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# 3D spheroids of the pancreatic beta cell line EndoC-βH5 for modelling diabetes mellitus in a microphysiological system

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## ABSTRACT

Type 2 diabetes mellitus (T2DM) is a complex multi-organ disease characterized by impaired glucose homeostasis. In healthy individuals, pancreatic β-cells respond to increased blood glucose concentration by secreting insulin. To date, multi-organ diseases, such as T2DM are not adequately reflected in animal models or in standard in vitro models. To address this challenge, we have recently shown functional coupling of human HepaRG™ spheroids and human islet microtissues for studying the interplay between the pancreas and the liver, two key organs in blood glucose regulation.[1,2] Here we show spheroids made of EndoC-βH5® cells as an alternative pancreas organ model. The human pancreatic β-cell line EndoC-βH5® exhibits a near-native β-cell phenotype. The cells are available as ready-to-use frozen stocks, which have high batch-to-batch reproducibility and maintain their function for weeks.[3]

We first optimized a protocol for spheroid formation and observed stable morphology and stable glucose-stimulated insulin secretion (GSIS). The EndoC-βH5® spheroids showed dose-dependent insulin response to glucose and, moreover, stimulation with the GLP-1 receptor analog exenatide led to a significantly increased insulin secretion. We then demonstrated that the EndoC- βH5® spheroid model can be co-cultivated together with HepaRG™ spheroids in the HUMIMIC Chip2 for up to 15 days. Liver function, as shown by albumin secretion, was stable over time. EndoC- βH5® cell functionality was demonstrated by insulin release into the culture medium as well as by GSIS of spheroids extracted from the chips at the end of the culture period. Organ cross-talk was investigated by an in vitro glucose tolerance test measuring glucose, lactate and insulin concentrations in response to a glucose load.

## ENDOC-BH5® SPHEROID MODEL

Although only Costar and 384 well plates show a homogeneous cluster of cells after centrifugation on DAY -4, EndoC-βH5® cells aggregate and form compact spheroids in all well plate types after a 4-day culture (1A). All conditions were confirmed to secrete insulin in response to glucose. Slightly lower amount of insulin was secreted from spheroids produced in 384 well plates (1B).

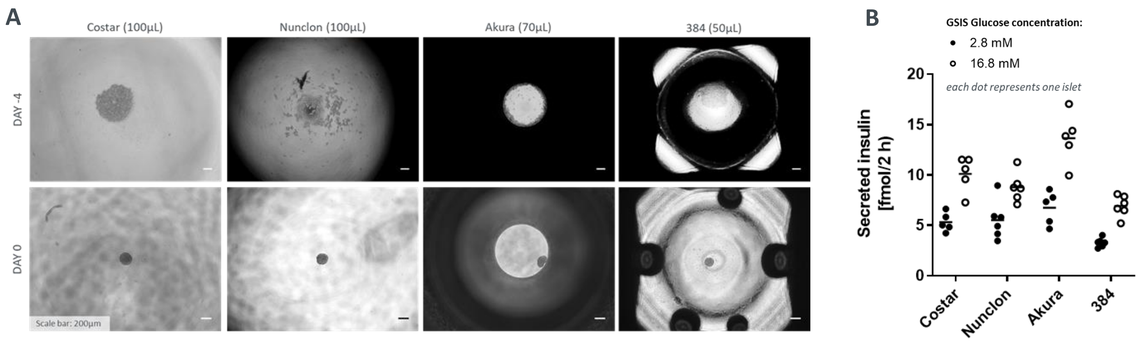


Fig. 1 EndoC-βH5® spheroid formation. Four different spheroid formation plates were tested. The **Costar** ULA 96-well round bottom plate (Corning; #7007); the **Nunclon**™ Sphera™ 96-well U-shaped bottom plate (Thermo Fisher; #15227905); the **Akura**™ 96 Spheroid Microplate (Insphero; #CS-09-004-03) and the **384**-well round bottom ULA plate (Corning; #3830). (A) Microscopic images of cell spheroids directly after seeding the 2000 cells into the well (DAY -4) and after 4 days of culture in the respective spheroid formation plate (DAY 0). Scale bar: 200µm. (B) Glucose stimulated insulin secretion (GSIS) analysis of 6 single EndoC-βH5® spheroids from each plate condition in low (black dots) and high (white dots) glucose.

## FUNCTIONAL CHARACTERIZATION (DOSE RESPONSE)

Insulin secretion increased with increasing glucose concentration (2A). Exenatide (GLP-1R agonist) dose response showed peak of insulin secretion between 1 nM and 10 nM Exenatide (2B).

