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Extracellular Matrix in Pancreatic Islets: Relevance to Scaffold Design and Transplantation

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Abstract

Intrahepatic islet transplantation provides a potentially more benign alternative to pancreatic transplantation. However, islet transplants are associated with limited engraftment potential. This inefficiency is likely at least partially attributable to the isolation process, which removes islets from their native environment. Isolation not only disrupts the internal vascularization and innervation of islets, but also fundamentally changes interactions between islet cells and macromolecules of the extracellular matrix (ECM). Signaling interactions between islet cells and ECM are known to regulate multiple aspects of islet physiology, including survival, proliferation, and insulin secretion. Although it is highly likely that disruptions to these interactions during isolation significantly affect transplant outcomes, the true implications of these conditions are not well understood. The following article reviews current understandings and uncertainties in islet–ECM interactions and explains their potential impact on posttransplant engraftment. Topics covered include matrix and receptor compositions in native islets, effects of isolation and culture on islet–ECM interactions, and potential for postisolation restoration of islet–ECM interactions. Greater understanding in these areas may help to reduce isolation and transplantation stresses and improve islet engraftment.

Keywords

Islet transplantation; β -Cells; Extracellular matrix; Basement membrane; Integrins

INTRODUCTION

Intrahepatic islet transplantation provides an alternative to pancreatic transplantation for amelioration of the diabetic state (94). However, islet transplants are associated with significantly lower engraftment efficiencies than whole pancreas transplants. The processes of isolation and culture disturb islet extracellular tissue and disrupt internal vascularization and innervation. While the true implications of these conditions are not well understood, it is likely that they significantly affect transplant outcomes. The following text provides an overview of

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interactions between islets and their surrounding extracellular tissue, and explains how greater understanding in this area may improve transplant outcomes.

NATIVE ISLET ANATOMY

The islets of Langerhans are small, discrete clusters of endocrine cells dispersed among a continuous medium of exocrine tissue. There are approximately 1 million islets in an adult human pancreas, with most measuring 50–200 μm in diameter and consisting of several thousand individual cells (22). The main secretory endocrine cells of islets are insulin-producing β -cells, glucagon-producing α -cells, and somatostatin-producing δ -cells, with lesser proportions of PP-cells that produce pancreatic polypeptide (20,63). Minority populations of ghrelin cells that produce the hormone ghrelin have also been identified in islets (112).

In human islets, α -cells, β -cells, and δ -cells are typically interspersed (16,20). Other types of mammalian islets used in research contain the same basic types of cells, but the compositional and cytoarchitectural relationships are often quite different. For example, mouse islets contain a higher β -cell/ α -cell ratio (16,20) and, similar to islets of other rodents, are typically organized such that β -cells form the inner core of the islet, with α -cells and δ -cells on the periphery (85). Regardless of species, it is important to note that there can be significant variation in the populations of cell types within islets of different regions of the pancreas (16,20,68). For example, it has been shown in rats that islets from the dorsal pancreas contain many α -cells and few PP-cells, while those of the ventral pancreas contain many PP-cells and very few α -cells (5).

Each individual islet is richly vascularized by direct arteriole blood flow. Arterioles enter islets and form fenestrated networks similar to renal glomeruli that promote bidirectional exchange between endocrine cells and blood (12,19). Although islets represent approximately 1–2% of pancreatic volume, they have been shown in experimental animals to receive as much as 15–20% of the blood flow (65,66). In addition to their extensive vascularization, islets are richly innervated by sympathetic and parasympathetic nerves that regulate hormone secretion and may also have trophic functions (1,22,89).

EXTRACELLULAR MATRIX

In natural tissue, cells carry out the majority of their life processes on a network of proteins and polysaccharides known as the extracellular matrix (ECM). Although the composition and organization of ECM varies widely among tissues in the body, its most basic functions are to provide support for cellular tissues and physical sites for cellular attachment. In addition to its structural functions, ECM is responsible for transmitting a wealth of chemical and mechanical signals that mediate key aspects of cellular physiology such as adhesion, migration, proliferation, differentiation, and death (69,79).

