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Review

Human Islet Isolation and Distribution Efforts for Clinical and Basic Research

Natasha Hui Jin Ng [1], Wei Xuan Tan [1], Ye Xin Koh [2], Adrian Kee Keong Teo [1, 3, 4, *]

1. Stem Cells and Diabetes Laboratory, Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore; E-Mails: nhjng@imcb.a-star.edu.sg; WTAN096@e.ntu.edu.sg; ateo@imcb.a-star.edu.sg; drainteo@gmail.com
2. Department of Hepatopancreatobiliary and Transplant Surgery, Singapore General Hospital, Singapore; E-Mail: koh.ye.xin@singhealth.com.sg
3. Department of Biochemistry and Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
4. School of Biological Sciences, Nanyang Technological University, Singapore

* **Correspondence:** Adrian Kee Keong Teo; E-Mails: ateo@imcb.a-star.edu.sg; drainteo@gmail.com

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Abstract

The ability to routinely and reproducibly obtain purified human islets has facilitated substantial progress in providing a safe and reliable treatment option for adult patients of type 1 diabetes. The availability of human islets for basic research has also significantly improved the understanding of the biology of human islets, and consequently the pathophysiology of diabetes. Presently, about 70 human islet isolation centers are known to exist around the world, in addition to multiple coordinated human islet distribution programs, that facilitate the exchange of knowledge and sharing of this precious resource. This review summarizes the steps involved in the isolation and dissemination of human islets, and discusses key considerations with respect to the major challenges faced during this

process. Another important objective of this review is to summarize the ongoing efforts for the distribution of human islets at the regional, national, and international levels. This review also highlights the value of human islets for clinical transplantation as well as basic research, particularly by highlighting selected studies that have significantly improved our understanding of human islet biology.

Keywords
Human islets; islet isolation; type 1 diabetes; islet transplantation; islet studies; islet biology

1. Introduction

The human pancreatic islets have proven to be a valuable tissue resource both as a therapeutic option for diabetes patients as well as for basic and clinical research. Type 1 diabetes (T1D) is an autoimmune disorder, wherein the immune system attacks and destroys the insulin-producing pancreatic beta cells, resulting in the loss of glycemic control and the need for insulin therapy. Since the first report on the ability to reverse diabetes in rats via transplantation of isolated rat islets [1], strategies to treat T1D in humans via the allogeneic transplantation of isolated human islets have been rapidly developed. In 1988, the invention of the Ricordi chamber, an automated islet digestion chamber, represented a breakthrough in this field and significantly increased the yield and purity of islets isolated from the human pancreas [2]. In 2000, Shapiro and colleagues developed the Edmonton protocol, which included several major improvements such as the use of a glucocorticoid-free immunosuppression regime and the infusion of a larger mass of islets (>13,000 IEQ [islet equivalents]/kg of body weight) to achieve better graft outcomes [3]. The authors reported that all seven T1D patients maintained insulin independence for a year following transplantation. The success of the Edmonton protocol was a significant improvement over the previous protocols, which were unable to achieve long-term insulin independence in T1D patients. Since then, islet transplantations have been performed across numerous centers globally, with comparable insulin independence rates to those of whole-pancreas-alone transplantations [4].

Primary human islets have also been extensively used for the study of islet biology in an *ex vivo* setting, substantially increasing our knowledge of islet composition and function, which would have been less achievable in cell lines of the individual islet cell types. The increasing value of human pancreatic islets for research has fueled the demand for these tissues, as rodent islets, being structurally and functionally different to human islets, are no longer sufficient for this purpose [5-8]. In order to cater to this demand, the past decade has witnessed a rise in the number of human islet clinical isolation facilities and coordination activities at the regional and global levels. Continuous efforts are made to improve the efficiency of the isolation and assessment of human islets in order to obtain better-quality material and facilitate more widespread adoption. Challenges faced during the use of human islets include the variation between tissue samples from different donors, subtle differences in islet isolation and processing techniques, and varying environmental conditions during the culture and distribution of islets. Some of these aspects are discussed in the subsequent sections.

2. The Process of Isolating Human Islets and Factors Influencing Islet Yield and Quality

Human islets are typically isolated from the pancreas of a cadaveric donor, although they can also be derived from live donors that have undergone partial pancreatectomy [9-11]. The isolation of islets from the human pancreas is a difficult and sensitive process that requires a specialized and licensed facility, as well as the combined efforts of multiple trained personnel. Each facility is subjected to strict regulations and must comply with the current Good Manufacturing Practice (cGMP) regulations if the isolated human islets are designated for clinical transplantation [12, 13]. Facilities that isolate human islets purely for research purposes are not obliged to adhere to the cGMP regulations [14]. Isolation of good-quality human islets is instrumental to the success of islet transplantation [15]. This section highlights the standard practices in assessing the isolated islets and discusses a number of key factors that affect islet yield, as well as challenges faced by the isolation facilities.

2.1 Overview of the Process of Isolating Human Islets

The NIH-sponsored Clinical Islet Transplantation Consortium has published a series of Standard Operating Procedures to standardize the large-scale isolation and purification of human islets across its participating centers and to ensure process efficiency and product consistency [16]. The enzymatic isolation procedure of human islets is now commonly followed, and the major steps are presented in Figure 1. Briefly, the pancreas is surgically extracted along with parts of the duodenum and spleen from a brain-dead cadaveric donor. The organ is transported to an isolation facility in a chilled organ preservation solution, typically the University of Wisconsin (UW) solution or histidine-tryptophan ketoglutarate (HTK) solution [4, 17]. At the islet isolation facility, a sample of the preservation liquid is taken for sterility tests. The pancreas is then carefully trimmed off from the surrounding spleen, duodenum, and fat tissues, before cannulation. Catheters are inserted into the main pancreatic duct of each of the pancreatic head and tail sections by the islet specialist [15]. The pancreas is then distended by injecting an enzyme blend consisting of collagenases and proteases through the cannulated pancreatic duct. The perfused pancreas is subsequently cut into smaller pieces and placed in an automated Ricordi digestion chamber [2]. Digestion occurs in the automated chamber in two phases. In the first phase, the enzyme solution is recirculated to progressively dissociate the pancreas. In the second phase, the enzyme solution is neutralized and diluted with EMEM dilution solution. The dissociated islets are then collected, washed with cold M199 wash solution, and resuspended in UW solution. The dissociated islets are purified by subjecting the tissue to continuous density gradient centrifugation, following which each gradient fraction is collected and the purity is quantified. The islets are typically pre-cultured in supplemented CMRL media in non-adherent conditions for up to 72 h for quality control, after which they may be distributed for transplantation or research purposes [4, 16]. Generally, most islet isolation procedures comprise of similar steps, and centers within the same consortium follow the same protocol for consistency. The performance of protocols with slight modifications is discussed in a later section.

