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Functional Assessment of the Induced Pluripotent Stem Cells (iPSC)-derived β -like Cells

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Background and Aim

Human β -like cells (BLCs) derived from induced Pluripotent Stem Cells (iPSC) are an emerging tool to study human β -cell development and function.

We established a functional assessment of each of our differentiations on the following properties:

- Secretory response (insulin secretion assay)
- Exocytosis kinetics (capacitance measurement)
- Ultrastructure (Electron microscopy)
- β -cells electrical coupling (Multi-Electrode Array, MEA)

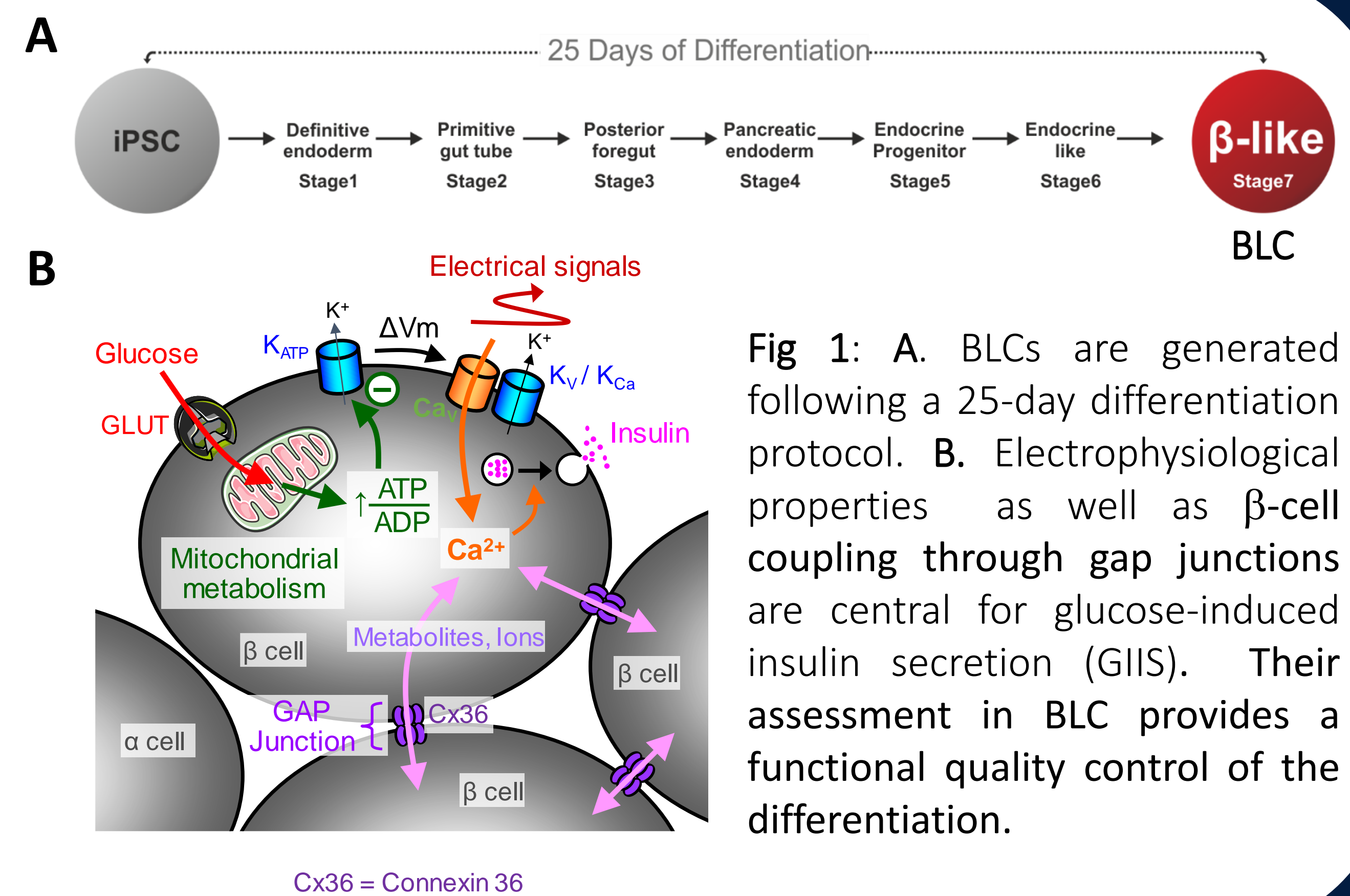


Fig 1: A. BLCs are generated following a 25-day differentiation protocol. B. Electrophysiological properties as well as β -cell coupling through gap junctions are central for glucose-induced insulin secretion (GIIS). Their assessment in BLC provides a functional quality control of the differentiation.

