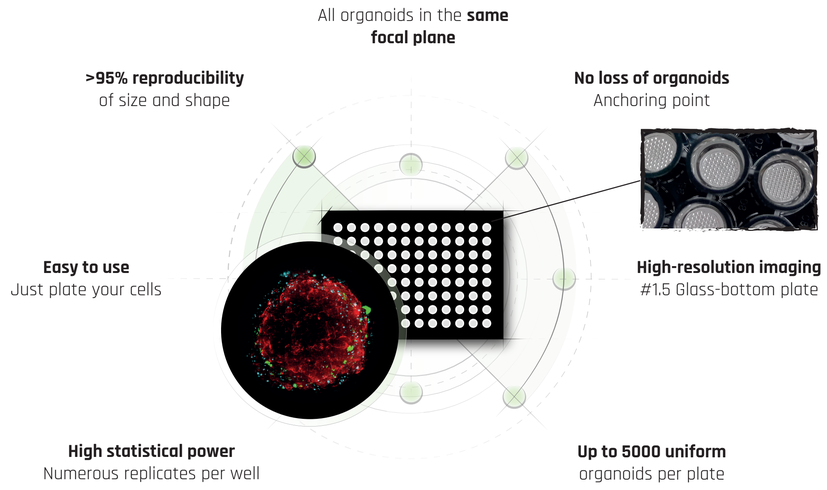
A high-throughput assay for the measurement of Ca^{2+}-oscillations and insulin release from uniformly sized β-cell spheroids

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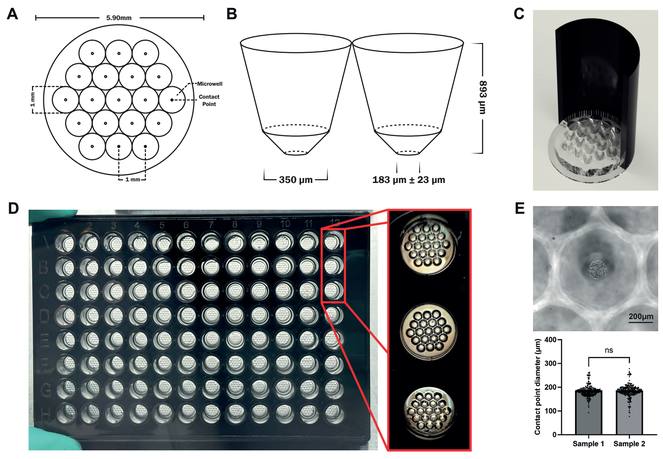
Abstract

Diabetes mellitus is a rapidly growing global health challenge, necessitating the development of more effective anti-diabetic therapies, including drugs that improve insulin release from pancreatic β-cells. Traditional high-throughput screening methods typically rely on 2D β-cell cultures, but such cultures do not mimic the 3D organisation and cell-to-cell communication of β-cells in pancreatic islets of Langerhans. Existing 3D β-cell culture models are hindered by high costs, technical complexity, and limited compatibility with high-throughput screening platforms. In this work, we developed an approach for generating 19 homogeneously shaped pancreatic β-cell spheroids in each well of a 96-well plate, using micropatterned polyethylene glycol (PEG)-based hydrogels and MIN6 insulinoma cells. The uniform shape and positioning of the individual spheroids enabled the simultaneous, real-time imaging of Ca2+ signals in up to 1824 independent spheroids in response to glucose and various test compounds. Using this approach, we show that increasing glucose causes concentration-dependent Ca2+ oscillations in individual spheroids, that these Ca2+ oscillations are sensitive to modulators of ATP-sensitive K+ channels, and that the frequency of Ca2+ oscillations correlate with insulin secretion. Finally, we demonstrate that the neurosteroid pregnenolone sulphate, an agonist of the cation channel TRPM3, increases the frequency of glucose-induced Ca2+ oscillations and enhances insulin release; the TRPM3 antagonist isosakuranetin inhibited these responses. In conclusion, we established a cost-effective and scalable 3D β-cell platform for high-throughput screening of insulin release-modifying compounds, with potential applications in drug development and personalized medicine for the management of diabetes mellitus.



