



# The Foundation for Engineering a Pancreatic Islet Niche

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Progress in diabetes research is hindered, in part, by deficiencies in current experimental systems to accurately model human pathophysiology and/or predict clinical outcomes. Engineering human-centric platforms that more closely mimic *in vivo* physiology, however, requires thoughtful and informed design. Summarizing our contemporary understanding of the unique and critical features of the pancreatic islet can inform engineering design criteria. Furthermore, a broad understanding of conventional experimental practices and their current advantages and limitations ensures that new models address key gaps. Improving beyond traditional cell culture, emerging platforms are combining diabetes-relevant cells within three-dimensional niches containing dynamic matrices and controlled fluidic flow. While highly promising, islet-on-a-chip prototypes must evolve their utility, adaptability, and adoptability to ensure broad and reproducible use. Here we propose a roadmap for engineers to craft biorelevant and accessible diabetes models. Concurrently, we seek to inspire biologists to leverage such tools to ask complex and nuanced questions. The progenies of such diabetes models should ultimately enable investigators to translate ambitious research expeditions from benchtop to the clinic.

**Keywords:** microphysiological systems, islet-on-a-chip, diabetes modeling, hydrogels, islet, islet physiology

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## 1 INTRODUCTION

Diabetes mellitus is considered one of the largest growing healthcare challenges of the 21<sup>st</sup> century, leaving an ominous global burden on public health. In the past two decades, the number of adults living with this disease has more than tripled and a continuous rise to more than half a billion adults with diabetes by the end of this decade is projected (1). The diagnosis of diabetes occurs when the human body is not able to produce adequate insulin or when cells fail to respond effectively to insulin, causing systemic dysregulation of blood glucose levels. Diabetes can be considered a syndrome that involves many organs/organ systems (*e.g.*, pancreas, liver, adipose tissue, and the immune system). Additionally, health complications can arise from sustained hyperglycemia, including severe damage to the heart, kidney, nerves, vasculature, and retina. This ultimately reduces the life expectancy of individuals with diabetes and imparts global socio-economic impacts. Current treatment options for diabetes range from administration of oral medications to experimental cell-based therapies.

**Abbreviations:** T1D, Type 1 diabetes; T2D, Type 2 diabetes; ECM, Extracellular matrix; BM, Basement membrane ECM; IM, Interstitial membrane ECM; ECs, Endothelial cells; ROS, Reactive oxygen species; hiPSC, human induced pluripotent stem cells; SC-βCs, Stem cell-derived β-like-cells; 3D, three-dimensional; MPS, microphysiological systems.

Biomedical research efforts, supported by governmental, philanthropic, and industry sectors, have made significant contributions to our understanding of the physiology and pathologies associated with diabetes; however, major knowledge gaps remain that negatively impact our progress towards a cure. Type 1 diabetes (T1D), for example, is an autoimmune condition where the body is unable to produce sufficient insulin because of a selective autoimmune-mediated attack on the insulin-producing  $\beta$ -cells. Genetics plays a major role in T1D, with over 150 loci linked to T1D susceptibility (2, 3). While genes such as Human Leukocyte Antigen Class II loci are clearly major players in T1D, at the time of writing, the exact role of the genes implicated in T1D pathogenesis has not been fully established. Furthermore, while researchers have gained insight into the peripheral immune and endocrine changes that precede disease diagnosis, the specifics of T1D initiation and subsequent propagation in the pancreas and pancreatic lymph nodes remain undetermined (4). Furthermore, it is unknown if  $\beta$ -cell dysfunction precedes lymphocytic interactions or whether this is a secondary effect. In Type 2 diabetes (T2D), the initiation, progression, and long-term mechanisms are also not well understood (5). People with T2D are challenged in the physiological control of glycemic homeostasis, at least partly due to insulin resistance. In the study of T2D pathophysiology, numerous questions remain, such as the degree of permanent  $\beta$ -cell mass loss, lipotoxicity kinetics in  $\beta$ -cell destruction, and the nuances of the timeline for insulin resistance. Evidently, diabetes is a complex disease initiated and propagated by unknown factors. Gaps in our knowledge delay efforts to find long-standing and effective treatments.

The endocrine organoids at the center of diabetes are the insulin-producing pancreatic islets of Langerhans. Islets are composed of multiple cells type and are responsible for maintaining blood glucose homeostasis. These manufacturing facilities secrete several endocrine hormones necessary for metabolic regulation. Examination of the scientific literature reveals a remarkable understanding of islet micro-architecture, including cellular composition, cell-cell junction physiology, and its surrounding peri-islet extracellular matrix (ECM). Access to such knowledge took decades of research, as methods for the investigation and/or interrogation of islets are limited by material access, species variance (*i.e.*, human vs. other animals) (6), and relevance to native physiology, as well as other factors such as efficiency, cost, and reproducibility. Key obstacles in diabetes research are at least in part due to the insufficiency of current models, such as animals and 2D cultures, in supporting clear experimental control and/or outputs when testing diabetes-relevant hypotheses. Thus, there is a need to engineer additional benchtop platforms that provide further insight into normal and diseased pancreatic tissues.

Designing new solutions to a problem using engineering design processes first demands conducting extensive background research. For biomedical engineering problems, this first starts from understanding the normal anatomy and physiology of the tissue of interest. This foundational knowledge can then be used to identify key design parameters and/or targets. Next, a

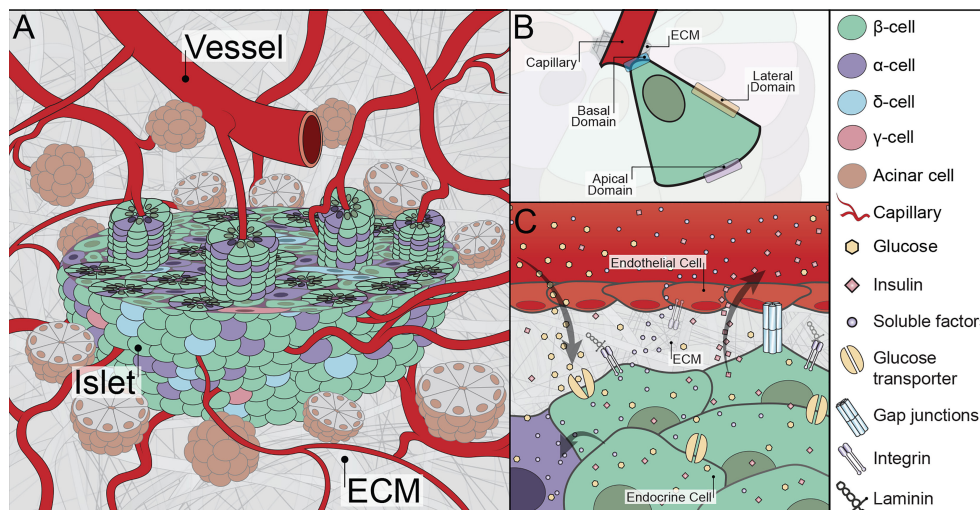
comprehensive understanding of conventionally available techniques and/or approaches is needed to identify their critical obstacles or deficiencies, as well as costs and market share. From this background knowledge, clear design requirements and engineering criteria for creating prototypes are identified. This review seeks to outline this said engineering process. The first section is dedicated to summarizing the native pancreatic niche, highlighting key unique features. Traditional methods of studying diabetes pathogenesis and potential therapies are also discussed, including their advantages and disadvantages. Subsequently, we summarize alternative strategies that elevate traditional culture methods by employing fluidics and 3D matrices. Lastly, we consider challenges in the adoption, scale-up, and translation of these platforms, as well as key future efforts in modeling the islet niche.

## 2 THE MICROENVIRONMENT OF THE PANCREATIC ISLET

### 2.1 General Islet Anatomy

The overall function of the pancreas is to support macronutrient uptake and homeostasis. The adult pancreas can be macroscopically segmented into four major sections: the head, neck, body, and tail. Within the organ, two key architectures are present: the acini and pancreatic ducts that provide the exocrine function to aid digestion in the small bowel; and islets that deliver endocrine function for carbohydrate regulation in the circulation. The majority of the pancreatic tissue is composed of acini, whose secreted digestive enzymes drain into the duodenum of the digestive system. Besides the exocrine tissue, other structures, including blood vessels (arteries, veins, and capillary systems), lymphatic vessels, and nerve fibers (sympathetic and parasympathetic) are also present (7). The interstitial space between each of these components is filled with an ECM. While a pancreas is considered a multi-functional organ, endocrine islets are the key pancreatic tissue of interest as it relates to diabetes.