Results

Secretory response

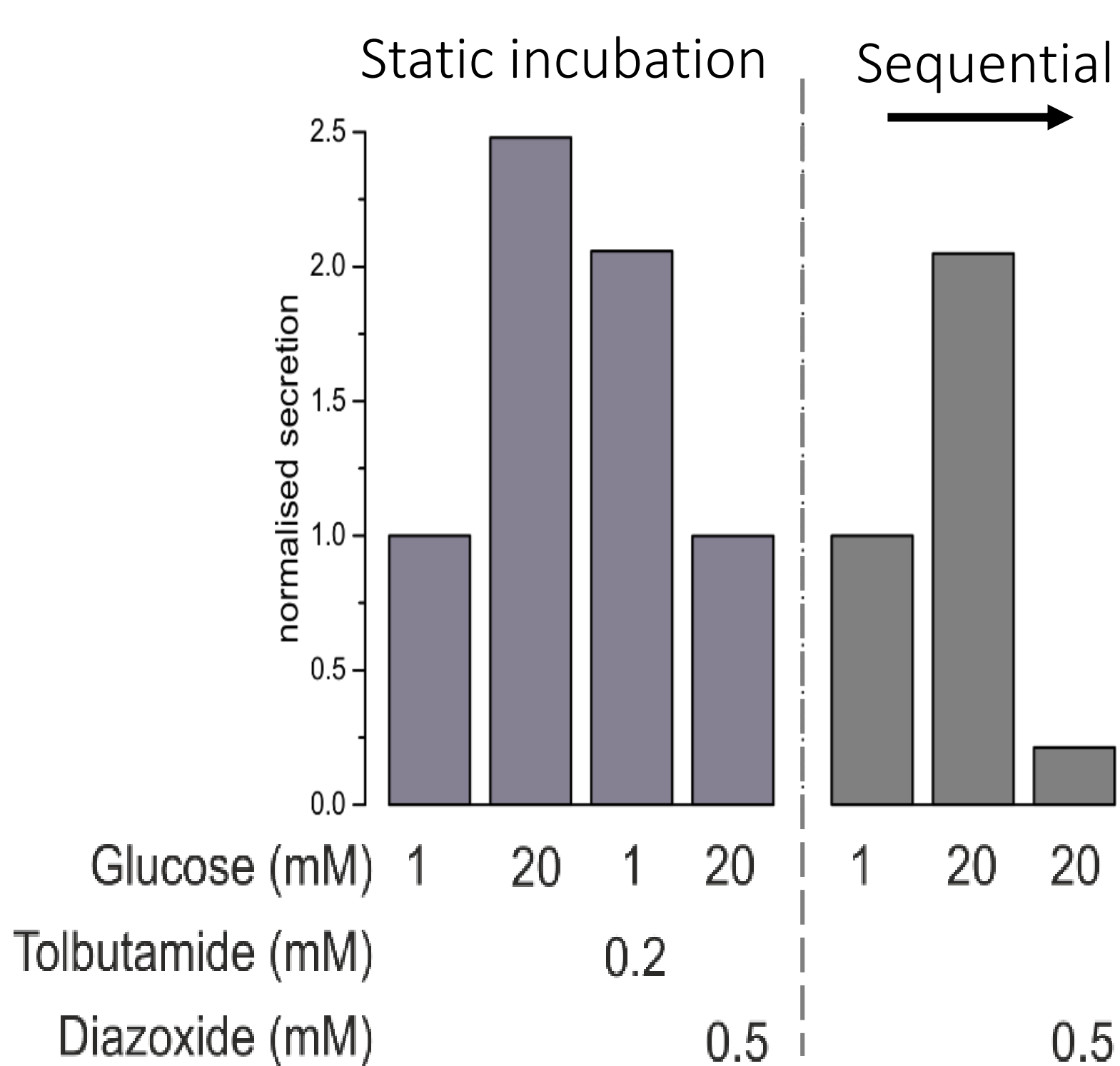


Fig 2: Representative GIIS induced by 1 and 20mM glucose and modulators of K_{ATP} channels activity (Tolbutamide/Diazoxide). The stimulations are either parallel static incubations (left), or sequential on the same BLC preparation (right).

