were retrieved. These cellular spheroids showed the fortified function and maintained viability. In conclusion, PECs treated with DER were found to have the potential to serve as a cell source for pseudo-islet generation following in vitro cellular expansion. These cells are both morphologically and genetically similar to naïve islets. And, we suggested the manufacture method of mixed cellular complex from two different origins. Using this construct, cell-cell interactions can be examined in vitro such as improvement of secretion ability and cell differentiation. BMSCs can be used as a replacement or additives for bioengineered pseudo-islets. As these cells were both morphologically and genetically similar to naïve islets, our new technique could be a potential method to overcome the scarcity of donor islets in the near future.

Key words : pancreas islet cell, mesenchymal stem cell, transplantation

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I. INTRODUCTION

Many studies of new treatments for diabetes mellitus are ongoing worldwide. Transplantation of pancreatic islets can confer complete and immediate control of blood glucose levels in diabetes mellitus. Pancreas islet cell transplantation, a new therapy for type 1 diabetes mellitus, in addition to supplying insulin, also replaces the intestinal endocrine system and can treat diabetes complications caused by persistently high sugar levels. Compared with pancreas transplantation, islet transplantation is technically much simpler, involving less surgical stress[1]. Much effort has been directed toward the clinical application of human islet transplantation following the report by the Edmonton group[2]. However, there are many obstacles precluding the use of islet transplantation as a conventional treatment. These obstacles include the lack of organ donors, immunosuppression, islet cell isolation, purification, and the destruction of islet cells after transplantation. In particular, insufficient islet mass may limit the use of this approach. Many groups have developed various

culture methods, including the use of scaffolds based on tissue engineering techniques or growth factors and different chemical supplements to enhance the functions of islets grown in vitro[3, 4, 5, 6]. Nevertheless, because they are artificial, scaffolds or supplements may cause side effects or other problems after implantation[3, 7]. Moreover, functional improvement of islets will not be achieved without a sufficient number of cells. The most common approach to isolating pancreatic islets for use in transplantation is the combination of mechanical and enzymatic digestive procedures. During this process, intra-islet endothelial cells lose their external vascular support, a disruption that contributes to dedifferentiation, apoptosis, and necrosis in islets during subsequent in vitro culture[1, 8, 9, 10].

We studied islet cell isolation and functionally assessed islet cells through various types of animal experiments to establish pancreatic islet cell transplantation. Recently, we developed other methods to overcome the functional loss of islet cells after isolation and transplantation. According to these study results, pancreatic endocrine cells (PECs), which are known to have no proliferation power, were generated via the expansion and reaggregation of islet cells. After developing a new method of DER, basic research was performed.

Many studies utilizing co-cultures of pancreatic islet and rat bone marrow mesenchymal stem cells (rBMSCs) have reported that undifferentiated BMSCs exerted positive effects on pancreatic islet function. BMSCs have been demonstrated to possess the ability to differentiate into distinct mesenchymal tissues, such as bone, tendons, muscle, adipose tissue, cartilage, and nerves; they have also been demonstrated to support hematopoiesis[11, 12]. In addition, MSCs exhibit immune-modulatory properties, and they have been reported to inhibit T-cell proliferative responses to mitogens and alloantigens in vitro and to prolong graft survival in vivo. The ability of MSCs infusions to minimize the risk of graft-versus-host disease after allogeneic transplantation has been

tested in patients[13, 14 15]. MSCs have also been demonstrated to secrete growth factors, such as vascular endothelial growth factor, which enhance the proliferation of endothelial and smooth muscle cells and promote angiogenesis both in vitro and in vivo[16, 17]. However, the effect of rBMSCs on differentiated pancreatic islet cells is not clear, and only a few reports have investigated the potential of MSCs to protect against cell damage in pancreatic islets. Additionally, highly differentiated cells have ability to induce the differentiation of under-differentiated cells into differentiated cells with functional phenotypes.

In this study, we have introduced a new technique to produce scaffold-free pseudo-islets with the minimal use of artificial substances as an alternative method to overcome donor scarcity. In addition, another purpose of this study was to clarify the mutualistic relationship between pancreatic islet cells and rBMSCs.

II. MATERIALS AND METHODS

1. Islet isolation

Male Lewis rats (250–350g) were maintained according to the ethical guidelines of our institution and our Animal Investigation Committee. The study was performed in accordance with institutional guidelines for animal research. Pancreata were subjected to enzymatic digestion using collagenase P (Roche, Indianapolis, IN, USA). Islets were purified using Histopaque 1119, 1083, and 1077 (Sigma Chemical Co., St. Louis, MO, USA) in a continuous density gradient process in a discontinuous gradient centrifuge. Cells were centrifuged for 10 min at 4°C/2000 rpm to obtain an islet layer. They were washed with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Invitrogen Co., Carlsbad, CA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen Co., Carlsbad, CA, USA). Using microscopy, islets were purified by hand-picking to reach 99% purity. Primary islet was defined as islet cells with no treatment after isolated from rat pancreas and isolated islets cell was obtained from primary isolated islet cell after EDTA-trypsin treatment.