The relationship between cells and ECM in living tissue is complex and interdependent. In living tissue, cells synthesize and deposit the macromolecules that provide the framework on which the tissue grows and remodels. This macromolecular framework, in turn, presents numerous signals to the cells that it supports. These signals influence many of the cell activities that ultimately determine tissue structure and function, including macromolecular synthesis and degradation.

Many such extracellular signals are encoded in ECM peptides and polysaccharides that interact directly with cell membrane receptors. The most widely characterized peptide signaling sequence is arginine-glycine-aspartic acid (RGD), which promotes cell–ECM adhesion in nearly all mammalian cells (88). Binding to RGD and many other types of cell–ECM interactions are mediated by heterodimeric transmembrane protein receptors known as

integrins. Integrins and other types of membrane-bound receptors play critical roles in cellular physiology because they transduce external signals to the cytoskeleton and initiate signaling cascades that ultimately affect genetic expression (41,51). This mode of signaling regulates multiple aspects of cell function, including adhesion, migration, proliferation, differentiation, and survival (62,69). For example, receptor-mediated adhesion is necessary to prevent anoikis, a type of apoptosis that occurs in unadhered cells (33,72,102). In addition to receptor-mediated interactions, ECM molecules also participate in less specific surface attachment to cells, where cationic heparin binding domains on adhesion proteins attach to negatively charged cell surface proteoglycans (70).

Another way in which ECM modulates cellular behavior is in the binding and storage of growth factors, cytokines, and other soluble signaling molecules (32,91). This selective binding to ECM regulates the activity of these signaling molecules by protecting them from degradation, facilitating their interactions with cellular receptors, and influencing their synthesis (31). In turn, the bound signaling molecules may provide signals to cells that regulate the expression of ECM molecules (45), cell surface receptors (44), or matrix degrading enzymes and their inhibitors (28). In this way, ECM is capable of modulating the biological activities of many key regulatory molecules and ultimately plays important roles in the growth, repair, and remodeling of tissues. Not all cell–ECM interactions are strictly biochemical in nature, however, as biomechanical interactions also contribute to signaling pathways that are critical to cell development and differentiation. For example, mechanical signals transmitted by ECM are believed to play important roles in tissue development and remodeling by regulating aspects such as cell migration (26) and tendencies towards proliferative or differentiated behavior (46).

ISLET-ECM INTERACTIONS

Although the architecture and physical interactions within and surrounding islets are complex and not completely understood, there is significant evidence that islets, like other tissues, are also heavily influenced by cell–ECM interactions. In mature, intact islets, interactions with ECM or synthetic matrix materials have been shown to regulate survival (7,67,77,78,90,102, 111,113), insulin secretion (7,67,76,78,111,113), and proliferation (7), and aid in the preservation and restoration of spherical islet morphology (67,78). Similarly, ECM-based materials have been shown to regulate survival (36,93), proliferation (39,86), and insulin secretion (14,37,54,87) in purified β -cell cultures. Perhaps most convincingly, incompletely isolated islets that retain some of their native ECM have been shown to have markedly reduced apoptosis rates (103) and significantly greater function with respect to insulin response in vitro than completely isolated and purified islets (2,103).

Matrix interactions also have been shown to be influential in cultured fetal islet tissue. They have been shown to regulate β -cell differentiation (8,24,25,47,48,82), proliferation (6,8), and insulin secretion (53,54,82), as well as migration that is specific for islet development (24, 52,53). Additionally, matrix interactions have also been implicated in the differentiation and development of islet-like tissue from sources such as adult pancreatic ductal tissue (13) and hepatic oval cells (64).

One especially interesting development among recent work is the role of β -cell–ECM interactions in the activation of NF- κ B signaling (37) and the subsequent production of cytokines (92). In this case, moderate NF- κ B activation due to β -cell–ECM interactions was shown to be essential for proper glucose-stimulated insulin secretion, but did not affect cell survival. In other work, integrin-based interactions with ECM were shown to modulate β -cell expression of fibroblast growth factor receptor-1 (FGFR1), a receptor that controls pathways

involved in β -cell survival, function, and insulin expression, and mediates the role of β -cells in vascular endothelial cell remodeling (56).