Electrical activity and exocytosis (single-cell)

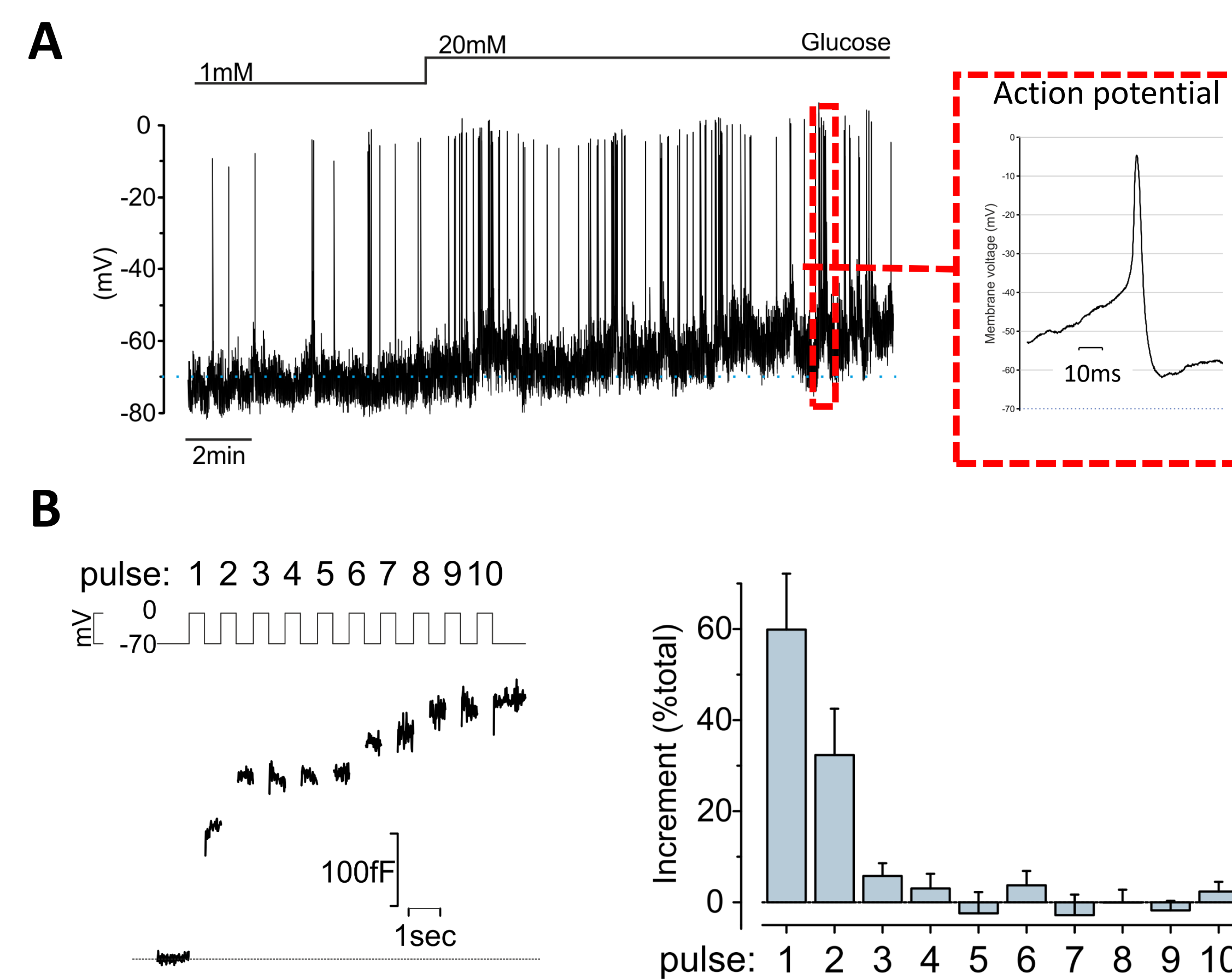


Fig 3: A. Glucose stimulation triggers action potential firing ($n=3$ cells). Action potential kinetics is similar to human primary β -cells (10ms duration). B. Exocytosis triggered by a train of 10 depolarisations to 0mV is biphasic as in human primary β -cells (Raw trace left, quantification right, $n=7$ cells).

