β-cell Exhaustion and Islet Microenvironment: The Role of Local Vascular Network

Esgotamento de Células β e Microambiente dos Ilhéus Pancreáticos: O Papel da Rede Vascular Local

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Abstract

The pancreas, a crucial organ of the endocrine system, is structurally organized in the islets of Langerhans. The islet microenvironment, comprising mesenchymal, vascular endothelial, neural, and immune cells embedded in an extracellular matrix (ECM), orchestrates important cellular interactions essential for maintaining islet homeostasis and proper β-cell function. This study underscores the intricate relationships between islet cells and their microenvironment, highlighting the vascular network's pivotal role in β-cell insulin secretion and its implications for diabetes pathophysiology. The ECM, primarily secreted by endothelial and endocrine cells, plays an important role in supporting β-cell survival, proliferation, and insulin secretion. Moreover, the dense capillary network within the islets enables efficient nutrient and hormone exchange essential for cell function whereas the pericytes regulate blood flow and β-cell function. Changes in the islet microenvironment, including alterations in ECM composition, vascular network dysfunction, or blood flow regulation affect β-cell function and contribute to dysfunctional hormone secretion, therefore contributing to diabetes pathogenesis. For instance, immune-mediated damage to the peri-islet basement membrane and abnormalities in microvasculature and pericyte function are linked to β-cell destruction in type 1 diabetes and β-cell dysfunction in type 2 diabetes, respectively. *In vitro* strategies to restore vascular cell mass and improve islet function, such as adding endothelial cells or ECM components to islets to improve revascularization and functional outcomes, hold promise in diabetes management and islet transplantation. Comprehending the intricate interactions within the islet microenvironment is key to devising innovative therapeutic interventions to restore β-cell function and enhance patient outcomes.

Keywords: β-cell function; diabetes pathophysiology; pancreatic islet microenviroment; vascular network

Resumo

O pâncreas, um órgão crucial do sistema endócrino, está estruturalmente organizado nos ilhéus de Langerhans. O microambiente desses ilhéus, composto por células mesenquimais, endoteliais vasculares, neurais e imunitárias envolvidas numa matriz extracelular (ECM), coordena interacções celulares essenciais para manter a homeostase e a função adequada das células β. Este estudo ressalta as complexas relações entre as células dos ilhéus e seu microambiente, destacando o papel fundamental da rede vascular na secreção de insulina e suas implicações na fisiopatologia da diabetes. A ECM, segregada principalmente por células endoteliais e endócrinas, é crucial para a sobrevivência, proliferação e secreção de insulina das células β. A densa rede capilar dentro dos ilhéus permite uma troca eficiente de nutrientes e hormonas essenciais, enquanto os pericitos regulam o fluxo sanguíneo e a função das células β. Mudanças na composição da ECM, a disfunção vascular ou a regulação do fluxo sanguíneo, afectam a função das células β e contribuem para uma secreção hormonal disfuncional, estando assim associadas à patogénese da diabetes. Por exemplo, danos imunomediados na membrana basal peri-ilhéu e anomalias na microvasculatura e função dos pericitos estão associados à destruição das células β na diabetes tipo 1 e à sua disfunção na diabetes tipo 2, respetivamente. Estratégias *in vitro* para promover a revascularização e resultados funcionais, como adição de células endoteliais ou componentes ECM, mostram-se promissoras no controle da diabetes e no transplante de ilhéus. Compreender essas complexas interações microambientais é fundamental para desenvolver intervenções inovadoras que, restaurando a função das células β, melhoram os resultados dos pacientes.

