CellR4 2016; 4 (4): e2120

Clinical intraocular islet transplantation is not a number issue

A. Shishido[1,2], A. Caicedo[1,4], R. Rodriguez-Diaz[4], A. Pileggi[1,2,3], P.-O. Berggren[1,5], M. H. Abdulreda[1,2]

[1]Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USA
[2]Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, USA
[3]Departments of Microbiology and Immunology and Biomedical Engineering, University of Miami, Miami, FL, USA
[4]Department of Medicine, Division of Endocrinology, University of Miami Miller School of Medicine, Miami, FL, USA
[5]Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, Stockholm, Sweden

Corresponding Author: Midhat H. Abdulreda, Ph.D; e-mail: mabdulreda@miami.edu
Per-Olof Berggren, Ph.D; e-mail: Per-Olof.Berggren@ki.se

Keywords: Islet transplantation, Beta cell replacement therapy, Intrahepatic, Liver, Eye, Anterior chamber, Immune privilege, Local tolerance, Peripheral tolerance, Intraocular, Islet number, Islet mass.

Abstract

It is now well established that beta cell replacement through pancreatic islet transplantation results in significant improvement in the quality-of-life of type 1 diabetes (T1D) patients. This is achieved through improved control and prevention of severe drops in blood sugar levels. Islet transplant therapy is on the verge of becoming standard-of-care in the USA. Yet, as with other established transplantation therapies, there remain hurdles to overcome to bring islet transplantation to full fruition as a long-lasting therapy of T1D. One of these hurdles is establishing reliable new sites, other than the liver, where durable efficacy and survival of transplanted islets can be achieved. In this article, we discuss the anterior chamber of the eye as a new site for clinical islet transplantation in the treatment of T1D. We specifically focus on the common conceptions, and preconceptions, on the requirements of islet mass, and whether or not the anterior chamber can accommodate sufficient islets to achieve meaningful efficacy and significant impact on hyperglycemia in clinical application.

Introduction

Recent results from the Clinical Islet Transplantation (CIT) Consortium trials showed that pancreatic islet transplantation in the liver improves blood sugar control significantly and reduces severe hypoglycemia episodes in type 1 diabetes (T1D) patients[1]. This leads to significant improvement in the patients' quality-of-life[2-4]. However, it has also become evident that due to the immediate blood-mediated immune reaction (IBMIR) and other liver-related issues (e.g., hypoxia, highly enzymatic and inflammatory environment, high drug levels, etc.)[5], the benefits of intrahepatic islet transplantation may be limited on the long-term[6]. Another concern, which is common to transplantation therapies in general, is about the serious and potentially life-threatening side effects associated with the required life-long systemic immunosuppression to avoid graft rejection[6,7]. Clinical evidence also shows that long-term success of islet transplantation therapy may be hindered by recurrent autoimmunity[8,9]. Therefore, to realize the full benefits of islet transplantation there remain two unmet critical needs to: 1) establish new islet transplantation sites with no added "strain" on the islet grafts as has been shown in the liver; and 2) achieve long-term efficacy and survival of islet grafts without the need for life-long systemic immunosuppression or its complications through induced graft immune tolerance.

New approaches to minimizing and/or eliminating immunosuppression are under investigation[10-16]; however, such approaches must be paired with new transplantation sites to ensure better engraftment and long-term function of transplanted islets. Consequently, different new sites for islet transplantation including but not limited to the omentum, subcutaneous, intramuscular, and the bone marrow have been investigated[17-20]. Consis-

tent with these efforts, we have been investigating the immune privileged anterior chamber of the eye as a potential site for clinical islet transplantation, where transplanted islets thrive during early engraftment due to high oxygen tension and can potentially survive long-term with minimal to no immunosuppression[21].

