



Asterand[®]

Partners in Human Tissue Research

The human *islet*OrganDOT™ system

A new approach to investigation of the effects of compounds
on glucose-stimulated insulin secretion

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Biochemical Pharmacology

- Introduction to Asterand
- Diabetes overview
- Isolated pancreatic islets for GSIS assays
- Asterand's human *islet*OrganDOT™ System



Asterand plc (LSE:ATD)

Human tissue research:

- Strong heritage and expertise based on years of experience in human tissue research (Pharmagene plc , Asterand Inc, Bioseek Inc)

Global operation :

- Tissue products business in Michigan US;



Fixed, frozen, fresh, diseased, non-diseased, tissues, biofluids, derivatives

- Services businesses in Royston UK and San Francisco US;



Target validation, phenotypic screening, compound profiling for efficacy and safety

- Global tissue procurement network of >100 active source sites
- Global customer base : Pharma, Biotech, Diagnostics, Government



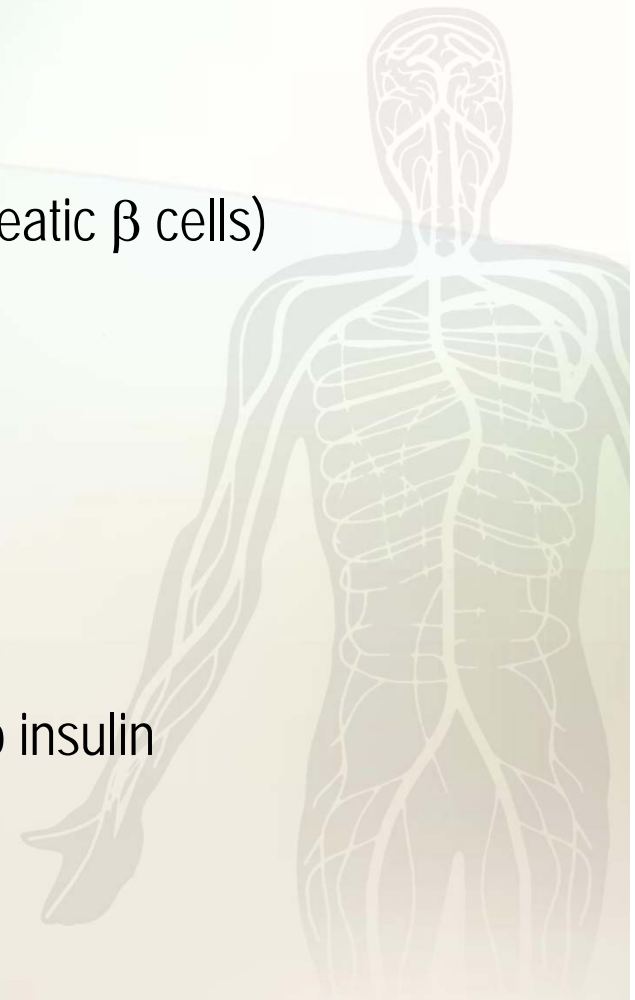
Asterand has established a variety of human cell based functional assays for evaluation of lead compounds in various therapeutic areas

- Mediator release
 - Alveolar macrophages
 - Bronchial epithelial cells
 - Whole blood
 - PBMCs
 - Monocytes
 - Neutrophils
 - Hepatocytes
- Neutrophil transmigration
- Lymphocyte proliferation
 - Whole blood
 - Purified B cells
- Hepatocytes
 - 11 β HSD-1 activity
 - Fatty acid oxidation
- Adipose
 - Lipolysis
- Cardiac toxicology
 - VICs proliferation assay
- BioMAP[®] platform
- *Islet*OrganDOT[™] System **NEW**

- Diabetes mellitus
 - Group of metabolic disorders characterised by hyperglycaemia as a result of defects in insulin secretion, insulin action, or both*.
 - Chronic hyperglycaemia is associated with long-term damage, dysfunction, and failure of various organs particularly the eyes, kidneys, nerves, heart, and blood vessels.
 - Almost 250 million people world-wide suffer from diabetes and over 3 million people die each year of diabetes associated complications.

*American Diabetes Association Expert Committee (Diabetes Care 26, S1, 2003)

- Two major forms of diabetes
 - Type 1 (approx. 5% of cases)
 - Autoimmune disease (destruction of pancreatic β cells)
 - Idiopathic
 - Type 2 (at least 80% of cases)
 - Heterogeneous metabolic disorder
 - Reduced insulin secretion
 - Reduced sensitivity of peripheral tissues to insulin



Common therapies for the treatment of type 2 diabetes

- Life-style changes

- Drug treatments

- Peripheral tissue sensitizers:

- biguanides (e.g. metformin)
- PPAR activators (e.g. pioglitazone)

