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The role of endothelial cells in pancreatic islet development, transplantation and culture

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Endothelial cells (ECs) play pivotal roles in the development and maintenance of tissue homeostasis. During development, vasculature actively involves in organ morphogenesis and functional maturation, through the secretion of angiocrine factors and extracellular matrix components. Islets of Langerhans, essential functional units of glucose homeostasis, are embedded in a dense endothelial capillary network. Islet vasculature not only supplies nutrients and oxygen to endocrine cells but also facilitate the rapid delivery of pancreatic hormones to target tissues, thereby ensuring precise glucose regulation. Diabetes mellitus is a major disease burden and is caused by islet dysfunction or depletion, often accompanied by vessel loss and dysregulation. Therefore, elucidating the regulatory mechanisms of ECs within islets hold profound implications for diabetes therapy. This review provides an overview of recent research advancements on the functional roles of ECs in islet biology, transplantation, and *in vitro* islet organoid culture.

KEYWORDS

endothelial cell (EC), pancreatic islet, diabetes mellitus, organoid culture, islet transplantation, β cells

1 Introduction

Pancreatic islets, also known as islets of Langerhans, are the primary functional unit for blood glucose regulation, through the finetuning of various secreted hormones. Islets consist of four main endocrine cell types: β cells, α cells, δ cells, and PP cells, which secrete insulin, glucagon, somatostatin, and pancreatic polypeptide, respectively (Nadal et al., 1999). Islets are embedded in dense capillary networks, which are established alongside embryonic islet formation and actively maintain islet function (Cleaver and Dor, 2012; Burganova et al., 2021; Hogan and Hull, 2017). Despite constituting only 1%–2% of pancreatic tissue volume, islets disproportionately receive 10%–15% of the pancreatic blood flow (Henderson and Moss, 1985), which highlights the critical function of islet vasculature in warranting efficient nutrient delivery, precise glucose sensing, and rapid hormone distribution.

Diabetes mellitus, characterized by insufficient insulin secretion or impaired insulin response, results in uncontrollable hyperglycemia. Conventional treatments, such as glucagon-like peptide-1 (GLP-1) receptor agonists and insulin injections,

require burdensome daily long-term intervention. Therefore, islet transplantation has emerged as a promising approach for restoring autonomous glycemic regulation. However, many challenges persist, including donor shortages, immune rejection, and the unpredictable survival of post-transplantation islets (Pepper et al., 2018; Shapiro et al., 2000; Lysy et al., 2013). ECs have been demonstrated to enhance islet survival post-transplantation, emphasizing the importance of vascularization in transplantation success (Bowers et al., 2019; Chen et al., 2023). In this review, we summarize recent studies explicating the role of ECs in islet biology, transplantation, and *in vitro* organoid culturing, combined with newly emerging technologies to further clinical translation for diabetes treatment.

2 ECs in pancreatic islet development

Signals derived from ECs are crucial for endocrine differentiation and islet functional maturation (Cleaver and Dor, 2012; Burganova et al., 2021; Hogan and Hull, 2017) (Figure 1). Here we enlist current studies illustrating the supporting role of ECs at various pancreatic developmental phases.

2.1 Role of ECs in islet embryonic development

As an endodermal origin, pancreas formation occurs alongside the major vessels (Cleaver and Dor, 2012). In mice, as early as embryonic day 8 (E8), direct contact was observed between dorsal aorta (red) and pancreatic foregut endoderm (yellow) (Figure 1A). Between E8.5 and E9.5, both dorsal and ventral pancreatic buds form and subsequently merge to generate the pancreas. Pancreatic epithelium underwent two distinct waves of endocrine differentiation. The initial wave, termed primary transition, begins around E9.5. During this phase, formed endocrine cells neither expressed mature markers nor assembled into functional islets. At around E13.5, secondary transition occurs, which marks a period of increased production and differentiation of endocrine cells with mature markers such as insulin by β cells and glucagon by α cells. By E18.5, these endocrine cells separate from ductal branches and migrate into the surrounding acinar tissue in aggregates to form mature islet structures (Pan and Wright, 2011; Gittes, 2009).

In humans, the dorsal and ventral pancreatic buds also arise from the foregut endoderm (Jennings et al., 2015), with their development initiating at 27–29 days post-conception (Jennings et al., 2013). Unlike in rodents, human pancreatic development involves only a single wave of endocrine cell formation. Furthermore, islet capillaries in humans are larger and less dense, with human islets containing five times fewer vessels per unit area compared to mouse islets (Brissova et al., 2015; Carlsson et al., 2002). A more comprehensive overview of ECs in human pancreas development can be found in this review (Henry et al., 2019). The mechanisms underlying the interaction between ECs and human islets remain unclear, as existing studies are primarily *in vitro* and lack *in vivo* validation.

During mouse embryonic development, ECs actively participated in pancreatic morphogenesis and endocrine

differentiation (Figures 1A, B). In 2001, Lammer et al. showed that dorsal aorta induced simultaneous expression of pancreatic duodenal homeobox 1 (Pdx1), a key pancreatic transcription factor, and insulin, marker of β cells, in cultured endodermal cells (Lammert et al., 2001). Consistent with this, removal of the dorsal aorta in *Xenopus laevis* embryos significantly decreased or even completely abolished the expression of pro-endocrine transcription factors and prevented the emergence of insulin-expressing cells. Furthermore, transgenic mice with ectopic vascularization in the posterior foregut led to ectopic insulin expression and consequently islet hyperplasia, with the ectopically insulin-expressing cells closely associated with the vascularized areas (Lammert et al., 2001).

Interestingly, the critical role of ECs is more biased towards dorsal pancreatic bud development compared to the ventral part. Experiments using fetal liver kinase one knockout ($Flk1^{-/-}$) (Vascular endothelial growth factor receptor 2, $Vegfr2^{-/-}$) mouse embryos, which failed to form ECs, resulted in severe impairment of dorsal pancreatic budding and Pdx1 expression, whereas ventral pancreatic development seemed unaffected (Yoshitomi and Zaret, 2004). Also, dorsal pancreatic epithelium in $Flk1^{-/-}$ embryos failed to activate pancreas-associated transcription factor 1a (Ptf1a) expression (Yoshitomi and Zaret, 2004), a key transcription factor for acquiring and maintaining pancreatic fate in progenitor cells (Kawaguchi et al., 2002).

In 2009, Crawford et al. discovered that connective tissue growth factor (Ctgf) is essential for establishing a normal ratio and architecture of islet endocrine cells during embryonic development (Crawford et al., 2009; Ivkovic et al., 2003). Specifically, *Ctgf* knockout mice displayed increased pancreatic Glucagon⁺ cells with concomitant decrease in Insulin⁺ cells, and disrupted separation of islets from the ductal epithelium (Crawford et al., 2009). Further study revealed that endothelial *Ctgf* deficiency led to reduced islet vascularization and decreased embryonic β cell proliferation (Guney et al., 2011) (Figure 1B).

ECs can also promote pancreatic development indirectly. Jacquemin et al. demonstrated that aortic ECs enhance the survival of mesenchymal cells surrounding the dorsal Islet1⁺ (a LIM-homeodomain transcription factor) cells, which secrete fibroblast growth factor 10 to promote *Ptf1a* expression in the pancreatic dorsal bud (Jacquemin et al., 2006). Kume and colleagues found that in chicken embryos, pancreatic endoderm cells secrete the C-X-C motif chemokine ligand 12, which recruits C-X-C chemokine receptor type 4-expressing angioblasts to migrate to the endodermal boundary, thereby inducing Pdx1 expression within the endoderm (Katsumoto and Kume, 2011). Overall, these studies elucidated ECs as an indispensable player in embryonic pancreas organogenesis.

2.2 Role of ECs in postnatal islet development

In 2006, Johansson et al. observed that the pronounced growth of islet endocrine cells during the first week after birth coincides with an even more significant increase in the proliferation of ECs, resulting in a marked increase in intra-islet vascular density. Furthermore, the proliferating endocrine cells were



Endothelial cell in pancreatic islet development (A) During embryonic days 8.5–9.5, dorsal and ventral foregut epithelia show budding protrusions, forming dorsal and ventral pancreatic buds. Endothelial cells secrete factors that induce the formation of Pdx1/Ptf1a⁺ cells. (B) During embryonic development from days 13.5–18.5, the pancreas undergoes a secondary transition where endocrine cells are produced and differentiated, and subsequently separate from the ducts, migrating to form islets. Endothelial cells can secrete Ctgf to promote the proliferation and migration of β cells. (C) At postnatal stage, endothelial cells not only secrete cell factors (Tsp-1, Ctgf) that regulate islet cell development but also participate in the regulation of β -cell function, proliferation, and expansion through various proteins (laminins/collagen IV) in the vascular basement membrane. As adults, the pancreas undergoes gradual updates, and the capacity of pancreatic beta cells is subject to dynamic changes. With metabolic changes in the body, such as pregnancy, aging, endocrine cells and endothelial cells also undergo corresponding changes. Endothelial cell (EC); Pancreatic duodenal homeobox 1 (Pdx1); Pancreas associated transcription factor 1a (Ptf1a); Connective tissue growth factor (Ctgf); Thrombospondin-1 (Tsp-1); Hepatocyte growth factor (Hgf); Gap junction protein 1 (Gja1); Number (No.); Proliferation (Prolif.).

located near the islet ECs, suggesting a supportive role of islet vasculature in this phase of islet growth (Johansson et al., 2006a) (Figure 1C).

ECs have been shown to regulate islet β cell function through secreted factors including Thrombospondin-1 (Tsp-1) and Ctgf. In 2011, Johan Olerud and colleagues investigated the role of Tsp-1 in islet morphology and β cell function (Olerud et al., 2011). Tsp-1, a glycoprotein primarily produced by islet ECs, is a potent inhibitor of angiogenesis (Jimenez et al., 2000; Dawson et al., 1997). The absence of Tsp-1 resulted in increased vascular and cellular proliferation within the islets, leading to significant glucose intolerance, reduced glucose-stimulated insulin secretion, and impaired (pro)insulin biosynthesis. These adverse effects in Tsp-1-deficient mice can be rescued by activating transforming growth factor β -1 (Tgf β 1) (Olerud et al., 2011; Drott et al., 2012; Crawford et al., 1998). Thus, Tsp-1 from ECs is crucial for β cell function through the activation of islet Tgfβ1. CD47, the receptor for TSP-1, is expressed in human islets (Erdem et al., 2023). Furthermore, Tsp-1 from ECs enhances β cell survival under lipotoxic (Cunha et al., 2016) and endoplasmic

reticulum (ER) stress conditions (Cunha et al., 2017). In the embryo, Ctgf is highly expressed in the pancreatic ductal epithelium and ECs, where it is essential for β cell proliferation. In adulthood, Ctgf is primarily expressed in ECs of the pancreatic islet (Crawford et al., 2009). Treatment of islets with partial β cell damaged using Ctgf has been shown to enhance β cell proliferation and restore islet mass by 50% (Riley et al., 2015).