Our extensive studies have demonstrated the feasibility and efficacy of intraocular islet transplantation in preclinical models[22-25], and this has recently led to a significant first step in the clinical implementation of this novel approach to islet transplantation. We have obtained FDA approval (IND 017007) to conduct a pilot clinical trial to assess primarily the safety and secondarily the efficacy of pancreatic islet transplantation into the anterior chamber of the eye of legally blind T1D patients with a stable kidney transplant (i.e., already on immunosuppression). The purpose of this article is to discuss in the context of preclinical and clinical evidence whether the human eye anterior chamber is big enough to accommodate sufficient islet mass in clinical application.

Materials and methods

All animal procedures were performed under protocols approved by the University of Miami IACUC.

Mice

C57BL/6J (B6) and AKITA mice were purchased from Jackson Laboratories (JAX) and housed under the supervision of the University of Miami's Department Veterinary Resources (DVR).

Diabetes induction

Acute induction of diabetes in B6 mice was achieved via single intravenous injection (150-220 mg/kg) of Streptozotocin (STZ). Frank diabetes was defined as 3 (three) consecutive readings of nonfasting glycemia ≥300 mg/dL. Heterozygous male AKITA mice developed severe hyperglycemia spontaneously during the first 3-6 weeks of life and no induction of diabetes was necessary.

Islet isolation and transplantation into the eye anterior chamber

Pancreatic islets were isolated from B6 donor mice as previously described[26]. Islet transplantation into the eye anterior chamber of was performed as previously described in details[23,27,28].

Glycemia and body weight monitoring

Glycemia was measured using portable glucometers (OneTouchUltra2; LifeScan, CA, USA) using a drop of blood from the tail vein.

Results

Based on clinical and preclinical experience in islet transplantation in the liver and other sites, research investigators have gravitated towards transplanting large numbers of islets to restore euglycemia in animal models of diabetes. Interestingly, an islet dose of 5,000 IEQ/kg under the kidney capsule, which corresponds to ~100 islets in an average mouse weighting 20 g, has been widely accepted as sufficient islet mass to restore euglycemia in mouse models of islet transplantation[26]. Moreover, since our introduction in 2008 of islet transplantation in the anterior chamber of the eye[23], we collected anecdotal evidence that even fewer islets may be sufficient to restore euglycemia in diabetic mice transplanted either under the kidney capsule or in the anterior chamber of the eye.

Therefore, to methodically and unequivocally address this issue we performed titration studies to assess the minimal islet mass required to restore euglycemia following intraocular islet transplantation (Figure 1A). We performed these studies in the streptozotocin (STZ)-induced diabetes C57BL/6 (B6) mouse model and in the spontaneously diabetic AKITA mouse. We transplanted 4-6 week old diabetic male AKITA mice in the anterior chamber of the eye with syngeneic (B6) 100 IEQ (~3,500 IEQ/kg at the onset of euglycemia post-transplant), and we monitored their nonfasting blood glucose levels and body weight longitudinally (Figure 1B). The median time to reverse diabetes (i.e., to achieve euglycemia; defined as 3 consecutive glycemia readings ≤200 mg/dL) following transplantation was 54 days; and 100% of the mice achieved stable euglycemia by 60 days post-transplant (Figure 1C). Notably, steady normalization of glycemia occurred despite a progressive and significant increase in the recipients' body weight (dashed lines in Figure 1B). Similarly, we transplanted STZ-induced diabetic B6 mice with as few as B6 75 IEQ (i.e., ~2,500 IEQ/Kg) and as many as 500 IEQ (i.e., ~17,000 IEQ/Kg); the mice transplanted with 75 or 150 IEQ had a median diabetes reversal time of 77 and 58.5 days, respectively, compared to 26 days in those transplanted with 300 and 500 IEQ (Figure 1D).