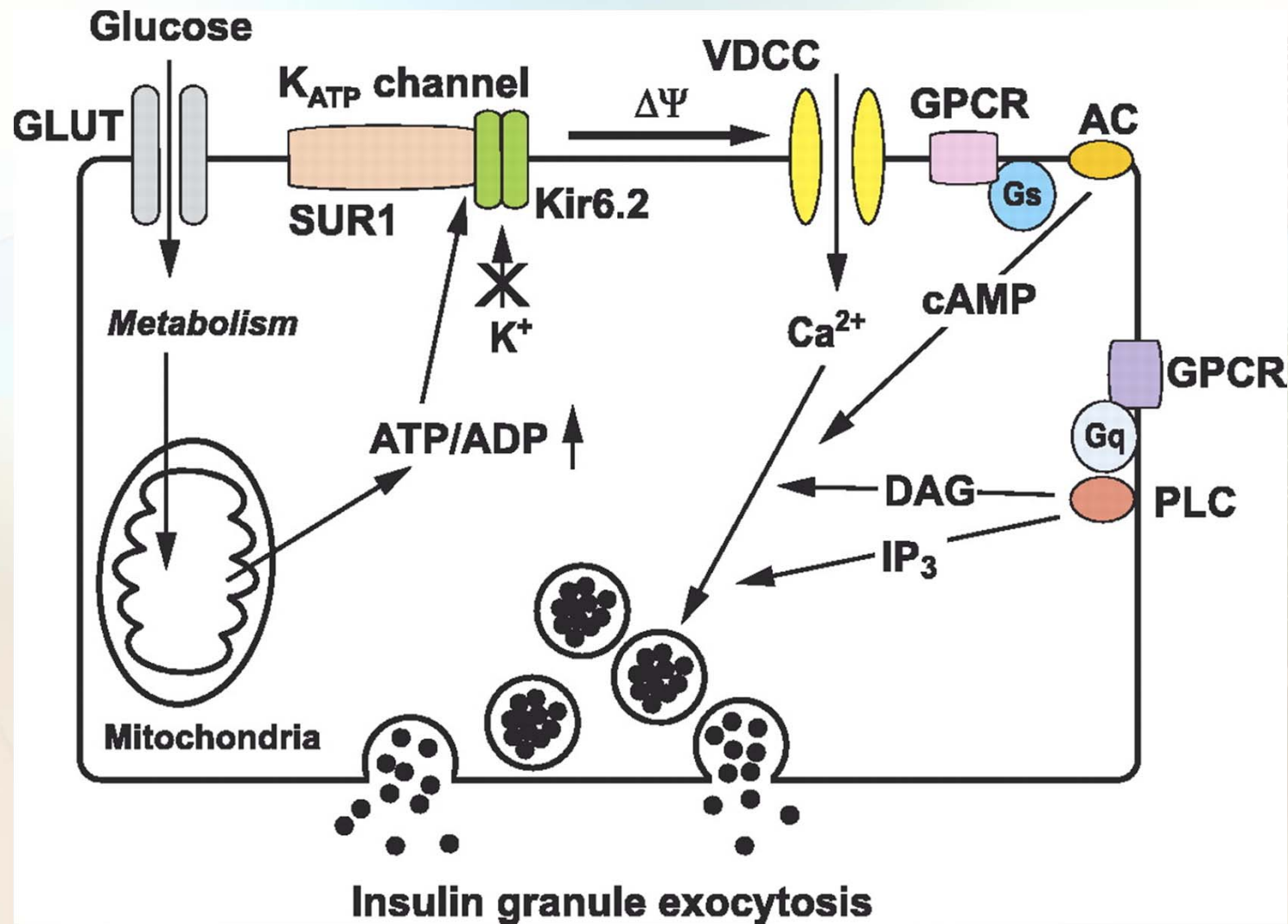


- Insulin secretagogues:

- Sulphonylureas and glinides (K_{ATP} channel blockers, e.g. glibenclamide)
- Incretin mimetics (GLP-1 receptor agonists, e.g. exenatide)
- Gliptins (DPP-4 inhibitors, e.g. sitagliptin)

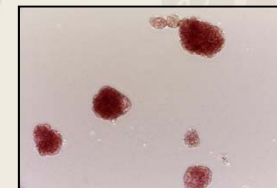


Mechanisms of insulin secretion



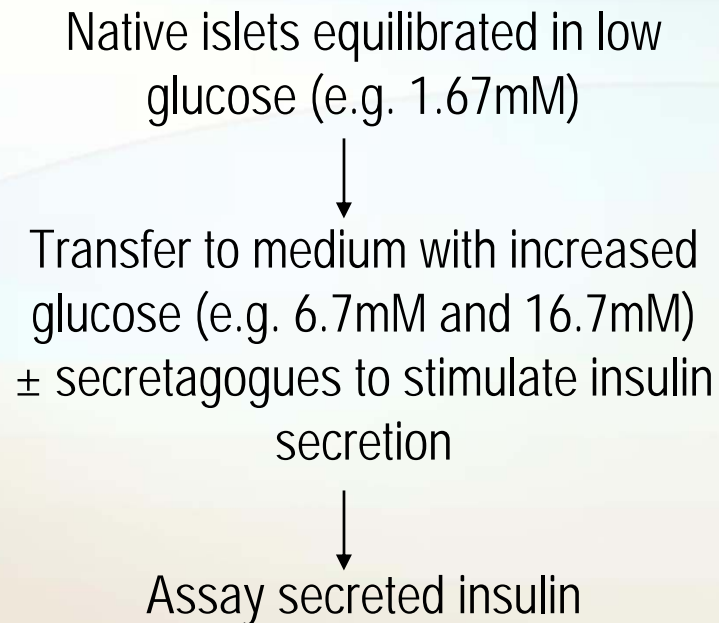
Models for investigation of insulin secretion *in vitro*

- Cell lines (e.g. mouse insulinoma cell line MIN6)
 - Partial β cell function
 - Loss of differentiated properties with long-term culture
- Freshly isolated rodent islets
 - Retain β cell function
 - Cellular architecture different to human islets
- Freshly isolated human islets
 - 'Gold standard' for clinically relevant studies of islet function and dysfunction
 - Limited supply; high cost; rapid loss of function



