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Collagen matrix support of pancreatic islet survival and function

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1. ABSTRACT

Diabetes mellitus is a chronic condition resulting from insufficient β-cell mass, which leads to improper glycemia regulation. Research efforts have focused on expanding islets and β-cells *in vitro* for use in cell-based therapies to cure diabetes. Collagens are triple-helix extracellular matrix proteins with widespread expression in mammals. With multiple functions, collagen can provide structural integrity in addition to mediating cellular signaling. In the pancreas, collagens I and IV are abundant and support cell structures while also stimulating cell surface receptors to influence pancreatic cell processes. Collagen-based materials and scaffolds have also been used to assist in the maintenance and expansion of islet cells *in vitro,* primarily through integrin and discoindin domain receptors. Islet transplantation using collagen-based scaffolds may improve long-term glycemic control, but progressive research efforts are required to realize this potential in humans. This review will outline the critical role played by native collagens I and IV and their receptors in maintaining islet function. The advantages of using collagens I and IV as culture gels/scaffolds and islet encapsulation vehicles for transplantation will be described.

2. INTRODUCTION

The extracellular matrix (ECM) is a complex network of scaffolds and glycoproteins that play a supportive role for cells via integrins and other cell adhesion receptors. Major supportive proteins in the ECM include collagens, laminins, fibronectins, tenascins, and proteoglycans (1). These proteins form a vast network that surrounds cells and influences a variety of processes, all while providing an internal structure to the organ. In the pancreas, islets are surrounded by ECM, which maintains islet cell insulin secretion (2), survival (3), differentiation (4), and morphology/architecture (5). Collagen contains triple-helix domains, is the most abundant type of protein in mammals, and has profound expression in the native pancreas. Of the 28 discovered types, collagens I and IV are unique in their supportive role of pancreatic islets. Major collagens I and IV binding receptors include integrin αβ1, and discoindin domain receptors 1 and 2 (DDR1 and DDR2), which mediate many different intracellular signaling cascades. Collagen I has been demonstrated to improve islet cell survival and maintain short-term insulin expression (2). Over time, however, the development of cystic structures occurs and insulin expression decreases (6). Collagen IV is normally found in the basement

membrane of islets. When cultured with collagen IV *in vitro*, islets demonstrate improved insulin secretion (7). Collagen-based scaffolds have also been developed that improve the supportive environment of islets and graft function during islet transplantation (8, 9). While these methods have shown a reversal of diabetes in multiple diabetic models, collagens remain limited in their ability to provide long-term glucose homeostasis. This review will focus on the use of collagen and collagen-based materials in support of islets.

3. EXTRACELLULAR MATRIX – COLLAGEN I AND IV

3.1. Collagen structure and distribution

Collagen is an essential ECM protein found in mammals. Twenty-eight different collagen types have been identified, of which the five most common are types I, II, III, IV, and V, which provide a variety of essential functions (10). Collagens are defined by their composition of triple-helix domains, yet each collagen type is composed of slightly different arrangements and types of helices (11). Fibrillogenesis begins with the synthesis of soluble procollagens, which is followed by proteinase-facilitated enzymatic modification into proteins that are primed for fibril formation. This is followed by spontaneous fibril self-assembly and further stabilization into collagen (for more detail, see (12, 13)). This process is not fully understood since integrin α2β1, RhoA and fibronectin assembly must be undisturbed in order to generate polymerized collagen I fibrils (14). Each triple-helix is composed of a large proportion of a specific amino acid motif: glycine-X-Y, with proline often occupying the X position. This sequence promotes helical conformation and stability (15, 16). The tertiary structure of collagen is described as a coiled-coil in which individual triple helices twist together to form a left-handed structure (17). Collagen can be very resistant to biodegradation after enhancing cross-linking, which is ideal for cell-based therapies that require long-term ECM support (18). Each collagen type has a complex and varied distribution; for instance, the basement membrane commonly includes collagens IV, VIII, XV and XVIII, while cartilage is composed of collagens II and XI (19).

The fibril-forming collagen, type I, provides vital structural integrity and is the most abundant collagen in mammals. Collagen I is present in connective tissues as well as bone, tendons and ligaments, and is formed by two α1(I) and one α2(I) chains (11). Primarily, collagen I is required for tensile strength of tissues but is also important in wound healing and distinct cell signaling (20). Meanwhile, collagen IV is a major component of the basement membrane and is critical for providing microstructural integrity and cell stimulation (11). Collagen IV is formed by a combination of the chains α1(IV), α2(IV), α3(IV), α4(IV), α5(IV), and α6(IV) depending on the tissue and developmental stage (19). Collagen IV also forms a three-dimensional (3D) structure that attaches to other essential basement membrane components including laminin, fibronectin, nidogen (21), usherin (22) and proteoglycans (23) in order to form a secure fenestrated basement membrane (24, 25). Indeed, mutations in the chains of collagen IV commonly affect the kidney, an organ that requires an intact basement membrane to function (25). In addition to a structural role, both collagens I and IV bind to cell surface receptors to mediate cellular effects.

