Insight into Microenvironment Remodeling in Pancreatic Endocrine Tissue Engineering: Biological and Biomaterial Approaches

Maryam Kaviani, Negar Azarpira*

Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

The treatment of diabetes mellitus, as a chronic and complicated disease, is a valuable purpose. Islet transplantation can provide metabolic stability and insulin independence in type 1 diabetes patients. Diet and insulin therapy are only diabetes controllers and cannot remove all of the diabetes complications. Moreover, islet transplantation is more promising treatment than whole pancreas transplantation because of lesser invasive surgical procedure and morbidity and mortality. According to the importance of extracellular matrix for islet viability and function, microenvironment remodeling of pancreatic endocrine tissue can lead to more success in diabetes treatment by pancreatic islets. Production of bioengineered pancreas and remodeling of pancreas extracellular matrix provide essential microenvironment for re-vascularization, re-innervation and signaling cascades triggering. Therefore, islets show better viability and function in these conditions. Researchers conduct various scaffolds with different biomaterials for the improvement of islet viability, function and transplantation outcome. The attention to normal pancreas anatomy, embryology and histology is critical to understand the pancreatic Langerhans islets niche and finally to achieve efficient engineered structure. Therefore, in the present study, the status and components of the islets niche is mentioned and fundamental issues related to the tissue engineering of this structure is considered. The purpose of this review article is summarization of recent progress in the endocrine pancreas tissue engineering and biomaterials and biological aspects of it.

Tissue Eng Regen Med 2016;13(5):475-484

Key Words: Diabetes mellitus; Islet transplantation; Extracellular matrix; Pancreatic endocrine tissue; Tissue engineering

INTRODUCTION

Cell therapy is a promising approach for the treatment of incurable and chronic diseases. Diabetes mellitus is an autoimmune and chronic disease that leads to insulin deficiency and several complications such as neuropathy, nephropathy, retinopathy, cardiovascular diseases [1-3]. According to World Health Organization reports, 347 million people worldwide have diabetes, the mortality of which will rise by more than 50% between 2005 and 2030. Insulin therapy has been accepted as a standard therapy since the advent of insulin. But, fluctuation of blood glucose during this therapy results in severe complications and death. The use of modified insulin formulations and delivery systems has improved the successful control of the dis-

ease. However, there is still the possibility of hypoglycemic episodes and complications related to insulin administration devices. It seems the only treatment for diabetes is pancreas transplantation [4]. Pancreas transplantation was carried out for the first time in 1966 [5]. Despite poor initial results, improvements in the surgical techniques and immunosuppressive regimens increased the efficacy and safety of this procedure. However, the whole organ transplantation needs invasive surgical procedures and is associated with significant morbidity and mortality. In diabetes patients, there is only need to receive endocrine pancreas that includes 2% of the whole pancreas. Delivery of the whole pancreas is associated with infection, thrombosis and pancreatitis. Therefore, pancreatic islet transplantation can be more desirable [6,7]. Pancreatic islet transplantation is less invasive and appears to be superior in the metabolic and immunologic aspects. According to recent Collaborative Islet Transplant Registry report, the complications of islet transplantation are 20 times lower than the whole pancreas transplantation. Another advantage of islet transplantation is possibility of isolation and transplantation of islets from inappropriate pancre-

Received: January 20, 2016

Revised: February 3, 2016

Accepted: February 5, 2016

***Corresponding author:** Negar Azarpira, Transplant Research Center, Shiraz University of Medical Sciences, Mohamad Rasulallah Research Tower, Khalili street, Shiraz 7193635899, Iran.

Tel: 98-71-36281529, Fax: 98-71-36281529

E-mail: negarazarpira@yahoo.com

as for the whole organ transplantation. Therefore, the shortage of organ donor somewhat reduces [7]. Efforts for the treatments of diabetes by islet transplantation has started since the 1970s [8,9]. Several islet transplantation clinical trials have been conducted to control diabetes complications [10-13]. While islet transplantation is a potential and promising method, there is evidence of islet dysfunction and loss of implant after engraftment [14-16].

Researchers have done pancreatic islet isolation in various centers [17-19]. Application of cells alone for the treatment of diseases creates some problems. After cell isolation extracellular matrix (ECM), internal vascularization and innervation is disrupted. During and after isolation of cells from native niche, the cells experience a stressful condition and undergo dysfunction, destruction and death over time [20-22]. Therefore, the establishment of appropriate scaffold for the cells can be helpful in this condition. Researchers have recently focused on the production of bioengineered pancreas and pancreas ECM remodeling [23-25].

Tissue engineering is an interesting approach for regeneration of ECM and maintenance of cell function and structure. This field of science is potential for control and treatment of diabetes. In this review, we first describe endocrine pancreas structure and niche and then summarize the advancement in recent studies on the remodeling of endocrine pancreas ECM and biomaterials and biological aspects of pancreatic endocrine tissue engineering.

MACROANATOMY AND MICROANATOMY OF THE PANCREAS

For success in the endocrine pancreas ECM remodeling, considering the native pancreas structure is essential. Pancreas is a secretory organ that lies in the abdominal cavity. The pancreas has an endodermal origin and forms from a ventral and a dorsal bud of the foregut [26]. The average size of this gland is about 16 cm long, 10.5 cm wide, 2.5 cm thick, and 75 g weight [27]. The pancreatic parenchyma is divided into distinct and incomplete lobes and lobules by a fibrous capsule that surrounds the

organ. Each lobule is composed of acini that form the functional unit of the pancreatic exocrine. The secretory cells of acini release pancreatic juice that are transported by ducts to the pancreatic duct and finally into the duodenum [26]. The endocrine part of the pancreas comprise 1–4% of the total pancreas volume [28]. The functional units of the endocrine pancreas are the islets of Langerhans and single endocrine cells scattered throughout the exocrine pancreas [29]. There are at least 5 types of hormone secreting cells in the islets. Beta cells, as the most pancreatic endocrine cells, constitute 50–70% of the total and secrete insulin. Alpha cells are glucagon producing and releasing cells, contributing to 20–40% of the total. Other endocrine cells are delta cells, PP cells, and epsilon cells that release somatostatin, pancreatic polypeptide and ghrelin, respectively. These endocrine cells constitute less than 20% of the cells [30,31]. Pericytes, macrophages and dendritic cells are other cells throughout the islets [28,32].