Glucose-stimulated insulin secretion using human islets *in vitro*

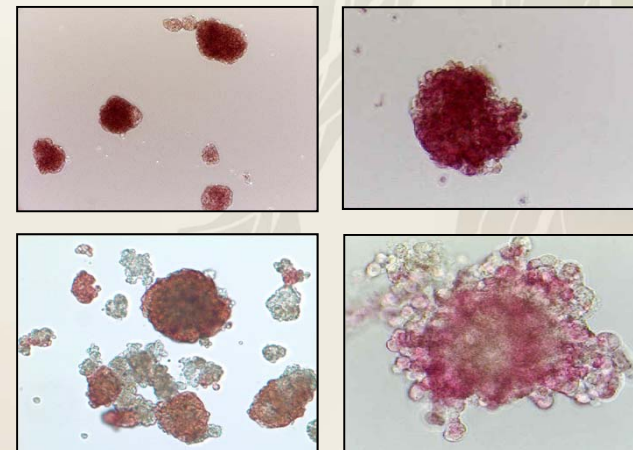
• Typical GSIS protocol



Dynamic (perifusion) GSIS assays are used but static GSIS assays are more common

• Issues

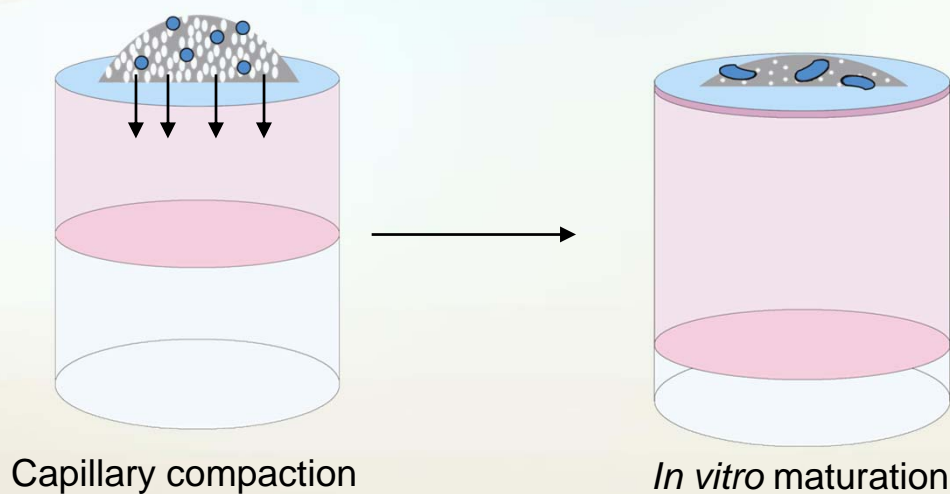
- Rapid loss of function post isolation
- Size heterogeneity
- Substantial apoptosis and necrosis of cells in centre of large islets
- Time consuming islet picking often necessary
- Low throughput



The human *islet*OrganDOT™ system

- OrganDOT™ methodology developed by Capsant Neurotechnologies Ltd.

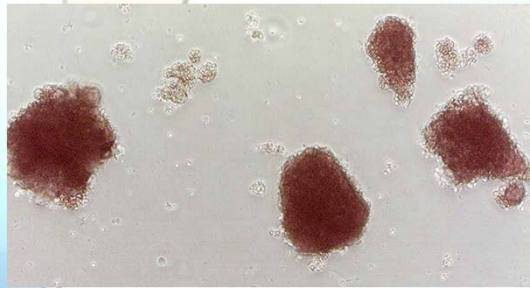
- Patented production method



- Asterand granted exclusive licence to use the OrganDOT system to develop *in vitro* models of pancreatic function