Despite the abundance of evidence supporting hypotheses on the importance of receptor–matrix interactions in islets, the current knowledge base leaves many unanswered questions regarding the specific details and implications of these interactions. In vitro ECM binding studies such as these are complicated by many factors, including fundamental difficulties in distinguishing between effects of specific binding events and those related to two- or three-dimensional culture. In addition, it is often difficult to attribute outcomes to specific matrix components because pure ECM preparations are difficult to obtain. Many of the studies cited above are based on binding to ECM preparations that contain multiple types of ECM macromolecules. Furthermore, these preparations also may bind or contain growth factors that complicate results.

Studies of islet–ECM interactions are also obscured by many factors at the cellular level. By architecture alone, islets are complex tissues, with multiple, interspersed cell types that contain a variety of receptors capable of interacting with numerous ECM components. Each receptor may recognize multiple ligands and each matrix protein may contain multiple cell binding domains, with each ligand–receptor binding combination potentially producing different cellular outcomes. The receptor and matrix compositions of islets also appear to vary significantly between developing and mature tissues (49), as well as between species (106, 107, 110). In addition, there are numerous nonendocrine cells in close proximity to islets that can complicate results. Ris and colleagues contend that even the purest islet preparations can contain nonislet passenger cells such as fibroblasts that rapidly proliferate in culture (93). Furthermore, receptor compositions of cultured islet cells are not necessarily stable, as integrin expression has been observed to decrease in suspension culture (110), but can be upregulated in the presence of certain matrix elements (55). Collectively, these factors complicate studies of islet–matrix interactions, and potentially lead to inconsistencies. Careful analysis of experimental conditions across multiple studies should ultimately provide a more complete understanding and aid in the standardization of pretransplant culture procedures.

ISLET ECM COMPOSITION

Adult human islets are usually surrounded by an incomplete capsule consisting of a single layer of fibroblasts and the collagen fibers that they produce. This capsule is closely associated with additional matrix proteins known as the periinsular basement membrane (BM) (107, 110). BM is a specific type of ECM associated with epithelium and endothelium, and consists of distinct layers of laminin (LM) and nonfibrillar collagen (Coll) linked by specific interactions with nidogen/entactin (47). The peripheral ECM of mature human islets has most often been reported to be composed of LM (73, 74, 87, 106, 108, 110) and Coll-IV (73, 74, 106, 110), although fibronectin (FN) (73, 74, 87) and Coll-I (73, 74, 107), Coll-III (73, 74, 107), Coll-V (107), and Coll-VI (74) have also been detected. Hughes et al. recently performed a quantitative evaluation of the peripheral matrix composition of human islets and reported that the prevalence of Coll-VI was significantly greater than that of Coll-I or Coll-IV (42).

The distribution of matrix proteins appears to be different in developing human islet tissue, where vitronectin (VN), FN, and Coll-IV have been identified in early precursor tissue emerging from pancreatic ducts, and Coll-IV and LM have been identified in developing islets, with very little, if any, FN and VN (24). The prospect that VN is only found in fetal islet tissue is supported by data indicating that VN receptor expression as well as motility on VN substrates are significantly greater in fetal β -cells than their adult counterparts (24, 52). These findings appear to be consistent with studies of other types of BM, where it is commonly found that VN is present during development, but absent in mature tissues (35).

The composition, thickness, and continuity of ECM on the islet periphery vary moderately across species. For example, canine islets generally have more substantial matrix coverage than rat and human islets, whereas porcine islets have minimal coverage and cell–cell interactions predominate at the exo/endocrine interface (106). Additional variation in capsule thickness may occur with age (75). Regardless of composition, thickness, and species of origin, however, the peripheral ECM of islets is almost completely lost to the enzymatic and mechanical stresses of isolation (110). It is usually replaced by a new, but not necessarily identical, layer of matrix proteins after several days of culture (110).