Endothelial cells have been found close to the endocrine cells of the islets. The endothelium is non-fenestrated and is supported discontinuously by the basal lamina [33]. The islets have an extensive vasculature that constitutes 7–8% of the total islet volume and receive blood about 10-times more than the exocrine part [34]. Arterioles enter each islet and form a dense network. Finally, endocrine secretions are delivered to the vessels and systemic circulation [35,36]. The pancreas receives a rich innervation from the sympathetic, parasympathetic, and afferent nerve fibers. These fibers pass through the pancreatic tissue and are closely associated with vascular network and endocrine cells [37]. Attention to rich vascular and neural networks in the pancreatic tissue and close communication of these networks with the structure and function of the pancreatic islet cells can be a useful guide in pancreatic tissue engineering.

NATIVE PANCREATIC ISLET MICROENVIRONMENT

ECM composition of the islet is varied in different stages of human development (Fig. 1). Adult pancreas consists of about

Figure 1. Pancreatic islet ECM proteins in different stages of development. Coll: collagen, LM: laminin, FN: fibronectin, VN: vitronectin.

one million islets and several thousand individual endocrine cells arranged in each islet [38]. A sheath of collagen and glia surround the islet [28]. A single layer of fibroblasts around the adult human islet produce collagen fibers [39]. Adult human islet ECM is composed of collagen (Coll)-I, -III, -IV, -V, -VI, laminin (LM) and fibronectin (FN) with various prevalence [40,41]. Early precursor tissue is arranged by Coll-IV, vitronectin (VN), and FN and Developing islets ECM is comprised of Coll-IV and LM and probably FN and VN [42].

Formation of fibrillar structures, networks and beaded filaments is associated with the presence of different types of collagen in the islets [43]. Coll-IV has a high prevalence and contributes to basement membrane network formation [41]. This type of collagen induces essential signaling for islet adhesion, proliferation and insulin secretion via interaction between the integrin and ECM [22,44,45]. Researchers reported that Coll-VI is a major component of human pancreas and the amount of this component is more than two times greater compared with Coll-I or Coll-IV [46].

LM is an important component of the basement membrane. There are different isoforms of LM around the islets [47]. LM-111 supports survival and differentiation of pancreatic islet cells [48]. It has been reported that LM-111 is absent in maturity [47]. LM-511 is the only isoform present in the human pancreas endocrine [39]. Analysis of molecular composition of human islet basement membrane has shown that human beta cells connect to LM-511 through integrin $\alpha 3\beta 1$ and Lutheran glycoprotein. *In vitro* studies demonstrated that LM-511 partially blocks dedifferentiation of beta cells [49].

FN plays essential structural and functional roles in ECM. RGD domains of FN, as a ligand for integrins, stimulate a conformational change in its receptor and conduct signaling cascades [50]. FN contributes to early precursor and adult islet ECM composition. In developing islets, little, if any, FN is present in the core of the islet clusters [42]. VN is another ECM protein that plays a role in cell adhesion [51]. Researchers suggest that VN positive cells are the source of endocrine progenitors [42].

Membrane-bound receptors, such as integrins, are important molecules in cellular function. These molecules initiate signaling cascades, leading to genetic expression and regulation of cellular characteristics and function [52]. Composition of human islets integrin receptors is controversial. The expression of α -3, -5, -v [53,54] and β -1, -3, -5 [42,45] subunits is more likely in human islets [41].

In cell-based studies for diabetes, application of both stem cells and pancreatic islet cells has been investigated [55-58]. Researchers showed that the culture of isolated islet cells on plastic surface without matrix support leads to glucose unre-

sponsiveness. However, maintenance of islet β -cell glucose responsiveness is attained by the culture of these cells on the ECM [59]. Three-dimensional differentiation of the embryonic stem cells into islet-like clusters within the collagen gel has been investigated. This model of differentiation provided a more efficient system to produce islet-like clusters than two-dimensional environment [60]. Attention to the pancreas niche and the presence of various protein components in different stages of pancreas development and then the selection of correct natural biomaterials can be effective in successful differentiation of the cells into viable and functional insulin-producing cells.

BIOENGINEERING APPROACHES TO IMPROVE ISLET MICROENVIRONMENT FOR ISLET TRANSPLANTATION

Mechanical and enzymatic digestion procedures for islet cells isolation resulted in disruption of the biological and mechanical support of the islets. Tissue engineering represents advanced methods for regeneration of interrupted ECM. Therefore, tissue engineering and regenerative medicine can be considered as a therapeutic option in islet transplantation. Recently, researchers have used different biomaterials and methods to access an efficient and optimal system for pancreas ECM remodeling and islet transplantation. The present review discusses the recent biomaterials and methods in pancreatic endocrine microenvironment remodeling.

BIOMATERIALS

Selection of appropriate natural and synthetic ECM analogues is an important key in successful bioengineering. The constructed scaffolds have to mimic native ECM. Synthetic biomaterials have many advantages including availability, specific degradation rate, nonimmunogenicity, good mechanical strength and controllable characteristics. Lack of biological activity in synthetic agents can be overcome by natural polymer application. Biological activity of these polymers results in cell adhesion, growth and differentiation. Induction of immune response, poor mechanical strength, and variations in degradation rates are disadvantages of these polymers [61,62].