- islet*OrganDOT cultures prepared on 24-insert plates

Dispersed human islet cells re-aggregate in OrganDOT™ culture



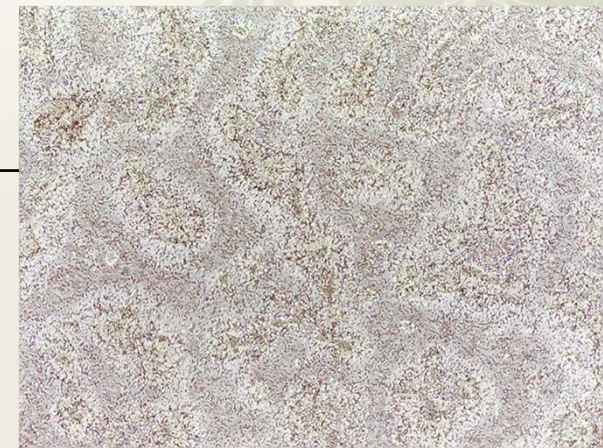
Intact islets



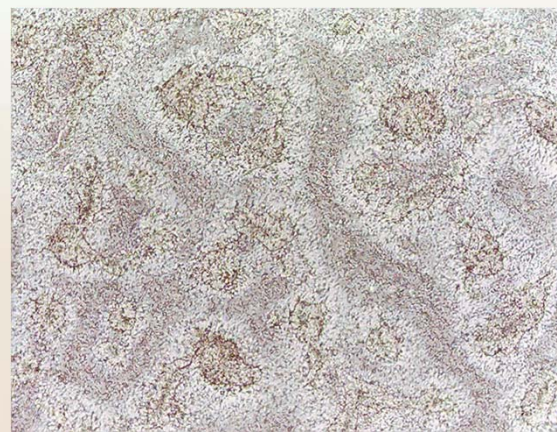
Day 0



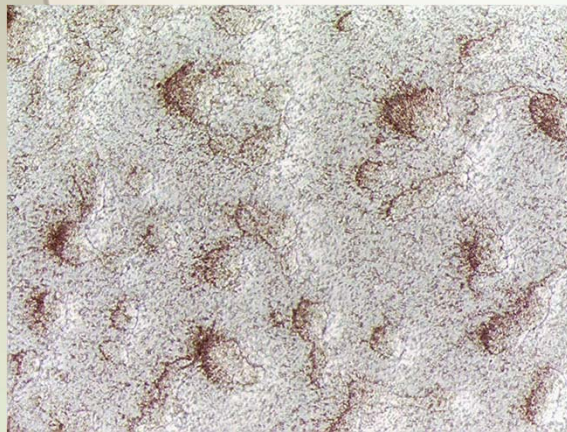
Day 3



Day 6

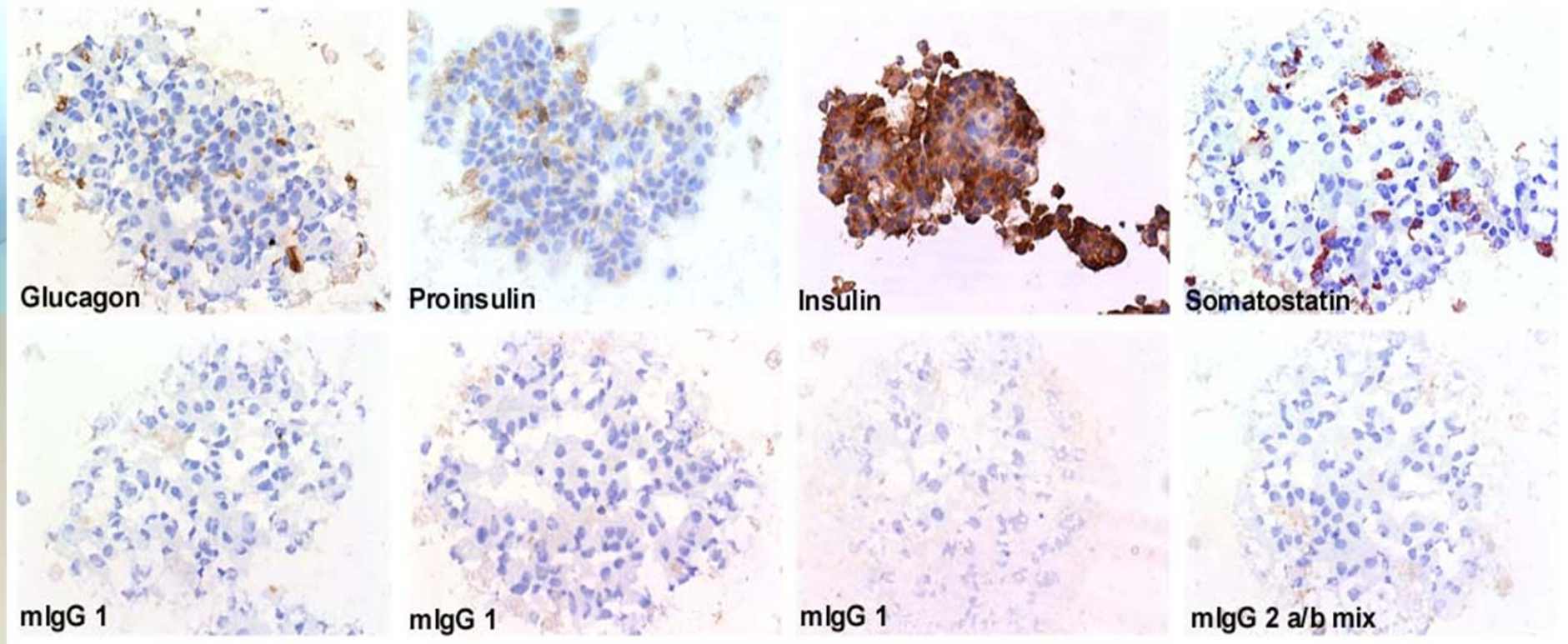


Day 13



Day 13

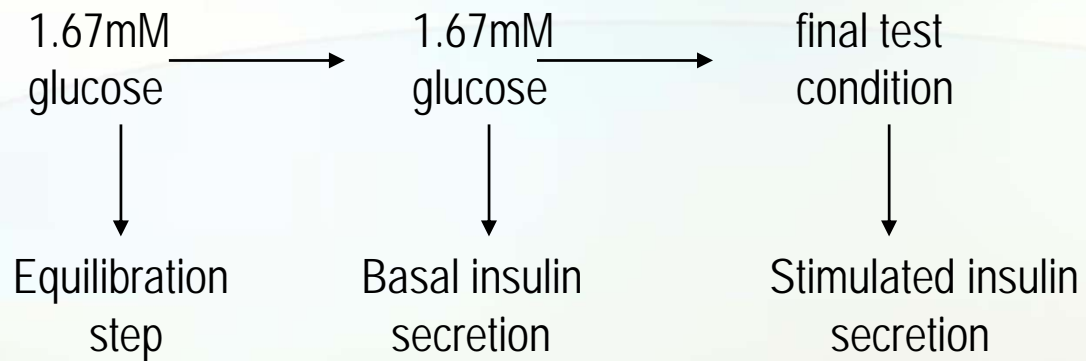
Human *islet*OrganDOT™ cultures contain α , β and δ cells