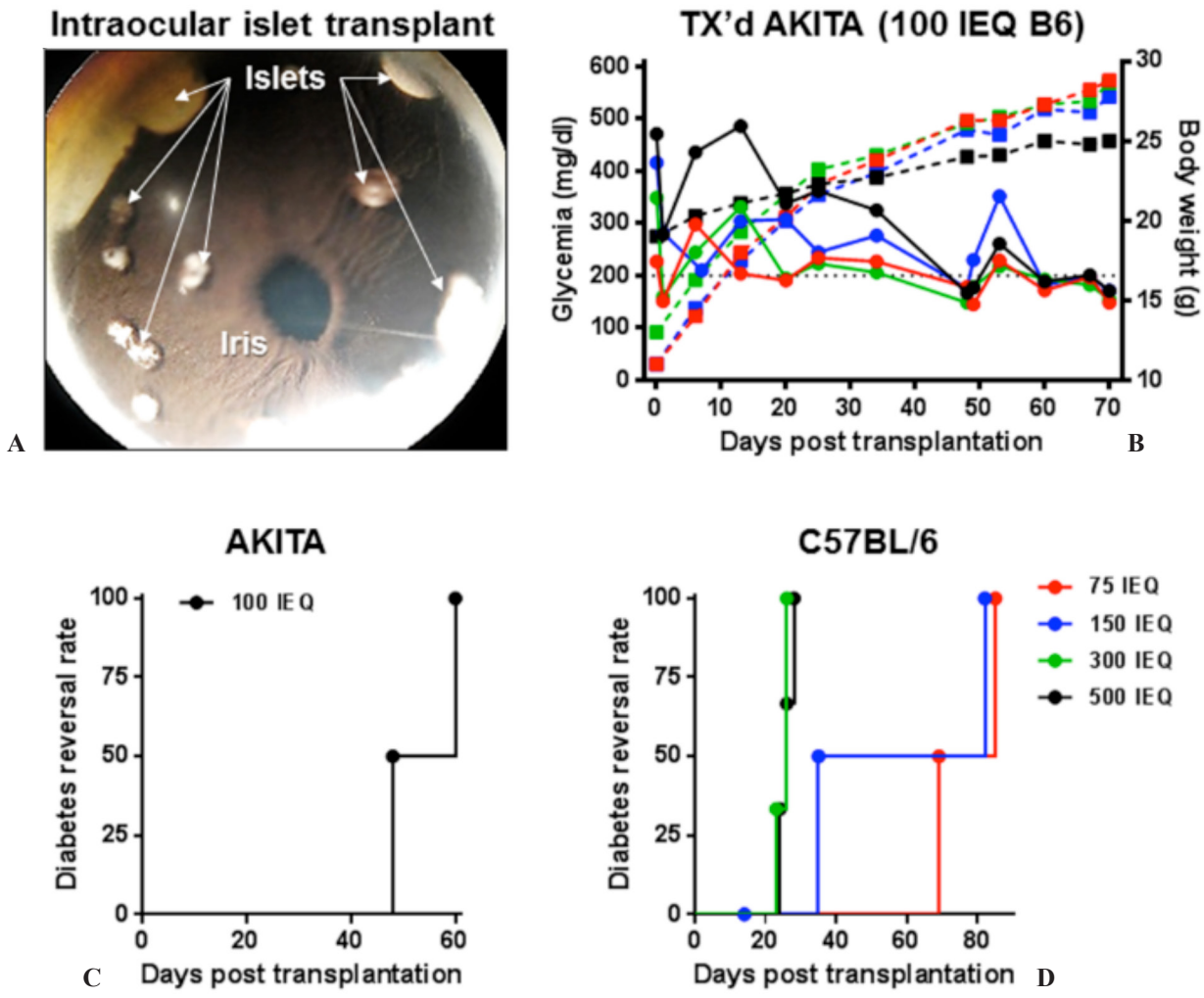

Figure 1. Pancreatic islet transplantation in the eye anterior chamber. (**A**) Photo of AKITA mouse eye transplanted with 100 IEQ showing islets engrafted on top of the iris individually or in clusters. (**B**) Longitudinal glycemia (solid lines; left Y axis) and body weight (dashed lines; right Y axis) record of diabetic male AKITA mice (n=4) which were transplanted with 100 IEQ B6 islets. Euglycemia was defined as 3 consecutive blood glucose readings ≤200 mg/dL (dotted line). (**C, D**) Kaplan-Meyer curves summarizing the % normoglycemic mice (expressed as "diabetes reversal rate") following transplantation in (**C**) AKITA (n=4) and (**D**) B6 recipients (n=2 for 75 IEQ and n=3 for 150, 300, and 500 IEQ groups).