Various studies have been conducted by natural ECM components. Researchers showed that Coll-I/-IV and FN cause cell adhesion and FN maintains the human islet structure and insulin content distribution [22]. Three-dimensional structures containing Coll-I can induce β -cell proliferation and insulin secretion [63]. Analysis of the Coll-IV impacts on human β -cell characteristics indicated that binding between $\alpha 1\beta 1$ integrin and Coll-IV is important for cell motility and glucose homeo-

stasis [44].

Alginate is an important biomaterial in bioengineering and biomedicine which belongs to the family of polysaccharides [64]. Application of alginate has some advantages as the most common biomaterial for islet encapsulation. Processing of the alginate capsules is possible at physiological conditions including body temperature and physiological pH and osmolality [65, 66]. This material is generally biocompatible and biodegradable. Alginate is useful for cell encapsulation and controlled delivery of biologically active agents [67]. Alginate can affect the cell growth, metabolism and insulin production [68-70].

Chitosan is another polysaccharide-based biomaterial in the bioengineered scaffold. Chitosan is known for biocompatibility with human cells, biodegradability, antimicrobial activity and hemostatic activity [71,72]. This natural polymer has been applied in endocrine pancreas tissue engineering. Reports suggest that application of collagen-chitosan hydrogel can be an efficient strategy to achieve the pro-angiogenic site for islet transplantation [73]. Chitosan hydrogels can show cytoprotection properties against the recipient's immune system in xenogeneic islet transplantation without effect on the insulin secretion. According to the safety and characteristics of chitosan, this hydrogel can be an alternative biomaterial for islet encapsulation in pre-clinical and clinical applications [74].

Heparin is a complex polymer which can be used for surface engineering of the pancreatic islets. Modification of the islet surface by heparin can provide inhibition of coagulation and complement activation [75]. Heparin plays several roles in angiogenesis and growth factor binding [76]. The affinity of heparin to a number of bioactive components has been employed for immobilization of heparin and growth factors on the islet surface [77]. Research has shown that the culture of the porcine islets with heparin protects them from damage [78]. Incorporation of synthetic biomaterials into heparin can affect the islet transplantation outcome [79]. Synthetic biomaterials can provide a natural environment without triggering an immune reaction [80].

Various synthetic biomaterials have been investigated for ECM remodeling of the pancreatic islets. The most commonly used biodegradable synthetic polymers belong to the polyester family. Poly-glycolic acid (PGA) is an aliphatic-polyester that possesses biocompatibility and reproducible mechanical properties. The degradation rate of this polymer is predictable and metabolization of its degradation product is possible. Therefore, the application of this polymer is interesting in tissue engineering [81]. Rat's islet cells have been successfully cultured on PGA scaffold. This scaffold showed positive effects on viability and insulin secretion of the islet cells [82]. Transplantation of the rat's islets in the presence of PGA scaffolds indicates appropriate cell adhesion to the scaffolds and improvement of the grafts'

morphology and function [83].

Poly-lactic acid (PLA) is another aliphatic-polyester with low tensile strength and rapid degradation rate. PLA possesses more hydrophobicity and slower degradation than PGA [84]. For access to benefits of PLA and PGA, their copolymer has been formed as poly-lactic-co-glycolic acid (PLGA). This copolymer indicates lower crystallinity and melting temperature than each individual homopolymer. The degradation rate and mechanical strength can be adjusted by the ratio of PLA/PGA for many tissue engineering objects [85,86]. Researchers demonstrated supportive function of PLGA scaffold in murine islet transplantation. Infiltration of the host cells into the scaffold, healthy morphology maintenance, support by host tissue and diabetes reversal were observed by this type of scaffold [87].

METHODS

Scaffold fabrication is an important stage of engineered tissue production. Researchers have applied various methods to produce efficient and adaptive three-dimensional environments for the pancreatic islet culture and transplantation. Recent biomaterials and biological approaches for endocrine pancreas ECM remodeling are summarized in Table 1.

Encapsulation is the most common approach in islet bioengineering. Immunoisolation of the islets using alginate-based capsule was firstly described by Lim and Sun [88]. Encapsulation can protect the cells against recipient immune system cells, but the islet survival and function are limited [89]. Different types of encapsulation devices have been defined. Macroencapsulation is the encapsulation process using a large number of islets in the form of hollow fiber, planar membrane or capsule. Microencapsulation is encapsulation of the single islet or a small number of islets within a thin layer of polymer gel. Conformal coating which covers individual islet and nonoencapsulation or layer by layer coating are another type of encapsulation [90,91]. Appropriate volume and permeability of these structures are essential for oxygen, nutrition, and glucose level access [92,93].

Researchers reported the effect of alginate encapsulation on human islets. They found that barium alginate has a limited effect on the cellular transcriptome. *In vivo* studies showed variability of the isolated islet quality center to center [94]. Viability and functionality of encapsulated human islets in alginate sheets have been investigated. *In vivo* studies showed that subcutaneously implanted sheets remained viable and efficient after 8 weeks [68]. Moreover, microencapsulation of human pancreatic islet-derived precursor cells in alginate device can lead to differentiation of the precursor cells into the functional beta cell-like cells. These findings show that alginate encapsulation may provide a favorable microenvironment for the islets and islet

Table 1. Recent biomaterials and biological approaches for pancreas ECM remodeling

