



SHORT COMMUNICATION

Biocompatible Scaffolds: An Effective Means for Improving Islet Cells Viability and Function

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Abstract

Islets of Langerhans are endocrine units of the pancreas. Local microenvironment of islets includes their immediate extracellular matrix and other molecules that surround islets within the exocrine pancreas. Transplantation of pancreatic islets, which is a promising method for treatment of type 1 diabetes, encounters several challenges including premature loss of grafted islets. The native islet extracellular matrix is disrupted during the process of enzymatic digestion of pancreas, which is required for isolation of islet from exocrine tissue. The lack of extracellular matrix then leads to a significant impairment of islet cell survival and function. This review outlines the significance of extracellular matrix in maintaining islet viability and integrity and summarizes the current body of knowledge regarding the role of different types of biocompatible extracellular matrices in improving islet cell viability and functionality in both *in vitro* and *in vivo*.

Introduction

Islets of Langerhans constitute about one to two percent of the volume of pancreas and are responsible for secretion of an array of metabolic hormones including insulin and glucagon [1]. Over the past couple of decades, transplantation of pancreatic islets has been advanced as a promising alternative to insulin replacement therapy for management type 1 diabetes mellitus [2]. Islet transplantation restores endogenous insulin and glucagon production and reinstates glucose homeostasis [3,4]. This therapeutic procedure, however, faces several challenges including shortage of islet sources and poor engraftment of transplanted islets [5]. Although in the first year post-transplantation

up to 80% of islet graft recipients enjoy their insulin independence, graft function declines to less than 50% by five years after transplantation even with vigorous anti-rejection regimens [6]. This graft survival statistics highlights the significance of non-immunological causes of islet graft loss. Additionally, more than one donor (up to three) is usually required per islet graft recipient to achieve sustained insulin independence [7]. In contrast to the islet transplantation, usually only one donor pancreas is sufficient to completely correct the metabolic abnormalities of diabetes with whole-organ pancreas transplantation [8]. As such, in the view of the limited supply of donor organs, the need for greater number of donor pancreas to achieve insulin independence per islet graft recipient is a major limitation for islet transplantation.

Damage to islets during the isolation procedure or loss of viable islets shortly after transplantation are possible explanations for non-immunological islet graft. Indeed, up to 70% of the transplanted islets may be damaged in the early post-transplant period even in the absence of immune attack [9,10]. This premature islet loss then leads to the requirement of multiple donors per each islet graft recipient.

Susceptibility to microenvironment disruption with subsequent functional impairment and islet cell apoptosis can result in loss of graft function [11]. Islet isolation process involves enzymatic digestion of pancreatic tissue, which inevitably results in the loss of peripheral

Table 1: Major islet ECM components and their corresponding receptors.

ECM protein	Location and Function	Receptors on islet cells
Type I collagen	Peri- and intra-islet ECM, provides tensile strength, structural integrity, and survival signalling.	$\alpha_v\beta_3$, $\alpha_2\beta_1$, DDR2
Type IV collagen	Basement membrane, provides microstructural integrity and cell stimulation, attaches to other basement membrane components.	$\alpha_1\beta_1$, DDR1
Fibronectin	Basement membrane, arranged in a 3D fibrillar mesh similar to collagen, plays import roles in cell adhesion and development.	$\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_5\beta_1$
Laminin	Basement membrane, glycoprotein with trimeric structure, important for cell binding, migration, and differentiation.	$\alpha_1\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$

basement membrane and Extracellular Matrix (ECM) surrounding the islets. These components are essential for islet integrity and damage to the islet native ECM triggers functional impairment of islets and eventual islet cell apoptosis [12-14]. In this view, reestablishment of the interactions between islet cells and the elements of their local microenvironment may preserve islet cell viability and function and eventually lead to improvement of the outcome of islet cells replacement therapies. To this end, an array of strategies aiming at protecting islet cells from early loss has been suggested to improve long-term islet graft function. This review will focus on some of these strategies particularly those that use Three-Dimensional (3D) biocompatible scaffolds to protect islets and improve their long-term viability and function.

Functional structure and composition of pancreatic islet ECM

Pancreatic islets are cell clusters that are composed of variety of cell types. The majority of islet cells are β cells, which produce insulin that lowers blood glucose. α cells are the second most abundant cells within islets and secrete glucagon, which through glycogen breakdown increases blood glucose. These two types of cells comprise more than 90% of islet cells. Other less abundant islet cells include δ -cells, ϵ -cells and PP-cells, which secrete other hormones and peptides [15]. These cells, alongside with capillaries and some stromal cells are organized within a spherical microarchitecture which is glued together and surrounded by a complex ECM.

ECM, in general, is composed of multiple proteins and glycoproteins that form a complex scaffold around cells. ECM plays an essential role in supporting cells both physically via structural proteins and biologically via integrins and other cell adhesion molecules [16]. ECM proteins mainly include different types of collagens, fibronectins, laminins, tenascins, elastin, and glycosaminoglycans [17]. Collagen is the most abundant ECM protein and contains triple-helix domains [18]. Twenty-eight different types of collagen have been identified in vertebrates with types I to V to be the most common ones [19]. Collagens provide tensile strength and contribute in cell adhesion and migration. Islet ECM is mainly composed of type I and IV collagens. These collagens play an important role in supporting pancreatic islets. Fibronectins and laminins are also important components

of islet ECM [12]. Fibronectins and laminins mainly connect cells to the underlying matrix and play key roles in cell adhesion and signalling. This ECM mediated signalling controls many cellular functions including differentiation, survival, and migration [20]. In particular, Islet cell-ECM crosstalk provides important survival and functional signals and maintains islet cell differentiation, viability, morphology, and function (e.g. insulin secretion) [21-24].