Discussion

Although extrapolation of preclinical findings, especially from small animals such as rodents, to the clinical setting is not straightforward, the above findings demonstrate that small numbers of islets are capable, albeit with delay, of achieving significant improvements in glycemic control following islet transplantation in the anterior chamber of the eye. This notion was further supported in our previous studies in the baboon[22]. Moreover, clinical experience shows that a substantial amount of islets is lost due to IBMIR, inflammation, and apoptosis following transplantation in the liver. Yet, benefits observed following intrahepatic islet transplantation, such as restored hypoglycemia awareness, have been shown to be retained by transplanted patients long after getting back on insulin therapy due to graft failure and/or rejection[29,30]. Notably, clinical evidence further indicates a lack of correlation between the number of transplanted islets and the clinical outcome[1]. Thus, the number of transplanted islets needed to meaningfully impact on hyperglycemia varies significantly among patients, and may not necessarily be as large as

commonly assumed. It is likely that the notion that a very large number of islets is needed has been reinforced by the mixed experience with intrahepatic islet transplantation, and it may not hold true in more islet-friendly transplantation sites.

Studies estimate the total number of islets in the human endocrine pancreas between 0.5-1 million islets[31]. Data from pancreatectomized patients also suggest that only 15-30% of the functional beta cell mass may be needed to maintain glucose homeostasis [32, 33]. Thus, the required number of islets to maintain euglycemia can be estimated at 125,000-250,000 islets; and in an average 80 kg human subject, this equates to ~1500-3000 IEQ/Kg. This is consistent with what may remain in the liver following a conservative 50% loss of infused islets due to IBMIR and initial inflammation/apoptosis; hence, an effective intrahepatic islet mass may be lower than 1,500-2,000 IEQ/kg with higher loss rates[34,35]. Importantly, an islet dose of 1,000-2,000 IEQ/kg in an average diabetic subject weighing 80 kg can be accommodated in the anterior chamber of one eye, which has an estimated volume of 200-300 μL in humans[36,37]. Thus, intraocular islet transplantation can be effective in conveying significant improvement in blood sugar control and prevention of severe hypoglycemia in T1D patients. Moreover, if safety is proven islets can also be transplanted into the second eye of the same individuals.

In summary, experimental evidence demonstrates the feasibility, safety, and efficacy of intraocular islet transplantation in preclinical models[22,23,25,38-41]. Importantly, the anticipated clinical trial will determine its safety profile and establish unequivocally if the anterior chamber of the human eye can host enough islets to sufficiently impact on hyperglycemia.

Acknowledgements

This work was supported by the Diabetes Research Institute Foundation (DRIF) (A.C., A.P., P.-O.B., M.H.A.); National Institutes of Health/ National Institute of Diabetes and Digestive and Kidney Diseases/National Institute of Allergy and Infectious Diseases Grants 5U19AI050864-10 (to A.P.), 1F32DK083226 and DK097194 (to M.H.A.), R03DK075487 and R01DK084321 (to A.C.), U01D K089538 (to P.-O.B. and A.P.), M01RR16587, 5U01D K70460-07, and R01DK55347-IU42RR016603, General Clinical Research Center, and Juvenile Diabetes Research Foundation International Grants 4-2004-361 (to P.-O.B., and A.P.) and 4-2008-811 and 17-2010-5 (to A.P.). We thank Drs. Damaris R. Molano, Victor L. Perez, Ying Wang, and Maged Hossameldin for their valuable help in this work. We also thank the Small Animal Core at the DRI for their help with animals.