Approaches (biomaterials/scaffold/transplantation)	Cell source	Results	Publication year, reference
Collagen gel with or without ECM components and microfabricated scaffold with ECM supplemented gel	Human islet	<i>In vitro</i> : -Improvement of long term islet culture, insulin secretion profile, pancreatic hormones, and pancreatic gene expression in ECM supplemented groups <i>In vivo</i> : -	2011 [63]
Alginate encapsulation -Transplantation into the intraperitoneal cavity of STZ-induced NOD/SCID mice	Human islets	<i>In vitro</i> : -Unchanged viability -Upregulation of genes involved in inflammation and vascularization -Unchanged protein expression -Little effect on cellular transcriptome -Normoglycaemia in all mice with the implantation of 3000 IEQ	2011 [94]
Alginate microcapsules -Transplantation into the intraperitoneal cavity of NOD/SCID mice	Human pancreatic islet-derived precursor cells	<i>In vitro</i> : -90% cell viability throughout 30 days <i>In vivo</i> : -Post transplant differentiation of hIPC -Beta cell-like function in the differentiated cells	2011 [95]
Alginate sheets -Transplantation in the subcutaneous space of rats	Human islets	<i>In vitro</i> : -Viability and functionality of islet sheets over 8 weeks <i>In vivo</i> : -Viability and functionality of islet sheets over 8 weeks	2011 [68]
Perfluorodecalin-enriched fibrin matrix	Human islets	<i>In vitro</i> : -Improvement of islet viability and function to controls -Level of hypoxia makers expression similar to control	2011 [105]
Microwell scaffolds from a thin polymer film or an electrospun mesh of (PEOT/PBT) block copolymer	Human islets	<i>In vitro</i> : -Higher glucose flux through electrospun scaffolds than thin film scaffolds -Similarity of insulin content and insulin secretion between islets cultured in scaffolds and free floating ones -Maintenance of islet morphology and insulin and glucagon expression during microwell culture <i>In vivo</i> :-	2013 [107]
Fibrin gel -Transplantation in the subcutaneous space of STZ-induced Balb/c mice	Rat islets co-culture with human ADSCs	<i>In vitro</i> : -Increased viability and insulin secretion after co-culture <i>In vivo</i> : -Normalization of the blood glucose levels -Protective effect of ADSCs against islet hypoxic damage -Effect of ADSCs on islet viability and new microvasculature regeneration	2013 [106]
Perfusion-decellularized mouse pancreas -Transplantation in the subcutaneous space of C57BL/6 mice	AR42J acinar cell line and MIN-6 beta cell	<i>In vitro</i> : -Effectively remove of cells -Successful cellular engraftment -Up-regulation of insulin gene expression in the graft <i>In vivo</i> : -Biocompatibility of decellularized scaffold	2013 [112]

Table 1. Recent biomaterials and biological approaches for pancreas ECM remodeling (continued)

Approaches (biomaterials/scaffold/transplantation)	Cell source	Results	Publication year, Reference
Alginate, alginate-chitosan and alginate-chitosan-PEG microencapsulation	Murine tumor pancreatic beta islet cell (CRI-D2)	<i>In vitro</i> : -Higher viability for alginate microcapsules -More insulin secretion in alginate microencapsulation group <i>In vivo</i> : -	2013 [96]
Collagen-alginate microfibers using a microfluidic chip -Transplantation into the intraperitoneal cavity of STZ-induced Balb/c mice	Rat islet single cells co-culture with rat ADSCs	<i>In vitro</i> : -Improvement of islet cell survival and function <i>In vivo</i> : -longer maintenance of blood glucose level in co-culture group	2014 [97]
Heparin mimetic peptide nanofiber gels -Transplantation in the omentum of STZ-induced Rat	Rat islets	<i>In vitro</i> : -Unchanged glucose stimulation index level compared to fresh islets <i>In vivo</i> : -Better maintenance of blood glucose levels -Better islet integrity and blood vessel density	2015 [108]

ECM: extracellular matrix, STZ: Streptozotocin, IEQ: islet equivalent, hIPC: human islet-derived stem/precursor cells, PEOT: poly (ethylene oxide terephthalate), PBT: poly(butylene terephthalate), ADSCs: adipose-derived stem cells

precursor cells [95]. In a comparative study, the effect of alginate, alginate-chitosan and alginate-chitosan-polyethylene glycol was investigated. Results showed higher viability and more insulin secretion in the alginate group [96]. Jun et al. [97] investigated encapsulation of co-cultured islet spheroids using microfluidic chip technology. Findings showed that adipose derived stem cells (ADSCs) have a protective effect on islet damage during culture and pre-transplantation. Transplantation of microfiber encapsulated islet spheroids resulted in blood glucose maintenance in the mouse model.

There are few clinical studies on engineered islets and almost all studies are with encapsulated islets. In these clinical trials encapsulated human or porcine islets were implanted in the peritoneal cavity or subcutaneous space in patients with type 1 diabetes mellitus [98-104]. In a recent report, sodium alginate encapsulated human islets were intraperitoneally transplanted in four cases of type 1 diabetes mellitus. The patients' follow-up showed positive serum C-peptide throughout 3 years. Moreover, stability of blood glucose without any immune response was observed [98].

Daoud et al. [63] report a suitable platform for long-term maintenance of human pancreatic islets. They evaluated different scaffolds, collagen gel with or without ECM components and microfabricated scaffold with ECM supplemented gel and concluded that incorporation of ECM components within the scaffolds improved the long-term islet culture. Moreover, presence of ECM

components in controlled pore structure microfabricated scaffold showed the responsiveness of glucose similar to fresh islets. Decrease of oxygen supply is a crucial condition during and after islet isolation. Researchers recruited perfluorodecalin-enriched fibrin matrix as a scaffold with diffusion enhancing medium against oxygen deficiency during islet culture. Results showed that this system can be effective on human islet viability and function *in vitro*. However, the level of the hypoxia markers remained similar to the controls [105]. Transplantation of human ADSCs and pancreatic islets through fibrin gel resulted in normalization of blood glucose levels in diabetic mice models. This co-culture system improved the islet's viability and regeneration of microvasculature [106]. Validation of micro-well scaffolds for human islet culture was done in a recent study. In this study, scaffolds were fabricated by synthetic copolymers of poly (ethylene oxide terephthalate)-poly(butylene terephthalate) in the form of thin film and electrospun mesh. Findings indicated the stability of islet' native morphology in the micro-well scaffold platform. Moreover, the insulin and glucagon expression and islet functionality were not impaired in this environment. However, the glucose flux occurred three times higher in the electrospun scaffolds [107]. Researchers reported heparin mimetic peptide nano-fiber gels containing growth factors can provide a suitable supportive environment for the rat's islets. The cultured islets under this condition showed a similar glucose stimulation index as the fresh islets. *In vivo* studies indicated better

maintenance of blood glucose level in animal models receiving nano-fibrous network containing islets [108].

