Functional coupling of human pancreatic islet microtissues and liver spheroids in a microphysiological system: Towards a novel human ex vivo model of Type 2 diabetes

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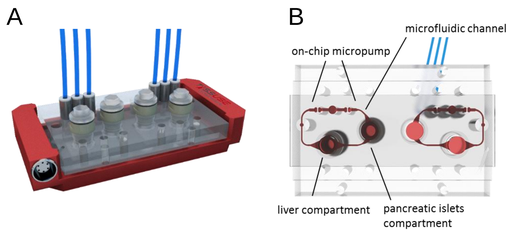
Introduction

Whole body glucose homeostasis is controlled through interaction between multiple organs. Particularly important is the feedback loop between liver and pancreatic islets that keeps blood glucose levels within a narrow range. This regulation is perturbed in type 2 diabetes. Here we present an organ-on-a-chip model to study the interplay between human pancreatic islet microtissues and human liver spheroids with the aim to not only study therapeutic intervention but also pathogenic factors in metabolic diseases like non-alcoholic fatty liver disease (NAFLD) and the metabolic syndrome.

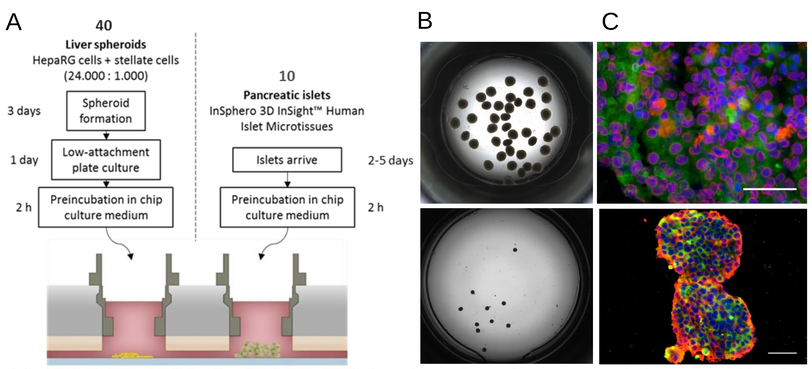
Experimental Set Up

The microfluidic two-organ-chip (2-OC) at a glance



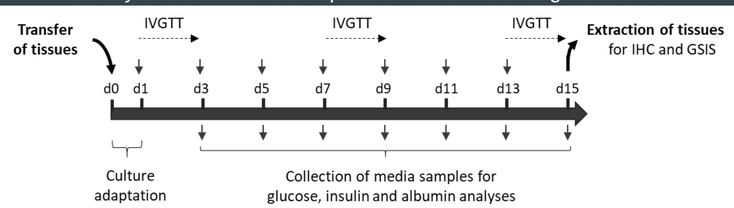
(A) A 3D view of the assembled 2-OC including air tube (blue) and temperature support (red). (B) Illustration of the view from underneath with media circuits, respective culture compartments and micropump valves highlighted in red.

Relevant organ equivalents for liver and pancreatic islets



(A) Standard tissue loading scheme of organ equivalents for 2-OC co-culture. Organ equivalents are combined in a physiological ratio of 1:100.000 compared to the human body. (B) 40 liver spheroids (top) and 10 pancreatic islets (bottom) in the respective 2-OC culture compartment. (C) Relevant protein expression of albumin (green) and CYP3A4 (red) in liver spheroids (top) and insulin (red) and glucagon (green) in pancreatic islets (bottom). Nuclei stained with DAPI (blue). Scale bar: 50 µm.

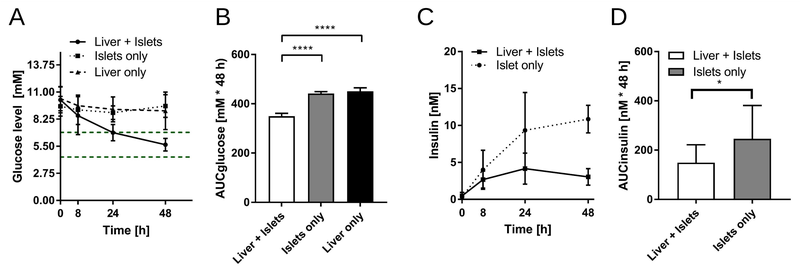
15-day 2-OC culture with repeated medium exchange and GTT



Medium is exchanged completely every 48 hours in both culture compartments (-300 µl, + 300 µl). An *in vitro* glucose tolerance test (IVGTT) can be performed during culture by additional sampling at 0, 8, 24 and 48 h for a glucose tolerance test.