Endocrine pancreatic islets are dense cellular islands intermingled within the exocrine pancreatic tissue and are easily distinguished by their high vascularization and a distinct layer of ECM coating (**Figure 1A**). Roughly, 1 – 15 million islets (8, 9) are distributed throughout the human pancreas without a clear pattern in terms of regional localization, orientation, and/or size (9, 10). An average islet is 150  $\mu\text{m}$  in diameter with roughly 1500 cells (11), but cluster sizes can vary from as small as tens of microns to as large as half a millimeter in diameter (12). Islet clusters contain a collection of endocrine cells with species-specific differences in their contribution and spatial position. For example, immunohistochemical analysis estimates 60-80%  $\beta$ -cells, 15-20%  $\alpha$ -cells, and <10%  $\delta$ -cells for mouse islets, while human islets contain 50-75%  $\beta$ -cells, 25-35%  $\alpha$ -cells, and 10%  $\delta$ -cells (6, 13–15). Additionally, the mouse  $\beta$ -cells appear to be centrally clustered within the islet, while in human islets  $\beta$ - and  $\alpha$ -cells exhibit a less-segregated distribution (15, 16). Cellular



**FIGURE 1** | Graphical illustration of relevant islet physiology. **(A)** The native peri-islet space consists of acinar cell clusters surrounding the pancreatic islets. The islets have an afferent blood vessel adjacent to their structure that quickly converts into tortuous capillary systems. A cross-section of an islet highlights the rosette-like architecture composed of endocrine cells wrapped around a capillary. The interstitial space adjacent to the islets also consists of ECM. **(B)** Intra-islet architectural organization in a rosette-like structure where distinct faces of an endocrine cell with respect to the capillary are visualized. **(C)** The subcellular interface of islets showing relevant molecules and cell-cell and cell-matrix interactions. Illustration not drawn to scale.

compositions can also change with respect to size and anatomical location within the pancreas. For instance, smaller islets contain predominantly  $\beta$ -cells, while larger islets are likely to have other cell types present (17). In rat pancreas,  $\alpha$ -cells are found largely in islets located at the tail of a pancreas while  $\gamma$ -cells are predominately found within islets from the head of the pancreas (18, 19). In addition to endocrine cells, islets also contain other cell types (e.g., vascular, mesenchymal stromal, immune, and neural cells; see **Table 1**). Globally, the variances in islet composition due to species, size, and location should be taken into consideration when conducting *in vitro* analysis of islet physiology.

In addition to cellular composition, the features of the ECM surrounding and within pancreatic islets are notable. Islet ECM is found within two distinct locations: 1) peri-islet ECM, which separates the intra-islet cells from the exocrine tissue; and 2) vascular ECM, which encases the intra-islet capillaries and serves as the interface between endocrine and endothelial cells (ECs) (see **Table 2**). The peri-islet ECM is comprised of both a basement membrane (BM) and an interstitial membrane (IM) ECM. The peri-islet BM is mainly comprised of collagen IV and laminins, with collagen providing structural integrity and

laminin imparting biological activity and cell signaling (16, 21, 28). Other ECM molecules are also present and contribute to its organization and function (**Table 2**) (16, 21, 27). For example, collagen and laminin self-assemble with either nidogen or perlecan to create a tight network (23). ECM subtypes within the peri-islet BM can be species-dependent. For example, laminin isoforms shift from LM-411 and LM-211 in mice to LM-511 and LM-211 in humans (20, 21, 23). Surrounding the peri-islet BM is the peri-islet IM, which is a looser network of proteins composed primarily of fibrillar collagens (i.e., col-I, col-III) with other matrix molecules (**Table 2**) (21, 24, 29).

Within the islet, ECM appears to be exclusively relegated to the outer coating of intra-islet capillaries (25). In mice, only a single layer of vascular BM is detected. In humans, two BM layers are present, with the outer layer arising from the invagination of the peri-islet BM. The major ECM components of the intra-islet ECM are collagens and laminins (16, 21, 22, 26) (**Table 2**). For human islets, the ECM components on either side of the double membrane vary. For example, the laminin composition interacting with endocrine cells is dominated by LM-511, while the side facing endothelial cells is a composite of LM-411, -421, -511, and -521 (26).

**TABLE 1** | Current understanding of different cells residing within the pancreatic islets. [Adapted from (17, 18)].

#### Cell Types within the Pancreatic Islet

##### Endocrine cells

##### Vascular cells

##### Mesenchymal stromal cells

##### Immune cells

##### Neural cells

$\alpha$ -,  $\beta$ -,  $\delta$ -,  $\gamma$  [pancreatic polypeptide (PP)],  $\epsilon$ -, ghrelin-cells  
 Endothelial cells (ECs), pericytes, vascular smooth muscle cells  
 Fibroblasts, myofibroblasts, adipocytes, duct cells  
 Granulocytes, lymphocytes, resident macrophages, dendritic cells, mast cells  
 Neurons, Schwann cells

**TABLE 2** | Currently identified major ECM types in adult pancreatic islets. Species-specific information is included in the table when appropriate with literature references.

Peri-Islet ECM		Vascular Intra-Islet ECM
<b>Peri-islet BM</b>	<b>Peri-Islet IM</b>	<b>Vascular BM (mice) and Vascular BM + Peri-islet BM (humans)</b>
Collagen-IV [ $\alpha 1$ & $\alpha 2$ in mouse islets (20)]	Collagen-I, -III, -VI (21)	Collagen-III, -IV, -VI (16, 21, 22)
Laminins [LM-211, -411 in mouse islets (21); LM-511, -521 in human islets (23)]	Fibrillin-2 (21)	Laminins (LM-211, -221, -411, -421, -511, -521 in human & mouse islets) (24–26)
Heparan Sulfate Proglycans ( <i>i.e.</i> , Perlecan) (21)	Tenascins (21)	
	Matrilin-2 (24)	
	Chondroitin (21)	
	Dermatan Sulfate	
	Proteoglycans (21)	
Nidogen-1, -2 (21)	Vitronectin (21)	
Fibronectin (27)	Fibronectin (21)	

There is a growing interest in understanding the cells primarily responsible for secreting ECM molecules within and around the pancreatic islet. Evidence suggests that ECM is secreted by ECs and not  $\beta$ -cells (25, 30–32). However, other vascular cells, mesenchymal stromal cells, or/and even neural cells (*i.e.*, pericytes, fibroblasts, myofibroblasts, or Schwann cells) may contribute to ECM production (21, 33).

It is well established that the interaction of islets with the ECM can regulate cell survival (34–37), proliferation (16, 38), and insulin secretion (39–41). The prompt loss of peri-islet ECM, *e.g.*, collagen IV, laminin, and perlecan, during the enzymatic digestion process of islet isolation leads to the induction of islet cytotoxicity and dysfunction (42). A correlation between the islet ECM and T1D has also been reported, where T1D pathogenesis is associated with both alterations in ECM composition and autoreactive T cell-mediated ECM degradation (21, 43). Overall, when designing systems for studying pancreatic islets, special care should be given to the preservation of its unique anatomical, cellular, and ECM components.

## 2.2 Pancreatic Islet Vascularization

Within the pancreas, islets are distinguished by their robust vascular network, which plays a key role in both nutritional support and function. Vascular perfusion of the islet is remarkable; globally islets receive up to 23% of the overall pancreatic blood flow despite comprising only 1–2% of an entire pancreatic mass (44–47). Studies in rat islets estimate an average islet blood flow rate of 69  $\mu\text{l}/\text{min}/\text{pancreas}$ , which increases up to 125  $\mu\text{l}/\text{min}/\text{pancreas}$  in response to elevated blood glucose (47, 48). Copious islet perfusion serves to provide optimal glucose sensing to facilitate insulin or glucagon release, as blood exiting the islet flows through the branches of the descending aorta and drains to the portal vein (49, 50). High blood flow capacity also supports the substantial metabolic demands of the islet (51, 52).