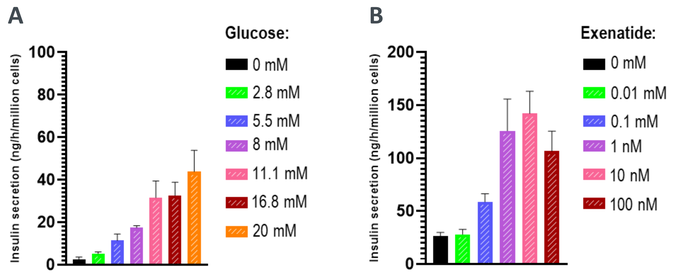


Fig. 2 Dose response of EndoC-βH5® spheroids to (A) Glucose and (B) Exenatide. GSIS assay was performed with single EndoC-βH5® spheroids incubated for 40 min in βKrebs®BSA buffer supplemented with the respective dose after over night incubation in ULTI-St® starvation medium (0.5mM glucose). Dose response of Exenatide (B) was performed in the presence of 11.1 mM glucose.

## MEDIUM COMPARISON

Increase of insulin secretion during GSIS assay in response to high glucose was only confirmed for ULT-Chip2 cultures. Exenatide significantly increased insulin secretion in all conditions, however ULT-Chip2 cultures showed the highest stimulation (3A). Insulin secretion measured during the culture increased over time in ULT cultures. CM1 and CM2 started with higher insulin levels at day 3 which decreased over time. At day 9, insulin secretion was at the same level for all conditions (2B).

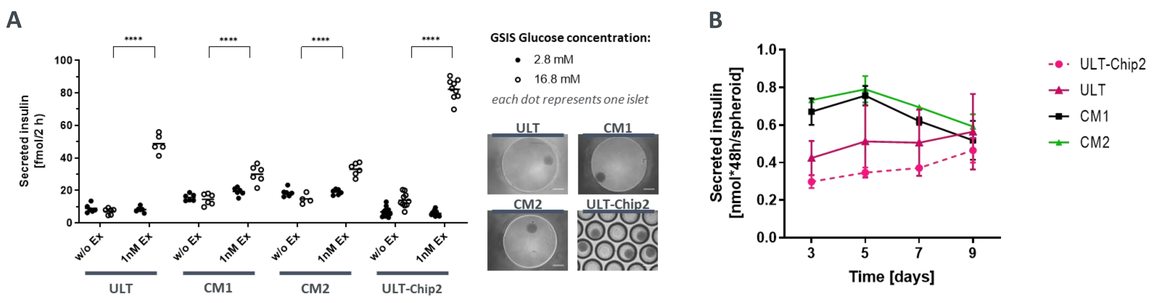


Fig. 3 Comparison of EndoC-βH5® spheroids cultured for 10 days in three different cell culture media. **ULT**Iβ1®medium: serum-free EndoC-βH5® specific suppliers medium (5.5 mM glucose), **CM1**: Co-culture medium 1 (11.1 mM glucose, 50 µM hydrocortisone), **CM2**: Co-culture medium 2 (5.5 mM glucose, 10 nM hydrocortisone). CM1 and CM2 are both based on WilliamsE with 10% FCS. ULT, CM1 and CM2 spheroids were cultured statically in Akura™ 96 well plates (1 spheroid per well). **ULT-Chip2**: EndoC-βH5® spheroids cultured in the HUMIMIC Chip2 96-well with ULT medium (10 spheroids per circuit inserted into ULA coated Dynarray© microcavities). (A) GSIS analysis of 6-10 single EndoC-βH5® spheroids from each condition in low (black dots) and high (white dots) glucose with and without the addition of 1nM Exenatide. Microscopic images show spheroids in the respective medium and culture condition at day 10. Scale bar: 200µm. (B) Secretion of insulin into the respective culture medium in 48h per spheroid during the 10-day culture.

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## FUNCTIONAL COUPLING WITH LIVER MODEL

40 HepaRG-based liver spheroids were combined with 10 EndoC-βH5® spheroids in the other organ compartment and co-cultured for 15 days in the HUMIMIC Chip2 96-well.