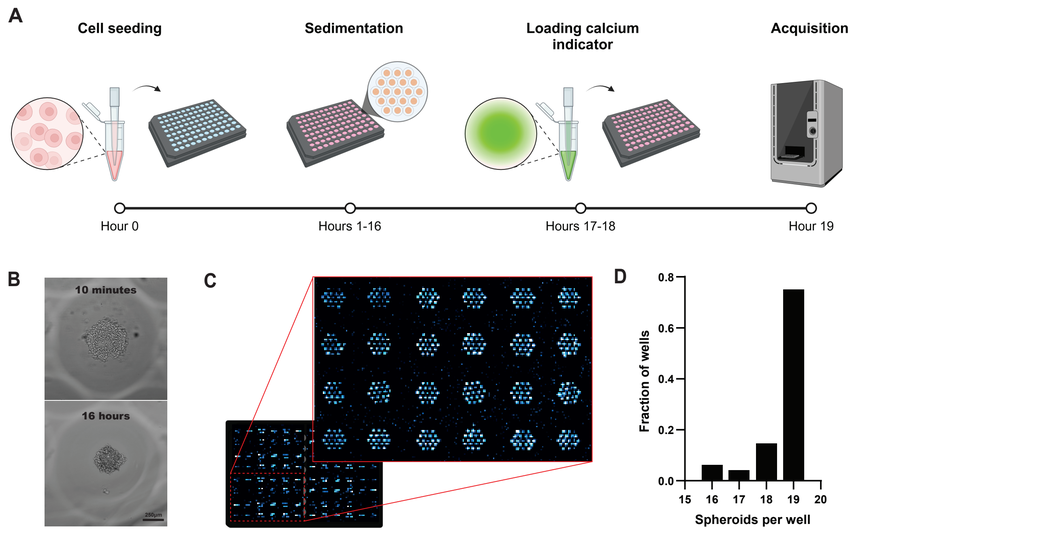
SmartSphero Plate Technology

The anti-adhesive properties of the hydrogel facilitate rapid self-assembly of cell aggregates into compact spheroids while the APT® (Anchoring Point Technology) anchors the spheroids in place. This prevents the loss of spheroids during typical manipulations (medium exchange, drug addition, etc), and makes the location of each spheroid predictable and on the same focal plane. As the hydrogel is optically transparent it is fully compatible with conventional microscopy.



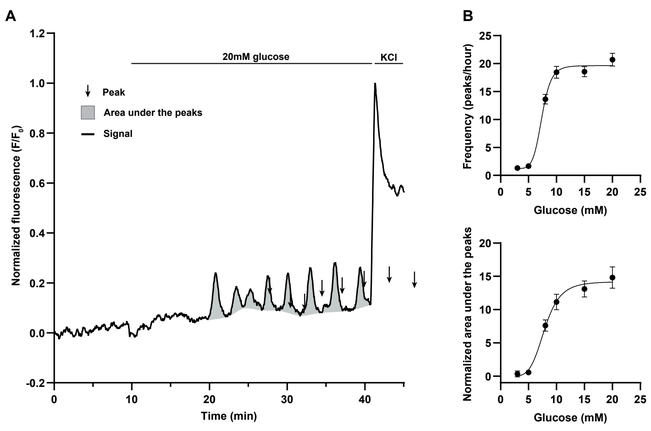
Calcium assay protocol established for 3D pancreatic β-cell spheroids

After validating the uniformity of microwells in the 96-well hydrogel plates, a protocol was established to measure calcium signaling in 3D MIN6 β-cell spheroids. Spheroids formed within 16 hours, were loaded with a Ca^{2} -sensitive dye, and imaged in real time. The hydrogel design ensured strong adhesion, minimizing loss during washing—75% of wells retained all 19 spheroids, with no well losing more than three.



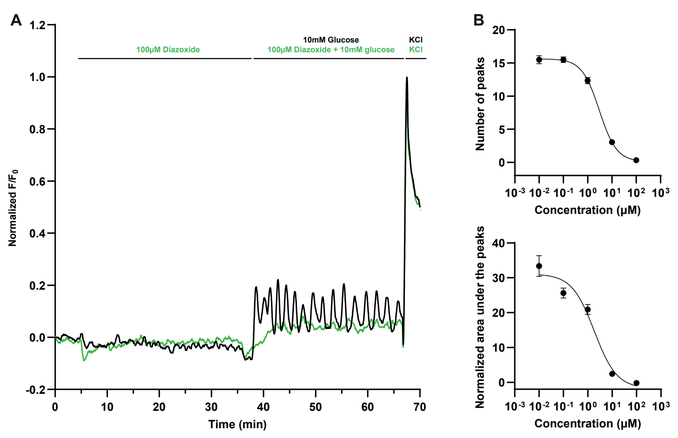
Increased levels of extracellular glucose induce Ca^{2+}-oscillatory behaviour

The Ca^{2+} response of spheroids to increasing extracellular glucose was assessed. While 3 mM glucose produced stable, non-oscillatory signals, concentrations above 5 mM triggered periodic oscillations, with frequency and amplitude increasing with glucose levels. Quantification showed a concentration-dependent rise in both frequency and area under the peaks (AuP), with EC_{50} values of 7.4 ± 0.4 mM and 7.9 ± 0.4 mM, respectively.



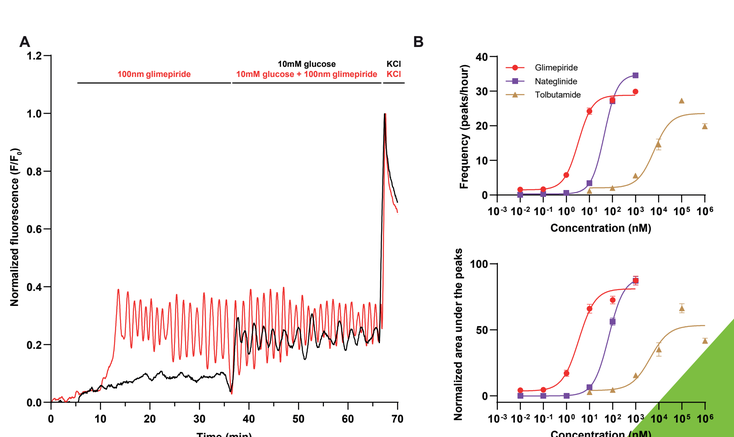
KATP-agonist diazoxide inhibits Ca^{2+}-oscillatory response to extracellular glucose

