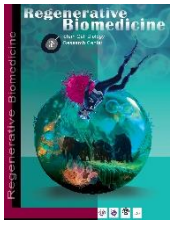

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Mini-Review Article

Stem Cell-based Approaches for Islet Replacement in Diabetes

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Abstract

Diabetes is a complex and multifactorial metabolic disease characterized by the autoimmune destruction of pancreatic beta cells or a combination of peripheral insulin resistance and impaired insulin secretion. Current therapeutic approaches are not curative and have their complications. Recently, stem cell-based therapies have emerged as a promising approach with the potential to restore normoglycemia and beta cell function, reverse diabetes, achieve long-term glycemic control, and prevent complications. Various stem cell sources are being investigated to find a potential solution for manufacturing unlimited transplantable insulin-secreting cells. Significant progress has been made in preclinical studies and the generation of islet-like endocrine clusters or organoids. However, the clinical translation of these cell therapies is still in the early stages. New strategies such as gene editing and tissue engineering may improve the safety and efficacy of stem cell-derived beta or progenitor cell transplantation. This review discusses the current state of islet transplantation, different cell-based therapies, and their potential for clinical translation, with a major focus on pancreatic organoids and associated technologies.

Keywords: Cell transplantation, Diabetes mellitus, Insulin-secreting cells, Pancreatic islets, Regenerative medicine

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Introduction

Type 1 and type 2 diabetes are complex, heterogeneous, and heritable metabolic conditions commonly characterized by hyperglycemia and disrupted glucose homeostasis. The inability to produce insulin and self-regulate blood levels of glucose causes type 1 diabetes (T1D), while type 2 diabetes (T2D) is due to a decrease in insulin sensitivity and function. When the immune system attacks the beta cells, the endogenous insulin-producing pancreatic islets are destroyed, resulting in T1D (1). T2D is the common form of diabetes that develops when organs such as muscle, liver, and adipose tissue become insulin resistant, leading to insulin hypersecretion that ultimately causes beta cell exhaustion and failure. T1D and long-standing T2D patients require exogenous insulin injections with continuous glucose monitoring. Despite different pharmaceutical therapies for T2D that reduce glucose levels, achieving normoglycemia is not always possible and is often associated with severe complications (2). Uncontrolled T1D can also result in additional complications, including an increased risk of heart attack, stroke, neuropathy, retinopathy, renal failure, and liver disease, impacting the quality of life. While insulin therapy achieves a degree of glycemic control and remains the main medication for treating T1D, to cure diabetes effectively, blood glucose control by beta cells must be fully recapitulated, and insulin production and glucose-dependent insulin secretion (GSIS) must be correctly controlled. In this regard, cell-replacement therapies have emerged as a promising approach with the potential to restore normoglycemia and beta cell function, reverse diabetes, achieve long-term glycemic control, and prevent complications. Over the past decades, cell-based therapy has been proposed as a potential therapy to replace insulin treatments (1, 2). To increase the accessibility of this therapy, a readily available supply of insulin-secreting cells or islets must be generated, and methods for preventing graft rejection must be improved. In recent years, efficient differentiation protocols have been developed to produce stem cell-derived insulin-secreting cells. Ongoing research on beta cell production from human pluripotent stem cells (hPSCs) promises that this field can be a transformative treatment for diabetes soon (3, 4). This review summarizes cell-based beta cell replacement for diabetes, pancreatic organoid technologies, and future perspectives.

Whole pancreas and islet transplantation

Whole pancreas transplantation has been performed since 1966 in T1D patients, effectively managing blood glucose and insulin independence in 76% of transplanted patients five years after transplantation (5). However, the mortality rate after five years is higher than 10% (6). As this procedure is frequently associated with serious complications of surgery, pancreatic islet transplantation has been developed to provide a less invasive procedure with low morbidity and mortality (7). A shift from pancreatic tissue extract transplantation to transplantation of purified islets was achieved in the 1960s after improvements in islet yield and quality by modifications in pancreatic tissue digestion, density gradient separation, and intraductal