In the interior of islets, there is a substantial amount of BM associated with the pervading microvasculature (53,106,110). Studies of mouse islets indicate that islet endocrine cells lack their own BM and interact directly with the vascular endothelial BM. This perivascular BM is also composed primarily of LM and Coll-IV (53,106,110), and is believed to be in close or direct contact with nearly all β -cells (11). In contrast to this single-layer arrangement, a recent study of human islets provides evidence for two distinct BM layers surrounding the islet microvasculature (108). In this case, a separate peri-islet BM layer surrounds the perivascular BM. The periislet BM is in direct contact with the endocrine tissue and contains different LM isoforms than the perivascular BM. Despite the abundance of Coll-IV in all forms of intraislet BM, receptor compositions of β -cells suggest that the majority of their binding interactions are with LM rather than Coll-IV (54). Interestingly, β -cells cultured on purified Coll-IV have shown greatly diminished insulin production and content (54).

In addition to direct, adhesive interactions with β -cells, perivascular BM influences islet health by maintaining the islet vasculature and providing the matrix to which endothelial cells attach and migrate. Intraislet endothelial cells and their associated matrix are known to play important roles in posttransplant islet revascularization, survival, and function (17,80). Perivascular BM also binds and releases vascular growth factors, which appear to be necessary for revascularization and the maintenance of islet-specific phenotypes (18,38,56,61,98). Despite its internal location, perivascular BM is likely to be affected by proteolysis during isolation as well as the disruption of the vascular supply and subsequent revascularization. Fundamental differences in the microvasculature of transplanted islets observed long after implantation (3, 21,71) suggest that it is unlikely that the perivascular BM is ever restored to its preisolation state.

Ultimately, the apparent lack of nonvascular ECM within mouse islets may indicate the relative importance of interendocrine cell interactions. Islet cells are coupled by gap junctions as well as cell adhesion molecules (CAMs) such as integrins, neural cell adhesion molecule (N-CAM), and E-cadherin (15,23,84,96). These intercellular connections are believed to be especially important in the transduction of signals related to processes such as islet development, glucose sensing, and insulin secretion. For example, expression of N-CAM and E-cadherin is heterogeneous within islets and is strongly correlated with several key aspects of islet cell functionality, including secretory capability (15,23). Recent work also indicates that Fas receptors on β -cells play an important role in secretory function in addition to their roles in apoptosis and proliferation (97).

ISLET INTEGRIN COMPOSITION

The integrin receptor composition of human islets is both complex and controversial (Table 1). In one of the most complete studies to date, Wang et al. report that cells in adult human islets were positive for $\alpha 3$, $\alpha 5$, αv , and $\beta 1$ integrin components, and negative for $\alpha 1$, $\alpha 2$, $\alpha 6$, and $\beta 2$ (110). Wang et al. also report the colocalization of $\alpha 3$, $\alpha 5$, $\alpha 6$, and $\beta 1$ integrin components with endocrine cell markers in fetal pancreatic tissue (109). These results for adult islet tissue are supported by the findings of Virtanen et al., which show that intraislet endocrine cells

express $\alpha 3$, αv , $\beta 1$, and $\beta 5$ integrin subunits, as well as the LM receptors α -dystroglycan and Lu glycoprotein (108). Neither $\alpha 2$ nor $\beta 3$ subunits were detected in this study, while $\alpha 6$ subunits were found exclusively on intra-islet vascular endothelial cells. In another recent study, Ris et al. confirm the presence of $\alpha 3$, $\alpha 5$, αv , and $\beta 1$ integrin subunits in adult human islet cells as well as purified β -cells, but also report detection of $\alpha 6$ (although its expression was significantly decreased in purified β -cells) (93). In addition, $\beta 4$ were detected in islet cells, but not purified β -cells (93). Studies by Kaido et al. add several more possibilities, as they report that purified human β -cells contain $\alpha v \beta 1$ (52), $\alpha v \beta 5$ (52), and $\alpha 1 \beta 1$ (53) integrins throughout development and adulthood, although $\alpha v \beta 1$ integrins are significantly downregulated upon maturation. These studies suggest that fetal β -cells depend heavily on $\alpha 1 \beta 1$ /Coll-IV interactions for motility during development so they can form islets with correct architecture (53), and that $\alpha v \beta 5$ is responsible for adhesion and may be associated with the maintenance of their differentiated form (52). In yet another study, Cirulli and colleagues report that developing human islet tissue is positive for $\alpha v \beta 5$, $\alpha v \beta 3$, and $\beta 1$ integrins, and negative for $\alpha 5 \beta 1$, and that the $\alpha v \beta 3$ and $\alpha v \beta 5$ integrins are downregulated after early stages of development (24). These results suggest that $\alpha v \beta 3$ and $\alpha v \beta 5$ are important for the emergence of islet cell progenitors from ductal epithelium, and that $\beta 1$ integrins are important for creating and maintaining islet architecture.