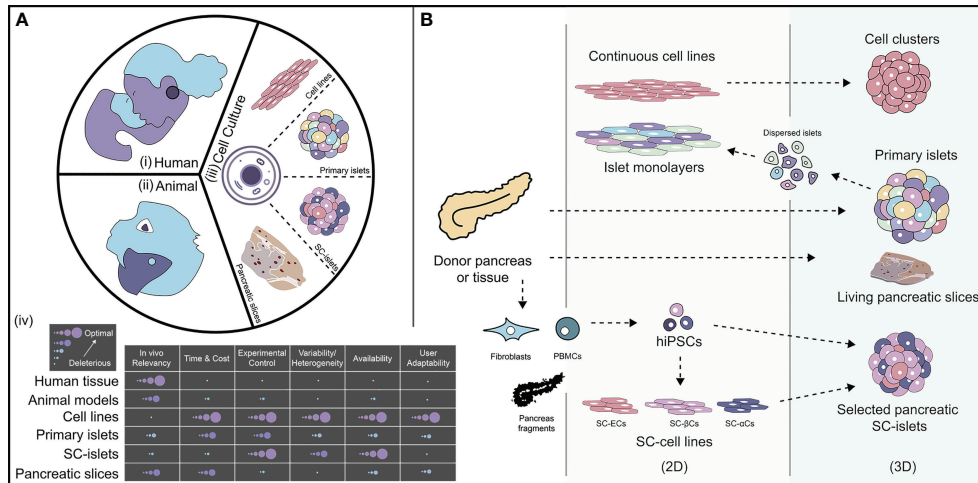
Beyond arterial and venous blood flow, interstitial flow between the individual cells of an islet further facilitates nutrient exchange and paracrine signaling. The interstitial flow velocity of physiological tissues is generally postulated to range from 0.1 – 2  $\mu\text{m}/\text{s}$  (53); however, islet-specific interstitial flow parameters have not been directly measured. From a fluid mechanics perspective, the shear stress experienced by

individual islet cells within their niche is expected to be orders of magnitude lower than the shear stress exerted on the ECs lining the vascular conduits (*i.e.*, arterioles and venules). Unlike the rest of the pancreas, however, islets may be largely deficient in lymphatic capillaries (54, 55). This could play a significant role in altering theoretical shear stresses and pressures within the islet interstitial space, particularly during a high glucose challenge.

The architectural design and cellular organization of the vascularized islet are distinctive. The afferent vascular arterioles entering an islet are converted to a dense and highly fenestrated network of capillaries (**Figure 1A**) (56). Capillaries are comprised of an inner layer of endothelial cells and an outer coating of pericytes and vascular ECM (57). These micro-vessels have 20–30% larger diameter than those in exocrine pancreatic tissue and are intertwined between the endocrine cells (18, 58). In rats,  $\beta$ -cells are reported to always be adjacent to at least one capillary ECs (59). In humans, this is less dogmatic, as intra-islet capillaries are primarily surrounded by  $\alpha$ -cells (60). Human  $\beta$ -cells can be found two or even three cell layers away from a capillary (61); however, the connection between human  $\beta$ -cells and ECs is dynamic, with some  $\beta$ -cells found extending between  $\alpha$ -cells to reach the outer capillary BM (61). The intimate interface of endocrine and endothelial cells is architecturally accomplished by their organization in a rosette-like structure around the blood capillary (**Figure 1B**) (17, 59, 62). In addition, another set of capillaries follows a tortuous path around the periphery of the rosette-like structure (59). It is presumed that the capillaries surrounding the rosette are arterial capillaries delivering metabolites, while the capillaries at the central lumen of the rosette are venous capillaries transporting waste away from the cells (59, 62); however, further definitive evaluation is required.

## 2.3 Distinct Islet Subcellular Interfaces

Intra-islet subcellular interfaces contain cell-cell junction proteins, transport channels, soluble factors, and ECM molecules that coordinate individual cellular responses into collective islet function (**Figure 1C**). For example,  $\beta$ -cells synchronize  $\text{Ca}^{2+}$  oscillations to efficiently initiate and promptly stop insulin secretion. Within the rosette structure surrounding the intra-islet capillaries, endocrine cells are



**FIGURE 2** | The landscape of how diabetes is studied and cell sources available for researchers. **[A (i-iii)]**: Currently available models to study diabetes, such as human clinical trials, animal pre-clinical models, and *in vitro* cell culture systems. **[A (iv)]** Relative breakdown of different characteristics of each available model for diabetes to highlight key advantages and disadvantages. **(B)** An outlook of available cell sources for diabetes modeling separated by 2D and 3D morphology. PBMCs, peripheral blood mononuclear cells; hiPSCs, human induced pluripotent stem cells; SC, Stem cell-derived; ECs, endothelial cells;  $\beta$ Cs,  $\beta$ -like-cells;  $\alpha$ Cs,  $\alpha$ -like-cells. Illustration not drawn to scale.

delegated into three distinct faces, with a corresponding physiology that reflects their architectural design: i) the edge near the ECs (the basal domain), ii) the membrane laterally connecting to neighboring endocrine cells (the lateral domain), and iii) the edge furthest away from the ECs (the apical domain) (**Figure 1B**) (63). The basal domain of the cell is unique in that it directly interacts with the vascular ECM. As such, cellular adhesion molecules are largely ECM binding receptors, such as integrin  $\beta$ 1 (23, 25, 64). It has been proposed that the majority of insulin granule fusion and release is targeted at the basal domain (65, 66). Furthermore, published reports indicate a link between the engagement of integrin  $\beta$ 1 and elevated  $\beta$ -cell proliferation and function (26, 64, 67). Moving away from the capillary and into the lateral domain, cell surface proteins shift to cadherins (*i.e.*, E-Cadherin), connexins (*i.e.*, Cx36, Cx30.2), and ephrin complexes (*i.e.*, ephrin-Eph), which are more common in cell-cell interactions (63, 68, 69). Both cadherins and ephrins play critical roles in the preservation of  $\beta$ -cell function. As disruption of these interactions, *e.g.*,  $\beta$ -cell dissociation, leads to loss and/or dysfunction in glucose-stimulated insulin secretion (69–71). Connexins, transmembrane proteins that form gap junction channels and provide intracellular communications, are also found within discrete locations in the pancreatic islet (68). Finally, the apical domain consists of primary cilia (*i.e.*, acetylated tubulin) and tight junction proteins (*i.e.*, ZO-1, PAR-3) (63). Select reports implicate cilia dysfunction in T2D development (72). Altogether, it is clear that the subcellular interactions within the islet are not random and appear coordinated around the capillary network.

The interface of  $\beta$ -cells with other endocrine cells contains transport channels that passively or actively facilitate the

diffusion of key molecules such as glucose, calcium, and sodium to support insulin secretion (**Figure 1C**). Classically known glucose transporters play key roles in the passive diffusion of glucose. Moreover, hormones released by non- $\beta$  endocrine cells not only contribute to whole-body glycemic regulation (*e.g.*, pancreatic polypeptide regulates food intake and glucagon directs liver-facilitated glucose release) but also provide local regulation of islet responses (*e.g.*, somatostatin release by  $\delta$ -cells inhibits insulin and glucagon secretion in  $\beta$ - and  $\alpha$ -cells, respectively). Additional non-hormonal soluble agents (*e.g.*, hepatocyte growth factors, angiogenic growth factor, and endothelin-1) are also found at the islet subcellular interface and contribute to cellular regulation (68, 73).

Gaining a clear understanding of pancreatic islet anatomy and physiology, ranging from the macroscopic niche to subcellular interfaces, provides a template for identifying key parameters that are important to preserve or mimic *in vitro*. As islets are complex micro-organs that are impacted by significant species-dependent heterogeneity in general anatomy, there is no such thing as a stereotypical islet. Within the native islet niche, islet cells are embedded within ECM that serves to anchor the tissues into 3D space for cellular migration and remodeling. Islet vasculature is key in supporting the metabolic demands of the cells and for optimal sensing and release of endocrine hormones. Lastly, subcellular features within islets and their polarity for specific interactions with respect to the vasculature provide critical cues in supporting islet functionality. When engineering the islet niche, these complexities will create difficulties in the generation of a wholly physiological system; however, hypothesis testing can allow for their refinement in assembling specific features to inform and/or predict *in vivo* outcomes.

### 3 HOW TO STUDY DIABETES?

The study of any disease generally starts with models as simple as cells in test tubes, which progress in complexity through small and large animal models and ultimately to the clinical setting with human subjects. Here, we outline key assets available in the study of pancreatic islet physiology and pathology (**Figure 2A**). We also provide historical perspectives, when relevant, and denote the advantages and disadvantages of each model.