injection (8). Although the first human islet transplantation was reported in 1977 by Najarian et al. (9), the islet auto-transplantation was conducted in 1980 for ten patients, with some of these patients successfully avoiding pancreatogenic diabetes after surgery (10). The first successful results of intrahepatic allogeneic islet transplantation in T1D patients were reported in the 1990s (11, 12). Up to the year 2000, several clinical trials were performed to achieve variable rates of success; however, only around 11% of patients achieved insulin independence beyond 1-year post-transplant (8). The first group to show a 100% insulin independence rate during the first year after allogeneic pancreatic islet transplantation was reported in 2000 by Shapiro et al (13), known as the Edmonton protocol. Afterward, although the Edmonton protocol was conducted in several islet centers around the world, substantial variability in success between centers was reported (14). In the next decade, several modifications of the original Edmonton protocol have improved the rates of insulin independence (15, 16). Recent data on islet transplantation in T1D patients show that insulin independence is gained in approximately 54% of the patients after the first-year follow-up. In contrast, at the end of the second year, 20% of the patients remain insulin-free. Eventually, they return to use some form of exogenous insulin within a few years after the transplantation (17). The limited availability of allogenic islets has tended to use scalable sources of insulin-producing cells (IPCs) such as stem cell-derived β cells (18). Considering the enormous potential of human pluripotent stem cells (hPSCs) in regenerative medicine, many studies have been conducted for the generation of scalable IPCs or islet organoids from these cells (19, 20).

Human Mesenchymal Stem Cells (MSCs)

Clinical studies have explored the safety and efficacy of mesenchymal stem cells (MSCs) transplantation in diabetic patients. These studies have used various sources of adult MSCs to understand the mechanisms of β cell protection provided by MSCs and to evaluate their efficacy in modulating the immune response (19). It has been suggested that MSCs transplantation may represent a safe and effective treatment for T1D patients (21, 22). However, the interpretation of early MSC-based clinical trials for T1D has been challenged by the small sample sizes and short follow-up periods that do not allow for proper statistical analysis (19, 20). MSC-based clinical trials for T2D have observed a significant increase in fasting C-peptide levels, improvement in HbA1c values, and reductions in required insulin dosage (19). Studies have focused on transplanting MSCs or MSC-derived insulin-producing cells to overcome the underlying T cell-mediated autoimmune dysfunction, restore immune homeostasis, preserve residual β cells, and replace damaged cells. However, clear evidence supporting these hypothetical mechanisms is currently lacking (21, 22). Two clinical trials have reported positive outcomes of in vitro generated MSC-derived pancreatic progenitors' transplantation; however, these studies lack clear evidence for the presence of mature functioning MSC-derived β cells. Therefore, it can be concluded that the use of MSCs can be supportive of islet cell survival in T2D patients, but the efficacy of the therapeutic use of MSCs in T1D clinical trials is

controversial. The ability of MSCs to differentiate into functional β cells in vitro is poor, and trans-differentiation in vivo does not seem to occur (19).

Human pluripotent stem cell-derived pancreatic progenitor or β-like cells

The advantages of unlimited self-renewability, accessibility, and the extreme potential to generate an alternative source of insulin-secreting cells in vitro make hPSC an important source to overcome major challenges of clinical islet transplantation (23, 24). Several publications have demonstrated the potential of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) to form functional mature insulin-producing β cells (19). However, so far, only a few clinical trials have used the administration of differentiated hESCs in T1D patients to evaluate the safety and efficacy of this new therapeutic approach (20, 25). The first-in-human phase I/II clinical trial (NCT02239354) was initiated in 2014 by ViaCyte Inc. with hESCs derived-pancreatic progenitor cells (PEC-01) within a closed encapsulation device called PEC-Encap (VC-01) in one T1D patient (26). However, the trial was suspended due to a foreign body reaction to the device component and its coverage with fibrous tissue (27, 28). Subsequently, ViaCyte initiated two further clinical trials (NCT03162926 and NCT03163511) implanting the same type of PEC-01 cells into diabetic patients in a modified encapsulation device. The outcomes showed that PEC-01 cells were able to survive and produce measurable C-peptide levels; however, the intended islet mass transplanted was insufficient to normalize HbA1c levels (29, 30). Vertex Pharmaceutical and Sigilon Therapeutics companies use hPSC-derived, fully differentiated β cells (SC-β cells), in their phase 1/2 clinical trials (19). Vertex transplanted the VX-880 cells intraportally without any encapsulation system (NCT04786262) (18). SC-β cells transplanted in patients with hypoglycemia unawareness syndrome were not fully mature β cells but were able to achieve glucose-responsive insulin secretion and improve HbA1c in two T1D patients (18, 23). CRISPR Therapeutics and ViaCyte have recently launched the first clinical trial based on gene-edited immune-evasive hPSCs-derived pancreatic progenitors (19). Patient-specific iPSCs potentially offer a promising alternative for autologous cell transplantation. A clinical trial conducted in 2017 in Japan showed the safety and feasibility of autologous iPSCs-derived pancreatic endoderm transplantation in a T1DM patient (24).