3.2. Distribution of collagen I and IV in islets

The endocrine pancreas is organized into groups of cells scattered throughout the length of the pancreas termed islets of Langerhans. These cells can regulate blood glucose through hormonal secretions that affect cellular uptake of glucose. In adults, islet cells have low proliferation, but under certain disease states, this can change. A prominent cell type in islets is the β-cell, which secretes insulin that lowers blood glucose. Meanwhile, α-cells secrete glucagon, which increases blood glucose by depleting glycogen stores. Other cells that are far less abundant than both β and α cells are the δ-cells, ε-cells and PP-cells, which secrete somatostatin, ghrelin and pancreatic polypeptide, respectively. The organization of endocrine cells within islets differs between species wherein mice and rabbits tend to have a core β-cell population with α-cells surrounding them and higher order species like humans and primates have β-cells and α-cells closely positioned throughout the islet (26). Humans have significantly less pancreatic β-cells per islet than monkeys, pigs, rabbits and mice; yet, the islet size distribution pattern of these species is quite similar (27). Islets also contain a vast number of endothelial and perivascular cells that provide substantially more blood to the islet cells than to the surrounding exocrine tissue. These and islet cells contribute to the secretion of numerous ECM proteins (28) that are critical for supporting pancreatic tissue architecture and cell function. Among the different ECM proteins, collagens have a varied distribution in the pancreas throughout development and adult life. In order to provide more insight, we have performed microarray analyses of human fetal pancreata at 8 to 21 weeks of age and determined the relative expression of various collagens during this period of development compared to levels in human adult islets (Table 1). Of 20 collagen genes analyzed by microarray, *COLLAGEN I-VI, IX, XII, XV, XVI, XVIII* and *XXI* showed expression levels significantly ($p<0.05$) higher in the fetal pancreata than in adult islets (Table 1). In juvenile and adult porcine islets, collagen IV and VI were intensely stained, while collagen I and V less abundant (29). Also, collagen I, III and IV were located in the porcine islet capsule (30). In the endocrine pancreas, type I, III and V collagens are present surrounding islets and near islet capillaries in various intensities between the pancreas of rat, dog, pig and human (31). Our studies indicate high expression of collagens I and IV in the developing human fetal pancreas and adult islets (Figure 1). Collagens I and IV can often be observed to co-localize with insulin, implying that β-cells produce both collagens I and IV (Figure 2). Collagen IV mainly localizes around the cell clusters in the basement membrane and near capillaries during pancreatic development, while in the adult human islets, collagen IV is present between insulin-positive cells and islet capillaries (Figure 1-2).

Table 1. Classification of collagen genes in the developing human fetal pancreas - microarray analysis[1]

Gene Symbol	Probe ID	Description	8-10 wks	19-21 wks
			n=5	n=6
COL1A1	202310_s_at	proalpha 1 (I) chain of type I	**43.61**	**19.25**
COL1A1	202312_s_at	collagen, type I, alpha 1	1.04	1.10
COL1A2	202404_s_at	collagen, type I, alpha 2	**60.15**	**38.15**
COL2A1	213492_at	pro-alpha 1 (II) collagen	**21.66**	**2.36**
COL2A1	217404_s_at	collagen, type II, alpha 1	**3.92**	1.23
COL3A1	201852_x_at	collagen, type III, alpha 1	**40.91**	**23.79**
COL4A1	211981_at	collagen, type IV, alpha 1	**2.65**	**2.35**
COL4A2	211964_at	collagen, type IV, alpha 2	**5.99**	**5.82**
COL4A3	214641_at	collagen, type IV, alpha 3	0.89	0.88
COL4A4	214602_at	collagen, type IV, alpha 4	0.91	0.91
COL4A5	213110_s_at	collagen, type IV, alpha 5	**17.43**	**7.35**
COL4A6	211473_s_at	collagen, type IV, alpha 6	1.06	0.97
COL5A1	203326_x_at	pro-alpha-1 (V) collagen	0.92	1.03
COL5A2	221730_at	collagen, type V, alpha 2	**34.36**	**9.66**
COL5A3	218975_at	collagen, type V, alpha 3	1.08	1.19
COL6A1	212937_s_at	collagen, type VI, alpha 1	0.92	1.12
COL6A2	209156_s_at	collagen, type VI, alpha 2	**9.69**	**4.19**
COL6A3	201438_at	collagen, type VI, alpha 3	**9.94**	**5.34**
COL7A1	204136_at	collagen, type VII, alpha 1	0.98	0.88
COL8A1	221152_at	collagen, type VIII, alpha 1	0.93	0.92
COL9A1	222008_at	collagen, type IX, alpha 1	1.56	1.22
COL9A2	213622_at	collagen, type IX, alpha 2	1.35	1.15
COL9A3	204724_s_at	collagen, type IX, alpha 3	**12.15**	**2.28**
COL10A1	217428_s_at	collagen, type X, alpha 1	0.98	0.95
COL11A1	204320_at	collagen, type XI, alpha 1	**3.54**	1.17
COL13A1	208535_x_at	collagen, type XIII, alpha 1	0.97	1.00
COL14A1	216865_at	collagen, type XIV, alpha 1	0.97	1.05
COL15A1	203477_at	collagen, type XV, alpha 1	**4.90**	**5.92**
COL16A1	204345_at	collagen, type XVI, alpha 1	**9.79**	**2.97**
COL17A1	204636_at	collagen, type XVII, alpha 1	0.95	0.97
COL18A1	209081_s_at	collagen, type XVIII, alpha 1	**3.72**	**2.57**
COL19A1	211011_at	collagen, type XIX, alpha 1	0.96	0.94
COL21A1	208096_s_at	collagen, type XXI, alpha 1	**20.40**	**18.19**

[1]Human fetal pancreata (8-21 weeks of fetal age) and isolated adult islets (aged 46–68 years) were collected according to protocols approved by the Health Sciences Research Ethics Board at the University of Western Ontario and the Research Ethics Boards of the McGill University Health Centre, in accordance with the Canadian Council on Health Sciences Research Involving Human Subjects guidelines. Fetal samples were grouped into: 8-10 weeks, 14-16 weeks and 19-21 weeks, with at least five replicates per group. Total RNA from human fetal pancreata and isolated adult islets was isolated using TRIZOL reagent (Invitrogen, Burlington, ON). Biotinylated cRNA was generated and hybridized to Affymetrix HG-U133A GeneChips [112]. Note: Collagen XX, XXII-XXVIII genes are not available on the chip. Data are expressed as mean fold changes ± SEM and normalized using the Robust Multichip Analysis to show expression relative to isolated human adult islet data. Significantly ($p<0.05$) up-regulated genes are highlighted in bold.