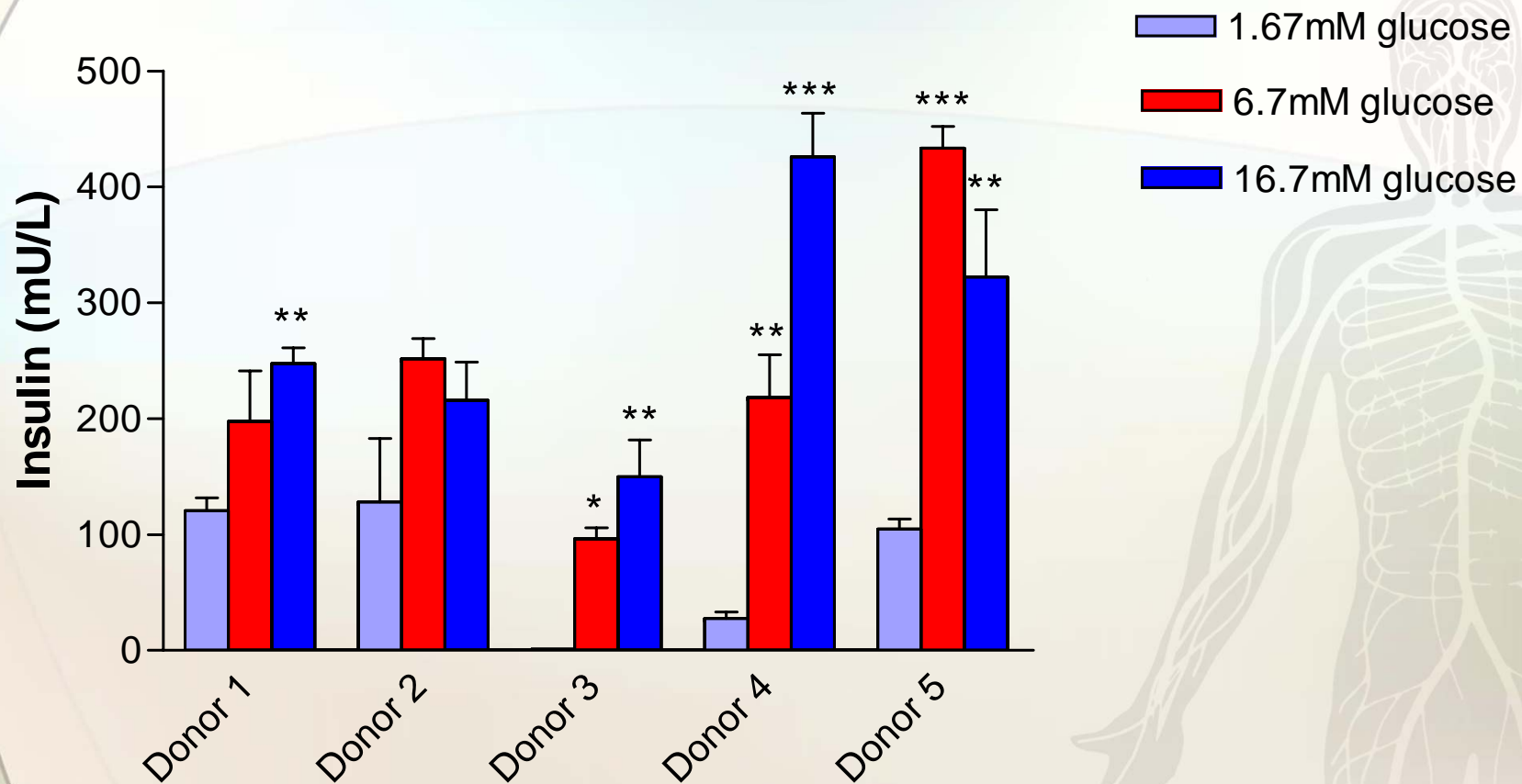
ICC stained using DAB end-point and counterstained with standard haematoxylin

Human *islet* OrganDOT™ GSIS assays

Each OrganDot culture is manoeuvred through solutions as follows:

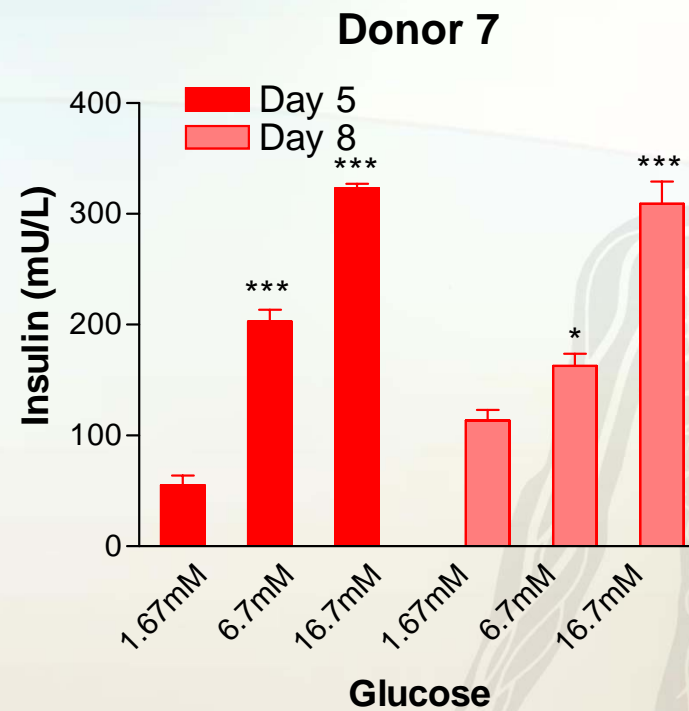
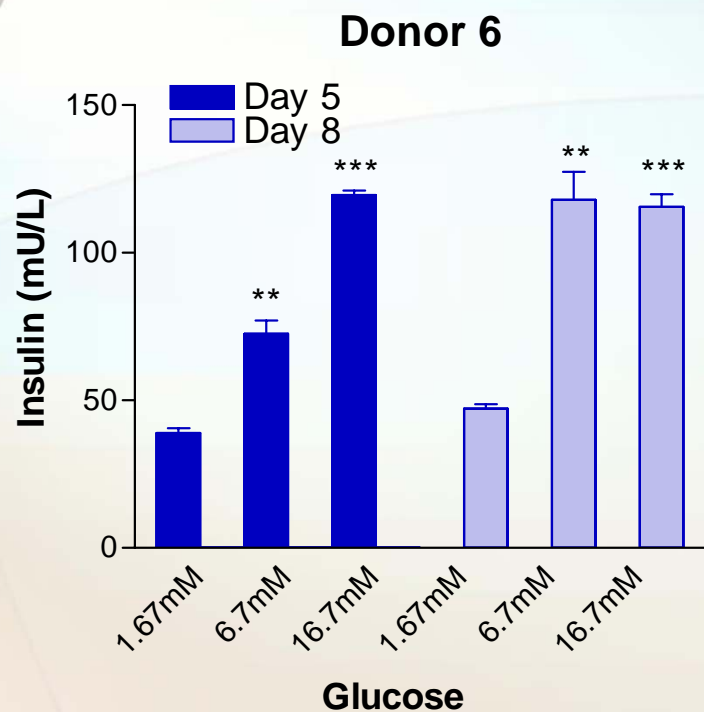