#### 3.1 Acquisition and Study of Human Samples

Within the United States, both government and private foundations have established resources for the distribution of human pancreatic islets procured from brain-dead organ donors [**Figure 2A(i)**]. The National Islet Cell Resource Center (ICR) consortium, founded in 2001, is the largest organized effort in the world and globally provides human islets for research (74). Since 2009, ICR operates as the Integrated Islet Distribution Program (IIDP), which is currently funded by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (75). The IIDP utilizes five U.S.-based certified islet isolation centers for the procurement of islets from cadaveric organ donors where, as of 2022, they have cumulatively made nearly 15,000 shipments of islets to researchers (76). Since 2016, participating investigators who receive human islets have access to The Human Pancreas Analysis Program (HPAP) database (77). Anonymized information available includes, clinical and donor islet phenotypic data (e.g., pre-shipment islet preparation information, histology, mass cytometry, oxygen consumption, functional perfusion, calcium imaging, and electrophysiological measurements), as well as epigenomic data (e.g., islet cell sequencing data and immune cell characterization), to better contextualize donor characteristics. The HPAP database is further sectioned into three branches: i) Pancreatlas (78), ii) The Human Islet Phenotyping Program (HIPP) (79), and iii) Diabetes Epigenome Atlas or The Human Islet Genotyping Initiative (HIGII) (80). An additional resource is The Human Protein Atlas, which provides proteomics information of the human pancreas and other tissues (81). Together, these portals serve as detailed and comprehensive resources of information regarding human islets procured from non-diabetic and diabetic donors (i.e., T1D, T2D, and monogenic forms of diabetes). Moreover, the Network of Pancreatic Organ Donors with Diabetes (nPOD) biobank provides live and fixed samples from the pancreas, spleen, lymph node (including pancreatic lymph node), thymus, and bone marrow from cadaveric organ donors, with, without, or at-risk of developing T1D (82). In addition, there are commercial-based sources of human islets and T1D-relevant tissues [e.g., Prodo Laboratories (83)]. Most importantly, access to these precious materials and the scientific knowledge held within is only possible due to altruistic donations. The scientific community expresses our highest gratitude to these donors and their families.

Studying diabetes using human samples can provide the most relevant information in regards to a human disease. However, the total number of donors is limited in terms of procurement,

costs, consistency of product, and capacity for durable interrogation [**Figure 2A (iv)**]. The primary source of human pancreatic tissue for pathophysiological studies is deceased donors, which become available at discreet and unpredictable time points. While biopsies from non-terminal patients undergoing partial pancreatectomy are occasionally available (84), this is an unreliable option for non-diseased or damaged pancreata. The elective procurement of pancreatic biopsies from otherwise healthy T1D individuals is problematic, as illustrated by the Diabetes Virus Detection Study (DiViD), which was terminated due to serious complications (85). Overall, mechanistic temporal studies of diabetes pathogenesis using living human models are limited to peripheral blood cells and proteins. Thus, there is a significant limitation in the type of queries that can be made using human subjects, as well as the supply and reliability of human T1D tissues (86).

#### 3.2 Animal Models

Animal models, including their explanted cells and tissues, have been used in basic science to study or understand biological phenomenon since the fourth century before the Common Era [**Figure 2A (ii)**]. In diabetes research, animal models have provided major discoveries resulting in clinical advances in the treatment of diabetes, as well as a basic understanding of disease initiation and propagation factors. Diabetes can be studied in animal models using either strains that spontaneously develop diabetes or genetic- or virally-induced models (87, 88). The most common strains for spontaneous T1D are the non-obese diabetic (NOD) mouse and the BioBreeding Diabetes-Prone (BB-DP) rat models (88, 89). These two strains, and their derivatives, exhibit genetic and environmental features that are relevant to human T1D, such as the essential role of specific MHC alleles and the emergence of  $\beta$ -cell-specific autoimmune antibodies against insulin, glutamic acid decarboxylase (GAD), and islet cell antigen 512 (IA-2), as well as autoreactive helper ( $CD4^+$ ) and cytotoxic ( $CD8^+$ ) T cells that recognize  $\beta$ -cell antigens similar to the human condition (90). The induction of T2D involves using obese and non-obese models that render insulin resistance and/or  $\beta$ -cell failure (87). Some commonly used spontaneously developed T2D models are strains carrying *Lepr<sup>db/db</sup>*, Kuo Kondo (KK)-, and New Zealand obese (NZO)-mice, which exhibit hyperglycemic symptoms and develop insulin resistance (87, 91). Diet-induced obesity is routinely used in mice, rats, and hamsters (e.g., Israeli sand rats); however, there are challenges with human relevancy with respect to diet and lifestyle. Alternatively, insulin-dependent diabetes can be induced *via*  $\beta$ -cell removal through chemical targeting and destruction with streptozotocin (STZ) or alloxan (AL), or through complete pancreatectomy (90, 92). Injurious  $\beta$ -cell killing or removal provides the advantage of inducing a predictable and overt diabetic state within any animal, which is useful for examining the efficacy of cell therapies and/or the study of long-term complications arising from hyperglycemia. However, this approach is not appropriate for the interrogation of autoimmune-related immunological pathways or for the study of  $\beta$ -cell dysfunction.

While not fully representative of human pathophysiology, the value of using animal models is their capacity to invasively investigate complex biological pathways that are not feasible in human subjects. Various mouse strains permit the interrogation of genetic risk, environmental cues, and therapeutic interventions within a model supportive of temporal analysis and enhanced experimental control [Figure 2A (iv)]. It also delivers a full spectrum of *in vivo* cues that are impossible to recapitulate *in vitro*. Furthermore, as imaging modality capacities are enhanced, unprecedented insight into temporal and *in situ* phenomenon is now feasible (93, 94). Research using T1D-relevant murine strains has resulted in significant contributions in the understanding of insulinitis,  $\beta$ -cell stress, and islet-relevant immunological responses, as well as in the optimization of cell therapy for the treatment of T1D (89, 95). Further advancements in the genetic engineering of murine strains, and in efforts to generate humanized mouse models, will enhance their utility and relevancy (96). Finally, the validation of the safety of therapeutic approaches in small and large animal models is typically necessary in the regulatory approval of clinical trials.

The use of animals, from their cells and tissue explants to whole organism models, for understanding human-based phenomenon has its limitations. Animal models possess key phenotypic and genotypic differences, along with variances in anatomy and physiology, metabolism, and immune responses. Cell distribution, composition, and function vary with respect to the animal species, which challenges the general extrapolation of results to humans (97, 98). Glycemic control, processing, and diet also play a role in the clear interpretation of impact (92). Furthermore, experimental handling challenges should be considered. Different cohorts of the same strain of mice exhibit variation in basal glucose levels, the sensitivity of STZ, insulin secretion abilities, and sex differences, while the immunological maturity of mice and rats bred within pathogen-free facilities is depressed (92, 99). Thus, care should be taken in designing an interventional study and interpreting its results. Finally, the detailed interrogation of cells and tissues within the living animal is limited. This has motivated the use of *in vitro* cellular models, which provide a different perspective in studying diabetes.

### 3.3 Cell Culture Models | Cell Source

A wide range of cells and tissue subtypes are grown in laboratory incubators, from two-dimensional (2D) monolayer cultures to complex multicellular 3D tissues. These biologic sources generally exhibit advantages of simplicity, low cost, reduced variability, ease in manipulation, and robustness in high throughput screening [Figure 2A (iii)]; however, the simple culture of isolated cells and tissues within basic cell culture flasks or dishes is insufficient to fully and durably support native cellular phenotype. Herein, we discuss categories of cell and tissue sources available for diabetes research and globally highlight their benefits and pitfalls (Figure 2B).

### 3.4 Continuous Cell Lines

As  $\beta$ -cells play a predominant role in the development of both forms of diabetes, numerous  $\beta$ -cell lines that mimic various features of glucose-responsive insulin secretion have been

established and/or engineered (100, 101). In general, most cell lines are tumor-derived or generated from the incorporation of viruses into fetal, neonatal, or adult pancreatic fragments (102). The advantages of these continuous  $\beta$ -cell lines are ease in culture and expansion, making them a convenient source for screening and testing new approaches or for the interrogation of distinct pathways (102).  $\beta$ -cell lines can be cultured as either 2D monolayers or 3D organoids, providing flexibility in culture formats. Many also exhibit sensitivity to immune-mediated cell death (102). Results from these cells, however, must be contextualized by their limitations. For example, the kinetics of insulin secretion and glucose sensing in continuous  $\beta$ -cells are generally non-physiological, as most exhibit either hypersensitive or insensitive insulin kinetics to glucose challenges. Their oncogenic nature also requires care in the direct extrapolation of results exploring  $\beta$ -cell stresses or cytotoxicity screens. Additionally,  $\beta$ -cell lines lack multi-cellularity and ECM, which likely contribute to native  $\beta$ -cell responses. Efforts to enhance cell line models include the integration of other cell types into the 2D monolayers or 3D organoids (103–105) and the culture of cells on ECM coated surfaces (64, 106). While continuous  $\beta$ -cell lines are highly useful and efficient for testing and screening, their lack of native human islet features ultimately requires results to be confirmed using primary pancreas tissues.