3.3. Collagen-binding integrin receptors

Integrins are a ubiquitously expressed group of cell and ECM protein adhesion receptors that mediate a variety of cellular effects. There exist 24 combinations of α and β subunits, including collagen-associated α1β1, α2β1, α3β1, α10β1 and α11β1 integrin receptors. Upon activation, these modulate cell proliferation, survival, motility and differentiation (more details reviewed in (32)). Early studies demonstrated a necessity of β1 integrin to mediate chondrocyte interactions with collagen I (33). Collagen I binds to α2β1 integrin to promote cell spreading and projections in Chinese hamster ovary cells (34), as well as osteoblastic differentiation of bone marrow cells (35). Interestingly, α2β1 is the likely receptor partner of collagen I fibrils, whereas α1β1 is limited to binding collagen I monomers (34) through the GFOGER (O = hydroxyproline) (36) or GLOGER (37) amino acid sequences in collagen. Integrin α2β1 is also required for collagen I assembly by vascular smooth muscle cells (14).

Although collagen IV is able to interact with both α1β1 and α2β1 integrins via the cyanogen bromide fragment, CB3, α1β1 was shown to interact with a higher affinity (38, 39). Indeed, human fetal β-cells cultured with collagen IV interact with α1β1 to improve insulin secretion (7), while deletion of α1 integrin leads to defective embryonic fibroblast migration and spreading on collagen IV (40). Furthermore, collagen IV, along with other ECM proteins are closely localized to integrins α3, α5 and α6β1 during human pancreatic development (41). Direct ligand binding assays showed no interactions between α3β1 and collagen IV in melanoma cell lines (42), while others showed increased keratinocyte binding to collagen IV upon α3 integrin ablation (43). These confounding studies suggest that integrin α3β1 may bind collagen IV only under certain conditions or in a tissue/cell specific manner.

3.4. Collagen-integrin interactions and downstream signaling pathway activity

Collagen-binding integrin receptors influence a variety of intracellular signaling pathways (Figure 3).

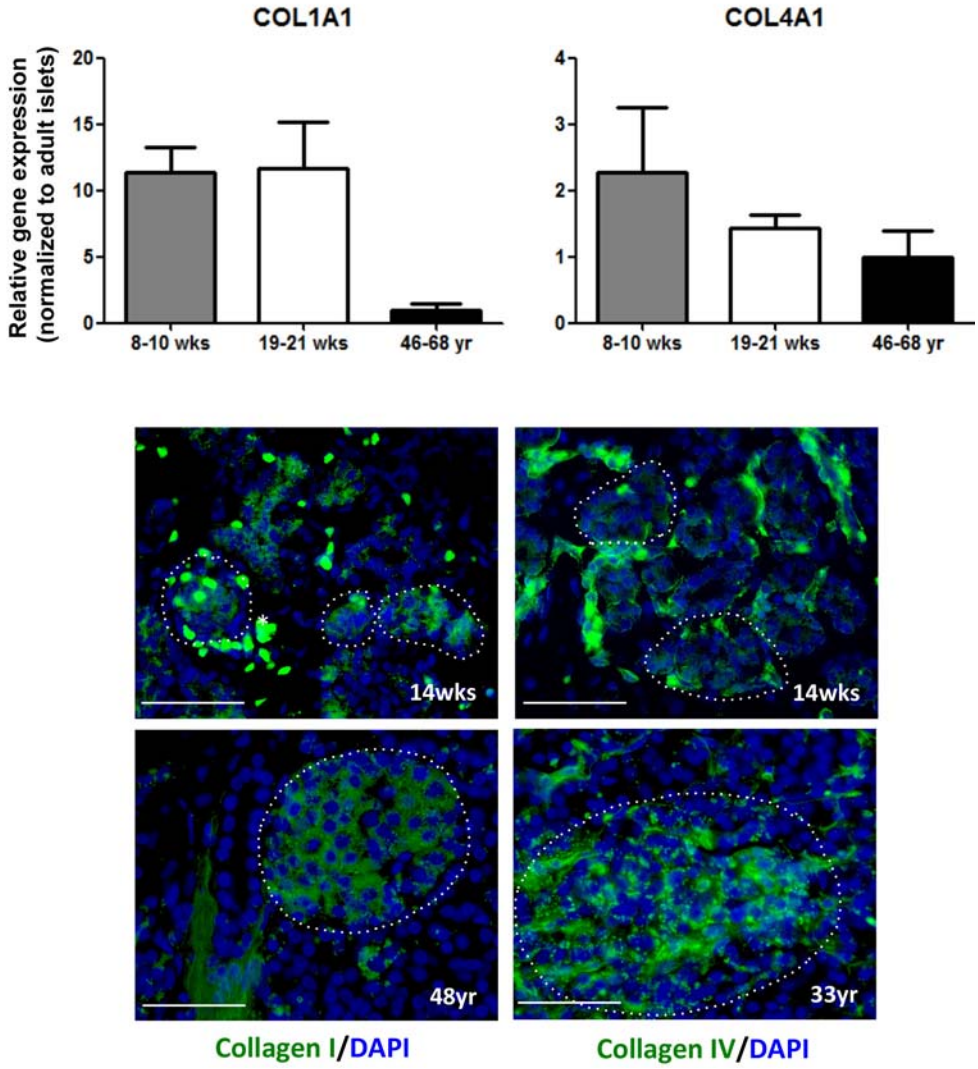

Figure 1. (a) Relative mRNA expression, evaluated by qRT-PCR, for COL1A1 and COL4A1 in human fetal pancreas and adult islets (*n=3*). Data normalized to adult islets, *p<0.05 **p<0.01. (b) Collagen I and IV (green) immunofluorescence staining in human fetal and adult pancreas. Nuclei stained with DAPI (blue). Dashed lines are the outline of islets. Scale bar: 50μm