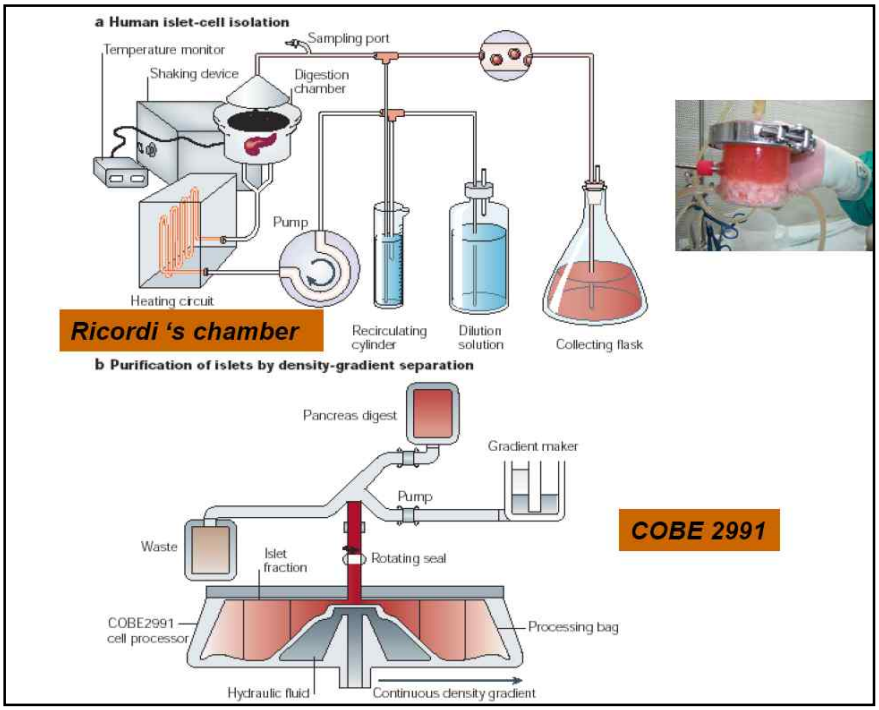

Figure 1. Schematic diagram of pancreas digestion and islet purification

2. Disaggregation-Expansion-Reaggregation

Islets isolated from 2 rats were digested with trypsin-EDTA (Invitrogen Co., Carlsbad, CA, USA) to prepare single PECs. These cells were seeded onto cell culture plates (BD Falcon Co., CA, USA) in DMEM/F-12 (WleGENE Inc., Daegu, Korea) medium supplemented with 20% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Plated cells were trypsinized at 90% confluence. The expansion process was continuously repeated for 2 weeks. Recovered cells from centrifugation were resuspended and shaken to yield high cell densities in suspension conditions. For shaking of the cultures, PECs suspended in 5 mL of DMEM-F12 medium (5×10^4 cells/mL) were cultured in a HydroCell (CellSeed Co., Tokyo, Japan) using a reciprocal shaker (Model: NA-201, Nissin Co., Tokyo, Japan) at 70 rpm for 2 days under standard culture conditions at 37°C and 5% CO_2.

3. Preparation of rBMSCs

Mesenchymal cells were isolated from the bone marrow (BM) of the femur of 8-week-old Lewis rats. The marrow sample was perfused and washed with phosphate-buffered saline or Dulbecco's modified Eagle's medium–low glucose (DMEM-LG; Invitrogen Co., Carlsbad, CA, USA).

Collected BM cells were centrifuged with 1200–1500 rpm, and isolated BM cells cultured in DMEM-LG supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin. After 7 days, the culture medium was changed, non-adherent cells were discarded, and adherent mono-nucleated cells were further expanded in culture for 3–4 weeks.

Afterward, isolated cells were identified and confirmed by FACS analysis: (+) CD44 & CD105/ (−) CD34 & CD45.

4. rBMSCs/PECs co-culture

PEC and rBMSC at a high density were plated onto low cell binding culture

dishes and maintained in a suspended state using a shaking culture.

5. Morphologic evaluation

Morphologic evaluation of pseudo-islets was performed using an Olympus IX51 phase-contrast microscope (Olympus Co, Tokyo, Japan).

6. Measurement of insulin secretion

Upon changing the medium every 24 h, supernatant was taken and immediately frozen at −70°C. Insulin secretion was determined in thawed samples using a commercially available insulin enzyme-linked immunosorbent assay kit (Alpco, Salem, IL, USA).