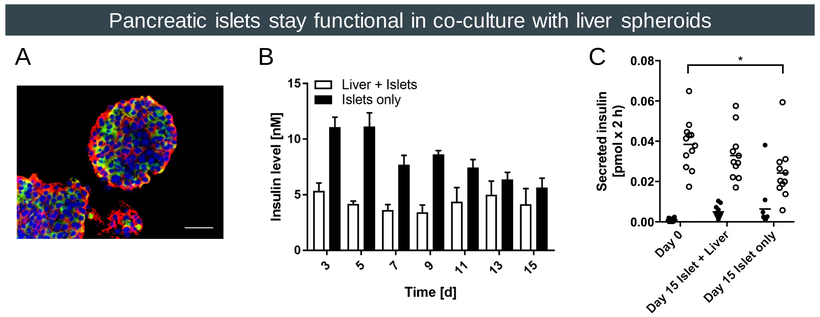
Results

Functional cross-talk between liver spheroids and pancreatic islet microtissues



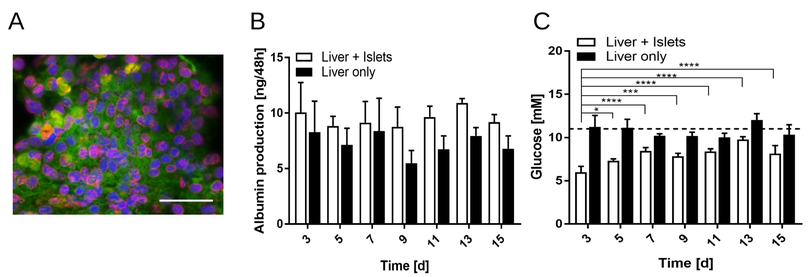
(A) Glucose utilisation profile in co- and single cultures on day 1. Dotted green line indicates physiological glucose range. (B) Area under the glucose utilization curve. (C) Insulin concentration profile on day 1. (D) Area under the insulin concentration profile. All data displayed as mean ± SD from n = 14 from three independent experiments with three donors of pancreatic islets run at two different laboratories. $p < 0.05$, ****$p < 0.0001$ using ANOVA with Tukey's multiple comparison post hoc test.

Pancreatic islets stay functional in co-culture with liver spheroids



(A) Protein expression of insulin (red) and glucagon (green) in co-cultured pancreatic islets on day 15. Scale bar: 50 µm. (B) Insulin accumulated over 48-h periods measured in the media of co-cultures and islets alone. Data shown as mean + SD, n = 5. (B) GSIS (Glucose-stimulated insulin secretion) of 11 single islet microtissues in low (2.8 mM, black dots) and high (16.8 mM, white dots) glucose. *$p < 0.05$ using ANOVA with Tukey's multiple comparison post hoc test.

Long-term functionality of liver spheroids



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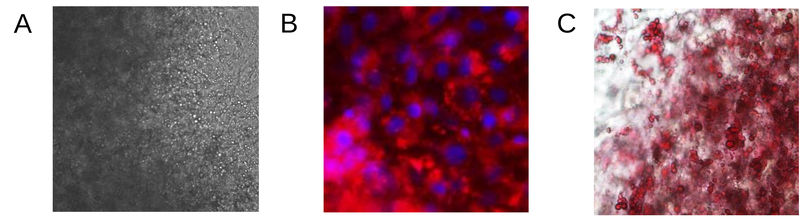
Lipid accumulation in liver spheroids might cause insulin resistance



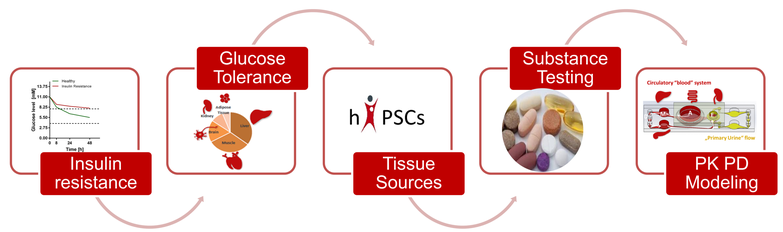
(A) Vesicles observed to grow in number and size during 2-OC cultivation. Vesicles are confirmed to be lipid vesicles by (B) Nile Red (red) and Dapi (blue) staining or (C) Oil Red O (red) staining. Scale bar to be added.

Conclusions

The results demonstrate that human pancreatic islets and human liver spheroids can be successfully cultivated in a common medium for up to 15 days. Islet-liver cross-talk can be studied by an *in vitro* glucose tolerance test showing insulin mediated glucose utilization and glucose level dependent insulin secretion. Islet function can be investigates by insulin release into the medium as well as by GSIS, which showed impaired islet function through prolonged hyperglycaemia in islet single cultures. Although liver functionality is stable over 15 days a reduced glucose utilisation over culture time indicates the development of insulin resistance. This might be explained by an excessive accumulation of lipids by the liver during culture, which was found before to cause insulin resistance.

Future Directions

The results encourage us now to develop the model further with the ultimate aim of developing an *in vitro* human type-2 diabetes model which is generated from patient-derived iPS cells. This will provide a tool that can be used for the study of pathomechanisms in the development of type 2 diabetes but also as a preclinical model for the identification of new drug targets and therapies[1].



Literature

1. Perry, R. J., Samuel, V. T., Petersen, K. F. & Shulman, G. I. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature* **510,** 84–91 (2014).

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