Palavras-chave: função das células β; fisiopatologia da diabetes; microambiente dos ilhéus pancreáticos; rede vascular

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> INTRODUCTION

The pancreas is a composite organ of the digestive and endocrine systems, serving a crucial role in the human body both as an exocrine and endocrine gland. The endocrine pancreas is architecturally organized into distinct clusters known as the Islets of Langerhans, which comprise specialized cellular entities. Adult human islets are composed of the most abundant group of insulin-secreting β-cells, followed by α-cells and δ-cells, secreting glucagon and somatostatin respectively. PP cells (y-cells) and ε-cells, producing pancreatic polypeptide and ghrelin respectively, are also present (Figure 1). Despite the key role of these hormones in regulating body glucose homeostasis the Islets of Langerhans occupy only 1-2% of the pancreatic mass. [1] The microenvironment surrounding pancreatic islets is a complex and diverse system, highly innervated and vascularized, comprising mesenchymal, vascular endothelial, neural, and immune cells embedded in an extracellular matrix (ECM) that support cellular functions (Figure 1). [2]

A body of evidence has drawn attention to the significance of intricate interplays between islet cells and islet microenvironment for their optimal functionality. [2] Particularly, the fundamental role of the vascular network in the insulin secretory capacity of β-cells has been investigated for its implications in diabetes pathophysiology. [1,3,4] Here, we will briefly discuss the pancreatic islet microenvironment, covering its interactions, and the vascular network's influence on β-cell function, aiming to illuminate their potential role in diabetes progression.

> PANCREATIC ISLET MICROENVIRONMENT

The intricate islet microenvironment plays an active role in supporting multiple endocrine and non-endocrine cell types within the islet. It provides mechanical and chemical signals that contribute to support and modulate cell differentiation, functional maturation, spatial organization, cell survival, and proliferation. [1] Additionally, it facilitates intercellular interactions involving paracrine and autocrine signaling mechanisms, which are essential for integrating multiple signals including glucose levels, hormones, neurotransmitters, and other factors. [4-6] This dynamic microenvironment system contributes to the overall functionality and homeostasis of pancreatic islets.

The ECM is a complex network of proteins, such as collagen, elastin, laminin, glycoproteins, proteoglycans and glycosaminoglycans, along with other cells such as fibroblasts and macrophages. It exists in two distinct locations within the pancreatic islet, namely the peri-islet and vascular ECM. In the peri-islet region, ECM is mainly composed of a basement membrane (BM) and an interstitial membrane (IM). The BM consists mostly of collagen IV and laminins. Human pancreatic islets contain two different BM layers, coating the intra-islet capillaries, with ECM components differing according to the side of the membrane. IM surrounds the BM, consisting of a less dense network of proteins composed of fibrillar collagens with other matrix molecules. [2,7,8] The interactions of islet endocrine cells with ECM can regulate several β-cell processes, including cell survival, proliferation, and insulin secretion. [4] Recent findings suggest that ECM is predominantly secreted by endothelial cells (EC) and endocrine cells, although other vascular, mesenchymal, and/or neural cells (i.e. fibroblasts, Schwann cells or pericytes) can also contribute to ECM formation. [9]

Fibroblasts play a crucial role in synthesizing several components of the ECM, thereby establishing the islet structural framework. These cells are typically located in the islet periphery, where they produce collagens forming the capsule. However, another fibroblast population is thought to be responsible for higher collagen and laminin levels surrounding the human islets' microvasculature. Islet fibroblasts can impact β-cell functions, including glucose sensing, insulin processing, and survival, both *in vitro* and after transplantation. [10] Despite abundant studies demonstrating the beneficial effects of ECM components added exogenously, little is known about the production of ECM molecules by endogenous cells and their interactions within the islet environment. [11]

Immune cells are also present in the pancreatic islet network. Particularly, islet macrophages exert dual roles in maintaining homeostasis, supporting β-cell mass and function through the secretion of signaling molecules, but also contributing to inflammation. [2,12] Tissue-resident macrophages are extremely heterogeneous, due to microenvironment-specific functions but can be functionally categorized in M1 and M2 groups. M1-like macrophages are activated by negative factors present in the microenvironment and induced in response to inflammatory signals. Islet-resident macrophages and β-cells respond to metabolic changes that can lead to a cycle of inflammation where an accumulation of M1-like macrophages increases cytokine production and exacerbates β-cell failure. [13] Despite heterogeneity, tissue-resident and M2-like macrophages have homeostatic functions, including the release of anti-inflammatory cytokines and matrix metalloproteinases, also being involved in β-cell proliferation. Their expression is reduced in obese and diabetic states, suggesting a potential