7. Glucose tolerance test

After 24 h of incubation with prepared pseudo-islets (200 islet equivalents (IEQ) purity > 80%), the function of each group was determined using a glucose challenge test. The test results are expressed as the stimulation index (SI).

8. Reverse transcription-polymerase chain reaction (RT-PCR)

Islet total RNA was extracted using the TRIZOL reagent (Invitrogen Co., Carlsbad, CA, USA) and an RNA Purification Mini Kit (QIAGEN Korea Ltd., Seoul, Korea). cDNA was synthesized from 500 ng of extracted RNA using a reverse transcriptase (RT) kit (Invitrogen) and oligo dT (BIOMEDIC, Buchun, Korea). The cDNAs were amplified by PCR using specific oligonucleotides for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), insulin, glucagon, pancreatic and duodenal homeobox gene 1 (PDX-1), and the glucose transporter 2 gene. The sequences of the primers were as follows:

(1) Rat GAPDH, 5′-TCCCTCAAGATTGTCAGCAA-3′ and

5′-AGATCCACAAACGGATACAT-3′;

(2) Rat insulin, 5′-CATCAGCAAGCAGGTCATTG-3′ and 5′-ACGGGACTTGGGTGTGTAGA-3′;

(3) Rat glucagon, 5′-ATGAACACCAAGAGGAACCG-3′ and 5′-TTCCTCAGCTATGGCGACTT-3′;

(4) Rat PDX-1, 3′-TACGCGGCCACACAGCTCTACAAGGAC-3′ and 5′-CCACTTCATGCGACGGTTTTGGAACCAGA-3′;

(5) Rat Slc2a2, 5′-GGACAAACTCGGAAGGATCA-3′ and 5′-CCAGTCCTGAAATTAGCCCA-3′.

9. Statistical analysis

Statistical analysis was performed using SPSS 13.0 for Windows (IBM SPSS Inc. Armonk, NY, USA). Data are shown as the mean ± standard deviation. Groups were compared by analysis of variance. Statistical significance was set at $P < 0.05$.

III. RESULTS

1. Islet isolation

The average yield from Lewis rats was 2128.16 ± 723.33 IEQ of high purity (Figure 2).

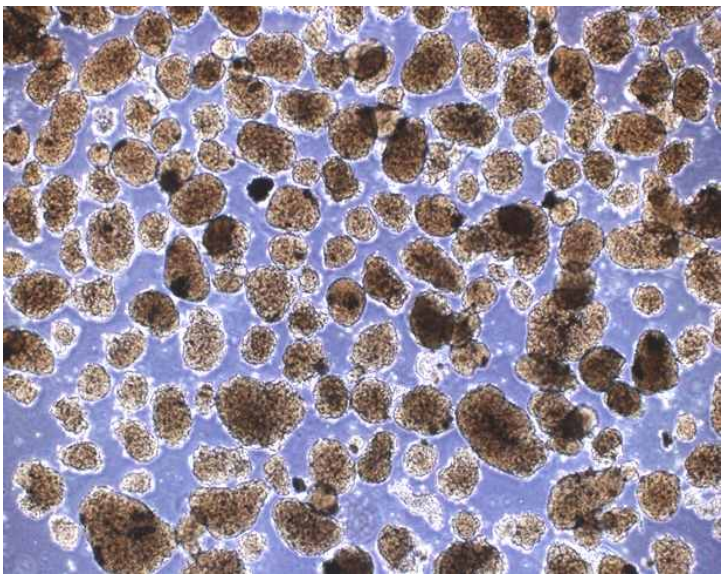

Figure 2. Primary islet from Lewis rat

2. Yield of pseudo-islets

The number of PECs ($7 \pm 1 \times 10^5$) yielded from isolated islets (350 ± 35.3 IEQ) expanded to $62.5 \pm 7.5 \times 10^5$. Moreover, the yield of pseudo-islets from these cells was 2500.13 ± 628.57 IEQ, which was approximately 7-fold greater than that of isolated islets.

3. Microscopic observations

Islets were examined under a phase-contrast microscope. Figure 3A shows sparsely dispersed cells attached to the culture plate after seeding single PEC. The number of PECs gradually increased in the culture dish. After serial passages, sufficient PECs were harvested to produce reaggregated pseudo-islets by the DER method (Fig 3B). PECs were suspended as a solution of individual cells. These cells were aggregated to complete the DER method. The aggregated cells (pseudo-islets) exhibited similar morphology and size to those of naïve islets (Fig 3C; 50–300 μm; Fig 3D).