Conflict of interest

Dr. Pileggi is currently employed at the National Institutes of Health (NIH). The opinions expressed in this article are the author's own and do not necessarily reflect the views of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

P-O.B. is cofounder and CEO of Biocrine AB, an unlisted biotech company that holds a patent on the intraocular transplantation technique and is using it as a research platform. M.H.A. is consultant of Biocrine AB.

References

1. Hering BJ, Clarke WR, Bridges ND, Eggerman TL, Alejandro R, Bellin MD, Chaloner K, Czarniecki CW, Goldstein JS, Hunsicker LG, Kaufman DB, Korsgren O, Larsen CP, Luo X, Markmann JF, Naji A, Oberholzer J, Posselt AM, Rickels MR, Ricordi C, Robien MA, Senior PA, Shapiro AM, Stock PG, Turgeon NA; Clinical Islet Transplantation Consortium. Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia. Diabetes Care 2016; 39: 1230-1240.
2. Tharavanij T, Betancourt A, Messinger S, Cure P, Leitao CB, Baidal DA, Froud T, Ricordi C, Alejandro R. Improved long-term health-related quality of life after islet transplantation. Transplantation 2008; 86(9): 1161-1167.
3. Poggioli R, Faradji RN, Ponte G, Betancourt A, Messinger S, Baidal DA, Froud T, Ricordi C, Alejandro R. Quality of life after islet transplantation. Am J Transplant 2006; 6(2): 371-378.
4. Piemonti L, Pileggi A. 25 years of the Ricordi automated method for islet isolation. CellR4 2013; 1(1): 8-22.
5. Naziruddin B, Iwahashi S, Kanak MA, Takita M, Itoh T, Levy MF. Evidence for instant blood-mediated inflammatory reaction in clinical autologous islet transplantation. Am J Transplant 2014; 14(2): 428-437.
6. Shapiro AM. Islet transplantation in type 1 diabetes: ongoing challenges, refined procedures, and long-term outcome. Rev Diabet Stud 2012; 9(4): 385-406.
7. Yu SH, Bordeaux JS, Baron ED. The immune system and skin cancer. Adv Exp Med Biol 2014; 810: 182-191.
8. Vendrame F, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, Diamantopoulos S, Standifer N, Geubtner K, Falk BA, Ichii H, Takahashi H, Snowhite I, Chen Z, Mendez A, Chen L, Sageshima J, Ruiz P, Ciancio G, Ricordi C, Reijonen H, Nepom GT, Burke GW 3rd, Pugliese A. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. Diabetes 2010; 59(4): 947-957.

