Insulin Secretion and Phenotype Analysis of Single Pancreatic Beta Cells Using Nanowells-in-Microwells

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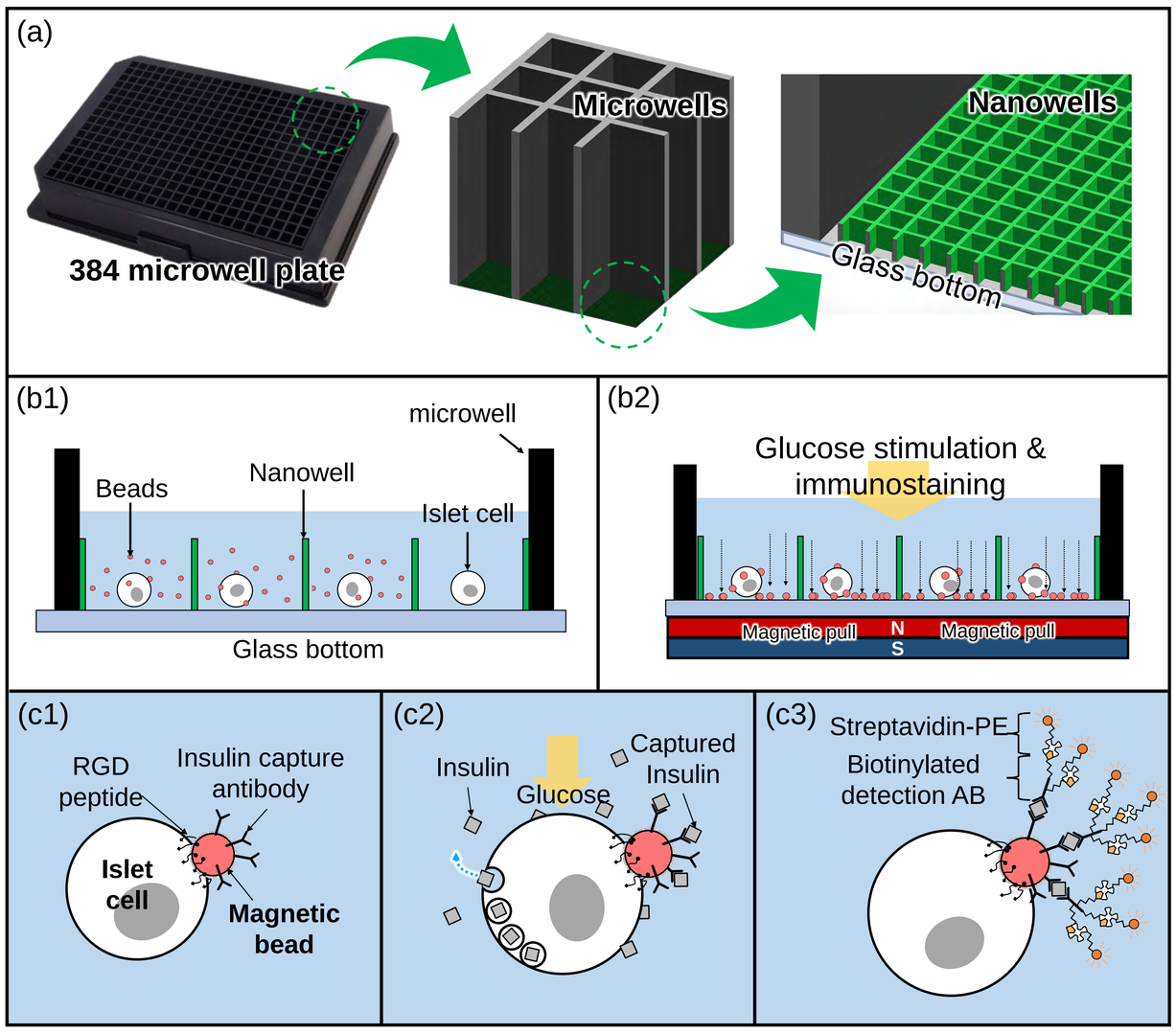
Introduction