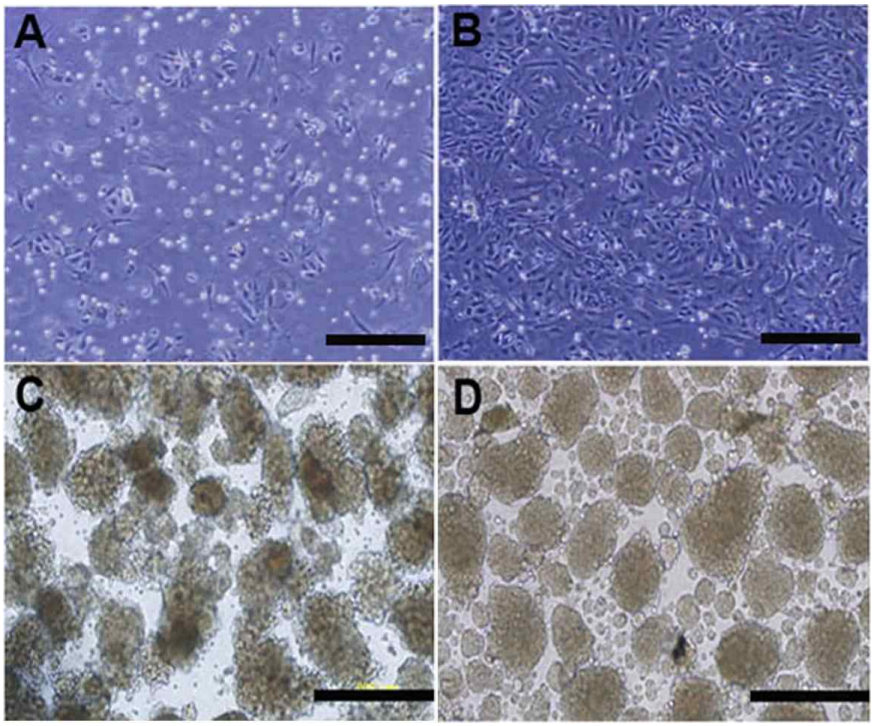

Figure 3. Microscopic observation and findings of islets after DER

A. PECs 3 days after the first seeding

B. PECs expanded to 90% confluence (Phase-contrast; original magnification, ×40)

C. Aggregated pseudo-islets by DER

D. Isolated islets exhibited similar morphology and size

* Bar in A and B = 1.0 mm, C and D = 200 μm.

4. Functional evaluations

Static incubation was performed 24 h after isolated islets or PECs were placed in a glucose challenge test culture. Insulin secretion depended on the change in the glucose level used as the stimulus. The SI values were 1.629 ± 0.13 for primary isolated islets and 1.295 ± 0.189 for pseudo-islets, results that were lower than those of primary isolated islets ($P < 0.001$). According to the result of RT-PCR, insulin secretion from pseudo-islets was similar to that from primary isolated islets; however, glucagon, PDX-1, and Slc2a2 expression was reduced in the pseudo-islets (Figure 4).

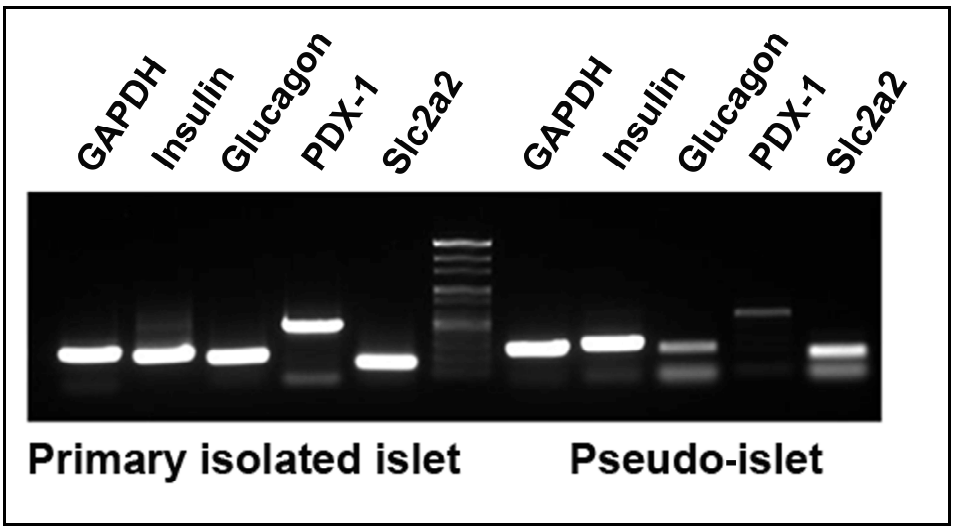

Figure 4. RT-PCR analysis of primary isolated islets and pseudo-islets

Insulin secretion from pseudo-islets was similar to that from primary isolated islets; however, glucagon, PDX-1, and Slc2a2 expression was reduced in the pseudo-islets.