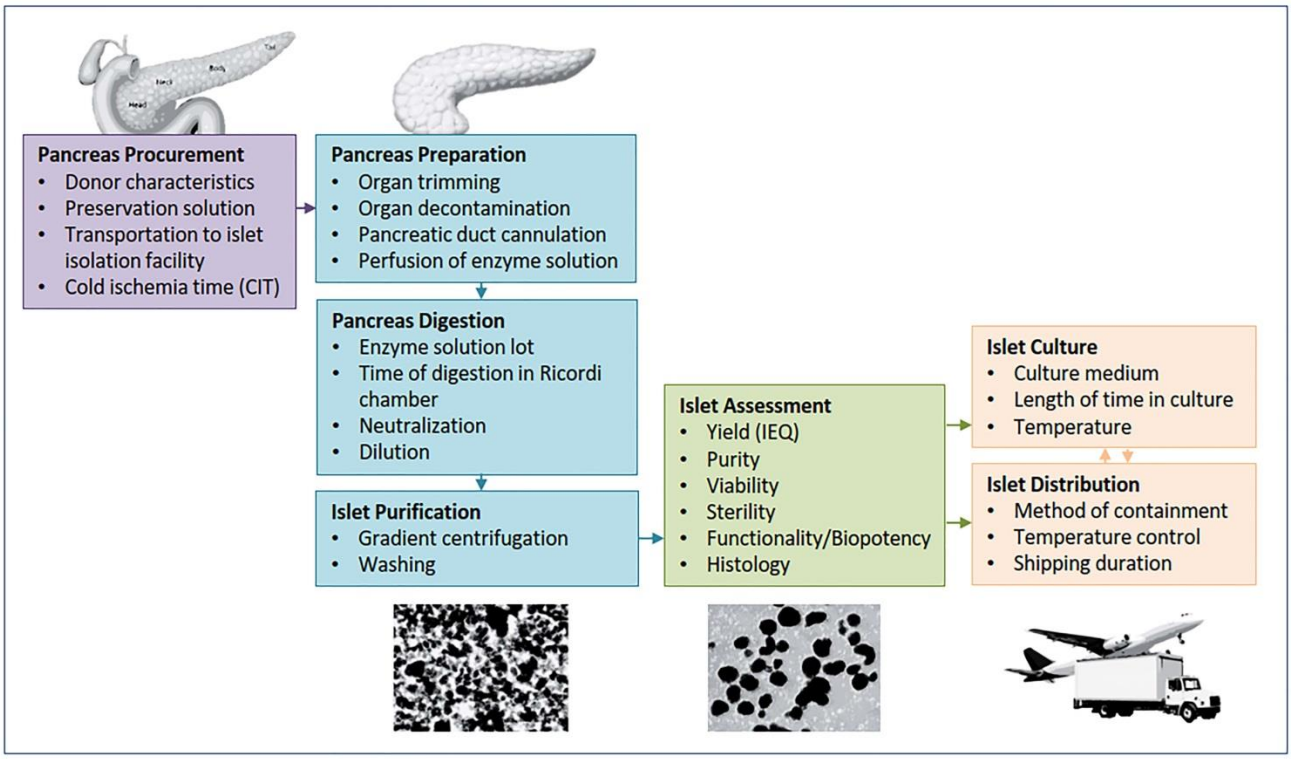

Figure 1 Overview of the key factors involved in the different steps of the human islet isolation and distribution process that can impact on eventual islet utility.

2.2 Parameters for the Evaluation of Isolated Human Islets

2.2.1 Yield

Islet yield or mass is one of the most important parameters in the assessment of islet recovery. It is most often described as islet equivalents (IEQs), as a standardized method for quantifying islets which exist in a variety of sizes and volumes. One IEQ represents a single spherical islet of 150 μm in diameter [18]. To quantify IEQs, zinc-binding stain dithizone (DTZ), which helps in the identification of islets by specifically staining the zinc-rich beta cells, is normally used. The islet cells are stained red, differentiating them from the surrounding exocrine tissues that appear brown. The size of islets is measured using a calibrated grid on the eyepiece of a bright-field microscope and the islets are categorized into different groups based on their diameter (from 50 μm to 400 μm, in 50 μm increments). The islet structures that are out of this range are disregarded. The number of islets in each category is then converted to IEQs using a fixed conversion factor. Although the manual counting method provides a straightforward way of quantifying islets, some researchers have questioned its reliability and accountability, and have proposed the use of digital image analysis to reduce user bias and variability [19, 20]. Nevertheless, IEQ counting presently remains the standard method for quantification of islet mass in the field.

For islet transplantation, the general consensus is that a minimum islet mass of 5,000 IEQ per kg body weight of the recipient is required to reverse diabetes in T1D patients [21]. However, it has been reported that transplanting a larger amount of islets (>11,000 IEQ per kg body weight of the recipient) is necessary to achieve long-term insulin independence in patients with severe diabetes [22]. This is due to a loss of up to 60% mass of the transplanted beta cells during the

initial period after islet infusion [23], which makes the availability of a larger surviving beta cell mass necessary to achieve insulin independence [21]. However, the IEQ dose is likely to be only one of several factors that influence the success of transplant engraftment. Other factors such as islet quality and donor characteristics may also contribute to eventual clinical outcomes.

2.2.2 Purity

Islet purity, defined as the proportion of islet cells relative to other pancreatic cell types such as the exocrine and ductal cells, is another aspect worth considering in every islet preparation [24]. Similar to islet counting, islet purity is assessed following DTZ staining to determine the ratio of islets to the exocrine portion. Purity level > 70% is considered to be highly pure [16]. Although high islet purity is generally preferred for islet transplantation, any correlation between islet purity and clinical outcome has not been clearly established yet. On the contrary, studies have indicated that transplantation of islets with lower purity may unexpectedly yield better clinical outcomes for T1D patients, as non-islet cells may have beneficial effects on islet graft function [24, 25]. In the context of basic research, islets are often manually hand-picked to ensure a pure islet population. However, the culture of islets in an impure preparation with surrounding exocrine tissues and dying cells may create a toxic environment that can compromise the integrity of islets.

2.2.3 Viability

Islet viability is another important factor in every islet preparation and is often measured using a cell-permeable fluorescent dye, fluorescein diacetate (FDA), and a cell-impermeable nucleic acid stain, propidium iodide (PI), which stain live and dead cells, respectively, based on their membrane permeability. However, these membrane integrity stains do not provide a comprehensive picture of cell viability. Studies have recommended the use of additional assays such as measurement of adenosine triphosphate (ATP), mitochondrial function, and oxygen consumption rate (OCR), which can contribute to more effective predictions of islet status after transplantation [26-28].

2.2.4 Functionality

For basic research, investigators tend to perform numerous assessments to evaluate islet quality, including morphological examination (islets that are spherical in shape and approximately 150 μm in diameter are desirable), histology, and functionality tests. Islet functionality, or biopotency, is assessed *in vitro* using a glucose-stimulated insulin secretion (GSIS) assay, followed by an immunoassay for insulin detection [16]. Functional validation prior to clinical islet transplantation is presently not mandatory, but doing so may increase the success of islet replacement therapy by transplanting only functional islets. However, several studies have suggested that the insulin responsiveness of isolated islets to glucose is not an accurate indicator of transplantation outcomes [25, 29]. This may partly be due to the presence of viable cells that do not respond to glucose *in vitro*, but recover and become functional after transplantation. Assays such as measurement of the OCR have been suggested to be better predictors of transplantation outcome [26]. Overall, a thorough assessment of the quality of isolated islets, which encompasses

a suite of assays for determining islet mass, viability, and insulin secretory response, where practical, is likely required to select the preparations that may result in better clinical outcomes.