Perfusion-decellularized organs probably make a suitable platform for cell culture and transplantation. This scaffold plays compositional, structural, and biomechanical roles of a native organ ECM [109]. Decellularization of the pancreas has been reported in animal models [110-112]. Perfusion-decellularization and recellularization of the mouse pancreas has been successfully done. After engraftment of MIN-6 b-cells in the scaffold, up-regulation of insulin gene expression has occurred. These results support the application of the whole pancreas ECM as a biocompatible and biological scaffold [112].

Overall, transplantation of the pancreatic islets is a promising approach for the treatment of type 1 diabetes. However, viability and efficiency of transplanted islets are restricted due to the destruction of ECM during and after cell isolation. Therefore, remodeling a suitable and efficient supportive platform is important for successful islet culture and transplantation.

CONCLUSION

Construction of a three-dimensional artificial matrix can lead to improvement of cell viability and function *in vitro* and act as a platform and carrier for cell transplantation. Therefore, regenerative medicine by tissue engineering technology has the potential to provide a suitable and lifelong method for the treatment of diabetes. To achieve this goal, intensive researches are essential.

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

REFERENCES

1. Kuehl M, Stevens MJ. Cardiovascular autonomic neuropathies as complications of diabetes mellitus. *Nat Rev Endocrinol* 2012;8:405-416.
2. Rask-Madsen C, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2012;32:2052-2059.
3. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev* 2013;93:137-188.
4. Narang AS, Mahato RI. Biological and biomaterial approaches for improved islet transplantation. *Pharmacol Rev* 2006;58:194-243.
5. Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery* 1967;61:827-837.
6. Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. *Diabetes Care* 2012;35:1436-1445.
7. Berney T, Johnson PR. Donor pancreata: evolving approaches to organ allocation for whole pancreas versus islet transplantation. *Transplantation* 2010;90:238-243.
8. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. *Surgery* 1972;72:175-186.
9. Gray BN, Watkins E Jr. Prevention of vascular complications of diabetes by pancreatic islet transplantation. *Arch Surg* 1976;111:254-257.
10. O'Connell PJ, Hawthorne WJ, Holmes-Walker DJ, Nankivell BJ, Gunton JE, Patel AT, et al. Clinical islet transplantation in type 1 diabetes mellitus: results of Australia's first trial. *Med J Aus* 2006;184:221-225.
11. Vantyghem MC, Kerr-Conte J, Arnalsteen L, Sergeant G, Defrance F, Gmyr V, et al. Primary graft function, metabolic control, and graft survival after islet transplantation. *Diabetes Care* 2009;32:1473-1478.
12. O'Connell PJ, Holmes-Walker DJ, Goodman D, Hawthorne WJ, Loudovaris T, Gunton JE, et al. Multicenter Australian trial of islet transplantation: improving accessibility and outcomes. *Am J Transplant* 2013;13:1850-1858.
13. D'Addio F, Maffi P, Vezzulli P, Vergani A, Mello A, Bassi R, et al. Islet transplantation stabilizes hemostatic abnormalities and cerebral metabolism in individuals with type 1 diabetes. *Diabetes Care* 2014;37:267-276.
14. Biarnés M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* 2002;51:66-72.
15. Ricordi C, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol* 2004;4:259-268.
16. Campbell PM, Senior PA, Salam A, Labranche K, Bigam DL, Kneteman NM, et al. High risk of sensitization after failed islet transplantation. *Am J Transplant* 2007;7:2311-2317.
17. Azarpira N, Aghdai MH, Nikeghbalian S, Geramizadeh B, Darai M, Esfandiari E, et al. Human islet cell isolation: the initial step in an islet transplanting program in Shiraz, Southern Iran. *Exp Clin Transplant* 2014;12:139-142.
18. Bucher P, Mathe Z, Morel P, Bosco D, Andres A, Kurfuest M, et al. Assessment of a novel two-component enzyme preparation for human islet isolation and transplantation. *Transplantation* 2005;79:91-97.
19. Ricordi C, Tzakis AG, Carroll PB, Zeng YJ, Rilo HL, Alejandro R, et al. Human islet isolation and allotransplantation in 22 consecutive cases. *Transplantation* 1992;53:407-414.
20. Ilieva A, Yuan S, Wang RN, Agapitos D, Hill DJ, Rosenberg L. Pancreatic islet cell survival following islet isolation: the role of cellular interactions in the pancreas. *J Endocrinol* 1999;161:357-364.
21. Wang RN, Rosenberg L. Maintenance of beta-cell function and survival following islet isolation requires re-establishment of the islet-matrix relationship. *J Endocrinol* 1999;163:181-190.
22. Daoud J, Petropavlovskaja M, Rosenberg L, Tabrizian M. The effect of extracellular matrix components on the preservation of human islet function *in vitro*. *Biomaterials* 2010;31:1676-1682.
23. Gao B, Wang L, Han S, Pingguan-Murphy B, Zhang X, Xu F. Engineering of microscale three-dimensional pancreatic islet models *in vitro* and their biomedical applications. *Crit Rev Biotechnol* 2016;36:619-629.
24. Riopel M, Trinder M, Wang R. Fibrin, a scaffold material for islet transplantation and pancreatic endocrine tissue engineering. *Tissue Eng Part B Rev* 2015;21:34-44.
25. Hamilton DC, Shih HH, Schubert RA, Michie SA, Staats PN, Kaplan DL, et al. A silk-based encapsulation platform for pancreatic islet transplantation improves islet function *in vivo*. *J Tissue Eng Regen Med*, 2015. doi: 10.1002/term.1990 [Epub ahead of print].
26. Drake R, Vogl AW, Mitchell A. Gray's anatomy for students. 3rd ed. New York: Churchill Livingstone; 2014.
27. Go VLW, Dimagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA. The pancreas, biology, pathobiology, and disease. 2nd ed. New York: Raven Press; 1993.
28. In't Veld P, Marichal M. Microscopic Anatomy of the Human Islet of Langerhans. In: Islam S, editor. The Islets of Langerhans: Volume 654 of