To test the role of K-ATP channel closure in glucose-induced Ca^{2+} oscillations, MIN6 β-cell spheroids were pre-treated with diazoxide, a K-ATP channel opener, before stimulation with 10 mM glucose. Diazoxide inhibited Ca^{2+} oscillations in a concentration-dependent manner, reducing both frequency and area under the peaks (AuP), with IC_{50} values of 2.9 ± 0.1 µM and 1.9 ± 1.71 µM, respectively.



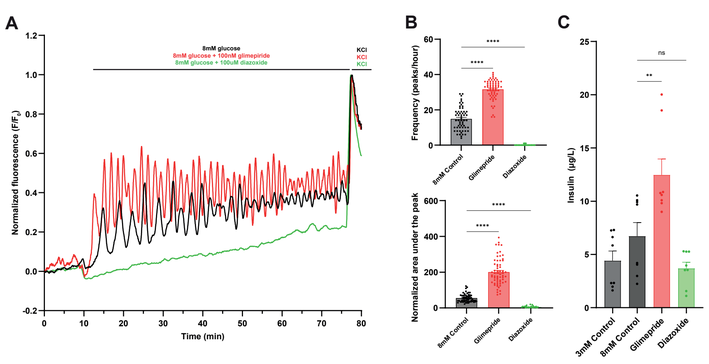
KATP-antagonists induce Ca^{2+}-oscillatory behaviour

K-ATP antagonists like glimepiride, tolbutamide, and nateglinide induced dose-dependent Ca^{2+} oscillations in MIN6 spheroids, even at low glucose. Glimepiride was the most potent, with EC_{50} values around 3.3 nM. The assay reliably quantifies K-ATP modulator effects on calcium signaling.



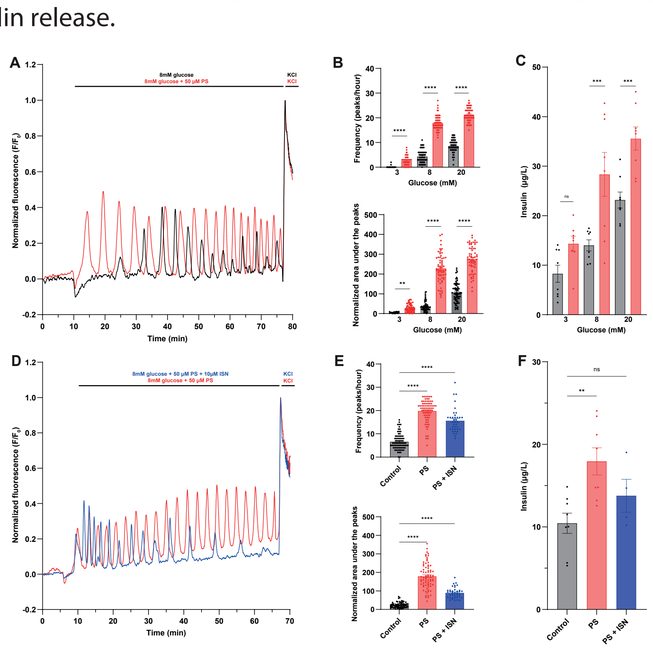
KATP-channel modulators impact insulin secretion by MIN6 spheroids

Calcium oscillations in MIN6 β-cell spheroids were shown to correlate with insulin secretion. At 8 mM glucose, both oscillations and insulin release increased. Glimepiride further enhanced both, while diazoxide abolished oscillations and suppressed insulin to baseline levels. This confirms a positive link between Ca^{2+} oscillation frequency and insulin secretion.



Pregnenolone sulfate (PS) induces Ca2+-oscillatory behaviour and increases insulin secretions

Pregnenolone sulfate (PS), a TRPM3 agonist, enhanced glucose-induced Ca^{2+} oscillations and insulin secretion in MIN6 spheroids at 50 µM. Co-treatment with the TRPM3 antagonist isosakuranetin partially reversed these effects, indicating that PS acts at least partly through TRPM3 to boost calcium signaling and insulin release.



Conclusions

- This study introduces a high-throughput, 96-well plate platform for generating uniformly sized MIN6 β-cell spheroids that synchronously exhibit glucose-induced Ca^{2+} oscillations and insulin secretion, enabling quantitative screening of small-molecule modulators.
- The micropatterned hydrogel design ensures spheroid adhesion, reproducibility, and compatibility with automated fluorescence imaging and microscopy, offering a scalable and physiologically relevant alternative to 2D cultures.
- The assay reliably mirrors known β-cell physiology and pharmacology, including responses to K_{ATP} modulators and pregnenolone sulfate, and can be extended to co-cultures or patient-derived cells for personalized drug screening.

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