Integrin α1 null fibroblasts showed a deficiency in adaptor protein Shc and mitogen-activated protein kinase activation (MAPK) when plated on collagen (44). However, the MAPK pathway members including the p38 and extracellular signal-regulated kinases (ERK) were activated in α1 integrin-deficient cells (45-47). Integrin α1β1 is also required for mesangial cell migration on collagen, via ERK1/2 activation (48). Endothelial cells need MAPK/p38 and phosphadityl inositol 3 kinase(PI3K)/AKT activation from integrin α1β1 for migration, tubulogenesis, and ERK-mediated proliferation (49). Integrin α1β1 can also negatively regulate α2β1 integrin in murine uteric bud epithelial cells and affect cord formation, adhesion and migration (50).

In pancreatic islet cells, β1 integrin is a well-established regulator of cell activity. Blocking β1 integrin function in human fetal islet cells decreased adhesion to collagen I, increased apoptosis and reduced expression of a critical β-cell transcription factor, PDX-1 (51). These changes were associated with reduced focal adhesion kinase (FAK) and ERK1/2 activation, which was overcome when cells were made to over-express FAK (51). Similar results were also demonstrated when β-cells were plated on ECM matrix produced by a rat bladder carcinoma cell line; whereby, blockade of β1 integrin led to inhibition of FAK activation and decreased glucose-stimulated insulin secretion (52). Furthermore, mice with conditional β1 integrin deficiency under control of the collagen Iα2 promoter had a significant impairment in islet vascularization and cell proliferation (labeled by Ki67), disrupted cell-cell contacts, as well reduced ECM products and Pdx-1 expression (53). Altered pancreatic islet function and survival under β1 integrin deficiency is associated with a significant reduction of FAK and ERK1/2 activation, but not the PI3K/Akt pathway (53). However, the PI3K/Akt

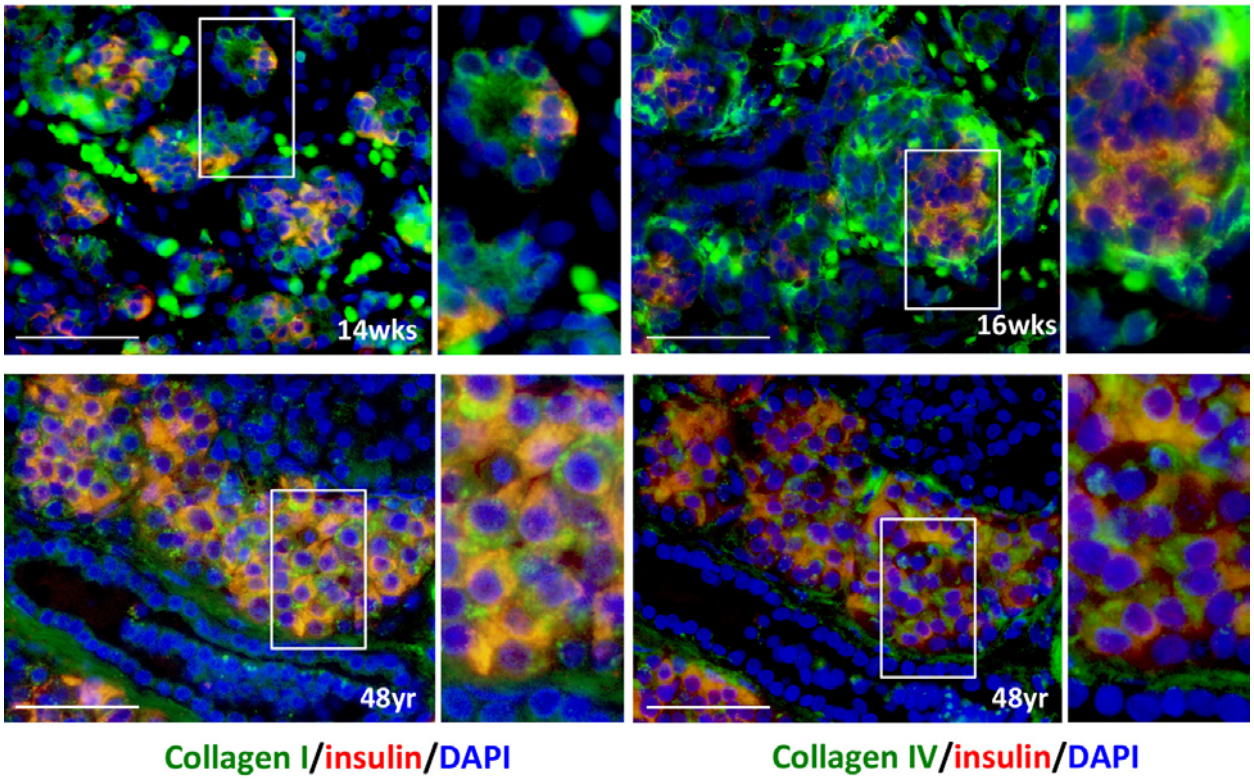

Figure 2. Human fetal and adult pancreas immunofluorescence stain for collagen I and IV (green) with insulin (red). Nuclei stained with DAPI (blue). Amplified (x3) regions are indicated by outline. Scale bar: 50μm