9. Azzi J, Geara AS, El-Sayegh S, Abdi R. Immunological aspects of pancreatic islet cell transplantation. Expert Rev Clin Immunol 2010; 6(1): 111-124.
10. Szot GL, Yadav M, Lang J, Kroon E, Kerr J, Kadoya K, Brandon EP, Baetge EE, Bour-Jordan H, Bluestone JA. Tolerance induction and reversal of diabetes in mice transplanted with human embryonic stem cell-derived pancreatic endoderm. Cell Stem Cell 2015; 16(2): 148-157.
11. Lee K, Nguyen V, Lee KM, Kang SM, Tang Q. Attenuation of donor-reactive T cells allows effective control of allograft rejection using regulatory T cell therapy. Am J Transplant 2014; 14(1): 27-38.
12. Stumpf M, Zhou X, Bluestone JA. The B7-independent isoform of CTLA-4 functions to regulate autoimmune diabetes. J Immunol 2013; 190(3): 961-969.
13. Cabello-Kindelan C, de la Barrera A, Malek TR, Bayer AL. In vivo environment necessary to support transplanted donor mouse T regulatory cells. Am J Transplant 2014;14(5):1032-45.
14. Rosenzwajg M, Churlaud G, Mallone R, Six A, Dérian N, Chaara W, Lorenzon R, Long SA, Buckner JH, Afonso G, Pham HP, Hartemann A, Yu A, Pugliese A, Malek TR, Klatzmann D. Low-dose interleukin-2 fosters a dose-dependent regulatory T cell tuned milieu in T1D patients. J Autoimmun 2015; 58: 48-58.
15. Rafael E, Tibell A, Rydén M, Lundgren T, Sävendahl L, Borgström B, Arnelo U, Isaksson B, Nilsson B, Korsgren O, Permert J. Intramuscular autotransplantation of pancreatic islets in a 7-year-old child: a 2-year follow-up. Am J Transplant 2008; 8(2): 458-462.
16. Sakata N, Aoki T, Yoshimatsu G, Tsuchiya H, Hata T, Katayose Y, Egawa S, Unno M. Strategy for clinical setting in intramuscular and subcutaneous islet transplantation. Diabetes Metab Res Rev 2014; 30(1): 1-10.
17. Berman DM, Molano RD, Fotino C, Ulissi U, Gimeno J, Mendez AJ, Kenyon NM, Kenyon NS, Andrews DM, Ricordi C, Pileggi A. Bioengineering the Endocrine Pancreas: Intraomental Islet Transplantation Within a Biologic Resorbable Scaffold. Diabetes 2016; 65(5): 1350-1361.
18. Wolf-van Buerck L, Schuster M, Baehr A, Mayr T, Guethoff S, Abicht J, Reichart B, Nam-Apostolopoulos YC, Klymiuk N, Wolf E, Seissler J. Engraftment and reversal of diabetes after intramuscular transplantation of neonatal porcine islet-like clusters. Xenotransplantation 2015; 22: 443-450.
19. Maffi P, Balzano G, Ponzoni M, Nano R, Sordi V, Melzi R, Mercalli A, Scavini M, Esposito A, Peccatori J, Cantarelli E, Messina C, Bernardi M, Del Maschio A, Staudacher C, Doglioni C, Ciceri F, Secchi A, Piemonti L. Autologous pancreatic islet transplantation in human bone marrow. Diabetes 2013; 62(10): 3523-3531.
20. Korsgren O, Nilsson B. Improving islet transplantation: a road map for a widespread application for the cure of persons with type I diabetes. Curr Opin Organ Transplant 2009; 14(6): 683-687.
21. Niederkorn JY. See no evil, hear no evil, do no evil: the lessons of immune privilege. Nat Immunol 2006; 7(4): 354-359.
22. Perez VL, Caicedo A, Berman DM, Arrieta E, Abdulreda MH, Rodriguez-Diaz R, Pileggi A, Hernandez E, Dubovy SR, Parel JM, Ricordi C, Kenyon NM, Kenyon NS, Berggren PO. The anterior chamber of the eye as a clinical transplantation site for the treatment of diabetes: a study in a baboon model of diabetes. Diabetologia 2011; 54(5): 1121-1126.
23. Speier S, Nyqvist D, Cabrera O, Yu J, Molano RD, Pileggi A, Moede T, Köhler M, Wilbertz J, Leibiger B, Ricordi C, Leibiger IB, Caicedo A, Berggren PO. Noninvasive in vivo imaging of pancreatic islet cell biology. Nat Med 2008; 14(5): 574-578.
24. Abdulreda MH, Faleo G, Molano RD, Lopez-Cabezas M, Molina J, Tan Y, Echeverria OA, Zahr-Akrawi E, Rodriguez-Diaz R, Edlund PK, Leibiger I, Bayer AL, Perez V, Ricordi C, Caicedo A, Pileggi A, Berggren PO. High-resolution, noninvasive longitudinal live imaging of immune responses. Proc Natl Acad Sci U S A 2011; 108(31): 12863-12868.
25. Abdulreda MH, Rodriguez-Diaz R, Caicedo A, Berggren PO. Liraglutide Compromises Pancreatic beta Cell Function in a Humanized Mouse Model. Cell Metab 2016.
26. Pileggi A, Molano R, Berney T, Cattan P, Vizzardelli C, Oliver R, Fraker C, Ricordi C, Pastori RL, Bach FH, Inverardi L. Heme oxygenase-1 induction in islet cells results in protection from apoptosis and improved in vivo function after transplantation. Diabetes 2001; 50(9): 1983-1991.
27. Speier S, Nyqvist D, Kohler M, Caicedo A, Leibiger IB, Berggren PO. Noninvasive high-resolution in vivo imaging of cell biology in the anterior chamber of the mouse eye. Nat Protoc 2008; 3(8): 1278-1286.
28. Abdulreda MH, Caicedo A, Berggren P-O. Transplantation into the Anterior Chamber of the Eye for Longitudinal, Non-invasive In vivo Imaging with Single-cell Resolution in Real-time. J Vis Exp 2013; (73): e50466.
29. Leitão CB, Tharavanij T, Cure P, Pileggi A, Baidal DA, Ricordi C, Alejandro R. Restoration of hypoglycemia awareness after islet transplantation. Diabetes Care 2008; 31(11): 2113-2115.
30. Baidal DA, Faradji RN, Messinger S, Froud T, Monroy K, Ricordi C, Alejandro R. Early metabolic markers of islet allograft dysfunction. Transplantation 2009; 87(5): 689-697.
31. Dolensek J, Rupnik MS, Stozer A. Structural similarities and differences between the human and the mouse pancreas. Islets 2015; 7(1): e1024405.
32. Daaboul J, Schatz D. Overview of prevention and intervention trials for type 1 diabetes. Rev Endocr Metab Disord 2003; 4(4): 317-323.
33. King J, Kazanjian K, Matsumoto J, Reber HA, Yeh MW, Hines OJ, Eibl G. Distal pancreatectomy: incidence of postoperative diabetes. J Gastrointest Surg 2008;12(9):1548-1553.
34. Bennet W, Groth CG, Larsson R, Nilsson B, Korsgren O. Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. Ups J Med Sci 2000; 105(2): 125-133.
35. van der Windt DJ, Bottino R, Casu A, Campanile N, Cooper DK. Rapid loss of intraportally transplanted islets: an overview of pathophysiology and preventive strategies. Xenotransplantation 2007; 14(4): 288-297.
36. Fontana ST, Brubaker RF. Volume and depth of the anterior chamber in the normal aging human eye. Arch Ophthalmol 1980; 98(10): 1803-1808.