Human *islet*OrganDOT™ cultures consistently respond to glucose



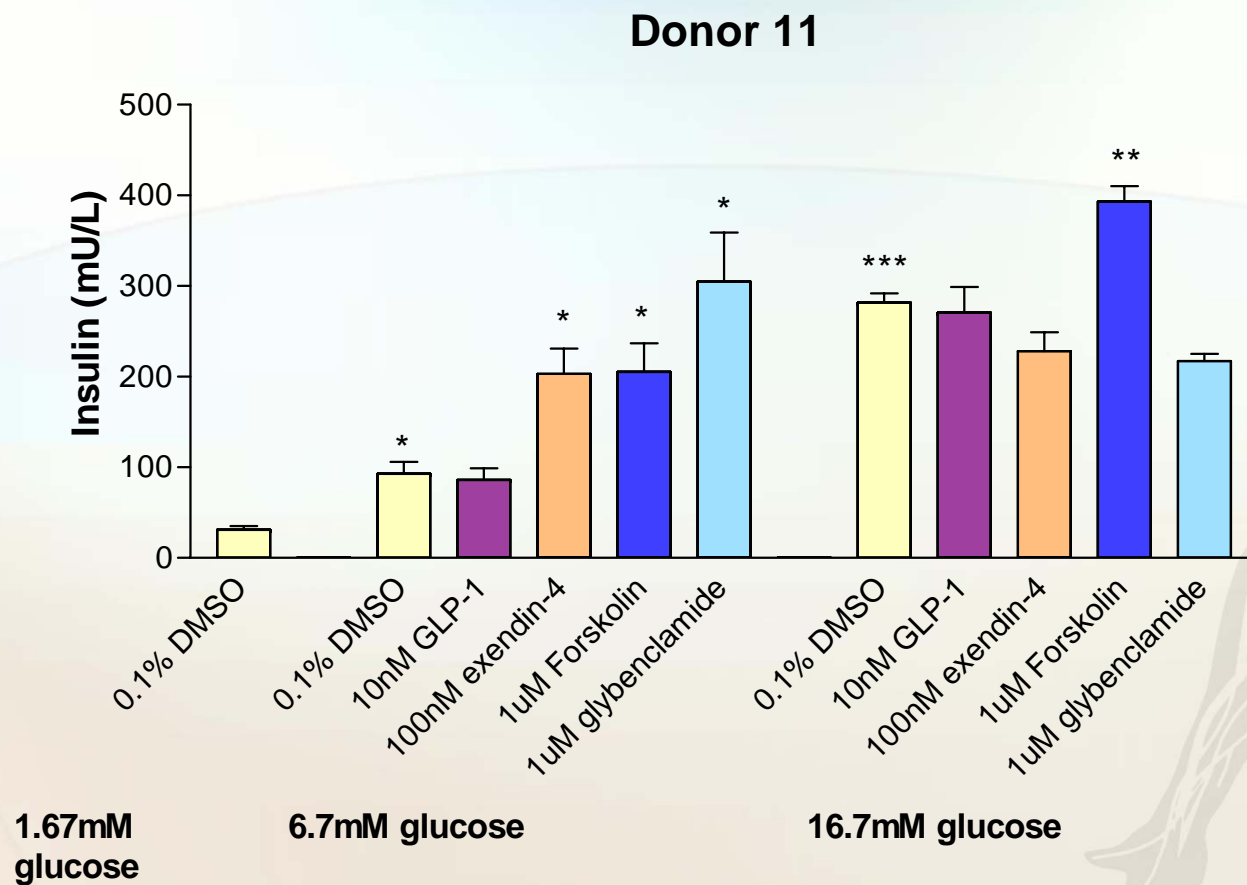
T-test performed against 1.67mM glucose.
 Data plotted as mean+sem (n=3)
 t-test: *<0.05, **<0.01; ***<0.001

Human *islet*OrganDOT™ cultures retain responses to glucose



T-test performed against 1.67mM glucose.
Data plotted as mean+sem (n=3)
t-test: * < 0.05, ** < 0.01; *** < 0.001