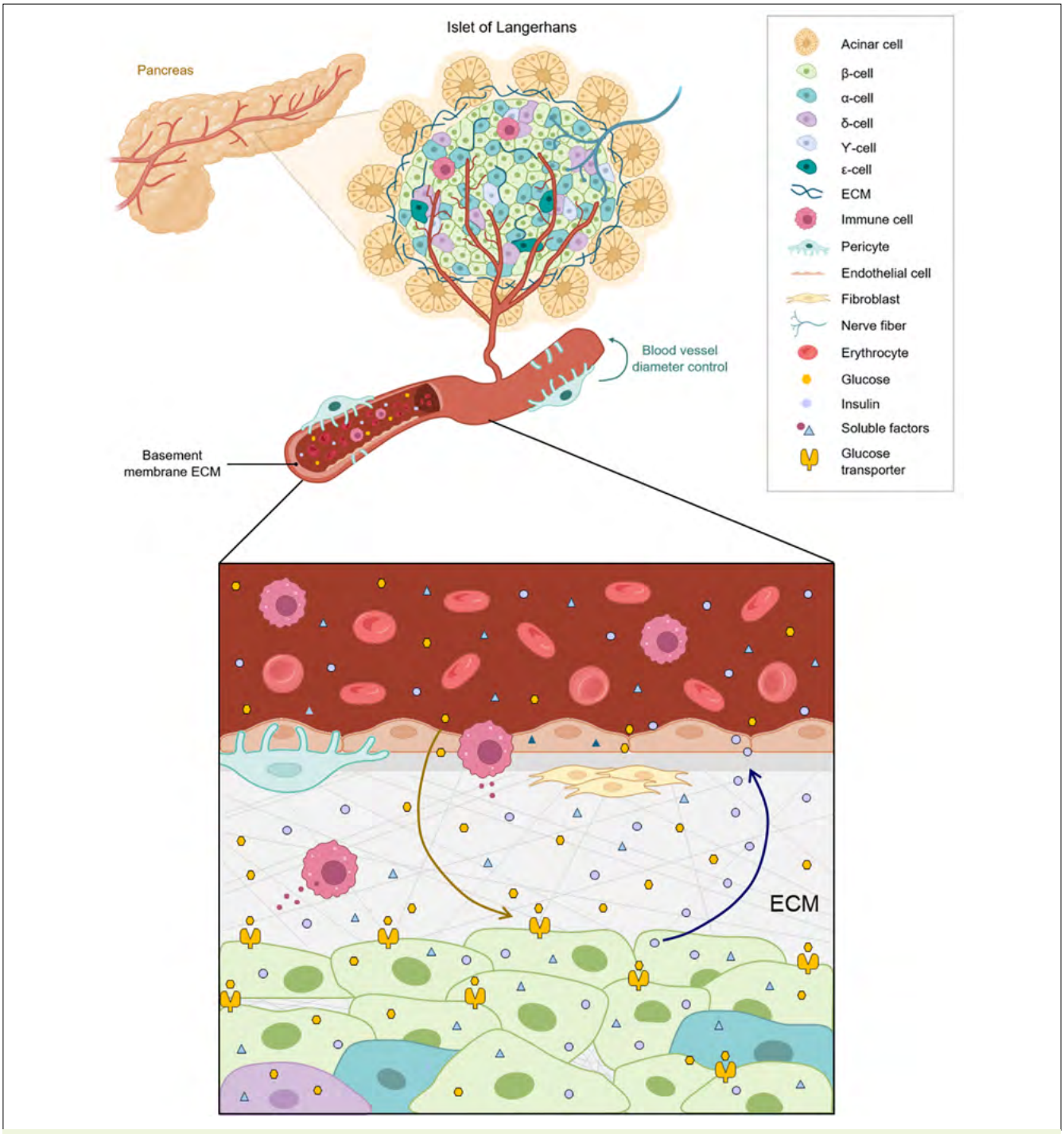

Figure 1 - The dynamic microenvironment of pancreatic islets. The pancreatic islet microenvironment is characterized by a highly vascularized and innervated milieu, featuring an extracellular matrix (ECM) composed predominantly of a vascular and endocrine cell-derived basement membrane. The peri-islet region comprises clusters of acinar cells enveloping the pancreatic islets. Adjacent to their structure, the islets are accompanied by an afferent blood vessel that swiftly transforms into convoluted capillary networks. The intricate network of tiny blood vessels within the endocrine cell cluster plays a vital role in supporting the viability of these cells and facilitating their proper function. Endocrine cell types within the islets include insulin-secreting β-cells, glucagon-secreting α-cells and somatostatin-secreting δ-cells. Although in smaller percentages, ε- and γ-cells are also present being responsible for ghrelin and pancreatic polypeptide secretion, respectively. Endocrine cells respond to blood circulating signals such as glucose levels, releasing hormones (e.g. insulin) into the intra-islet capillaries. Islet-specific resident macrophages are also found, being implicated in normal β-cell development and function. The cross-talk between endocrine cells, macrophages and endothelial cells has been described as an important event for islet development and function. Penetrating nerve fibers may also interact with endocrine cells. Pericytes located within pancreatic islets serve as a reservoir of nourishing factors aiding in the differentiation, maturation, and proliferation of β-cells. Additionally, through their contractile processes, they regulate the local dynamics of islet capillaries and the blood flow.

link to disease progression. [14] Additionally, resident tissue macrophages contribute to ECM remodeling and composition changes through the production of enzymes involved in ECM breakdown. [12,15]
Endocrine cell function can also be regulated by neural cells, which also influence islet structure and cell mass. Pancreatic islets are richly innervated by both sympathetic and parasympathetic nervous systems. These inputs modulate the potentiation of insulin secretion, blood flow, and enzyme/hormone secretion. Studies suggest the presence of glial-like cells in pancreatic islets that may modulate neural signaling and influence islet function. [1,16]