### 3.5 Primary Islets

Our present knowledge of  $\beta$ -cell pathophysiology has mostly stemmed from the interrogation of isolated pancreatic islets. The possibility of isolating islets was first suggested as early as 1911 (107), but the isolation process was not fully optimized and automated until closer to the late 1980s (108–111). While the primary  $\beta$ -cell is of main interest to diabetes researchers,  $\beta$ -cell function is better retained when cells are kept within the 3D primary islet structure. Primary pancreatic islets are typically cultured in static systems on non-adherent plates, in order to retain their multicellular, 3D architecture. The dispersion of primary islets and subsequent culture of primary  $\beta$ -cells in 2D monolayers results in dysfunction of insulin secretion, likely due to loss of key cell-cell interactions (69–71). Although, the culture of dispersed islet monolayers onto defined surfaces can provide single-cell resolution of key subcellular interactions (112). Islets can also be dispersed, sorted and/or virally transduced, and reassembled, which provides insight into the roles of cell-cell and/or cell-matrix interactions (113); however, this process typically leads to significant cell loss. In general, isolated islets provide intimate investigation and unique insight into diabetes-related hypotheses *via* knock-down studies, genetic manipulations, or procurement from donors at specific disease states.

The utilization of primary pancreatic islets to address questions related to native or disease physiology, however, is limited by the deleterious pathways activated at the start of the islet isolation process. Specifically, isolated islets quickly exhibit a loss in islet structural integrity (114), up-regulation of stress genes and cytokines (115, 116), increased reactive oxygen species (ROS) production (117), and activation of inflammatory (118) and apoptotic pathways (119). Islet decline is unresolved in

culture, as the lack of the ECM niche and ideal culture conditions creates anoikis-initiated apoptosis and insulin dysfunction (120, 121). Thus, the utility of primary islets is extremely limited by temporal constraints, with most islet studies conducted within less than one week post-isolation. The accessibility of primary human islets is also limited to the availability of donor tissues. While animal tissues are more easily procured, key genotypic and phenotypic mismatches must be considered when extrapolating results. Overall, the utilization of primary islets can be a powerful resource in the pipeline of tools to understand native physiology and disease progression, but significant improvement in culture parameters is necessary to facilitate stable long-term islet culture.

### 3.6 Stem Cell-Derived Islet Organoids

A promising approach to enhance both the access and relevance of cell cultures to human physiology is the development of stem cell-derived organoids, which can theoretically provide an infinite supply of cells. Human endocrine and non-endocrine cells can be generated from both human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs), which are typically procured from living donors (**Figure 2B**) (95, 122). Stem cell-derived  $\beta$ -like-cells (SC- $\beta$ Cs) created from earlier differentiation protocols were insufficiently mature *in vitro* and required animal implantation to elevate their functionality (123–125). More recent SC- $\beta$ Cs differentiation protocols have shown improved *in vitro* maturation with increased insulin secretion to glucose challenges (e.g., exhibition of both first and second phases of insulin secretion) and functionally mature mitochondria (126–131). As advances are made in recreating islet-like organoids *in vitro*, unique research questions can be explored. For example, one can examine patient- and disease-specific differences from their respectively derived SC- $\beta$ Cs (132). Cells can be genetically manipulated to enhance cell fate mapping and impart distinct risk or protective alleles. Moreover, other islet-resident cells can be incorporated, such as ECs,  $\alpha$ -cells, and  $\delta$ -cells, to mimic the native islet environment (122). The potential to completely customize the final stem cell-derived islet organoid provides unprecedented control to experimental studies.

Limitations of these cells still exist, as they lack full functional maturity (133). For example, key  $\beta$ -cell transcription factors are decreased in SC- $\beta$ Cs, when compared to isolated islets (134). While some differentiated protocols exhibit biphasic insulin secretion profiles, the magnitude of the second phase insulin secretion is still much lower than native islets, which may lead to insufficient efficiency in blood glucose clearance. From the perspective of scalability and manufacturing, there are still challenges with limited shelf-life and inter- and intra-batch reproducibility of the final product. Efforts in creating cell culture methods that more closely recapitulate key features of the native *in vivo* environment (e.g., oxygen gradients and ECM niche), as well as efforts that support their long-term culture to enhance maturity, may serve to resolve these issues (41, 135). Overall, advancements in islet stem cell biology create a promising avenue for researchers to incorporate these cells within their proposed disease models.

### 3.7 Living Pancreatic Slices

Given the challenges in islet isolation and SC- $\beta$ Cs differentiation, the emergence of organ pancreatic slices provides a distinct *in vitro* tool, where islets are retained within their 3D pancreatic niche and cultured as a pancreatic tissue segment. Inspired by brain tissue slicing techniques, pancreatic slices are created by encasing the organ in agarose and sectioning 100 – 200  $\mu$ m thick slices using a vibratome (136, 137). Slices are then typically cultured free-floating in media. With this approach, the islets and their peri-islet niche, including ECM, vasculature, lymphocyte, and neural cells are generally preserved, permitting *in situ* assessments of not just endocrine and exocrine cells but also of the additional contributing peripheral cells. Common readouts obtained from these slices are dynamic live imaging confocal microscopy of the identified islets within the slices, indirect measurements of hormones released in perfusion experiments (138), and/or electrophysiological characterization using patch-clamp technology (139). Numerous queries can be made across the translational spectrum by using pancreas slices from animal and human donors both in healthy and diabetic states. For example, T1D pathogenesis was analyzed using slices from human pancreatic donors by showing loss of  $\beta$ -cells and immune cell infiltration (140). T2D human slices were utilized to show that  $\beta$ -cell dysfunction transpired even when  $\beta$ -cell mass was intact, providing evidence of features exhibited during early T2D pathogenesis (84). Additional emerging research is using these slices to explore diabetes pathogenesis and the islet-immune interface (138).

While this approach serves as a great platform, slices are challenged by their inherent complexity and restricted access, particularly from human source. The heterogeneity of human donors is extremely high, as the slices are obtained from 0.5 cm (3) blocks and are procured from cadaveric donors. Slices are delicate and their durability in culture under traditional static conditions is typically limited to only a few days, although more advanced culture platforms, such as oxygen-enhanced transwell systems and the use of protease inhibitors, indicate the potential to extend their culture (141). Enhancing the duration of culture will open up opportunities for more advanced temporal multi-omics studies. The unpredictable distribution and size of the islets impart heterogeneity and bias of these studies, as slices rich in pancreatic islets are typically selected for study and larger islets are challenging to examine due to slice thickness. The impact of the mechanical slice on activation of stress factors within the cells is also unknown at this time. Finally, in the study of T1D pathophysiology, care must be taken in the contextualization of observed immunological processes, as cells and pathways identified within these slices at the time of examination are biased by the duration of disease. In general, living pancreatic slices have the potential to subjugate diabetes research efforts, as this relatively new sample type provides a unique *in vitro* window into the pancreatic islet niche.

To conclude, diabetes research has typically relied on conventional human, animal, and cell culture models; however, these cells and tissues exhibit unique features and demands that must be considered prior to the extrapolation of results. To select



a specific source for study, one must consider and balance various parameters such as *in vivo* relevancy, time, cost, manipulability, tissue availability, and user adaptability [Figure 2A (iv)]. Species source is also a critical choice. While human models are likely the most relevant, access and manipulation are extremely limited. Murine models are comparatively easy to obtain and are more supportive of customization; however, they are restricted in their translatability to mimic a human disease. SC- $\beta$ Cs are flexible for manipulation and can be generated from heterogeneous donor sources, but suffer from complexities in differentiation and may lack consistency and maturity. Finally, pancreatic slices exhibit the advantages of *in situ* study, but elevate the challenges of stable culture. Overall, not one viable source provides the perfect model for studying T1D and multiple cell sources should be used and compared to elevate *in vitro* diabetes research and disease modeling. Furthermore, to elevate the physiological relevance of these cells, additional culture strategies for more closely mimicking the unique islet niche should be integrated.

## 4 STRATEGIES TO ENGINEER AN ISLET NICHE

When examining the breadth of current diabetes disease models on a simple grid of relevancy versus time and cost, it is obvious that standard culture models and whole organism animal models fall into two extreme locations. Traditional cell cultures are highly simplistic in their static 2D environment, while the complexity of animals impairs full control and interrogation capacity. This large gap in biological platforms supports the critical need and the value proposition to engineer more efficient and effective disease models. To provide clear value, engineered models must not sacrifice biological relevancy; concurrently, they must accelerate the scientific discovery timeline while being economically viable. Building these *in vitro* engineered islet models also demands a clear identification of what key aspects of the native peri-islet space are crucial to mimic. Given that the two key features needed for recreating an islet-supportive niche are a 3D microenvironment and dynamic perfusion, subsequent sections highlight the integration of 3D hydrogel systems and the utilization of suitable microphysiological systems (MPS). We then explore how these systems can be customized to provide distinct insight into diabetes-relevant hypotheses.