ECs can also produce basement proteins (extracellular matrix (ECM) components) (Nikolova et al., 2006; Kragl and Lammert, 2010), such as laminin and type IV collagen, which constitute the basement membrane of islets (Nikolova et al., 2006; Vartanian et al., 2009). In 2004, Kaido et al. demonstrated that islet ECs secrete type IV collagen, which interacts with integrin $\alpha 1\beta 1$ expressed in both fetal and adult β cells, thereby enhancing insulin secretion (Kaido et al., 2004). Additionally, research by Nikolova and Sakhneny et al. revealed that laminins produced by ECs promoted β cell proliferation and increased expression of functional markers such as insulin 1 (Ins1), MAF bZIP transcription factor A (Mafa), and glucose transporter 2 (Glut2), as

well as significantly improved glucose-stimulated insulin secretion (Nikolova et al., 2006; Sakhneny et al., 2021).

Berclaz et al. (2016) utilized extended-focus Fourier domain Optical Coherence Microscopy to create a labelfree, three-dimensional imaging method for observing islets, which also enables a three-dimensional (3D) livevisualization of vascular network. With the availability of this technique, we anticipate better elucidating the collaboration between pancreatic islets and surrounding vasculature (Berclaz et al., 2016).

2.3 Role of islet ECs in pregnancy, aging, and diabetes

Adult islets in general have slow overall turnover rate. However, islet mass changes in response to altered metabolic demands. Increased islet mass is observed in pregnancy, obesity, or insulin resistance (Bonner-Weir, 2000; Sorenson and Brelje, 1997; Meier et al., 2006), conversely, aging is often associated with notable decline in β cell mass (Tschen et al., 2009; Rankin and Kushner, 2009; Kushner, 2013) (Figure 1C). In all these cases, ECs have been identified to play a part.

Maternal pancreatic islets enlarge during pregnancy (Sorenson and Brelje, 1997; Johansson et al., 2006b), in accordance with and likely result from elevated levels of placental prolactin, prolactin, and growth hormone. ECs not only mediate these drastic hormonal changes, but also support β cell proliferation, survival, and function through paracrine effects (Sorenson and Brelje, 1997; Brelje et al., 1993; Vasavada et al., 2000). It has been shown that EC proliferation occurs even before islet cell proliferation (Sorenson and Brelje, 1997; Johansson et al., 2006b). Johansson et al. found that proliferating ECs promote islet cell proliferation by secreting hepatocyte growth factor (Hgf), and reciprocally, islet cells produce vascular endothelial growth factor-A (Vegfa) and insulin to stimulate EC secretion (Johansson et al., 2006b).

Aging islets display reduced cell proliferation and regeneration, leading to decreased β cell mass (Tschen et al., 2009; Rankin and Kushner, 2009; Kushner, 2013; Chen et al., 2011). In 2021, Chen et al. showed a sparser vascular network within the aging mice islets, while the surrounding exocrine vessel density appeared unchanged. This study also identified a specific subset of ECs within the islets (CD31⁺ Emcn^{hi}) that promote β cell proliferation. The CD31⁺ Emcn^{hi} ECs population declines with aging, which is attributed to increased expression of gap junction protein 1 (Gja1). Targeted ablation of Gja1 restored EC proliferation and islet vessel density, consequently increasing β cell proliferation, β cell mass, and insulin production in aged islets (Chen et al., 2021). Taken together, it is plausible that decreased islet ECs disrupt islet homeostasis and lead to β cell loss with aging. In light of this study, targeted interventions aimed at restoring aging islet vasculature may prevent β cell loss, preserve islet function and potentially delay the onset of diabetes.

Diabetes can cause islet microvascular complications, including hyperplasia, basement membrane thickening, vessel dilation, rupture and impaired vasomotion (Mateus Gonçalves et al., 2023; Okajima et al., 2022). Under glycemic conditions, ECs within the pancreas express inflammatory and activation markers, which may lead to their dysfunction and consequently affect insulin secretion by β cells. The alterations observed in ECs in the context of diabetes have been comprehensively summarized in a previous review (Hogan and Hull, 2017), thus; they will not be reiterated in this discussion. The prevention of vascular damage could be beneficial for diabetes management. Studies have shown that metformin (Zou et al., 2022), rutin (David et al., 2023), and glycine supplementation (Wang B. et al., 2024) exhibit protective effects against vascular endothelium dysfunction in type 1 diabetes mellitus (T1DM). Further elucidation of the mechanisms by which pancreatic ECs support the survival and function of β cells may provide new therapeutic targets for diabetes treatment.

3 ECs for islet transplantation

Conventional treatment for diabetes relies primarily on oral hypoglycemic agents or daily insulin injections (Mathieu et al., 2021). However, these approaches can only ameliorate hyperglycemia temporarily but fail to restore autonomous glycemic control, until the first successful islet transplantation (Kobayashi, 2008; Bellin and Dunn, 2020; Powers, 2021; Shapiro et al., 2017; Rickels and Robertson, 2019; Paget et al., 2022; Wang Q. et al., 2024). Besides being a promising cure, islet transplantation has its own hitch, that is the unpredictable graft failure incidences. It is presumed that up to 60%–80% of islet cells may be lost within 48 h after transplantation (Davalli et al., 1995; Eriksson et al., 2009; Biarnes et al., 2002). To improve post-transplantation islet survival, many groups have tried accelerating revascularization of the transplanted islets (Figure 2).

3.1 Local release of pro-angiogenic factors

Employing local growth-promoting factors have been demonstrated to expedite the vascularization of transplanted grafts (Relevant studies are summarized in Table 1; Figure 2A). Vegf is a pivotal stimulator of angiogenesis and plays a crucial role in islet revascularization (Yancopoulos et al., 2000; Ferrara and Davis-Smyth, 1997). However, Vegf expression in islet cells significantly decline 2-3 days post-transplantation (Vasir et al., 2001). Studies aimed at overexpressing Vegf in transplanted islets have shown enhanced angiogenesis, increased vascular density, and improved graft blood flow (Lai et al., 2005; Zhang et al., 2004). Studies by Zhang et al. (2004) and Lai et al. (2005) demonstrated that Vegf overexpression not only promoted β cell proliferation and survival but also improved glycemic outcome in diabetic mice (Lai et al., 2005; Zhang et al., 2004). Furthermore, Golocheikine et al. (2010) reported that embedding mouse islets in a Vegf and Hgf enriched matrix gel has been reported to induce angiogenesis, reverse diabetes, and enhance the expression of adhesion molecules (Icam, Vcam) and signaling pathways (P-FAK, P-ERK1/2), supporting stable engraftment (Golocheikine et al., 2010). García A. J's team developed an injectable VEGF-releasing polyethylene glycol hydrogel (Phelps et al., 2013; Phelps et al., 2015; Weaver et al., 2017; Weaver et al., 2018), which significantly enhanced islet vascularization and engraftment in T1DM mouse model. This



FIGURE 2

Endothelial cell for islet transplantation (A) Local factor release system: Vascularization of the graft is facilitated through the release of vascular growth factors. This can involve strategies such as viral-mediated overexpression of these factors in organoids or islets, or transplantation after coating the islets with a matrix gel or hydrogel infused with growth factors. (B) EC-islet co-TP system: ECs were co-transplanted with islets or organoids, including single ECs, multicellular types containing ECs, and functional microvascular co-transplantation systems. (C) Vascular Engineering for TP: Biomaterials were used to promote subcutaneous pre-vascularization in advance, or vascularization networks were constructed *in vitro* before islet or organoid transplantation. Endothelial cells (ECs); Transplantation (TP); Co-transplantation (co-TP); Endothelial progenitor cells (EPCs); Vascular endothelial cells (HUVEC); Mesenchymal stem cells (MSCs); Human amniotic epithelial cells (hAECs); Human adipose derived stem cells (hADSCs); Microvascular fragments (MVFs).

VEGF-A protein hydrogel reduced the required number of islets by 40%, improved body weight and glucose responsiveness, and promoted intra-islet vascularization. In 2018, Gebe et al. introduced an islet implant scaffold, which can locally and controllably release Vegfa, and showed reduced early islet necrosis and improved graft performance (Gebe et al., 2018). Recent studies also highlight the role of endothelial cilia in regulating islet vascularization via the Vegfa/Vegfr2 pathway. Disruption of this pathway, as seen in $Bbs4^{-/-}$ islets, delays revascularization and impairs vascular permeability, ultimately hindering glucose delivery and islet function (Xiong et al., 2020).

Besides Vegf/Vegfr2 signaling, other angiogenic pathways have also been explored. Su et al. (2007) investigated the role of angiopoietin-1 (Ang-1), a factor that promotes angiogenesis and inhibits apoptosis, in pancreatic islet transplantation. The study found that islet cells expressing Ang-1 exhibited significantly increased microvascular density, improved cell viability, and enhanced insulin secretion in response to glucose stimulation (Su et al., 2007). More recently, Karanth et al. (2021) revealed that angiopoietins, including Ang-1 and Ang-2, promote the generation of islets from induced pluripotent stem cells (iPSCs) with elevated glucose responsiveness and enhanced expression of all islet hormones. Islets stimulated by Ang-2 were capable of modulating insulin exocytosis in response to glucose stimulation through the dynamic process of actin filament polymerization and depolymerization. This regulation likely occurs via the CDC42-RAC1-gelsolin-mediated insulin secretion signaling pathway (Karanth et al., 2021). Overall, these findings indicate that pro-angiogenic growth factors can effectively enhance transplant vascularization, protect islet cells, and support the function of pancreatic islet transplants.

3.2 Supportive ECs for islets co-transplantation

Co-transplantation of islets with ECs has been explored in order to achieve rapid blood perfusion of transplanted islets with the host, thereby improving islet cell survival, function, and long-term graft viability (Relevant studies are summarized in Table 2) (Figure 2B).

