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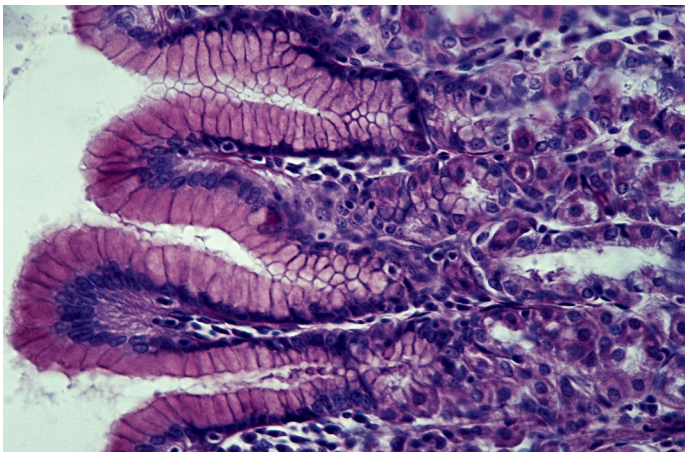
Pancreatic islet cell replacement therapy is a promising strategy for patients with type 1 diabetes mellitus, but the scarcity of organ donors and need for immunosuppression hamper its wide application. Huang and colleagues developed an efficient method to redirect the fate of primary human gastric stem cells, generated from stomach tissue, towards insulin-producing β-cells.

REFERS TO Huang, X. et al. Stomach-derived human insulin-secreting organoids restore glucose homeostasis. *Nat. Cell Biol.* **25**, 778–786 (2023).



Over the past 20 years the shortage of organ donors for allogeneic pancreas or islet transplantation in patients with type 1 diabetes mellitus has sparked the search for alternative sources of insulin-producing cells. Human pluripotent stem cells (hPSCs) are currently at the forefront of this field, and exciting clinical developments from the past 2 years have revealed the first evidence of functional insulin-producing cells after implantation[1,2]. However, long-term safety and efficacy in humans still needs to be established. In a recent report published in *Nature Cell Biology*, Huang et al. describe a potential alternative to hPSC-derived β-cells, in a three-stage protocol to generate islet-size cell clusters that contain 70% insulin-positive cells from primary human adult gastric tissue[3].

Biopsies of gastric mucosa can be easily obtained using a flexible endoscope. The authors showed that SOX9$^+$ human gastric stem cells (hGSCs) derived from the corpus section of the stomach can be grown as 2D culture on post-mitotic feeder cells (mouse embryonic fibroblasts) and that these cultures can be expanded to contain billions of cells within a couple of months. Previous work from the authors[4] had shown in vivo reprogramming of mouse intestine and stomach cells to insulin-producing cells using overexpression of a combination of three key transcription factors that are involved in β-cell differentiation (PDX1, NEUROG3 (also known as NGN3) and MAFA; collectively, 'PNM'). Building on this foundation, the authors adapted their strategy to direct the conversion of primary hGSCs in vitro. Combinations of these factors had previously been reported to induce the conversion of other cell types into insulin-producing cells, including exocrine cells[5] and α-cells[6].

The first step in Huang et al.'s protocol consists of lentivirus-based overexpression of the key endocrine progenitor marker NEUROG3 for 2 days. This step is followed by the co-expression (also lentivirus-based) of the key transcription factors PDX1 and MAFA. The cells are then cultured as a monolayer on a combination of Matrigel and fibronectin. At day 6, the cells are detached and reaggregated in 3D cell clusters that are homogeneous in size (about 1,000 cells each) using microwell plates.

In addition to their potential use for research, hGSC-derived β-cells represent a possible option for future clinical β-cell replacement therapy. Currently, hPSCs are centre stage as an alternative cell source in the context of islet replacement therapy. When compared with hPSCs, hGSCs display a number of attractive characteristics.

> "hGSC-derived β-cells represent a possible option for future clinical β-cell replacement therapy"

First, the yield of insulin-positive cells after differentiation (60–70%) appears to be at least as good as that reported for hPSC-derived β-cells. The hGSC-derived β-like cells displayed some functionality in vitro, and the best glucose-induced insulin secretion was obtained 10–20 days after differentiation. Additionally, cells responded adequately to a glucagon-like peptide 1 analogue. Functionality was further shown by testing the opening (with glibenclamide) and closure (with diazoxide) of ATP-dependent potassium channels, which are an essential component of glucose-stimulated insulin secretion in β-cells. Upon transplantation into an immunodeficient mouse model of severe β-cell failure and diabetes mellitus, the cells can revert hyperglycaemia for over 3 months.