Organoids: a future source for islet transplantation

Recent advances in three-dimensional (3D) culture methods have led to the development of organoid culture systems, which allow researchers to culture cells in vitro for extended periods. Organoids are 3D cellular structures created through the self-organization of tissue-specific cells that can mimic the structure and function of the original organ (31). Due to their ability to better simulate the structure of islets, islet organoids provide better conditions than two-dimensional pancreatic beta cell differentiation protocols. However, islet organoids do not fully restore glucose-stimulated insulin secretion (32). The use of

disease-specific pancreatic organoids facilitates an understanding of the molecular mechanisms of diseases (disease modeling), the identification of potential biomarkers, drug screening, drug production, and the development of personalized medicine for drug testing or toxicology studies (33). The success of organoid generation from adult cells was due to the discovery of Lgr5-positive stem cells in mice intestinal crypts, which led to the in-vitro production of intestinal organoids (34). Since then, organoid culture methods have been developed for the stomach, liver, pancreas, brain, lungs, and other organs and tissues (35). This development led to the hypothesis that the culture of adult stem cells from the human pancreas could be suitable for pancreatic regeneration studies and beta cell replacement therapy. Human pancreatic ducts can proliferate and expand in vitro, forming hollow 3D structures when cultured in collagen or Matrigel, making them suitable for regenerative medicine studies (36). Several groups have succeeded in generating pancreatic organoids from adult human pancreas. These organoids containing hollow spheres cannot form complex apical-truncal structures or any spontaneous differentiation towards endocrine cells (37, 38). In 2015, hESC-derived PDX1 and NKX6.1 positive pancreatic progenitor cells were cultured in a 3D Matrigel culture system. The resulting spheroids were able to be passaged and maintained for 12 days in 3D culture and continued to express progenitor cell markers such as SOX9, PDX1, and NKX6.1 (39). The production of islet organoids using targeted differentiation of pluripotent cells in 3D culture conditions has been associated with many successes. The islet-like organoids that were produced from hESCs (40) or the spontaneous accumulation of endocrine cells (41) could secrete insulin in response to glucose in vitro and in vivo. Researchers are seeking to produce complex organoids from the residues of the human pancreas islet isolation procedure. The first reported attempt for GMP-compliant, large-scale pancreatic organoids manufacturing from adult human cells comes from Dossena et al., who were able to expand islet-depleted pancreatic digest from a single cadaver donor to form up to 250×10^6 pancreatic organoids, reaching a total number of approximately 250×10^9 cells (42). However, more research is necessary to develop a truly efficient and safe approach. Currently, the clinical application of adult pancreatic organoids as a cell source for diabetes cell-based therapies is still far from realization due to the complicated access to this potential cell source, doubtful existence of bipotential progenitors in the adult pancreas, inaccessibility of the proper pancreatic niche, inherent variability among different donors, safety concerns, and technical difficulties associated with up-scaling organoid culture (25).

Strategies for the generation of pancreatic organoids

There are still gaps regarding the actual effect of pancreatic epithelial and non-epithelial cells in human pancreas development to produce pancreatic organoids that mimic the structure of the islets of Langerhans. Simulating cell interactions during pancreas development provides a suitable model. Analysis of signaling pathways has shown the importance of tissue-specific stromal cell influence.

Therefore, by incorporating these cells, the niche of pancreas development is better mimicked (45-43). Co-cultivating ESC-derived pancreatic progenitors with pancreas-derived endothelial or mesenchymal cells increases the possibility of their proliferation and self-renewal. This strategy may also promote their in vivo differentiation into insulin-secreting cells after transplantation (46-48). Studies have emphasized the importance of mimicking pancreas development and recapitulating pancreatic cell-cell interactions to generate more mature pancreatic organoids through pancreatic non-epithelial cell co-culture (49, 50). Despite progress in producing pancreatic organoids, there is still room for improvement, and applying new methods can enhance this knowledge. During development, cells can be influenced by various environmental factors, including soluble factors (such as small molecules, cytokines, and growth factors), properties of the extracellular matrix, intercellular interactions, and mechanical forces. This highlights the necessity of using three-dimensional co-culture systems for the formation of multicellular structures (51). Various bioengineering approaches, such as extracellular matrix design, engineered vascularization, genetic manipulation of signaling molecules at the cell surface, bioprinting, pancreas-on-a-chip, and microfluidic technologies can engineer cellular interactions to generate more potent pancreatic organoids or produce mature and functional pseudo islets (52-54).