3.2.1 Co-transplantation of islets with ECs

Bone marrow-derived endothelial progenitor cells (EPCs) have been used in multiple studies to facilitate the reconstruction of the graft's vascular network (Krenning et al., 2009; Garikipati and Kishore, 2017; Critser and Yoder, 2010) (Relevant studies are summarized in Table 2). Kang et al. (2012) co-transplanted porcine islets with human umbilical cord blood-derived EPCs into diabetic nude mice (Kang et al., 2012), resulting in the accelerated normalization of blood glucose levels within approximately 11 days (islet-only group failed to achieve normoglycemia over 5 weeks) and higher serum porcine insulin levels. The authors attributed this significant improvement to increased Vegf/Hgf secretion and basement membrane production from the co-transplanted umbilical cord blood-derived EPCs (Kang et al., 2012).

Similarly, Quaranta et al. (2014) co-transplanted islets with EPCs into the portal vein of diabetic rats, achieving sustained normal blood glucose levels for up to 180 days, while the control group remained below the diabetic threshold throughout the observation period (Quaranta et al., 2014). Further studies by Oh et al. (2013) and Penko et al. (2015) demonstrated that co-transplanting mouse islets with bone marrow-derived EPCs into the renal capsule of diabetic mice improved glucose tolerance, serum insulin levels, and diabetes reversal rates compared to islet

	Contributions	 Vegf production in islets stimulates graft angiogenesis and enhances islet revascularization A novel strategy to accelerate islet revascularization and improve islet survival 	 A method for optimizing angiogenesis of islet transplants 	 Vegf and Hgf synergistically enhance angiogenesis after islet transplantation leading to stable engraftment 	• Establishes a simple biomaterial strategy for islet transplantation to promote enhanced islet engraftment and function	(Continued on the following page)
	Organoid/islet function after TP	 GTT↑ GSIS (insulin secretion) ↑ β Cell mass↑ Glycenic control in diabetic mice (16 days) ↑ 	 Insulin secretion ↑ β Cell mass (14 days) ↑ β Cell proliferation ↑ Percentage of normoglycemia ↑ 	 Survival ↑ Suboptimal (250) islet equivalents could restore normoglycenia (30 days) Islet apoptosis ↓ Insulin staining ↑ 	 Insulin/C-peptide↑ GTT↑ GTT↑ Superior glycemic function compared with hepatic portal vein transplantation Reverse diabetes (<35 days)↑ 	
	EC function after TP (characterization/ period observation)	 Vascular density f CD31*cell f E C marker: CD31 staining 16 days post-TP 	 EC sprout formation ↑ Vascular density ↑ Blood flow ↑ EC marker: BS-1 staining 	 Vascular density ↑ Stable blood vessel formation EC marker: H&E staining 15 days post-TP 	 Vascularization in extrahepatic transplant sites f transplant sites and engraftment and engraftment EC marker: 2-4 weeks post-TP 	
	Transplant model	 Virus infection Kidney capsule STZ-induced diabetic mice 	 Virus infection Diabetic mice Hypoxic conditions 	 A matrix gel containing VEGF and HGF Subcutaneous transplantation Diabetic mice 	 Engineered Vegf- releasing-PEG hydrogel Hepatic portal vein/epididymal fat pad/S.c. site/small bowel Diabetic mice 	
S.	The source of the b-cell	Mice fresh islets (Infected with Ad-VEGF)	Mice fresh islets (RIP-VEGF-A mice)	Mice fresh islets	Mice fresh islets	
lease of pro-angiogenic factor	The source and isolation of the EC	MousePrimaryAutogenous EC	 Mouse Primary Autogenous EC	 Mouse Primary Autogenous EC 	 Mouse Primary Autogenous EC 	
TABLE 1 Summary of local re	References	Zhang et al. (2004)	Lai et al. (2005)	Golocheikine et al. (2010)	Phelps et al. (2013); Weaver et al. (2017); Weaver et al. (2018)	

Contributions	 The scaffolded islet implant can reverse diabetes in SC sites without prevascularization Low-dose Vegf released from the implant helps reducing islet stress post-grafting 	 Ang-1 confers a cytoprotective effect on islets, enhancing islet engraftment and preserving functional islet mass in transplants 	 First revealed that angiopotetins, including Ang-1 and Ang-2, enable iPSCs to generate islets with enhanced glucose responsiveness, a key feature of mature islets
Organoid/islet function after TP	 Percentage of normoglycemia 1 Time to normoglycemia 1 GTT 1 Necrotic area 1 <45 days 	 Islet survival f β Cell Mass f Bood glucose J GSIS (insulin secretion) f GTT f Reverse diabetes (<30 days) 	 In vitro Pancreatic progenitor markers [†] Iselt hormone markers [†] Insulin release [†] GTT [†]
EC function after TP (characterization/	 period observation) Necrotic area ↓ Vascularized area Vascular density ↑ EC marker: H&E staining 12 days post-TP 	 Vascular density ↑ Intra-islet vessels ↑ EC marker: CD31 staining 30 days post-TP 	 Generation of ECs and pericytes f EC marker: VE- cadherin/NG2 staining 22-29 days (<i>in vitro</i>)
Transplant model	 A scaffolded islet implant-Vegf releasing Subcutaneous transplantation Diabetic mice 	 Kidney capsule Diabetic mice 	• In vitroculture
The source of the b-cell	Mice fresh islets	Mice fresh islets (transduction by Ad-Ang1)	iPSCs induced islet organoid islet organoid
The source and isolation of the EC	 Mouse Primary Aurogenous EC 	 Mouse Primary Autogenous EC 	 Human iPSCs-induced ECs
References	Gebe et al. (2018)	Su et al. (2007)	Karanth et al. (2021)

TABLE 1 (Continued) Summary of local release of pro-angiogenic factors.

5 Endotticulat cells (ECS): Y ascular endotticulat growth lactor (VESET); Italispiantauon (LFF); Streptozotocul (SLE); Bouthylatet badretera sumpluciona (SE Hepatocyte growth factor (Hgf); Hematoxylin and eosin (H&E); angiopoietin-1 (Ang-1); angiopoietin-2 (Ang-2); Induced pluripotent stem cells (iPSG).

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transplantation alone (Oh et al., 2013; Penko et al., 2015). This approach also increased vascular density from both donor and recipient sources (Oh et al., 2013). Overall, co-transplantation with EPCs showed improved islet function. *In vitro* studies have shown that EPCs reduced the expression of connexin 36 on β cell and enhanced glucose-stimulated insulin release (Penko et al., 2015).

Alternative EC sources for co-transplantation with islets have also been explored. In 2016, Barba-Gutierrez et al. encapsulated mouse islets with pancreatic-derived vascular ECs and transplanted them into streptozotocin-induced diabetic mice. This approach improved islet structures maintenance, enhanced vessel integration, reduced TUNEL⁺ apoptotic cells, and accelerated normoglycemia after transplantation compared to islets alone (Barba-Gutierrez et al., 2016). Similarly, Vlahos et al. (2017) embedded rat islets within collagen modules containing human umbilical vein ECs (HUVECs) and transplanted them subcutaneously into diabetic mice. This approach resulted in sustained glucose normalization for up to 21 days, a result not achieved with an equivalent number of free islets, and improved islet revascularization and host integration. Mechanistically, collagen-embedded islets promoted a shift toward an M2-like macrophage response, which is associated with vascularization and tissue remodeling (Vlahos et al., 2017).

3.2.2 Co-transplantation with other cell types

In addition to ECs, the co-transplantation of islets with other supportive cells has also been explored (Relevant studies are summarized in Table 2). In 2008, Johansson et al. co-cultured human mesenchymal stem cells (MSCs) and dermal microvascular ECs with human islets in vitro, promoting the formation of vessel-like structures before transplantation, which potentially enhanced islet engraftment success (Johansson et al., 2008). In 2014, Taniguchi's team developed a dynamic self-condensation technique to create tissue organoids from dissociated organ progenitor cells in the presence of ECs and MSCs (Takebe et al., 2014). Subsequently, Takahashi et al. (2018) cocultured dissociated murine β cell lines (MIN6), human and murine islets with HUVECs and human MSCs to form self-organizing vascularized islets (Takahashi et al., 2018a; Takebe et al., 2015; Takahashi et al., 2018b). This method not only improved cell survival and functionality in vitro but also significantly enhanced therapeutic outcomes post-transplantation (Takahashi et al., 2018b; Homma et al., 2023).

In 2019, Soltanian reported the generation of a type of pancreatic organoids by assembling various cell types derived from embryonic stem (ES) cells, including human ES-derived PDX1⁺ cells, MSCs, and ECs. The human pancreatic organoids exhibited enhanced vascularization, characterized by greater vessel density and quantity, a higher count of Insulin⁺ cells, and improved secretion of human C-peptide, with glycemic effects persisting for 90 days post-transplantation (Soltanian et al., 2019). In 2020, Yoshihara et al. used human iPSCs to generate human islet-like organoids by incorporating human adipose-derived stem cells (hADSCs) and HUVECs into multicellular spheroids. These organoids, containing insulin-producing cells, were transplanted into diabetic mice and maintained glucose homeostasis for approximately 40 days, with similar effects to human islets (Yoshihara et al., 2020). In 2022, Wassmer et al. developed and transplanted prevascularized isletlike clusters made from rat pancreatic islets, human amniotic epithelial cells (hAECs), and HUVECs. Compared to fresh islets, these pre-vascularized islet-like clusters improved engraftment and vascularization in mouse models. This improvement is likely attributed to the interaction between hAECs, HUVECs, and islet cells, through the upregulation of angiogenesis-promoting genes (Vegfa) and β cell function-related genes (Glp1r, Pdx1) (Wassmer et al., 2021). More recently, (Naqvi and Naqvi, 2023) demonstrated improved glucose clearance by transplanting a mixture of mouse MSCs, EPCs, and islets (Naqvi and Naqvi, 2023).

Collectively, these studies suggest that creating a prevascularized niche with ECs and other supportive cells can significantly improve glycemic function of transplanted islets or islet-like organoids (Takahashi et al., 2018b).

3.2.3 Co-transplantation with microvascular fragments (MVFs)

The above-mentioned studies typically use only ECs for cotransplantation with islets; still, on average it takes over a week to achieve integration with the host (Laschke and Menger, 2016). In contrast, MVFs isolated from adipose tissue retain intact endothelialized lumens and are covered with vessel mural cells, providing a rich source of angiogenic factors. It has been shown that MVFs can rapidly reassemble into functional microvascular networks and integrate with host blood vessels, ensuring rapid and sustained blood perfusion of the graft (Shepherd et al., 2004; Sun et al., 2020; Laschke and Menger, 2015; Nalbach et al., 2021a). Hence, MVFs appear to be potent vascularization units for tissue engineering and regenerative medicine (Laschke and Menger, 2015; Nalbach et al., 2021a; Laschke and Menger, 2022; Sun et al., 2022; Frueh et al., 2017a; Frueh et al., 2017b) (Relevant studies are summarized in Table 2).