pathway was significantly altered when the rat insulinoma cell line, INS-1, had a3 integrin blocked, and not β1 integrin (54). Collagen - integrin interactions play a key role in maintaining pancreatic islet architecture, proliferation and survival via the MAPK or PI3K pathway. These effects can occur directly by phospho-ERK1/2 activation of the insulin promoter or indirectly by phospho-ERK1/2 phosphorylation of transcription factors Beta2 or Pdx-1, which are essential to preserve islet function (55, 56) Pdx-1 activation/stabilization can also be influenced by MAPK/p38 (57) and PI3K/Akt (58). ERK1/2 has been shown to maintain glucose-stimulated β-cell survival, which is associated with cAMP-responsive element-binding protein levels and activation (59). Akt can also promote β-cell survival (reviewed in (60)), as well as induce β-cell proliferation by inducing cyclin D1, cyclin D2, p21 and cyclin-dependent kinase 4 expression (61).

3.5. Collagen-binding discoidin domain receptors

Besides integrins, discoidin domain receptors (DDR) are a group of receptor tyrosine kinases that bind ECM proteins. Two types exist, DDR1 and DDR2, which are differentially expressed but both activated by fibril-forming collagens (62). DDR2 was shown to transduce signals when bound by collagen I in human embryonic kidney 293 cells, while collagen IV activates DDR1 (62, 63). As well, the hydrophobic pocket of DDR2 binds to collagen via GVMGFO motifs (64). Activation of DDRs has profound effects on cell differentiation, proliferation, survival, adhesion, spreading, migration, invasion, and cell-cell interactions (reviewed extensively in (65)). DDR1 activation is distinct and independent of β1 integrin (66) and requires Src for DDR1-mediated migration of smooth muscle cells (67) (Figure 3). While integrins signal via FAK, DDR1 uses Pyk2 to mediate its effects and also requires Rap1 (68). Rap1 can then affect pancreatic islet cell function, while also promoting proliferation via Ribosomal Protein S6 (69). DDR1 is implicated in cancer metastasis, whereby inhibition of DDR1 impairs tumour invasion of bone (70). Importantly, it has been observed that DDR1 is highly expressed and activated in breast and non-small cell lung cancer (71, 72). In exocrine tissues, DDR1-deficient female mice had abnormal mammary duct branching and were unable to lactate, indicating a key role of DDR1 in breast development (73). DDR1 is expressed in mouse islets (74) and early progenitors, which can be used to identify, sort, and isolate pancreatic endocrine progenitor cells using FACS analysis (75). DDR1-expressing cells were localized to a region of the fetal pancreas known to harbour endocrine progenitors, and also expressed important endocrine cell development transcriptions factors NK6 homeobox 1 (Nkx6.1) and neurogenin-3 (Ngn3) (75). In the developing human pancreas, DDR1 expressing cells also expressed NKX6.1, and had weak colocalization with CD133, a cell surface marker of hematopeotic stem cells and fetal pancreatic ductal epithelial cells (76). DDR1 expression is also significantly increased in malignant human pancreatic endocrine tumours (77).

