博 士 論 文 の 内 容 の 要 旨

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学位名	博士（学術）
学位授与年月日	2021 年　3 月　20 日
論文題目	Cryopreservation and transplantation of rat pancreatic islets for diabetes therapy model **(糖尿病治療モデル確立に向けたラット膵ランゲルハンス島の超低温保存と移植に関する研究)**

(博士論文の内容の要旨)

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, which can cause serious long-term complications such as retinopathy, nephropathy, and peripheral neuropathy, if left untreated. As of 2019, an estimate of 463 million patients had diabetes worldwide, resulting in approximately 4.2 million deaths. Transplantation of isolated pancreatic islets is a promising treatment alternative to frequent insulin injections. It can restore normoglycemia in patients with type-1 diabetes and, at least in part, type-2 diabetes. However, a huge number of islets is required for clinical transplantation, because the considerable loss of transplanted islets occurs due to the instant blood-mediated inflammatory reaction, hypoxia and low nutrient availability until vascularization into engrafts. Cryopreservation of pancreatic islets without functional loss can overcome the inevitable shortage of donors and allow time to investigate immunological tissue matching between donors and recipients. Therefore, this study was designed to establish a novel cryopreservation protocol reliable for a higher yield of functional pancreatic islets in the rat diabetes therapy model (Chapter-I). Two cryopreservation protocols (Bicell® freezing versus Cryotop® vitrification) were compared for their potential to recover the functional rat pancreatic islets. Immediate cryosurvival rates of post-thaw and post-warm Brown-Norway (BN) rat islets were comparable. Most of the dead cells were detected in the peripheral area of post-warm islets, and were removed after subsequent 24 h culture. On the other hand, the dead cells in post-thaw islets distributed not only in their peripheral area but also in the center. Quantitative PCR analysis showed that Bicell® freezing compromised expression of genes relating to β-cell function, but not to one of the apoptotic pathways. Expression of these genes was maintained in islets before and after the Cryotop® vitrification. Stimulation index (SI) values in glucose-stimulated insulin secretion (GSIS) assay, as a parameter of insulin secretion potential, were 6.7, 1.9 and 3.9 in fresh control, post-thaw and post-warm islets, respectively. Larger islets had comparable survival rates, but lower SI values after Cryotop® vitrification than smaller counterparts (Chapter-II). Different cryodevices, such as Cryotop®, open-pulled straws, and hollow fiber, have been employed for the vitrification of isolated rodent islets. However, these vitrification protocols have a technical limit as large quantities of islets cannot be loaded onto or into each cryodevice. The limitation depends on necessary treatments, such as equilibration or dilution of cryoprotectants, and exposure or minimization of vitrification solution, which are difficult to achieve within the required timeframe. The nylon mesh sheet was processed as a developed figure of a triangular pyramid for islet vitrification. When a unit of 10 islets was applied to each cryodevice, both the immediate cryosurvival rate of post-warm islets and the SI values in the GSIS assay were comparable between the nylon mesh and the Cryotop® vitrification groups. Increased numbers of islets per nylon mesh cryodevice (up to 100) had no adverse effects on the cryosurvival and the insulin secretion potential (Chapter-III). While the islet insulin secretion potential measured in vitro by the GSIS assay is closely related to the in vivo response for glycemic control of diabetic rodents, the adaptability of

vitrified-warmed islets to cure hyperglycemia of diabetes model rats needs to be confirmed strictly by islet transplantation. Diabetic BN rats were prepared by a single intravenous injection of streptozotocin. One hundred islets were loaded on a nylon mesh cryodevice, and then vitrified-warmed. Each diabetic recipient rat received 800 fresh control or vitrified-warmed islets beneath the kidney capsule could achieve normoglycemia within 3 weeks after the subrenal transplantation, at similar success rates (87.5 and 83.3% in fresh control and nylon mesh vitrification groups, respectively). Intraperitoneal glucose tolerance test showed similar 2-h responses to the glucose uptake of cured rats between vitrification and fresh control groups. Day-70 nephrectomy confirmed the successful engraftment of transplants through the subsequent diabetes reversal and H&E staining (Chapter-IV). In conclusion, the nylon mesh vitrification seems to be the best promising through both the in vitro and in vivo assays when compared to the other cryopreservation protocols so far reported. The simple and easy operation to perform the nylon mesh vitrification enables us to handle the large quantities of isolated rat islets. The progress of the rodent model system would help promote the development of future innovative technology for clinical diabetes therapy (Chapter-V).