> ROLE OF LOCAL VASCULAR NETWORK

Comprising highly endocrine vascularized clusters, the islets of Langerhans exhibit a distinct capillary network, receiving up to 20% of the direct arterial blood flow to the pancreas. [1] The characteristics of afferent islet microvasculature depend on islet size, with small islets being typically served by a single arteriole, while large islets may receive the blood from up to three arterioles. Inside the islet, arterioles branch into thick capillaries forming a glomerular-like network five to ten times denser than that observed in the exocrine pancreas. [17,18] Given the increased vascularization, almost all β-cells come into contact with a highly permeable capillary, a phenotype that is believed to optimize the fine-tuning of blood glucose fluctuations and the outflow of hormones into the bloodstream. Such contact, however, does not appear to occur directly, being vascular and β-cells separated by a glycoprotein-rich vascular basement membrane. [3] In contrast to the rest of the pancreas, islets may exhibit a scarcity of lymphatic capillaries. This could play a crucial role in modifying shear stresses and pressures within the interstitial space of the islet, particularly under conditions of hyperglycemia. [19]
Recent studies have revealed that roughly 40% of the islet microvascular area is covered by pericytes. Pericytes are abluminal mural cells that exhibit contractile cytoplasmic processes along the endothelial tube. Using such structures they control vascular diameter and capillary blood flow through vasodilation and vasoconstriction. It is known that pericytes display a remarkable plasticity capable of adopting distinct phenotypes, however, their specific role in adult pancreatic islets remains a developing area of study. [2,20] Evidence of pericyte involvement in regulating β-cell function and mass is now starting to emerge. [6,20,21] In the context of type 2 diabetes (T2D), recent evidence highlights the importance of pericytes in glycemic control and points them out as potential targets for therapeutic approaches. [3] However, further studies are needed to elucidate the mechanisms underlying pericyte function, their interactions with the different pancreatic cell types, and the impact of pericyte dysfunction on diabetes development. [22]
Intra-islet capillaries, which connect endocrine cells to the blood supply, are adapted to facilitate proper gas exchange, waste removal and transport of nutrients, metabolites, and hormones from islets to the bloodstream. [1] Perfusion experiments with horseradish peroxidase have shown that endothelial fenestrae are sites through which proteins quickly permeate, [23] highlighting the critical importance of these regions in enabling the rapid entry of insulin into circulation upon a glucose stimulus. Beyond metabolic support, ECs also contribute to the distinctive microenvironment of the islet and deeply influence nearby cell function. Intra-islet ECs establish tight interactions with endocrine cells during embryogenesis and throughout life, providing reciprocal functional and trophic support. This cross-talk between the different cell types holds significant implications for regulating critical physiological processes associated with specialized islet cell phenotypes. [3] In that regard, blood vessels represent an important source of developmental signals that are involved in different stages of pancreatic organogenesis. Vascular Endothelial Growth Factor (VEGF) is a key player in this process, as evidenced by the abundance of VEGF receptor 2-positive ECs in the early embryonic mesenchyme. Inhibition of the VEGF receptor leads to abnormal epithelial growth, while overexpression of VEGF disrupts pancreatic growth and islet structure. [24,25] Intra-islet ECs are also described to secrete other mediators including vasoactive factors (e.g. endothelin-1), connective tissue growth factor (CTGF), thrombospondin (TSP)-1, and hepatocyte growth factor (HGF), thus contributing to β-cell survival, proliferation and function by insulin gene expression and secretion. [26,28] CTGF is known to drive β-cell expansion during embryogenesis in an autocrine manner, while HGF influences β-cell function through exocrine signaling. [29] In humans, the expression of the anti-angiogenic protein TSP-1 is upregulated in response to hyperglycemia. TSP-1 deficiency has been associated with pancreatic hyperplasia, glucose intolerance, and impaired glucose-stimulated insulin secretion (GSIS). [27] Additionally, both ECs and pericytes also produce an array of ECM components (e.g. laminins, collagen IV, proteoglycans, and nidogen) that are important for the synthesis and maintenance of islet BM. This is quite important since β-cells are unlikely to produce

their own ECM constituents. [3] Conversely, on the flip side, the secretion of the angiogenic vascular endothelial growth factor A by endocrine β-cells stimulates the formation of a dense and highly fenestrated vascular network, promoting the rapid secretion of islet hormones into the circulation. [30]

Notably, several reports have also described the role of islet vascular cells in regulating tissue inflammation and immune response. Endothelial cells attract macrophages to the islet surrounding area, which subsequently promote the proliferation and regeneration of β-cells. [1,3] Despite that, the precise role of pancreatic vascular endothelium as the gatekeeper of pancreatic tissue inflammation, particularly at diabetes onset, remains poorly explored.

> IMPLICATIONS FOR β-CELL FUNCTION

Dysfunction of islet microvasculature and impaired regulation of islet blood flow are thought to compromise exchanges between β-cells and the circulation, leading to deficient hormone secretion. Corroborating this, several studies have described structural alterations in the human islet vascular network and ECM composition during type 1 diabetes (T1D) and T2D. [3] In a hyperglycemic state, the presence of advanced glycation end-products in pancreatic islets augments ECM crosslinking and stiffness, thus affecting the local islet microenvironment and inhibiting cellular signaling and behavior. [3,29] In T1D, increased deposition of glycosaminoglycans and degradation of ECM components can enhance or inhibit immune cell infiltration, damaging the peri-islet BM and contributing to inflammation and β-cell destruction characteristic of T1D. [31] Changes in the regulation of pancreatic vascular permeability were described to augment leukocyte recruitment and extravasation into the islets of T1D mice, accelerating disease progression by promoting β-cell apoptosis and reducing insulin levels. [32] On the other hand, in T2D islets, it has been reported the observation of a less ramified microvasculature. [33]