2.3 Factors Affecting the Quality of Human Islets

There is a multitude of factors that can affect the quality of islet preparations (Figure 1). Donor characteristics have been commonly reported to play a major role in influencing the quality of islet preparations [14, 30-32]. As expected, hyperglycemia or high glycated hemoglobin (HbA1c) levels have been shown to negatively impact the yield, purity, and biopotency of islets [14]. Other variables such as the age of the donor remain controversial. While some studies have reported that old age negatively impacts the yield, purity, and function of islets [14, 33], another study has reported that pancreatic tissues from older donors gave rise to higher islet yield [34]. Some others reported no difference in the measured isolation outcomes of islets between old and young donors, although the latter resulted in better graft functions after transplant [35, 36]. Some studies have also revealed that donors with higher BMI yielded higher IEQs [31, 32, 34]. However, Lyon *et al.* observed that higher donor BMI correlated with larger islet size but not islet yield, purity, or function [14]. These conflicting results with regards to donor characteristics suggest that other factors might play an important role in determining the isolation and graft outcomes of human islets.

One such example is the cold ischemia time (CIT) of the tissue, defined as the length of time from the procurement and preservation of donor pancreas to its processing at the islet isolation facility, which is known to impact islet yield [12, 14]. Human pancreatic tissues that are designated for clinical islet transplantation are strictly required to have a CIT of less than 12 h [16, 31], which significantly limits the number of samples that can be accepted for transplantation, particularly in areas where the nearest isolation facility is located at a distance [37]. Extended pancreas CIT (up to 24 h) has been shown to moderately impact islet yield, purity, and total insulin content, with no changes to the exocytotic properties of beta cells [14]. This suggests that donor pancreas with extended CIT may remain suitable for islet isolations, especially for basic research.

Another critical factor influencing islet quality involves the digestion of the pancreas, which has been widely acknowledged as a highly inconsistent step in the process of human islet isolation [38-40]. Digestion of the human pancreas requires a cocktail of dissociation enzymes, including class I and II collagenases and other proteases. Poor pancreas digestion can result in poor yield and variation in the quality of the islets. Despite substantial efforts to optimize enzymatic digestion [41, 42], enzyme blends are also highly variable due to differences between batches. Kin *et al.* proposed the use of an in-house collagenase assay to pre-determine the enzymatic activity of each batch before organ perfusion [38]. A recent comparison between different enzyme blends currently used in the market revealed similar efficiencies in islet yield, purity, and viability, but with some differences in the insulin secretory function of the isolated islets [43]. Islet purification using Ficoll-based density gradient centrifugation has been considered to be the gold standard and is widely employed. Some investigators have also recommended the use of an OptiPrep-based density gradient purification method to reduce the production of cytokines/chemokines from islet preparations and improve the survival of beta cells [44]. Overall, reducing the inconsistencies and inefficiencies in the islet isolation procedures will continue to benefit the community and correspondingly facilitate the islet transplantation and research activities.

2.4 Challenges Faced in Human Islet Distribution

One major challenge faced in the human islet distribution pipeline is the shortage of islets for both transplantation and research. This can be attributed to at least two factors: (1) high operating costs of a human islet facility [45, 46] and (2) the lack of pancreas donors [45, 47]. It was previously reported that the cost of establishing a cGMP-compliant human islet isolation facility ranged from USD 1–7 million, while the annual maintenance costs ranged from USD 0.8–3 million [45]. The high cost and low pancreas donor rates present constraints that have prevented several countries from establishing their own human islet isolation facilities, thereby increasing the dependence on international human islet distribution platforms. In this case, reliable transportation of human islet material becomes particularly important, especially when shipping to geographically distant locations.

The conditions of transportation of the isolated islets are crucial in preserving islet quality. Islets may be contained in conical tubes, blood transfusion bags, gas-permeable bags, or silicone rubber membranes. Although several studies have advocated the use of gas-permeable bags to transport clinical-grade islets in order to reduce hypoxia and maintain islet quality, the advantages of these bags are not entirely consistent across studies [48-51]. Rotary systems have also been proposed to circumvent the problem of diffused oxygen and nutrient gradients in static systems [52, 53].

Another common factor influencing islet quality is fluctuations in temperature during transportation. Islets transported in a commercial airline may be exposed to temperature fluctuations of up to ±20 °C [51]. Exposure of islets to temperatures beyond their physiological range for an extended period of time negatively impacts their quality. It has been shown that hypothermic conditions (1–4 °C) drastically reduce the viability of mammalian cells [54], while high temperatures (43 °C) decrease the survival rates of early xenografts of pig islets in mice [55]. The Integrated Islet Distribution Program (IIDP) provides ColdMark® and WarmMark® indicators to detect undesirable temperature changes. Methods to monitor and prevent temperature fluctuations during the shipment of temperature-sensitive goods will be valuable for increasing confidence in the distribution of high-quality islets.

Another challenge faced during islet distribution involves the extended duration of islet culture. Studies have demonstrated that culturing islets has decreased its efficacy [56, 57]. Islet culture may also not be favorable for the maintenance of resident immune cells, blood cells, endothelial cells, and other cell types that are important for islet function [58, 59]. However, recent evidence has suggested that culture or preservation of islets offers various advantages, such as allowing time for testing of sterility and functionality, providing patients with more time to travel to transplant sites, providing sufficient time to administer immunosuppressive drugs to patients, improving islet morphology, and selecting islets with better purity and viability [13, 17, 60-62]. Therefore, optimizing strategies to preserve islets in culture will be essential to prolong their survival and function *in vitro*.

3. Human Islet Distribution Efforts around the World

Presently, there are a few large consortia or programs that currently coordinate or have recently coordinated the distribution of human islets for clinical transplantation, research, or both.

These include the Alberta Islet Distribution Program (AIDP) [63], IsletCore program at the Alberta Diabetes Institute [64], IIDP [45], European Consortium for Islet Transplantation (ECIT) [65], and the Swiss-French GRAGIL Consortium [66]. Commercial sources include Prodo Laboratories (as part of the Scharp-Lacy Research Institute) and Lonza. To achieve a better understanding of the level of human islet coordination activities across the world, we attempted a literature search and compiled a list of searchable human islet isolation centers for both clinical transplantation and research, and the status of their membership within any islet distribution networks (if any) (Table 1, Figure 2). It is immediately apparent that several human islet isolation facilities are based in North America, especially in the United States (US), with a good number also present in Europe. The number of human islet isolation facilities in other geographical regions such as South America and the Asia Pacific are considerably lower. However, this list may possibly be under-represented, as there may be facilities that are not reported on the internet. Thanks to the efficiency of islet distribution programs, many organ transplant centers are able to perform islet transplantation without establishing their own islet isolation facility [22, 67]. To optimize the safety and effectiveness of islet transplantation for T1D patients worldwide, the Collaborative Islet Transplant Registry (CITR) (https://citregistry.org/) was founded as a centralized platform to facilitate the data collection and analysis pertaining to islet transplantation. According to the CITR-reported data, which were collected from 1999 until the end of 2018 from participating transplant centers in North America, Europe, Australia, and Asia, over 2000 islet transplantations have taken place. However, it may be possible that a large number of human islet transplantation centers are not registered under the CITR. This section provides an overview of the ongoing efforts for the distribution of human islets to different regions around the world.