37. Labiris G, Gkika M, Katsanos A, Fanariotis M, Alvanos E, Kozobolis V. Anterior chamber volume measurements with Visante optical coherence tomography and Pentacam: repeatability and level of agreement. Clin Experiment Ophthalmol 2009; 37: 772-774.
38. Nyqvist D, Speier S, Rodriguez-Diaz R, Molano RD, Lipovsek S, Rupnik M, Dicker A, Ilegems E, Zahr-Akrawi E, Molina J, Lopez-Cabeza M, Villate S, Abdulreda MH, Ricordi C, Caicedo A, Pileggi A, Berggren PO. Donor islet endothelial cells in pancreatic islet revascularization. Diabetes 2011; 60(10): 2571-2577.
39. Rodriguez-Diaz R, Speier S, Molano RD, Formoso A, Gans I, Abdulreda MH, Cabrera O, Molina J, Fachado A, Ricordi C, Leibiger I, Pileggi A, Berggren PO, Caicedo A. Noninvasive in vivo model demonstrating the effects of autonomic innervation on pancreatic islet function. Proc Natl Acad Sci U S A 2012; 109(52): 21456-21461.
40. Ilegems E, Dicker A, Speier S, Sharma A, Bahow A, Edlund PK, Leibiger IB, Berggren PO. Reporter islets in the eye reveal the plasticity of the endocrine pancreas. Proc Natl Acad Sci U S A 2013; 110: 20581-20586.
41. Abdulreda MH, Caicedo A, Berggren PO. A natural body window to study human pancreatic islet function and survival. CellR4 2013; 1(2): 111-122.