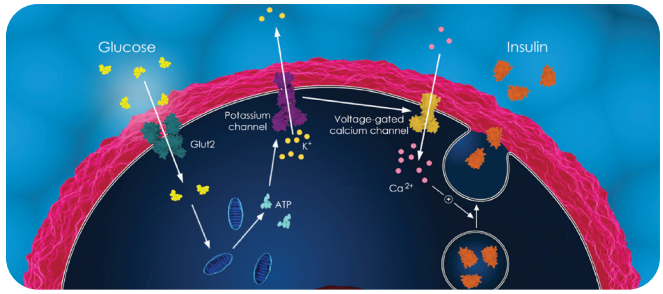
Prolonging β cell proliferation with a 3D culture system

Introduction

Within diabetes research, an evolving area of interest is the identification and development of an efficacious treatment for β cell loss. Cell-replacement therapies including the use of pluripotent stem cell-derived β cells offer a conceptual solution, yet provide a low proliferative capacity. Finding small molecule drug candidates poses challenges due to low β cell specificity leading to proliferation in systemic cells, and cytotoxicity. In order to investigate the efficacy of small molecule candidates, a system representative of the intra and extracellular environment is required. 3D cell culture systems offer the complexity required to assess functionality of the drug candidate at the cell and tissue level, yet lack the simplicity and high throughput screening capabilities of conventional 2D systems.

As β cells exhibit significantly higher levels of zinc than other cell types, the researchers designed a zinc-binding prodrug (ZnPD) with the ability to deliver drugs to β cells in a highly specific and sustained manner based on zinc ion chelation as a mechanism for controlled release. For compound screening using the new ZnPD a cell culture platform is needed exhibiting high zinc ion levels equivalent to physiological conditions. However, standard 2D models lack the level of zinc required to create an appropriately representative model of β cells.



The herein introduced Disque Platform (DP) is a platform technology that bridges the gap between the complexity of the 3D model with the cost and simplicity of a 2D platform. Cell discs are formed by seeding stem cell-derived β cells (SC β cells) in a circular mold where a semipermeable hydrophilic polytetrafluoroethylene (PTFE) membrane coated with a vasculature extracellular matrix is attached. The cell discs are, placed in a 96 well, flat bottom plate. Important features, including cell-cell and cell-matrix junctions, insulin secretion, and gene expression, were maintained in the hybrid 2D simplistic DP.

Validation of disque platform

Validation of the disque platform for cell culture and screening was performed with a dual comparison to a standard 2D format and 3D format using the gold-standard suspension flask culture system (SF). Investigation of DP's ability to reestablish cell-cell interactions and thus higher intracellular zinc ion levels was first performed by assessing the relative expression of e-cadherin (E-cad), connexin 36 (CX36), two junction proteins, and zinc transporter 8 (ZnT8) via quantitative real time polymerase chain reaction (qRT-PCR). As exhibited in Figure 1E, expression levels between DP and SF were consistent whereas the 2D system exhibited significantly lower levels. To determine the long-term cell culture capabilities of DP, pancreatic progenitor cells (PP) were assessed at day 5 and day 18 for cell viability and differentiation. DP and SF exhibited similar results, with expression levels of important β cell markers remaining consistent across platforms. As a means of evaluating β cell proliferation, inhibition of the Notch junction signaling pathway was used to determine whether DP, like SF, could represent PP cell to SC β cell differentiation. SF and DP demonstrated an ability to recapitulate Notch inhibition, whereas the 2D model exhibited no response.