Conclusion

There are several ongoing studies and clinical trials focused on stem cell–derived beta or progenitor cells for the treatment of diabetes mellitus. Alongside significant advancements in stem cell biology and islet transplantation, other advanced therapeutic approaches such as gene manipulation, immunotherapy, and cell encapsulation have also garnered attention in the realm of diabetes treatment. Currently, beta cell-derived stem cells do not fully replicate their endogenous counterparts. However, with improvements in beta cell engraftment and progenitor cell differentiation protocols, the number of cells required for transplantation can be reduced. Stem cell-derived islets offer a potentially limitless resource to increase accessibility to islet transplantation. Advancements in single-cell technologies for better characterization of the produced cells have shed light on differentiation processes, aiding in the development of more efficient protocols that result in increased insulin secretion in the final product while minimizing the presence of off-target cell types. Progress in the generation of immune-evading stem cell–derived beta or progenitor cells may enhance the efficacy and safety of cell transplantation and reduce the need for immunosuppressive drugs. In the near future, immune-protected stem cell-derived beta or progenitor cells may be achievable through genetic engineering approaches that enable cells to evade the immune system and resist rejection post-transplantation. Additionally, candidate biomaterials could induce local immune tolerance post-transplantation, improving graft viability and reducing the required number of cells for transplantation. It is important to recognize that a combination of these therapeutic approaches may be necessary for optimal outcomes, as various aspects of transplantation need to be

considered. Nevertheless, the rapid progress in scientific and technological aspects of cell-based therapies, makes this field a promising area of research for the treatment of diabetes mellitus.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

References

1. Singh A, Afshan N, Singh A, Singh SK, Yadav S, Kumar M, et al. Recent trends and advances in type 1 diabetes therapeutics: A comprehensive review. 2023:151329.

2. Ashe S, Hebrok M, editors. Role of Cell-Based Therapies in T2D. Seminars in Nephrology; 2023: Elsevier.

3. Cochrane VA, Hebrok MJNRE. Stem cell-derived islet therapy: is this the end of the beginning? 2023;19(12):681-2.

4.Hogrebe NJ, Ishahak M, Millman JRJCSC. Developments in stem cell-derived islet replacement therapy for treating type 1 diabetes. 2023;30(5):530-48.

5. Sharples E, Mittal S, Friend PJAd. Challenges in pancreas transplantation. 2016;53:871-8.

6. Takaki T, Shimoda MJGH, Medicine. Pancreatic islet transplantation: toward definitive treatment for diabetes mellitus. 2020;2(4):200-11.

7. Perez-Basterrechea M, Esteban MM, Vega JA, Obaya AJJB, bioengineering. Tissue-engineering approaches in pancreatic islet transplantation. 2018;115(12):3009-29.

8. Marfil-Garza BA, Shapiro AMJ, Kin TJJoHBPS. Clinical islet transplantation: current progress and new frontiers. 2021;28(3):243-54.

9. Najarian JJTP. Human islets transplantation. A preliminary report. 1977;9:233-6.

10. Najarian JS, Sutherland D, Baumgartner D, Burke B, Rynasiewicz JJ, Matas AJ, Goetz FCJAos. Total or near total pancreatectomy and islet autotransplantation for treatment of chronic pancreatitis. 1980;192(4):526.

11. Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Falqui L, et al. Insulin independence after islet transplantation into type I diabetic patient. 1990;39(4):515-8.

12. Tzakis AG, Zeng Y, Fung J, Todo S, Demetris A, Starzl T, et al. Pancreatic islet transplantation after upper abdominal exeriteration and liver replacement. 1990;336(8712):402-5.

13. Shapiro AJ, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. 2000;343(4):230-8.

14. Matsumoto S, Shimoda MJJoD. Current situation of clinical islet transplantation from allogeneic toward xenogeneic. 2020;12(10):733-41.