In 2021, Nalbach et al. and Salamone et al. successfully generated vascularized pancreatic organoids by co-culturing islet cells with functional MVFs in vitro (Nalbach et al., 2021b; Salamone et al., 2021). These vascularized pancreatic organoids exhibited significantly enhanced angiogenic capacity and quickly integrated with host blood vessels as early as day 3, mediated by paracrine signaling between ß cells and ECs through Hgf/c-Met/Ho-1 pathway (Nalbach et al., 2021b). In the same year, Aghazadeh et al. found that co-transplantation of rat MVFs markedly improved cell survival and glucose responsiveness of human islets and human embryonic stem cell (hESC)-derived pancreatic organoids in three different T1DM mouse models, leading to a rapid reversal of diabetes (Sun et al., 2022; Aghazadeh et al., 2021; Powers and Brissova, 2021; Hoesli and Kieffer, 2021). Similarly, Wrublewsky (2022) and Nalbach et al. (2021) co-transplanted mouse islets and adipose tissue-derived MVFs into recipient mice. This combined approach accelerated blood glucose recovery in diabetic mice compared to islet transplantation alone, allowing effective glucose reduction with fewer islets (Nalbach et al., 2021b; Wrublewsky et al., 2022).

Studies have shown that co-transplanting islets with MVFs accelerates vascular integration with the host, reducing graft ischemic time and enhancing transplantation success. This approach holds promise for using recipient-derived adipose tissue microvasculature to potentially decrease the required islet mass, improve graft survival, and expedite the achievement of normoglycemia. However, a key challenge remains obtaining sufficient quantities of host-derived microvasculature for clinical application.

3.3 Vascular engineering for islet transplantation

The standard method for islet transplantation involves infusing islets into the hepatic portal vein. However, many donor islets are lost due to immediate blood-mediated inflammatory response (IBMIR) and ischemia (Pepper et al., 2018; Shapiro et al., 2017; Naziruddin et al., 2014). Patients often require multiple islet infusions to achieve insulin independence. Finding a minimally invasive site that can support the survival of transplanted islets is necessary. Subcutaneous pancreatic islet transplantation seems like a promising option but is limited by poor vascularization, which fails to provide adequate oxygen and nutrients for islet survival and function (Vlahos et al., 2017; Lacy et al., 1991), thereby limiting its application. With rapid advancements in biomaterials and bioengineering strategies, researchers are exploring the possibility of using these to address vascular deficiencies in subcutaneous islet implantation (Relevant studies are summarized in Supplementary Table 1) (Figure 2C).

3.3.1 Utilizing biomaterials to promote subcutaneous neovascularization

Promoting subcutaneous vascularization prior to islet transplantation is expected to reduce the time required for circulation connection. Initially, research has focused on using biomaterials to enhance neovascularization at the subcutaneous site. In 2017, Mahou et al. developed a semi-interpenetrating polymer network (SIPN) that facilitated blood vessel growth at the subcutaneous implantation site. Islets embedded in SIPN remained viable and responsive to glucose stimulation in vitro, and transplanted diabetic mouse models showed a progressive return to normoglycemia (Mahou et al., 2017). Two other studies aimed to ensure islet transplantation success by employing basic fibroblast growth factor (bFGF) to induce neovascularization at subcutaneous sites. In 2000 and 2001, Kawakami et al. implanted a bFGFreleasing device 1 week before transplantation, resulting in a wellvascularized capsule and maintenance of normoglycemia for over a month post-transplantation (Kawakami et al., 2000; Kawakami et al., 2001). Similarly, in 2014, agarose rods containing bFGF and heparin were implanted 1 week before surgery in dorsal subcutaneous sites. After removing the rods, islets were transplanted into the prevascularized sites, quickly reversing hyperglycemia in diabetic rats within 1-3 days. Notably, allogeneic islets transplanted to these pre-vascularized sites demonstrated long-term graft survival and function compared to those transplanted into the hepatic portal vein (Luan and Iwata, 2014). These findings suggest the prevascularizing subcutaneous sites prior to islet transplantation can improve graft function by ensuring a sufficient blood supply, thereby reducing the time required for islet engraftment and even reducing immune rejection.

3.3.2 Utilizing bio-devices to promote subcutaneous neovascularization

The use of bio-devices to promote subcutaneous neovascularization has also been explored. For instance, Craig et al. (2005) and Pileggi et al. (2006) pre-implanted a cylindrical stainless-steel mesh to facilitate the formation of blood vessels and connective tissue around the device (Craig et al., 2005;

Pileggi et al., 2006). When syngeneic islets were transplanted into these vascularized devices, they restored normal blood glucose levels and maintained long-term functionality, comparable to portal vein transplantation (Craig et al., 2005; Pileggi et al., 2006). Upon removal of the device, hyperglycemia was observed, while the preserved islets and intense vascular network were evident in the retrieved grafts (Pileggi et al., 2006). In 2005, Andrew et al. used a subcutaneous cell pouch device implanted 3–4 weeks prior to induce neovascularized tissue chambers. Islets transplanted into these chambers in mice restored blood glucose control rapidly and responded well to glucose challenges, similar to intrarenal subcapsular islet transplantation (Andrew R Pepper et al., 2015).

Recent studies suggest that the use of biological devices can prevent immune rejection in subcutaneous transplantation. Sorenby et al. (2008) utilized TheraCyte immunoprotective devices implanted subcutaneously to induce vascularization. This approach reduced the required dose of encapsulated islets by approximately 10-fold, while also achieving normoglycemia comparable to sole islets kidney encapsulation (Sorenby et al., 2008). Similarly, Smink et al. (2017) described a novel prevascularized, subcutaneously implanted, retrievable poly (D, Llactide-co-ɛ-caprolactone) scaffold, which protected the viability and function of islets, enhancing their engraftment in subcutaneous sites (Smink et al., 2017). In 2020, Liu et al. modified a prevascularized tissue-engineered chamber (TEC) to improve the viability and function of the seeded islets in vivo by providing a microvascular network before transplantation (Liu et al., 2020). Notably, xenogeneic islets within TECs maintained long-term recipient-specific immune tolerance (Liu et al., 2020). Recently, Shapiro's team (2023) implanted a hollow nylon catheter into the subcutaneous space, inducing a controlled inflammatory response in the host that created a vascularized pocket several weeks later (Pepper et al., 2015). After catheter withdrawal, a thread-like alginate-based islet-encapsulation device was transplanted into this modified pocket (An et al., 2018). This SHEATH system enabled sustained diabetes reversal, significantly increasing oxygenation, physiological glucose responsiveness, and islet survival rates. Moreover, it allowed for in situ replacement of damaged devices and rapid restoration of normal blood glucose levels. This device system holds promise for facilitating the clinical translation of immunosuppression-free subcutaneous islet transplantation (Wang et al., 2023). In 2019, Song et al. developed a functional microvascular mesh using an anchored self-assembly (ASA) strategy. These microvascular meshes significantly enhanced the vascularization of rat islets transplanted under the skin and achieved a 3-month correction of diabetic mice (Song et al., 2019).

These results suggest that subcutaneous vascularization devices, in combination with various biomaterials, serve as promising therapeutic assistance to evade immune attack. However, these studies rely on mouse models only, and lack long-term observation. Supportive data for preparing clinical translation such as extent of vascularization, immune infiltration, as well as long-term cell survival and function stability. Most importantly, the scalability of the current technique remains unknown. So far, only Shapiro's team assessed the scalability of their SHEATH system using a minipig model, demonstrating that the implantation, removal, and replacement are minimally invasive, technically feasible, and scalable, for clinical application (Wang et al., 2023).

Contributions (Advantages-A, Limitations-L, Scalability-S)		A: EPC Co-TP can enhance islet engraftment by rapid revascularization L: Only basic research S: Not strong	A: Co-TP of EPCs and islets induced a stable rescue of glycemic control lasting for a significant fraction of the animal life span L: Only basic research S: Not strong	A: BM-EPCs Co-TP with islets can improve revascularization and organization of islet grafts L: Only basic research S: Not strong	A: VECs could be functionally integrated into isolated islets A: Islets that are coated with VECs increased their capacity to engraft L: Only basic research S: Not strong
Organoid/islet function after TP		 Better glycemic control Reversal of diabetes (42 days) β Cell proliferation ↑ lslet survival ↑ 	 Reversal of diabetes (180 days) GTT ↑ Faster reversal of diabetes 	 GTT ↑ Insulin creation ↑ Diabetes reversal rate ↑ Faster reversal of diabetes 28 days observation 	 A well-defined rounded shape TUNEL-positive cells ↓ Reversal of diabetes ↑ 30 days observation
EC function after TP (characterization/ period observation)		 Rapid revascularization Capillary/vessel density ↑ Functional vessel ↑ E C marker: VE-cadherin/Col IV/BS1-lectin staining 3/14/21/35 days after TP 	 Endothelial thickness ↑ Vessel density ↑ EC markeer: PECAM-1 staining 30/180 days after TP 	 Vessel number ↑ Graft revascularization ↑ EC marker: CD31 staining 28 days after TP 	 CD31⁺cell ↑ Functional integrated into isolated islets EC marker: CD31 staining 3/7/10/30 days after TP
Transplant model		 Co-TP EPCs with Porcine islets Kidney capsule Diabetic mice (STZ) 	 Co-TP of EPCs with rat islet Portal vein Diabetic rat (STZ) 	 Co-TP BM-EPCs with islets Kidney capsule Diabetic mice (STZ) 	 Integration of islets coated with VECs Kidney capsule Diabetic mice (STZ)
The source of the b-cell		Porcine islets	Lewis rat islets	Fresh mice islets	Mice islets
The source and isolation of the EC	ition of islets with ECs	 Human EPCs Primary (umbilical cord blood- derived (Hur et al., 2004)) 	 Rat EPCs Primary (BM-derived (Dobson et al., 1999)) 	 Mouse EPCs Primary (BM-derived (De Falco et al., 2004)) 	 Mouse VECs Primary (Pancreas)
References	2.2.1 Co-transplanta	Kang et al. (2012)	Quaranta et al. (2014)	Oh et al. (2013); Penko et al. (2015)	Barba-Gutierrez et al. (2016)

(Continued on the following page)