Effect of various secretagogues on GSIS



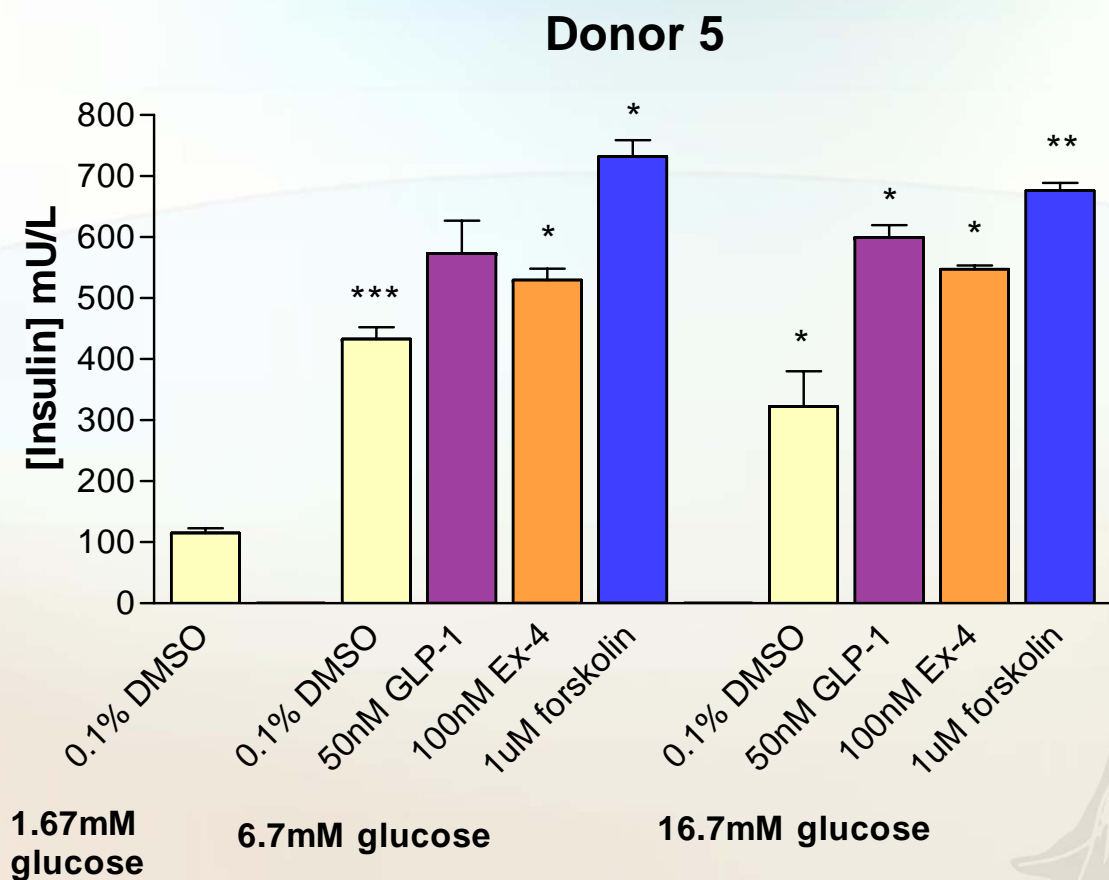
T-test for glucose only controls are performed against 1.67mM glucose

T-test for the glucose + secretagogue samples were performed against the respective glucose only control

Data plotted as mean+sem (n=3)

t-test: * < 0.05, ** < 0.01; *** < 0.001

Effect of GLP-1, exendin-4 and forskolin on GSIS



T-test for glucose only controls are performed against 1.67mM glucose

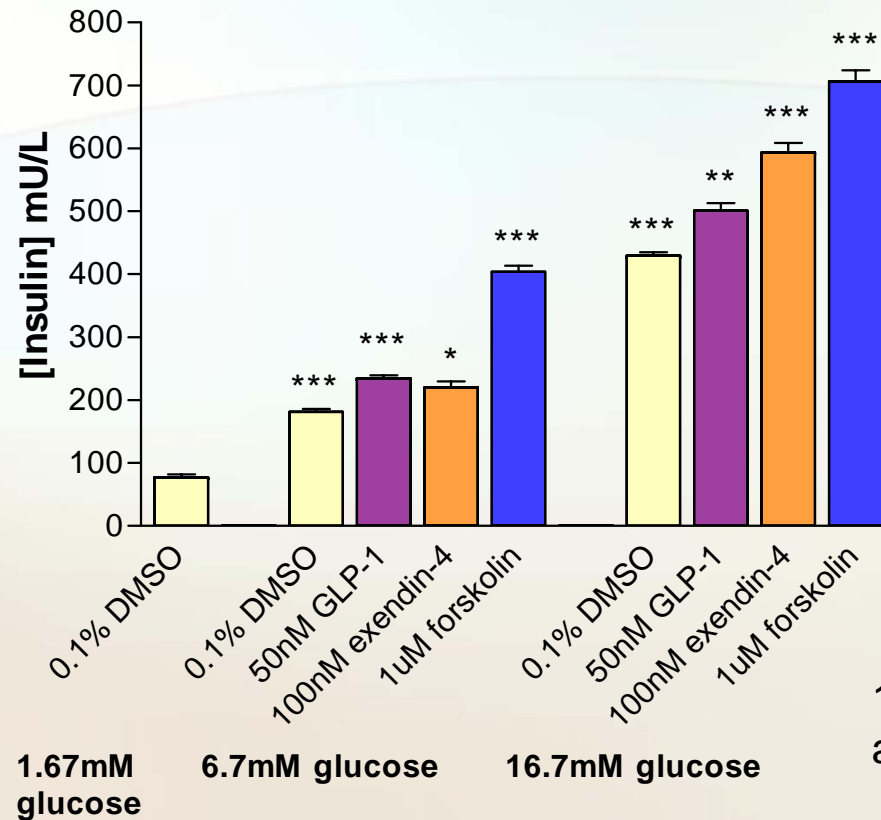
T-test for the glucose + secretagogue samples were performed against the respective glucose only control

Data plotted as mean+sem (n=3)

t-test: * < 0.05, ** < 0.01; *** < 0.001

Effect of GLP-1, exendin-4 and forskolin on GSIS using enhanced culture conditions