- the series *Advances in Experimental Medicine and Biology*. Netherlands: Springer; 2010. p. 1-19.
29. Bouwens L, Pipeleers DG. Extra-insular beta cells associated with ductules are frequent in adult human pancreas. *Diabetologia* 1998;41:629-633.
30. Andralojc KM, Mercalli A, Nowak KW, Albarello L, Calcagno R, Luzi L, et al. Ghrelin-producing epsilon cells in the developing and adult human pancreas. *Diabetologia* 2009;52:486-493.
31. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, et al. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 2005; 53:1087-1097.
32. Dolenšek J, Rupnik MS, Stožer A. Structural similarities and differences between the human and the mouse pancreas. *Islets* 2015;7:e1024405.
33. O'Morchoe CC. Lymphatic system of the pancreas. *Microsc Res Tech* 1997;37:456-477.
34. Henderson JR, Moss MC. A morphometric study of the endocrine and exocrine capillaries of the pancreas. *Q J Exp Physiol* 1985;70:347-356.
35. Konstantinova I, Lammert E. Microvascular development: learning from pancreatic islets. *Bioessays* 2004;26:1069-1075.
36. El-Gohary Y, Sims-Lucas S, Lath N, Tulachan S, Guo P, Xiao X, et al. Three-dimensional analysis of the islet vasculature. *Anat Rec (Hoboken)* 2012;295:1473-1481.
37. Åhrén B. Autonomic regulation of islet hormone secretion--implications for health and disease. *Diabetologia* 2000;43:393-410.
38. Carroll PB. Anatomy and physiology of islets of Langerhans. In: Ricordi C, editor. *Pancreatic islet cell transplantation; 1892-1992 one century of transplantation for diabetes*. Austin, TX: R. G. Landes Co.; 1992. p. 7-18.
39. Otonkoski T, Banerjee M, Korsgren O, Thornell LE, Virtanen I. Unique basement membrane structure of human pancreatic islets: implications for beta-cell growth and differentiation. *Diabetes Obes Metab* 2008;10 Suppl 4:119-127.
40. Van Deijnen JH, Van Suylichem PT, Wolters GH, Van Schilfgaarde R. Distribution of collagens type I, type III and type V in the pancreas of rat, dog, pig and man. *Cell Tissue Res* 1994;277:115-121.
41. Stendahl JC, Kaufman DB, Stupp SI. Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation. *Cell Transplant* 2009;18:1-12.
42. Cirulli V, Beattie GM, Klier G, Ellisman M, Ricordi C, Quaranta V, et al. Expression and function of alpha(v)beta(3) and alpha(v)beta(5) integrins in the developing pancreas: roles in the adhesion and migration of putative endocrine progenitor cells. *J Cell Biol* 2000;150:1445-1460.
43. Heino J. Cellular signaling by collagen-binding integrins. In: Gullberg D, editor. *I Domain Integrins*. New York: Springer; 2014.
44. Kaido T, Yebra M, Cirulli V, Montgomery AM. Regulation of human beta-cell adhesion, motility, and insulin secretion by collagen IV and its receptor alpha1beta1. *J Biol Chem* 2004;279:53762-53769.
45. Kaido T, Perez B, Yebra M, Hill J, Cirulli V, Hayek A, et al. Alphav-integrin utilization in human beta-cell adhesion, spreading, and motility. *J Biol Chem* 2004;279:17731-17737.
46. Hughes SJ, Clark A, McShane P, Contractor HH, Gray DW, Johnson PR. Characterisation of collagen VI within the islet-exocrine interface of the human pancreas: implications for clinical islet isolation? *Transplantation* 2006;81:423-426.
47. Jiang FX, Naselli G, Harrison LC. Distinct distribution of laminin and its integrin receptors in the pancreas. *J Histochem Cytochem* 2002;50: 1625-1632.
48. Jiang FX, Cram DS, DeAizpurua HJ, Harrison LC. Laminin-1 promotes differentiation of fetal mouse pancreatic beta-cells. *Diabetes* 1999;48: 722-730.
49. Banerjee M, Virtanen I, Palgi J, Korsgren O, Otonkoski T. Proliferation and plasticity of human beta cells on physiologically occurring laminin isoforms. *Mol Cell Endocrinol* 2012;355:78-86.