Alongside, aberrant pericyte phenotype and gradual loss of pericytic coverage of islet capillaries have been linked to defective β-cell function during the disease. Conversion of pancreatic pericytes to myofibroblasts negatively impacts ECM production and promotes tissue fibrosis, leading to β-cell dysfunction. [34] In what concerns the loss of pericytes, this event is known to be accompanied by EC hyperplasia, capillary dilation, and vascular leakage. [35] Moreover, *in vivo* ablation of pancreatic pericytes results in the reduction of islet insulin content and secretion, and in the decreased expression of β-cell function and maturity-related genes. Importantly, the removal of pericytes from isolated islets was shown to induce β-cell dedifferentiation. [36] This suggests that pericytes may play a role within the islet microenvironment in supporting β-cell performance, regardless of their function in regulating blood flow, either by the release of vital signals or through direct cell-to--cell communication.

It is of utmost importance to reiterate that most of the *in vitro* studies assessing the pancreatic islet function rely on the use of cells derived from isolated islets, acquired by enzymatic degradation of ECM structure. During this process, and immediately after the isolation, pancreatic islets can maintain some endothelial cell expression, however, after two days in culture only around 15% of ECs survive. [3,37] Such reduction adversely affects the endocrine function of isolated islets, highlighting the need to develop strategies to restore vascular cell mass. Concerning this issue, exposure of cultured β-cells to endothelial- or pericyte-conditioned medium enhanced GSIS and β-cell proliferation, [21,38] demonstrating once again the importance of vascular cell-derived mediators in endocrine cell function. In the islet transplantation context, supplementing islets with additional ECs leads to better revascularization and improved functional results compared to individual islets. [39,40] This can be explained by the fact that after transplantation, islet grafts undergo a notable decrease in vascular supply. Hence, the restoration of islet function heavily depends on the presence of functional ECs and the development of new blood capillaries, within the transplanted islets, to obtain metabolic supply from the host's vasculature. [41] The introduction of BM proteins, such as laminins, into *in vitro* culture β-cells also showed to improve insulin gene expression and GSIS. [42]

> CONCLUSIONS

Overall, the pancreas plays a key role in maintaining metabolic homeostasis by the secretion of hormones like insulin, produced in specialized cells within the pancreatic islets. These islets, though small in proportion to the pancreatic mass, contain a diverse microenvironment comprising ECs, mesenchymal, vascular endothelial, neural cells, and immune cells, involved in an ECM network. The ECM offers structural support and modulates essential cellular processes like survival, proliferation, and hormone secretion within the islets. Neural and immune cells influence endocrine cell function and islet structure, further emphasizing the complexity and importance of the islet microenvironment. Moreover, the local vascular ne-

twork ensures the efficient exchange of nutrients, oxygen, and hormones critical for islet function.

Disruptions within the islet microenvironment contribute to the pathogenesis of diabetes, highlighting the importance of understanding the mechanisms and interplay within pancreatic islets. Alterations in ECM composition, islet vascular network, or in the regulation of blood flow, affect β-cell function and contribute to dysfunctional hormone secretion. For instance, damage to the peri-islet BM by immune cells is linked to β-cell destruction in T1D. In T2D, a less ramified microvasculature and aberrant pericyte phenotype contribute to defective β-cell function.

Strategies aimed at restoring vascular cell mass and enhancing islet function are promising in diabetes management and islet transplantation. These include adding ECs or ECM components to islets to improve revascularization and functional outcomes. Additionally, a better understanding of the intricate interactions established within the islet microenvironment is essential for unraveling diabetes pathophysiology and developing innovative targeted therapeutic interventions aimed at restoring β-cell function and improving patient outcomes. <

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