	Contributions (Advantages-A, Limitations-L, Scalability-S)	 A: Sufficient to restore and maintain normoglycemia for 21 days A: Offer a suitable option for Subcutaneous islet transplantation L: Only basic research S: Not strong 		A: The composite EC-MSC islets as a means of improving islet engraftment L: Lack of long-term observation, only <i>in vitro</i> study S: Not strong	A: A method to make organoids by mixing epithelial, mesenchymal and vascular cells L: Lack of long-term observation S: Not strong	A: Self-condensation culture enables endothelialization of diverse tissue fragments A: Vascularized islet transplant enhances therapeutic potential against diabetes L: Lack of long-term observation L: The use of two distinct stronal progenitors especially stronal verton S: Not strong	(Continued on the following page)
	Organoid/islet function after TP	 Restoration of Normoglycemia (21 days) GTT↑ M2-like macrophages ↑ 		only <i>in vitro</i> culture • Insulin release ↑ • 6 days observation	 Restoration of Normoglycemia (10 days) Rapid normalization of body weight and blood glucose levels 	 Restoration of Normoglycemia (35 days) Therapeutic Capability ↑ Insulin secret ↑ GTT ↑ 	
	EC function after TP (characterization/period observation)	 CD31⁺number ↑ Intraislet vascularization ↑ EC marker: CD31/a-SMA staining 7/14/21 days after TP 		 In vitro EC coverage 1 ECs proliferation Vascular sprouts 1 EC marker: CD31/UEA-1 staining 2-6 days (<i>in vitro</i>) 	 Rapid reperfusion ↑ Vascularity ↑ Functional microvascular networks ↑ EC marker: CD31/Dextran staining 10 days after TP 	 Rapidly re-perfused <i>in vivo</i> Enhances engrafiment EC marker: CD31/Dextran staining 7/14/35 days after TP 	
	Transplant model	 Islet embedded in HUVEC coated modules Diabetic mice (STZ) 		 Coating of islets with ECs and MSCs <i>In vitro</i>culture 	 Generation of 3D vascularized organ buds <i>in vitro</i> Kidney capsule Diabetic mice 	 Self-Condensation Culture: HUVEC, MSCs and islet (3 days) Kidney capsule Diabetic mice 	
ets co-transplantation.	The source of the b-cell	Fresh rat islets		Human islets	MIN6	Human and mice islets	
Imary of supportive ECs for isle	The source and isolation of the EC	 Human HUVEC Primary (Lonza) 	ion with other cell types	 Human MSCs; ECs Primary (Dermis microvascular ECs) 	 Human MSCs, HUVEC Primary (Lonza) 	 Human HUVEC; MSCs Primary (Lonza) 	
TABLE 2 (Continued) Sun	References	Vlahos et al. (2017)	2.2.2 Co-transplantat	Johansson et al. (2008)	Takebe et al. (2015)	Takahashi et al. (2018a)	

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	Contributions (Advantages-A, Limitations-L, Scalability-S)	A: The first report showing considerable capacity of hPSC derivatives (ES-PPs, ES-ECs, and ES-MSCs) for formation of functional PO. L: Only basic research S: Not strong	A: HILOs contain endocrine-like cell types that, upon transplantation, rapidly reestablish glucose homeostasis in diabetic mice L: Lack of long-term observation S: Not strong	A: Better engraffment and improved vascularization <i>in</i> <i>vivo</i> in a murine model L: Lack of long-term observation L: Difficult to generate large numbers of uniform and functional prevascularized islet organoids S: Not strong	A: Co-TP of MSCs and EPCs along with islet to improvise the maximum survival time of mice islets to restore normoglycemia L: Only basic research S: Not strong
	Organoid/islet function after TP	 Insulin⁺cell f β cell maturation f C-peptide release f Transplantation for 90 days 	 Restoration of Normoglycemia (40 days) Rapidly reestablish glucose homeostasis Insulin secretion <i>in vitro</i>[†] GTT 	 Islet function <i>in vitro</i> f β cell function (Glp-1r, Pdx1) f Glycemic Control 30 days observation 	 Restoration of normoglycemia C-peptide 1 GTT 1 100 days observation
	EC function after TP (characterization/period observation)	 Vessel area ↑ Number of vessels ↑ EC marker: CD31/CD144/vWF staining (<i>in vitro</i>) 	Z	 Better engraftment and improved vascularization <i>in vivo</i> Angiogenesis factor (Vegf-a) EC marker: CD34/Lectin staining 30 days after TP 	 CD31⁺cell ↑ Mice endothelial markers expressed induction of angiogenic factors argiogenic factors EC marker: CD144/CD31/CD106 staining 7days (<i>in vitro</i>)
	Transplant model	 3D-printed tissue trapper device Intraperitoneal transplantation Nude mice 	 Form organ-like and vascular •structures in 3D Kidney capsules Diabetic mice (STZ) 	 Aggregation of hAECs and HUVECs with islets <i>in vitro</i>(5 days) Epididymal fat pad Diabetic mice (STZ) 	 Self-condensation of MSCs and EPCs with mice islets Omental pouch Diabetic mice (STZ)
ts co-transplantation.	The source of the b-cell	ES-PP	iPSCs	Rat islet cells	Fresh mice islets
mary of supportive ECs for isle	The source and isolation of the EC	HumanES-EC; ES-MSCCell line	 Human hADSCs; HUVECs Primary 	 Human hAECs; HUVECs Baudin et al., 2007 	 Mouse MSCs, EPCs Primary (BM-derived)
TABLE 2 (Continued) Sum	References	Soltanian et al. (2019)	Yoshihara et al. (2020)	Wassmer et al. (2021)	Naqvi and Naqvi (2023)

(Continued on the following page)

Contributions (Advantages-A, Limitations-L, Scalability-S)		A: Ad-MVF-islet of Langerhans coculture to improve the islets vascularization L: Only <i>in vitro</i> study S: Not strong	 A: The prevascularized islet organoids are rapidly blood-perfused and interconceted host ressels A: A lower number of islet grafts are required to restore normoglycemia in diabetic mice L: This technique is time-consuming S: Difficult to implement into clinical practice 	A: Ready-made MV's are superior to single endothelial cells in TP A: Ready-made MV's improve hESC-derived pancreatic cells and human islet engraftment L: The maturation of β cells <i>in</i> <i>vivo</i> S: Has certain clinical translational potential	A: A low number of subcutaneously transplanted islets can reverse diabetic mice L: only basic research S: Not strong	dothelial progenitor cells (EPCs); s); von Willebrand factor (vWF);
Organoid/islet function after TP		 only <i>in vitro</i>culture A vascularized islet Basal lamina surrounded islet GSIS - Cultured for 8 days <i>in vitro</i> 	 Organoid viability ↑ Insulin secretion ↑ GTT ↑ Reversal of diabetes (28 days) 	 Blood glucose ↓ GTT ↑ GTT ↑ Islet survival ↑ Islet survival ↑ β cell maturation ↑ Long-term glycemic control Reversal of diabetes (15 weeks) 	 Islet survival f β Cell Mass f Blood glucose ↓ GSIS f Reversal of diabetes (105 days) 	elial progenitor cells (BM-EPCs); En ECs); Mesenchymal stem cells (MSC
EC function after TP (characterization/period observation)		 Angiogenesis factors [†] Forming tubes, branches, and entire capillary networks EC marker: CD31/CD90 staining & days (<i>in vitro</i>) 	 Microvasculature angiogenic activity functional capillary density ↑ EC marker: CD31/IDextran staining 14/28 days 	 Vessel perfusion f E C number f E C marker: CD31/Lectin/acSMA staining 1/7/14 weeks after TP 	 CD31⁺cell î EC marker: CD31 staining 28/105 days after TP 	n (TP); Bone marrow-derived early endoth EA-1); Human amniotic epithelial cells (hA e stimulated insulin release (GSIS).
Transplant model		 Coculture into Type-I collagen hydrogel <i>In vitro</i>culture (28 days) 	 Liquid overlay coculture (Prevascularized islet organoids) Kidney capsule Diabetic mice (STZ) 	 Co-transplantation of islets and MVs in collagens I Subcutaneous transplantation Diabetic mice (STZ) 	 Co-transplantation of islets and MVs Kidney Kidney capsule/subcutaneous transplantation Diabetic mice (STZ) 	 Bone marrow (BM); Transplantatio /ECs); Ulex europeaus agglutinin-1 (U ancreatic progenitors (ES-PP); Glucos.
The source of the b-cell		Rat islets	Mice islets/Human islets	HESC-derived pancreatic cells/Fresh human islets	Fresh mice islets	otinylated Bandereira simplicifolia (BS) n umbilical vein Endothelial cells (HU [*] 's); Embryonic stem (ES) cell-derived <u>j</u>
The source and isolation of the EC	ion with MVFs	. Rat . MVFs . Primary (Adipose tissue)	 Mice MVFs MVFs Primary (Epididymal fat pads Fruch et al., 2017b) 	 Rat MVFs Primary (Epididymal adipose tissue) 	 Mouse MVFs Primary (Epididymal adipose tissue) 	ntation (TP); Streptozotocin (STZ); bi scular endothelial cells (VECs); huma ?SCs); Microvascular fragments (MVF
References	2.2.3 Co-transplantat	Salamone et al. (2021)	Nalbach et al. (2021a)	Aghazadeh et al. (2021)	Wrublewsky et al. (2022)	Endothelial cells (ECs); Transpla Glucose tolerance test (GTT); Va Induced pluripotent stem cells (il

TABLE 2 (Continued) Summary of supportive ECs for islets co-transplantation.

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4 ECs in stem cell-derived β cells and islet organoids co-culture

The general implementation of islet transplantation is hindered by the scarcity of organ donors as well as laborious isolation procedures. One approach to solve the islet supply shortage is to obtain an adequate quantity of functional insulin-secreting cells *ex vivo*.

The ongoing advancements in differentiating functional β cells from human pluripotent stem cells (hPSCs) and islet organoid technologies hold immense potential to revolutionize traditional diabetes treatments. Over the past decade, researchers have investigated and optimized various protocols for expanding and inducing pancreatic β cells *ex vivo*. These methodologies encompass the utilization of ESCs, iPSCs, pancreatic islet progenitor cells derived from embryonic or adult tissues, and re-differentiation or trans-differentiation from exocrine cells (Hogrebe et al., 2023; de Klerk and Hebrok, 2021; Yu and Xu, 2020; Casamitjana et al., 2022; Jiang et al., 2022; Zhou et al., 2008; Al-Hasani et al., 2024; Li et al., 2014; Pagliuca et al., 2014; Rezania et al., 2014; Wang et al., 2020). At the same time, incorporation of ECs in coculture systems has been shown to significantly improve cell yields and organoid functions (Relevant studies are summarized in Table 3).