Collectively, these studies suggest that mature human islet cells express $\alpha 3$, $\alpha 5$, αv , $\beta 1$, $\beta 3$, and $\beta 5$ integrin components, with possibilities for $\alpha 1$ and $\alpha 6$, although their presence is debated. Regardless of specific make-up, it appears that many different integrins are expressed in human islets, and that their compositions are developmentally regulated. Although the matrix binding properties of these integrin components have been studied extensively in many nonislet cell types, it is nevertheless difficult to speculate about the specific consequences of binding in islets because certain integrins are known to show marked affinity differences across cell types (30).

In addition to these uncertainties, further questions arise about the relationship between human islet integrin compositions and those of research animals. For example, a recent study reports that adult mouse islets express $\alpha 3$, $\beta 1$, and $\beta 4$ integrin subunits, but do not contain $\alpha 6$ (49). Studies in phylogenetically related species reveal more differences, as purified rat β -cells have been shown to express $\alpha 6 \beta 1$ (14) and $\alpha 3 \beta 1$ (14,55) without $\beta 4$ subunits (87), while hamster islets have been shown to express $\alpha 3$ and $\alpha 5$, but not $\alpha 2$ and $\beta 1$, integrin subunits, and begin to express αv after several days culture (110). Differences in receptor and matrix compositions may ultimately explain species specific variation in the survival and performance of cultured and transplanted islets. For example, Ris et al. report that dissociated cultures of human islet cells are not as hardy as dissociated rodent islet cells (93). Purified rat β -cells also show significantly greater potential for proliferation in vitro than primary human β -cells and have been observed to increase their rate of proliferation when cultured on ECM preparations (86). If anything, existing receptor composition data suggest that compositions of rodent islets are less complicated than that of human islets.

PROPERTIES OF ISLET MATRIX ELEMENTS

Fibronectins and Other RGD-Containing Components

Fibronectins (FNs) are high molecular weight glycoproteins that are common elements of ECM (43). Interactions between FN and integrins are among the most well-characterized cell-matrix interactions. FN binds a variety of integrins through recognition of the RGD motif (59). RGD is presented in FN as an extended, flexible loop between two β -strands (43). PHSRN, a sequence that binds synergistically with RGD, is located on an adjacent region of FN and is close enough to be recognized by the same integrin (4). Numerous other adhesive matrix elements also express active RGD motifs, and among those possibly relevant to islets are VN and nidogen/entactin, a smaller BM glycoprotein that interacts with LM and Coll-IV (59). Of

the integrins listed above that are potentially found in adult human islets, most bind RGD. All α_v integrins recognize RGD (52), as well as $\alpha_5\beta_1$ (59) and $\alpha_3\beta_1$ (95), although $\alpha_3\beta_1$ -RGD interactions are weak. FN also contains additional adhesive sequences such as LDV, IDAPS, and REDV, although these sequences do not appear relevant because they are recognized by integrins that are generally not associated with islets (59).