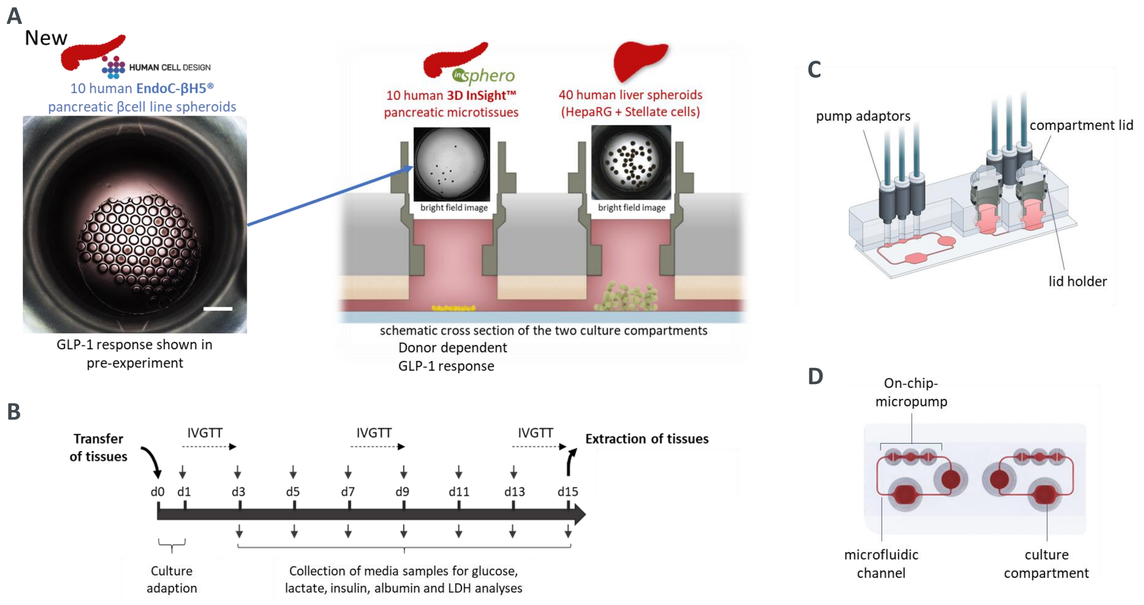


Fig. 4 Experimental setup of HUMIMIC Chip2 96-well co-culture. (A) Standard tissue loading scheme of organ equivalents. Scale bar: 1mm. (B) 15-day chip culture scheme with repeated complete medium exchange every 48h. An *in vitro* glucose tollerance test (IVGTT) was performed during the culture by additional sampling at 0, 24 and 48h. (C) 3D view and (D) bottom view illustration of the HUMIMIC Chip2 96-well shows the microfluidic circuits in red with the on-chip micropump and the culture compartments.

## FUNCTIONAL COUPLING WITH LIVER MODEL

During the 48h IVGTT, insulin levels rose steadily in all cultures, however once glucose levels had dropped in the co-cultures also insulin secretion of the EndoC-βH5® spheroids decreased. This islet-liver cross-talk seen here with the EndoC-βH5® spheroids was comparable with the cross-talk seen in the previous study with the primary islet microtisues[1]. Low LDH release (<10% of positive control) and stable albumin production by the HepaRG™ spheroids in normoglycemic and hyperglycemic medium indicates cultures to be viable and functional.

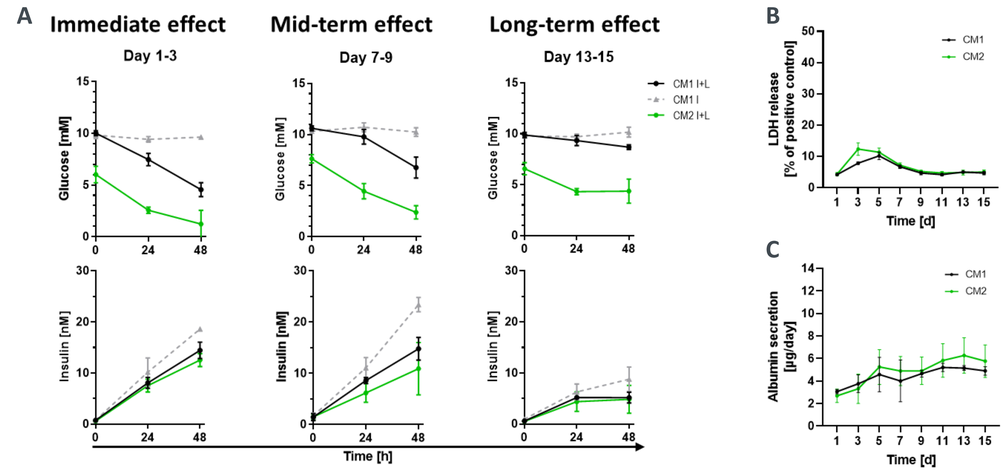


Fig. 5 (A) *In vitro* glucose tolerance test (IVGTT), (B) LDH release and (C) Albumin production measured in islet-liver co-cultures and islet only cultures in the HUMIMIC Chip2 96-well. Hyperglycemic medium with either islet and liver co-cultures (**CM1 I+L**; black) or islet only cultures (**CM1 I**; grey) was compared with co-cultures in normoglycemic medium (**CM2 I+L**; green).

## SUMMARY & CONCLUSION

The newly developed 3D spheroid model of EndoC- βH5® cells represents a reproducible and functional human β-cell model to study the liver-pancreas axis in vitro. In the future, the established co-culture model could be used as a platform for studying human islet biology in both healthy and T2DM as well as for development of new therapies.

**References 1-** Bauer, S. et al. Sci Rep 7, 14620 (2017). **2-** Casas, B. et al. PLoS computational biology 18, e1010587 (2022). **3-** Olleik, H. and Blanchi, B. Diabetes 71(Supplement_1):254-LB (2022).