4.1 Formation of pseudoislets from β cell lines and ECs

The co-culture system is an efficient way to study the interaction between islet cells and endothelium. Pseudoislets, formed by the aggregation of β cells, are used *in vitro* to study β cells in a 3D structure and can be employed to investigate the interactions between β cells and ECs. Over the years, various studies have adopted diverse approaches to generate and utilize these pseudoislets (Relevant studies are summarized in Table 3).

ECs can significantly enhance the functionality of pseudoislets and insulin secretion. In 2011, Penko et al. first described mosaic pseudoislets, created in vitro by aggregating rat pancreatic cells with rat endothelial progenitor cells. These pseudoislets exhibited improved pancreatic function and insulin secretion compared to traditional methods (Penko et al., 2011). In 2020, Urbanczyk et al. created two-layered pseudoislets composed of human cell linebased β-cells (EndoC-βH3) and HUVECs. A well-defined spatial distribution of the pseudoislets was achieved by controlling the aggregation process using magnetic levitation. The pseudoislets exhibited higher insulin secretory function and a greater number of CD31⁺ HUVECs compared to those formed by spontaneous aggregation (Urbanczyk et al., 2020). In 2023, Porter et al. constructed pseudoislet spheroids coculturing the non-proliferative EndoC- β H5 human β cell line with HUVECs. The 1:3 β cell to HUVEC coculture spheroids displayed higher insulin release (Porter et al., 2023). In 2024, Wang et al. constructed 3D pseudoislets by co-culturing of MIN6 cells and HUVECs in a 2:1 ratio. Functional experiments conducted after 3 days of culture showed increased insulin secretion (Wang et al., 2024a).

ECs also provide protective effects on pseudoislets against stress conditions. In 2021, Wieland et al. cocultured mouse α cells, mouse β cells, and HUVECs in a 1:9:5 ratio to form pseudoislets.

After 5 days in culture, oxidative stress was induced using H2O2. The presence of HUVECs significantly reduced oxidative stress in both α and β cells, suggesting a protective effect of ECs on islets (Wieland et al., 2021). Amin et al. (2021) demonstrated that immortalized HUVECs (EA.hy926), when pretreated with the Nrf2 activator rosolic acid (RA) and cocultured with ER-stressed MIN6 cells, exhibited increased Nrf2 levels and reduced ER stress in MIN6 cells. This indicates that RA-sensitized ECs provide protection to β cells (Amin et al., 2021).

ECs have been found to regulate gene expression and signaling pathways of co-cultured islets. Spelios et al. (2018) found that cocultured pseudoislets enhanced the expression of genes and proteins related to the GLP-1 pathway, partially explaining the improved glucose sensitivity (Spelios, 2018). Daniel et al. (2021) demonstrated that conditioned medium from rat islet microcapillary EC (IMEC) primary cultures could attenuate the first and second phase glucose stimulated insulin release (GSIS) in freshly isolated rat islets and the INS-1E insulinoma cell line. They identified the enzyme triosephosphate isomerase (TPI) as an attenuating factor in the endothelial conditioned medium that promotes insulin secretion via the TPI-sulfonylurea receptor-KATP channel (SUR1-Kir6.2) complex (Daniel et al., 2021). Wang et al. (2024a) demonstrated MIN6 cells cocultured with HUVECs upregulated the expression of E-cadherin, Connexin36, Pdx1 and Mafa, and promoted the secretion of betacellulin (BTC) by MIN6 cells, activating the EGFR-mediated JAK-STAT pathway to enhance MIN6 function (Wang et al., 2024b).

Reciprocally, islet cells were reported to enhance the growth and migration of co-cultured ECs. In 2022, Jung et al. cocultured β -TC6 cells and pancreatic islet endothelial MS1 cells, and found that RNA binding protein HuD in β -TC6 cells promoted the proliferation and migratory behavior of ECs by regulating the expression of Endostatin and Serpin E1 (Jung et al., 2022).

4.2 Induction of pancreatic β cells from hPSCs in the presence of ECs

ECs co-culture has been shown to improve the generation of pancreatic β cells derived from hPSCs (Relevant studies are summarized in Table 3). In 2011 and 2016, Talavera-Adame et al. utilized an in vitro self-coagulation culture system to generate functional pancreatic ß cells from embryoid bodies (EBs) derived from hPSCs, in the presence of human microvascular ECs (HMECs). Compared to controls, EBs cocultured with ECs exhibited higher expression of mature β cell markers and improved insulin secretion in vitro (Talavera-Adame et al., 2016; Talavera-Adame et al., 2011). Transplantation of these EBs into diabetic mice resulted in increased C-peptide secretion and significantly lowered blood glucose levels (Talavera-Adame et al., 2016). BMP pathway was activated at the EB-EC interface. EC effects in coculture were mimicked by BMP-2 and inhibited by NOGGIN, indicating BMP pathway activation is central to this process (Talavera-Adame et al., 2016; Talavera-Adame et al., 2011). These findings highlight the beneficial effect of coculture with ECs in enhancing the differentiation and function of hPSCs derived β cells.

The presence of ECs can promote the formation of functional islet cell aggregates. With the continuous improvement of β cell

	Contributions		 Mosaic pseudoislets were formed by pancreatic β cells and EPC <i>in vitro</i>for the first time Mosaic pseudoislets maintained function <i>in vitro</i> 	 The first study that introduce <i>in vitro</i> testing systems based on human cell lines and ECs Investigate the impact of the spatial distribution on cocultures of human-cells and ECs 	- Ratio of β cell/EC affects insulin release	• ECs promote islet cell function through the BTC-EGFR-JAK/STAT signaling pathway	 Provide important insights into the roles of α and ECs in protecting against oxidative stress 	(Continued on the following page)
	EC function (characterization/ period observation)		 Tube formation <i>in vitro</i> Uptake of acetylated low density lipoprotein EC marker: Vegfr2, CD31 and Vcam1 were expressed 14 days (<i>in vitro</i>) 	 EC marker: CD31 staining 2/5 days (<i>in vitro</i>) 	 EC marker: CD31 staining 2 days (<i>in vitro</i>) 	• EC marker: CD31 staining	Z	
	Organoids' function		 Viability of cell ↑ Islet function ↑ GSIS (insulin secretion) ↑ 	 CD31⁺ cell ↑ Cell viability ↑ GSIS ↑ β cells surrounded by HUVECs express significantly more E-cadherin 	• GSIS ↑ Metabolic activity ↑	 GSIS ↑ β cell markers ↑ After TP GTT ↑ 	• Oxidative Stress J	-
and Islet Organoids.	Cultivation method		 Re-aggregation technique An embryoid body forming medium (BPEL) and a spin protocol <7 days 	 Magnetic levitation to create two-layered heterotypic spheroids "1:1"; "ECs inside"; "β -cells inside" 25 days 	 100% β cells, 1: 1 β cell/endothelial, and 1: 3 β cell/endothelial 7 days 	 Transwell Min6 cells to HUVEC cells was 1:2 3 days 	• $\alpha : \beta (1:14), \alpha : \beta : EC (1:9:5),$ $1 \times \alpha : \beta : EC (1:9:2), 2 \times \alpha : \beta : EC (1:9:5) and 4 \times \alpha : \beta : EC (1:9:20)$ • 5 days	
n the Generation of β -like Cells	The source of the β cell	and ECs	Rat islet cells	EndoC-βH3	EndoC-βH5	MIN6	INSIE	-
lothelial Cell Coculture Used ir	The source and isolation of the EC	Indoise the second sec	. Rat . EPCs Primary (Bonder et al., 2009)	 Human HUVEC Primary (PromoCell) 	HumanHUVECPrimary (Lonza)	 Human HUVEC Primary (Lonza) 	 Human HUVEC Primary (Lonza) 	
TABLE 3 Summary of Enc	References	3.1 Formation of Pseu	Penko et al. (2011)	Urbanczyk et al. (2020)	Porter et al. (2023)	Wang et al. (2024a)	Wieland et al. (2021)	

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	Contributions	 RA sensitized ECs confer protection to β cells 	 The organoids increase endocrine marker expression and insultn secretion The organoids increase GLP-1 receptor and phosphokinase A expression 	• An effective paracrine interaction exists between IMEC and β cells and modulates glucose-induced insulin secretion via TPI-sulfonylurea receptor-KATP channel (SUR1-Kir6.2) complex attenuating interactions
	EC function (characterization/ period observation)	 Levels of growth factors like VEGF, EGF and FGF reduced in ER stress-induced ECs; treatment with RA restored their expression 	• EC marker: BS1 staining	 Glycolytic enzyme triosephosphate isomerase was secreted from ECs
S.	Organoids' function	 Nrf2 ↑ Heme oxygenase-1 (HO-1) and NADPH-quinone oxidoreductase-1 (NQO-1) ↑ ER stress ↓ Glucose-regulated protein (GRP) 78 and C/ERB homologous protein (CHOP) ↓ 	 Cell viability ↑ Insulin expression ↑ Insulin secretion ↑ β cell associated Gene ↑ GLP1/phosphokinase A expression ↑ 	• GSIS 1
f β -like Cells and Islet Organoid	Cultivation method	 ECs pre-treated with different concentrations RA thapsigargin-induced ER stressed pancreatic β-cells 	 Uncoated 6-well tissue culture plates Self-Condensation 5-14 days 	 Condition medium from IMEC 1 day
Iture Used in the Generation of	The source of the β cell	MIN6	Human EndoC- H1-cells	INSIE
imary of Endothelial Cell Cocu	The source and isolation of the EC	 Human Immortalized HUVECs (EA.hy926) Cell line 	• Mouse • MS1 Cell line	 Rat IMEC Primary (Islets microcapillary)
TABLE 3 (Continued) Sun	References	Amin et al. (2021)	Spelios, (2018)	Daniel et al. (2021)

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(Continued on the following page)