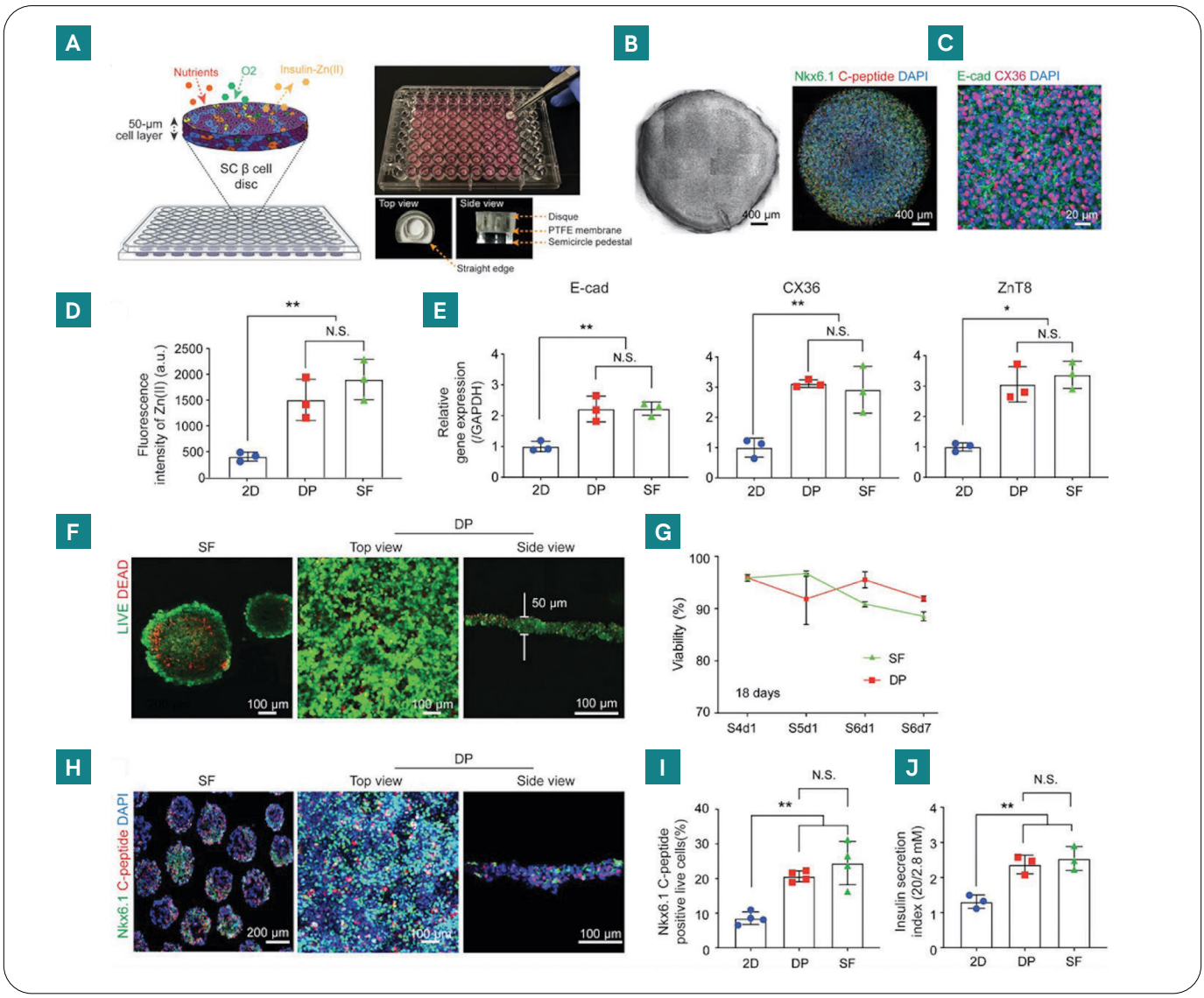


Figure 1: Investigating the validity of the disque platform. Additional method details can be found in the method and figure legend of https://advances.sciencemag.org/content/6/47/eabc3207/tab-figures-data.

Development of ZnPDs for targeted β cell proliferation

As a mechanism of promoting β cell proliferation, Harmine, a DYRK1A inhibitor, was used in conjunction with ZNPD. A three part drug compound including inactivated harmine, a Zn(II)-binding ligand, and a self-immolative linker was created, forming a zinc binding scaffold. Zn(II) binding results in hydrolization of the linker, and an active form of harmine is released into the β cell promoting proliferation. A fluorescent cargo tag was conjugated to the drug to confirm the release of the drug into β cells. Fluorescence could be observed within an hour of Zn(II) binding in a does dependent manner. A lack of fluorescence in PP cells suggests that binding is specific to SC β cells.

High throughput screening of different formulations of the drug were assessed to determine differences in proliferation and cytotoxicity. HTS imaging was performed on Revvity Opera Phenix®, which offers reliable data with speed, sensitivity and high throughput automated workflow systems. Data suggested that rapid delivery and release of harmine within β cells was linked to an increase in cytotoxicity. Using the DP platform to evaluate differences in a side-by-side comparison allowed researchers to generate a drug formulation, ZnPD6, that favored prolonged local concentrations of harmine, decreasing cytotoxicity.

Conclusion

Data evaluating the DP when compared to 3D SF, gold-standard suspension flask culture system, and the standard 2D platform supports the validity of its technology for the evaluation of small molecule drugs for β cell proliferation. DP offers a hybrid platform option, compatible with HTS imaging platforms, with the means of evaluating multiple drug candidates in tandem for efficacy, cell-cell and cell-matrix interactions, with the rapid functionality and cost effectiveness of a 2D system.

References

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