5. Primary isolated islet cells with rBMSCs

The cellular aggregates of pancreatic islet cells and rBMSCs were retrieved in the shape of a spheroid through a high-density suspension shaking culture. These cellular spheroids exhibited fortified function and maintained viability (Fig 5)

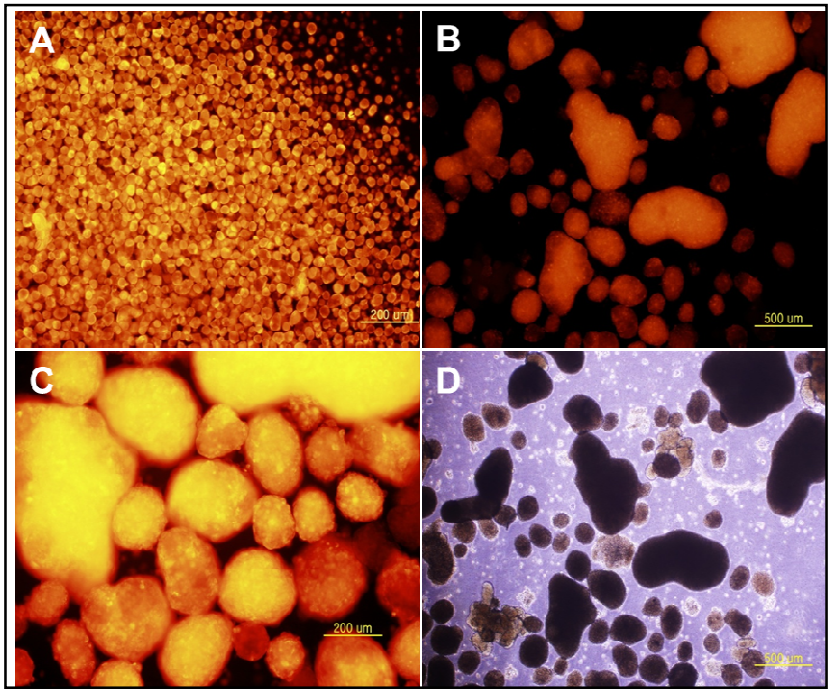

Figure 5. PKH staining result of islet and rBMSC co-culture

A. rBMSCs (approximately 2.1×10^6 cells/cm^2 each)

B and C. Islet (200 islet cells) + rBMSC cell staining (PKH26 staining)

D. Islet cells + rBMSCs

After 24 h of incubation with prepared primary isolated islets (50 islet cells, purity > 80%), single islet cells + rBMSCs, and primary isolated islet cells + rBMSCs, the function of each group was examined by a glucose challenge test, and the results were expressed as the SI [SI = (insulin secretion at 400 mg glucose/dl)/(insulin secretion at 100 mg glucose/dl) 1/10 dilution result] (Figure 6).

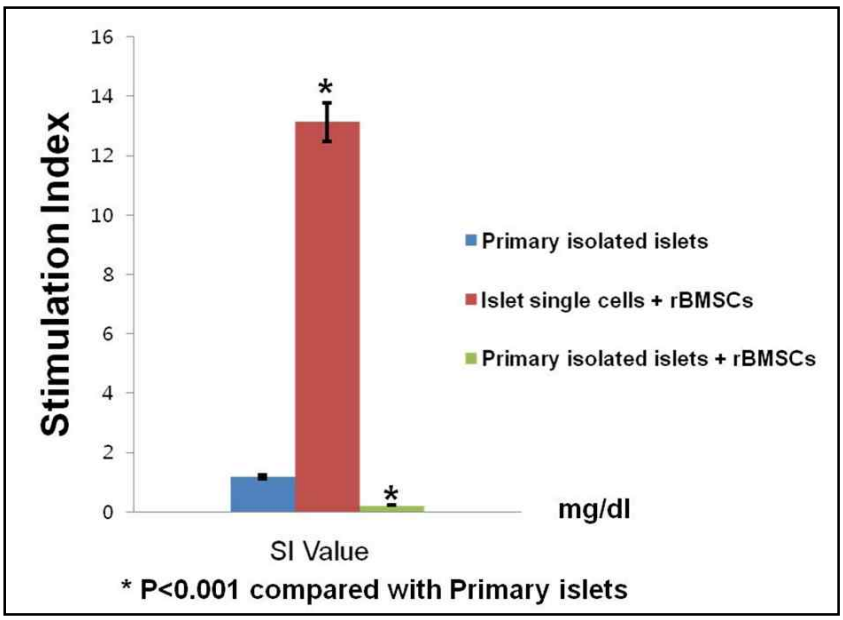

Figure 6. Results of the insulin secretion test

The RT-PCR results revealed no difference in expression pattern among the samples (Figure 7).