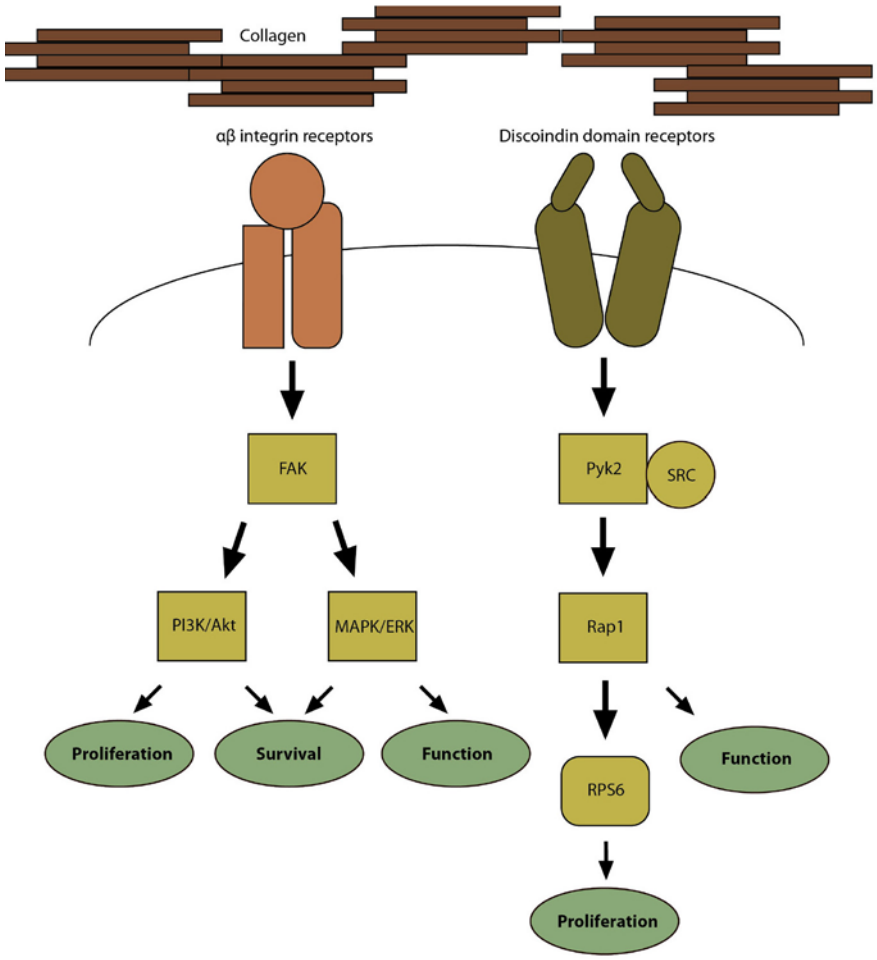

Figure 3. Integrin and discoindin domain receptors bind to collagen and activate downstream signaling pathways. Integrins activate focal adhesion kinase to promote proliferation and survival via the PI3K/Akt pathway, and function and survival via the MAPK/ERK pathway. Discoindin domain receptors phosphorylate Pyk2, which promotes the activation of the small GTPase Rap1. This activity improves β-cell function, and promotes β-cell proliferation via RPS6.

Through these two ECM binding receptor groups, collagens I and IV can influence a variety of cell activities, which suggests that collagen may be important to include in islet cell culture systems.

4. COLLAGEN-BASED ISLET CELL CULTURE

4.1. Collagen I-based culture

In the native rat pancreas, collagen I is present in perivascular islet capillaries, similar to the human pancreas (31). However, the human pancreas also contains collagen I immunoreactivity in and around islets with α3β1 integrin co-localization (Figure 4) (31). Therefore, much research has gone into investigating whether *in vitro* replacement of collagen I could maintain islet differentiation, proliferation, survival and function. In particular, culturing isolated rat islets with a collagen I matrix led to a significant reduction in islet cell death after 5 days (3) and an improvement in glucose-stimulated insulin secretion (GSIS) after culture for 11 days (2). Using the rat insulinoma cell line, INS-1, our research group has found that INS-1 cells exposed to collagens I and IV demonstrated significant increases in adhesion, spreading and cell proliferation when compared to cells cultured on fibronectin, laminin and tissue-culture polystyrene (78). Perturbation of α3β1 integrin interactions with collagen I and IV led to significantly decreased FAK, ERK1/2 and Akt phosphorylation and significantly increased cleaved caspase-3, indicating that binding between collagen I and IV to α3β1 integrin is essential for supporting the survival of INS-1 cells, human fetal pancreas and human adult islets (54, 78). Analysis of canine islets cultured on collagen I showed a significant improvement in adhesion, GSIS, and increased insulin content, all while significantly reducing islet cell death when compared to controls (79). Human adult islets cultured on a collagen I matrix had significantly improved cellular response to acute glucose challenge after 3 days and were viable for longer than 8 weeks (80). Meanwhile, studies in human fetal pancreatic islet cells demonstrated that β1 integrin blockade resulted in significantly decreased mRNA and protein expression of essential islet genes (insulin, glucagon and PDX-1), along with down-regulation

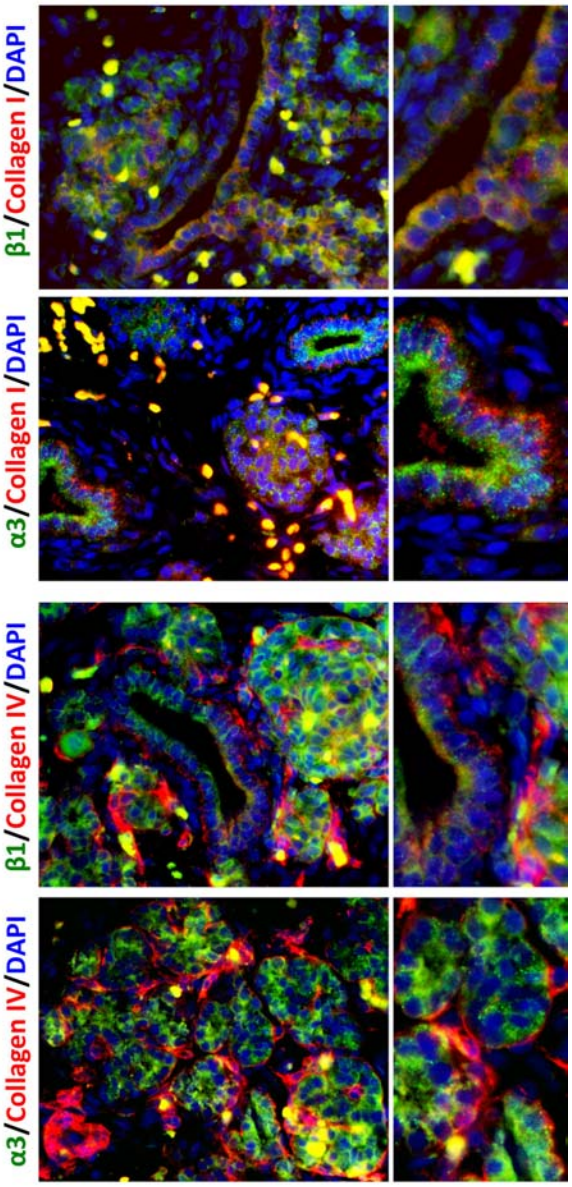

Figure 4. Co-localization of collagen I and IV (red) with α3 and β1 integrins (green) in human fetal pancreas. Nuclei stained with DAPI (blue). Amplified (x3) regions are indicated by outline. Scale bar: 50μm