### 4.1 3D Hydrogels

Hydrogels are 3D structures formed from physically or chemically cross-linked polymers that retain large amounts of water (typically > 90% v/v). While these hydrating gels are more commonly employed in T1D for pre-clinical and clinical cell therapy approaches (142, 143), here we discuss the utility of 3D hydrogels for use within an *in vitro* islet niche. Hydrogels employed for islet support can be globally sorted into four main categories: i) bio-inert hydrogels; ii) functionalized bio-inert hydrogels; iii) hydrogels made from discreet soluble ECM

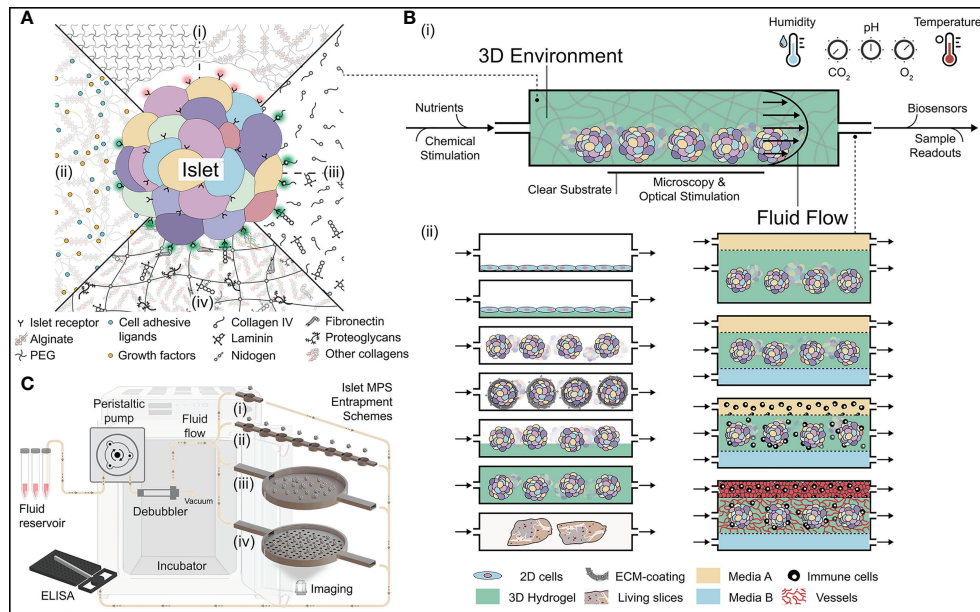
components; and iv) decellularized ECM (dECM) hydrogels (Figure 3A). When used as islet niches, these matrices can support 3D distribution, mechanical protection, and cell-matrix interactions. In addition, they can support the generation of more complex 3D structures, such as vascular networks.

#### 4.1.1 Bio-Inert Hydrogels

Bio-inert hydrogels lack natural interactions with cells, thus they are typically non-adherent. These 3D hydrogels are made by cross-linking either synthetic or naturally derived materials [Figure 3A (i)]. Bio-inert hydrogels offer the advantages of minimal immunogenicity and protein absorption, decreased cell degradation and/or remodeling, and control over degradation profiles and release kinetics (144, 145). Hydrogels made from synthetic polymers typically exhibit stability and reproducibility, as well as more tunable control of viscoelastic and mechanical properties to mimic tissue-like characteristics. Commonly used 3D synthetic hydrogels for encapsulation of islets are polyethylene glycol (PEG) (143) and poly(vinyl alcohol) (PVA) (146). Alternatives to synthetic-sourced materials are naturally derived polysaccharide bio-inert hydrogels, such as alginate and agarose hydrogels. The low toxicity, ease of gelation, mechanical stability, and transparency of these materials make them appealing for islet-based applications, particularly for cellular encapsulation (142, 147–149). Both alginate and agarose can support islet culture (150–155) and permit islet immobilization for cell-specific spatiotemporal tracking (156). The use of bio-inert hydrogels can be challenged by their non-adhesive and inert nature. It can induce apoptotic pathways which can be further exacerbated during long-term islet culture (145, 157). Collectively, the ease of stability of bio-inert hydrogels provides a robust option for creating 3D islet micro-niche.

#### 4.1.2 Functionalized Bio-Inert Hydrogels

Bio-inert hydrogels can be customized to impart specific biological features, such as the incorporation of growth factors, enzyme-sensitive peptide sequences, and/or discreet cell attachment sites to enhance cell proliferation and function [Figure 3A (ii)] (158). The incorporation of specific features into synthetic hydrogels has improved islet function, stability, and viability *in vitro*. For example, the functionalization of PEG with arginyl-glycyl-aspartic acid (RGD) peptide or with collagen IV and laminin increased islet insulin secretion (159, 160), whereas the conjugation of PEG with vascular endothelial growth factor (VEGF) promoted graft vascularization (144). While a bit more arduous than synthetic polymers, alginate can also be functionalized to provide various ECM components to improve cell survival and reduce cell apoptosis (161, 162). Adding collagen to alginate hydrogel may further support islet functionality by providing elevated cell-matrix interactions (163). While the selection of key bio-active features and their stable integration into the material can be a challenge, these materials can provide discrete control over the customization of the hydrogel, supporting the clear investigation of a specific material parameter on islet culture.



**FIGURE 3** | Approaches in building the next generation of 3D islet niche. **(A)** Schematic of different types of 3D hydrogels for culturing islets. **[A-(i)]** Bioinert hydrogels that are naturally non-adhesive to islets. **[A-(ii)]** Functionalized bioinert hydrogels with the incorporation of cell adhesion ligands and growth factors. **[A-(iii)]** Hydrogels with soluble ECM. They could be made either using singular or complementary sets of ECM molecules. **[A-(iv)]** Multi-component decellularized 3D hydrogels with extensive adhesion capabilities to the islets. **(B)** Fundamental features of islet microphysiological systems (MPS). **[B-(i)]** Housing islets within a 3D space require the orchestration of several parameters. **[B-(ii)]** Various MPS schemes for housing islets with hydrogels and other cells/tissue types. **[C (i-iv)]** Different types of islet entrapment schemes using architectural design. Various accessories needed for culturing islets within the MPS are also visualized. Illustration not drawn to scale.

#### 4.1.3 Discreet Soluble ECM Hydrogels

Singular or combinatory soluble ECM hydrogels is another approach for improving islet culture, as pancreatic islets are naturally embedded in a peri-islet ECM comprised of both basement and interstitial matrix proteins [Figure 3A (iii)]. Leveraging what is known about the native peri-islet ECM, hydrogels made of soluble collagen I, III, and IV have been created that exhibited preserved function and suppression of cell death (164–166). A drawback of integrating distinct and soluble ECM proteins into bulk hydrogels is that their resulting conformations and interactions with the encapsulated islets are likely non-physiological. For instance, collagen IV chains are naturally formed in the presence of laminins and nidogens (167); thus, collagen-only hydrogels may lack its native 3D chain configuration. Fibrin is another ECM-based hydrogel that can maintain islet viability and function *in vitro* (34, 168–170). As an easily cell-degradable material, fibrin may also be suitable for providing a dynamic environment for islets to remodel within a 3D space (171). In addition, fibrin is a common material used to support the formation of *in vitro* vascular networks, which could be combined with islets to form vascular structures around these spheroids (172, 173). Combinatory ECM-based hydrogels can also be made to provide further customization (e.g., hyaluronic acid and collagen hydrogels) (174). While reports on the survival (36), insulin gene expression (25), and morphological changes (112) of  $\beta$ -cells are varied and subjugated by the diversity of ECM molecules, the use of soluble ECM-based hydrogels can

nonetheless create a distinct islet niche that not only support islets, but provides dynamic flexibility to support other cell types and/or tissue structures.

#### 4.1.4 Decellularized ECM Hydrogels

Multi-component ECM, sourced from decellularized organs, is another hydrogel option for islet culture [Figure 3A (iv)] (29, 175, 176). In contrast to artificially combining distinct ECM proteins into a hydrogel, dECM naturally contains a diverse spectrum of protein molecules to provide embedded cells with physiological signals from multiple proteins, peptides, and factors. While some groups utilize the 3D decellularized structure to seed cells and serve as a conduit for implantation (177), an alternative approach is to grind the dECM into a fine powder. Rehydration of dECM powder results in a self-assembled and nano-fibrous 3D dECM hydrogel with customized mechanical and biochemical parameters *via* modulation of matrix concentration, decellularization process, tissue source, and composition (41, 178, 179). For example, the concentration of the matrix can be modified to provide distinct viscoelastic properties and even mimic selected mechanical features of the native pancreas (41).