50. Johansson S, Svineng G, Wennerberg K, Armulik A, Lohikangas L. Fibronectin-integrin interactions. *Front Biosci* 1997;2:d126-d146.
51. Britland S, Perridge C, Denyer M, Morgan H, Curtis A, Wilkinson C. Morphogenetic guidance cues can interact synergistically and hierarchically in steering nerve cell growth. In: Bridges CR, Sanders D, Curtis A, editors. *EBO-Experimental Biology Online Annual 1996/97*. Vol. 1996/1997. Heidelberg: Springer; 1998. p. 15-34.
52. Larsen M, Artym VV, Green JA, Yamada KM. The matrix reorganized: extracellular matrix remodeling and integrin signaling. *Curr Opin Cell Biol* 2006;18:463-471.
53. Ris F, Hammar E, Bosco D, Pilloud C, Maedler K, Donath MY, et al. Impact of integrin-matrix matching and inhibition of apoptosis on the survival of purified human beta-cells in vitro. *Diabetologia* 2002;45:841-850.
54. Wang RN, Paraskevas S, Rosenberg L. Characterization of integrin expression in islets isolated from hamster, canine, porcine, and human pancreas. *J Histochem Cytochem* 1999;47:499-506.
55. Nekoei SM, Azarpira N, Sadeghi L, Kamalifar S. In vitro differentiation of human umbilical cord Wharton's jelly mesenchymal stromal cells to insulin producing clusters. *World J Clin Cases* 2015;3:640-649.
56. Shaer A, Azarpira N, Karimi MH. Differentiation of human induced pluripotent stem cells into insulin-like cell clusters with miR-186 and miR-375 by using chemical transfection. *Appl Biochem Biotechnol* 2014; 174:242-258.
57. Shaer A, Azarpira N, Vahdati A, Karimi MH, Shariati M. miR-375 induces human decidua basalis-derived stromal cells to become insulin-producing cells. *Cell Mol Biol Lett* 2014;19:483-499.
58. Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells* 2007;25:2837-2844.
59. Bosco D, Meda P, Halban PA, Rouiller DG. Importance of cell-matrix interactions in rat islet beta-cell secretion in vitro: role of alpha6beta1 integrin. *Diabetes* 2000;49:233-243.
60. Wang X, Ye K. Three-dimensional differentiation of embryonic stem cells into islet-like insulin-producing clusters. *Tissue Eng Part A* 2009;15: 1941-1952.
61. O'Brien FJ. Biomaterials & scaffolds for tissue engineering. *Mater Today* 2011;14:88-95.
62. Barnes CP, Sell SA, Boland ED, Simpson DG, Bowlin GL. Nanofiber technology: designing the next generation of tissue engineering scaffolds. *Adv Drug Deliv Rev* 2007;59:1413-1433.
63. Daoud JT, Petropavlovskaja MS, Patapas JM, Degrandpré CE, Diraddo RW, Rosenberg L, et al. Long-term in vitro human pancreatic islet culture using three-dimensional microfabricated scaffolds. *Biomaterials* 2011; 32:1536-1542.
64. Pawar SN, Edgar KJ. Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 2012;33:3279-3305.
65. Sandler S, Andersson A, Eizirik DL, Hellerström C, Espevik T, Kulseng B, et al. Assessment of insulin secretion in vitro from microencapsulated fetal porcine islet-like cell clusters and rat, mouse, and human pancreatic islets. *Transplantation* 1997;63:1712-1718.
66. de Vos P, Faas MM, Strand B, Calafiore R. Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials* 2006;27:5603-5617.
67. Ha TLB, Quan TM, Vu D, et al. Naturally derived biomaterials: Preparation and application. In: Andrades JA, editor. *Regenerative Medicine and Tissue Engineering*. Croatia: InTech, Chapters Published; 2013. p. 247-274.
68. Lamb M, Storrs R, Li S, Liang O, Laugenour K, Dorian R, et al. Function and viability of human islets encapsulated in alginate sheets: in vitro and in vivo culture. *Transplant Proc* 2011;43:3265-3266.
69. Qi M, Strand BL, Mørch Y, Lacić I, Wang Y, Salehi P, et al. Encapsulation of human islets in novel inhomogeneous alginate-ca²⁺/ba²⁺ microbeads: in vitro and in vivo function. *Artif Cells Blood Substit Immo-*