Table 1 List of human islet isolation centers and distribution programs (current and recent) around the world. This table has been updated as of 4th May 2019. Abbreviations: ITC: Clinical Islet Transplantation Consortium; ECIT: European Consortium for Islet Transplantation; GRAGIL: Groupe Rhin-Rhône-Alpes-Genève pour la Transplantation d'Îlots de Langerhans; OXCIT: Oxford Consortium for Islet Transplantation; AIDP: Alberta Islet Distribution Program; AHN: Allegheny Health Network; nPod-IIP: Network for Pancreatic Organ Donors with Diabetes-Islet Isolation Program; IIDP: Integrated Islet Distribution Program. *No publications were found to report human islet isolation activity at the Yale University, although such activity has been reported on the university's webpage (https://medicine.yale.edu/intmed/drc/cores/biology.aspx). Disclaimer: We apologize for inadvertently missing out on any human islet isolation centers or any misrepresentation of the use of human islets.

	Country	**IsolationCenters for Human Islets**	**Distribution Programs for Human Islets (if any)**	**Known Use of Isolated Human Islets**	**Representative Reference**
	Islet Isolation Facilities in Asia				
1	China	Changzheng Hospital, Shanghai		Basic research and clinical transplantation	PMID: 28290605
2		China-Japan Friendship			PMID:

		Hospital			24268157
3		Fuzhou General Hospital, Xiamen University			PMID: 18633105
4		Shanghai First People's Hospital			PMID: 17042977
5		Tianjin First Center Hospital			PMID: 28659588
6		Zhongda Hospital, Southeast University			PMID: 26546984
7	Japan	Fukuoka University		Clinical transplantation	PMID: 16298616
8		Fukushima Medical University			PMID: 16298616
9		Kobe University			PMID: 16298616
10		Kyoto University Hospital			PMID: 16298616
11		National Hospital Organization Chiba-East Hospital			PMID: 16298616
12	Singapore	Lee Kong Chian School of Medicine at Nanyang Technological University		Basic research	PMID: 28694456
13	South Korea	Seoul St Mary's Hospital		Clinical transplantation	PMID: 26130966
14		Sungkyunkwan University School of Medicine			PMID: 15940047
	Islet Isolation Facilities in Europe				
15	Belgium	Vrije Universiteit Brussels	ECIT	Basic research and Clinical transplantation	PMID: 9421388
16	Czech Republic	Institute for Clinical and Experimental Medicine		Basic research	PMID: 27803935
17		Prague University		Clinical transplantation	PMID: 21404489
18	France	Grenoble University Hospital	Swiss-French GRAGIL Network		PMID: 26068866
19		Lille University Hospital	ECIT Basic Research Program	Basic research and clinical transplantation	PMID: 22911383
20	Germany	University of Dresden			PMID: 25083718
21	Italy	San Raffaele Diabetes Research Institute	ECIT Basic Research Program		PMID: 23040067

22		University of Pisa		Basic research	PMID: 22412385
23	Netherlands	Leiden University		Basic research and clinical transplantation	PMID: 21257658
24	Norway	Oslo University Hospital	CITC		PMID: 27862341
25	Spain	Carlos Haya University		Clinical transplantation	PMID: 16298608
26	Sweden	Uppsala University Hospital	CITC, ECIT Basic Research Program, Nordic Network for Islet Transplantation	Basic research and clinical transplantation	PMID: 25422108
27	Switzerland	Geneva University Hospital	ECIT Basic Research Program, Swiss-French GRAGIL Network		PMID: 23040067
28		Lonza		Basic research	PMID: 30074059
29		University Hospital Zurich		Clinical transplantation	PMID: 17327426
30	United Kingdom	Oxford DRWF Human Islet Isolation Facility	OXCIT	Basic research and clinical transplantation	PMID: 29412141
31		King's College London			PMID: 29205751
32		University of Edinburgh			PMID: 24119216
33		Worcester Acute Hospitals NHS Trust			PMID: 17469038
	Islet Isolation Facilities in the Middle East				
34	Iran	Tehran University of Medical Sciences		Basic research and clinical transplantation	PMID: 21818570
35		Shiraz University of Medical Sciences			PMID: 23477484
	Islet Isolation Facilities in North America				
36	Canada	Clinical Islet Laboratory at Alberta Health Services, University of Alberta	AIDP, CITC	Basic research and clinical transplantation	PMID: 22099755
37		IsletCore from the Alberta Diabetes Institute, University of	IsletCore Program	Basic research	PMID: 26653569

		Alberta			
38		Banting and Best Diabetes Center, University of Toronto			PMID: 29436377
39		McGill University Health Center Human Islet Transplant Laboratory (MHITL)		Basic research and clinical transplantation	PMID: 26280028
40		Toronto General Hospital			PMID: 30520247
41		Transplant Quebec			PMID: 30520247
42		University of British Columbia			PMID: 23262664
43	United States	Allegheny Health Network's Islet Isolation Laboratory, Allegheny General Hospital		Clinical transplantation	PMID: 30057900
44		Baylor Research Institute		Basic research and clinical transplantation	PMID: 20944753
45		Columbia University			PMID:17229069
46		Dartmouth College			PMID: 25630865
47		Emory University	CITC		PMID: 25648831
48		Icahn School of Medicine at Mount Sinai		Basic research	PMID: 27941248
49		Joslin Diabetes Center			PMID:17229069
50		Massachusetts General Hospital	CITC	Basic research and clinical transplantation	PMID: 23321263
51		National Institutes of Health		Basic research	PMID: 25648831
52		Northwestern University	CITC	Basic research and clinical transplantation	PMID: 25648831
53		Scharp-Lacy Research Institute (Prodo Laboratories)	IIDP	Basic Research	PMID: 26452321
54		Southern California Islet Cell Resources Center	IIDP	Basic research and clinical	PMID: 20098276

				transplantation	
55		Stanford Islet Research Core at Stanford University		Basic research	PMID: 17389914
56		University of Alabama			PMID: 25648831
57		University of California, San Francisco	CITC	Basic research and clinical transplantation	PMID: 26922947
58		University of Chicago		Basic research	PMID: 26922947
59		University of Colorado			PMID: 25648831
60		University of Florida	nPOD-IIP		PMID:24325575
61		University of Illinois	CITC	Basic research and clinical transplantation	PMID: 25648831
62		University of Louisville		Basic research	PMID: 27500247
63		University of Massachusetts			PMID: 25648831
64		University of Miami	IIDP, CITC	Basic research and clinical transplantation	PMID: 29723228
65		University of Minnesota	CITC		PMID: 25648831
66		University of Pennsylvania	IIDP, CITC		PMID: 27920090
67		University of Pittsburgh			PMID: 27920090
68		University of Virginia			PMID: 23040067
69		University of Wisconsin	IIDP, CITC		PMID: 25648831
70		Washington University		Basic research	PMID: 25648831
71		Yale University		Basic research and clinical transplantation	*
	Islet Isolation Facilities in Oceania				
72	Australia	St Vincent's Hospital	Australian Islet Transplant Consortium	Basic research and clinical transplantation	PMID: 29483160