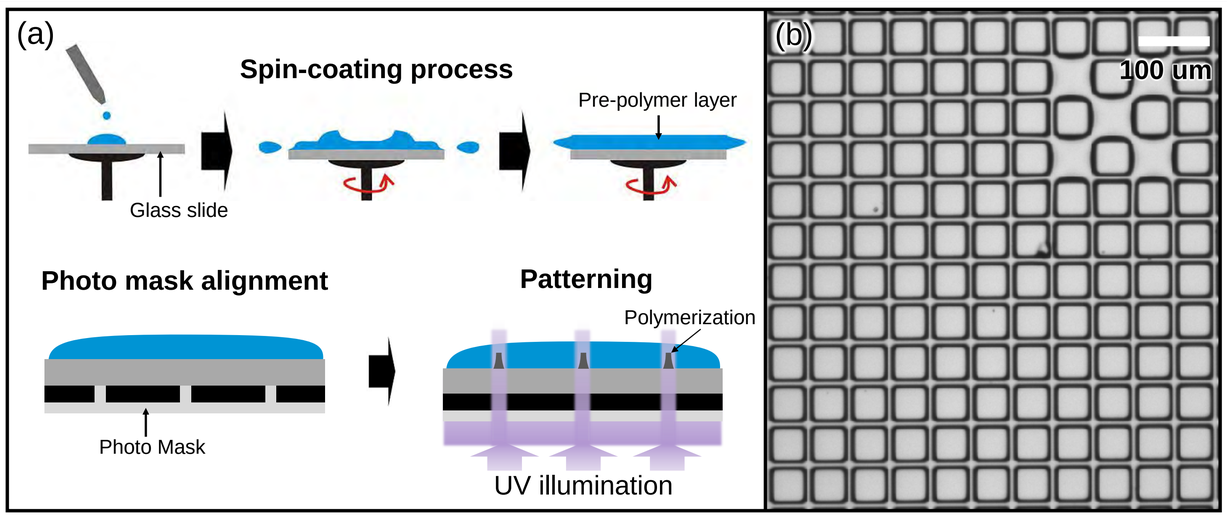
- Understanding the heterogeneity of single pancreatic beta-cells insulin secretion is important to discover the fundamental mechanism of beta-cell failure in diabetes.
- One of the approaches for measuring secretion in single-cell units is to confine individual cells in arrays of nanoliter wells (nanowells) which can provide the advantage of high-throughput analysis.
- we propose the nanowell-based approach to investigate the heterogeneity of single pancreatic beta cells insulin secretion.

Concept

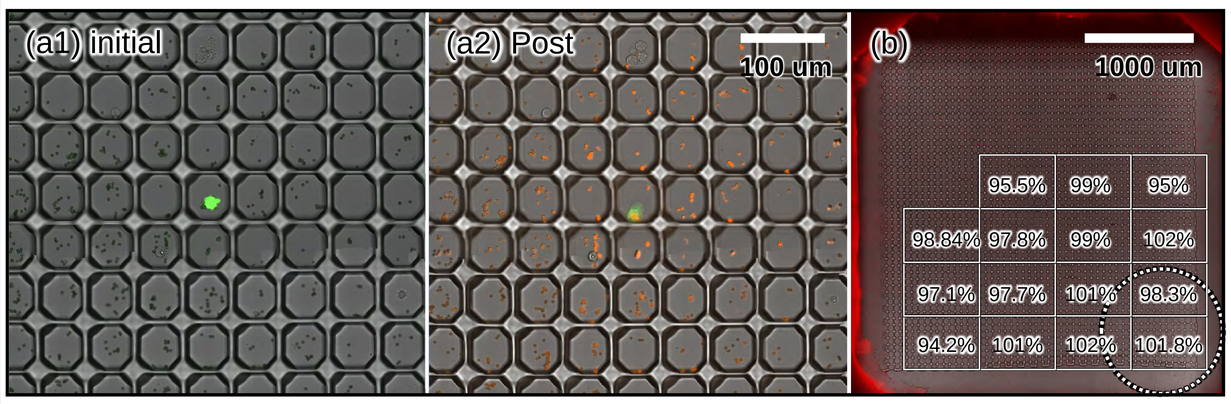


Concept of the proposed nanowell-based approach to investigate the heterogeneity of single pancreatic beta cells insulin secretion. Schematic diagram of (a) nanowell arrays in a microwell; (b) glucose-stimulated insulin secretion (GSIS) assay using nanowells in a microwell; (c) enzyme-linked immunoassay (ELISA)

Nanowell



(a) Fabrication process of nanowell arrays using photolithography process. (b) Fabricated nanowell array.



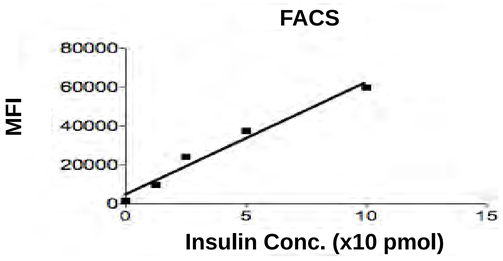
Cell & bead retention test in nanowell while the assay. (a) Capture images of cells and beads seeded in nanowell arrays before and after the assay. (b) bead retention rate of each region in nanowell. note that (the doted circle indicates where the reagents are aspirated and dispensed