15. Bellin MD, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm S-H, et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. 2008;8(11):2463-70.

16. Vantyghem M-C, Kerr-Conte J, Arnalsteen L, Sergent G, Defrance F, Gmyr V, et al. Primary graft function, metabolic control, and graft survival after islet transplantation. 2009;32(8):1473-8.

17. Kabakchieva P, Assyov Y, Gerasoudis S, Vasilev G, Peshevska-Sekulovska M, Sekulovski M, et al. Islet transplantation-immunological challenges and current perspectives. 2023;13(4):107-21.

18. Berney T, Wassmer CH, Lebreton F, Bellofatto K, Fonseca LM, Bignard J, et al. From islet of Langerhans transplantation to the bioartificial pancreas. 2022;51(4):104139.

19. De Klerk E, Hebrok MJFie. Stem cell-based clinical trials for diabetes mellitus. 2021;12:631463.

20. Chen S, Du K, Zou CJSCR, Therapy. Current progress in stem cell therapy for type 1 diabetes mellitus. 2020;11(1):1-13.

21. Pavathuparambil Abdul Manaph N, Sivanathan KN, Nitschke J, Zhou X-F, Coates PT, Drogemuller CJJScr, therapy. An overview on small molecule-induced differentiation of mesenchymal stem cells into beta cells for diabetic therapy. 2019;10(1):1-18.

22. Stiner R, Alexander M, Liu G, Liao W, Liu Y, Yu J, et al. Transplantation of stem cells from umbilical cord blood as therapy for type I diabetes. 2019;378:155-62.

23. Jiang H, Jiang F-XJWJoSC. Human pluripotent stem cell-derived β cells: Truly immature islet β cells for type 1 diabetes therapy? 2023;15(4):182.

24. Dadheech N, James Shapiro AJCB, Translational Medicine VSCTStT. Human induced pluripotent stem cells in the curative treatment of diabetes and potential impediments ahead. 2019:25-35.

25. Bittenglova K, Habart D, Saudek F, Koblas TJI. The Potential of Pancreatic Organoids for Diabetes Research and Therapy. 2021;13(5-6):85-105.

26. Henry RR, Pettus J, Wilensky J, SHAPIRO AJ, Senior PA, Roep B, et al. Initial clinical evaluation of VC-01TM combination product—a stem cell–derived islet replacement for type 1 diabetes (T1D). 2018;67(Supplement_1).

27. Butler PC, Gale EA. Reversing type 1 diabetes with stem cell-derived islets: a step closer to the dream? J Clin Invest. 2022;132(3).

28. Henry RR, Pettus J, Wilensky J, SHAPIRO AJ, Senior PA, Roep B, et al. Initial clinical evaluation of VC-01TM combination product—a stem cell–derived islet replacement for type 1 diabetes (T1D). Diabetes. 2018;67(Supplement_1).

29. Shapiro AJ, Thompson D, Donner TW, Bellin MD, Hsueh W, Pettus J, et al. Insulin expression and C-peptide in type 1 diabetes subjects implanted with stem cell-derived pancreatic endoderm cells in an encapsulation device. 2021;2(12).

30. Shapiro A, Thompson D, Donner TW, Bellin MD, Hsueh W, Pettus JH, et al. Insulin expression and glucose-responsive circulating C-peptide in type 1 diabetes patients implanted subcutaneously with pluripotent stem cell-derived pancreatic endoderm cells in a macro-device. 2019;1.

31. Balak JRA, Juksar J, Carlotti F, Lo Nigro A, de Koning EJP. Organoids from the Human Fetal and Adult Pancreas. Current diabetes reports. 2019;19(12):160.

32. Tsakmaki A, Fonseca Pedro P, Bewick GA. Diabetes through a 3D lens: organoid models. Diabetologia. 2020;63(6):1093-102.

33. Funata M, Nio Y, Erion DM, Thompson WL, Takebe T. The promise of human organoids in the digestive system. Cell death and differentiation. 2021;28(1):84-94.

34. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459(7244):262-5.

35. Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. Nature cell biology. 2016;18(3):246-54.

36. Kerr-Conte J, Pattou F, Lecomte-Houcke M, Xia Y, Boilly B, Proye C, Lefebvre J. Ductal cyst formation in collagen-embedded adult human islet preparations. A means to the reproduction of nesidioblastosis in vitro. Diabetes. 1996;45(8):1108-14.