73		Westmead Hospital		Clinical transplantation	PMID: 23668890
	Islet Isolation Facilities in Other Regions				
74	Argentina	Hospital Italiano de Buenos Aires		Clinical transplantation	PMID: 9532053
75	Brazil	Hospital de Clinicas de Porto Alegre			PMID: 25993680
76		PUC-Paraná University			PMID: 25993680
77		University of Sao Paulo			PMID: 25993680

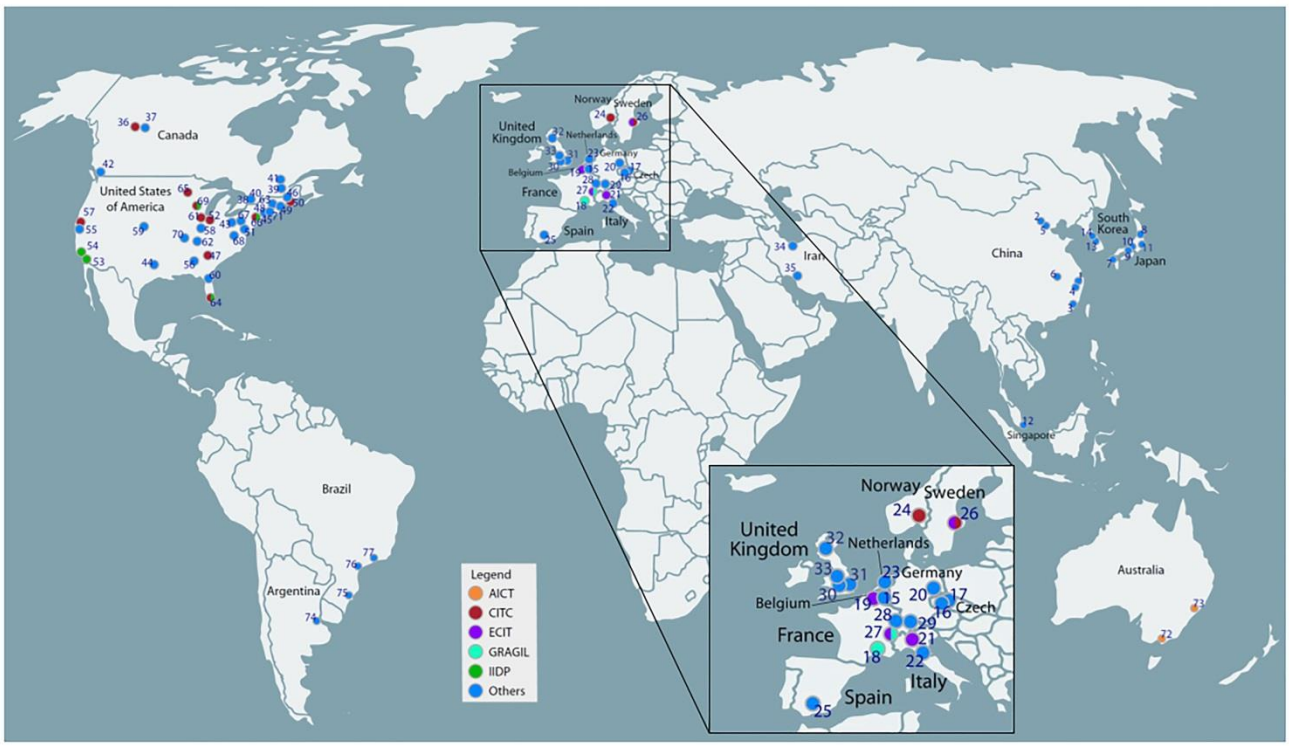

Figure 2 A schematic map of isolation centers for human islets around the world and the islet distribution networks. Centers are numbered with reference to Table 1. Circles are color-coded as stipulated in the Legend. AICT: Australian Islet Transplant Consortium; CITC: Clinical Islet Transplantation Consortium; ECIT: European Consortium for Islet Transplantation; GRAGIL: Groupe Rhin-Rhône-Alpes-Genève pour la Transplantation d'Îlots de Langerhans; IIDP: Integrated Islet Distribution Program. Disclaimer: We apologize for any human islet isolation centers that we may have missed out or any misrepresentation of islet isolation facilities.

3.1 North America

The University of Alberta in Canada, which has contributed to many pioneering efforts in human islet isolation, including the development of the widely-adopted Edmonton Protocol [3], is considered to be a leader in islet isolation and transplantation. It currently has two of the most active human islet isolation facilities in the world– the Clinical Islet Laboratory (CIL) and IsletCore

Laboratory. Under the AIDP, the CIL had distributed 6.3 million IEQs to eight local and international research institutions through 127 shipments in 2010 [63]. The AIDP now distributes islets to investigators in North America, Middle East, Europe, and Asia. The primary objective of the CIL is to isolate human islets for clinical transplantation. This is different from the IsletCore program at the Alberta Diabetes Institute, which isolates, stores, and distributes islets solely for research purposes. IsletCore was founded to address the widening gap between the demand and supply of human islets [65, 68]. With a facility dedicated to isolating islets solely for research, the operational costs are reduced without the need to adhere to cGMP regulations [14]. The IsletCore has provided a major resource and biobank of human islets for research worldwide. According to the IsletCore 2018 Annual Report, the program has isolated 76 million IEQs from 297 donor pancreatic tissues since its inception in 2011 until the end of 2018, with more than 10 million IEQs in 2018 alone. About 25% of the donors were Type 2 Diabetes (T2D) patients and a small number were T1D patients. Of the 76 million IEQs, more than 32 million IEQs were distributed, while the rest were cryopreserved or banked. The IsletCore now distributes islets to a network of 86 research laboratories worldwide. The IsletCore also isolates human islets from diabetic donors, providing a valuable resource of human tissue for the study of the pathophysiology of diabetes.

In the US, the National Institutes of Health (NIH)-sponsored IIDP is the largest research-only human islet distribution center. Notably, the IIDP has shipped over 168 million IEQs since 2009, with more than 8 million IEQs in 2018 alone. Most of the five centers that are affiliated with IIDP isolate islets from non-diabetic cadaveric donors and occasionally from T1D or T2D diabetic cadaveric donors. In particular, the University of Florida, as part of the Juvenile Diabetes Research Foundation (JDRF) Network for Pancreatic Organ Donors with Diabetes (nPOD)-Islet Isolation Program (IIP), isolates islets from T1D donors to serve the T1D research community. The US has more human islet isolation facilities than any other country in the world (Figure 2), which could be attributed to greater availability of funding sources to support islet isolation facilities, a larger pool of pancreas donors, and better infrastructure and expertise to support the preparation of high-quality human islets. However, despite the substantial number of islet isolation centers and a well-coordinated consortium for islet distribution, there is still an insufficient supply of human islets to meet the international research demand [69].

3.2 Europe

The ECIT, that consists of six isolation centers across Europe, provides human islets for both clinical transplantation and basic research (Table 1). As of 2014, the ECIT had received requests amounting to 31 million islets [65]. The Swiss-French GRAGIL network was formed to coordinate islet transplantation for T1D patients. It distributes islets only within France and Switzerland, from its two main islet isolation centers at the Geneva University Hospital and Grenoble University Hospital [70]. In Scandinavia, the Nordic Network for Islet Transplantation was founded to support islet transplantation for T1D patients. It distributes islets largely within the Scandinavian countries, with the Uppsala University in Sweden being the only islet isolation facility in the region. In the UK, there are three islet isolation facilities, at the University of Oxford, King's College London, and the University of Edinburgh. These form a network to supply human islets for both clinical transplantation and research. The presence of coordinated efforts in a few regions of Europe ensures efficient distribution of islets and minimizes the need for more isolation facilities within

these regions. However, coverage in other parts of Europe is still visibly lacking. A number of individual isolation facilities were identified in other European countries, including the Czech Republic and Germany. These centers are not registered under the CITR.