The encapsulation of human and rat islets within the dECM hydrogels, sourced from porcine lung, bladder, or pancreas, retained islet functionality in culture and altered the secretion of cytokines and metabolic hormones, when compared to standard 2D static culture (41). Others observed correlations between ECM

composition and the functionality of  $\beta$ -cells and porcine islets (178, 179). For stem cell-differentiation towards the  $\beta$ -cell fate, the co-culture of pancreatic progenitors with dECM appears to improve their  $\beta$ -cell maturation (180, 181), although a recent report indicates that this is likely driven by collagen V (182).

Key challenges in the use of this material relate to the processing method, which can vary depending on the tissue type, the purity and state of the tissue at the time of processing, and the targeted ECM final product components. Most decellularization processes disrupt ECM integrity to a certain extent to remove nuclear content and the processing of the dECM to create reconstituted hydrogels can further impact ECM integrity and activity (176, 183). Altogether, combinatory ECM protein hydrogels promote a dynamic reciprocity that can be highly beneficial for understanding cell-cell and cell-matrix interactions within an islet organoid and supporting the stable long-term culture of islets.

## 4.2 Microphysiological Systems

The static culture of multi-cellular islet organoids is challenged by insufficient nutritional gradients created by their high metabolic activity and reliance on diffusion-based transport (184). Transport efficiency is further dampened when islets are placed within 3D hydrogels. Culture systems such as stirred flasks, rotating wall vessels, hollow fibers, and direct perfusion chambers, can support 3D culture by promoting convective-based mass transport for a more effective exchange of nutrients and waste. While likely desirable for the scale-up of cell cultures for translational use, these techniques require larger culture media volumes when compared to typical 2D culture. Further, they exhibit limited capacities for discreet built-in assays for studying islet pathophysiology, pharmaceutical agent screenings, or conducting mechanism of action studies. Microphysiological systems (MPS), also known as tissue chips or organs-on-chips, impart discreet convective flow at a small culture scale, while also facilitating the integration of sensors, outputs, and optical monitoring within a highly defined and controlled microenvironment. MPS mimicking key aspects of organs such as the lung, heart, kidney, and vasculature are becoming widely available within the scientific community (185, 186).

The *in vitro* recreation of an islet-niche demands the precise control of various culture conditions, ranging from temperature, pH, humidity, and oxygenation. In addition, a predictable fluidic flow ensures efficient nutrient delivery, as well as provides biomechanical cues and forces onto the cells within the larger islet cluster [Figure 3B (i)]. Beyond delivering more physiological and reproducible culture conditions, this deliberate control provides expansive capacities to precisely perturb and interrogate the islet within the MPS. Various cell culture conditions and geometries are feasible within the islet MPS, which facilitate the incorporation of diverse cell types and morphology (*i.e.*, homogenous versus heterogeneous cell population, monolayers versus 3D clusters), 3D hydrogels capable of supporting both islet clusters and/or vascular structures, and fluidic shear stress (*i.e.*, gravity-based flow, peristaltic- or pressure-based flow) [Figure 3B (ii)]. For instance, 2D homogeneous or heterogeneous cell

monolayers can be cultured within an MPS to interrogate cell-cell and cell-matrix observations at a subcellular resolution with the added complexity of cell shear stress. Furthermore, monolayers can be cultured onto standard plastic or ECM-coated surfaces to investigate the impact of 2D cell-matrix interactions. Alternatively, in accord with their native 3D structure, islet 3D clusters can be freely cultured within the MPS niche. Culturing islets alone within the MPS can provide convective delivery of nutrients and removal of waste, as well as permit temporal tracking of cellular dynamics. The inclusion of ECM cues and 3D support to islets can further bio-augment the system to support the dynamic complex structures required for cell adhesion, growth, and responsiveness. Islet-matrix interactions can be integrated by either coating the islet surface with relevant ECM (187), submerging islets within a thin layer of hydrogel (188), or fully encasing the islets within a hydrogel (41) [Figure 3B (ii)]. Conceivably, when culturing hydrogel-embedded islets, the presence of flow plays a crucial part in an effective nutrient exchange, as deleterious diffusional gradients can be created by a macroscale hydrogel (156). To provide modularity in the selection of geometric, cellular, and matrix features to support broad hypothesis testing, MPS design features should be highly adaptable.

Tailoring the culturing capacity of islets within an MPS is not a frivolous feat. Isolated islets cultured in adhesive cell culture platforms lose their 3D morphology and function due to cell spreading. Alternatively, if they are incubated on a geometrically straight/flat non-adhesive space, then they could wash right out with the fluidic flow. Thus, an islet MPS must contain a design where the islets can be entrapped and immobilized within a niche (Figure 3C). The entrapment of islets can be achieved by using device geometry or/and 3D hydrogel encapsulation. Single-well geometries (189–195) or the manipulation of channel heights (196) can be used to physically entrap single and encapsulated islets as they are loaded into the system, which permits the intimate interrogation of single islets [Figure 3C (i)]. This approach can be scaled up to create multiple traps, that are geometrically aligned [Figure 3C (ii)] (197–204). While this can increase the culture capacity of the system, the total number of islets that can be cultured is limited to the order of tens of islets. Alternatively, a millimeter-scale – open-well system approach can assess larger scales (*i.e.*, 10 to 100 islets) [Figure 3C (iii)] (156, 188, 198, 205–209). In this approach, more aggregated metrics are collected, although single islet imaging is still feasible. Islet retrievability is also feasible in this open well system, where samples can be used for post-culture analysis. A challenge with this approach is mitigating the clumping of clusters over time, but this can be resolved with the use of 3D hydrogels (156) or an open well system with small geometric well-traps that capture single clusters [Figure 3C (iv)] (210–212). Overall, the architectural features within the islet MPS is a critical parameter in its and is riven by the key metrics of interest (*e.g.*, single cluster or aggregate-based), as well as the driving hypothesis of the work.

The materials used for manufacturing an islet MPS is a key parameter of interest because the surface chemistry of the device can influence the cell biology, hydrodynamics, and the transport and absorption of compounds. One of the most commonly used

materials for fabricating MPS is polydimethylsiloxane (PDMS), due to its ease in fabricating custom molds, optical transparency, and gas permeability (185, 213, 214). For islet culture, several PDMS-based MPS have been reported (188, 196, 199, 206, 210, 212). Despite its widespread use, PDMS material properties, such as adsorption of small molecules and the leaching of uncrosslinked oligomers, restrict its utility for scientific discovery applications (215–217). The photolithographic fabrication of PDMS-based MPS can also impart inherent size restrictions. While this does not create a problem for 2D cell culture techniques, it does restrict the utilization of MPS for 3D organoids with sizes ranging from 50  $\mu\text{m}$  to 1 mm (111, 188). Glass is an alternative material used to fabricate MPS, providing the advantages of supporting surface chemistry pretreatments, glass etching, and electroosmotic flow (190, 191, 193, 218); however, the use of this material, particularly for rapid prototyping and scalable MPS manufacturing, can be arduous. Alternatively, thermoplastics, such as polystyrene, poly(methylmethacrylate), and polycarbonate, support scalable manufacturing and avoid the challenges of biofouling (219). Islet-specific MPS made from thermoplastics have demonstrated preservation of islet function while supporting *in situ* imaging and measurements of secreted hormones (156, 200, 205). With that in mind, the material selected for the fabrication of an islet MPS should serve to permit rapid prototyping, large-scale manufacturing, and durable use without sacrificing its intended use for culturing islet organoids.

Another key MPS advantage is the integration of built-in assays that conveniently report the current health status of the residing cells. *In situ* and off-line measurements of cells is essential in the study of endocrine tissues, as they rely on accurate measurements of secreted hormones and cellular readouts. Thus, the value of a next-generation islet MPS can be greatly extended by incorporating *in situ* spatio-temporal interrogation of micro-assays to monitor cells within the engineered niche (**Figure 3B**). This can be accomplished by designing MPS prototypes that support high-resolution imaging modalities. Such imaging capacities can track cellular destruction, functional readings, as well as interactions with the surrounding matrix or other cell types, in real-time while under continuous perfusion. In engineering an islet MPS, it is recommended that desired readouts are first defined, as the MPS architectural design and the material of choice can sometimes dictate the feasibility of assay integration. For example, an islet MPS made from thermoplastics with a desire to measure fluorescently-labeled insulin required the modification of the islet niche to include borosilicate glass (200). An essential assay for most platforms is the capacity to monitor the dynamic responses of islets to physiologic stimuli, such as a glucose challenge. To date, methods of on-demand monitoring of islet function can be achieved through capillary electrophoresis immunoassays (220–222) or intracellular calcium oscillation monitoring as a surrogate for insulin secretion (138, 223). Recent attraction in leveraging bioreporters to design cellular biosensors is another great avenue to dynamically monitor the biological activities of islets during long-term culture. In general, cells can be virally transfected or genetically altered to induce modifications in proteins that “bioreport” specific activities. These biosensor cells

can be added to the culture platform or the islets themselves can be modified to serve as biosensors. For instance, islets can be transfected with GCaMP6 adenovirus for *in situ* reporting of intra-cellular calcium activities (113, 156). Alternatively, distinct biosensors (*i.e.*, cell lines) that report specific soluble metabolites can be incorporated into the downstream flowstream. Islet-specific offline biosensors can monitor hormones and neurotransmitters such as serotonin (224), GABA ( $\gamma$ -aminobutyric acid) (225), glutamate (226), acetylcholine (227), and ROS (228). Additional resources for cell-based biosensors and electrochemical biosensors are nicely reviewed elsewhere (229, 230). The desire to include built-in assays and biosensors must be considered cautiously to not abolish the functionality, durability, and robustness of the islet MPS. Often, they require a separate set of reagents and ploys that may not be easily incorporated within the niche being built for the islet organoids. In that case, approaches that support independent or exchangeable modules may avoid the conflict. Islet MPS built to include such modular assay kits can be of high value to the islet biologists and immunologists to interrogate key biological questions as it pertains to islet biology and pathophysiology.