	Contributions	 HuD has the potential to regulate the crosstalk between β cells and islet endothelial cells by regulating Endostatin and Serpin E1 expression, thereby contributing to the maintenance of homeostasis in the islet microenvironment 		 Cocultured EBs had a higher expression of mature cells markers and enhanced insulin secretion <i>in vitro</i> Cocultured EBs remain long-term existence 	 The organoids express β cell and other endocrine markers and are functional 	 Organoids express β cell markers Organoids secrete functional insulin production in response to glucose challenge
	EC function (characterization/ period observation)	• EC marker: Pecam 1 staining		• EC marker: CD31 staining	 EC maker: CD31 expression The assembled tubule network was formed 	Z
	Organoids' function	 HuD downregulation duft f)TC6 cells inhibited the growth and migration of MS1 cells 		 β cell marker f Expansion <i>in vitro</i> f GSIS f(C-peptide and insulin secretion) After TP After TP C-peptide f β cells survival f Blood glucose level ↓ Long-term function and existence (>120 days) 	 Viability of β cell ↑ GSIS ↑ (insulin secretion) β cell marker ↑ 	 β cell marker (PDX1/NKX6.1)[†] Insulin Release [†] Islet maturation [†]
f β -like Cells and Islet Organoids.	Cultivation method	• 1 day		 Self-condensation culture system Collagen-laminin gels About 7 passages 	 Hydrogel platform (aggregates) Three-dimensional assemblies 1-20 days 	 A hydrogel-based platform Spontaneous aggregation About 7 days
Iture Used in the Generation o	The source of the β cell	BITC6	n the Presence of ECs	EBs derived from hPSC	hESC	hESC
Imary of Endothelial Cell Cocu	The source and isolation of the EC	• Mouse • MS1 Cell line	reatic β -cells from hPSCs ir	 Human HMEC Cell line 	 Human HUVEC Cell line 	 Human HUVEC Cell line
TABLE 3 (Continued) Sum	References	Jung et al. (2022)	3.2 Induction of Panc	Talavera-Adame et al. (2011); Talavera-Adame et al. (2016)	Augsornworawat et al. (2019)	Candiello et al. (2018)

d Islet Organoids Cells of R-libo -G ţ .: 7 lle litu dothelial Cell Co fΕr U S I ÿ

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	Contributions	• This study shows that pancreatic microenvironment- derived factors can mimic <i>in vivo</i> conditions <i>in vitro</i> to generate authentic β cells for translational applications WNT5A activates the non-canonical (JNK/c-JUN) WNT signaling and works with Grenlin1 to inhibit the BMP pathway during β cell maturation	• Proinflammatory macrophages induce β cell pyroptosis through the TNFSF12- TNFRSF12A pathway		 Coculture of freshly isolated rat islets with ECs enhances their sur vival and function in vitro 	 First isolation and culture of pancreatic blood vessels A novel explant culture model for angiogenesis and tissue engineering research 	(Continued on the following page)
	EC function (characterization/ period observation)	 EC markers: PECAMI, CFIII, FLKI, CDH5, ICAM, VWF were expressed The endothelial gene expression decreased after 8 passages 	 EC marker: CD31 staining (<i>in</i> <i>vitro</i>: 7/14 days) Uptake of acetylated low-density lipoprotein 		 The morphology showed a paved monolayer with a periplast showing a characteristic buffy positive reaction 	• EC marker: CD31expression (<i>in</i> <i>vitro</i> , 8 days)	
	Organoids' function	 C-peptide + cell ↑ Ins + cell ↑ C-peptide secretion ↑ GSIS ↑ 	 GSIS ↑ β cell markers ↑ β cell proliferation, survival, and maturation ↑ 		 Islet viability >90% Insulin release [↑] 	 Islets remained viable Insulin expression¹ Slight growth of ß-cells Microvessels were encapsulate islets 	
eta-like Cells and Islet Organoids	Cultivation method	 A hydrogel-based platform A stepwise manner About 14 days 	 VMI organoids, +differentiated H9 hESCs toward macrophages 14 days 		 Cocultures with thoracic aorta 7 days 	 3D culture system Embedd in Rat tail type 1 collagen 8 days 	
lture Used in the Generation of	The source of the β cell	hESC	Differentiated MEL-11 ^{NS/GFP} hESCs into pancreatic endocrine cells	ds from Adult Stem Cells	Rat islets	mice islets	
mary of Endothelial Cell Cocu	The source and isolation of the EC	 Human MSC and EC (fetal pancreas, duodenum, and spleen), HUVEC and MS1 Primary cell and cell line 	 Human hESC-derived Cell line 	lishing Pancreatic Organoi	RatThoracic aorta ECPrimary	 Mouse pancreatic blood vessels Primary 	
TABLE 3 (Continued) Sum	References	Chmielowiec et al. (2022)	Yang et al. (2024)	3.3 ECs Assist in Estab	Song et al. (2009)	Lehmann et al. (2019)	

Contributions	 First identification of pancreatic islet progenitors Long-term expansion and maturation in vitro The organoids can reverse diabetes in vivo 		 Develop a bioengineered platform to generate implantable and functional endocrine Neo-Pancreases 	 A commercially available mi- crofluidic platform to generate perfusable microvascular networks, and by incorporating pancreatic islet. 	 A geometrically controlled pancreatic pseudo-tissue model The MIN6 cells formed islet-like clusters surrounded by an endothelial MS1 cell monolayer, and show higher insulin secretion
EC function (characterization/ period observation)	 EC marker:CD31 staining (<i>in vitro</i>, 28 days) Endothelial networks were formed ECs require fresh isolation after each passage 		Z	 Interconnected networks were formed Microvascular networks formed perfusable lumens 	 A monolayer of a vessel-like structure was formed
Organoids' function	 Long-term expansion (>100 days) Maturation <i>in vitro</i> C-peptide and insulin secretion f Glucose responsive f After TP Maturation GTT f Insulin secretion f Reverse diabetes (<125 days) 		• GSIS †	 Microvascular networks form perfusable lumens Microvascular network recruitment to islets 	 Blood vessel-like structure formation Cell viability of over 85% for about 2 weeks Insulin secretion [↑]
Cultivation method	 Re-aggregation 2.5D Matrigel culture system Long-term expansion (>100 days) 		 Decellularization of rat liver and re-endothelialization 3 days 	 A commercially available microfluidic platform to generate perfusable microvascular networks 6 days 	 Cell-laden collagen sheet 15 days
The source of the β cell	Procr + endocrine progenitor cells	ulture of EC and Islet Cells	Primary ratislets	Rat islets	Min6
The source and isolation of the EC	• Mouse • ECs • Primary (mammary gland/skin)	ingineered Models for Coci	RatRAECCell line	HumanHUVECCell line	• Mouse • MS1 Cell line
References	Wang et al. (2020); Wang et al. (2022)	3.4 Development of E	Everwien et al. (2020)	Rambol et al. (2020)	Seo et al. (2020)

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Geng et al.

	tion EC function Contributions (characterization/ period observation)	 Morphologically, cells Morphologically, cells The possibility of addin displayed typical elliptic shape endothelial cells for the endothelial cells for the neo-vascularization of x wF/CD31/CD144 staining Over a period of 6 h implementation of β cereplacement therapies in more favourable transplantation sites than the liver 	 EC marker: Providing ECs into the CO21 hydrogel improv CD31 staining CD11 hydrogel improv β cell response as well a the expression of the expression of relevant BM proteins 	ECmarker: The device with the
ds.	Organoids' fund	 GSIS ↑ After TP Glycemic control ↑ Graft revascularizati 	 GSIS ↑ Basement membrane proteins 	 Cell viability ↑
f β -like Cells and Islet Organoic	Cultivation method	 +Human amniotic epithelial cells ICs, HUVECs and hAEC at a ratio of 5:4:1 4 days 	8 days • Encapsulated in COL1	A hydrogel-based
Iture Used in the Generation o	The source of the β cell	Primary ratislets	INSIE	β-TC3
mary of Endothelial Cell Cocul	The source and isolation of the EC	 Human HUVEC Primary Baudin et al., (2007) 	 Human HUVEC Primary (PromoCell) 	• Rat
TABLE 3 (Continued) Sum	References	Wassmer et al. (2021)	Zbinden et al. (2021)	Hospodiuk- Vormensis of al (2002)

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Bone marrow (BM); Transplantation (TP); Endothelial progenitor cells (EPCs); Human microwascular endothelial cells (HMECs); Human pluripotent stem cells (hPSCs); human embryonic stem cells (hESCs); Mouse pancreatic islet endothelial cell line (MS1); Endothelial cells (ECs); Dissociated murine β cells (MIN6); Glucose stimulated insulin release (GSIS); Human umbilical vein endothelial cells (HUVEC); Mesenchymal stem cells (IMSCs); Human cell line-based β-cells (EndoC-βH3); Mouse insulinoma β cells (β-TC3 cells); Rat heart microvessel endothelial cells (RHMVECs); Primary rat islets microcapillary endothelial cells (IMEC); Bandeiraea simplicifolia (BS-1); von Willebrand factor (vWF).

ibility of adding

ial cells for the cereting grafts nent therapies in

ntation of β cell

rdrogel improves

ponse as well as

tissues and sets the stage

to establish organoid

advanced microfluidics

perfusions using

This microphysiological

viable organ-on-chip diverse biological 3D

system represents a

were formed, covered by

 Vessel-like structures pericytes and smooth

GSIS ↑

(EndoC-βH5:FMA73:HUVEC-RFP, 10,000 cells per well)

30 days

 A ratio of 6:1:1 +Fibroblasts

EndoC-βH5

• HUVEC Cell line Human

Quintard et al. (2024)

exhibited the expression of tight junction protein

muscle cells, and

ZO-1 and adherens

junction protein

VE-cadherin staining (in

EC marker: CD31

vitro,1-30 days)

model to vascularize

Cell line

pancreatic-like spheroids

was 3D bioprintable and

perfusable

cell-related genes

• VEGF-A, Endothelin-1, and NOS3 expression ↑ - Insulin secretion \uparrow Sprouting number and length ↑

The endothelial

and NOS3 were expressed VEGF-A, Endothelin-1,

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differentiation technologies using hPSCs, Candiello (2018) and Augsornworawat (2019) developed a strategy for assembling hPSCsderived pancreatic cell aggregates with ECs on hydrogel platforms. The heterogeneous islet organoids expressed β cell and other endocrine markers, demonstrated functionality, and were able to secrete C-peptide and functional insulin in response to glucose stimulation (Augsornworawat et al., 2019; Candiello et al., 2018).

ECs have been reported to regulate β cell fate decisions. In 2022, Chmielowiec et al. cocultured hESC-derived β cells with human primary mesenchymal cells and ECs derived from fetal pancreatic tissues between week 9 and 20 of gestation. They identified week 17 and week 20 as critical time points in the microenvironment that favors β cell differentiation. They found that both ECM and soluble factors secreted by mesenchymal and ECs play a pivotal role in promoting β cell fate decisions. Among these factors, MSCs and ECs express WNT5A, which cooperates with GREMLIN1 to inhibit the BMP pathway during β cell maturation, and endothelial-derived Endocan was shown to promote the differentiation of SST⁺ δ cells (Chmielowiec et al., 2022).

