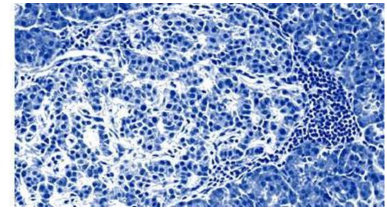
nPOD

Network for Pancreatic Organ Donors with Diabetes



# Human pancreas tissue slices for the study of alpha cell physiology in type 1 diabetes pathogenesis

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## PURPOSE

Dysregulated glucagon release contributes to type 1 diabetes (T1D) pathogenesis; however the underlying mechanisms of alpha cell dysfunction, increased glucagon secretion or reduced suppression of glucagon release in response to hypoglycemia are still uncertain. Human pancreatic tissue slices offer a valuable platform to study intact islet cell biology in situ, at the same time overcoming enzymatic and mechanical distress involved in the conventional islet isolation procedures. Here we characterize alpha cell function, physiology and mass using human pancreatic tissue slices prepared at the nPOD/OPPC at the University of Florida in Gainesville.

## METHODS

Pancreata from non-diabetic (ND), autoantibody-positive (Aab+) and T1D organ donors were processed at the University of Florida (UF) in Gainesville in line with nPOD/OPPC SOPs. Tissue slices of 120 µm thickness were prepared and viability was evaluated by immunofluorescence staining. Glucagon secretion was assessed acutely after slice preparation and measured with a commercially available ELISA kit. In addition, slices were rested overnight and calcium responses were recorded from single cells within islets (identified by backscatter) using an inverted Leica SP8i confocal microscope. After functional assessment of alpha cells at UF, slices were fixed and shipped to the Paul Langerhans Institute Dresden, Germany (PLID) in order to assess 3D slice morphometry. Fixed slices were stained for endocrine cell markers (insulin, glucagon and somatostatin) and whole slice imaging was performed on a Zeiss LSM 780 NLO equipped with an automated stage. Image processing and analysis was accomplished semi-automatically using Fiji and MorphoLibJ.

## SUMMARY OF RESULTS

Here we show dynamic glucagon and insulin secretion patterns using pancreas tissue slices from organ donors. Glucagon release was assessed in response to specific stimuli (kainate, epinephrine or arginine) and KCl for cell membrane depolarization. We characterized glucagon secretion kinetics in slices from ND, Aab+ and T1D pancreata. Our preliminary analysis shows that in the ‘prediabetic’ state, glucagon output in response to all applied stimuli is slightly decreased. However, stimulation index in the two groups is comparable, suggesting that alpha cells are functional in Aab+ state. Slices from T1D showed only small changes in glucagon secretion in comparison to ND. Tissue slices were then further investigated by 3D morphometry and assessed for calcium dynamics in alpha cells by live cell imaging. Analysis of the acquired data is still ongoing.

## CONCLUSIONS

This work provides proof that alpha cell physiology can be investigated using pancreas tissue slices prepared from organ donor pancreata. Here we show a first time characterization of alpha cell function, physiology and mass within the same tissue sample. These studies will provide insight into the mechanism underlying aberrant glucagon release in diabetes pathogenesis.