Ultra structure

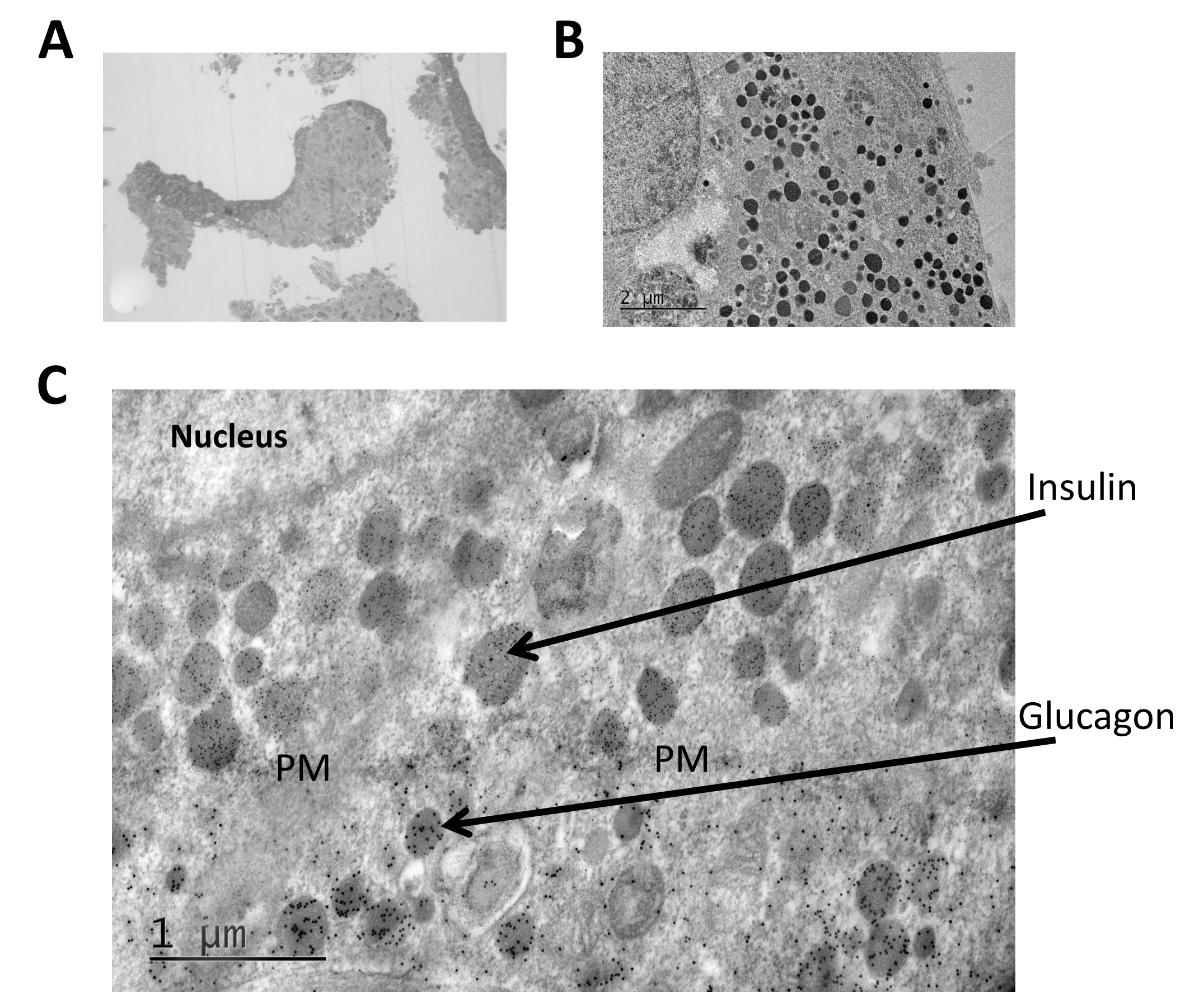


Fig 4: A. Semi thin section showing cell heterogeneity. B. Ultra structure of BLC. Scale bar: 2 μ m C. Immunogold labelling of insulin (BLC, 10nm gold particles, top) and glucagon (15nm gold particle, alpha-like cell, bottom). PM: Plasma Membrane. Scale bar: 1 μ m.