The combination of macrophages and ECs can efficiently promote maturation of β cells efficiently. In 2024, Yang et al. developed vascularized macrophage-islet (VMI) organoids from hPSCs. They differentiated MEL-1INS/GFP human embryonic stem cells (hESCs) into pancreatic endocrine cells, H9 hESCs into macrophages, and H1 hESCs into ECs by overexpressing ETV2. The VMI organoids exhibited enhanced insulin secretion and calcium mobilization upon high glucose stimulation, indicating the β cells within VMI organoids are more mature than those cultured separately (Yang et al., 2024).

Overall, hPSC-derived β cells offer significant promise for future diabetes therapies, with ECs playing a crucial role in enhancing the development and functional maturation of these cells.

4.3 ECs assist in establishing pancreatic organoids from adult stem cells

With the discovery of adult stem cells in various tissues, attempts have been made to utilize pancreatic adult stem cells to generate functional pancreatic organoids in vitro (Relevant studies are summarized in Table 3). In 2009, Song et al. isolated ECs from the rat thoracic aorta and cocultured them with freshly isolated rat islets. They found ECs enhanced islet survival and function in vitro within 7 days (Song et al., 2009). Similarly, Lehmann et al. (2019) developed a coculture system with mouse islets and cultured pancreatic blood vessels. They first developed a method to isolate and culture pancreatic blood vessels, which could maintain endothelial markers in vitro. In the coculture system, the vessels frequently encapsulated the islets. The cocultured islets remained viable, expressed insulin for up to 8 days, and exhibited slight outgrowth (Lehmann et al., 2019). However, both studies only focused on short-term in vitro culture and did not involve in vivo transplantation.

In 2020, Wang et al. identified a population of stem/progenitor cells characterized by the surface expression of protein C receptor (Procr) within the pancreatic islets of adult mice. This study further developed a methodology for expanding these progenitors and differentiating them into functional islet organoids through coculture with freshly isolated ECs. The resultant islet organoids contained an abundance of β -like cells, along with smaller numbers of α -, δ - and PP-like cells, mirroring the cellular composition of mouse islets. The ECs used for the coculture were isolated from various mouse tissues, such as inguinal fat pads or the skin (Wang et al., 2020; Wang et al., 2022; Misra and Nostro, 2020). This study not only identified islet progenitor cells in the adult mouse pancreas but also achieved their long-term expansion and maturation into functional islet organoids *in vitro*. Importantly, it provides theoretical and technical support for the *in vitro* generation of large quantities of functional pancreatic β cells from islet progenitor cells, opening new avenues for diabetes treatment.

4.4 Development of engineered models for coculture of EC and islet cells

With significant advancements in tissue engineering, researchers are now developing sophisticated engineered models to coculture ECs and islet cells, aiming to enhance the functionality and integration of islet transplants (Relevant studies are summarized in Table 3).

Using ECs to create a biocompatible scaffold enhanced the engraftment of islets. Everwien et al. (2020) created a decellularized, re-endothelialized, and endocrine-repopulated rat liver, referred to as the Neo-Pancreas. In their study, ECs formed a monolayer during cultivation. After 3 days of cultivation, the Neo-Pancreas exhibited increased insulin secretion in response to high glucose stimulation (Everwien et al., 2020).

Thorough vascular penetration is required with EC-lined scaffolds to sustain islet function. In 2020, Rambøl successfully developed an in vitro microfluidic platform to study the interactions between islets and the microvascular network. By utilizing MSCs and HUVECs, a perfusable microvascular network was constructed. In vitro studies revealed that rat islets could locally recruit microvessels; however, within 5 days, these microvessels enveloped only the islet surface without penetrating the interior (Rambol et al., 2020). In the same year, Seo et al. used a cell-laden collagen sheet to coculture MIN6 and MS1 cells, forming blood vessel-like structures and islet-like clusters. These cells maintained over 85% viability within 2 weeks and exhibited higher levels of insulin secretion (Seo et al., 2020). Wassmer et al. (2021) generated and transplanted pre-vascularized insulin-secreting organoids composed of rat primary islet cells, HUVECs, and hAECs. The pre-vascularized islet organoids exhibited enhanced function, better engraftment and improved vascularization (Wassmer et al., 2021).

ECs have been shown to promote the expression of relevant basement membrane proteins, which support β cells survival and serve as a primary source of ECM proteins. These proteins facilitate cell engraftment and the overall stability and function of the engineered tissue (Zbinden et al., 2021).

Organs-on-chips meticulously incorporate cells, tissueto-tissue interfaces, fluidic dynamics, and other biomimetic microenvironments, thereby faithfully replicating organ-specific functions (Tao et al., 2019) and inter-organ interactions (Tao et al., 2022). The vascularization process within the chip significantly enhanced the growth, maturation, and functional performance of the islet organoids. Hospodiuk-Karwowski et al. (2022) developed a hydrogel-based perfusable, vascularized pancreas-on-a-chip device using rat heart microvessel ECs and mouse insulinoma beta (β)TC3 cells (Hospodiuk-Karwowski et al., 2022). In 2024, Quintard et al. developed a microfluidic platform to establish and monitor the formation of endothelial networks around islet spheroids, which were cultured on-chip for up to 30 days. The islet organoid growth, maturation, and function were enhanced when vascularized on-chip (Quintard et al., 2024).

The aim of co-culturing ECs and β cells in organoid systems is to improve β cell differentiation and maturity, more accurately study the islet microenvironment, and enhance vascularization in mouse organoids and human islets prior to transplantation (Beydag-Tasöz et al., 2023). Overall, these engineered models present promising approaches for developing functional and stably vascularized islets that could potentially improve current clinical shortcomings in islet transplantation. These findings highlight ECs as an important factor supporting the generation and maintenance of functional pancreatic islet organoids in culture.

4.5 Regional EC heterogeneity

ECs exhibit significant phenotypic heterogeneity across different tissues, reflected in their morphology, molecular markers, and functional adaptations to the microenvironment. This heterogeneity is also observed in ECs in the pancreatic vasculature (Chen et al., 2021; Khan et al., 2024). Specifically, intra-islet ECs differ markedly from exocrine ECs in their physiological and functional roles. In mice, intra-islet ECs create a unique microenvironment by expressing specific markers (CD31⁺ Emcn^{hi}) and secreting higher level of paracrine factors (like Hgf and Igf1/2), which support the development, function, and survival of β cells (Chen et al., 2021). Pancreatic ECs exhibit high permeability and specialized intercellular junctions, facilitating efficient nutrient and hormone transport essential for β cell metabolic needs and insulin secretion (Khan et al., 2024).

Coculturing hESC-derived pancreatic progenitors with primary mesenchymal cells and ECs increased C-peptide⁺ cells proportion compared to coculture with HUVECs and MEFs. Urbanczyk et al. demonstrated that co-culturing isolated human islets with human pancreatic microvascular ECs significantly improved islet functionality compared to co-culture with HUVECs (Urbanczyk et al., 2024). A study comparing EC from different portions of pancreas also found mesenchymal cells and ECs from the head of the pancreas exhibited a seemingly more superior supportive capacity and could induce more C-peptide⁺ cells (Chmielowiec et al., 2022). Besides, Aghazadeh, (2021) showed that ready-made microvessels were more effective than single HUVEC in connecting with the host vasculature, ensuring successful islet transplantation (Aghazadeh et al., 2021).

In conclusion, co-culture with organ-specific and primary ECs showed improved organoid function and islet transplantation outcome. Future research should focus on deciphering the molecular cues ECs exert, in order to benefit clinical transplantation.

5 Discussion

In 2000, Shapiro's team pioneered a new era of islet transplantation for the treatment of T1DM, freeing all seven recipients from exogenous insulin (Shapiro et al., 2000). Still, challenges remain to be resolved including donor shortage, graft survival, immune rejection, and long-term function maintenance.

To address the donor shortage issue, many groups developed differentiation protocols to generate stem cell derived islets. For example, in the VX-880 clinical trial conducted by Vertex, 11 out of 12 patients transplanted with ESC-derived islets discontinued insulin use after a period of time (Vertex, 2024; Medicine, 2025). In another study, researchers used chemically induced stem cellderived islets to restore glucose regulation in a T1DM patient, and resulted in complete insulin independence starting from day 78 after transplantation (Wang et al., 2024c). Despite the recent advancement in cell therapy, it seems that up to 3 months are required for the graft to reach optimal function (devoid of exogenous insulin use). Hence, whether the use of EC co-culture could accelerate the functional maturation of transplanted grafts is definitely worth investigating. To move forward, considerations on EC source, purity, phenotype, scalability, mode of administration and respective quality assessments should be addressed to ensure their safety and efficacy in clinical applications.

Conventional transplantation through the portal vein is both invasive and difficult to monitor. Subcutaneous transplantation has been an attractive alternative for islet transplantation, due to its simplicity of surgical procedures, minimal invasiveness, capacity to accommodate relatively large graft volumes, ease of monitoring, removal or replacement. For this purpose, various subcutaneous vascularization devices are developed in order to protect graft from immune attack while still able to nourish through circulation. But scalability has always been a challenge for such devices. Recent advances in biomaterials and bioengineering have enabled successful pre-vascularization of subcutaneous sites and the development of immunosuppression-free strategies (Wang et al., 2023). The SHEATH system has explored the potential for scalability using a large animal (minipig) model and the recently launched clinical trial (NCT05073302) on prevascularization technology, which will provide valuable insights to guide further optimization and clinical translation of the SHEATH system. Besides subcutaneous transplantation, recent studies from Deng's lab have proposed abdominal anterior rectus sheath as an alternative transplantation site for its abundant vasculature (Wang et al., 2024d; Liang et al., 2023). Whether combining a pre-vascularization device will yield better results awaits further investigation. Ultimately, the scalability of cell replacement therapy is crucial for achieving clinical benefits, and requires interdisciplinary collaboration and technological innovation.

This review summarizes the recent studies on the role of vascular ECs in islet development, transplantation, and organoid culture. Continued exploration of these areas holds promise for enhancing the efficacy of islet transplantation and is undoubtedly useful for developing novel therapeutic strategies.

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AG: Funding acquisition, Project administration, Validation, Writing-original draft. SY: Validation, Writing-original draft. QY: Supervision, Writing-review and editing. YZ: Funding acquisition, Conceptualization, Supervision, Writing-review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

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that could be construed as a potential conflict of interest.

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Supplementary material

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