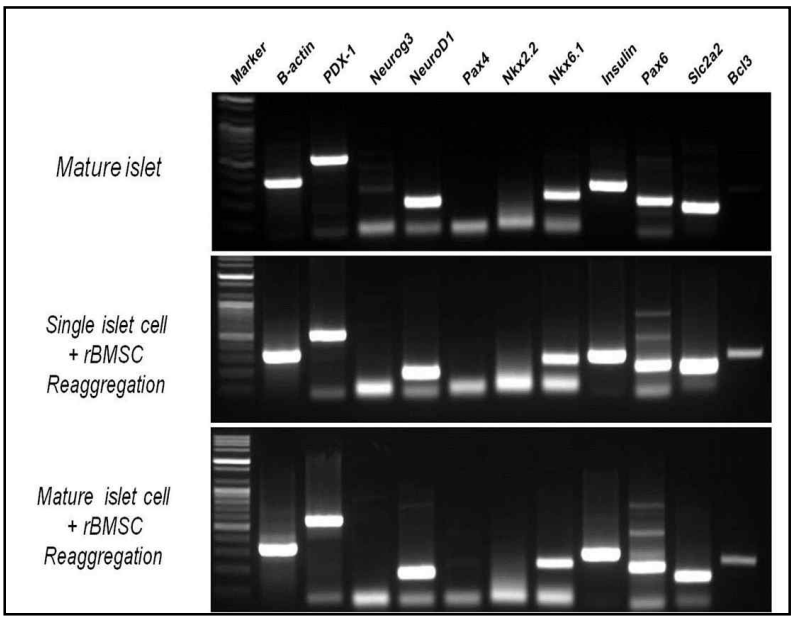

Figure 7. The RT-PCR results of primary isolated islets, single islet cells + rBMSCs, and primary isolated islet cells + rBMSCs

IV. DISCUSSION

Islet transplantation as an alternative to pancreas replacement has important implications for the treatment of diabetes mellitus. Compared with whole pancreas transplantation, islet transplantation is a much simpler procedure, imposing less surgical stress on the patient. Thus, a system for preserving isolated islet cells without reducing their viability or function could be invaluable. The present results indicate that co-culture with BMSCs improves islet survival and function.

Considering that an average of approximately 2000 IEQ can be obtained from a rat, our findings are important because approximately 2500 IEQ of pseudo-islets were produced after a 2-week expansion from 300 islets at initial seeding. The size and shape of the pseudo-islets were similar to those of control isolated islets. Insulin secretion depended on the level of glucose as the stimulus. This finding demonstrated that secretion varied according to the stimulus; therefore, pseudo-islets maintained islet functions. However, when compared with isolated islets, pseudo-islets had functionally imperfect SI values. RT-PCR analysis revealed similar patterns of gene expression in pseudo-islets and isolated control islets. Nevertheless, enzyme-linked immunosorbent assays performed after the glucose challenge test revealed that the SI values for pseudo-islets were lower than those of control islets. In addition, RT-PCR data indicated reduced expression of glucagon, PDX-1, and Slc2a2 in pseudo-islets. Glucagon is involved in the endocrine control of blood glucose levels, and PDX-1 is involved in differentiation[18]. Hence, high expression of these genes suggests the formation of a pancreas, insulin production, and beta cells responses to glucose stimuli[19]. Expression of Slc2a2, a glucose transporter, was reduced in pseudo-islets. The reduced expression of PDX-1 and Slc2a2 suggested that pseudo-islets maintain limited function as islets.

Limited function can be overcome by transplanting more islets. To verify this hypothesis, glucagon secretion capacity, in vitro analysis of various gene expression levels, histologic examination of pseudo-islets, and in vivo transplantation of pseudo-islets into diabetic animal models shall be performed in future studies[19].

The DER method retains PECs at a high density in suspension for a short period and yields 3-dimensional artificial islets. Frequent cell–cell contact, as permitted by growth at high density, stimulates the formation of aggregates. DER technology may have an advantage in clinics because it is scaffold-free and uses a limited number of artificial substances.

Recent reports have indicated that co-culture of islets with other cells preserves islet mass. For example, Miki et al.[20] demonstrated that cells of the MNNK-1 human islet-derived fibroblast cell line helped maintain the morphology and insulin-secretory function of islets co-cultured using inserts for 7 days. Murray et al.[21] reported that pancreatic-derived epithelial cells sustained human islet insulin function in a rotational culture for 10 days, and Chao et al.[8] used human stem cells in a co-culture system, demonstrating that these cells protected human fetal islet-like cell clusters.

We were able to produce a combined cellular complex of 2 different types of cells via cultivation in highly dense, floating condition. For single cell islets + rBMSCs in particular, substantial insulin secretion capacity was observed. Mature islets + BMSCs displayed lower insulin secretion. It appears that insulin secretion was not complete due to rBMSCs surrounding the surface of islets. In single cell islets + rBMSCs, islets were tightly surrounded by rBMSC, whereas islets in the mature islets + rBMSC, lacked cell-to-cell contact.