## 5 THE PROGENIES OF 3D ISLET MPS

At the time of writing, there is no “has-it-all” approach when engineering a diabetes disease model. Thus, it becomes essential for engineers to define the aspect(s) of the native islet niche that are being modeled or recreated. This review, in conjunction with other key review papers recently reported (65, 231–233), should provide guidance for identifying important aspects of islet physiology to include in a given engineered islet niche. Altogether, the augmentation of the next generation of islet MPS demands inclusion of the following criteria: i) purposeful geometric designs, ii) 3D hydrogels, iii) controlled perfusion, and iv) robust workflow and interrogation capacities customized to the specific needs for the islet.

Specifically, islet MPS designs cannot encompass flat geometries with straight paths and channels that are common for 2D cultures, as 2D monolayers of dissociated islets exhibit poor physiological phenotype and function when compared to their 3D counterparts. Culturing of 3D islets demands sophisticated designs that immobilize the clusters from washing out while they are subjected to continuous flow. The height of the islet MPS device must support the culture of organoids in the range of 100–500  $\mu\text{m}$  in diameter. The surface topography of the device must also be non-adherent over extended culture periods; otherwise, islets may adhere and lose their native 3D morphology. Long-term cultures may require additional attention, as even non-adherent surfaces can foul over time and become adherent.

Next, the incorporation of certain aspects of native 3D architectures by using hydrogels is important for durable *in vitro* culture. 3D hydrogels can provide attachment sites for cells and can serve as a platform for the inclusion or development of additional 3D structures, such as vasculature networks [**Figure 3B** (ii)]. Hydrogels can also enhance 3D imaging, as they stably

immobilize the 3D cell cluster. Hydrogel selection is likely driven by the specific hypothesis or goals of the MPS. For example, a hydrogel supportive of vascular development is distinctly different from one seeking to support durable 3D islet encapsulation. The need to study additional dynamic features, for example, the tracking islet-immune interactions, would require a 3D hydrogel that is supportive to both islet suspension and T cell motility. Finally, while there is an increased interest in developing multi-component hydrogels that resemble the heterogenic nature of the native islet-BM niche, the development of the next generation hydrogels is limited by our knowledge of identifying true proportions and compositions of the islet-specific ECM protein niche. By filling this knowledge gap, we would be able to engineer superior hydrogels for enhanced islet culture.

The islet MPS must allow for the controlled perfusion of islets with fully developed laminar flow. Islets are highly metabolically active clusters that require a robust supply of nutrients and oxygen, as well as an effective means to clear metabolic waste. This is especially critical for impure batches of islets or for living pancreatic slices, where acinar tissue can accumulate enzymes proximal to the islet niche. A carefully crafted islet MPS niche should present continuous convective forces to support this goal. The fluidic stream should contact the islet surface rather than flow from a distance away. Such exposure can present mechano-transduction forces on the surface of single cells within an islet. Inducing shear stresses on the islet surface can initiate a cascade of focal adhesion kinases, which could reduce activation of apoptosis, and ultimately support islet function (234). The scale of shear stresses induced in the MPS must be carefully tailored, however, as there are risks of shear-induced  $\beta$ -cell damage (212, 235). The customized perfusion of islets can give additional insight into the dynamics of key hormones and metabolites with temporal readout resolutions. One can stimulate islet organoids with physio-mimetic challenges and evaluate their performance. It can also allow for the exploration of gradient impact versus local toxicity effects of deleterious agents (e.g., cytokines, ROS) near the islets. Overall, the controlled perfusion of islets can provide discreet and controlled flow with the presence of heterogenic features of the peripheral islet microenvironment and can serve as a gold standard tool to perform basic scientific research.

The progenies of 3D islet MPS must have robust handling and *in situ* imaging capacity. The islet MPS should allow the adaptable and customizable workflow to the users. Considerations must be made to ease the islet loading processes and, concurrently, retrievability should be enabled for access to genomic-, flow-cytometry based-, and/or super-resolution imaging- data. Integration of *in situ* imaging capacities must be furnished to enable insightful spatiotemporal tracking and/or optical stimulation capacity while simultaneously under perfusion and within an environmentally controlled chamber. The MPS should also allow ease in introducing a series of media, drugs, and/or cells to imitate hypothesized biological events. The fabrication and use of islet MPS should also be reasonable for its scalable production sustainably and economically without sacrificing its utility. Altogether, a carefully orchestrated islet MPS design should deliver a “work-horse” platform for testing a broad spectrum of hypotheses.

The 3D islet MPS can be further equipped with added features and functionality by including multiple cell types, automation processes, and expansive assay capacities. Features that permit the incorporation of different solutes, media, and multiple cell types can elevate the impact of the islet MPS (**Figure 3B (ii)**). Continuous recirculation of different solutes (*i.e.*, drug molecules) or even cells (*i.e.*, immune cells) may enable dynamic interrogation of islet pharmacokinetics and disease pathology. For example, in the case of understanding islet-immune attack during diabetes pathogenesis, such advanced models can highlight and impart phenomenon that may be completely different when studied under static culture conditions or even under a gravity-based flow. Another attractive component of creating a relevant 3D islet niche is the incorporation of a fully vascularized cavity surrounding the cultured islets [**Figure 3B (ii)**]. A vascularized 3D islet MPS could support the investigation into the role of ECs and islet connections on islet physiology *in vitro*. Anastomosis of engineered vascular network with the resident intra-islet capillaries could further lead to biorelevant islet MPS models. Such models could be used to investigate the role of capillary flow in  $\beta$ -cell stimulation/secretion as well as for our understanding of how immune cells transverse through micro-vessels and migrate towards targeted islet cells. An additional transformative resource is the utilization of isogenic cells within an advanced 3D islet MPS. Using hiPSCs, the feasibility of creating a multi-cellular repertoire from the same human source opens new avenues for creating patient-specific disease models containing not only pancreatic islet cells, but immune cell components (e.g., macrophages, T cells, and B cells) (122). In addition, automation strategies for inputs and outputs for cells, hydrogels, and media can streamline MPS utility by increasing robustness and user adaptability. Lastly, expansive assay capacities either encompassing “all-in-one” islet MPS or completely modular interrogation schemes can boost the impact of a 3D islet MPS. For example, *in situ* oxygen-sensing strategies combined with hormonal or stress-signal measurements *via* biosensors can have broader applications in islet research for not only the temporal *in situ* monitoring of islet health but also for understanding factors that regulate their fate. Modularity in biosensor platforms can also be designed such that they can be assembled for an on-demand interrogation.

## 6 CONCLUSION

Overall, the next era of islet research is being driven by ambitious endeavors supported by engineers, biologists, and immunologists. By bridging the gap between different stakeholders to ultimately progress diabetes research, it is the hope that this roadmap provides a guide for engineers to craft progenies of an islet MPS that are bio-relevant and easily adaptable by the broader scientific community. Concurrently, we hope that biologists and immunologists are inspired to use engineering principles and tools to ask sophisticated questions. Since the revelation of insulin nearly a century ago, undoubtedly, there have been prominent efforts made in understanding diabetes. As the next century unfolds itself with the revolution

in life sciences and biotechnology, we posit that a focus on engineering sophisticated benchtop platforms for studying islets will accelerate the timeline for realizing a cure for diabetes.

## AUTHOR CONTRIBUTIONS

SNP conceived the idea for the review paper. SNP and CLS wrote the manuscript with critical input from CEM. SNP designed the overall visualization of the figures with feedback from CLS. RC

illustrated the native islet figure with feedback from SNP and CLS. All authors read and approved the final version of the manuscript.

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