37. Lee J, Sugiyama T, Liu Y, Wang J, Gu X, Lei J, et al. Expansion and conversion of human pancreatic ductal cells into insulin-secreting endocrine cells. eLife. 2013;2:e00940.

38. Boj SF, Hwang CI, Baker LA, Chio, II, Engle DD, Corbo V, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell. 2015;160(1-2):324-38.

39. Huang L, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. Nature medicine. 2015;21(11):1364-71.

40. Shim JH, Kim J, Han J, An SY, Jang YJ, Son J, et al. Pancreatic Islet-Like Three-Dimensional Aggregates Derived From Human Embryonic Stem Cells Ameliorate Hyperglycemia in Streptozotocin-Induced Diabetic Mice. Cell transplantation. 2015;24(10):2155-68.

41. Kim Y, Kim H, Ko UH, Oh Y, Lim A, Sohn JW, et al. Islet-like organoids derived from human pluripotent stem cells efficiently function in the glucose responsiveness in vitro and in vivo. Scientific reports. 2016;6:35145.

42. Dossena M, Piras R, Cherubini A, Barilani M, Dugnani E, Salanitro F, et al. Standardized GMP-compliant scalable production of human pancreas organoids. 2020;11(1):1-12.

43. Banerjee I, Sharma N, Yarmush M. Impact of co-culture on pancreatic differentiation of embryonic stem cells. Journal of tissue engineering and regenerative medicine. 2011;5(4):313-23.

44. Jaramillo M, Mathew S, Mamiya H, Goh SK, Banerjee I. Endothelial cells mediate islet-specific maturation of human embryonic stem cell-derived pancreatic progenitor cells. Tissue Engineering Part A. 2015;21(1-2):14-25.

45. Jaramillo M, Banerjee I. Endothelial cell co-culture mediates maturation of human embryonic stem cell to pancreatic insulin producing cells in a directed differentiation approach. JoVE (Journal of Visualized Experiments). 2012(61):e3759.

46. Sneddon JB, Borowiak M, Melton DA. Self-renewal of embryonic-stem-cell-derived progenitors by organ-matched mesenchyme. Nature. 2012;491(7426):765-8.

47. Oh BJ, Oh SH, Choi JM, Jin SM, Shim WY, Lee MS, et al. Co-culture with mature islet cells augments the differentiation of insulin-producing cells from pluripotent stem cells. Stem cell reviews and reports. 2015;11(1):62-74.

48. Yilmaz I, Sariboyaci AE, Subasi C, Karaoz E. Differentiation Potential of Mouse Embryonic Stem Cells into Insulin Producing Cells in Pancreatic Islet Microenvironment. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association. 2016;124(2):120-9.

49. Soltanian A, Ghezelayagh Z, Mazidi Z, Halvaei M, Mardpour S, Ashtiani MK, et al. Generation of functional human pancreatic organoids by transplants of embryonic stem cell derivatives in a 3D-printed tissue trapper. Journal of cellular physiology. 2019;234(6):9564-76.

50. Ghezelayagh Z, Zabihi M, Zarkesh I, Gonçalves CAC, Larsen M, Hagh-Parast N, et al. Improved Differentiation of hESC-Derived Pancreatic Progenitors by Using Human Fetal Pancreatic Mesenchymal Cells in a Micro-scalable Three-Dimensional Co-culture System. Stem cell reviews and reports. 2022;18(1):360-77.

51. Murrow LM, Weber RJ, Gartner ZJ. Dissecting the stem cell niche with organoid models: an engineering-based approach. Development (Cambridge, England). 2017;144(6):998-1007.

52. Hakobyan D, Médina C, Dusserre N, Stachowicz ML, Handschin C, Fricain JC, et al. Laser-assisted 3D bioprinting of exocrine pancreas spheroid models for cancer initiation study. Biofabrication. 2020;12(3):035001.

53. Lin M, Gao M, Pandalai PK, Cavnar MJ, Kim J. An Organotypic Microcosm for the Pancreatic Tumor Microenvironment. Cancers. 2020;12(4).

54. Lai Benjamin FL, Lu Rick X, Hu Y, Davenport HL, Dou W, Wang EY, et al. Recapitulating pancreatic tumor microenvironment through synergistic use of patient organoids and organ-on-a-chip vasculature. Advanced functional materials. 2020;30(48).