Second, the process of isolating hGSCs and differentiating them into β-cells is faster than generating hPSC-derived β-cells. The differentiation of hPSCs towards β-cells routinely takes about a month,

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whereas hGSC-derived β-cells are generated in about 10 days. The difference is even greater when considering an autologous approach, given that the reprogramming of patient-specific somatic cells to induced pluripotent stem cells takes months (whereas autologous hGSCs can be obtained quickly via biopsy).

Finally, another crucial benefit of hGSC-derived β-cells from a safety point of view is the fact that there is no proliferation found in the grafts even after 6 months in vivo. This is in contrast with some reports of the formation of cystic structures upon transplantation of hPSC-derived islets[7]. Even more importantly, undifferentiated hPSCs can form a tumour (teratoma) when transplanted in vivo, whereas the authors show in this study that undifferentiated hGSCs do not survive when engrafted in vivo — even in a well-vascularized environment, such as the subrenal capsular space.

However, similar to hPSC-derived β-cells, hGSC-derived β-cells generated in vitro display some level of immaturity, including a limited secretory capacity. This reduced secretory capacity is associated with an immature morphology of insulin granules and low levels of *SLC30A8*, which encodes ZNT8 (a zinc transporter that is necessary for insulin storage and secretion). Additionally, stimulus–secretion coupling remains to be further assessed in these cells. Despite the fact that the cells express the maturity marker MAFA, it is surprising that the key β-cell transcription factor NKX6.1 (which is believed to be required for differentiation to a β-cell fate[8,9]) is not upregulated. In fact, the authors show that forced expression of NKX6.1 does not improve the differentiation efficiency. Furthermore, the authors performed single-cell transcriptomics and mapped the steps of the differentiation path that include SOX4$^+$ and galanin$^+$ progenitors. Although a SOX4-expressing progenitor is known to be involved during the development of the pancreas[10], cells that express the neuropeptide galanin are not yet known as major regulators of the pancreatic fate. These apparent discrepancies with classical pancreas development might be related to the source of cells and/or to the developmental trajectory followed in this protocol. Finally, hGSC-derived β-cells display a residual gastric gene signature, which — together with the lack of NKX6.1 and other signs of immaturity — has unknown consequences for the long-term stability of the phenotype of the cells when challenged by stress conditions (for example).

> **"hGSC-derived β-cells generated in vitro display some level of immaturity"**

To conclude, the proof-of-concept study described by Huang and colleagues proposes gastric stem cells as an interesting alternative renewable source of insulin-producing cells for β-cell replacement therapy. Nevertheless, the robustness of the approach needs to be confirmed by independent research groups. Also it will be essential to determine whether the differentiation of lines derived from a greater number of donors can be achieved. Furthermore, important hurdles remain for clinical translation including adherence to good manufacturing practice regulations to ensure the safety of the cell product. hGSC expansion and differentiation culture conditions need further optimization (for example, excluding serum, feeder cells and Matrigel) and, ideally, genome-disrupting lentivirus-based overexpression of transcription factors should be replaced by a small molecule-based protocol.

Finally, other questions that are common to all cell sources for β-cell replacement therapy remain unanswered. Off-the-shelf allogeneic (third-party) cell products might be more interesting than autologous cell products from a cost perspective, but the prevention of immune rejection after transplantation of allogeneic cells requires additional strategies (such as immunosuppressive treatment), and there would be important ethical and legal issues related to ownership and commercial use of allogeneic hGSC products. In addition, remaining questions include how to tackle the issue of β-cell autoimmunity in type 1 diabetes mellitus (especially in the context of autologous transplantation) and identifying the best transplantation site. Furthermore, the procedure should be minimally invasive and the site should allow the engrafted cells to be retrieved in case of adverse reactions. And finally, the long-term safety and efficacy of the cell product should be further established in preclinical models.

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Competing interests

The authors declare no competing interests.