Donor 15
Day 13



10nM GLP-1 + 100nM diprotinin added to culture medium

T-test for glucose only controls are performed against 1.67mM glucose

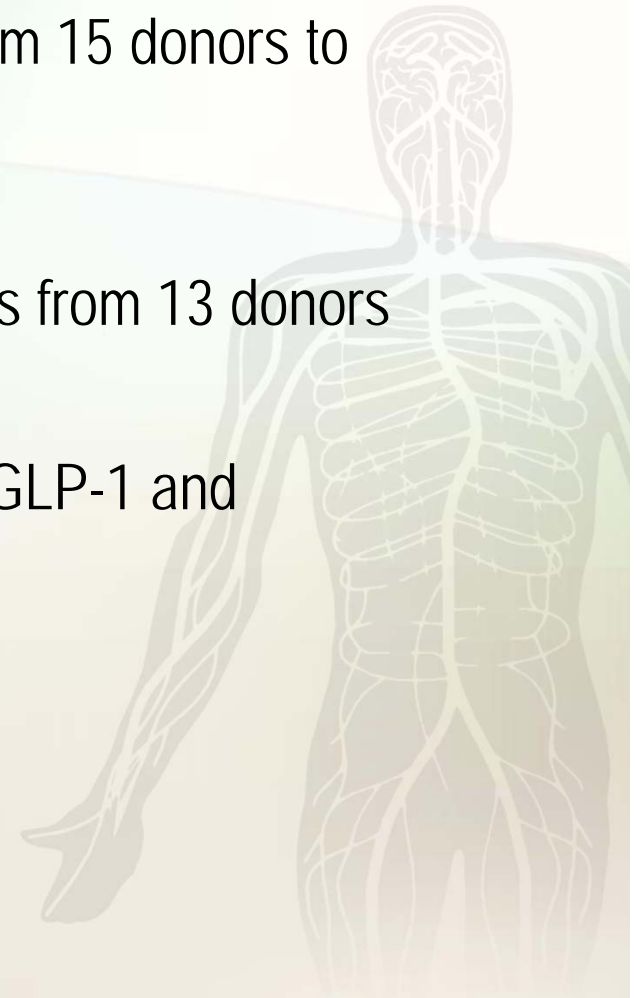
T-test for the glucose + secretagogue samples were performed against the respective glucose only control

Data plotted as mean+sem (n=3)

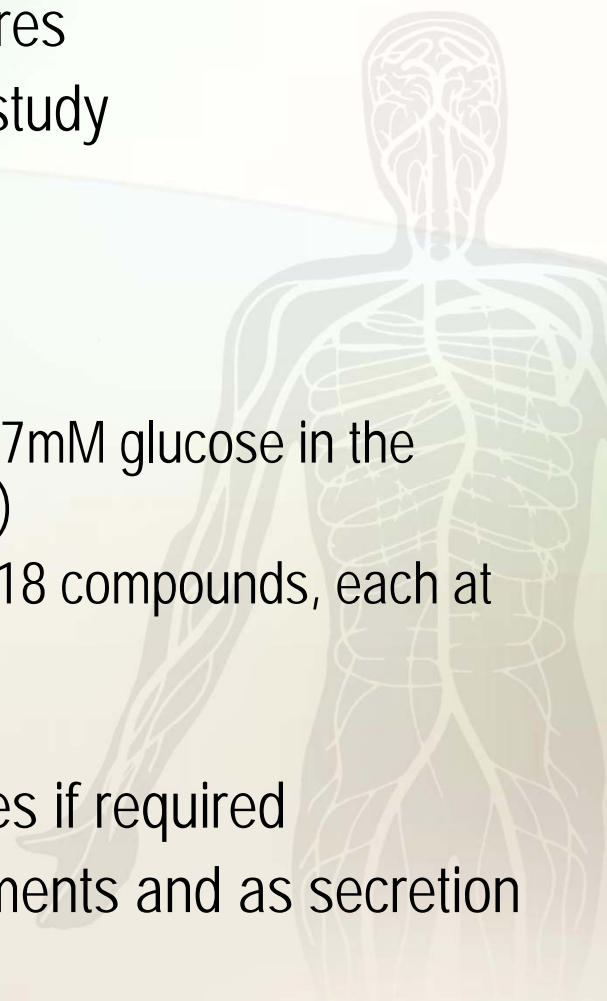
t-test: *<0.05, **<0.01; ***<0.001

Summary of GSIS results

- Islet OrganDOT cultures prepared from islets from 15 donors to date
- Increased insulin secretion obtained with cultures from 13 donors
- Donor- to- donor variation in responsiveness to GLP-1 and exendin-4 but consistent response to forskolin



Format for testing effects of compounds on GSIS

- 24-insert plates used for islet OrganDOT cultures
 - Up to 10 x 24-well plates can be run for each study
 - N=4 for each treatment
 - Flexible options for testing compounds:
 - Controls are typically 1.67mM, 6.7mM, and 16.7mM glucose in the presence or absence of 50nM GLP-1 (24 wells)
 - 54 test slots available in a 10-plate study (e.g. 18 compounds, each at 3 concentrations)
 - Repeat testing on the same OrganDOT cultures if required
 - Results reported as absolute insulin measurements and as secretion indexes
- 

Conclusions

- Human islet OrganDOT culture offers a robust platform for testing the effects of lead compounds on glucose-stimulated insulin secretion
- Potential to evaluate effects of compounds on the secretion of other mediators, e.g. glucagon or somatostatin
- System amenable for investigation of many aspects of islet cell biology, e.g. β cell proliferation, apoptosis, or responses to inflammatory mediators



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Please visit the Asterand stand in the exhibition hall