Interestingly, physical contact was more effective in sustaining islet morphology, survival and secretory function in long-term culture[21]. In the single cell islets + rBMSCs group, rBMSC aggregated around the islets and formed a capsule-like structure that appeared to preserve the overall morphology of islets. These observations indicate that contact co-culture with rBMSC may provide a microenvironment suitable for the repair of islet injury and support of islet function. Previous study results indicated that contact co-culture is the major contributing factor for islet survival, maintenance of cell morphology and glucose-responsive insulin secretion[22]. However, there might also be a synergistic effect resulting from the regulation of inflammatory cytokine production and cell-to-cell contact. Hence, it is believed that immunostaining assays will be needed for further investigation.

Nevertheless, there was no distinguishable difference among the 3 groups at the genetic level. It appears that single cell islets, unlike primary islets, interacted with rBMSCs and experienced increased insulin secretion in consequence. Additional investigation will be necessary.

V. CONCLUSION

We suggest the production method consisting of a mixed cellular complex of 2 different origins. Using this construct, cell-cell interactions can be examined in vitro such as the improvement of secretion ability and cell differentiation. rBMSCs can be used as a replacement or additives for bioengineered pseudo-islets. As these cells were both morphologically and genetically similar to naïve islets, our new technique could be a potential method to overcome the scarcity of donor islets in the near future.

REFERENCES

1. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant 2005; 5: 2037-46.
2. Kodama S, Kojima K, Furuta S, Chambers M, Paz AC, Vacanti CA. Engineering functional islets from cultured cells. Tissue Eng Part A, 2009; 15: 3321-9.
3. Lim JY, Min BH, Kim BG, Shin JS, Park CS, Yoon TW, et al. Combinations of growth factors enhances the potency of islets in vitro. Pancreas 2009; 38: 447-53.
4. Morikawa M, Kimura T, Murakami M, Katayama K, Terada S, Yamaguchi A. Rat islet culture in serum-free medium containing silk protein sericin. J Hepatobiliary Pancreat Surg 2009; 16: 223-8.
5. Teramura Y, Iwata H. Islet encapsulation with living cells for improvement of biocompatibility. Biomaterials 2009; 30: 2270-5.
6. Noel A, Jost M, Maquoi E. Matrix metalloproteinases at cancer tumor-host interface. Semin Cell Dev Biol 2008; 19 :52-60.
7. Piemonti L, Leone BE, Nano R, Saccani A, Monti P, Maffi P, et al. Human pancreatic islets produce and secrete MCP-1/CCL2: relevance in human islet transplantation. Diabetes 2002; 51: 55–65.
8. Chao KC, Chao KF, Chen CF, Liu SH. A novel human stem cell coculture system that maintains the survival and function of culture islet-like cell clusters. Cell Transplant 2008; 17: 657–64.
9. Cross SE, Richards SK, Clark A, Benest AV, Bates DO, Mathieson PW, et al. Vascular endothelial growth factor as a survival factor for human islets: effect of immunosuppressive drugs. Diabetologia 2007; 50: 1423–32.
10. Kim DH, Yoo KH, Yim YS, Choi J, Lee SH, Jung HL, et al.

Cotransplanted bone marrow derived mesenchymal stem cells (MSC) enhanced engraftment of hematopoietic stem cells in a MSC-dose dependent manner in NOD/SCID mice. J Korean Med Sci 2006; 21: 1000–4.

11. Brohlin M, Mahay D, Novikov LN, Terenghi G, Wiberg M, Shawcross SG, et al. Characterisation of human mesenchymal stem cells following differentiation into Schwann cell-like cells. Neurosci Res 2009; 64: 41–9.
12. Popp FC, Renner P, Eggenhofer E, Slowik P, Geissler EK, Piso P, et al. Mesenchymal stem cells as immunomodulators after liver transplantation. Liver Transpl 2009; 15: 1192–8.
13. Bacigalupo A, Valle M, Podestà M, Pitto A, Zocchi E, De Flora A, et al. T-cell suppression mediated by mesenchymal stem cells is defi cient in patients with severe aplastic anemia. Exp Hematol 2005; 33: 819–27.
14. Rasmusson I, Ringdé n O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp Cell Res 2005; 305: 33–41.
15. Komori M, Tsuji S, Tsujii M, Murata H, Iijima H, Yasumaru M, et al. Efficiency of bone marrow-derived cells in regeneration of the stomach after induction of ethanol-induced ulcers in rats. J Gastroenterol 2005; 40: 591–9.
16. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 2007; 25: 2648–59.
17. Ashizawa S, Brunicardi FC, Wang XP. PDX-1 and the pancreas. Pancreas 2004; 28: 109-20.
18. Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H. Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature 1997; 385: 257-60.
19. Jeong JH, Lee JI, Ju MK, Joo DJ, Huh KH, Kim MS, et al. Proliferation

of pancreatic endocrine cells using disaggregation – expansion – reaggregation technology in isolated rat islets. Transplant Proc 2010; 42: 907-10.