Multicellular activity assessed by MEA and its automatic analysis in hard real-time

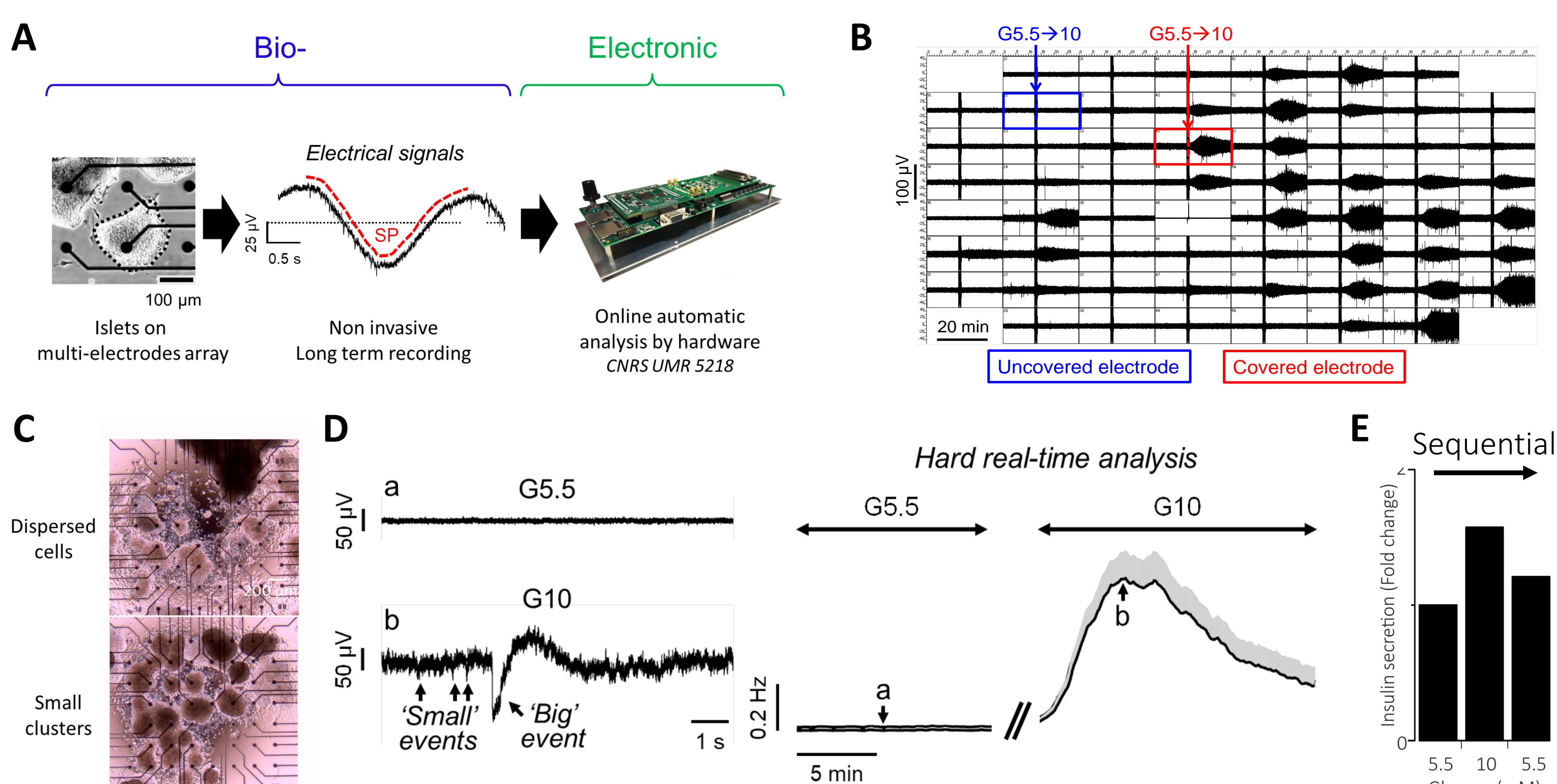


Fig 5: A. Principle of the approach: islets on MEA generate slow potentials (SP) analysed by a microelectronic circuit. SPs are β cell-specific coupling signals. B. 60 recording channels showing β -like cells activity. C. Images of preparations. D. Representative traces at 5.5 and 10mM glucose and real-time analysis of SP frequencies (means+SEM, $n=29$ electrodes). E. Corresponding insulin secretions.

Conclusions

β -like cells derived from iPSC exhibit critical human β -cell features:

- Glucose-induced insulin secretion
- Electrical excitability and exocytosis
- Electrical coupling

BLCs allow functional studies on human β -cell differentiation. The emergence of new differentiation protocols should improve their homogeneity and ultrastructure.

This pipeline will support the characterization of T2D-risk alleles on human β -cell function.