Despite the apparent abundance of RGD binding integrins within islets, there is still significant uncertainty surrounding the interactions of islets with RGD and FN. In developing pancreatic tissue, α_3 and α_5 integrins are reported to be colocalized with FN (109). RGD-dependent adhesion has been demonstrated in mature canine islets (111) and has been shown to inhibit apoptosis in mature human islets (90). Addition of soluble FN to islet suspension cultures has also been implicated in islet survival and preservation of integrin expression, and has been shown to produce insulin stimulation indices that are more than twice those of islets in unsupplemented suspension cultures or attached to collagen-coated dishes (111). In contrast to this, dissociated porcine islet cells have been shown to exhibit strong adhesion and spreading on FN-coated substrates, but not improved function (27). Other studies with purified human β -cells have demonstrated adherence to FN (24,53,93), but also report lack of spreading and survival in adult cells (93), as well as lack of migration in fetal and adult cells (53). Unfavorable results have also been reported for β -cells cultured on VN, where adhesion was shown to reduce insulin content in fetal cells and diminish insulin gene transcription in both fetal and adult cells (54).

One of the most interesting results pertaining to RGD was borne out in vivo, where treatment of human fetal islet precursor tissue with a competitive RGD blocking peptide significantly reduced the number of insulin-positive cells after transplantation into nude mice (24). Accordingly, RGD is apparently critical to cell migration in early islet development, and data from multiple studies suggest that this interaction may be mediated via RGD sequences contained in FN and/or VN (24,52). In contrast, non-RGD-based interactions with BM molecules such as LM may be implicated in creating and maintaining islet architecture (24).

Ultimately, it appears that most or all islet cell types are capable of recognizing RGD, although it remains to be seen whether RGD is a prominent ligand in the native adult pancreas and whether its interactions with islet cells have desirable outcomes. While existing data suggest RGD has important implications in the developing pancreas, its contributions in adult islets and implications in culture and transplantation are less clear.

Collagens

Collagens are structural ECM proteins that typically form triple-helical domains (40). This tendency is attributed to the gly-x-x structure of collagens in which glycine frequently repeats as every third amino acid residue (30). Among the collagens potentially found in islets, Coll-I, Coll-III, and Coll-V form fibrillar structures, Coll-IV forms networks, and Coll-VI forms beaded filaments (40). Further discussion will focus on Coll-IV, because it is the collagenous component most frequently associated with islets. Unlike fibrillar collagens that form long fibers in connective tissue, Coll-IV assembles into the planar hexagonal networks that comprise BM. These “chicken wire”-like structures form from antiparallel tetramers of triple-helical Coll-IV subunits that are linked to other tetramers through interactions at N-terminus extensions (30).

The main integrin that binds Coll-IV is $\alpha_1\beta_1$, although $\alpha_3\beta_1$ (109) and $\alpha_2\beta_1$ (30) have also been shown to bind. While the presence of α_3 integrins in adult human islets is well documented (93,109,110), only one study cites the presence of $\alpha_1\beta_1$ integrins in human β -cells (53) and another reports that islets do not contain either α_1 or α_2 (110). The $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins have been shown to bind to the GFOGER and GAOGER sequences in Coll-IV (30). Most cell

binding sequences in collagens are of the basic pattern GXXGER (30), although other active sequences have been identified in Coll-IV, such as TAGSCLRKFSTM (104), which has been shown to bind endothelial cells. In addition, Coll-IV can interact indirectly with cells by binding other matrix elements such as heparan sulfate proteoglycans via direct interaction, and LM via the intermediary glycoprotein nidogen/entactin (83). Although most collagen types contain several RGD domains, they are generally considered inactive in native conformations because the sequences are partially concealed by helicity and are presented at significantly lower curvatures than the preferred “flexible loop” conformation (30). These collagen RGD domains may become accessible upon denaturation or enzymatic digestion, which may explain why other receptors such as $\alpha 5\beta 1$ and $\alpha v\beta 3$ have been reported to bind collagen.

Although Coll-IV is prevalent in the peripheral ECM and perivascular BM of islets, its role in islet physiology remains less than clear. While Coll-IV has been shown to be significantly more effective in promoting survival in intact islets than Coll-I (90), it has also been shown to decrease insulin production and secretion in purified β -cells (54). Ultimately, the lack of a clear Coll-IV binding integrin pathway in islets may indicate that islet cells and Coll IV have limited interactions despite their close proximity.