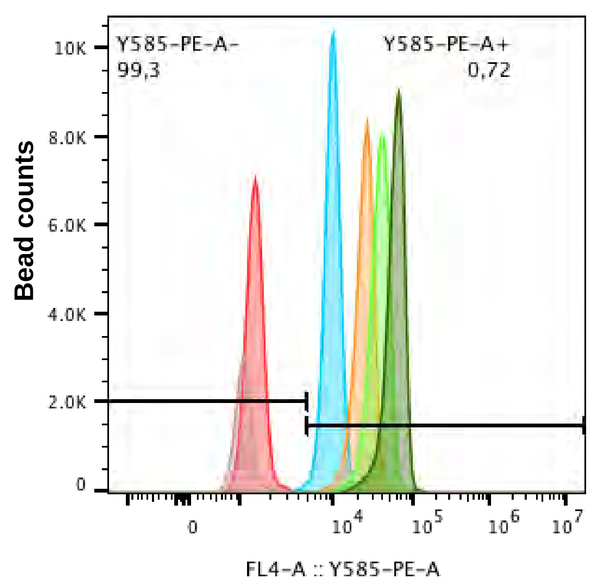
Insulin detection bead

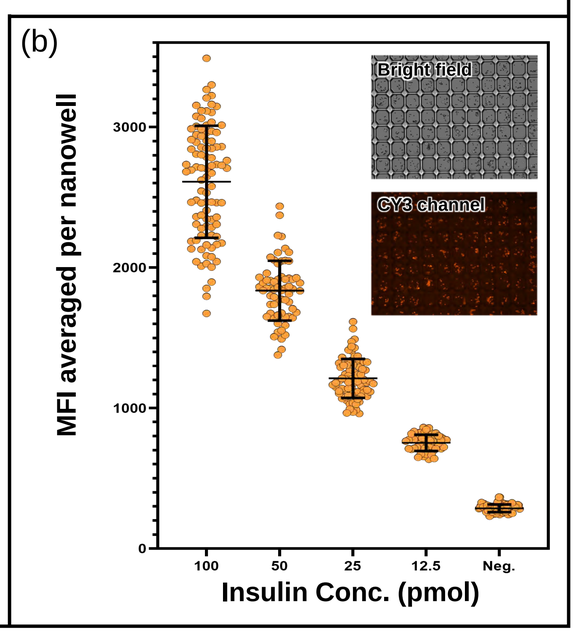
(a)

Color	Case	Bead counts	MFI*
	unstained	35043	1109
	Neg.	82988	1436
	Insulin 12.5 pmol	108117	9686
	Insulin 25 pmol	98691	24110
	Insulin 50 pmol	104540	37499
	Insulin 100 pmol	102332	59857

*Mean Fluorescent Intensity



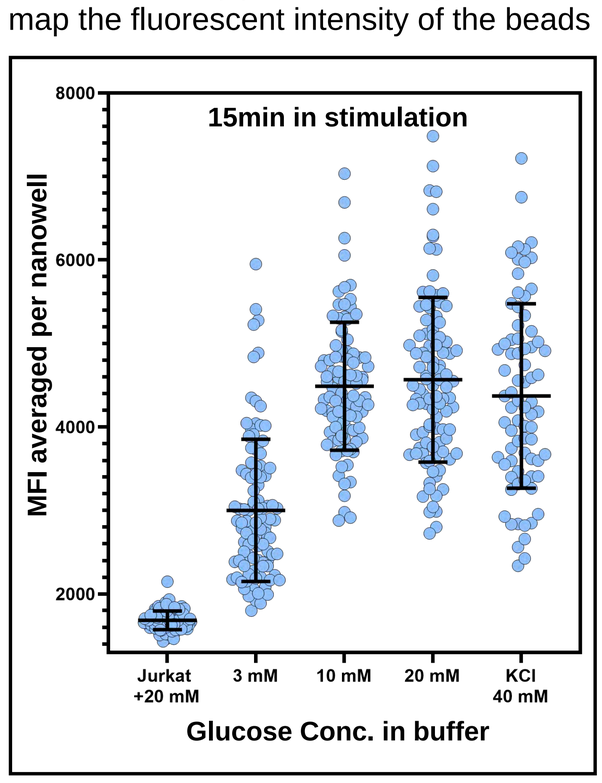




Insulin capture capability of the detection beads: (a) Flow cytometry result; (b) Staining standard curve of insulin-doped beads in nanowells. The beads were stained in tubes and distributed to nanowell arrays

GSIS assay using nanowells in a microwell

A sample result of GSIS assay using nanowells in a microwell (from the case of 10 mM glucose stimulation for 15 min). (a) Initial position of cells and beads seeded in nanowell array. (b) Cells and beads after GSIS assay including ELISA; (c) imaged in CY3 channel; (d) 2D heat map of the fluorescent intensity of the beads; (e) 3D heat map the fluorescent intensity of the beads



The measurement of the signal of the captured insulin after GSIS assay including ELISA. Stem cell-derived β-cells were used for the GSIS assay. Note that glucose stimulation time was 15 min at incubator.

Conclusion

This work present the nanowell-based approach to investigate the heterogeneity of single pancreatic b-cell insulin secretion and phenotype. Owing to the nanowells capable of confining cells and detection bead, it was able to conduct simultaneous profiling of b-cells insulin secretion. These results demonstrate the potential to use this approach for high-throughput secretion and phenotype analysis on single cells.

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