The way to the heart of diabetes is through your gut

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The gut is famously known for its function in food digestion and absorption, but what if we told you that it is also involved in diseases like diabetes? Diabetes is a metabolic disease, which affects over 460 million adults worldwide, where the body struggles to regulate blood glucose levels. The gut releases hormones that help control glucose levels and, when severely obese patients with type 2 diabetes undergo a bypass surgery that rearranges their gut, they see independent weight loss and glucose improvements. Although various treatments are available for patients who suffer from diabetes, there are still many unresolved questions concerning its pathology which means we are yet to find a cure. This article explores how organoids, a 3D stem cell-derived model also known as a mini-organ, might be employed to study the gut’s role in diabetes. Intestinal organoids serve as an effective new model to better understand the disease, thanks to its ability in enabling the cells to arrange in a way that closely resembles the human gut. Using intestinal organoids in diabetes research could lead to new treatment options which are necessary in order to improve the lives of those whose are affected by diabetes every day.

What goes wrong in diabetes?

Diabetes mellitus is a disease characterized by an inability to effectively manage the body’s blood sugar levels. Diabetes-like symptoms were first reported as far back as the Egyptian time period, where physicians reported a strange phenomenon of emaciation, frequent urination and ants being attracted to the patients’ urine owing to excess glucose being excreted. Despite the vast progress of its understanding in today’s modern society, there are still many missing pieces concerning this complex disease which has resulted in the lack of a cure. Both type 1 (T1D) and type 2 diabetes (T2D) are becoming more frequent worldwide and it is expected that by 2025 there will be over 5 million people living with diabetes in the UK. T1D is an autoimmune disorder in which the insulin-producing β-cells of the pancreatic islets of Langerhans are destroyed by the body’s own cytotoxic immune cells. Insulin is released from β-cells into the blood in response to elevated glucose levels following a meal. Importantly, it allows your cells to take in glucose and either use it as an energy source or store it for later use. This process is the key to glucose regulation and without insulin the body remains exposed to continual high glucose concentrations, also known as diabetes. As a result of β-cell death, type 1 diabetics have to inject insulin and monitor their glucose levels for the rest of their life. In contrast, T2D often develops from obesity; here the body becomes insulin resistant, reducing its effectiveness. To compensate, the β-cells work harder to produce more insulin in an attempt to overcome the resistance, which over time causes β-cell failure leading to elevated glucose. Prolonged exposure to increased blood glucose damages blood vessels causing heart disease, eye damage, kidney damage and poor circulation. Ways to manage T2D include weight/diet management, therapeutics that reduce blood glucose levels and gut bypass surgery. However, no current treatment is considered a cure.

The importance of the gut’s role in glucose control/diabetes

The small and large intestines, or the gut, are organs that are part of the gastrointestinal tract whose key role is the digestion and absorption of food to provide energy for living (Figure 1). Although the gut is mostly associated with food digestion and absorption, it has plenty of other exciting functions. For example, the gut acts as a defence barrier and protects the body from pathogens by secreting mucus and antimicrobial molecules from specialized cells that are in contact with the immune system. Moreover, the gut is home to 100 trillion of bacteria, as well as viruses, fungi and protozoa! Together they make up the gut microbiome, but not to worry because the microbiota aid digestion, produce important fermentation products and synthesize key vitamins. It also interacts with the immune system and the brain. Microbiota dysbiosis is believed to be linked to diseases including obesity, T1D...
Diabetes and T2D through mechanisms of glucose metabolism, inflammation and fat metabolism.

Feeling full after your breakfast this morning? This is mostly thanks to your gut which releases satiety signals from its enteroendocrine cells (EECs), specialized solitary hormone-producing cells which are scattered throughout the intestinal tract and endow the crown of largest endocrine organ to the gut. These cells sit in the gut epithelial layer and produce over 20 different peptide hormones. They monitor the luminal contents and convey information regarding nutrient availability, the presence of pathogens and other cues to peripheral tissues, the immune system and the brain (gut–brain axis; Figure 2). In this way EECs help to co-ordinate fuel absorption and utilization but also help protect the body too. As part of this the gut has a key role in glucose regulation; it releases two hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinohiprotic peptide (GIP), which help β-cells secrete an appropriate amount of insulin. Current T2D treatments exenatide and liraglutide take advantage of this mechanism by mimicking the actions of GLP-1. GLP-1 also helps with weight loss by acting on satiety regions in the brain; this in turn ameliorates insulin sensitivity and highlights the important role of GLP-1 in maintaining glucose control. Moreover, gastric bypass is a surgical technique for severely obese patients in which the stomach is reduced in size and part of the gut is rearranged so that it bypasses the first segment of the small intestine, the duodenum. Interestingly, obese patients with T2D show improved glucose sensitivity independent of weight loss. This suggests that the surgery could produce a glucose-lowering signal or signals which if identified could be targeted to design new therapeutics. Although there are various treatment options for people with diabetes, there is still the necessity to better understand the disease in order to fill the treatment gaps and develop a cure.

Organoids: from cells to a mini-organ

Disease modelling in a dish, or in vitro work, is a model of choice for many researchers and its use has contributed to numerous scientific discoveries. Despite its success, growing cells in a dish in 2D is not the most physiologically relevant model, as these cultures lack interactions with other cell types and tissues. It is, therefore, not representative of the environment found in the body. Organoids or mini-organs offer a solution to these limitations, they are a group of stem-cell-derived cells which resemble an organ and form a 3D structure. Today, there are a diverse range of organoids used by scientists; name the organ and the organoid model probably exists. However, the conditions to generate and culture organoids were only discovered a decade ago by Clevers, Sato and colleagues. Their innovative organoid technology was initially developed from adult intestinal crypts and Lgr5+ stem cells that formed spontaneous gut organoids when key factors from the stem cell niche...
were recreated in a dish. These include special growth factors and a 3D matrix which all serve to make the stem cells happy. Importantly, organoids closely resemble the landscape of the normal gut epithelium: being a single cell layer maintained by proliferating stem cells and containing all other expected cell types, including EECs. This single cell layer forms around a central lumen and organizes into distinct crypt and villus regions just like our own intestines (Figures 3 and 4).