of the FAK/MAPK/ERK signaling cascade when cells were plated on collagen I gels (51). Islet survival, however, was significantly increased when isolated islets were cultured on collagen IV, laminin and fibronectin when compared to collagen I (81). Further, adding a combination of soluble laminin or fibronectin to the culture media of islets cultured on collagen I prevented islet-epithelial transformation (6). Culturing neonatal islets on collagen I gel led to significantly increased cell proliferation and expansion of c-Kit-expressing cells, which was maintained for up to 8 weeks (82). Past work has also shown that collagen I supports differentiation and maturation of pancreatic precursor cells into β-cells when cultured in 3D polyethylene glycol hydrogels (83). The use of a collagen I-based 3D entrapment method improved adult islet morphology, viability and function with no defects in oxygen consumption when compared to controls (84). Collagen I gels supplemented with other ECM proteins also proved to support long-term human islet culture demonstrating similar insulin stimulation indexes to freshly isolated islets (85). Despite 3D culture more closely mimicking the endogenous islet environment, other groups found that human islet cells in a 3D collagen I matrix developed into cystic structures (86), began expressing the ductal cell marker, CK-19, and abated insulin expression (87). These results suggest that collagen I plays a role in regulating islet cell proliferation, function, and survival; however, islets from humans differ from other organisms in their response to collagen I-based cell culture.

4.2. Collagen IV-based culture

The basement membrane surrounding islets includes a range of proteins including collagen IV (Figure 1) (88). Many studies have investigated the importance of collagen IV *in vivo,* and as a supportive gel *in vitro.* Injecting streptozotocin (STZ) into neonatal Wistar rats had a significant impact on glucose tolerance, and after recovery, collagen IV expression remained deficient, suggesting that collagen may be required to facilitate β-cell growth and function (89). Collagen IV is also critically important for β-cell motility and insulin secretion via α1β1 integrin (7). Likewise, collagen IV improved adult β-cell insulin secretion in an ERK-dependent manner (90), which likely occurs through increased FGFR1 expression (91). Culturing islets on collagen IV also prevented apoptosis when compared to controls (81). We have demonstrated that collagen IV improves human islet and INS-1 cell adhesion, spreading, survival and function by interacting with integrin α3β1 (Figure 4) (78). RIN-5F cells, however, showed minimal changes in cell adhesion and proliferation when cultured on plates coated with either collagen I or IV compared to controls (92). Using encapsulation methods, collagen IV was able to improve GSIS, with a synergistic improvement when collagen IV was mixed with laminin (93). Three dimensional culture of human adult islets in collagens I and IV led to higher insulin gene expression compared to fibronectin or laminin (94). Another 3D culture system was developed using poly(lactic-co-glycolic acid)-collagen hybrid scaffolds, which supported RIN-5F cell proliferation, function and differentiation (95).

Both collagen I and IV have substantial potential for improving islet health. Despite the benefits associated with collagen gels, dedifferentiation of islet cells must be avoided.

5. COLLAGEN-ASSOCIATED ISLET DEDIFFERENTIATION

Despite collagen being an improvement upon tissue culture polystyrene and some ECM proteins, islets cultured in collagen gels tend to dedifferentiate to non-insulin producing cells. When isolated adult male Sprague-Dawley islets were cultured in collagen gels (produced from rat tails) with growth factors, there was a time-

dependent decrease in insulin secretion as well as increased expression of cytokeratin-19, a marker of ductal cells (96). These results suggest that collagen may not be able to maintain the differentiated state of islets, and instead supports a ductal-cell phenotype. Indeed, a similar finding was observed when culturing isolated adult human (86) and canine (97) islets in three-dimensional rat tail collagen gels. Development of these duct-like epithelial cystic structures is initially associated with a significant increase in islet cAMP, JNK signaling and cleaved caspase-3 levels, with reduced ERK/Akt signaling, giving a net effect of increased apoptosis. Upon ductal-like structure formation, there is a reduction in JNK signaling and cleaved caspase-3 with an increase in ERK/Akt signaling resulting in duct-like cell proliferation (6, 98). Interestingly, this dedifferentiation could be reduced, in a dose-dependent manner, by the addition of transforming growth factor β (99). Another research group cultured islets in a collagen matrix and showed a concentration-dependent effect of EGF on islet dedifferentiation via the AKT and ERK signaling pathways (100). Meanwhile, when these collagen I-cultured islet-derived duct-like epithelial cells were treated with the islet neogenesis-associated protein, they re-differentiated back to islet-like structures including four endocrine cell types (101). Conversely, human islet culture on collagen molecules adhered to tissue culture polystyrene did not lead to dedifferentiation, but rather to maintenance of insulin gene expression (94). Therefore, it appears that scaffolds provided by collagen gels play an important role in islet dedifferentiation.

6. COLLAGEN-BASED ISLET TRANSPLANTATION

Since the development of the Edmonton protocol (102), researchers have been dedicated to investigating ways to improve and maximize the function and survival of islets, both pre- and post-transplantation. Under the current conditions, transplanted islets are unable to maintain long-term insulin independence in human patients (103). However, islet transplantation protocols that include ECM protein-encapsulation of islets may assist in protecting islets and maintaining their function *in vivo* (104).

