SRP Diabetes Postdoctoral Fellowship Program 2025

Project title: *In vivo evaluation* of anti-diabetic drugs using sensor-engineered human islet organoids transplanted into the anterior chamber of the eye

Background

With the increasing number of patients suffering from diabetes with a high risk of developing serious secondary complications leading to reduced life expectancy, there is a worldwide interest in finding more effective treatments. However, a major challenge is how human pancreatic islet cells/β-cells can be studied in the living organism. Therefore, we have developed an *in vivo* imaging platform for microscopic analysis that allows a multi-parameter-assessment of islet cell/β-cell function and survival by employing the anterior chamber of the eye (ACE) as a transplantation site for islets and utilizing the cornea as a natural body window for imaging (1). Islets engrafted in the ACE become vascularized and innervated and multiple aspects of islet cell/β-cell morphology and function, and effects of different interventions, can be imaged non-invasively *in vivo* (2-5). The ACE-platform uniquely enables intravital study of human islets and islet organoids, which is not possible to do in living humans.

GLP-1 receptor agonists (GLP-1Ra) were first developed for the treatment of type-2 diabetes (T2D) and, subsequently, obesity (6). Although there is a lot of knowledge, the mechanisms through which GLP-1R signalling affects normal and diabetic human β-cells are incompletely understood. It probably involves crosstalk between membrane ion channels, intracellular glucose metabolism, but maybe also between the different endocrine islet cells. In order to optimize the use of already registered drugs, and in the development of new drugs, it is important to be able to study the direct effects *in vivo* on human islets under both normal and diabetic conditions.

Objectives

Aim 1: Develop and optimize sensor islet organoid *in vitro* for β-cell function readouts. Engineer sensor human islet organoids with fluorescent proteins to monitor important β-cell functions such as calcium handling and glucose responsiveness. Perform *in vitro* optimization of organoid culture and transduction protocols to ensure robust and reproducible sensor performance.

Aim 2: Refine and validate *in vivo* the sensor islet organoids. Evaluate the long-term sustainability, functionality and reproducibility of sensor islet organoids *in vivo* by transplanting them into the ACE of immunocompromised diabetic mouse models.

Aim 3: *In vivo* investigations of the cellular effects of GLP-1Ra. The effect of GLP-1Ra will be studied in human islet organoids transplanted into the ACE under different metabolic conditions.

Methodology

Related to aim 1: Human pancreatic islets from cadaveric donors, sourced via the Nordic Network for Islet Transplantation, will be used to engineer islet organoids expressing fluorescent proteins, like GCaMP for calcium dynamics or β-FLUOMETRI for glucose responsiveness. After dissociation and transduction with adenovirus encoding fluorescent biosensors, islet organoids will be reaggregated. Organoid size, density and morphology will be optimized for uniform expression and sensor performance. Transfection efficiency and real-time β-cell function and connectivity will be evaluated by *in vitro* confocal imaging.

Related to aim 2: Human islet sensor organoids will be transplanted into the ACE of immunocompromised $Rag1^{-/-}$ mice. One eye will receive a metabolic transplant of 400 human islets, while the contralateral eye will host 10 reporter sensor islet organoids for *in vivo* imaging. Mouse pancreatic β-cells will be destroyed with streptozotocin (STZ) 6 weeks post-transplantation, after which the transplanted human islets in the ACE will be the sole source of insulin. Longitudinal *in vivo* assessments of calcium handling and glucose responsiveness will be performed monthly for three

months at single-cell resolution. Metabolic status will be evaluated through body weight monitoring, random glucose measurements and intraperitoneal glucose tolerance tests (IPGTTs).
Related to aim 3: To evaluate human β-cell function *in vivo*, we will transplant human sensor islet organoids into the ACE of immunocompromised $Rag1^{-/-}$ mice. Simultaneously, 400 human islets will be transplanted into the contralateral eye of the same animals. Six weeks after transplantation, the endogenous pancreatic β-cells of the mice will be destroyed with STZ, and the mice will be dependent on the insulin from the human islets. The mice will then be divided into four experimental groups: 1) mice on a normal chow diet treated with placebo, 2) mice on normal chow diet treated with GLP-1Ra, 3) mice on a high-fat, high-sucrose diet (HFHSD) treated with placebo, and 4) HFHSD-fed mice treated with GLP-1Ra. We will monitor *in vivo*, longitudinally and at single cell resolution the functionality of the human islet organoids using confocal imaging. Monthly evaluations of biosensor responses, including calcium dynamics and glucose responsiveness, will be conducted over a three-month period. Metabolic status will be assessed by monitoring body weight, performing random glucose measurements, and conducting IPGTTs.

Work plan (a clear description of the work which the postdoc fellow will perform)
-Aim 1 (4 months): Engineer sensor human islet organoids with fluorescent proteins, optimize culture and transduction protocols, and assess β-cell function via confocal imaging.
-Aim 2 (8 months): Transplant sensor organoids into the ACE of diabetic mice, evaluate long-term functionality, and perform longitudinal *in vivo* imaging of β-cell function.
-Aim 3 (12 months): Transplant organoids into the ACE, test GLP-1Ra under different metabolic conditions, and monitor islet and β-cell function and glucose responsiveness through longitudinal confocal imaging and metabolic assessments.

Relevance of project for diabetes
It is well known that there is a difference between human and rodent islets and a major problem has been how to be able to *in vivo* study human islets. In this project human pancreatic islets will be used to engineer islet organoids expressing fluorescent biosensors. By transplanting these organoids into the ACE, we will be able to dynamically, longitudinally, with a high-resolution, monitor human islets and β-cell function in real time. This innovative approach will make it possible to evaluate *in vivo,* in human islets, the efficacy of new drugs, but also to investigate unknown mechanisms and effects of already used medications.

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