70. Köllmer M, Appel AA, Somo SI, Brey EM. Long-term function of alginate-encapsulated islets. *Tissue Eng Part B Rev* 2015 [Epub ahead of print].
71. Prasitsilp M, Jenwithisuk R, Kongsuwan K, Damrongchai N, Watts P. Cellular responses to chitosan in vitro: the importance of deacetylation. *J Mater Sci Mater Med* 2000;11:773-778.
72. Dutta PK, Dutta J, Tripathi V. Chitin and chitosan: chemistry, properties and applications. *J Sci Ind Res* 2004;63:20-31.
73. McBane JE, Vulesevic B, Padavan DT, McEwan KA, Korbitt GS, Suuronen EJ. Evaluation of a collagen-chitosan hydrogel for potential use as a pro-angiogenic site for islet transplantation. *PLoS One* 2013;8:e77538.
74. Yang KC, Qi Z, Wu CC, Shirouza Y, Lin FH, Yanai G, et al. The cytoprotection of chitosan based hydrogels in xenogeneic islet transplantation: an in vivo study in streptozotocin-induced diabetic mouse. *Biochem Biophys Res Commun* 2010;393:818-823.
75. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* 1999;48:1907-1914.
76. Rak J, Weitz JI. Heparin and angiogenesis: size matters! *Arterioscler Thromb Vasc Biol* 2003;23:1954-1955.
77. Chow LW, Wang LJ, Kaufman DB, Stupp SI. Self-assembling nanostructures to deliver angiogenic factors to pancreatic islets. *Biomaterials* 2010;31:6154-6161.
78. Bennet W, Sundberg B, Lundgren T, Tibell A, Groth CG, Richards A, et al. Damage to porcine islets of Langerhans after exposure to human blood in vitro, or after intraportal transplantation to cynomolgus monkeys: protective effects of sCR1 and heparin. *Transplantation* 2000;69:711-719.
79. Freudenberger U, Hermann A, Welzel PB, Stirl K, Schwarz SC, Grimmer M, et al. A star-PEG-heparin hydrogel platform to aid cell replacement therapies for neurodegenerative diseases. *Biomaterials* 2009;30:5049-5060.
80. Borg DJ, Bonifacio E. The use of biomaterials in islet transplantation. *Curr Diab Rep* 2011;11:434-444.
81. Wolfe PS, Sell SA, Bowlin GL. Natural and Synthetic Scaffolds. In: Palua N, Suscheck CV, editors. *In Tissue Engineering: From lab to Clinic*. Berlin: Springer-Verlag; 2011. p. 41-67.
82. Chun S, Huang Y, Xie WJ, Hou Y, Huang RP, Song YM, et al. Adhesive growth of pancreatic islet cells on a polyglycolic acid fibrous scaffold. *Transplant Proc* 2008;40:1658-1663.
83. Hou Y, Song C, Xie WJ, Wei Z, Huang RP, Liu W, et al. Excellent effect of three-dimensional culture condition on pancreatic islets. *Diabetes Res Clin Pract* 2009;86:11-15.
84. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Del Rev* 2012;64:72-82.
85. Park TG. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* 1995;16:1123-1130.
86. Athanasiou KA, Niederauer GG, Agrawal CM. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* 1996;17:93-102.
87. Gibly RF, Zhang X, Graham ML, Hering BJ, Kaufman DB, Lowe WL Jr, et al. Extrahepatic islet transplantation with microporous polymer scaffolds in syngeneic mouse and allogeneic porcine models. *Biomaterials* 2011;32:9677-9684.
88. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980;210:908-910.
89. Vériter S, Gianello P, Dufrane D. Bioengineered sites for islet cell transplantation. *Curr Diab Rep* 2013;13:745-755.
90. Kozlovskaya V, Kharlampieva E, Zavgorodnya O. Encapsulation and surface engineering of pancreatic islets: advances and challenges. In: Lin C, editor. *Biomedicine*. Croatia: InTech, Chapters Published; 2012.
91. Yang HK, Yoon KH. Current status of encapsulated islet transplantation. *J Diabetes Complications* 2015;29:737-743.
92. Schrezenmeier J, Kirchgessner J, Gerö L, Kunz LA, Beyer J, Mueller-Klieser W. Effect of microencapsulation on oxygen distribution in islets organs. *Transplantation* 1994;57:1308-1314.
93. Beck J, Angus R, Madsen B, Britt D, Vernon B, Nguyen KT. Islet encapsulation: strategies to enhance islet cell functions. *Tissue Eng* 2007;13:589-599.
94. Vaithilingam V, Quayum N, Joglekar MV, Jensen J, Hardikar AA, Oberholzer J, et al. Effect of alginate encapsulation on the cellular transcriptome of human islets. *Biomaterials* 2011;32:8416-8425.
95. Montanucci P, Pennoni I, Pescara T, Blasi P, Bistoni G, Basta G, et al. The functional performance of microencapsulated human pancreatic islet-derived precursor cells. *Biomaterials* 2011;32:9254-9262.
96. Roshanbifar K, Salahshour Kordestani S. Encapsulating beta islet cells in alginate, alginate-chitosan and alginate-chitosan-PEG microcapsules and investigation of insulin secretion. *J Biomater Tissue Eng* 2013;3:185-189.
97. Jun Y, Kang AR, Lee JS, Park SJ, Lee DY, Moon SH, et al. Microchip-based engineering of super-pancreatic islets supported by adipose-derived stem cells. *Biomaterials* 2014;35:4815-4826.
98. Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, et al. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care* 2011;34:2406-2409.
99. Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. *Diabetes Care* 2006;29:137-138.
100. Elliott RB. Transplantation of microencapsulated neonatal porcine islets in patients with type 1 diabetes: safety and efficacy. 70th Scientific Sessions. Alexandria: American Diabetes Association; 2010. Abstract.
101. Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation* 2007;14:157-161.
102. Prochorov AV, Tretjak SI, Goranov VA, Glinnik AA, Goltsev MV. Treatment of insulin dependent diabetes mellitus with intravascular transplantation of pancreatic islet cells without immunosuppressive therapy. *Adv Med Sci* 2008;53:240-244.
103. Tuch BE, Keogh GW, Williams LJ, Wu W, Foster JL, Vaithilingam V, et al. Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care* 2009;32:1887-1889.
104. Valdés-González RA, Dorantes LM, Garibay GN, Bracho-Blanchet E, Mendez AJ, Dávila-Pérez R, et al. Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur J Endocrinol* 2005;153:419-427.
105. Maillard E, Juszczak MT, Clark A, Hughes SJ, Gray DR, Johnson PR. Perfluorodecalin-enriched fibrin matrix for human islet culture. *Biomaterials* 2011;32:9282-9289.
106. Bhang SH, Jung MJ, Shin JY, La WG, Hwang YH, Kim MJ, et al. Mutual effect of subcutaneously transplanted human adipose-derived stem cells and pancreatic islets within fibrin gel. *Biomaterials* 2013;34:7247-7256.
107. Buitinga M, Truckenmüller R, Engelse MA, Moroni L, Ten Hoopen HW, van Blitterswijk CA, et al. Microwell scaffolds for the extrahepatic transplantation of islets of Langerhans. *PLoS One* 2013;8:e64772.
108. Uzunalli G, Tümtas Y, Delibasi T, Yasa O, Mercan S, Güler MO, et al. Improving pancreatic islet in vitro functionality and transplantation efficiency by using heparin mimetic peptide nanofiber gels. *Acta Biomater* 2015;22:8-18.
109. Song JJ, Ott HC. Organ engineering based on decellularized matrix scaffolds. *Trends Mol Med* 2011;17:424-432.
110. Conrad C, Schuetz C, Clippinger B, Vacanti JP, Markmann JF, Ott HC. Bio-engineered endocrine pancreas based on decellularized pancreatic matrix and mesenchymal stem cell/islet cell coculture. *J Am Coll Surg*

- 2010;211:S62.
111. Mirmalek-Sani SH, Orlando G, McQuilling JP, Pareta R, Mack DL, Salvatori M, et al. Porcine pancreas extracellular matrix as a platform for endocrine pancreas bioengineering. *Biomaterials* 2013;34:5488-5495.
112. Goh SK, Bertera S, Olsen P, Candiello JE, Halfter W, Uechi G, et al. Perfusion-decellularized pancreas as a natural 3D scaffold for pancreatic tissue and whole organ engineering. *Biomaterials* 2013;34:6760-6772.