The crosstalk between islet cells and ECM is generally mediated by a group of cell surface receptors known as integrins, which trigger many different intracellular signalling pathways [25]. Each component of the islet ECM has selective affinity to different integrins. For instance, collagen I mainly binds to $\alpha_v\beta_3$ and $\alpha_2\beta_1$ integrins while collagen IV preferentially binds to $\alpha_1\beta_1$ [26,27]. Discoidin Domain Receptors (DDR) are another family of cell surface receptors that can bind to ECM proteins. DDRs are a group of receptor tyrosine kinases that their activation can trigger a wide array of cellular responses including differentiation, adhesion, proliferation, and survival [28]. Studies have shown that Collagen I mainly activates DDR2 while DDR1 is more responsive to collagen IV [29,30]. Main Islet ECM proteins and their corresponding islet cell surface receptors are summarized in Table 1.

As noted above, interaction of islets with their ECM provides important survival and functional signals [31-33]. As such, when this interaction is disrupted because of enzymatic digestion of the ECM during the islet isolation process, islet cell viability and function is jeopardized. This phenomenon is known as matrix signaling mediated cell death or anoikis [34,35]. Indeed, it has been shown that anoikis after islet isolation significantly limit functional and viable islet mass [36,37].

Models for reinstatement of islet-ECM interaction

To address the problem of premature cell death and malfunction in ECM-deprived islets, many groups have tried several types of natural and synthetic scaffolds to re-establish vital islet-ECM crosstalk. These strategies mainly include entrapment of islets within various types of extracellular matrices. Entrapment of islets within an ECM generally results in improvement of islet cell survival and enhances glucose stimulated insulin secretory capacity. For instance, Weber, et al. showed that β -cell survival was significantly improved in hydrogels

containing collagen type I, collagen type IV, fibrinogen, fibronectin, laminin, and vitronectin. Glucose stimulated insulin secretion was also improved in gels containing collagen type IV or laminins [38]. Further, Yep, et al. showed that collagen IV-modified scaffolds could promote islet cell viability, decreased early-stage islet cell apoptosis, improved islet metabolic function and insulin release, and eventually restored euglycemia significantly faster [39]. The role of collagen based ECMs in support of islet viability and function was reviewed by Riopel and Wang and they concluded that embedding islets within a mixture of native ECM components that are commonly found in the pancreas is required to preserve islet cell survival and function [12]. In a separate strategy, synthetic peptide sequences have been identified and synthesized to imitate the effect of ECM proteins in stimulating the integrin receptors. In particular, Arginine-glycine-aspartic acid (RGD) peptide has been shown to reduce islet cell the apoptosis [33].

Our research group has developed a biocompatible 3D scaffold to improve islet cell viability and function *in vitro* and *in vivo* [40]. To keep the model as simple as possible, our matrix is composed of collagen type I and glycosaminoglycan. We then populated this scaffold with fibroblasts. While collagen provides a favourable initial ECM for islets, fibroblasts support and maintain the matrix integrity, produce other essential ECM components (e.g. Collagen IV, fibronectins, and laminins), and additionally support islets by producing various growth and angiogenic factors [41-44]. We found that a collagen gel that is seeded with dermal fibroblasts (known as Fibroblast Populated Collagen Matrix, FPCM) can function as a very favorable extracellular matrix for islet cells, resulting in significant preservation and improvement of islet cell viability and function [40,45]. Further, we showed that embedding islet within a FPCM significantly reduced the islet mass required for induction and maintenance of euglycemia in diabetic mice [40]. In another study, we demonstrated that FPCM enhanced survival and function of cultured human islets and reduced islet amyloid formation. Islet amyloid is formed by aggregation of islet amyloid polypeptide, which is secreted by islet PP cells. Islet amyloid plaques, which are a common pathological finding in type 2 diabetes, contribute to progressive β cell dysfunction and death [46]. There is a growing body of evidence showing that toxic amyloid aggregates also form in cultured and transplanted human islets [47,48]. Consequently, with a similar mechanism seen in type 2 diabetes, amyloid formation results in accelerated β cell dysfunction and apoptosis both in cultured and transplanted human islets, leading to early islet graft failure [46,49,50]. Our study showed that when human islets are embedded within a FPCM not only viability and function of islets were well preserved, islet amyloid formation and apoptosis was significantly reduced [51]. These studies collectively offer a new strategy to improve the viability of

human islets, which can potentially improve the outcome of clinical islet transplantation.

Conclusion

ECM deprivation is a major cause of progressive islet cell dysfunction following islet isolation process, which consequently results in premature islet graft failure. Collagen based 3D matrices have been proven to significantly improve islet cell viability and function. Fibroblasts are the key role players in production and maintenance of ECM. They synthesize ECM elements including collagens, fibronectins, and other matrix components. As such, fibroblasts can provide an indispensable support for islets and maintain the integrity of the collagen-based matrices.

In summary, the body of evidence presented here strongly suggest that embedding islets within a collagen I matrix, particularly when populated with fibroblasts, significantly promotes islet cell viability and functionality, enhances engraftment of islet grafts, decreases the critical islet mass needed to treat diabetes in a murine islet transplantation model, and finally can prevent human islet amyloid deposition. These promising findings offer a valuable novel approach to reducing the number of islet donors per recipient and improving islet transplant outcome.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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