20. Miki A, Narushima M, Okitsu T, Takeno Y, Soto-Gutierrez A, Rivas-Carrillo JD, et al. Maintenance of mouse, rat, and pig pancreatic islet functions by coculture with human islet-derived fibroblasts. Cell Transplant 2006; 15: 325–34.
21. Murray HE, Paget MB, Bailey CJ, Downing R. Sustained insulin secretory response in human islets co-cultured with pancreatic duct-derived epithelial cells within a rotational cell culture system. Diabetologia. 2009; 52: 477–85.
22. Jung EJ, Kim SC, Wee YM, Kim YH, Choi MY, Jeong SH, Lee J, et al. Bone marrow-derived mesenchymal stromal cells support rat pancreatic islet survival and insulin secretory function in vitro. Cytotherapy. 2011; 13: 19-29.

ABSTRACT(IN KOREAN)

췌장 내분비 세포를 이용한 유사 췌도의 제작 및 기능평가

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주 만 기

췌장 섬 세포 이식은 1 형 당뇨병의 새로운 치료방법이다 하지만, 실제 임상에서 치료법으로 사용하기에는 많은 장애가 있다. 특히 충분한 양의 이식 가능한 췌장 섬 세포의 확보가 어려워 임상치료로 한계가 있어 왔다. 이에 저자 등은 이전 실험을 통해 pseudo-lets을 생산 할 수 있는 Disaggregation-Expansion-Reaggregation (DER) 배양법을 개발 하였다. 또한 최근 연구 보고에 의하면 중간엽줄기세포와 췌장 섬 세포를 공동 배양 시 성장과 분화에 긍정적인 효과를 보여준 것으로 보고 되었다. 본 연구의 목적은 새로 만들어진 pseudo-islets의 형태 및 기능적인 평가를 통해 췌도이식에 있어 이러한 세포의 사용 가능성을 테스트하고 췌장 섬 세포와 중간엽줄기세포간의 공동 배양 시 상호 효과를 증명하기 위해 수행되었다. Lewis rat으로부터 분리된 췌장은 collagenase 및 trypsin 처치를 통하여 단일 췌장 섬세포로 분리되었다. 이러한 골수 중간엽줄기세포의 수집은 Lewis ra의 대퇴부에서 천자하여 세포배양을 통해 증식 하였다. 이후, 배양된 pseudo-islet과 중간엽 줄기세포를 같이 배양하였다. 공동 배양된 pseudo-islet은 각각 glucose challenge test, ELISA, PCR, 및 면역화학 염색을 통해 기능 및 특성을 평가했다. 위상차현미경으로 관찰 하였을 때 pseudo-islet은 구형의 모양과 크기가 최초 분리된 췌장 섬세포와 비슷하였다. PCR 검사에서도 인슐린, 글루카곤 및 PDX-1발현을 보였으나 췌장 섬세포와 비교할 때 글루카곤과 PDX-1의 발현은

저하되어 있었다. ELISA 검사에서도 유사한 인슐린 분비를 보여주었다. 췌장 섬세포와 중간엽줄기세포 공동 배양을 통해 두 세포의 구형 집합체를 얻을 수 있었고 이러한 구형 세포 집합체는 pseudo-islet 세포의 기능을 유지 및 강화 시키는 가능성을 보여주었다. 결론적으로, 새로운 췌장 섬세포 배양 방법인 DER을 통해 모양과 기능을 유지한 psudo-islet 세포의 고효율의 배양 성적을 얻을 수 있었고 또한 이러한 pseudo-islet 세포와 중간엽줄기세포의 공동 배양을 통해서는 보다 강화된 인슐린 분비기능을 가진 pseudo-islet-중간엽줄기세포 집합체를 얻을 수 있었다. 이 두 가지 세포배양법 모두 최소한의 첨가제와 인위적인 조작을 통해 이루어짐으로써 악성세포 발현 등의 원하지 않는 결과를 막을 수 있었다. 이처럼 모양과 기능을 유지한 pseudo-islet의 고효율 배양법은 췌도이식을 위한 췌장 섬세포 부족을 해결할 수 있는 잠재적인 방법이 될 수 있을 것으로 생각된다.

핵심되는 말 : 췌장 섬세포, 중간엽 줄기세포, 췌도이식

PUBLICATION LIST

1. Jeong JH, Lee JI, Ju MK, Joo DJ, Huh KH, Kim MS, et al. Proliferation of pancreatic endocrine cells using disaggregation-expansion-reaggregation technology in isolated rat islets. Transplant Proc. 2010: 42; 907-10.