6.1. Collagen-based scaffolds

Collagen is a useful matrix protein demonstrated to promote islet health. This can be taken advantage of in a clinical setting of islet transplantation. Murine islets embedded in a fibroblast-populated collagen matrix significantly improved graft survival after transplantation (9). Diabetes reversal occurred and required less islet mass compared to non-collagen supported controls (9). Likewise, transplanted collagen IV-adsorbed scaffolds containing islets significantly improved glycemia faster than scaffolds coated with either fibronectin, laminin, or serum (8). Furthermore, when a sub-optimal number of human islets were seeded in collagen IV-containing scaffolds, they had improved graft function compared to controls (105). Collagen implants containing a central islet mass surrounded by preformed vasculature were able to maintain islet survival and insulin release *in vivo* (106). The site of transplantation can also affect the outcome of collagen-based islet grafts, wherein subrenal capsule islet transplantation was significantly better at improving glucose homeostasis than subcutaneous islet transplantation (107). This may be due to deficient revascularization of embedded islets when transplanted subcutaneously (107). As well, encapsulating islets using collagen may protect them against immune destruction (108). Taken together, the use of collagen as a vehicle for islet transplantation greatly improves islet survival, vascular formation and glucose homeostasis and may protect islets from host immune destruction. However, collagen must be used in human islet transplantation to investigate its usefulness in treating diabetes.

6.2. Collagen-coated biomaterials

Using biomaterials made of collagen and other compounds, many studies have demonstrated improved islet function and differentiation (108-110). A collagen-alginate composite to encapsulate dispersed islet spheroids was able to improve glucose tolerance after transplantation, unlike controls (108). Likewise, when collagen IV was passively adsorbed to polysulfone surfaces, significantly more islet cells adhered to the surface (109). Use of an expanded polytetrafluoroethylene solid support system that was coated with type IV collagen to transplant islets proved useful since animals that received it had greater engraftment success compared to controls (110).

6.3. Growth factors and collagen-based islet transplantation

To further promote transplanted islet cell survival and function using collagen-based materials, researchers attempted to promote blood vessel formation in the donor graft by encapsulating islets with collagen in the presence of vascular endothelial growth factor (VEGF) (111). Islets under these conditions significantly improved the glucose homeostasis of STZ-induced diabetic mice for up to 28 days when compared to controls (111). Furthermore, an implant containing a polyvinyl alcohol sponge infused with a type I collagen hydrogel, VEGF, and islets, was able to normalize blood glucose levels in STZ-treated mice (112). Alternatively, the use of collagen sponges and microspheres with basic fibroblast growth factor allowed diabetic rats to return to normal blood glucose for over 40 days where controls did not (113). Other studies made use of collagen gels derived from transgenic mice fibroblasts expressing an immunomodulatory factor (indoleamine 2,3-dioxygenase) to improve islet graft function long-term (114).

6.4. Benefits of collagen use in islet transplantation

Collagen can be used to improve outcomes of islet transplantation in three direct ways: (1) islets can be cultured on collagen or embedded in 3D collagen gels (likely with a combination of factors without serum) to temporarily maintain their differentiation and function before transplantation (94); (2) after the culture period, islets along with the collagen gels and factors can be transplanted into a recipient host to provide a scaffold for revascularization; (3) islets can be encapsulated with collagen immediately after isolation and transplanted into a recipient host (108). In each case, islets would ideally be transplanted subcutaneously to avoid immune attack by the

host. Revascularization of the graft could be promoted by adding angiogenic factors to the collagen gels (111).

Further application of islet transplantation is the bioartificial endocrine pancreas – a device that connects to a host vasculature or embeds in a tissue that contains islets in an enclosed capsule that diffuses nutrients but does not cause an immunologic reaction and physiologically maintains blood glucose levels (115). Since collagen has low immunogenicity, absorbability, and biodegradation, yet promotes cell communication, it is a prime candidate for support within a bioartificial endocrine pancreas (116, 117). Indeed, when collagen-based devices were implanted with fibroblast growth factor 2, vascularization along with islet function and survival was improved (118, 119).

7. CONCLUSION & FUTURE DIRECTION

Collagens I and IV are essential endogenous ECM proteins that fulfill necessary structural and stimulatory functions for tissues and cells. Collagen I is necessary for tensile strength of organs, and stimulates integrin α2β1 and DDR2, while collagen IV is located in the basement membrane and binds α1β1 and DDR1 to affect cell survival, differentiation and function. Islet culture in collagens are able to extend cell survival and function, however the risk of dedifferentiation into a ductal phenotype exists. Collagen encapsulation methods are effective at enhancing islet graft survival and function, yet must be attempted in humans to ensure safety.

Based on current research, collagen alone is insufficient to replicate the natural ECM environment in which islets reside. Therefore, ideal future experiments would include culturing islets with a mixture of ECM proteins that are commonly found in the native pancreas. Indeed, culture of fetal porcine islet-like cell clusters with matrigel (a hetergenous mixture of ECM proteins secreted by sarcoma cells) was able to enhance long-term insulin secretion (120); however, a standardized, non-tumour derived ECM gel is preferred for any clinical application. Additionally, a comprehensive understanding of the collagen-binding receptors that mediate cellular effects is required in order to investigate ulterior methods to stimulate islets and maintain their differentiation and function. In differentiation protocols, for instance, small molecules have been generated that can more effectively differentiate embryonic stem cells to definitive endoderm than growth factors (121). Using similar technology to stimulate collagen-binding receptors on islets may prove to be a great success in preserving long-term islet function and survival. Finally, islet transplantation remains ineffective at maintaining long-term glucose homeostasis. The use of collagen islet encapsulation is attractive because fewer islets are needed to normalize glycemia in diabetic rodents (9, 105) and immune destruction may be reduced, but it must be combined with other ECM proteins (122), growth factors (111) and/or cell types (123) to preserve islet health. The synergistic effects of these combinations could demonstrate effective islet survival and allow successful long-term islet transplantation.

8. ACKNOWLEDGEMENTS

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