Laminins

Laminins are cross-shaped heterotrimeric glycoproteins composed of three polypeptide chains joined by disulfide bonds (105). To date, at least 12 different LM isoforms have been identified (29). The specific expression and distribution of LM isoforms in islets is not well understood and many studies identify the peripheral and perivascular matrix component as “laminin” (73,74,106,110). Recent studies by Jiang et al. indicate that LM-111 (formerly called LM-1) is the primary isoform present in the developing mouse pancreas (47) and that it is completely replaced by LM-511 (formerly called LM-10) at maturity (49). In other recent work, Parnaud et al. report that LM-332 (formerly called LM-5) is present in rat and human islets and is mainly associated with α -cells (87). Virtanen et al. report various isoforms of LM-411/421 (formerly LM-8/9) and LM-511/521 (LM-10/11) among the double BM layer of human islets (108).

Multiple cell membrane receptors adhere to LM molecules, including integrins containing $\alpha 6$, $\alpha 3$, $\beta 1$, and $\beta 4$ subunits (9,60). Not surprisingly, these subunits represent a majority of the integrins that were mentioned above as possibly being expressed on islets. Various nonintegrin receptors have also been shown to bind LM, so interactions with islets may not necessarily be integrin based (60). Recent studies report that LM is colocalized with $\alpha 6$ integrins in the developing pancreas (109) and has been shown promote in vitro islet survival (90).

Although multiple integrin binding regions have been identified in LM, most have only been narrowed to lengthy fragments of the molecule (60). Among the shorter integrin and nonintegrin binding fragments that have been identified, many are located on the $\beta 1$ chain that is common to multiple LM isoforms, including LM-111 and LM-511. Among these sequences are YIGSR (34), PDSGR (57), RYVVLPR (57), and LGTIPG (34). Several other adhesive sequences are present in the $\alpha 1$ chain of LM-111, including IKVAV (58), VAYI (81), and IKLLI (101). Little is known about the interactions of any of these ligands with islet cells, however.

In addition, many LM isoforms contain RGD sequences. Although the activity of these regions has been debated, they are generally considered to be inaccessible and nonreactive in native LM conformations (60). Similar to those in collagens, these “cryptic” RGD sequences may have implications in tissue repair or remodeling because they are exposed only after degradation or denaturation.

SIGNIFICANCE OF ISLET–ECM INTERACTIONS IN TRANSPLANTS

Donor islets face a perilous journey from organ procurement to final engraftment. During the multihour isolation process, islets are exposed to mechanical stresses, hyperosmolarity, and tissue-digesting enzymes, only to be transplanted to a foreign environment where they are exposed to hypoxia, immune attack, and systemic toxins, including those stemming from immunosuppressive therapy. Transplanted islets must adapt to their new surroundings without the internal vascularization and innervation that they had in the pancreas, as well as most or all of their native peripheral ECM. At the current time, the implications of isolation-related ECM damage and subsequent implantation in a foreign extracellular environment are not well understood. Considerations to address this problem will be discussed below.

Although multiple *in vitro* studies indicate that matrix contact restoration can be advantageous for vitality and function in isolated islets (7,67,76,77,111), the mechanisms of action remain unclear. A large portion of β -cells are on the interior of islets and do not have direct contact with the peripheral ECM, so any benefits they derive from matrix restoration must be transmitted indirectly. For example, intercellular junctions may transmit signals from peripheral cells attached to the ECM, or the ECM may bind and release growth factors that later diffuse to the islet interior. Attachment of peripheral islet cells to ECM may also benefit β -cells by maintaining islet architecture and preserving specific intercellular relationships.