3.3 Asia

There appear to be significantly fewer human islet isolation centers in Asia as compared to North America and Europe, with a majority of such centers being concentrated in China, South Korea, and Japan, and only one in Singapore (Figure 2). To the best of our knowledge, there are no coordinating efforts or networks that facilitate the distribution of human islets in a country or to other countries in Asia. In China, a number of facilities, such as the Tianjin First Center Hospital, are known to isolate human islets for both research and clinical transplantation [71, 72]. However, the list in Table 1 is likely to be an underestimate, especially for China, as information regarding certain centers in China may not be well-documented in English. Human islet isolations are largely reserved for clinical use in Japan and South Korea. In Singapore, the Lee Kong Chian School of Medicine isolates human islets for research purposes [73]. In the Middle East, islet isolation facilities have so far only been reported in Iran [74]. The major reasons for the relatively low level of activity in Asia include the lack of pancreas donors, possibly due to cultural reasons [47], as well as insufficient resources allocated to establish and maintain the human islet isolation infrastructure. Investigators in Asia may, therefore, rely on established distribution programs such as the AIDP, IsletCore, IIDP, or commercial sources (Prodo Laboratories and Lonza) to obtain access to human islets for clinical transplantation or research. However, a major downside of obtaining islets from international distributors is the lengthy transportation time, often taking up to several days. The quality of islets post-shipment may be compromised due to the variability in handling procedures and temperature fluctuations during long-distance shipping [75]. The deterioration of islets in culture over time is particularly evident for islet preparations of lower purity. Considering the shortage of donors in Asia, access to islets from diabetic donors of Asian descent are even more limited, and they remain recognized as a rare and valuable resource due to the genetic and epigenetic differences between different ethnic groups [76].

3.4 Other Regions

In Australia, the Australian Islet Transplant Consortium facilitates the distribution of human islets across Australia and New Zealand. There are three islet transplant centers in Australia (Westmead Hospital, St Vincent's Hospital, and Royal Adelaide Hospital) and none in New Zealand. The transplant centers obtain islets that are isolated from two islet isolation facilities situated in Melbourne and Sydney (Table 1). According to the 2017 annual report by the Australia and New Zealand Islet and Pancreas Transplant Registry (ANZIPTR), a total of six islet transplants have been performed since 2016.

Little is known about islet transplantation activities in South America and other parts of the world. Three islet isolation facilities have been reported in Brazil, and one in Argentina, with activities pertaining to clinical islet transplantations [77-79]. None of these are registered under the CITR.

4. Human Islets for Beta Cell Replacement Therapy and Research

The availability of human islets and the continuous improvements in protocols and strategies over the past two decades have resulted in substantial clinical impact and scientific development. This section briefly highlights the ongoing research on islet transplantation outcomes and provides a summary of the leading basic science research that has enabled unprecedented investigations into islet biology, based on work carried out during the past ten years.

4.1 Human Islets for Clinical Transplantation

Clinical islet transplantation, also known as beta cell replacement therapy, is currently used to treat some patients with T1D. Allogeneic islet cell transplantation is safe and has yielded success as measured by graft function, absence of hypoglycemic unawareness, normalized glycemic control, and partial to complete insulin independence in T1D patients across multiple centers. However, not all centers have been able to achieve the same level of success, especially in the long term [80]. The rate of success has been observed to be influenced by the experience of the personnel managing the islet isolation and transplantation procedures [81]. A long-term (>10 years) follow up of T1D patients that had undergone allogeneic islet cell transplantation revealed that the treated patients maintained some level of islet graft function, although this function decreased considerably over time [82]. A subset of patients required multiple islet transplants and very few of them had complete insulin independence at the completion of the 12-year follow-up. Therefore, trials have demonstrated that the islet grafts are safe over the long term but their function declines over time, possibly due to inadequate initial transplanted islet mass and a suboptimal transplantation site (liver) that may not be ideal for long term viability [4, 80]. As such, issues remain with the transplantation of allogeneic islets, including the need for multiple islet transplants and a continuous requirement of the patient to undergo heavy long-term immunosuppression to maintain islet graft function, resulting in an increased risk of side effects. Unlike Canada, parts of Europe, and Australia, allogeneic islet transplantation is considered experimental in the US and is not used as standard therapy. The lack of reimbursement and research funding for allogeneic islet transplants might contribute to the decline in reported cases of allogeneic islet transplants and an increase in autologous islet transplants in North America [80].

Autologous islet transplantation is performed to prevent post-surgical diabetes, following a total pancreatectomy in patients with chronic pancreatitis or certain pancreatic tumors. The benefits of autologous islet transplantation are widely acknowledged and this form of transplantation is considered to be a safe and effective treatment option for chronic pancreatitis as well as for the preservation of islet function [83, 84]. Total pancreatectomy with transplantation of autologous islets is increasingly being performed in the US [85]. However, patients may still lose insulin independence after some years, possibly due to the development of autoimmunity after autologous transplant. A recent study demonstrated that transplantation of autologous islets may also be beneficial for other conditions apart from chronic pancreatitis, such as pancreatectomy for the treatment of severe pancreatic fistulas or extensive distal pancreatectomy for neoplasms of the pancreatic body and neck. Furthermore, this option helps the clinician avoid the high risk of pancreatic anastomosis in case of unfavorable pancreata. Balzano *et al.* suggested that the procedure should be recommended to patients undergoing pancreatic surgery for both non-

malignant and malignant conditions [86, 87]. Clinical transplantation of islets is now not only exclusive to institutions with on-site isolation facilities but is also made possible by the sharing of expeditious off-site islet isolation facilities [88], which enables the outsourcing of islet isolation after a pancreatectomy if the same site is not capable of doing so.

Another active area of development in islet transplantation involves the use of alternative sites for transplantation. Infusion of islet cells through the portal vein for engraftment in the liver is the current gold standard [89]. However, intrahepatic islet transplantation is associated with several limitations, including a limited volume of transplanted tissue and high exposure to immunosuppressive drugs as well as the recipient's immune system [90]. A number of secondary sites have been investigated, including omental islet transplantation, which has been reported to be effective at maintaining euglycemia without exogenous insulin [91]. Other possible sites of implantation include the peritoneum, subcutaneous tissue, and kidney capsule [85, 92, 93]. Long-term data in large patient cohorts will be required to determine the safety and feasibility of these alternative transplantation sites.