But how do you create an organoid model in the lab? Organoids can originate from either adult-tissue-derived stem cells (ASCs) or induced pluripotent stem cells (iPSCs). ASCs are adult cells which retain their tissue-specific, stem cell potential. For example, intestinal organoids can be produced from gut biopsies using any model organism including humans as a source, whereas iPSCs are genetically reprogrammed from specialized adult cells to undifferentiated stem cells and can then be directed to become the tissue of choice. Samples are then cultured with growth factors and suspended in a 3D matrix to copy the endogenous stem cell niche.

Additionally, dependent on which factors the organoids are exposed to, one can control which type of epithelial cell it predominantly differentiates into. This technology has now been adapted by scientists working on other tissues. The power of organoid technology lies in their close resemblance to the tissue they represent both functionally and structurally, the ability to grow cultures over many months to years and their amenability to standard experimental techniques.

Applications of pancreas and gut organoids in diabetes research

Organoid technology opens many different areas of diabetes research ranging from molecular biology (looking at gene regulation) to medically translatable research (transplantation potential and drug discovery). For example, type 1 diabetics lose their β-cells at a young age and current research is trying to produce insulin-secreting organoids, in the hope that these could ultimately serve as a transplantation cure for humans. There are various sources of tissues which have this potential, mainly the pancreas and the gut. Several groups have successfully created insulin-producing organoids from pancreatic tissue; these organoids have shown improved glucose homeostasis when transplanted into diabetic mice. In one of

Figure 3. (a) Diagram that depicts an intestinal organoid. The budding structures are crypts, and the epithelium shows a brush border with villi that are facing the lumen. The different colours represent different cell types expressed by the epithelium. (b) Image of an intestinal organoid in culture, similarly the budding crypts and lumen are visible.

Figure 4. Intestinal organoid stained for the cell membrane (blue) and for endocrine cell markers (pink). The image is taken with a confocal microscope as this microscopy technique enables us to see the organoid in a 3D plane.
these studies from 2018, Takahashi and colleagues co-cultured three different cell types with stem cells which resulted in a vascularized islet-like organoid. The group transplanted these into diabetic mice and found a reduction in blood glucose concentrations which were sustained until graft removal.

The gut and the pancreas have the same embryological landscape, and there is research that focuses on using reprogrammed gut organoids to secrete insulin. Chen and colleagues did just this in 2014 by transducing the transcription factors Ngn3, Pdx-1 and MafA into intestinal crypts; these transcription factors are vital for β-cell development, and they were able to form “neo-islets” with β-cell characteristics. Transplantation of these organoids into diabetic mice resulted in glucose-mediated insulin release. If this were to be successful in humans, it would provide the exciting possibility of taking patient biopsies and reprogram their own cells to produce insulin.

Moreover, intestinal organoids allow the scientific community to better understand the pathology of the human gut in obesity and diabetes. Biopsies obtained from people with obesity and diabetes or from people pre- and post-gut surgery can now be cultured long term in a dish providing experimental access which has not been possible before. For example, the effect of obesity on the function of human gut EECs can be modelled for the first time or the functional benefits accrued by the epithelium following gut surgery might be identified.

As previously mentioned, alterations in the microbiome have been associated with T2D and obesity. Several taxa, including *Bacteroides*, have shown a decreased bacterial diversity in type 2 diabetics compared to healthy controls. Other research has shown the products of microbiota, such as propionate, can trigger the secretion of GLP-1. Intestinal organoids serve as novel tool to study the microbiome, as selected microorganisms can be directly added to the culturing media or injected into the lumen of the organoid. This has the potential to add mechanistic colour to microbiome research in the diabetes field, which to date has relied on associations and correlations.

Furthermore, the prolonged exposure to excess glucose in diabetes leads to vascular damage which can compromise eyesight and kidney function and increase the risk of cardiovascular disease, termed diabetic complications. Organoids derived from the retina or kidney will serve as important models to study the pathology of these complications and identify novel treatments.

**Future direction**

Although organoids have greatly advanced bench side research, they are still not equivalent to an organ in a human body. A recently developed technology, organ-on-a-chip is a platform that provides a human-like engineered environment. The fundamental concept of the chip is to apply a more realistic environment for the desired engineered organ. For example, the gut consists of an epithelium, the microbiota, vasculature and an immune system and experiences mechanical forces from both peristalsis (rhythmic muscular movements which move food through the gut) and flow from lumen contents passing by. With an organ-on-a-chip it is possible to add all of the different tissues within a chamber as well as apply the mechanical forces. These features often bring the model’s functionality closer to that found in the body. The initial organ-on-a-chip models used 2D cell lines and scientists are now trying to use organoids instead. Additionally, there are labs that are striving to combine different organs-on-a-chip to create a body-on-a-chip. To do this, multiple organs can be linked using an engineered vasculature so that they can communicate with each other as they would in you or me. It may one day be possible to model a patient with diabetes on a chip and determine the true role of the gut in diabetes. Science is progressing at an incredibly fast pace; the hope is that by deploying more accurate models of human disease we will be able to better understand disease pathologies including those in diabetes and identify new treatments and cures.
Further Reading


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