The importance of peri-insular BM–islet interactions raises several potentially important considerations in transplant procedures. In terms of transplant site selection, it may be beneficial to select sites that offer tissues similar in matrix composition to the pancreas. Islets may also benefit from more sophisticated means of determining isolation endpoints to avoid damage to their cells and ECM. In addition, it may be possible to provide islets with surrogate ECM materials that furnish the same chemical signals that preserve differentiated function and protect against cell death in native tissue. Proteins (7,67,76,77,111), synthetic polymers (50), and even self-assembled materials (78) that express relevant ligands (100) or deliver matrix binding growth factors (99) are among materials that may be appropriate for this purpose. These materials could be delivered during transplantation in the form of degradable scaffolds, or during pretransplant culture in the form of solid or soluble substrates.

Clearly, scaffold materials that present some of the same ligands as the ECM of native islets are most likely to succeed. Matrix components must be carefully selected, however, as some islet–ECM binding interactions have been shown to yield outcomes that appear to be undesirable for transplantation (6,8,27,54). Short peptides potentially offer greater purity and less chance of immunogenicity than intact matrix proteins, although selection of ligands is a difficult issue. While it is likely that RGD is not the principle signaling ligand in mature islets, less is known about the interactions that islets may have with the short adhesive sequences in Coll-IV and LM. Ultimately, it is quite likely that optimal islet ECM replacements will incorporate multiple matrix-based signals, including growth factors or other soluble entities that are bound and released in a controlled fashion.

The potential significance of interactions between β -cells and the BM surrounding the internal vasculature must not be overlooked, either. Because nearly all β -cells in native islets are believed to be in contact with this BM, these interactions may be most critical in the preservation of functional islet mass. While isolation and transplantation do not completely upset this relationship, the disruptions that they cause may significantly affect islet engraftment and performance. Given the internal nature of these β -cell–BM interactions, it is clear that attempting postisolation restoration with scaffolds poses significant challenges. Furthermore, dissociating islets for the purpose of restoring these interactions would likely have costs that far outweigh any possible gains. In this case, soluble ECM fragments and self- assembling

peptide-based materials (10,99) raise interesting possibilities for materials that could infiltrate islet cores and provide temporary perivascular BM surrogates. In addition, external scaffolds that store and release vascular growth factors normally stored in the perivascular BM may be especially beneficial (99).

In the large scheme, islet–BM interactions likely represent only a few of many factors that influence engraftment. While other aspects such as transplant site vascularity are of unquestionable importance, the true implications of islet–BM interactions are simply not well understood. Given the architecture of islets, it is quite likely that many signals relevant to engraftment are also transmitted by soluble factors and cell–cell interactions. While the relative roles of soluble factors, cell–cell interactions, and cell–ECM interactions in intact islets remain to be determined, it is clear that dissociated cells such as purified β -cells, islet progenitor cells, or stem cells present a different set of circumstances. In these cases, engraftment would likely suffer if the individual cells were not localized and provided with extracellular sites for attachment prior to implantation. Indeed, these types of cell transplants may illustrate the true utility of scaffolds in diabetes management, as interactions with surrogate ECM materials may help to maintain viability and differentiated function, and aid in the formation of islet-like clusters. In these situations, intrahepatic transplantation may not be a viable option because tissue volumes may be prohibitively large and the small, dissociated cells may be difficult to localize in the liver. Moreover, scaffolds may be especially important in transplants of stem cells and progenitor cells, as many studies indicate that ECM serves as a key determinant in islet differentiation through presentation of matrix-bound signals and regulated delivery of soluble factors (8,24,25,47,48,82).

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Table 1

Summary of Integrin Subunit Expression in Human Islets as Reported in the Literature

Expression	Subunit	Source
Highly likely	$\alpha 3$	93,108,110
	$\alpha 5$	93,110
	αv	93,108,110 (24,52: expression downregulated in adulthood)
	$\beta 1$	24,52,53,93,108,110
	$\beta 3$	24 (low expression in adulthood), 108 (shows no expression in mature islets)
	$\beta 5$	24,52 (low expression in adulthood)
Possible or very low level	$\alpha 1$	53 (positive), 110 (negative)
	$\alpha 6$	93 (positive; low expression in β -cells), 108 (vascular endothelial cells only), 110 (negative)
	$\beta 4$	93 (non- β -cells)
Unlikely	$\alpha 2$	108,110
	$\beta 2$	110