Novel islet encapsulation systems are also being developed and tested in pre-clinical and clinical settings to confer immuno-protection and prevent rejection of islet allografts. Approaches for encapsulation include macro-encapsulation, a device-based system that houses several islets within a semi-permeable barrier, or micro-encapsulation, for microscale capsules that coat individual islets. These approaches typically utilize polymer- or alginate-based semi-permeable membrane barriers that selectively enable nutrient exchange but block immune components [94, 95]. Macro-encapsulation strategies that have so far been applied in clinical trials include the oxygenated βAir device developed by Beta-O_2 and the Encaptra device by ViaCyte. The results from a phase-1 study on a small number of patients revealed that although the peritoneally-transplanted βAir device was safe and supported the survival of the islet allografts for several months, the transplanted islets retained limited insulin secretion function [96]. The subcutaneously-delivered Encaptra delivery system, which encapsulated stem cell-derived pancreatic progenitor cells instead of human islets, appeared to be well-tolerated but was limited by low rates of engraftment. The studies on microencapsulation by Diatranz Otsuka involved the peritoneal transplantation of alginate-encapsulated porcine islets into T1D patients without immunosuppression [97, 98]. Some patients were reported to have improved HbA1c levels and reduced hypoglycemic events, although the reduction in the dependence on exogenous insulin was marginal [98]. Overall, continued improvements in encapsulation technologies and biomaterials are still required, and the clinical outcomes of other ongoing trials that have employed different encapsulation strategies are yet to be seen. This approach, when proven effective, will promote the widespread adoption of allogeneic islet transplantation with reduced need for immunosuppression.

4.2 Unique Findings on Human Islet Biology and Pathogenesis of Diabetes Based on Human Islet Studies

Research activities on pancreatic islet cell biology and its applications in health and disease are increasing rapidly, and this trend is expected to continue over the next few years. This is fueled by multiple factors, including the increasing global prevalence of diabetes and the resulting intensified focus on the disease mechanisms of diabetes. The widespread adoption of the human

beta cell line EndoC-βH1 [99], increased accessibility to human islets as a research tool, as well as rapid advances in stem cell platforms to generate insulin-producing beta cells from human pluripotent stem cells, have also driven the progress made in studying beta cell functions. Human islets can reveal important biological aspects that are not possible in other isolated cell models. For example, human islets are known to comprise of 30–40% alpha cells interspersed among the beta cells, whereas in rodents, alpha cells only constitute ~10% of the islets and are found on the periphery of a beta cell core [8].

4.2.1 Regeneration of Human Beta Cells

Mature adult human beta cells are known to have particularly low proliferation rates, possibly due to age-related changes in cell cycle blockage and silencing of proliferative signaling molecules and genes that are essential for cell replication [100, 101]. Several groups have attempted to develop strategies to regenerate human beta cells to tackle diabetes. One study used human islets to show that beta cells can be induced to proliferate at a faster rate both *in vitro* and *in vivo* by synergistic inhibition of the DYRK1A and TGFβ superfamily signaling pathways. The authors demonstrated that the inhibition of these pathways by pharmacological agents may not only increase the number of beta cells but also their differentiation markers, in islets from both non-diabetic and T2D donors [102]. The detailed mechanistic studies using human islets highlighted an increase in the proliferation rate of beta cells following the disruption of SMAD-mediated activation of the cell cycle inhibitors *CDKN1A* and *CDKN1C*. The authors also demonstrated that the combination of DYRK1A and TGFβ superfamily inhibitors induced proliferation in an *in vivo* setting, when the human islets were transplanted into mice. However, as these small molecules also target cell types other than beta cells, development of beta cell-specific compounds is needed to avoid off-target adverse effects. Other approaches for the regeneration of endogenous beta cells include genetic silencing of the cell cycle inhibitors p18 and p21 in human islet cells to promote the entry of quiescent adult human beta cells into the cell cycle [103]. Another strategy for the regeneration of beta cells involves the conversion of non-beta cells such as alpha cells and ductal cells into beta cells, although these studies have mainly been validated only in animal models [104, 105]. Together, these studies have highlighted the possibility of restoring the mass and function of beta cells in T2D. In several cases, primary human islets remain the gold standard cell model for study. However, as the data from human islets tend to be noisy due variabilities in islet preparations, the functional or mechanistic studies using human islets are often supplemented with other models, or involve the use of very large sample sizes. In a study by Wang *et al.*, more than 100 human islet samples (obtained from IIDP) were used [102].

4.2.2 Understanding the Regulation of the Glycemic Set Point in Humans

A study by Rodriguez *et al.* demonstrated that non-human islet cells do not necessarily recapitulate human biology. This study involved the use of human islets to determine the glycemic set point in humans [106]. As different animal species have different glycemic set points, the study showed that it was necessary to use human islets to determine the mechanisms regulating the glycemic set point in humans. The study compared the outcomes from experimental xenotransplantation of islets from human donors, mice, and cynomolgus monkeys into immunodeficient nude mice. The authors demonstrated that the pancreatic islets alone were

enough to robustly impose its glycemic set point; in other words, they established their target normoglycemic value in the recipient species. Strikingly, islets from all three species imposed different non-fasting glycemic levels. In contrast to mouse islets, human islets required the cooperation and interaction of both alpha cells and beta cells to fine-tune insulin secretion and maintain normoglycemia.

4.2.3 Human Beta and Alpha Cell Function in Disease States

In addition to the use of islets from healthy cadaveric donors, access to islets from diabetic donors presents an immensely valuable opportunity. Brissova *et al.* were able to obtain islets from eight T1D donors to study the molecular and functional profiles of beta and alpha cells from T1D patients [107]. The study reported that the rare residual beta cells that were found in the islets appeared to retain their insulin secretory function at a similar level to that of the normal islets. On the other hand, glucagon secretion from alpha cells was impaired, possibly due to changes in the expression of genes governing the identity and exocytotic machinery of alpha cells. Thus, diabetic islets facilitated the study of the functional capacity and molecular alterations of the alpha cells and residual beta cells in the context of T1D, which would otherwise have been impossible in non-diabetic islets.

Another study performed RNA sequencing of human islets from normoglycemic donors (n=81) and donors that were chronically exposed to hyperglycemia in pre-diabetes or T2D (n=35), in order to identify changes in the gene expression profile as a result of chronic hyperglycemia [108]. The authors also exposed a subset of these islets to short-term (up to 24 h) high glucose concentrations *in vitro*, in order to rule out genes that were affected by acute glucose exposure. They reported that genes such as *ERO1B*, which encodes an oxidoreductase that controlled protein folding in the endoplasmic reticulum (ER), may be causally involved in T2D pathogenesis. These data provided a catalog of gene expression changes in human pancreatic islets upon acute and chronic glucose exposures, facilitating future studies of causal mechanisms in T2D.

