Over 400 million people worldwide are affected by **diabetes** and over 1.5 million people die yearly due to diabetes-induced complications. The direct cause of diabetes is loss or dysfunction of pancreatic **β-cells**, the only cells in the body that can produce insulin, a hormone responsible for controlling blood sugar level. Currently used therapies require regular insulin injections, which unfortunately are not perfect in mimicking natural body response in sugar level stabilization. For that reason, huge efforts are put into developing a cell replacement therapy, where β-cells derived from **human pluripotent stem cells (hPSCs)** in the laboratory could be transplanted into patients. Still, so far β-cell generation methods do not deliver β-cells in numbers and quality needed for large-scale application in therapy.

Directed differentiation of hPSCs into pancreatic β-cells aims to mimic natural developmental pathways by culturing hPSCs *in vitro* with certain factors precisely regulating this process. *In vivo*, those factors are provided by the **microenvironment** that surrounds pancreatic cells. Yet, there is an incomplete understanding of how exactly microenvironment regulates β-cells development and function. First, the microenvironment is complex and dynamically changes over time; its components, their origin and function remain unclear. Next, the possibilities to study the pancreatic microenvironment in humans are limited to research with hPSCs, yet their use is focused on production of β-cells for therapy and thus lack the microenvironment component.

In this project, we hypothesize that: 1) Mesenchyme, the main cellular component of the pancreatic microenvironment, has multiple subtypes within the pancreas, and is organ-specific. We suggest that the **mesenchyme subtypes** have different roles during pancreas development. **2)** Addition of microenvironment components to pancreatic cells during hPSC differentiation would restore their mutual interactions in a way which better reflects *in vivo* development.

To confirm these hypotheses, we will first identify **mesenchyme subtypes** in mouse embryonic pancreas using single-cell RNA sequencing (scRNA-Seq) that allows a simultaneous insight into gene expression profiles of thousands of single cells. Based on this, we will look at the differences between mesenchyme subtypes to infer how they communicate with other cells and what their function is. Moreover, we will create a "**roadmap**" of how pancreatic mesenchyme forms. Next, using advanced light-sheet fluorescence microscopy we will create a **3D map of spatial relationships** between those cell types, to confirm putative interactions between cell types. We will then explore experimentally the identified mesenchyme subtype ancestry using sophisticated transgenic mice. Finally, we will use the gathered data to identify an ancestry pancreatic mesenchyme subtype that would support generation of hPSC-derived β-cells in the laboratory. We will then develop a method to recreate this specific mesenchymal subtype by hPSC differentiation. Further, we will join hPSC-derived pancreatic progenitors and hPSC-derived mesenchyme to create a co-culture miniorgan system closely reflecting *in vivo* developmental processes driving generation of functional β-cells. We anticipate that in such a system, pancreatic cells will spatially organize into structures resembling natural development. As a proof-of-concept of our miniorgan system, we will focus on the first steps of β-cell development, called peninsula formation, which has not been thoroughly studied. Using advanced microscopy techniques and scRNA-Seq, we will study in detail how peninsulas are formed.

Our results will facilitate technological improvements to create β-cells for therapy. Our miniorgan system will be the first all-hPSC pancreatic system that includes organ-specific microenvironment cells. Importantly, the miniorgan system would be invaluable for **drug testing and disease modeling**, as it would recapitulate human endocrine pancreas more faithfully than existing models.