4.2.4 Omics Studies of Human Islets

The characterization of human islets using systems biology approaches has also progressed rapidly in recent years. Researchers have established comprehensive biobanks of human islets from organ donors, often including both non-diabetic and diabetic individuals to facilitate the analysis of genetics, transcriptomics, and epigenomics, in order to understand the physiology and pathophysiology of beta cells. The IMIDIA (Innovative Medicines Initiative for Diabetes) consortium in Europe performed comparative analyses of a large collection of islet transcriptome data collected from 116 non-diabetic and 55 T2D donors, and identified novel and known gene targets that were dysregulated in T2D and that influenced beta cell insulin secretion[10]. Network analyses identified additional transcription factors that were potential drivers of the dysregulated beta cell gene signatures in T2D. Access to a large biobank of human islets with next-generation sequencing and phenotypic characterization data also facilitated the targeted studies of diabetes-associated genetic variants in islets [109-111]. For instance, RNA sequencing and high density genotyping were performed on 118 human islet samples (from Oxford and Edmonton) to generate expression quantitative trait locus (eQTL) data [111]. The overlapping of a subset of these islet eQTLs with known T2D risk and/or glycemic trait loci from genome-wide association studies

(GWAS) revealed putative effector transcripts whose expression levels could be altered to influence T2D risk. The authors then focused on one such candidate, *ZMIZ1*, and showed that its upregulation reduced glucose-induced insulin secretion due to the impairment of insulin exocytosis. In another study on the T2D-associated locus *PAM*, insulin secretion was found to be significantly reduced in islets from carriers that were heterozygous for the low-frequency rs35658696 risk variant, compared to matched controls (n=16 in each group) [109]. The measurements of exocytosis from dispersed human islets revealed that beta cells from risk variant carriers showed altered insulin granule release dynamics under glucose stimulatory conditions. These studies on human islets helped establish the previously unknown mechanisms through which the T2D-linked effector transcripts influence beta cell function, thus contributing to T2D risk. Therefore, access to tissues from donors of Asian descent [73] and other minorities may provide new opportunities to study ethnicity-specific disease-associated variants.

Several studies have now reported chromatin maps and other islet-specific epigenetic signatures from human islets, including chromatin state [112, 113], DNA methylation [114, 115], histone marks [116, 117], and transcription factor binding sites [117]. Together with the available genomic and transcriptomic data, this information can now be integrated to form a comprehensive picture of the genomic and epigenomic landscape of islets, and identify the regulatory pathways through which genetic variation can influence the T2D susceptibility in relevant tissues.

4.2.5 Findings from Single Cell RNA Sequencing Studies

Apart from bulk analyses of human islets, advances in high-throughput single cell analysis techniques have facilitated the investigation of a gene or protein expression patterns at a single cell level. The islet is an interesting structure to study because of its composition of multiple endocrine cell types (beta, alpha, delta, PP, and epsilon cells) and the still incompletely-understood complex interactions between these cell types that contribute to the overall functionality of the islet. Recently, single-cell RNA-sequencing (scRNA-Seq) has been used by various groups of researchers to identify cell-type-specific as well as overlapping transcriptional profiles within the islet, and to compare patterns of gene expression between healthy and T2D states [73, 118-121]. One group also used mass cytometry with up to 24 different labels to simultaneously quantify the expression of different endocrine cell type markers and proliferation markers at protein level [122]. In this study, the authors evaluated the single-cell expression patterns from 20 human islet samples of different age groups, and reported that alpha cells exhibited higher basal proliferation and replication potential in response to mitogenic signals, compared to the other major endocrine cell types [122]. The authors also identified multiple beta cell states, including a small cluster showing high expression of the proliferative marker Ki67. There has also been growing interest in the characterization of additional pancreatic cell types (such as acinar and ductal cells) from the same human donor in order to improve the overall understanding of human pancreas function. Segerstolpe *et al.* identified two subpopulations of acinar cells, one of which was marked by the high expression of inflammatory response genes, which could be responsible for immunological responses to environmental signals [119]. Furthermore, Baron *et al.* reported the presence of multiple subpopulations of ductal cells that

were also spatially separated and were thus likely to correspond to different functional specializations in the human pancreas [123].

Importantly, it has been consistently reported across studies on single cells that each cell type population, especially the beta cells, possess remarkable heterogeneity. A better understanding of beta cell heterogeneity and how different beta cell subtypes influence beta cell function or regeneration might be critical to tackling diabetes [124]. Studies analyzing the differential gene expression in pancreatic cells of healthy and T2D subjects have highlighted ER stress pathways and other genes that were not known to be involved in islet cell growth or function but are now potential candidates for therapeutic targeting, and have described the previously underappreciated role of delta cells in islet dysfunction in T2D [118-120, 123].

4.2.6 Functional Imaging of Human Beta Cells

Functional imaging is another method that has been frequently employed to study the biology of human islets at single-cell resolution in both intact and dispersed islets. As both insulin and glucagon exocytosis from the beta cells and alpha cells respectively is preceded by calcium influx, calcium flux assays using calcium dyes or recombinant calcium probes are performed to determine the dynamics of calcium in response to changes in glucose levels as a proxy for islet cell function [125, 126]. Exocytotic events may also be studied using total internal reflection fluorescence (TIRF) microscopy to track individual insulin granule fusion in the beta cells of intact human islets [127, 128]. Another approach that facilitates the longitudinal and non-invasive functional imaging of islet cells in an *in vivo* environment involves the transplantation of isolated human islets into the anterior chamber (AC) of the mouse eye followed by microscopic analysis [129]. This technique allows the engraftment and subsequent innervation and vascularization of islets, at least in rodent models. One study that employed this methodology was able to characterize the 3-dimensional architecture and intra-islet arrangement of alpha and beta cells with respect to the vascular network and surrounding endothelial cells [130]. These powerful imaging techniques have facilitated investigations of individual islet cell function, with spatial as well as a temporal resolution within the architecture of the entire islet. Future studies will likely focus on more functional imaging of the islets and on the combination of functional studies with multi-omics analyses.

5. Concluding Remarks

It is evident that there is an increasing demand for human islets for both clinical transplantation and research, and although there are presently more facilities than ever before, setup costs are high and there remains a shortage in supply. Despite efforts to standardize the protocols for the isolation and assessment of human islets, the overall quality of islet preparations and the reported characteristics of these islets can be highly variable. These variations contribute to differences in clinical outcomes after transplantation and difficulties in interpreting and comparing data across different research studies [64, 131, 132]. Therefore, emphasis should be placed on developing more sophisticated standards and criteria to standardize the isolation procedures and assessment metrics [131], and to establish larger collaborative multi-center networks around core islet isolation facilities, in order to circumvent the inconsistency issues [22]. Such efforts will encourage more collaborative studies and enable human islet experiments to be validated across multiple

centers [133]. As we improve our understanding of the constitution of a functional islet, researchers are also using this information to actively develop novel strategies to improve islet function, and devise alternatives to human islets for therapy and/or research by using human pluripotent stem cell platforms to 'manufacture' human islets [134] or through xenotransplantation [98, 135]. Overall, numerous outstanding clinical and basic research studies on human islets have been carried out, some of which were beyond the scope of this review. Continued accessibility to functional human islets of high quality and novel initiatives that facilitate the sharing of knowledge and expertise may significantly improve global patient and research outcomes.

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Competing Interests

The authors have declared that no competing interests exist.

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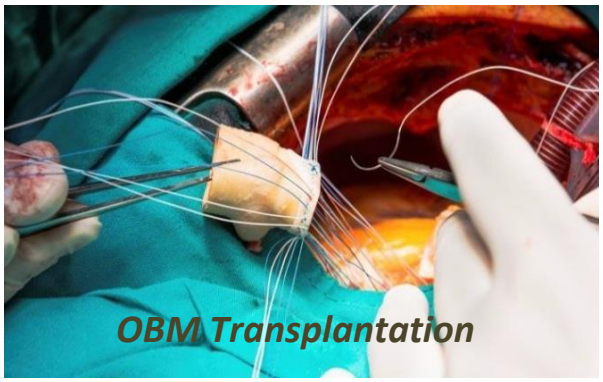

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